The management of diamondback moth and other crucifer pests

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Editors
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Editors Nancy M Endersby and Peter M Ridland

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Preface

The 4th International Workshop on the Management of Diamondback Moth and other Crucifer Pests was held in Melbourne from 26th to 29th November 2001. Following the tradition of the previous three workshops in this series, entomologists and others involved with diamondback moth (*Plutella xylostella*) and the *Brassica* industry came together from many parts of the world.

The Workshop provided a forum to review the approaches taken to management of diamondback moth in many countries and, as the fourth workshop in a series, also provided a valuable opportunity for renewing acquaintances and extending the international research community that has developed around the study of this insect. Delegates considered some of the long-standing challenges involved with implementing integrated pest management and managing insecticide resistance, but also had the opportunity to discuss emerging issues such as the use of genetically modified *Brassica* plants and use of molecular methods to characterise diamondback moth populations to study origins and dispersal.

Diamondback moth has become a key pest in Australian horticulture in the last 15 years due to the development of resistance to synthetic pyrethroid insecticides. Most recently, Australian broadacre *Brassica* crops (canola in Western Australia, New South Wales and South Australia; forage crops in Victoria and Tasmania) have also suffered extensive damage due to diamondback moth, particularly in times of drought. The Australian research effort on diamondback moth has increased in response to these challenges. In the vegetable industry, progress has been made in development and implementation of Integrated Pest Management programs. More challenges lie ahead as we endeavour to guide broadacre *Brassica* producers away from the insecticide treadmill, by directing research to more sustainable control methods and integrated systems. From this point of view, the year 2001 was a very appropriate time for the Workshop to be held in our country.

In these Proceedings, the Workshop papers have been put into a standard format where possible. While papers have not been extensively peer-reviewed, some required a major effort of editing and every attempt has been made to retain the original meaning and views of the authors. All claims of commercial products and processes as well as opinions expressed do not imply endorsement by the editors or the organisers. The papers are presented in the following sections: Population variation and dispersal, Biology of diamondback moth, Insect-plant interactions, Forecasting and sampling, Biological control, Insecticide resistance, Insecticides and Implementation of IPM.

The Workshop Organising Committee is very grateful to the sponsors of our Workshop, namely Dow AgroSciences LLC, Victorian Department of Natural Resources and Environment - Enhancing Science Networks Program, Syngenta Crop Protection Pty Ltd, Caltex Australia - Crop Protection Division, Organic Crop Protectants Pty Ltd, Amvac Chemical Corporation and Rotam Australasia. Their funds assisted us to bring eminent DBM researchers from around the world to attend as invited speakers. We thank Southcorp Wines Pty Ltd for providing a selection of fine Australian wines which were enjoyed by delegates at the Poster Session.

We also thank our Conference Secretariat: Bronwen Hewitt, Dominique Azzopardi, Jason Hewitt, Fiona Campbell, Michael Sullivan, Conference Management, The University of Melbourne. Thanks to Claire Braund and Roger Johnson of The Regional Institute Limited for publishing our Proceedings in printed form, on CD and on the web.

Most importantly we thank the 93 delegates from 23 countries whose participation resulted in a rewarding and productive workshop. We hope this volume will be a useful reference for both new and seasoned researchers of the remarkable insect, the diamondback moth.

Nancy Endersby, Chief Editor
Peter Ridland, Workshop Convener

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Foreword

The papers in this volume report on a wide range of research and related activities concerning the diamondback moth and other pests of cruciferous vegetables. The foundation of an effective pest management program is a thorough understanding of the targeted pests. Hence, a substantial portion of the research reported at the Workshop focused on the biology and ecology of the diamondback moth, especially its genetics, host finding behaviour, movement and interactions with natural enemies. Building upon this foundation of understanding, researchers reported on the development, implementation and evaluation of integrated pest management programs. This collection of papers provides an overview of the current understanding of the diamondback moth and approaches for its management. It is a testament to the achievements of all of the contributors, whose success will ultimately be measured in the fields of farmers around the world.

The scientific program was organised by Greg Baker, Michael Keller, Jianhua Mo, Peter Ridland, Nancy Endersby, Bronwyn Walsh and Richard Vickers. The aim was to develop a program that looked forward to the development of sustainable systems of pest management for cruciferous vegetable crops. Although not recorded here, Rick Roush, Myron Zalucki and Tony Shelton led discussions that facilitated the exchange of ideas among the delegates and stimulated the search for new approaches to pest management for the diamondback moth and other pests.

Greg Baker and Michael Keller
Scientific Program Conveners

Management of the diamondback moth: déjà vu all over again?

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Abstract

The diamondback moth, *Plutella xylostella* (L.), is considered the most universally distributed of all Lepidoptera and the main insect pest of crucifers worldwide. Although *P. xylostella* is confined to feeding on plants within the Cruciferae, this diverse family contains a wide host of weed species and cultivated plants that occur throughout the world. Over the last several decades the importance of crucifers in the human diet has increased, resulting in increased crucifer production and changing management practices. Where once crucifers were part of smaller diversified cropping systems, they are often now grown as monocultures under intensive cultivation practices, including year-round production. In areas of China, south-east Asia and other areas where crucifers are important to the human diet, there may be more than 20 generations of *P. xylostella* annually. As production practices have changed and the demand for damage-free produce has intensified, farmers have relied on intensive use of insecticides which, in turn, has contributed to insecticide resistance, reduced effectiveness of natural enemies, frequent control failures, and some environmental and human health concerns. Because of the health benefits and cultural significance of crucifers, especially in Asian populations, production will continue to increase. What have we learned from the past that will allow us to provide better management of *P. xylostella* in the future? A central element will be to develop preventative tactics and 'rescue' treatments which conserve natural enemies, promote resistance management strategies, and utilize cultural control practices that rely on understanding the agroecology of *P. xylostella*. Most importantly, successful management of *P. xylostella* will rely on farmers and scientists taking a more 'regional perspective' that includes information on *P. xylostella* movement and the effects of management tactics on their behaviour and population genetics on a community level.

Keywords

Plutella xylostella

The importance of the diamondback moth

Throughout the world the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is considered the main insect pest of crucifers, particularly cabbages, broccoli and cauliflowers (Talekar & Shelton 1993). *Plutella xylostella* is considered the most widely distributed lepidopteran pest, and occurs in diverse small scale and large scale agricultural production areas, as well as on weeds. A decade ago, Javier (1992) estimated the cost for controlling it at US$1 billion annually, but it is unclear how this cost was calculated.

The economic impact of *P. xylostella* can be evaluated by several methods. If one determines the potential value of the crop, the potential loss incurred if insects made the plant totally unmarketable, the cost of control to prevent it from being unmarketable, then the actual value of the crop minus the cost of control can be calculated as the loss caused by the insect. However, a plant will often not be made fully unmarketable, but may be reduced in yield or quality and require additional trimming to remove the damage. Furthermore, additional complications arise due to the large number of insecticides used against *P. xylostella*, their variable costs, the variable number of applications and their effectiveness. Further complications arise since some applications on crucifers may be targeted against other insect pests such as aphids or other Lepidoptera. Thus, there does not appear to be any reliable data on the total worldwide value of crucifers nor the losses incurred by *P. xylostella*. However, the following examples give some indication of the importance of *P. xylostella* in selected regions of the world (Shelton 2001).

China has the largest population in the world and cruciferous vegetables make up an important part of the Chinese diet. The area of cabbage and cauliflower grown in 1999 in China was 1.2 million ha. (FAO 2001) and *P. xylostella* is widespread in most provinces. There are five or six generations in Jilin Province in Northeastern China and up to 20 generations in Guangdong Province in Southern China. *Plutella xylostella* has been the most important insect pest of cruciferous vegetables especially in Southern China and the Changjiang River Valley in the last 20 years. When no sprays were applied to control *P. xylostella*, the losses

of summer cabbage in Shanghai were 99% in 1992 and 80% in 1994, compared with the plots treated with insecticides (Zhao *et al*. 1996). The estimated control costs are *ca.* US$100/ha for each crop for the peak periods (in April/May and September/October).

In the United States and Canada, the importance of *P. xylostella* is variable. Nearly 6 million ha of canola are grown in Canada and, in 1995, *ca.* 1.25 million ha were sprayed to control an outbreak of *P. xylostella* at an estimated cost of CN $50 million (Dosdall *et al*. 2004). In Texas it has been suggested that 100% of cabbage and at least 20% of broccoli would be unmarketable if it were not treated (T.X. Liu, personal communication). This translates to losses of $40M - $70M for cabbage and *ca.* $400K for broccoli. A similar situation also occurs in Florida where *P. xylostella* is the main pest of crucifers. In the more northerly latitudes of the US, the situation is different because of less pressure by *P. xylostella*. Cathy Eastman in Illinois (personal communication) notes that cruciferous vegetable crops are grown on *ca*. 30,000 acres in the Midwest and > 80% of the acreage needs to be treated at least once for the *P. xylostella* and *P. rapae* complex. Depending on the season, most growers may treat 2-3 times for this complex. This is the same situation noted by A. M. Shelton in New York, although during hot, dry years *P. xylostella* will be a much more difficult problem and, if no treatments are applied, much of the *ca*. $80 million cabbage crop would be unmarketable for fresh market cabbage. California is a main US producer of fresh market broccoli where it was grown on nearly 50,000 ha with a farm gate value of *ca.* $500 million. A severe infestation by *P. xylostella* in 1997 resulted in crop losses estimated to be > $6 million (Shelton *et al.* 2000).

Mexico is a major producer of broccoli and related crucifers used for processing and export to the United States. Most production is located in the 'El Bajio' region where more than 30,000 ha of broccoli are produced with a total farm gate value of >US $63 million. *Plutella xylostella* greatly reduces the yield and quality of the crop and accounts for the majority of insecticide use in crucifer production (Diaz-Gomez *et al.* 2000). If no sprays were applied for control of *P. xylostella*, it is reasonable to conclude that all plants would be unmarketable.

In Australia, Greg Baker (personal communication) notes that *P. xylostella* attacks the 136,000 hectares of major *Brassica* vegetable crops and is considered the chief insect pest. Crop loss due to *P. xylostella* damage in an average year is estimated to be *ca.* $A 8 million and control costs $A 12 million. Another important crop attacked by *P. xylostella* is canola and in Australia there is *ca.* 1 million ha. The crop loss in canola due to *P. xylostella* is estimated to be *ca.* $A 3 million and control cost $A 6 million.

Throughout Europe, *P. xylostella* attacks *Brassica* vegetables and field crops such as canola on a regular basis. These crops are grown throughout Germany with high concentrations of cabbage and canola in the northern parts of Germany. Cruciferous vegetables amount to one third of the total field vegetable growing area in Germany. There are no comprehensive data on yield losses due to *P. xylostella* attack but, in most years, the attack level by *P. xylostella* will be below an injury level and the pest will be controlled by spraying against the other two main lepidopteran pests, *Pieris rapae* and *Mamestra brassicae* (Martin Hammes, personal communication). In some years, particularly during hot, dry weather conditions, heavy attack and corresponding high yield losses can be observed. This situation is very similar to the Netherlands where 8,500 ha of cabbages and cauliflower are grown.

Other large producers of cabbages and cauliflower are India (530,000 ha), Russian federation (162,700 ha), South America (7,000 ha) and the combined area of Indonesia, Thailand and Vietnam which have a total of 78,655 ha (FAO 2001). Other cruciferous crops are also attacked by *P. xylostella*, but the value of these crops is unknown. Losses caused by *P. xylostella* in all these areas, especially in southeast Asia, can be very severe since *P. xylostella* has developed resistance to many insecticides (Talekar & Shelton 1993).

Because of the importance of *P. xylostella*, the pest was the subject of three international conferences prior to this meeting. The first two were held in Taiwan in 1985 and 1990, respectively, while the third was held in 1996 in Malaysia. It is important to note the changing emphasis of the talks over the three conferences with a marked trend for more presentations on biological control, insecticide resistance and IPM. The third and this present conference continue that appropriate trend.

Crucifers, humans and the history of *P. xylostella* problems

The history of *P. xylostella* is associated with the history of its diverse host crops. *Plutella xylostella* is restricted to the plant family Cruciferae, although a recent report has indicated that a population in Kenya

can survive on peas (Bernhard Löhr, personal communication). However, this example seems like an anomaly due to growing peas nearby crucifers. There are 220 genera of crucifers, which include many wild and cultivated plants, and they occur on all continents. Crucifers are characterised by a wide range of secondary plant compounds (glucosinolates) toxic to many insects, although the specialist *P. xylostella* has come to rely on some of them for host location and feeding. The glucosides sinigrin, sinalbin and glucocheirolin act as specific feeding stimulants for *P. xylostella* and 40 plant species containing one or more of these chemicals serve as hosts (Talekar & Shelton 1993). Non-host plants may contain these stimulants, but also contain feeding inhibitors or toxins (Gupta & Thorsteinson 1960). Allyl isothiocyanate also stimulates egg production in *P. xylostella* adults (Hillyer & Thorsteinson 1969).

Cruciferous vegetables, primarily brassicas, are important components of the human diet and are grown on small subsistence farms as well as large scale farms. In 2000, over 3.5 million ha of cabbages were harvested worldwide and these cabbages included Chinese cabbage, mustard cabbage, pak choi (*Brassica chinensis*), white and red cabbages, Savoy cabbages, Brussels sprouts, collards, kale and kohlrabi (FAO 2001). An additional 834,000 ha of broccoli were produced. These cruciferous vegetables are important component of the Asian diet and nearly 50% of them are produced in Asia, where loose-leafed cabbages are preferred. Modern hard-headed cabbages descended from wild cabbages that originated in the Mediterranean and Asia Minor. Wild cabbages were called "gifts from the Gods" and the Celts and Romans spread them throughout Europe. These cabbages were introduced into North America by early explorers in 1541. More recently, canola or rapeseed has become a major cruciferous crop with greater than 12 million metric tonnes produced in 2000 (FAO 2001). Products from canola/rapeseed include oils for human consumption, industrial oils and animal feeds. With the growth in area of canola/rapeseed, reports of problems of *P. xylostella* management have been reported in Canada, Australia and Europe.

While *P. xylostella* and crucifers have existed for centuries, it is clear that in many areas of the world there is greater concern about *P. xylostella* causing economic damage to crops. There are several potential reasons for this. In the US it has been estimated that 39% of our pests were brought over with the new crops, while in South Africa it has been estimated that 68% of the 188 arthropod species on 14 introduced crops switched hosts once another suitable host was introduced (Van Driesche & Bellows 1996). Clearly, the introduction of a new crop into an area can lead to insect pest problems. However, it is unlikely, for a number of social and economic reasons, that growing plants in a potentially suitable region will be restricted in the future so this will not be a realistic method for *P. xylostella* management. However, other human agricultural practices have caused large consequences for *P. xylostella*, and these are the ones that we may be able to help manage in the future. We have abundant evidence that *P. xylostella* problems have been exacerbated by the use of year-round crucifer cultivation which eliminates a break in the insect cycle. This practice, combined with an overuse of insecticides, often leads to control failures.

There are more than 500 cases of arthropods becoming resistance to a particular pesticide (Georghiou & Lagunes-Tejeda 1991) and *P. xylostella* is one of the 'leaders' in this area. *Plutella xylostella* is the first agricultural insect to have developed resistance to DDT (Ankersmit 1953, Johnson 1953) and, since that time, some populations of *P. xylostella* in certain areas have developed resistance to all known classes of insecticides. This includes resistance to Cry1A and Cry1C toxins of the bacterium, *Bacillus thuringiensis*, in many places (Shelton & Roush 2000) and, to a far lesser extent, to spinosad in specific areas of Hawaii (Zhao *et al.* 2002).

Prior to the introduction of pyrethroids in the early 1980s, the main classes of insecticides used for *P. xylostella* control were organophosphate and carbamate insecticides. Although pyrethroids have a higher level of safety for humans, there are other problems. Pyrethroids are fairly broad spectrum and their use often disrupts natural enemies because they are usually more susceptible than the pest species (Croft & Brown 1975, Croft 1990). In one of our recent works, we examined the potential to integrate biological and insecticidal control of *P. xylostella* with *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae), one of the most important parasitoids of *P. xylostella* in North America (Harcourt 1986, Lasota & Kok 1986, Hu *et al.* 1998, Shelton *et al*. 2002). Our results (Xu *et al.* 2001) indicate that *D. insulare* increases its tolerance to permethrin much more slowly than *P. xylostella* and appears limited in the extent of resistance it can develop. For example, we can often find populations of *P. xylostella* with several hundred-fold resistance to permethrin, but we were only able to detect a 5-fold level of tolerance in *D. insulare*. Thus, rather than building up tolerance in a parasitoid or predator of *P. xylostella*, it may be more appropriate to use an insecticide which will have less impact on the parasitoid in the first place. Compared with the organophosphates, carbamates and pyrethroids, materials such as Bt, spinosad, indoxacarb and emamectin

benzoate are generally considered less harmful to natural enemies. Additional studies are being performed in various laboratories to determine their impact on natural enemies of *P. xylostella* under field conditions and this type of information is sorely needed.

The future for managing *P. xylostella*

As has been written, "Past experiences with diamondback moth management have reinforced the belief that single component strategies will fail" and we need to rely on other cultural and biological controls to a greater degree (Talekar & Shelton 1993). What is needed is an integrated approach using host plant resistance, biological controls, cultural controls, behavioural management and judicious use of insecticides. None alone will work sufficiently, but together they can complement each other and lead to a more sustainable system. However, underlying all of these strategies must be a more complete understanding of the movement patterns of *P. xylostella* and the causes that influence movement. To develop sound management strategies for *P. xylostella* will require an understanding of the ecology of the landscape in which *P. xylostella* and its natural enemies interact with the rest of this agroecosystem.

We really know very little about movement of *P. xylostella,* although there are scattered reports that it can move long distances (Chu 1986). For *P. xylostella* adults, the spatial and temporal relationship of cruciferous weeds and crops, and wind patterns will influence movement and the probability of infestation. The scale of the movement can be local (within an individual field or between adjacent fields), or regional or intercontinental. Perhaps little can be done to prevent regional movement of *P. xylostella* other than to ensure they are not being transported on transplants (Shelton *et al.* 1996), but movement between plantings or within a region can be manipulated with an understanding of the insect's biology and some careful planning.

Local movement can have a profound impact on pest management practices. As was seen with the development of resistance to spinosad in Hawaii (Zhao *et al.* 2002), growers essentially treated individual crucifer plantings within a field as discrete populations of *P. xylostella* when, in fact, the real population travelled freely between all the plantings and was continuously being selected for resistance every time one of the plantings was treated. If this field population did not also have susceptible *P. xylostella* immigrating into it to dilute resistant alleles, development of resistance would be further exacerbated. Likewise, it is important to understand adult movement patterns of *P. xylostella* to assess the potential for mating disruption using pheromones. Previous studies have shown mixed results (McLaughlin *et al.* 1994, Schroeder *et al.* 1999), perhaps not only because of the pheromone blend but also because of a lack of knowledge of adult movement patterns. Likewise, the dissemination of microbial-infected *P. xylostella* adults (Vickers *et al.* 2001) will depend on the propensity of infected adults to move and infect other adults. In a similar fashion, an understanding of movement behaviour will be essential to understand whether trap cropping can be an effective tool for arresting the movement of *P. xylostella* adults as they seek oviposition sites. However, it is not only the movement patterns of adult *P. xylostella* that should be examined, but also the movement patterns of their natural enemies (Schellhorn & Silberbauer 2002).

Putting the parts together: an example from Mexico

One size will not fit all, and management practices appropriate for a small farmer in Thailand may not be suitable for a large-scale grower in Australia, but each can learn something from the other. In Mexico there is a large scale *P. xylostella* management program that tries to implement the latest developments in technology using a landscape management approach, but it does not get much 'publicity' since it is a commercial operation. The 'El Bajio' region in central Mexico has been in broccoli production for over 40 years and grows up to 40,000 ha of broccoli for processing. Since much of this production is exported to the US, the quality standards are high. There are seven processors who work with the growers who supply the raw product, and these processors cooperate to a large extent because they work in the same region, often in adjacent fields, and what affects one processor will affect the others.

In the late 1980s, growers in El Bajio began to have control failures because of resistance to pyrethroids and they sought advice from people who were familiar with crucifer production. The general advice was to back away from pyrethroids to allow natural enemies to exert some control, start using Bts but make sure coverage is good, sample the fields regularly and set a threshold, monitor for resistance on a regular basis and, most importantly, have a host-free period to break the life cycle of *P. xylostella* in that region. Generally, these recommendations were followed and the number of sprays declined by >60% in a single season without any loss in product quality. However, now growers are reporting instances of some

populations of *P. xylostella* becoming tolerant to Bt, but it is still generally effective and the situation is not nearly as bad as it was with pyrethroid resistance.

As the growers and processors assess their current situation, their goals are to decrease insecticide use while maintaining or increasing the quality of the product, conserve the effectiveness of the new classes of insecticides (spinosad, emamectin benzoate and indoxacarb) and the older classes as well, enhance the use of biological control and cultural controls, and implement novel strategies if they prove effective in small trials. To achieve these goals will require something unusual in business - cooperation between companies. The seven Mexican processors can be viewed as the 'funnel' through which the raw product flows and they must cooperate to have a regional strategy that can be followed by the individual growers. The processors must be the ones to work together to ensure that the host-free period is followed since it is the foundation for the regional management. Likewise, the processors must implement a regional resistance management program for the newer insecticides which uses a 'window strategy' so that selection pressure for resistance to one insecticide is not constant. While they recognise the need for this (even more so now that resistance to spinosad has occurred in Hawaii), they are struggling to determine how they can do this over 40,000 ha which encompasses hundreds of different growers and for insecticides that have variable costs and efficacy. Through experience they have come to respect insecticide resistance and value the use of other tactics such as conserving natural enemies and even making augmentative releases of parasitoids. Fundamentally, they have begun to take a landscape perspective on managing *P. xylostella* and have initiated efforts to combine tactics and share data and knowledge more than ever before. It is too soon to determine the outcome, but it is an important program from which we can learn.

The future needs of the *P. xylostella* research community

It is important to ask what we have learned from these previous three conferences as we begin the fourth conference. One central point is that there is a wealth of information about *P. xylostella* and we need to communicate this knowledge within the *P. xylostella* research community. Secondly, nearly all of us who work in *P. xylostella* management have experienced control failures and these appear to have been due to one or more of the following: insecticide resistance, changing cultivation practices and the lack of effective biological control. Each of these causes may be influenced by the other. Third, management of *P. xylostella* will require an integrated approach since reliance on any single method is sure to fail in the long (and often short) run. Finally, it has become apparent that there is still much to be learned and that we must work as an international community so that we can develop and implement more sustainable and reliable strategies for managing *P. xylostella* in the future. What we don't want to see is *déjà vu* all over again.

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Brassica IPM adoption: progress and constraints in south-east Asia

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Abstract

The Integrated Pest Management (IPM) programme for brassicas in many countries of south-east Asia follows a generic model whose basic components are: (i) Regular scouting of pests, namely the diamondback moth (DBM) and its major natural enemies to ascertain their population levels to justify insecticidal treatments; (ii) Use of pre-determined economic threshold levels (ETLs) and (iii) Incorporation of other non-chemical control measures, such as the release and conservation of parasitoids, use of trap crop and yellow sticky traps. The current empirical level of adoption by growers of this programme ranges between 50 to 100% and reasons given by growers for adopting IPM include: (i) Financial gains (higher net returns, decreased costs of control), (ii) Reduction in damage by pests, (iii) No unwarranted problems due to pesticide residues and (iv) Reduction of risks in terms of yield. However, despite the widely acknowledged benefits of the programme, the grower adoption rate in many countries has been rather slow. The major constraints to adoption were (i) the difficulty to comply with procedures determining economic threshold levels (ETLs), (ii) lack of management consideration for the other pests complex besides DBM and (iii) the weak support provided by extension agencies. The strategies to increase adoption have been outlined and these include overcoming the various current technical and non technical constraints, such as simplifying the ETLs, strengthening the linkage between research and extension and formulating technological baskets rather than technological packages. The need to crystallise inputs from social and behavioural scientists at the formulation stage of the programme was underscored.

Introduction

The *Brassica* crop system and components of the IPM programme

In south-east Asia, a wide range of *Brassica* vegetables is cultivated practically throughout the year both in the highlands and specialised areas in the lowlands (Talekar & Shelton 1993). Although these vegetables are traditionally grown in the open, recently, in many countries, cultivation has shifted to rain or insect protected structures. Irrespective of the system, growers use intensive cultivation practices to produce the vegetables in areas averaging less than two acres.

The *Brassica* Integrated Pest Management (IPM) programme is amongst the most well-worked IPM programmes for a particular pest problem (in this case, the diamondback moth and associated pests) in the world. In south-east Asian countries, the diamondback moth, *Plutella xylostella* (L.) is a pest of common concern and, considering the magnitude of its resistance problem against a wide range of synthetic pesticides, it was only natural that some of the earliest efforts in IPM were initiated in countries namely Malaysia (Loke *et al.* 1997, Sivapragasam *et al.* 1985), Indonesia (Sudarwohadi 1996), Thailand (Piyarat *et al.* 1997), Vietnam (Lim 1992) and the Philippines (Eusebio & Rejesus 1997). Some of the leading countries in south-east Asia and the key components of their *Brassica* IPM programmes are shown in Table 1.

Table 1. Key components of the *Brassica* IPM programme in major *Brassica* growing countries of south-east Asia

Country	Key Components
Malaysia	Economic thresholds, incorporation of parasitoids, cultural practices, use of biopesticide, *Bacillus thuringiensis* (Bt)
Philippines	Economic thresholds, incorporation of parasitoids, IPM selective insecticides
Thailand	Economic thresholds, incorporation of parasitoids, use of microbial insecticides, yellow sticky traps
Indonesia	Economic thresholds, incorporation of parasitoids, use of microbial pesticides
Vietnam	Economic thresholds, Bt and other microbials, intercropping, crop rotation (cucurbits and crucifers) and cultural practices

Essentially, the IPM programme in many of these countries follows a generic model and relies on the basic components such as: (i) regular scouting of pests, namely the diamondback moth (DBM) and its major natural enemies, to ascertain their population levels to justify insecticidal treatments; (ii) use of pre-determined economic threshold levels (ETLs) and, in some cases, (iii) the incorporation of other non-chemical control measures, such as the release and conservation of parasitoids, use of trap crops and yellow sticky traps. Besides these key components, other control components have been gradually added to the IPM 'basket' or repertoire, depending on the local pest requirements and geographical location, i.e. whether it is highlands or lowlands.

Status of *Brassica* IPM adoption

Although studies on adoption of agricultural innovations are many (see Rogers 1968), there are only a few studies done investigating the adoption of IPM innovations by farmers (Grieshop *et al.* 1988). However, despite the abundance of information on *Brassica* IPM, studies investigating the adoption process of the *Brassica* IPM programme by growers in the countries of south-east Asia are significantly lacking. Thus, to gauge the number of farmers adopting the IPM approach, a cursory survey was done by way of providing the relevant questions via a questionnaire to key IPM personnel in the countries of the region. Responses obtained from the four major countries are shown in Table 2.

Table 2. Initial and current adoption values of *Brassica* IPM in four south-east Asian countries

Country	Initial	Current
Malaysia	Low (30–49%)	Moderate (50–79%)
Indonesia	High (80–100%)	Moderate (50–79%)
Thailand	Moderate (50–79%)	High (80–100%)
Philippines	Moderate (50–70%)	High (80–100%)

Depending on the country, the current empirical level of adoption by growers, as perceived by the implementers from each country surveyed, ranged between 50 to 100%. Detailed studies, however, need to be initiated to quantify whether the values given actually reflect the situation on the ground, especially for countries such as Thailand and Philippines which indicated high adoption values.

These values do not categorise the farmers on the level of adoption based on the number of components farmers use in their programme. Based on the United States Department of Agriculture (USDA) standards, in IPM-based fields, adoption was categorised into three levels namely, (1) low adoption where scouting (S) and pesticide applications based on thresholds (T) for one type of pest are advocated; (2) medium IPM adoption which involved S + T plus 1 or 2 additional IPM practices being implemented and (3) high IPM adoption when S + T plus 3 or more additional IPM practices are used within the farm (Benbrook *et al.* 1996). Based on these criteria, the level of adoption of *Brassica* IPM tended to be moderate with most of the countries using at least 1 or 2 other additional components besides S and T (Table 1). With the exception of Malaysia *(vide infra*), no specific data are available in the literature to ascertain any trend in the adoption rate (e.g. *S*-curve, Rogers 1968) since the beginning of the IPM programme. Based on the survey, the reasons given by growers for adopting the IPM programme (% of respondents) include: (i) financial gains such as higher net returns and decreased costs of control (20%), (ii) reduction in damage by pests (27%), (iii) personal interest (13.5%), (iv) easy to use (6%), (v) little risk involved in terms of yield etc. (27%) and (vi) other reasons such as no residue of pesticides (6%). It is interesting to note that the latter reason did not feature prominently with the growers as, to most researchers and government policy makers, the problem of residues is an important driving force for initiating the IPM programme.

Constraints to adoption

Some of the reasons from respondents for the lack of interest shown by farmers in adopting the IPM programme included: (i) lack of confidence in the technology, (ii) difficulty in complying with the procedure of monitoring and counting insects based on the pre-determined economic threshold levels (ETLs), (iii) time consuming procedure and (iv) lack of understanding of the benefits of the programme.

Difficulty in complying with scouting for pests and their natural enemies

Likewise in many other IPM programmes, sampling populations and treating them according to the pre-determined ETLs is basic to the implementation of the *Brassica* IPM programme. However, counting of insects and determining the ETLs were noted to be significant problems faced by growers. Essentially, the system of monitoring insects should not be too laborious in nature since regular scouting requires labour and trained personnel–resources that are in short supply in many developing countries. In fact, the *Brassica* IPM programme in many countries has moved towards one of increasing complexity towards the determination of ETLs. This is exemplified by the programme in Malaysia whereby the system has evolved from one of basic threshold determination based on pest counts only (e.g. DBM larvae) (Sivapragasam *et al.* 1985), to that which included the counts of parasitoids (Loke *et al.* 1992) and eventually to one that incorporated the other key pests besides DBM (Jusoh 1997). Although these features evolved out of the inevitable necessity to portray as realistically as possible the vagaries of the *Brassica* system, unfortunately this process of ETL determination poses a crucial impediment to the wide adoption of the *Brassica* IPM programme in that country. Compounding this, Talekar and Shelton (1993) suggested that in many countries, the adoption of *Brassica* IPM is also hindered because many farmers cannot differentiate pests and beneficial organisms. Although alternatives to physical counting had been looked into, such as the yellow trap and pheromone trap, these tended to have limited predictive utility.

Dearth of consideration to counter a complex of pests

Another constraint inherent in most of the programmes is that these programmes are skewed towards the specific management of the diamondback moth (DBM) and lack the necessary control technological inputs to manage the other pests and pathogens. Therefore, a holistic approach to tackle the other occasional and recurrent pests will be crucial if the ultimate objective of maximising yields is to be achieved. For example, in Malaysia, *Brassica,* especially head cabbage cultivation, is limited in the lowlands by the presence of the cabbage webworm, *Hellula undalis,* flea beetle, *Phyllotreta* spp. and recently, *Spodoptera exigua.* A similar situation exists in the other Southeast Asian countries such as Thailand and the Philippines where *H. undalis* is a major problem. In addition, pathogens, *Erwinia craccivora* causing bacterial soft rot and *Xanthomonas* spp. causing bacterial wilt are major problems to *Brassica* cultivation both in the lowlands and highlands in many of these countries.

Weak and non-sustainable extension link and transfer of technology

One of the major constraints faced by IPM implementers is the difficulty to sustain the interest of those already ‘converted’ to the programme. The situation is compounded by pesticide sales pressure, lack of sustained support from the relevant change agents and dearth of external funds. More recently, probably as a result of increasing costs for legal pesticides, there are rampant sales of cheap illegal pesticides in the market. One effective mechanism to counter many of these problems will be to strengthen the existing extension component of the IPM programme. Wearing (1988) stated that “problems with the transfer of IPM technology are today identified as a principal bottleneck limiting progress with IPM worldwide despite rising pesticide costs and resistance problems.” He also stated that the lack of extensive educational programmes is a major barrier to IPM adoption. It has been underscored that promoting area-wide adoption involves key elements of training, extension and transfer of technology (Saharan *et al.* 1996). The pertinence of a strong extension component in the adoption process is exemplified by the situation in the Cameron Highlands, Malaysia. Figure 1 shows the rate of adoption by growers of the *Brassica* IPM programme initiated on an area-wide basis with strong extension support.

The general trend of the adoption curve, up until 1994, revealed the typical S-shape (Rogers 1968), but after that time decreased and has remained somewhat at that level ever since. One of the key contributing factors to the downward trend is, by and large, related to the significant absence of the extension component which resulted from the termination of the IPM project in that area with the desirable management of the pesticide residue problem. Currently, it is common to see growers applying pesticides on a routine basis and the IPM adoption level stands at around 30% from a peak of almost 70% with the significant presence of the extension component. It seems obvious that the strengthening of the extension component within the adoption process could alleviate some of the major constraints faced by farmers such as their lack of confidence in the programme and its perceived benefits.

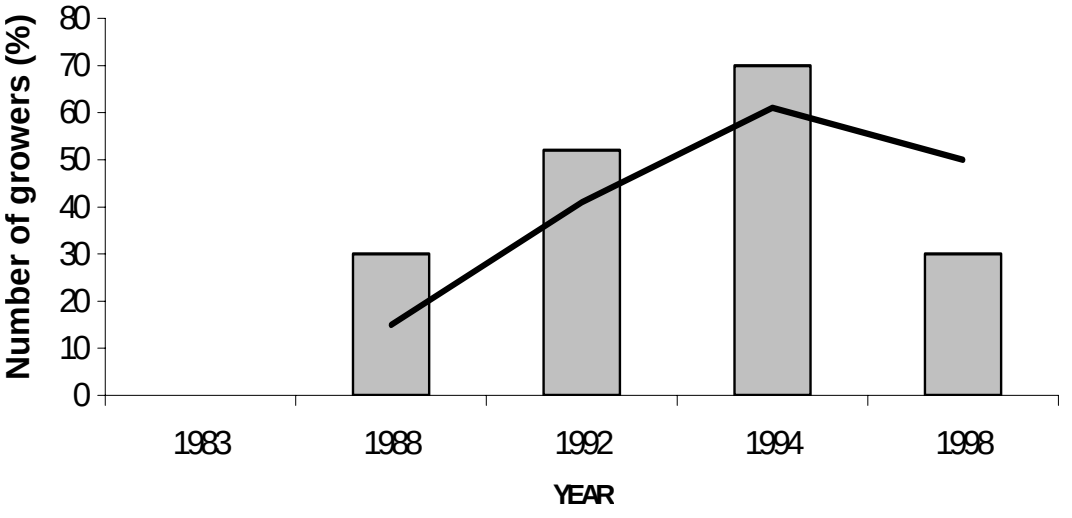

Figure 1. Rate of adoption of the *Brassica* IPM programme by farmers in the Cameron Highlands, Malaysia. Trend curve (------) is best curve by computer.

Recommendations and needed shifts to increase adoption

It is clear that not all growers adopted the *Brassica* IPM programme. In fact, in some cases, the adoption rate is on the decline and growers continue to apply pesticides on a routine basis. This is despite the numerous advantages shown in studies with regards to the benefits of the *Brassica* IPM programme (Sudarwohadi 1996, Eusebio & Rejesus 1996, Loke *et al.* 1992, Sivapragasam *et al.* 1985). The benefits include, decreased or the same costs of control, higher net returns and reduction in risks as measured by variability in quality or average level of net return is the same as or lower than that found with the conventional approach. Norton (1982) underlined that research and development in pest management does not always lead to practical improvements. The issues generally fall into two categories, namely, (1) design, whereby R&D is aimed at the wrong questions or at developing inappropriate practices and (2) delivery, whereby despite the product being well targeted, the results are not getting through to be implemented by the pest managers and their advisers (Figure 2).

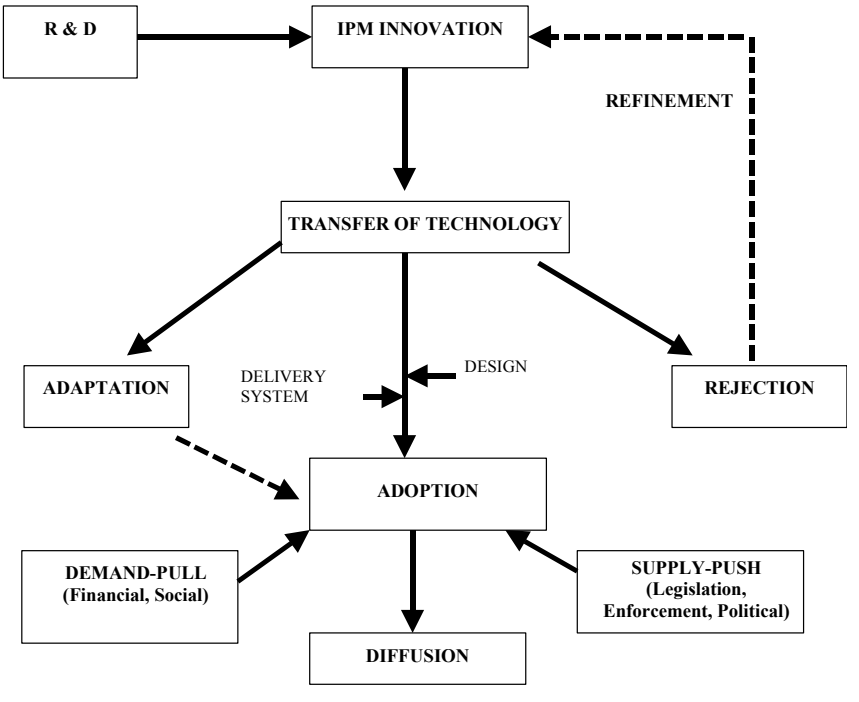

Figure 2. Process flow from IPM development to adoption and diffusion

Both these issues are relevant in the current context of the *Brassica* IPM programme. Besides the fact that some farmers do refine and adapt the technology to suit their specific needs, two of the key determinants for increasing the adoption of the *Brassica* IPM programme are in the refining of the design (e.g. simplifying

ETLs, considering pests complex) and the delivery mode of the IPM programme. In the latter instance, which could be pivotal to sustain the desired level of adoption, it is necessary to either apply the Supply-Push strategy (e.g. pesticide residues and health related issues) through legislation, enforcement or simply political will or the Demand-Pull strategy whereby the inherent advantages of adopting the IPM programme (financial, risks reduction etc.) are perceived by the farmer as desirable towards meeting their farming objectives (Figure 2).

It is also important to recognise the fact that IPM involves "*a complex set of behaviour, decision-making procedures and methods, technology and values organised to provide efficient alternative methods to pest management"* (Apple & Smith 1976). Therefore, to improve the level of adoption, it will be necessary to understand the social aspect, namely the perception of growers and the adoption process which could prove useful for designing and dissemination techniques relevant to IPM. Figure 3 shows the perceptive factors involved in the grower's decision making process (Heong 1981) which need to be harmonised during the formulation of the IPM programme if the farmer's ultimate objectives of yield and net revenue are to be met.

Besides these, the socio-economic profile of the growers also provide pertinent information on their perceptions, actions and needs. As pointed out by Grieshop *et al.* (1988), using the Tomato IPM programme as a model, factors such as age, education level, size of farm holding, land ownership, type of enterprise (whether family owned or otherwise), growers sources of information and previous experience with IPM, are important socio-economic considerations that affect the decision making process and the eventual adoption rate of the IPM programme. For example, land ownership, whether it is owner cultivated or rented based on the temporary occupation of land (T.O.L.) scheme could be a major determinant on the way in which that particular land is used. Usually, farmers on T.O.L., unlike owners, tend to intensify and maximise their yield output from the unit land given their limited time frame, without consideration to the entailing risks of pollution by pesticides.

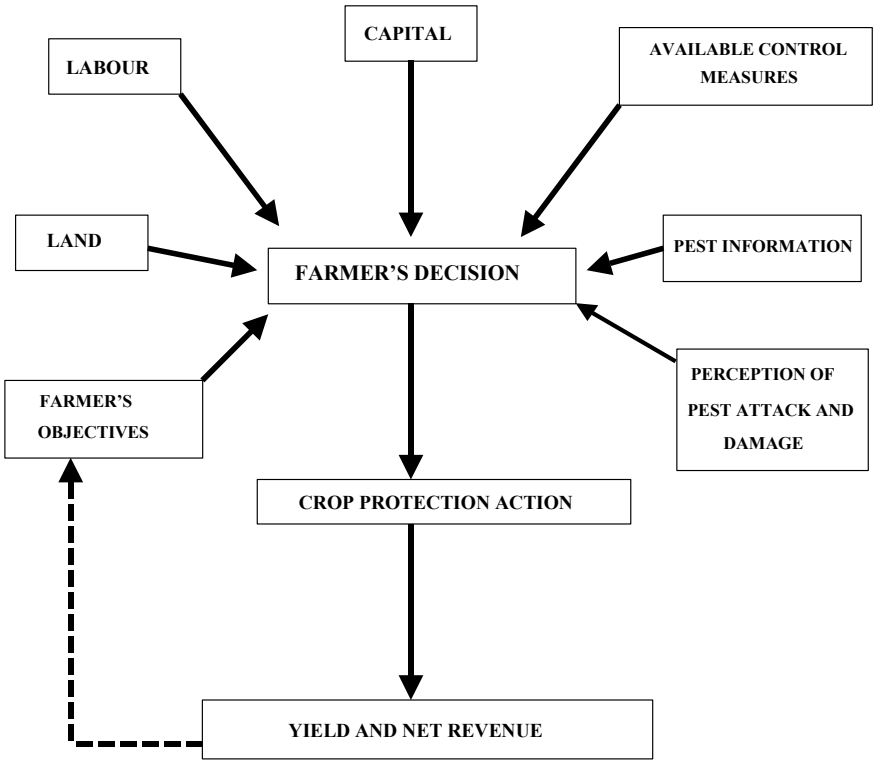

Figure 3. Factors influencing farmer's decision making (after Heong 1981)

To date, many evaluations of the effectiveness of IPM by research personnel typically have focused on the economic considerations. However, Allen and Rajotte (1990) added that, although economic considerations are significant in the decision making process related to the use of IPM, non-economic factors such as the complexity of the innovation (as with the use of ETLs), compatibility of control measures (compatibility of pesticides against pests), ease of use and the ability to respond to the risks of uncertainty to pest attacks are

important to the adoption of an innovation such as IPM. It is, therefore, not surprising that insecticides, which are a product unlike IPM which could be construed as a process or information, fit well into the mould of a good innovation easily adoptable by growers.

To improve the adoption of the *Brassica* IPM programme, some of the following shifts could be considered:

Technology packages to technology baskets

To tackle the problem of a complex of pests in a realistic manner, there is a need to consider the management of a multiple pest complex present on the crop. Thus, there is a need to apply the system analysis and decision tools approach to develop 'technology-baskets' rather than 'technology packages' which are suitable and flexible for problem solving at different locations. In this context, therefore, there is need to equip farmers with a repertoire of knowledge to manage these multiple pests in an integrated manner. Intricately linked to this is the grower's inability to distinguish between the pests and beneficial organisms. Currently, there is an abundance of information on the various components of the *Brassica* IPM programme. The challenge is in the intelligent application of those that are already present in a sound political, social and economic manner. In this context, there is a need to ensure that the IPM programme is well beyond the technical realm and needs to be complemented by social, political scientists and resource economists.

Naturally, factors outside the realm of pests should also be considered in the formulation and eventual implementation of the IPM programme. In this context, a shift towards a crop-based management system will, by and large, encompass the varied needs of all stakeholders of the *Brassica* system. This may eventually lead to the sustainability of the *Brassica* system portrayed as Level III of IPM integration based on Kogan (1998) whereby there is integration of multiple pest impacts and the methods for their control within the context of total cropping system.

Complex ETLs to action thresholds

The difficulty and complexity of determining ETLs could be overcome if adequate training is provided to farmers and if professional services are used, just like in the developed countries. The use of simplified action thresholds based on level of pest damage may be an alternative to actual pest counts and is worth considering (Walker *et al.* 2004).

Broad spectrum to selective insecticide use

Successful *Brassica* DBM-IPM programmes have been dependent on key parasitoids such as *Diadegma semiclausum* and *Cotesia plutellae.* Therefore, if the programme is to succeed with parasitoids, a concerted effort should be made to use insecticides that are compatible with these natural enemies. Otherwise, problems seen in Indonesia for the control of the cabbage head caterpillar, *Crocidolomia binotalis,* using insecticides which induced DBM outbreaks as a result of mortality of *D. semiclausum* (Shepard & Schellhorn 1997), could be repeated. For the moment, *B. thuringiensis* is still used widely. However, with the impending concern about resistance to this microbial insecticide, there is a need to look at compatible alternatives.

Top-down issue focus to bottom-up farmer focus

Since successful IPM programmes generally have a 'farmer-focus,' besides the strengthening of the extension arm of the implementation model, non-formal education methods such as farmer field schools (FFSs) and the Area Field Laboratory (ARF) (Ooi 1998) need to be promoted to make farmers literate in pest management practice. Zainal *et al.* (1994) suggested that a strategy to help growers understand reasons for possibly using less agricultural chemicals and inorganic fertilisers is shown to be the single most effective approach for promoting the more widespread adoption of sustainable practices for cabbage production. Research should not only confine itself to the priorities determined based on 'top-down' manner. As suggested earlier, it should also encompass the needs, perceptions and socio-economic milieu of the growers, be less compartmentalised and 'bottom up' participatory and feedback oriented in nature. The primary focus therefore is to concentrate on the elements of transfer, i.e. simplifying and delivering the *Brassica* IPM programme to achieve a desirable level of adoption. For a start, the strategies include modifying the current implementation method, either as "adaptive implementation" (Wearing 1988) such as refining the current ETLs determination procedures for ease of use by the grower or through "programmed implementation" (Wearing 1988) in which the grower is "educated" adequately to understand the crop and

its environment as it is done based on the participatory approach advocated in the FFSs approach. Unless appropriate IPM component tactics are assembled together into pragmatic IPM programmes, the integrated approach towards pest management will not fully materialise and eventually its rate of adoption will be affected.

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Improving the integration of pest management practices: theoretical and practical challenges

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Abstract

In order to improve the level of adoption of integrated pest management (IPM), it is important to understand the benefits of integrating IPM practices. Integrated systems are less prone to failure and, when they incorporate natural enemies, they are also more resilient than systems that rely on a single method of pest suppression. The impact of changing IPM practices on the pest population dynamics was evaluated in a qualitative manner by varying the parameters of the Lotka-Volterra Model. Factors that reduced the mean population density, reduced the amplitude of pest population fluctuations or increased the interval between pest population peaks were considered to improve IPM systems. Practices that reduce the net reproduction of pests, like resistant plant varieties and promotion of generalist natural enemies, are one way to improve IPM systems. Providing food resources for specialist natural enemies can also improve the level of control. Both broad-spectrum and selective insecticides can disrupt biological control systems that involve specialist natural enemies, so it is important to use pesticides only as a last resort. Even when IPM practices deliver only a fraction of the overall level of control, they can contribute to an effective IPM system. There are many practices that can be incorporated into integrated systems directed at management of the diamondback moth (DBM). Further theoretical and empirical research is needed to assist farmers with the implementation of integrated systems for management of DBM and other pests.

Keywords

Lotka-Volterra Model, conservation biological control, integrated pest management

Introduction

There is a growing awareness that the potential of integrated pest management (IPM) is not being realised in many crops (Ehler & Bottrell 2000, Stephenson *et al.* 2001). Elements of IPM practice, such as the use of economic thresholds in making decisions, are widely recognised as important tools for the management of pests. However, biologically-based IPM systems have not been developed in many instances, in part because of poor understanding of the ecological complexity of farming systems (Ehler & Bottrell 2000).

The integration of pest management systems can occur at several levels (Kogan 1988). At the lowest level, the methods used against a single pest can be carefully combined to deliver more effective pest suppression. At higher levels, integration can include the methods used against several to many pests in different classes of organism (insects, weeds, plant pathogens) and also can involve the concerted efforts of farmers, advisers, researchers and others involved in IPM.

The integration of pest management methods can enhance the level and reliability of pest suppression. Such integration has strong theoretical foundations. With a clearer understanding of these principles, better integrated management systems can be developed for use against the diamondback moth and other crucifer pests. These are the subjects of this paper.

Why integrate pest management methods?

Integrated systems of pest management are potentially more reliable and robust than those based on a single method of pest suppression. This can be understood by considering three hypothetical pest management systems used against a lepidopteran pest. In the first, a single method is used to control the target pest, for example, an insecticide spray that delivers 95% control. In the second, partial host plant resistance and augmentation with egg parasitoids each deliver 78% control. In the third, generalist egg predators and parasitic wasps that attack small larvae and sprays of *Bacillus thuringiensis* directed against large larvae, each deliver 63% control. If it is assumed that there are no interactions that affect the efficacy of the methods, then it is easy to see that all three systems deliver the same overall level of mortality (Figure 1). These examples illustrate the value of using methods that do not completely control pests when they are combined in integrated pest management systems.

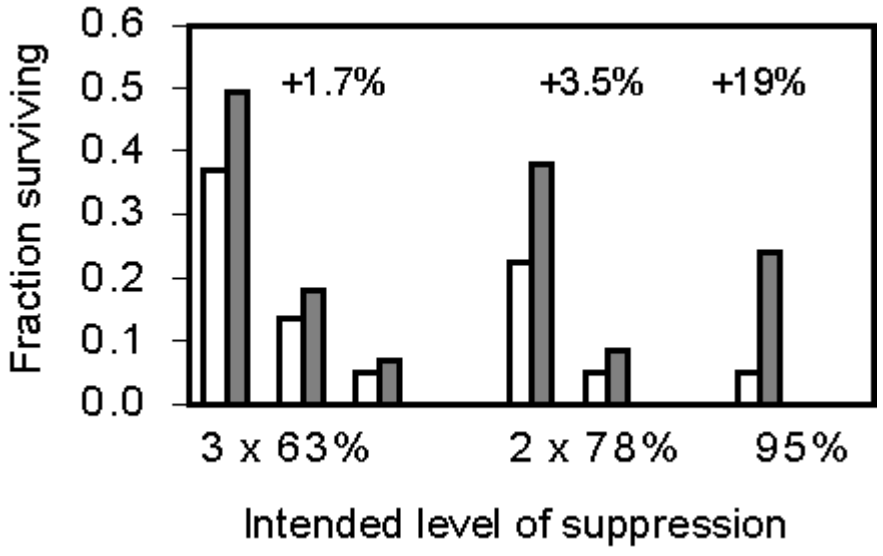

Figure 1. A comparison of the effectiveness of three different pest management systems. To achieve 95% pest suppression, it is possible to use one highly effective method that delivers the full 95% reduction in numbers (right), two methods that reduce numbers by 78% each (middle) or three methods that reduce numbers by 63% each (left; the fraction surviving each practice is shown in successive open bars). However, if the first method employed in each system is 20% less effective, then the overall level of control is poorest if only one method is used (19% greater pest survival) compared with systems that employ two (+3.5%) or three (+1.7%) control methods (grey bars).

The efficacy of pest management methods can vary. For example, rainfall after an insecticide spray can wash some of the active ingredients off treated plants. To see how such variation can influence the overall degree of control in integrated pest management systems, consider the previously-discussed hypothetical systems of pest management. In particular, assume that the first method in each alternative system was 20% less effective, i.e. 20% fewer pests died. In this case, the single insecticide spray would allow 19% more pests to survive while only 1.7% more pests would survive if the combination of three integrated methods was used (Figure 1). This example shows that integrated pest management systems are more robust, i.e. they are less prone to failure.

Biologically-based IPM systems are also resilient. Following natural or human disturbances, natural enemies and other biotic factors that limit pest damage can recover through the combined effects of reproduction and immigration. The development of resilient and robust pest management systems should be a primary goal of pest management practitioners.

The theoretical foundation of integration

There are three ways to reduce pest numbers within an agricultural production system: reduce the net reproduction of the pest, increase the pest's mortality or inhibit immigration. The latter is not considered theoretically here, but where knowledge of the factors that influence pest movement is available, inhibition of immigration into crops should be pursued as a management method, e.g. by planting trap crops (Hokkanen 1991, Mitchell 2000).

The predictions of the Lotka-Volterra model illustrate in a qualitative way how pest management methods can influence the degree of pest suppression and the frequency at which pest populations reach damaging densities. This is a relatively simple mathematical model (Wilson & Bossert 1971), but the predictions of the model reflect how changing biological characteristics and circumstances affect pest population dynamics when a *specific* natural enemy contributes to pest suppression in an IPM system. The Lotka-Volterra model is given by two equations:

$$\frac{dN}{dt} = a_1 N - b_1 NP \quad (1)$$

$$\frac{dP}{dt} = a_2 NP - b_2 P \quad (2)$$

where N is the population size of the prey,
P is the population size of the predator,
a_1 is the instantaneous *per capita* reproduction rate of the prey in the absence of the predator,
b_1 describes the instantaneous attack rate of the predator on the prey,
a_2 describes the efficiency of conversion of prey resources into predator reproduction,
b_2 is the instantaneous *per capita* death rate of the predator.

The model can be modified by incorporating density-dependent prey population growth in the absence of predation in order to aid in the interpretation of the model's predictions. A term for logistic population growth can be added to equation (1):

$$\frac{dN}{dt} = a_1\left(1 - \frac{N}{k}\right)N - b_1 NP \quad (3)$$

where k is the carrying capacity of the prey population.

This model predicts delayed density-dependent fluctuations of the prey (= pest in this context) and predator populations that asymptotically approach a stable equilibrium (Figure 2a).

The effects of different management methods can be predicted by changing the model parameters. For example, if the net reproduction of the prey decreases (decrease the value of a_1), the intervals between population peaks of the prey are longer and the amplitude of fluctuations in the prey population is smaller (Figure 2b). The net reproduction parameter in this model represents the net difference between the production of offspring and density independent mortality factors. Thus damaging pest densities should occur less frequently if either individual reproduction is reduced by factors like host plant resistance or non-specific mortality factors like generalist predators are enhanced. Another way to improve the degree of control is to enhance the activities of the specific natural enemy. While it may not be practical to increase the attack rate of the predator, it should be possible to increase predator longevity (decrease the value of b_2 in the model). For example, the longevity of parasitic wasps can be increased by providing floral nectar in agricultural systems (Idris & Grafius 1995, Landis *et al.* 2000). This leads to a lower mean prey density and longer intervals between peak densities (Figure 2c).

The Lotka-Volterra model can also be used to show how insecticides can affect biological control systems. Consider a situation where an insecticide spray kills 80% of both the pest and its specific predator (Figure 3a). Following the spray, the prey population recovers more quickly than the predator population. This illustrates the resurgence of pests that often follows insecticide applications (DeBach & Rosen 1991). Pest resurgence arises as a result of "Volterra's Principle" (Wilson & Bossert 1971). Following simultaneous harvest or removal of both prey and predator, the prey population will always recover its original numbers more quickly than the prey. Inspection of the model equations shows why. The net reproduction of the prey depends only on its own numbers (model term a_1N in equation 1), while the reproduction of the predator depends on the numbers of both the prey and the predator (model term a_2NP in equation 2). The effects of the insecticide are multiplied for the predator population. In many situations, pesticides are more deadly to natural enemies than to pests, which accentuates the effects of Volterra's Principle.

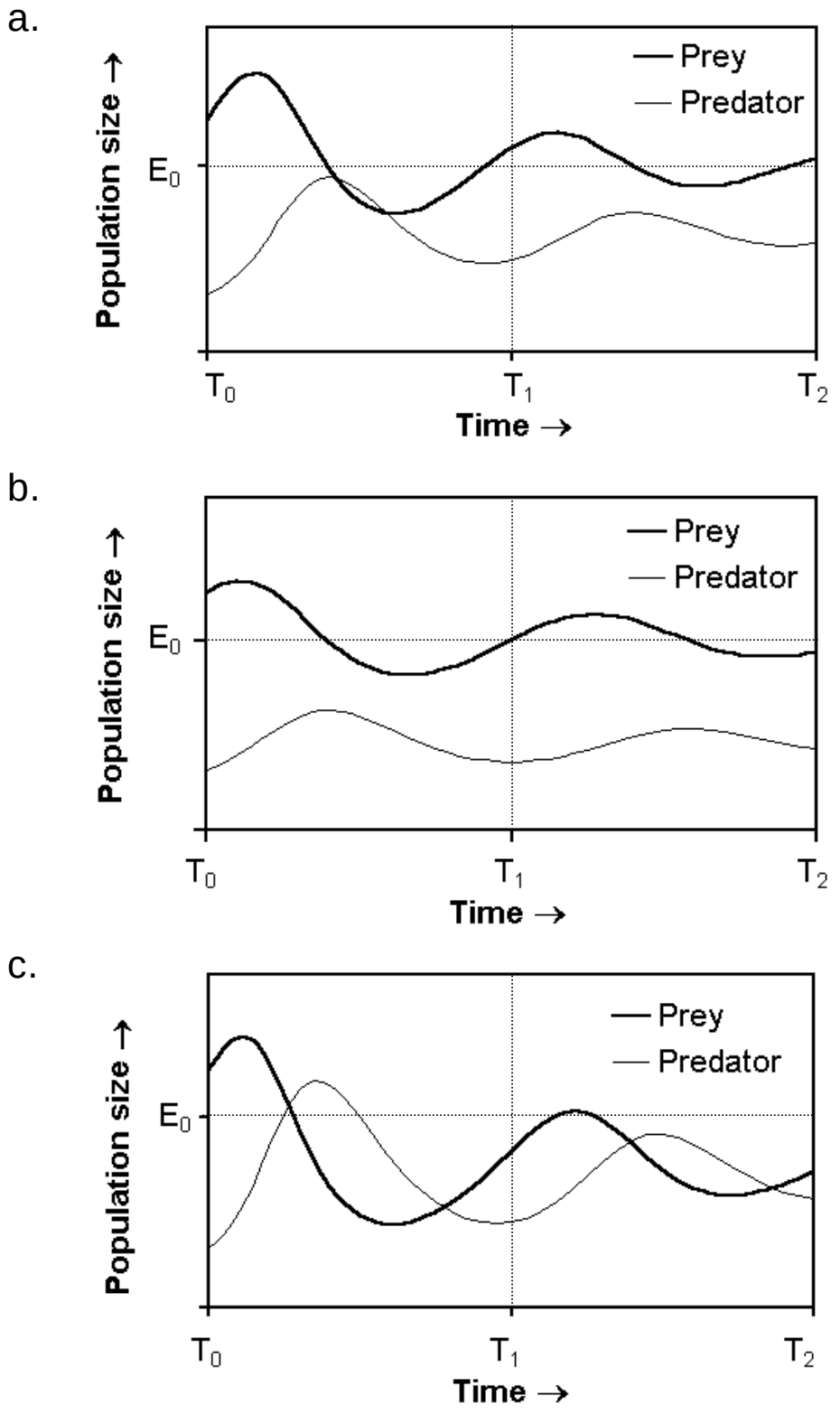

Figure 2. Predictions of the modified Lotka-Volterra Model, which incorporates density dependent population growth of the prey in the absence of the predator. (a) Predictions of the basic model. (b) Effects of a reduction of the net reproduction of the prey on numbers of prey. (c) Effects of a decrease in the mortality rate of the predator on numbers of prey (predator numbers not shown). E_0 is the equilibrium density of the prey in the basic model. T_0, T_1 and T_2 are arbitrarily selected times. Initial conditions are the same in each model.

Some selective "soft" insecticides are relatively benign to predators and parasitoids, which limits the level of predator mortality (Singh & Varma 1986, Udayagiri *et al.* 2000). However, the Lotka-Volterra model predicts that soft insecticides can still disrupt biological control (Figure 3b). When prey numbers decline, predator reproduction is still reduced. The result is a decline in populations of specific predators. Thus, pest resurgence can still occur following the application of a "soft" insecticide. Pesticides often have a disruptive effect on biological control systems. The primary benefit of selective pesticides is that interactions among non-target species are not affected by them.

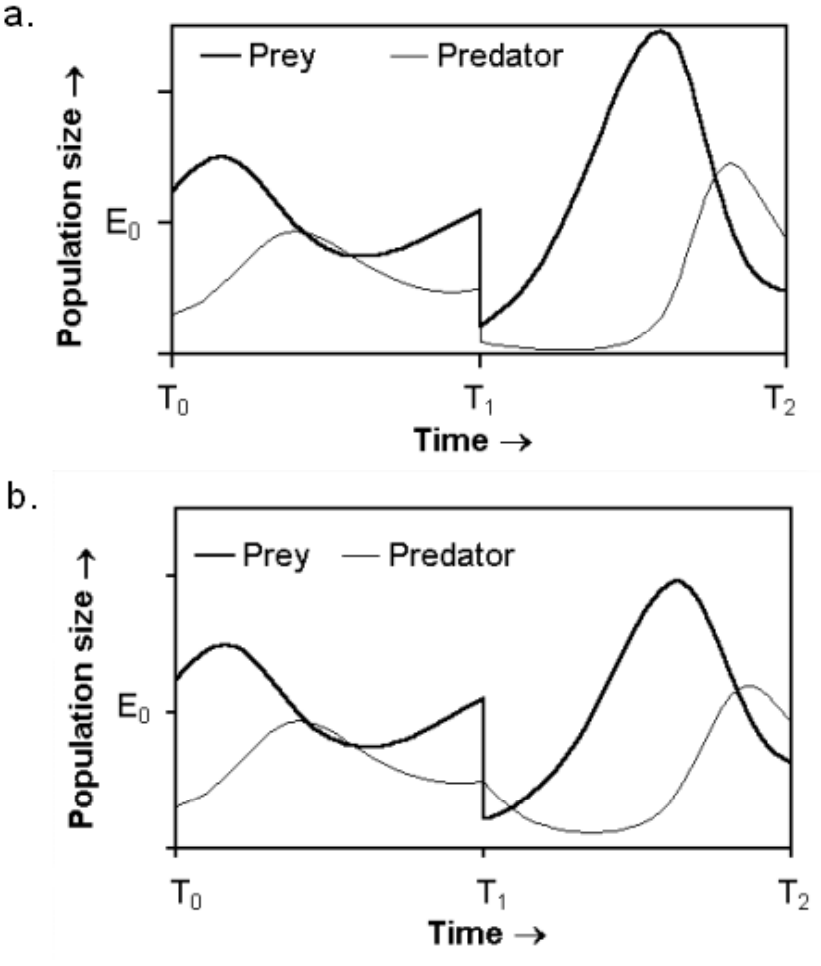

Figure 3. Effects of pesticide sprays on the dynamics of predator and prey populations as predicted by the modified Lotka-Volterra Model. (a) Pesticide kills 80% of both predator and prey populations at time T_1. (b) Pesticide kills 80% of prey population only at time T_1. E_0 is the equilibrium density of the prey.

General population models like the Lotka-Volterra model can offer insight into how various pest management practices can influence the population dynamics of pests. However, these predictions are only qualitative since they depend on simplifying assumptions. The impact of pesticides on parasitoid-host interactions can also be influenced by other biological factors and more specific predictions about the impact of pesticides on parasitoid-host population dynamics have been made (Hassell 1984). In the future, more detailed studies of how management activities affect the dynamics of real pest and natural-enemy populations are needed in order to gain a more quantitative understanding of the interactions that influence the outcome of pest management programs. Inevitably such studies will be more complex, so it is imperative that scientists distil the key outcomes of such studies into simpler recommendations in order to persuade farmers to change their farming methods.

Practical approaches to the integration of pest management methods

Reducing the net reproduction of pests

The net reproduction of pest populations can be reduced by directly inhibiting growth, development and individual reproduction and indirectly by increasing density-independent mortality levels. In addition to the

use of resistant plant varieties to reduce the net reproduction of pests, plant health can be managed to reduce their fitness. For example, when high rates of nitrogen fertiliser are used to produce host plants, the diamondback moth develops more quickly and reaches a larger adult size (Fox *et al.* 1990). Since body size is correlated with fecundity in insects (Honek 1993, Nylin & Gotthard 1998), this suggests that the rate of population increase in diamondback moth is probably greater when high levels of nitrogen are used to produce host plants. Oviposition by a range of pests can be inhibited by under-sowing crops with plants like clover (Finch & Kienegger 1997). This should also reduce the net reproduction of the pest.

Conserving and enhancing natural enemies

Both generalist and specific natural enemies can contribute to the effectiveness of an IPM system. Although the population dynamics of generalist predators are not tightly linked to the pest, they can act as a buffer against pest incursions and can reduce the population growth by reducing the net reproduction of pest populations. Generalist predators can be conserved by providing alternative prey, non-prey foods like pollen and nectar (syrphids and coccinellids) and seasonal refuges (Wratten 1996, Gurr & Wratten 1999). The longevity of parasitic wasps can be prolonged if they feed on sugar sources (Idris & Grafius 1995). Floral and extrafloral nectar and homopteran honeydew can all contribute sugar food for parasitoids. Floral diversity in and around crops can be enhanced by planting selected species around crop borders, along irrigation and tractor alleys within crops and by under-sowing with a cover crop. Care must be taken when selecting flowering plants because the structure of some flowers prevents parasitoids and predators from gaining access to nectar and pollen (Jervis *et al.* 1993), while some species provide food that can enhance pest reproduction (Baggen & Gurr 1998).

Avoiding and minimising negative interactions

While there are many methods that could be combined in an IPM system, it is important to avoid the negative interactions that may occur. The potentially negative impacts of insecticides on the subsequent reproduction of predators and parasitoids has already been described. The sudden mortality caused by insecticidal pathogens could cause the same negative effects. This could be minimised by using lower "IPM rates" of insecticides. For example, pirimicarb (Pirimor®) is applied at 10% of the recommended rate in Australian citrus to control aphids (Smith *et al.* 1997). This tends to reduce aphid numbers to tolerable levels while conserving natural enemies of aphids and other insect pests. Although pesticide sprays directly kill pests, sprays may have unintended effects on their behaviour. The surfactants used to increase the coverage of insecticide sprays are known to stimulate oviposition by diamondback moth (Rigginbucci *et al.* 1998). It seems that this is due to a change in the wax structure on the leaf surface and several different types of surfactants have the same stimulatory effect on oviposition. Negative interaction can also occur when certain resistant plant varieties interfere with the behaviour and survival of predators and parasitoids (van Lenteren *et al.* 1995). Negative interactions can be unpredictable, so it is important to monitor populations of pests and natural enemies when new methods are introduced into pest management systems.

There is a need to better understand how mortality caused by predators and parasitoids influences the population dynamics of pests and the damage they cause. Such information could be used to develop more comprehensive action thresholds for the use of selected insecticidal agents. For example, Loke *et al.* (1992) developed action thresholds for control of the diamondback moth that incorporated information about larval densities, plant growth stages and levels of parasitism. Their work was the result of empirical research on management of DBM. Unfortunately there is no body of theoretical work that can guide the development of action thresholds that incorporate the activities of natural enemies. This is a fertile area for future theoretical and empirical research.

If the mortality caused by natural enemies is to be reliably incorporated into IPM decision-making advice, then it is important to have available methods to assess their activities. Dissection of larval DBM is easy and can indicate the presence of parasitoid eggs and larvae. All that is needed is a low magnification dissecting microscope, two fine forceps and a dish of water with a dark background. After grasping the head and tail of a larva with the forceps, it can be pulled apart to reveal eggs and larvae floating freely in the haemocele. Where dissection is not practical, yellow sticky traps (Idris & Grafius 1998) and traps baited with virgin female parasitoids (Decker *et al.* 1993) can be used to indicate parasitoid activity. The numbers of insects collected in traps would only give an index of activity rather than a measure of the mortality they cause, but trap catches may be sufficient to convince farmers that natural enemies are active in their crops.

Practical integration of pest management methods

The integration of pest management methods should commence when planting a crop is first planned and should continue after harvest. For transplanted vegetable *Brassica* crops, this spans the production of seedlings, transplanting and crop establishment, vegetative crop growth, the final period when the harvested plant parts are produced and beyond harvest. There are many methods that could be integrated into a pest management program (Talekar & Shelton 1993).

When planning a crop, the choice of plant variety can influence the likelihood of pest damage. Consumer preferences for certain vegetable characteristics tend to be more important in the selection of varieties than resistance to insect attack, so resistance has not been a primary objective of plant breeders. This might change with the introduction of transgenic crops, but consumers must be convinced of the safety of transgenic crops before they will buy them. Seedlings could be produced under screens to physically prevent infestation by pests like diamondback moth, thereby limiting selection for resistance. In addition, natural enemies could be seeded on plants in the nursery before they are transplanted (Hofsvang & Hagvar 1979), thereby ensuring that biological control is more reliable. Both of these methods highlight the advantage of farmers and nurseries forming a partnership in the development of an IPM system, which would raise the level of human integration.

Once seedlings reach the farm, there are many possible methods that can be incorporated into a pest management system. For example, crops could be under-sown with clover or another plant to inhibit colonisation by pests (Finch & Kienegger 1997), flowers could be planted to conserve and enhance the activities of predators and parasitoids (Landis *et al.* 2000) and soil fertility could be managed to avoid high levels of nitrogen in plants. Colonisation of the crop by DBM could be inhibited by planting a trap crop (Mitchell *et al.* 2000), or a "dead-end trap crop" could be grown that attracts oviposition by DBM, but does not support larval development (A. Shelton, personal communication). In the longer term, farmers may begin to manage the landscape surrounding their fields to achieve an ecological integration that more comprehensively reduces pest activities (Landis *et al.* 2000). To succeed, the farmer must anticipate pest activity and respond by adopting methods that reduce the likelihood of crop damage.

Once the crop is transplanted, monitoring and the use of selective control methods are important components of an integrated pest management system (Talekar & Shelton 1993). Monitoring can indicate the densities of pests, the incidence of parasitism and the efficacy of the pest management program. Monitoring of pest activity and damage using pheromone traps and direct plant inspection should be done at intervals related to prevailing weather. When warm and dry conditions prevail, DBM develops more quickly and typically has greater survival (Harcourt 1986), hence monitoring should be more frequent at these times. If the potential for damage is indicated by monitoring, then farmers should preferentially use a soft insecticide like *Bacillus thuringiensis* or one of the newer selective insecticides. There should be no need to use older products like organophosphates or synthetic pyrethroids that have a broader spectrum of activity and are known to disrupt biological controls.

Conclusion

Farmers are concerned with how effectively a pest management system suppresses pest populations and thereby reduces damage caused by pests. There are clear benefits from integrating pest management practices to achieve this aim. Pest management systems can be more robust and resilient when the means of pest suppression are carefully selected and integrated. An understanding of how various practices interact can guide the development of pest management systems. However, farmers must also weigh up the practicality and cost-effectiveness of their pest management practices. The challenge for those who develop pest management systems is to develop better theoretical understanding of the benefits of integrating pest management practices and to translate this into practical systems that will be adopted by farmers.

Acknowledgements

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Diamondback moth resistance to Bt: relevance of genetics and molecular biology to detection and management

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Abstract

Diamondback moth is the only species that has evolved resistance to *Bacillus thuringiensis* in open field populations. Resistance has appeared several times and involves more than one mechanism, yet one type of resistance designated "Mode 1" occurs often in diamondback moth and other lepidopteran species. This type of resistance is very potent, shows recessive inheritance, displays a characteristic cross-resistance spectrum and is associated with reduced toxin binding to the midgut; thereby exhibiting many properties expected of a mutation in the toxin binding target. Recently, the molecular mechanism causing "Mode 1" resistance to Cry1A toxins in another lepidopteran was shown to be due to a knockout of a cadherin-superfamily gene, implying that the cadherin is an important Bt toxin binding target. If it eventually becomes possible to diagnose this type of resistance using DNA-based tests for the cadherin gene, it is important to develop methods that can provide growers with timely information so that Bt deployment strategies can change as needed. Actual implementation of a resistance management plan still remains the major challenge in ensuring continued efficacy of Bt-based control strategies, whether in spray formulations or transgenic plants.

Introduction

Insecticidal toxins from the bacterium *Bacillus thuringiensis* Berliner (Bt) have played an important role in controlling crop damage by the diamondback moth (DBM, *Plutella xylostella* (L.), Lepidoptera: Plutellidae) and other pests. Unlike most chemical insecticides, which target the nervous system, Bt toxins have a unique mode of action. Upon sporulation, certain strains of Bt produce crystalline (Cry) protein inclusions. More than 100 Cry proteins have been identified. When a susceptible insect larva ingests a Bt crystal, the crystal dissolves and the Cry protoxins go into solution in the midgut lumen. Partial digestion by the insect's own proteases is required to convert the protoxins to activated toxins. These bind to certain sites on the brush border membrane of the midgut, causing formation of pores in the membrane, lysing the epithelial cells and eventually killing the insect.

The earliest Bt formulations for pest control were spore/crystal mixtures isolated from large-scale fermentation cultures and are still in use. More recently, genes encoding the Cry proteins have been inserted into crop plants, which produce the toxin in their tissue and are thereby protected from insect feeding damage. Most Cry proteins have a limited host range which is believed to be due to the specificity of the binding step. Because Cry proteins are not toxic to predatory or parasitic insects or to mammals, they have many environmental advantages over chemical insecticides. The single most important threat to their continued efficacy is the evolution of resistance in insect pests and this consideration has dictated deployment strategies for Bt-transgenic plants.

As DBM is still the only species with Bt resistance in open field populations, it has a very rich literature on the topic. Tabashnik (1994) provides an overview of the earliest Bt resistance studies in insects and Ferré *et al.* (1995) and Ferré and Van Rie (2002) focus on physiological and genetic mechanisms of resistance. Talekar and Shelton (1993) cover general pest control strategies for DBM, Tabashnik *et al.* (1996) deal specifically with Bt resistance in DBM and Tabashnik *et al.* (1998) introduce the concept of "Mode 1" resistance. Here we provide an overview of some of the key studies on Bt resistance in DBM from 1990 to 2001. We also evaluate the prospects for application of DNA-based markers for field detection of resistance and its application for Bt resistance management in DBM.

Resistance in field populations

Most Bt spray formulations used on DBM are spore-crystal mixtures derived from *B. thuringiensis* subsp. *kurstaki* (or Btk, with Cry1Aa, Cry1Ab, Cry1Ac, Cry2A, and Cry2B toxins) and *B.thuringiensis* subsp. *aizawai* (or Bta, with Cry1Aa, Cry1Ab, Cry1C, and Cry1D and Cry2B). A few are based on a single toxin such as Cry1Ac. Bt spray formulations were widely adopted for DBM control in the late 1980s and were initially quite successful. Loss of effectiveness was noted in many locations where field applications were most intensive and this was eventually shown to be due to resistance.

Resistance is defined as a genetically-based decrease in susceptibility of a population over time, in response to long-term exposure to an insecticide. Establishing that resistance has occurred (and not faulty insecticide application, for example) requires determination of insecticide susceptibility under laboratory conditions. DBM is the first (and still the only) species to evolve Bt resistance in open field populations. Early reports include Hawaii (Tabashnik *et al.* 1990), the Philippines (Ferré *et al.* 1991), Florida (Shelton *et al.* 1993) and Japan (Morishita *et al.* 1992). More recently Malaysia (Verkerk & Wright 1997), Central America (Pérez & Shelton 1997), and Mexico (Díaz-Gomez *et al.* 1994, Díaz-Gomez *et al.* 2000) have been added to the list. Resistance to Btk products dates from the earliest reports; loss of Bta efficacy is more recent but increasing.

The evolution of Bt resistance in DBM followed the classical pattern seen with chemical insecticides in this species. Over-reliance on a single type of control, combined with year-round growing conditions and a rapid generation time have provided the optimal conditions for resistance evolution time and time again. In retrospect this should not have been surprising, but it must be remembered in the 1980s Bt was still regarded in some quarters as “naturally resistance-proof” because it was derived from a micro-organism that had been in the environment for a very long time as opposed to recently-developed chemical insecticides. If not for the “reality check” of Bt resistance popping up all over the globe in DBM, it is unlikely that the development of pro-active resistance management plans for Bt-transgenic crops would have been pursued with as much enthusiasm. For this reason, DBM has been called “the moth heard 'round the world” (Tabashnik *et al.* 1996).

Laboratory studies of resistant strains

The ready availability of so many resistant DBM field populations has provided an unparalleled opportunity for laboratory studies. Initially it was not clear whether resistance was due to a physiologically-based decrease in susceptibility or a newly-acquired ability to avoid Bt in the environment. A number of studies tested female oviposition and larval feeding preferences. Although there is a tendency for larvae (resistant or susceptible) to avoid food containing Bt if given a choice, to date there is no evidence that behavioural avoidance is a mechanism of Bt resistance in DBM.

Early studies showed how DBM responded to continuation and relaxation of selection with Bt in the laboratory. Of primary interest was the speed of further resistance evolution and the maximum level attainable under continued, controlled selection in the laboratory, which could establish a “worst-case” scenario for future behaviour of field populations. Once a certain resistance level was attained by selection, its stability was also assessed, to determine whether that level could be maintained without further selection, or would spontaneously decline. Representative studies include populations from Hawaii (Tabashnik *et al.* 1990, Tabashnik *et al.* 1994a), the Philippines (González-Cabrera *et al.* 2001), Malaysia (Iqbal *et al.* 1996, Sayyed *et al.* 2000a), Japan (Maruyama *et al.* 1999) and the continental USA (Zhao *et al.* 2000). In all cases studied, laboratory selection of field-collected resistant strains indicated that much higher resistance levels were attainable, but that susceptibility usually increased when selection was halted. We now turn to possible reasons for this decline.

Fitness costs in resistant strains

If resistance involves a physiological change that decreases susceptibility, this may entail a fitness cost. Susceptibility decreases slowly at first under selection because resistance genes are initially rare; presumably because they are selectively disadvantageous in the absence of the insecticide and only reappear in the population by mutation. Once field-collected resistant strains are established in the laboratory, they usually increase in susceptibility if Bt selection is not applied, showing that the initial decrease is reversible. This “instability” suggested a fitness cost, so it was of great interest to find which components of the life cycle were most affected. The practical implications would be that resistance might be delayed or reversed

in the field as well by withholding Bt applications for long enough for the population to return to a susceptible level.

Several fitness components appear to be reduced in some, but not all, resistant strains. Egg production, hatchability and adult viability were lower in resistant Hawaiian strains (Groeters *et al.* 1994). A strain from Japan showed lower egg hatchability, longer larval and pupal stage duration, lower larval, pupal, and adult survivorship, and lower fecundity (Shirai *et al.* 1998). However, selected and unselected sub-lines of a Malaysian strain were found to vary in fecundity, egg viability, growth rate and development time, but the variation was compensatory such that the intrinsic rate of increase of all the strains was the same (Sayyed & Wright 2001).

Mechanisms of resistance

Much effort has been expended on determining the biochemical or physiological changes underlying Bt resistance. Two early papers, one on Indianmeal moth (Van Rie *et al.* 1990) and the other on DBM (Ferré *et al.* 1991), established reduced toxin binding to the midgut epithelium as an important resistance mechanism. Although most attention since those studies has been paid to reduced binding, a few other studies, e.g. (Liu *et al.* 2000, Sayyed *et al.* 2001b) provide evidence of reduced proteolytic activation as well; based on the observation that susceptibility of the activated toxin is much more than to the protoxin.

Studies on Philippine strains were the first to establish the connection between high levels of resistance and reduced toxin binding in DBM (Ferré *et al.* 1991). The BL strain, derived from a field population exposed to Btk, was 200-fold resistant to Cry1Ab, but not Cry1B or Cry1C. Binding of midgut BBMVs (brush border membrane vesicles) to Cry1Ab was greatly reduced in BL relative to a susceptible strain, while Cry1B and Cry1C binding were the same in the two strains. Strains from Hawaii confirmed and extended this correlation. Originally resistant strains that had reverted to susceptibility while being maintained in the laboratory without selection, showed levels of BBMV binding to Cry1Ac equivalent to susceptible strains with no prior exposure to Bt. BBMVs prepared several generations later, after renewed laboratory selection with Bt had restored high levels of resistance, no longer bound to Cry1Ac (Tabashnik *et al.* 1994b). Strains from Florida (Tang *et al.* 1996) and Malaysia (Wright *et al.* 1997, Sayyed *et al.* 2000b) also showed reduced binding, and this phenomenon (with some variations) is often found with resistance to the Cry1A-type of toxins. In contrast, Cry1C-resistant strains have not generally displayed marked reduction of BBMV binding (Wright *et al.* 1997, Zhao *et al.* 2000).

A series of competitive binding experiments and previous results have been integrated into a model of four Cry1 toxin binding sites and their properties in DBM (Ballester *et al.* 1999, Ferré & Van Rie 2002). Site 3 (Cry1B-binding) and Site 4 (Cry1C-binding) are distinct and do not interact with other toxins. Site 1 binds Cry1Aa only; and Site 2 binds Cry1Ab, Cry1Ac, Cry1F and, to a much lesser extent, Cry1Aa (Granero *et al.* 1996). Two distinct types of Site 2 modification have occurred in resistance: "Type 1" reduces only Cry1Ab binding, while "Type 2" abolishes binding to Cry1Aa, Cry1Ab, Cry1Ac and probably Cry1F. Both types may correspond to the "Mode 1" syndrome defined by Tabashnik *et al.* (1998).

Most studies measure equilibrium or endpoint toxin binding to BBMV in suspension. Other methods (surface plasmon resonance, Masson *et al.* 1995; or binding to tissue sections, Escriche *et al.* 1995), while sometimes corroborating the BBMV results, often fail to show binding and the significance of these discrepancies is not clear.

Studies in other Lepidoptera have identified one class of Cry1A-binding proteins from larval midguts to be aminopeptidase N (APN), digestive enzymes that cleave amino acids from the N-terminus of proteins. The first APN to be cloned on the basis of its Bt toxin-binding ability was from *Manduca sexta* (L.) (Lepidoptera: Sphingidae) (Ms-APN1, (Knight *et al.* 1995)). Subsequently, a 120 kDa Cry1Ac-binding APN was identified in DBM (Luo *et al.* 1997). However, the protein isolated from resistant (NO-QA) and susceptible strains bound Cry1Ac equally well, making it unlikely to be involved in resistance. Denolf *et al.* (1997) cloned the first APN from DBM (Px-APN1) and the second from *Manduca* (Ms-APN2). A second APN (Px-APNA) was cloned from DBM (Chang *et al.* 1999); its discovery provided evidence of family of aminopeptidases in Lepidoptera and several other genes have now been cloned. A third APN from DBM (Px-APN2) has been cloned (GenBank AJ222699). It is not known whether any of the three cloned APNs correspond to the 120 kDa protein studied by Luo *et al.* (1997).

Cross-resistance

Cross-resistance is a decreased susceptibility of a population to an insecticide, caused by selection with a different insecticide. Significant progress in Bt cross-resistance studies was made only after individual toxins were purified, often at great labour and expense, and tested separately. The practical application of measuring cross-resistance patterns is to predict whether selection with one particular toxin or formulation will endanger the future efficacy of other toxins or formulations.

The early use of formulations based on the two subspecies, Btk and Bta, gave rise to the first observations of cross-resistance. In Hawaii, strains selected with Btk to >1,000-fold resistance levels were resistant to its constituent toxins Cry1Aa, Cry1Ab, Cry1Ac, and Cry2A, but not to Cry1C which is absent from Btk (Tabashnik *et al.* 1993a). Three-fold cross-resistance to Bta was observed, and suggested to be due to the presence of Cry1Aa and Cry1Ab in both formulations. Some strains from Malaysia selected with Bta exhibited slight cross-resistance to Btk, but not *vice-versa* (Iqbal *et al.* 1996); however other strains selected with Btk or Bta had no apparent cross-resistance to the other formulation (Wright *et al.* 1997). In strains selected to a high level of Cry1C resistance; if Cry1A resistance is also present, it appears to be under different genetic control (Liu *et al.* 1996, Zhao *et al.* 2001).

Generalising the results from these and other experiments; resistance to one of the Cry1A toxins is often accompanied by resistance to the others and to Cry1F and Cry1J, but not Cry1C; and resistance to Cry1C does not confer high cross-resistance to the others. An interesting Philippine strain provided a variant on the first statement, in which resistance to Cry1Ab but not Cry1Aa or Cry1Ac was (at least initially) the case. Cry1C resistance may confer a slight amount of cross-resistance to Cry1A, enough to respond to selection, but has a different genetic mechanism.

Synergism

Another practical matter is whether a given Bt resistance mechanism can be overcome by other pest control methods or compounds such as synergists. Synergism occurs when the combined potency of two compounds is greater than would be predicted by their separate potencies when administered singly. There are two issues here: whether a given compound synergise Bt against susceptible DBM and whether this synergistic action is enhanced or reduced in resistant strains. Enhancement should occur if the synergist specifically interferes with the resistance mechanism.

Since proteolytic activation of the protoxin is required for toxicity, one might expect that protease inhibitors would synergise Bt toxins. But in an early study, two serine-protease inhibitors failed to synergise Bt in either susceptible or resistant Hawaiian strains (Tabashnik *et al.* 1992a). Transgenic *Arabidopsis* expressing potato protease inhibitor PI2 suffered more feeding damage by Bt-susceptible DBM because they increased their consumption rate to compensate for the lower protein intake. This actually reduced the efficacy of Bt in *Arabidopsis* expressing both proteins (Winterer & Bergelson 2001).

In addition to the Cry proteins, other compounds produced by Bt have toxic effects. The Cyt1A protein of *B. thuringiensis* subsp. *israelensis* has no structural similarity to the Cry proteins and appears to have a different mode of action. In resistant strains of a mosquito and a beetle, Cyt1A synergised certain Bt toxins (Wirth *et al.* 1997, Federici & Bauer 1998). Similar synergism of Cyt1A against resistant DBM occurred in one study (Sayyed *et al.* 2001a), but not in another (Meyer *et al.* 2001).

Ungerminated Bt spores, when added to purified Cry proteins, increase their toxicity. The response depends on many factors; including the specific Bt toxin, spore type, and DBM strain. In one study, HD-1 spore/crystal preparations synergised Cry1A toxins in the resistant strain and Cry1C in resistant and susceptible strains, but not Cry2A in either strain (Tang *et al.* 1996). In another study, Btk spores synergised Btk crystals against both susceptible and resistant larvae; and the latter effect could be blocked by addition of streptomycin to inhibit spore germination. However, Bta spores did not synergise Bta crystals; and Btk spores synergised Cry1C against both resistant and susceptible strains, but synergised Cry2A against susceptible larvae only (Liu *et al.* 1998).

Inheritance of resistance

After the response to laboratory selection with Bt has stabilised, a strain is likely to be fairly homogeneous genetically and the inheritance of resistance can then be characterised. The first step requires crossing

resistant (R) and susceptible (S) strains to produce an F_1 generation, and measuring its concentration-mortality curve. If reciprocal F_1 curves coincide with each other and with the S curve, resistance is autosomal and recessive. If the F_1 and R curves are coincident, resistance is dominant. Usually the situation is more complex and terms like "incompletely recessive" are used when the F_1 curve is substantially closer to the S curve; however these concepts can become quite complex when the concentration administered and other environmental factors influence the response (Bourguet *et al.* 2000). To estimate the number of different genes contributing to resistance, the concentration-mortality response of backcross or F_2 generation is compared with predicted mortality predicted under various hypotheses. If there is a good chi-squared fit to the simplest model, most investigators conclude that resistance is monogenic. A more rigorous approach is to compare the fit among models with 1, 2, or more genes, but it can be difficult to discriminate among such models with bioassay data alone (Tabashnik 1991).

Generally, DBM resistance to the Cry1A toxins has been found to be completely or partially recessive (Hama *et al.* 1992, Tabashnik *et al.* 1992b, Martínez-Ramírez *et al.* 1995, Tabashnik *et al.* 1997a, Tang *et al.* 1997, Sayyed *et al.* 2000a). It has never been found to be dominant; although a Thai strain appeared to have polyfactorial control of resistance (Imai & Mori 1999). Crosses between three strains with recessive resistance to Cry1Ab (from Pennsylvania, the Philippines and Hawaii) enabled a three-way test for allelism revealing a common Cry1Ab resistance gene. This was the first evidence that Bt resistance arising in different geographical areas could have the same genetic basis. The Philippine strain also had a second gene controlling Cry1Ab resistance (Tabashnik *et al.* 1997b).

Recessivity of Cry1C resistance appears to show more dependence on the concentrations tested. A Hawaiian strain displayed achieved 60-fold resistance under autosomal control, with more pronounced dominance at lower concentrations. A strain from South Carolina selected to >60,000-fold resistance to Cry1C displayed autosomal, incompletely recessive inheritance when evaluated by a leaf-dip bioassay and completely recessive behaviour on Cry1C-expressing transgenic broccoli (Zhao *et al.* 2000).

Experimental tests of IRM strategies with resistant DBM strains

Insecticide resistance management (IRM) aims to use a combination of control strategies to retard or contain the spread of resistance in the field. The large number of potential strategies includes mixtures of different toxins, incorporation of synergists, application in spatial mosaics, temporal rotations of toxins, ultrahigh concentrations to ensure all heterozygotes are killed and refuges that permit a certain fraction of the population to escape selection by the toxin (Tabashnik 1994). There is a large modelling literature addressed to the advantages and disadvantages of these, but very few experimental tests. The availability of Bt resistant strains in the field is a unique advantage of DBM in providing data directly relevant to these issues.

Resistant strains have been used for two main purposes. The first is as a standard—a realistic upper bound to accurately compare and calibrate the results of laboratory and field studies (Tabashnik *et al.* 1993b, Pérez *et al.* 1997a). This includes the first laboratory experimental test of the F_2 screen for rare resistance alleles (Zhao *et al.* 2002). The second main use of Bt-resistant strains is in experiments that compare different IRM plans. In a laboratory selection experiment designed to simulate the rate of resistance evolution under various conditions, the presence of refuges was shown to delay the rate of increase of resistance to Bta (Liu & Tabashnik 1997). In field cage tests in Honduras (Pérez *et al.* 1997b), the effects of different rates of Btk application and the presence or absence of a 25% refuge (unsprayed row) was examined on the rate of resistance evolution, mortality and fraction of marketable cabbage produced. Open-field tests with different deployment strategies of transgenic Cry1Ac-expressing broccoli and field-collected resistant insects explored the effects of different physical placement of refuge (nontransgenic) plants (Shelton *et al.* 2000). Greenhouse cage experiments employing different refuge sizes with transgenic Cry1Ac-expressing broccoli and different DBM resistance allele frequencies showed that larger refuges delayed resistance more, compared separate refuges with mixed ones and highlighted the importance of larval movement (Tang *et al.* 2001). Recent greenhouse cage tests indicated that, compared with sequential or mosaic deployment of Cry1Ac and Cry1C toxins in transgenic broccoli, pyramided two-gene plants could significantly delay resistance development to each or both toxins while providing good control of the DBM population (JZ Zhao & AM Shelton, unpublished data).

A genetic mapping approach to Bt resistance in diamondback moth

It would be very useful to identify the genes responsible for the resistance phenomena discussed here, for basic and applied reasons. It would resolve some of the conflicting data from different approaches and

enhance our basic understanding of the fundamental mode of action of Bt toxins. It would also enable the development of DNA-based diagnostic tests for resistance in the field. Resistance genes found in one species of Lepidoptera could well be relevant to other species. Recently the gene conferring "Mode 1" resistance (Tabashnik *et al.* 1998) in tobacco budworm *Heliothis virescens* F. (Lepidoptera: Noctuidae) was identified using a genetic approach. An 11-domain cadherin protein, homologous to proteins from other Lepidoptera that bind Cry1A toxins, mapped to exactly the same chromosomal location as the resistance gene. Cloning of the cadherin gene from a resistant strain showed that it was interrupted by a retrotransposon insertion, preventing synthesis of a full-length protein capable of binding Bt toxins. Thus a knockout of the cadherin conferred resistance, strongly implicating it as the major binding protein of Cry1A toxins in *H. virescens* (Gahan *et al.* 2001).

We are now applying the same methods to identify resistance genes by linkage mapping in DBM. Using about 200 AFLP markers, sufficient to mark all of the 31 chromosomes, we showed that a single gene accounted for most of the Cry1Ac resistance in the NO-QA strain from Hawaii (Heckel *et al.* 1999). By cloning and sequencing one of the AFLPs near that gene we developed a polymorphic marker that can be used for linkage tests in other strains. The aminopeptidase Px-APNA was mapped to a different linkage group, excluding it as a candidate for the resistance gene. We are now testing additional APNs from DBM, as well as the homologue of the *H. virescens* cadherin, to see if they map to the same linkage group as resistance.

We are also analysing Cry1Ac and Cry1C resistance mechanisms in a South Carolina strain (Zhao *et al.* 2001). The same linkage group associated with Cry1Ac resistance in NO-QA is the only one associated with Cry1Ac resistance in this strain. It is intriguing to speculate that this linkage group might be homologous to the one in *H. virescens,* as Herrero *et al.* (2001) have shown an association between the marker MPI (linked to resistance in *H. virescens*) and Cry1Ab resistance in a Philippine strain of DBM. In the South Carolina strain, two different linkage groups are associated with Cry1C resistance, confirming earlier indications of a different genetic basis to resistance against the two toxins. So far, no APNs have been found to map to these linkage groups (S. Baxter, unpublished data).

Based on this progress, it is likely that molecular methods of diagnosing Bt resistance in field populations of DBM will be available soon. DNA-based methods will not replace conventional bioassay methods, but instead offer a valuable complement to them. Only larvae can be bioassayed, but any life stage can provide DNA for analysis. Living, healthy individuals are required for bioassay, but frozen or ethanol-preserved specimens can provide DNA. Bioassay can only detect resistance in a population if the resistance allele frequency is high enough to shift the concentration-mortality curve of the entire population; but the DNA detection basis works on an individual basis and could detect resistant alleles at a frequency of one in ten thousand; if ten thousand individuals were screened and one positive were found. When resistance is recessive, bioassays can only detect resistant homozygotes, but DNA-based methods can detect heterozygotes as well. The detection of resistance at low frequencies will probably be the most useful aspect of DNA-based methods. An independent approach at detecting rare recessive alleles, the F_2 screen, indicates that their frequency is likely to be less than 0.001 in an Australian population of DBM (Ahmad & Roush 1999).

Conclusion

Applications to Bt resistance management in the field

The potential benefits of DNA diagnostic methods for detection of resistance to Bt-transgenic crops are constrained by several factors in some systems. Bt-expressing maize and cotton in large monocultures present a strong, uniform selection pressure which the target pests have not yet overcome. The goal is to prevent resistance occurring in the field. The "high-dose/refuge strategy" attempts to do this by ensuring that a certain fraction of the crop does not express Bt. This "refuge" from Bt selection allows the production of susceptible individuals which mate with the few Bt resistant survivors of the transgenic crop, producing heterozygous offspring that are killed by the high dose in the next generation. DNA-based detection would be most useful for validating that strategy or giving an early warning if it starts to fail. Predicting which of many possible resistance mutations will occur in the field first, based on laboratory studies, can be difficult (but not impossible, as shown by studies in *H. virescens* (Gould *et al.* 1997, Gahan *et al.* 2001). If resistance is detected early by the DNA methods, "soft" options such as increasing the refuge size or refraining from planting transgenic crops must be delayed to the next planting season; although the "harder" responses of crop destruction or massive chemical control could be implemented immediately.

In contrast to pests of transgenic cotton and maize, application of DNA diagnostic methods in DBM may be less constrained and more flexible for two reasons. The first relates to the existing distribution of resistance genes. The worldwide selection pressure on DBM has provided a variety of different outcomes and the goal is to manage resistance mechanisms that already occur in the field rather than to anticipate the unknown. Genetic characterisation of these mechanisms is therefore possible because they already exist and are thus guaranteed to be relevant. Some, but not all, of these will be the same in different populations, facilitating screening efforts. Migration will have already distributed resistance genes over a wide area. Overall, historical effects will be less important, although not negligible. The prudent approach will be to supplement DNA methods with bioassays at first, with each new geographical area to be screened.

The second factor in favour of the DNA approach for DBM is the manner in which Bt is used still exclusively in spray formulations. This enables a much more flexible response to any findings of DNA monitoring than possible with transgenic plants. Changing the frequency or timing of sprays, or switching to a different Bt formulation with other toxins, or resorting to chemicals with an entirely different mode of action, are all possible. If contingency plans were put into place in advance, switching strategies might be accomplished very rapidly. If Bt were one component in a pre-determined rotating series of different compounds, DNA monitoring could verify that susceptibility had returned to a level sufficient to obtain effective control when Bt was ready to be used again.

On the other hand, some factors weigh against the utility of DNA monitoring in DBM. The technology is too complicated for most growers and consultants, and diagnostics would have to be centrally done. Resistance management is less effective when resistance is already common; but the advantage of DNA approaches is greater when resistance is rare. Economically, the stakes are lower with sprays than with transgenic plants. Moreover, in many cases Bt sprays have been a relatively minor component in crop protection overall because of their low field persistence and difficulty of getting the insecticide to the insect, compared with other options.

Although Bt-transgenic plants are not currently in commercial use for DBM control, future adoption may enhance some opportunities for resistance management and the concomitant benefits of DNA diagnostics. The high-dose/refuge strategy, or “pyramided” plants expressing two different toxins, could be very effective in both pest control and resistance management. Alternating between different Bt genes in plants within a region could also be done on a yearly basis, similar to the window strategy with foliar sprays. Industry control of availability of seeds expressing different Bt toxins may be a more effective way of promoting grower compliance with such a rotational strategy than control of availability of different sprays, which can be stored and used whenever desired.

The biggest challenge of DNA diagnostics remains to provide the information to growers, consultants and the pest control industry in a timely and useful manner so that it can be used in decision-making in the context of a previously devised and accepted integrated pest management plan with a variety of options. Ideally, an area-wide Bt resistance monitoring program (e.g. Carrière *et al.* 2001) would be coordinated with a long-term assessment of DBM movement, population structure and gene flow. The use of DNA-based markers for both purposes would be an efficient use of sampled individuals and could be extended to screening for chemical insecticide resistance by examining genes for targets of the pyrethroids, cyclodienes and organophosphates.

In summary, just as diamondback moth has furnished the greatest variety of resistance phenomena to *Bacillus thuringiensis,* so may it provide the greatest number of opportunities to successfully manage the resistance problem. Keeping Bt as an active ingredient in the arsenal against this cosmopolitan pest is achievable; only time will tell whether it is achieved.

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New insect control agents: modes of action and selectivity

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Abstract

Insect control agents remain an important part of most insect pest management (IPM) programs. Over the past decade, a variety of new insect and mite control agents have been developed, or are being developed, that may fit a variety of IPM needs, and many of these have novel modes of action. Several new acaricides acting at complex I of the mitochondrial transport system and chlorfenapyr, a proinsecticidal uncoupler, are representative of new compounds targeting insect respiration. Several diacylhydrazines (e.g. methoxyfenozide, an ecdysone agonist), along with pyriproxyfen, a juvenoid, are relatively new insect control agents that act on the insect endocrine system. Several new acaricides/insecticides (etoxazole, spirodiclofen) also disrupt mite and insect developmental processes. Among the new neurally active insect control agents, ethiprole (a phenylpyrazole) and emamectin benzoate both act on GABA gated chloride channel, although in very different ways, while indoxacarb acts at a novel site in the voltage gated sodium channel. Pymetrozine and IKI-220 act on the (aphid) insect nervous system in novel ways to disrupt feeding. There are several new neo-nicotinoids including thiacloprid, thiamethoxam and clothianidin that, like imidacloprid, are potent agonists for insect nicotinic acetylcholine receptors. The novel macrolide, spinosad, appears to alter the function of the nicotinic as well as GABA gated chloride channels in a novel manner. These recent new insect control agents demonstrate a variety of new and under-utilized modes of action. In many cases these new insect control agents also couple field efficacy with improved selectivity (compared to older chemistries) towards beneficial insects, especially predators. The preservation of a beneficial insect component in any IPM program is an important non-chemical source of selection pressure against a pest population, resulting in not only in an overall enhancement in pest control, but a reduced likelihood of resistance development.

Keywords

Insecticide mode of action, mammalian toxicity, beneficial insects

Introduction

The goal of any crop protection program is to provide the grower with effective, reliable and, most importantly, a cost effective means to address pest problems. The grower's tool-box of insect control options includes a variety of approaches such as biological control, cultural practices, transgenic crops, host plant resistance, as well as insect control agents. However, there are no "silver bullets" when it comes to pest insect control, since no one technology is appropriate or suitable to every pest problem. Because insect control agents provide a predictable, effective and timely means to address pest problems, they are likely to remain a key component of integrated pest management (IPM) programs for the diamondback moth and most other important insect pest species. As such, the availability of effective, affordable and safe synthetic organic insect control agents is thus critical. The last decade has seen a far wider variety of new chemistries with new modes of action and enhanced selectivity become available than at any other time since the advent of DDT. Compared with organophosphates, carbamates and pyrethroids, many of these new insect control agents provide improved environmental/mammalian toxicology profiles along with greater opportunities to integrate multiple control tactics. In light of the diamondback moth's (*Plutella xylostella*, DBM) long history of resistance development, a true integration of control tactics is essential to the long-term availability of control options for DBM. This enhanced selectivity not only encompasses mammalian safety, but also a trend towards improved safety to beneficial insects. As such, preserving beneficial insects provides a valuable adjunct for insect control agents, increasing options for pest control and providing an important alternative selection pressure on the pest to reduce the chances for resistance development. The following is a very brief overview of selected examples of these newer insect control agents and their respective modes of action.

Inhibitors of respiration

The disruption of mitochondrial respiration can, like the nervous system, be an effective target for insect control agents. If mitochondrial electron transport (MET) is blocked or if oxidative phosphorylation is uncoupled, the production of ATP is stopped, ultimately leading to death.

MET inhibitors - MET involves the re-oxidation of NADH by transferring electrons through a chain of carriers to oxygen (Fukami 1985). A chemically diverse group of acaricides including fenazaquin, fenpyroximate, pyridaben, tebufenpyrad (Figure 1) and pyrimidifen, all appear to act at the same site (complex I) as rotenone of the MET system (Hollingworth & Ahammadsahib 1995, Hollingworth 2001). Although these compounds are primarily acaricides, studies have shown that the spectrum can be altered and expanded (Hackler *et al*. 1998) to include Lepidoptera (e.g. tolfenpyrad, Figure 1). In addition to site I inhibitors, many patents point to methoxyacrylate chemistry as potential insecticides. NA-83 (fluacrypyrin) is an example of a methoxyacrylate site III-based acaricide currently under development (Smith 2001).

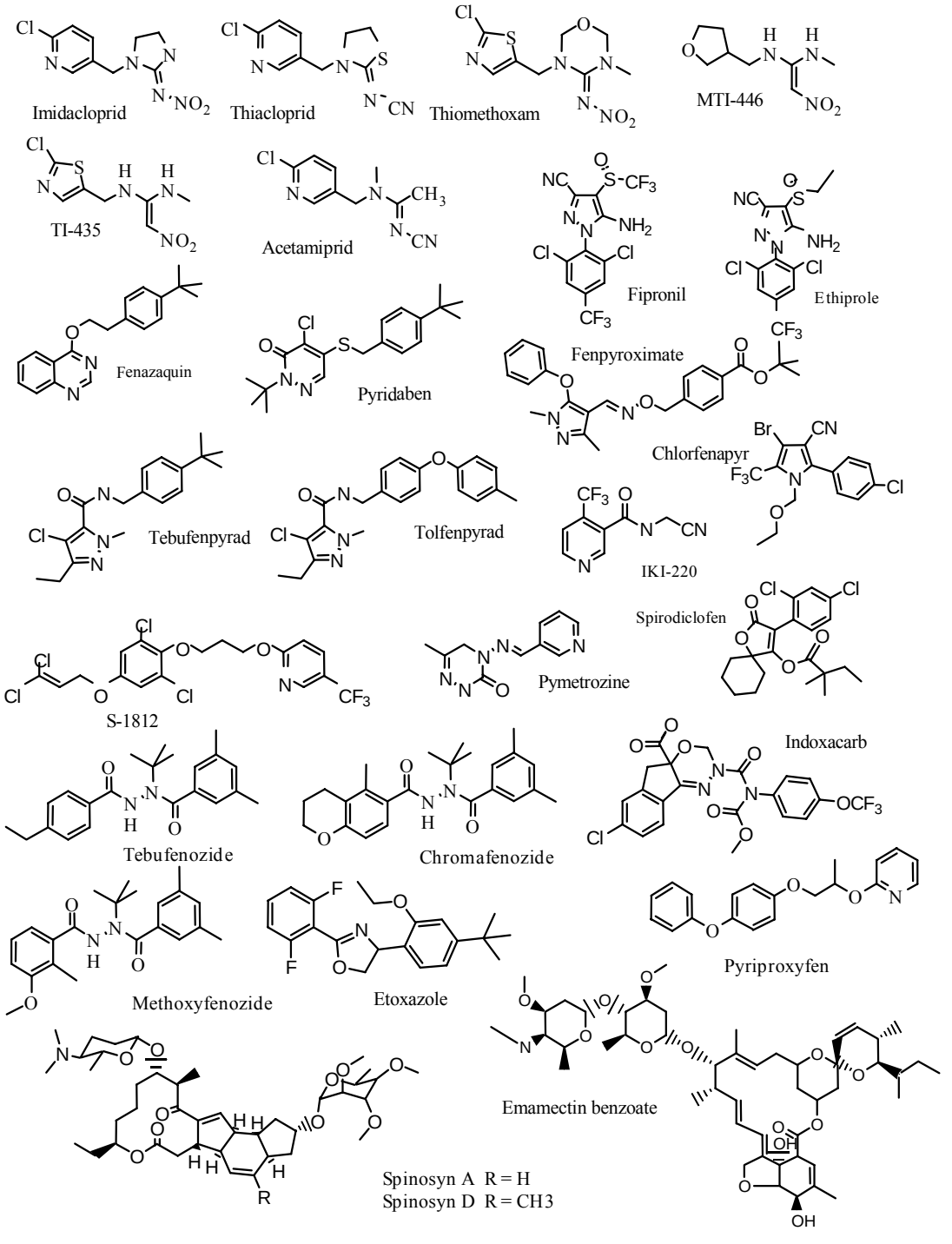

Figure 1. Structures of selected insect control agents

Pyrroles – Unlike the MET acaricides, the insecticidal pyrroles function by the uncoupling of oxidative phosphorylation from the MET chain. Chlorfenapyr is a pro-insecticide requiring biological activation before it can function (Hunt & Treacy 1998). The pro-insecticidal nature of chlorfenapyr contributes to a favourable mammalian toxicity profile (Kuhn *et al*. 1993, Table 1).

Insect growth regulators

Juvenoids - Juvenoids are compounds that mimic the action of juvenile hormone, thereby disrupting metamorphosis leading to a variety of deleterious effects (Sparks 1990, Dhadialla *et al*. 1998). While juvenoids have had limited impact on IPM programs, the introduction of new, more active and more photostable juvenoids such as pyriproxyfen (Figure 1, Miyamoto *et al*. 1993, Dhadialla *et al*. 1998) may yet change this trend, especially for the control of pests such as whiteflies and aphids. As exemplified by pyriproxyfen, the juvenoids tend to have highly favourable mammalian toxicity profiles (Table 1).

Diacylhydrazines - The diacylhydrazines are a novel class of IGRs (Hsu 1991) which in the Lepidoptera function as ecdysone agonists, disrupting the moulting process by mimicking the action of 20-hydroxyecdysone (Wing & Aller 1990). Tebufenozide is effective in the control of a variety of lepidopterous insect pests (Hsu 1991, Heller *et al*. 1992), as is methoxyfenozide. Two other additions to this class of chemistry include halofenozide (Dhadialla *et al*. 1998) and chromafenozide (Yanagi *et al*. 2000, Smith 2001, Figure 1). In part, due to their novel mode of action, the diacylhydrazines display a very favourable mammalian and environmental toxicity profile (Table 1) as well as good selectivity towards beneficial insects (Table 2).

Chitin synthesis inhibitors and other developmental inhibitors - In addition to the now classical acylureas such as diflubenzuron and hexaflumuron, other types of compounds that inhibit chitin synthesis or other related processes are making their way to the marketplace. One example is etoxazole (Suzuki *et al*. 2001, Figure 1), a highly effective acaricide with a very favourable mammalian and avian toxicity profile (Table 1). Another developmental inhibitor that is also an acaricide that is effective on a variety of resistant mite species is spirodiclofin (Wachendorff *et al*. 2000, Figure 1).

Compounds acting on the insect nervous system

Due to the many sites available for disruption, the insect nervous system continues to be the favoured target for most insect control agents. A majority of the insect control agents in use today, including organophosphate, carbamate and pyrethroid insecticides. All act via disruption of nervous transmission.

Avermectins - Abamectin is a fermentation derived insecticide that acts on the insect nervous system functioning to open chloride channels, acting as a *gamma*-aminobutyric acid (GABA) agonist at binding sites, and/or enhancing the action of GABA at the receptor site or stimulating the presynaptic release of GABA (Fisher 1993, Miller & Chambers 1987, Lasota & Dybas 1991, Turner & Schaeffer 1989). Extensive structure activity studies identified the semi-synthetic derivative, emamectin benzoate (Figure 1), as a very effective insecticide for Lepidoptera (Fisher 1993). Given the novel mode of action and high degree of efficacy, emamectin benzoate should be a very useful tool in IPM programs for DBM and other lepidopterous pests.

Phenylpyrazoles - The phenylpyrazole, fipronil (Figure 1), is the first of a new class of broad-spectrum insect control agents that act to block the GABA gated chloride channel in the insect nervous system (Gant *et al*. 1998). This mode of action is similar to the cyclodienes, however, the exact binding site for fipronil in the GABA gated chloride channel may be distinct from that of the cyclodienes and picrotoxinin (Gant *et al*. 1998). Ethiprole (Smith 2001), another phenylpyrazole in development, is structurally very similar to fipronil, but has improved mammalian safety (Table 2).

Neo-nicotinoids - In the insect nervous system, nicotinic acetylcholine receptors appear to predominate while in mammalian systems, muscarinic receptors predominate (Eldefrawi & Eldefrawi 1990, Eto 1992) providing a potential source of selectivity. The neo-nicotinoid, imidacloprid (Figure1) acts as an acetylcholine agonist on the nicotinic receptor (Mullins 1992). In addition to imidacloprid, several other neo-nicotinoids (Figure 1) including acetamiprid, thiamethoxam, thiocloprid, TI-435 (clothianidin) and MTI-0446 have been or are being developed, some possessing an expanded spectrum (Nakayama & Sukekawa 1998, Elbert *et al*. 2000, Smith 2001). The development of two neo-nicotinoids (nitenpyram and

AKD-1022) has been dropped for agricultural use (Smith 2001). The neo-nicotinoids exhibit generally favourable environmental and toxicological profiles (Table 1) coupled to reasonable selectivity towards beneficial insects (Table 2) all contribute to the expanding use of this chemistry in vegetable and other crop IPM programs.

Oxadiazines - Indoxacarb (Figure 1), an oxadiazine, is bioactivated through the action of an esterase/amidase to a highly potent blocker of voltage gated sodium channels at a site distinct from that of the pyrethroids (Wing *et al*. 1998). Indoxacarb is effective against a variety of insect pests, especially lepidopterous larvae and appears to possess an excellent environmental profile (Harder *et al*. 1996, Table 1). Indoxacarb is also selective towards beneficial insects (Table 2) providing a very good fit for DBM IPM programs.

Table 1. Toxicity values (technical material) for selected insect control agents

	Rat /Mouse LD_{50} (mg/kg)		LD_{50} (mg/kg)	LC_{50} (ppm)	
Compound	**Oral**	**Dermal**	**Avian**	**Fish**	**Daphnia**
Standards					
DDT	87-500	1931	611	0.08	0.0047
Dieldrin	40-100	52-117	37	0.0012	0.00024
Aldicarb	0.9	2.5 – 5	594	0.61	0.061
Carbofuran	8	2550	190	0.38	--
Methyl parathion	9-42	63-72	90	3.7	0.00014
Chlorpyrifos	82-245	202-2000	940	0.0071	0.0017
Profenofos	400	472-1610	--	0.024	0.0014
Cypermethrin	247	>2000	>10000	0.025	0.0013
Respiratory					
Fenazaquin	130-140	>5000	1747->2000	0.004-0.034	0.004
Pyridaben	820-1350	--	>2250	0.001-0.008	0.0006
Tebufenpyrad	100-600	>2000	>2000	0.073	1.2
Tolfenpyrad	>100	--	--	--	--
Chlorfenapyr	>626	>2000	10 – 34	7.4-11.6	--
Developmental					
Pyriproxyfen	>5000	>2000	>2000	0.45-2.7	0.40
Tebufenozide	>5000	--	>2150	3.0-5.7	3.8
Methoxyfenozide	>5000	>2000	>5620	>4.3	3.7
Chromafenozide	>5000	>2000	>5000	>18.9 - >47	>94.5
Etoxazole	>5000	>2000	>2000	7.8	>40
Spirodiclofin	>2500	>2000	>2000	--	--
Neural Actives					
Imidacloprid	450	>5000	31-5000	211	32-85
Thiacloprid	444-836	>2000	2716	30.5	>85
Thiamethoxam	1563	>2000	576-1552	>114-125	--
Acetamiprid	146-217	>2000	--	>100	>100
MTI-0446	>2000	>2000	1000->2000	>40->1000	--
Fipronil	100	>2000	31-2150	0.25-0.43	0.19
Ethiprole	>2000	>2000	--	--	--
Emamectin benzoate	70	--	--	--	--
Indoxacarb	>5000	>2000	>2250	0.5	--
Spinosad	>3683	>5000	>1333	6-30	14
Pymetrozine	5820	--	--	--	--
IKI-220	884-1768	>5000	--	>92 ->100	>100

Data adapted from Larson *et al*. 1985, Hollingworth 2001, Smith 2001, Ware 1982, Elbert *et al*. 2000, Yanagi *et al*. 2000

Dihalopropenoxy aryl ethers – S-1812 (pyridanil, Figure 1, Smith 2001) is a recent novel class of chemistry. It is a broad spectrum lepidopteran material that also shows a high degree of safety towards beneficial insects (Table 2).

Pymetrozine and IKI-220 - Aphids treated with pymetrozine (Figure 1) simply cease feeding, usually within a short time of treatment, with the ultimate effect being the insects starve to death (Kristinsson 1994, Harrewijn & Kayser 1997). This feeding disruption appears to be the result of a direct, novel effect on the insect nervous system (Harrewijn & Kayser 1997). IKI-220 (Figure 1) has a different structure than pymetrozine, but has similar effects on aphids, in that it acts on the nervous system to shut down feeding (Morita *et al*. 2000).

Spinosyns – Spinosad (a mixture of spinosyns A and D, Figure 1) represents a new class of fermentation-derived tetracyclic macrolides (Kirst *et al*. 1992, Sparks *et al*. 1999). The spinosyns, which act via the insect nervous system, are especially active against a variety of lepidopterous pests (DeAmicis *et al*. 1997, Sparks *et al*.1999) and possess very favourable mammalian toxicity and environmental profiles (Sparks *et al*. 1999, Crouse & Sparks 1998, Table 1). The mode of action appears to involve alteration of nicotinic receptor function as well as the function of GABA gated chloride channels (Salgado *et al*. 1997, Watson 2001). Additionally, spinosad also exhibits a great deal of selectivity towards beneficial insects, especially predators (Table 2) enabling a great deal of utility in DBM and other IPM programs.

Table 2. Relative selectivity of selected compounds towards beneficial insects

Compound	Predators					Parasitoids	
	Orius	***Geocoris***	***Chrysopa***	***Hippodamia convergens***	***Coccinella***	***Cotesia marginiventis***	***Trichogramma***
Tebufenozide	* * * *	* * * *			* * * *		
Methoxyfenozide	* * *	* * * *					
Spinosad	* * *	* * * *	* * * *	* * * *	* * * *	*	*
Indoxacarb	* * * *	* * * *	* * * *		* * * *	* * * *	* * * *
S-1812	* * *	* * * *	* * * *	* * * *		* * * *	* * *
Imidacloprid	* * *	* * *		* *			
Emamectin benzoate	*						
Abamectin	*						
Fipronil	*	*		* * * *			
Chlorfenapyr	**	*		* * * *			
Cyhalothrin	*	*	* * * *	* * *	* * * *	* *	* * *
Cyfluthrin	**	* * * *		*			
Cypermethrin							* * *
Profenofos	*	* * * *		* * *			* *
Malathion	*	*		* *			
Endosulfan		* * * *		* * *			

Rating is a relative scale with * being not very selective, while **** indicates a relatively selective material. Data adapted from Tillman *et al*. 1998; Ruberson & Tillman 1999; Tillman & Mulrooney 2000; Elzen 2000, 2001; Studebaker & Kring 2001.

Summary

Insect control agents remain a critical component of most IPM programs. The availability of insect control agents that act on new or under-exploited target sites reduces the potential for target site-based (but perhaps not metabolism-based) cross-resistance to existing products and provides growers with new, and in many cases safer and/or more selective, insect control choices. The modes of action demonstrated by some of these new insect control agents represent new chemistry on known, but generally under-utilised, target sites (MET acaricides, pyrroles, phenylpyrazoles, neo-nicotinoids, juvenoids), while others represent new target sites within known systems (diacylhydrazines, oxadiazines). In some cases, it is clear that the insect nervous system is the target, but the actions of these new insect control agents are incompletely understood (pymetrozine, spinosyns). Regardless, all of the above insect control agents certainly possess modes of action outside of the mainstream (inhibition of acetylcholinesterase - organophosphates and carbamates; and opening of voltage-gated sodium channels - pyrethroids) and thus present new opportunities for IPM and insect resistance management programs.

In addition to new modes of action, and in some cases directly attributable to a novel mode of action, many of the new insect control agents also possess very favourable mammalian and environmental profiles (Table 1), as well as high levels of selectivity towards a variety of beneficial insects (Table 2). Thus, many of these new chemistries present growers and the scientific community with new opportunities to address many of the IPM and resistance management needs of critical insect pests such as the diamondback moth. However, at the same time, there is a real need/responsibility to make the best use of these new tools as is possible. For a variety of reasons, including the continuing rapid consolidation of the agrochemical industry worldwide, the future replacement of any of these new tools is increasingly problematic. Thus the loss of any of these new chemistries potentially represents an increasingly irreplaceable resource that may be difficult or even impossible to replace in the not too distant future.

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Host plant finding by insects - undersowing crop plants with clover reveals the missing link

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Abstract

Characteristic volatile chemicals are credited with the major role of guiding herbivorous insects to their host plants. Studies comparing how insects find host plants growing in backgrounds of clover and bare soil indicated that it is not chemical, but visual stimuli that govern where the insects land. A mechanism of host-plant finding is proposed in which chemical cues start and end the process of host-plant selection and in which the missing (central) link, based on "appropriate/inappropriate" landings, is governed by visual stimuli. This missing link operates via the insects landing indiscriminately on green surfaces but avoiding brown surfaces, such as soil. Hence, host plants growing in bare soil are colonized by more insects than similar plants growing amongst green non-host plants. Five earlier mechanisms of host-plant finding, plus the "Resource concentration hypothesis" and the "Enemies hypothesis" are discounted. The new theory answers also why wild host-plants growing under natural conditions are not decimated by pest-insects, a question that has puzzled entomologists for decades.

Keywords

Brassica pests, intercropping, deterrent chemicals, Resource Concentration Hypothesis, appropriate/inappropriate landing theory

Introduction

Pliny the Younger (23-79AD) wrote in his *Naturalis Historiae* that when rape (*Brassica napus* L.) and vetch (*Vicia sativa* L.) were grown together, many insects that occurred normally on these plants were not found (Schoonhoven *et al.* 1998). This was probably one of the earliest clues that by increasing plant diversity within a cropping system it might be possible to lower the numbers of pest insects that find crop plants.

In recent years, many researchers have shown that the numbers of herbivorous insects found on crop plants are reduced considerably when the background of the crop is allowed to become weedy, when the crop is intercropped with another plant species, or when the crop is undersown with a living mulch (Finch & Collier 2000).

It has been suggested that when diverse backgrounds of the types mentioned above “disrupt” (Vandermeer 1989) the searching insects, the action is mediated through 1) the non-host plants physically impeding the searching insects (Perrin 1977); 2) visual camouflage (Smith 1976); 3) root exudates from the non-host plants altering the physiology of the host-plant (Theunissen 1994); 4) odours of the non-host plants directly deterring the searching insect (Uvah & Coaker 1984); or 5) the odours of the non-host plants "masking" those of the host plant (Tahvanainen & Root 1972). Two general hypotheses, have also been proposed to explain these reductions in insect numbers. The "Resource concentration hypothesis" (Root 1973), which indicates that more insects are found where the "resource" (host plants) is most concentrated and the "Enemies hypothesis" (Root 1973), which indicates that fewer herbivorous insects are found on host-plants growing in diverse backgrounds, because many of the herbivorous insects are eaten by the higher numbers of predators arrested also at such sites.

In this review, we will discuss briefly the seven hypotheses put forward to date. We will then go on to describe a theory based on “appropriate/inappropriate” landings which we believe is the key, or “missing link”, to host-plant selection by herbivorous insects. A more detailed description of the theory can be found in our original review (Finch & Collier 2000). This greatly shortened version has been included in the current proceedings for completeness, as it was the main subject the senior author was asked to address at the meeting.

Materials and methods

Laboratory and field experiments were done to determine how growing cabbage plants (*Brassica oleracea* L.) (Cruciferae) in backgrounds of bare soil and subterranean clover (*Trifolium subterraneum* L.) (Papilionaceae), affected host-plant finding by eight pest species belonging to four insect orders.

All insects were produced in the Insect Rearing Unit at HRI Wellesbourne. The insects tested were the small white butterfly (*Pieris rapae* L.), the large white butterfly (*P. brassicae* L.), the cabbage root fly (*Delia radicum* L.), the mustard beetle (*Phaedon cochleariae* Fab.), the diamondback moth (*Plutella xylostella* (L.)), the garden pebble moth (*Evergestis forficalis* L.) the cabbage moth (*Mamestra brassicae* L.) and the cabbage aphid (*Brevicoryne brassicae* L.). Depending upon species, between 30-200 insects were used per replicate in each experiment. The cabbage (*Brassica oleracea* var. *capitata* Alep.) plants were grown in 7.5cm pots, tested at the "five true-leaf" stage, and left in their pots throughout the experiments. The laboratory experiments were done in a large rotating cage or in smaller Perspex® cages. The rotating cage (160 cm x 160 cm x 63 cm high) contained a 145 cm diameter turntable, which rotated once every four minutes. The rotation ensured that all treatments placed on the turntables were exposed equally to the insects, which aggregated near the strip lights used to illuminate the test chamber. The Perspex® cages were sufficiently large (80 cm x 48 cm x 54 cm) to house one seed-tray of clover and one of soil. Field experiments were done in large (600 cm x 315 cm x 180 cm high) field cages and in the open field.

In each laboratory test, a pot containing a test plant was inserted in the centre of each seed-tray of clover or bare soil. In each field-cage, 32 host plants were arranged, at 50 cm spacing, in four rows of eight plants; alternate plants being surrounded by either clover or bare soil. Most experiments lasted five to ten days and involved at least ten replicates. The insect eggs were counted daily. To reduce bias, host-plants removed from one background were placed into the opposite background when re-introduced into a test cage. The field experiments were done using sixteen $25m^2$ plots, arranged as a 4 x 4 Latin square. The four treatments, each replicated four times, were plots of cabbage plants undersown with subterranean clover, plots undersown with white clover (*Trifolium repens* L.) and two treatments in which the plants were surrounded by bare soil. One of these last two treatments was subjected to the full insecticide and fungicide schedule (positive control) and the other was left unsprayed (negative control).

Results

In all experiments and for all eight species, fewer (P=0.05) eggs (colonies for the cabbage aphid) were found on cabbage plants (15-20 cm tall) surrounded by green clover (10-12 cm tall) than on similar plants surrounded by bare soil (Finch & Kienegger 1997). The percentage reductions in egg numbers ranged from 39±5% for the diamondback moth to 94±3% for the cabbage moth (Finch & Kienegger 1997). When the small white butterfly was presented with cabbage plants of different sizes, the clover (10-12cm tall) reduced the numbers of eggs laid by only 48±4% on 25cm tall cabbage plants and had no effect on 35 cm tall plants. In addition, the numbers of eggs laid by the cabbage root fly, the diamondback moth and the large white butterfly on host plants presented in brown (dead) clover (230±33, 87±9 and 98±15, respectively) did not differ (P=0.05) from those laid (255±46, 81±9 and 94±14, respectively) on host-plants presented in bare soil (Finch & Kienegger 1997).

Results similar to those mentioned above were obtained in field experiments, in which undersowing with clover enabled commercially-acceptable cabbage plants to be harvested, without having to apply insecticide, fungicide or herbicide.

Discussion of the earlier hypotheses

Although authors indicated that diverse backgrounds affected host-plant selection in the ways described earlier, it is hard from both the current results and published data to refute the view that all species are affected similarly (Finch & Collier 2000). Of the earlier proposals, "visual camouflage" implied "concealing an object" (Smith 1976), whereas our mechanism (see later) is dependent on all surfaces being clearly visible to the searching insect. It is doubtful also whether physical interference *per se* (Perrin 1977) contributes greatly, as the brown clover had the same plant architecture as the green clover, but did not deter the searching insects. Similarly, by leaving the test plants in their pots throughout the experiments, root exudates from the non-host plants could not cause physiological changes in the host plants (Theunissen 1994). In addition, no evidence has been produced during the last 18 years to support the suggestion (Uvah & Coaker 1984) that the non-host plants produce their effects through chemical deterrence. Even

backgrounds of plants such as sage (Dover 1986), thyme (Dover 1986) and onions (Uvah & Coaker 1984), selected specifically for their pungent odours, have failed to deter insects from landing on their host plants. The host-plant odour being "masked" by that of the non-host plant (Tahvanainen & Root 1972) also does not seem to be the mechanism, as similar effects were obtained when host plants of the cabbage root fly were surrounded by weeds, spurrey (*Spergula arvensis* L.), peas (*Pisum sativum* L.) or clover, all of which have different odour profiles (Finch & Collier 2000). More striking, however, is that the effect was produced also when host plants were surrounded by green, but not brown, plant models (Kostal & Finch 1994), or by sheets of green paper (Ryan *et al.* 1980; Kostal & Finch 1994 & 1996), neither of which release plant odours. The current differences also cannot be explained by the "Resource concentration hypothesis" (Root 1973), as the host plants are at the same density in both situations. Similarly, the "Enemies hypothesis" (Root 1973) is difficult to support, as it would be against established principles, to suggest that predators are found mainly on the host-plants in the clover when most prey colonize the host-plants in the bare soil. Contrary to the earlier claims (see Altieri 1994), differences in colonization alone appear sufficient to account for the lower numbers of pest-insects found when host plants are grown in diverse backgrounds.

The theory

Instead of the seven hypotheses described previously, we believe that a mechanism that we have described as "appropriate/inappropriate landings" is the central link in host-plant selection by insects. Our theory of host-plant selection can be divided into a chain of actions involving just three inextricably-bound links. In the first link, volatile chemicals emanating from plants indicate to flying receptive insects that they are passing over suitable host-plants (Figure 1-1). Once the odour of the host-plant in the air becomes sufficiently concentrated, it induces the insect to land (Figure 1-2). In this way, the volatile chemicals bring the insects into the close vicinity of the host plants. However, during the last few milliseconds, when the insects are only a short (often < 1m) distance away from the plant, instead of maintaining their directed response to volatile stimuli, herbivorous insects switch to a directed response to green objects, which in most cases means to plant leaves. It is not surprising that vision takes over at this stage, as most flying animals use vision to "pin-point" a suitable object on which to land. Therefore, insects that fly over plants growing in bare soil will be stimulated to land on host plants, the only green objects available to them (Figure 1-3a), as most herbivorous insects avoid landing on brown surfaces, such as soil.

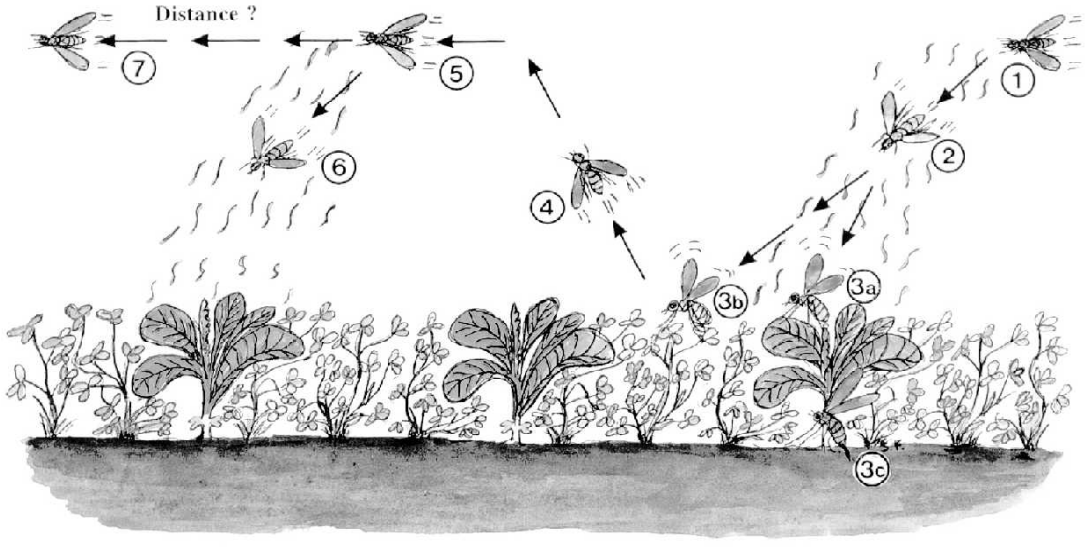

Figure 1. Schematic diagram to illustrate how diverse backgrounds, here represented by clover (*Trifolium* spp.), influence host plant finding by the cabbage root fly. Numbers represent insect actions 1-7 (see text). Figure copied from Finch & Collier 2000.

When host plants are growing in bare soil, most landings will be what we have classed as "appropriate" and so the host-plants will in effect "concentrate" the insects. In contrast, insects flying over host plants surrounded by clover, land in proportion to the relative areas occupied by leaves of the host (Figure 1-3a) and non-host (Figure 1-3b) plants, as specialist herbivorous insects do not discriminate between the two when both are green. Hence, any landings made on the non-host plant, here represented by clover (Figure 1-3b), are classed as "inappropriate". The amount of time the insects spend on the leaves of the non-host plants before taking off again is governed by whether the insects receive acceptable or antagonistic stimuli

through their tarsal receptors. Once the insects are again airborne (Figure 1-4), if they are stimulated to land after flying only a relatively short distance (Figure 1-5 & 6), they could land on a host plant. In all situations, however, the plant on which the insect first lands, even if it is a "host plant", may not stimulate the insect sufficiently, via its contact chemoreceptors on the tarsae or head appendages, to arrest it, and the overall process will be repeated. If this represented the complete system, then under “no-choice” situations in the field, it could just be a matter of time before the numbers of eggs laid on host plants growing in diverse backgrounds were similar to those laid on host plants growing in bare soil. However, this does not occur, as there is a second phase to host plant finding.

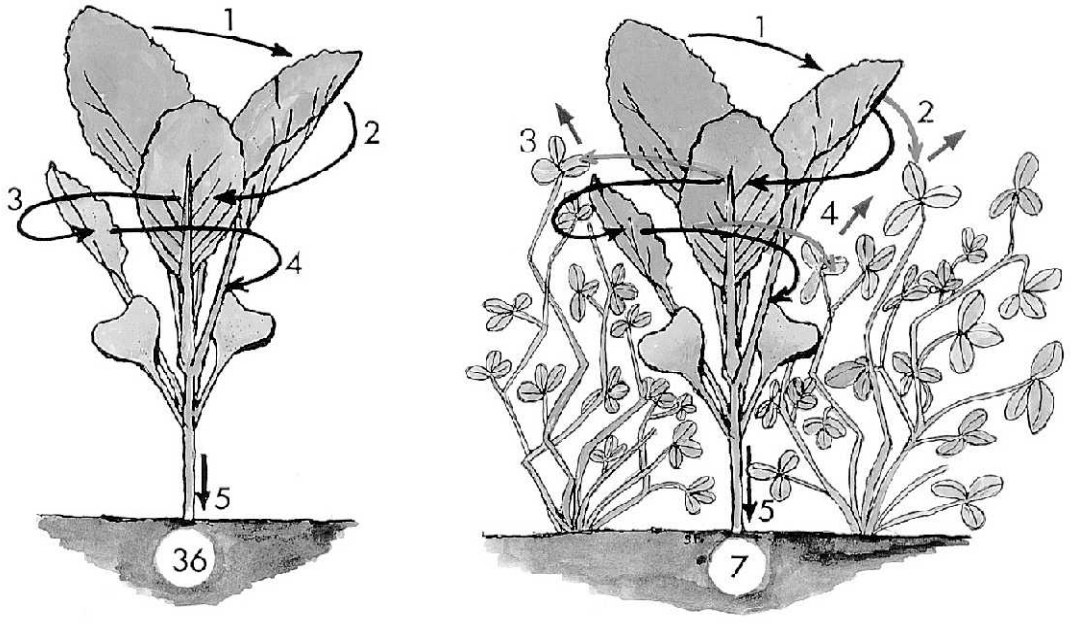

Figure 2. Schematic diagram to illustrate how diverse backgrounds, here represented by clover (*Trifolium* spp.), influence host plant acceptance by the cabbage root fly. Numbers represent the four (mean no.) leaf-to-leaf flights made by the fly to ascertain whether the plant is a suitable substrate around which to lay its eggs. Figure copied from Finch & Collier 2000.

This second phase can be illustrated (Figure 2) most clearly by data collected from a detailed study of the cabbage root fly. The figure shows that before accepting a host plant as a suitable site for oviposition, receptive female cabbage root flies make, on average, four spiral flights before laying eggs alongside the plant (see Figure 1-3c). Hence, the insects stand a much greater chance of “losing” the host plant in a diverse background as, on average, they repeat the initial appropriate/inappropriate landing procedure a further three times. Observations under laboratory conditions showed that for every 100 females that landed on a *Brassica* plant surrounded by bare soil, thirty-six (Figure 2 - left) received sufficient stimulation from the plant to be induced to lay eggs. In contrast, only seven (Figure 2 - right) out of 100 females that landed on host plants surrounded by clover managed to lay eggs. Fewer flies managed to lay in this situation, because following each short spiral flight, a proportion of the flies landed on the leaves of the surrounding clover plants. This failure to re-contact a leaf of a host plant after any spiral flight prevented the females from accumulating, within the allotted time, sufficient stimulation from the host-plant to be induced to lay eggs. Hence, the barrier that this fly faces when its host plants are grown in diverse backgrounds is not chemical nor mechanical, but behavioural, simply because during the innate series of spiral flights the fly must continue to accumulate more positive host-plant stimuli each time it lands.

Discussion

The maximum distance recorded for insect orientation to host-plant volatiles in the field is only a few metres (Finch 1980). The amounts of volatile chemical needed to induce directed responses are invariably several orders of magnitude greater than those released naturally (Finch 1980). For example, in the relative laminar airflow of a wind tunnel, cabbage root fly could be induced to fly upwind when host-plant odour was released at 2.5g/day (Hawkes & Coaker 1979). Such an amount (2.5g/day) is equivalent to the volatile chemicals released daily from 100,000 plants (Finch 1980), or 2 ha of a commercial crop, concentrated into a point source. Furthermore, although the receptive flies moved towards the odour source, the results were

unexpected, as more than 90% of the "flights" were shorter than 50 cm (Hawkes & Coaker 1979). Such behaviour supports the suggestion that the cue from volatile plant chemicals is to stimulate the insects to land. Finally, even when large amounts of chemical are released from insect traps in the field, many of the insects responding miss the trap on landing (Finch 1980, Prokopy *et al.* 1983) and do not enter subsequently (Finch 1995, Kostal & Finch 1996) a further indication that the insect uses visual rather than chemical stimuli when selecting its landing site.

The current "appropriate/inappropriate landings" hypothesis, which involves visual stimuli as the pivotal link, seems more convincing than mechanisms based solely on chemical cues. Its great advantage is that once an insect has landed, it has by-passed the major difficulty of obtaining directional cues from odours while still in flight (Murlis *et al.* 1992). In addition, disruptive air movements around host plants (Murlis *et al.* 1992), the small amounts of volatile chemical released (Finch 1980), the short distance over which the insect responds (Finch 1995), the closing speed of the flying insect (Finch 1980) and the fact that many receptive insects miss the "target" (Finch 1995; Kostal & Finch 1996), all suggest that the central link in host-plant finding is not governed by volatile chemicals but by visual stimuli. Hence, as only visual stimuli are important in the critical central link, there should be an infinite number of plant combinations that could be used to "deter" pest insects. In addition, it is still debateable whether it is easier for insects to locate a source of odour once they have landed. For example, of the Colorado potato beetles (*Leptinotarsa decemlineata* Say) that came within the 60 cm radius of detection of an individual potato plant, only half were “attracted” to the plant (Jermy *et al.* 1988).

To ensure that a high proportion of the searching insects land on non-host plants, the foliage of both plant types must be in the insect's field of vision at the time it lands. Hence, to obtain the maximum impact from undersowing in crop protection, the relative height of the two plant types is crucial to the success of the overall system. When the outline of the host plant is made obvious, either by mowing the intercrop to reduce plant competition (Theunissen *et al.* 1995, Finch & Kienegger 1997) or by allowing the host plants to protrude well above the background crop (Finch & Kienegger 1997), then the effect is lost.

Finally, before intercropping can be considered a viable alternative to applying insecticides for pest control in large-scale commercial production, further work is required to study in detail the effects of the selected plant combinations on crop pathogens and weeds, and to generate the agronomy required to ensure that the main crop receives adequate nutrients and water.

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Developing Trichogramma *(*Hymenoptera: Trichogrammatidae*)* as a pest management tool

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Abstract

The egg parasitoid, Trichogramma (Hymenoptera: *Trichogrammatidae*), is used extensively around the world as a biological control agent for the control of lepidopterous pests. Wasps are either released to augment an existing population "inoculative release," or they are released in large numbers to coincide with maximum pest pressure "inundative release." Field releases however, have had variable success. This has been attributed to wasp quality and issues relating to the release and integration of wasps into an agricultural setting. Wasp quality can be split into genetic and environmental components. Here, genetic quality is discussed in terms of the identification and maintenance of species/strains most suited to the particular situation. Environmental effects that are thought to impact upon wasp quality include rearing host effects; rearing conditions i.e. under constant environmental conditions, or by acclimation; and storage conditions. Release and integration issues that are considered important for the development and maintenance of a successful IPM approach include host/parasitoid synchrony, pesticide choice and timing of application as well as weather conditions at the time of release. In this paper we focus on and discuss quality issues, both genetic and environmental, as well as consider information pertaining to optimal release conditions, in relation to the development and maintenance of Trichogramma as an effective biological control agent.

Introduction

Parasitoids can have a major impact in natural and agricultural ecosystems where they influence or regulate the population density of many of their hosts (Godfray 1994). Trichogramma and other egg parasitoids are generally part of the local ecosystem and often contribute to the control of lepidopterous pests in the absence of disruptive pesticides. There are many examples of pest control by naturally occurring Trichogramma such as the control of *Helicoverpa* eggs in corn in Brazil (De Sa & Parra 1994), control of the noctuids, *Brusseola fasca* and *Jesamina calamistis,* and pyraloids, *Chilopartellus orichalociliellus* and *Eladana saccharins,* which attack maize in east Africa (Bonhof *et al.* 1997) and control of the cranberry fruitworm, *Acrobasis vaccinii,* in the U.S.A. (Simser 1994).

Naturally occurring predators and parasites are often not present in sufficient numbers at the right time to keep pest species within an economically sustainable limit. Trichogramma release programs can be used to overcome these limitations. There are two ways to use Trichogramma release in pest control: "inoculative" releases to maintain and augment an existing population, or "inundative" releases to introduce large numbers of insectary reared Trichogramma to coincide with maximum host presence. Both approaches aim to increase Trichogramma parasitism of the pest to reduce crop losses.

Trichogramma have been used in inundative releases more than any other natural enemy (Stinner 1977). Situations where Trichogramma are used to control lepidopterous pests include grapes (Glenn & Hoffmann 1997), tomatoes in greenhouses (Shipp & Wang 1998), tomatoes in the field (Consoli *et al.* 1998) and sugar cane (Greenberg *et al.* 1998a). Trichogramma are also used against *Helicoverpa armigera* on a variety of crops in India (Romeis & Shanower 1996) and on sweet corn in Australia (Scholz *et al.* 1998). Trichogramma is an effective biological control agent against the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae), throughout Europe (e.g. Mertz *et al.* 1995) and North America (Andow *et al.* 1995). They are used in nonfood crops such as cotton (Naranjo 1993) and to provide foliage protection in forests (Bai *et al.* 1995). Trichogramma are even used against Lepidoptera in stored grain, where *Trichogramma evanescans* and *T. embryophaga* attack *Ephestia kuehniella* and *E. elutella* (Scholler *et al.* 1996).

Despite the widespread use of Trichogramma, there are relatively few cases where the successful control of a pest can be unequivocally ascribed to releases of these parasitoids. There are many documented failures of Trichogramma releases despite a few notable successes (Twine & Lloyd 1982, Smith *et al.* 1987, Li 1994).

The variable success of Trichogramma field releases can be attributed to a number of factors (Smith 1996). These include pesticide applications, weather conditions at the time of release, lack of host parasitoid synchrony and the quality of the release material. This last area is complex and encompasses diverse issues such as technical aspects related to rearing, storage and shipping of the release material as well as the characteristics of the Trichogramma chosen for release programs.

In this paper we focus on quality issues and consider three main questions. Firstly, how do we ensure that the Trichogramma strain or species is suited to release conditions? This requires effective screening of genetic variability. Once suitable strains are identified, mass reared populations need to be monitored to ensure that undesirable characteristics are not selected. Secondly, which environmental factors can influence quality and how do we manipulate these factors to ensure that the quality of released wasps is maximised? Most environmental factors relate to the nature of the rearing host, which can influence the size and behaviour of the adult wasps. Finally, what release conditions influence the success of Trichogramma? Releases should ideally be integrated into all aspects of the target pest environment using a system approach (Lewis *et al.* 1997).

Quality issues: genetic and environmental components

Phenotypic variation can have genetic and environmental components. Environmental variation is often mediated via the size of the host egg, which in turn affects the size of the Trichogramma (e.g. Bennett & Hoffmann 1998, Glenn & Hoffmann 1997). In addition, there are genetic factors that can act independent of size. For example, Bigler *et al.* (1988) and Dutton *et al.* (1996) found that walking speed was affected by genetic factors independent of size.

Genetic quality: identification and maintenance of appropriate strains

Successful commercial release programs require Trichogramma that are effective against target hosts under field conditions. The overall release quality of Trichogramma will be influenced by many traits. Identifying which traits are related to optimal release quality is a major problem when screening for suitable strains. It depends on the specific field situation in which the parasitoids are being used as to which trait(s) will be important. Once an effective strain has been identified, quality needs to be maintained, avoiding selection for undesirable traits in mass rearing conditions.

There are many examples where Trichogramma species and strains of the same species differ in traits that may be related to field performance. For instance, the ability of foraging females to discover hosts has been found to vary among strains. Foraging ability is likely to be related to the surface area that females prospect per unit time (Bigler *et al.* 1993) and walking speed (Bigler *et al.* 1988, Dutton *et al.* 1996). Strain variation has also been found for longevity (Bigler *et al.* 1993, Dutton *et al.* 1996), emergence rates (Dutton *et al.* 1996, Frei & Bigler 1993), sex ratio (Bigler *et al.* 1993) and fecundity on both natural and factitious hosts (Bigler *et al.* 1993, Dutton *et al.* 1996, Frei & Bigler 1993).

Trichogramma strains can also differ in the extent to which they respond to environmental change. Variation has been found between species and strains for their ability to survive and parasitise under different temperatures (Pak & Van Heiningen 1985). The variation in Trichogramma strains for cold tolerance and adaptability to low temperatures makes this a useful criterion for evaluation of candidate strains for inundative releases in cold conditions (Voegele *et al.* 1988). Flight propensity (Forsse *et al.* 1992, Prasad *et al.* 1999), walking speed and activity (Suverkropp *et al.* 2001) and rates of travel (Boldt 1974) are also affected by temperature and may represent useful traits for screening.

While it is clear that variation exists among Trichogramma strains and species for a number of traits that may contribute to the success of field releases, there are three questions that need to be addressed when assessing the potential impact of this variability.

What is the relative contribution of genetic versus environmental factors to the phenotypic variation?

Identifying strain differences within one generation represents a first step in a genetic analysis, but provides no information as to whether variation persists across generations particularly if environmental conditions change. Genetic studies need to encompass more than one generation and should ideally consider the heritability of traits across different environments. Examples of genetic studies on parasitoids encompassing more than one generation include Wajnberg and Colazzo (1998) and Bennett and Hoffmann (1998).

What are the effects of mass rearing on phenotypic variation?

Once useful strains have been identified, phenotypes can still change due to commercial conditions. Mass rearing facilities usually have constant temperature and regulated periods of dark and light, with high parasitoid host densities and limited need for host searching behaviours such as walking or flying. The presence of genetic variation within species means that Trichogramma may readily adapt to conditions used in mass rearing facilities (Sorati *et al.* 1996). This appears to be one of the major problems encountered in quality control of Trichogramma for mass release (van Bergelijk *et al.* 1989). Inbreeding effects may also cause a decline in quality in commercial cultures. However, as Trichogramma are haplodiploid, major inbreeding effects are unlikely. Sorati *et al.* (1996) suggest that the absence of inbreeding effects in Trichogramma could be used in the maintenance of mass reared colonies. If the strains selected for mass release could be intensively inbred to fix useful genes and minimise their loss during adaptation to artificial environments, desirable traits could be maintained.

Which traits are relevant for field success?

There is likely to be an association between locomotion and parasitism in the field. Variation in travel speed has been used to estimate capacity for host location and the efficiency of *T. maidis* strains for inundative biological control programs (Bigler *et al.* 1988). There have also been attempts to combine quality parameters in devising an index for quality (Liu & Smith 2000). In particular, Dutton *et al.* (1996) measured four quality parameters: walking speed, lifespan and fecundity on the natural host as well as the factitious host. However, fecundity on the factitious host was found to be a better predictor of success in the field than the quality index. More recently, fluctuating asymmetry has been looked at as an additional indicator of wasp quality. Hewa-Kapuge and Hoffmann (2001) found that although there was no association between the asymmetry of individual traits and wasp fitness, the ability of *Trichogramma* nr. *brassicae* to successfully find host eggs could be predicted by the combination of asymmetries of nine different traits.

Environmental effects: rearing host

To rear Trichogramma on a commercial scale, it is necessary to use a factitious rearing host, such as *Ephestia kuehniella* or *Sitotroga cerealella,* rather than the natural or target host. The choice of factitious host is often dictated by the ease of rearing and not necessarily by any factors related to the likely success of the wasps being produced. Factitious hosts are selected on the simplicity of their mass production, mechanisation of rearing processes and cost of production compared with that of using the target pest (Greenberg *et al.* 1998b).

There are several potential effects of rearing parasitoid biocontrol agents on a non-target host. Rearing host can affect qualities such as development time (Bai *et al.* 1995), travel speed (Boldt 1974), longevity, percent emergence and sex ratio (Corrigan & Laing 1994). The size of the factitious host egg could alter the size of the emerged wasp, which could have effects on wasp attributes such as target host acceptance and fecundity. Often Trichogramma reared from one host will be used to target several pests, introducing many potential complications, as the same wasps may not be suitable across several different hosts.

There appear to be only minor changes in acceptance of the target host when Trichogramma are reared on a factitious host. Bjorksten and Hoffmann (1995) found that oviposition experience had a stronger effect on host preference than pre-adult experience (learning through development in rearing host). Bjorksten and Hoffmann (1998) found experience effects in *T.* nr. *brassicae* due to rearing host and oviposition by females, but these effects only influenced the likelihood of parasitism in low ranked hosts and not in high ranked hosts that would comprise the target pests in a mass release program.

There is evidence that rearing host can cause variation in size of emerged wasps (Bai *et al.* 1995). *T. carverae* reared on *E. postvittana* eggs are significantly larger than those reared on *S. cerealella* (Glenn & Hoffmann 1997) and the size of mass produced *T. pretiosum* and *T. minutum* adult females is dependent on the size of the rearing host egg in which the insect develops (Greenberg *et al.* 1998c). But does size matter? There are reports of increased size being associated with increased fitness in parasitoids, measured as success in locating hosts (Bennett & Hoffmann 1998, Kazmer & Luck 1995) and with increased fecundity (Bai *et al.* 1995, Greenberg *et al.* 1998c) although this is not always the case. Bigler *et al.* (1993) found that wasps emerged from *E. kuehniella* were bigger than those emerged from *S. cerealella*, but there was no perceived quality difference.

Because results may not be consistent across studies, it is necessary to examine factors influencing field performance in chosen species/strains. For instance, where size may be a good indicator of success of *T. carverae* released in grapevines (Bennett & Hoffmann 1998), this may not be true of other species/systems. Fecundity on the factitious host may be species/strain specific so it is necessary to compare fecundity on the rearing host to the target host before a comprehensive cost-benefit analysis of mass rearing-release system can be made (Corrigan & Laing 1994, Greenberg *et al.* 1998c).

Other environmental effects

Apart from host effects, other environmental factors may also impact on wasp quality. In particular, wasps can become acclimated to particular conditions. Mass production facilities rear the wasps at constant temperature (usually 25ºC). It is possible that wasps reared under constant conditions in insectaries are of low quality because they fail to survive extreme temperature fluctuations encountered in the field (Scott *et al.* 1997). This may reduce the effectiveness of releases in cold weather. Scott *et al.* (1997) reared *T. carverae* at three temperatures (14ºC, 25ºC and 30ºC) and found that only wasps reared at 14ºC parasitised eggs at that temperature. The substantial reduction in parasitism of the sugarcane borer, *Diatrae sacharalis,* by *T. galloi* in the winter months may be related to a thermal alternation shock, as the wasps are mass reared at constant temperatures (Consoli & Parra 1995).

Responses to high temperature can also be improved via acclimation and may be useful in improving the quality of beneficial insects (Huey & Berrigan 1996). There is some experimental evidence that heat resistance can be increased by acclimation. Scott *et al.* (1997) found that heat hardening *T. carverae* adult wasps at 33ºC or 35ºC for 1–2 hours increased survivorship at 40ºC. Maisonhaute *et al.* (1999) and Hoffmann and Hewa-Kapuge (2000) also found that high temperatures could protect against subsequent lethal heat shocks. Unfortunately any benefits of acclimation could be offset by fitness costs of the acclimation process (Hoffmann 1995, Huey & Berrigan 1996). Gunie and Lauge (1997) found that temperature shocks affected the emergence rate and fecundity of females. Even a short low amplitude shock of 32ºC to *T. brassicae* had a strong negative effect on emergence rate and fecundity under benign conditions. However, in both *T.* nr. *brassicae* and *T. carverae*, acclimation conditions can be set so that there are no deleterious effects on parasitism rate and longevity (Hoffmann & Hewa-Kapuge 2000, Thomson *et al.* 2001). Acclimation seems a promising procedure for releases when temperature extremes are unavoidable, particularly as acclimation has been shown to increase parasitism under field conditions (Thomson *et al.* 2001). But specific research is needed to determine the optimum temperature, stage of development and length of exposure for different species/strains to improve performance at high temperatures while minimising the costs.

Quality control

To ensure a high quality product is delivered to the grower, optimum conditions for rearing, storage and shipment are imperative. It is important that producers become aware of the negative effects of poor handling of Trichogramma (Dutton & Bigler 1995). Bigler *et al.* (1993) tested the quality of commercially available Trichogramma and concluded that more elaborate product control systems were necessary to increase reliability of the product.

Product quality is recognised as one of the most important reasons for failure of the biological control agent *T. chilonis* against *H. armigera* (Romeis *et al.* 1998). O'Neil *et al.* (1998) evaluated the quality of four commercially available natural enemies including *T. pretiosum.* The post shipment quality from ten companies was assessed for emergence rates, sex ratio, survivorship, species identity, reproduction and parasitism. Considerable differences in the number received, survivorship and emergence rates were found. Field studies using commercially available *T. brassicae* against *O. nubilalis* in sweet corn differed in emergence profiles in two different years (Mertz *et al.* 1995). When several commercially available species of Trichogramma used against *Plutella xylostella* were compared, inconsistent responses were observed within most of the products indicating potential problems with quality control (Vasquez *et al.* 1997).

The insects need to be reared on an industrial scale and cold storage makes possible the management of large quantities of living material for intensive periods of use. It is necessary for rearing facilities to have reliable systems for storing eggs, pupae or adults (Chang *et al.* 1996). Diapause and quiescence constitute major physiological adaptations for sustaining survival during environmental extremes and both adaptations can have practical applications in storage during mass rearing (Chang *et al.* 1996, Zaslavski & Umarova 1990).

Unfortunately, cold storage may impact on the success or efficiency of the organisms to be released. These effects include reduced fecundity (Chang *et al.* 1996, Frei & Bigler 1993), poor flight activity (Dutton & Bigler 1995), reduced longevity (Jalali & Singh 1992) and reduced emergence rate (Cerutti & Bigler 1995, Jalali & Singh 1992).

Release and integration issues

After selection of the optimum species or strain for the target host and ensuring the best product possible after storage and shipment to the point of release, release conditions need to be considered to ensure maximum parasitism. The grower has some control in ensuring host parasitoid synchrony, avoiding contact with harmful pesticides, controlling the stage of development at the time of release, providing protection from predation and releasing in optimal weather conditions.

Host parasitoid synchrony

Host availability is the key to realising the highest possible level of parasitism. For successful augmentative releases, monitoring of the pest species is essential to ensure application of the Trichogramma when host eggs are available (Hassan 1994). Large numbers of released organisms need to be synchronised closely with the start of oviposition in the pest (Smith 1994). Monitoring of the target host must be continued through the growing season of the crop and further inundative applications made when appropriate. It is also necessary to ensure that target eggs in the area at the time of release will be acceptable to the released Trichogramma. A high rate of parasitism will be achieved only if the wasps reach the host eggs when they are susceptible to parasitism (Glenn & Hoffmann 1997). Reports vary as to the acceptability of host eggs of different ages to Trichogramma species. Some eggs are acceptable for most of their development time (Glenn & Hoffmann 1997), but other studies show the age of host eggs will affect performance (Shipp & Wang 1998, Monje *et al.* 1999).

Pesticides

Whether attempts to improve levels of Trichogramma parasitism are by inoculative or inundative methods, there is a high potential for failure if there is associated pesticide use. Trichogramma is generally sensitive to pesticides (Franz *et al.* 1980). Chemicals can be immediately toxic and cause death (contact toxicity) and this effect can persist (toxicity of dried residue) (Hassan *et al.* 1998, Thomson *et al.* 2000). Contact with pesticides at the less susceptible life stage (parasitoids within hosts) can cause prolonged development time of immature stages and reduced rates of emergence, fecundity, parasitism capacity, adult longevity and mating likelihood (Franz *et al.* 1980, Consoli *et al.* 1998, Hassan *et al.* 1994, Brunner *et al.* 2001, Takada *et al.* 2001).

Exposure of Trichogramma adults to pesticides may also interfere with mating. Delpuech *et al.* (1999) found that pyrethroids and chlorpyrifos at sublethal doses interfered with sex pheromone communication. Male *T. brassicae* treated with a very low dose of the insecticide deltamethrin failed to respond to females and untreated male response to treated females was also significantly decreased. In Trichogramma, like other insects, sex pheromonal communication probably involves nervous transmission both for the reception and emission of the pheromone. Even if insecticides do not provoke mortality, sublethal doses may be a threat for species and population equilibria by modifying physiological or behavioural parameters such as pheromonal communication.

There is a changing perception of pesticide use as resistance and effects of chemicals are recognised, leading to ways to minimise chemical use and combine pesticide use with protection of introduction of beneficial organisms in IPM programs. As it becomes more apparent that biological control is an important component of pest management, there has been a change in consumer attitude which is leading to the development of suitable new pesticides. Research is currently identifying pesticides suitable for use in conjunction with biological control, as it is clearly preferable to use chemicals that have minimum toxicity to beneficial organisms (Franz *et al.* 1980). To determine which chemicals will be most suitable for inclusion in an IPM program, insecticides must be tested for their effect on beneficial species. A battery of standardised laboratory and semi field test protocols based on lethal and sublethal effects as evaluation criteria for the side effects of pesticides on beneficial organisms have been developed by the IOBC/WPRS working group 'Pesticides and Beneficial Organisms' (Hassan *et al.* 1994, Hassan *et al.* 1998). The results showed that preparations greatly differ in initial toxicity as well as persistence.

Reducing pesticide resistance and replacing insecticide use with inoculative or inundative releases of Trichogramma is complicated by the fact that each crop is sprayed with many chemicals. For this reason, rather than approach biological control as a replacement for chemical control, a more conservative integrated pest management (IPM) approach is often used. In such a program efforts are made to reduce the use of pesticides, to test and use less toxic pesticides to beneficial species and to remove releases of beneficial organisms as far as possible from spray applications (Thomson *et al.* 2000). In fact several researchers report higher parasitoid activity and better control of pests without the use of pesticides (Scholz *et al.* 1998, Simser 1994). For instance, in *Helicoverpa armigera* on sweet corn in Australia, an application of deltamethrin reduced the action of a large natural population of *T. pretiosum* wasps and resulted in higher larval infestation and significantly more cob damage (Scholz *et al.* 1998).

Weather conditions at the time of release

Weather is one of the most important factors influencing the performance of biological control agents (Naranjo 1993, Wang *et al.* 1997, Shipp & Wang 1998, Fournier & Boivin 2000). Climatic factors, particularly temperature extremes, must be considered in any project involving pest management with beneficial insects. In India, several species of Trichogramma have gained importance against different lepidopteran pests, but the performance of these parasitoids under some climatic conditions seems erratic (Ramesh & Baskaran 1996). *Trichogramma galloi* is the most common egg parasitoid of the sugar cane borer, *Diatraea saccharalis,* in São Paulo, Brazil. Field releases indicate a substantial reduction in parasitism capacity of *T. galloi* during the winter months (Consoli & Parra 1995). The likely weather conditions at the precise time of release also need to be considered particularly where Trichogramma may encounter daily extreme temperatures. Ambient air temperatures in desert cotton growing areas of California and Arizona where Trichogramma are released to control pink bollworm, *Pectinophora gossypiella* Saunders, frequently exceed 40ºC (Naranjo 1993). *T. brassicae* released to control European corn borer, *O. nubilalis,* in south east France may be exposed to temperatures as high as 44ºC during dispersal in enclosed cardboard capsules (Chihrane & Lauge 1996).

Concluding remarks

To address the inherent variability, each pest system must be studied both in the laboratory and in the field before a cost benefit analysis can be completed. Maximum fecundity on the target host, competence at the required temperature (is acclimation appropriate?) and optimum rearing host to produce wasps of high fitness and fecundity are key considerations. Different rearing procedures can result in important variation in the quality of the Trichogramma. This variation as well as genetic changes in continuous laboratory cultures may have significant effects on laboratory reared parasites to be released in the field. The economics of production may favour producing and releasing greater numbers of less efficient parasitoids to obtain the same degree of control although this may not compensate for low quality Trichogramma (Dutton *et al.* 1996). Low numbers of wasps should prove equally effective if large individuals with a high fitness are released (e.g. Bennett & Hoffmann 1998). The cost benefit analysis for each system must include the cost of producing the optimum Trichogramma for the situation and investigate whether the same result can be achieved more economically by producing more of a less effective wasp. Field trials are essential to confirm that laboratory tests of suitability are confirmed prior to release. Finally research is needed to understand methods of storage and dispersal of wasps to ensure the best product possible is released at the point of use.

In summary, improvement in the success of Trichogramma as a biological control agent requires optimisation of the materials used, particularly choice of rearing host for provision of a high quality product. This product cannot be affected by inappropriate cold storage or poor conditions during transit and needs to be released at the optimum time to maximise the number of Trichogramma at the time of maximum host density. Releases should be appropriately timed to minimise interaction with toxic pesticides or else relatively harmless pesticides must be chosen. Most importantly, each system must be studied with the optimal species or strain chosen and the potential of acclimation assessed. Obviously not all these parameters can be chosen to fit a specific case, but in a cost benefit analysis, the cost of each choice can be considered in terms of maximising benefit. A more sophisticated/integrated approach is required where all aspects of the host/parasitoid interaction as well as the physical conditions such as temperature or presence of pesticides need to be considered.

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Improvement of crucifer IPM in the Changjiang River Valley, China: from research to practice

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Abstract

Crucifers comprise a major group of vegetable crops in the Changjiang River Valley, China. The control of insect pests on crucifer vegetable crops has largely relied on the heavy use of chemical insecticides in the last 30 years, resulting in serious consequences of insecticide resistance, increased costs of control and insecticide residues hazardous to human health. A group of Chinese and Australian scientists have undertaken a joint venture to develop practical and sustainable IPM strategies for crucifer vegetable crops in this region. The work consists of three overlapping and ongoing phases: problem definition, research and development, and implementation. Natural enemies were surveyed and evaluated. Biological and selective insecticides were screened through bioassays and field tests. Damage relationships by various insect pests were assessed by artificial defoliation and natural infestation of plants. It was found that cabbages could endure some defoliation without reduction of head weight, but that the level of compensation varied with the growth stages being attacked. Plants at the pre-heading or mid-late heading stages could endure substantial damage while plants at transplant or cupping to early heading stage were sensitive to damage. These findings on various components interrelated to IPM were used to develop management strategies at the crop system level, which were tested in the field. Field IPM trials across several seasons and localities showed that the new strategy could offer effective control of all insect pests. Compared with conventional methods, IPM practices could usually reduce insecticide input by as much as 30–70%, with little risk of crop loss. Implementation activities included grower involvement in field trials, field days and participatory workshops as well as frequent dissemination of fact sheets. Evidence shows that a substantial improvement in farmers' knowledge, attitude and approaches towards IPM has been achieved in the project areas. The future challenges to and opportunities for improving crucifer IPM in China are discussed.

Keywords

crucifer vegetables, action thresholds, natural enemies, biological insecticides, integrated pest management

Introduction

Crucifers constitute one major group of vegetables in China. The area under vegetable cultivation has increased about 5-fold in the last 20 years, reaching over 14 million ha in 2000, based on single crops. The proportion of crucifers usually accounts for 40–55% of all vegetable crops, depending on the region. In Zhejiang province, the proportion of crucifer vegetables has been decreasing in recent years due to an increase in other crops, but remains over 40%.

In the Changjiang River Valley, crucifer vegetables are mostly grown by small landholders (<0.5 ha) around urban centres, in highlands, and in specialised production areas. The crop systems are complex and erratic, revolving as they often do around intercropping (growing more than one crop on a small piece of land at the same time) throughout the year. A complex of insect pests attacks crucifer vegetable crops. As an example, Figure 1 shows the major insect pests that the crucifer growers usually have to deal with through the seasons in a year in Hangzhou, Zhejiang.

In the last 30 years, the control of insect pests on crucifer vegetable crops in China has largely relied on heavy use of chemical insecticides. This has had serious consequences of insecticide resistance, increase of control costs, and most importantly pesticide residues hazardous to human health. Here we report a joint effort by farmers, extension officers and researchers that has helped to improve the situation in some

localities. We then discuss briefly the challenges to and opportunities for improving integrated pest management (IPM) in crucifer vegetable crops in the Changjiang River Valley, China.

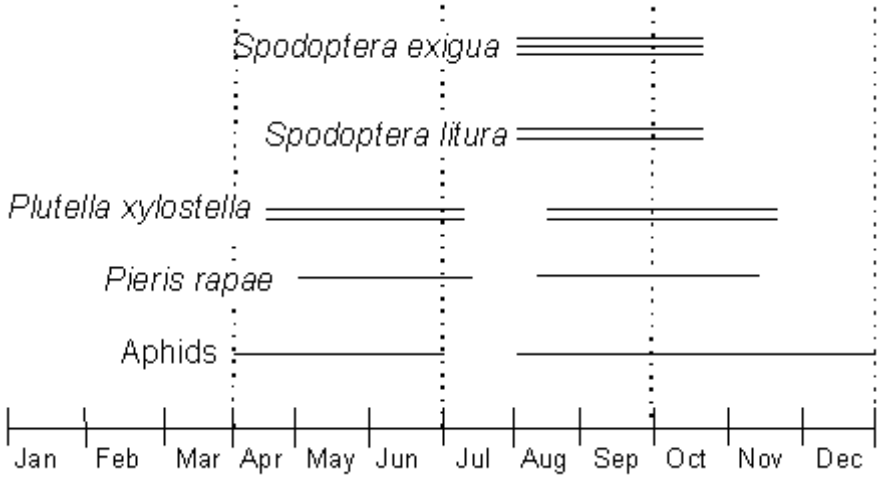

Figure 1. Occurrence of insect pests that may cause serious damage to crucifer vegetable crops during various periods of the year in Hangzhou, China.

A joint venture in improving crucifer IPM

This project was started in 1995 to build on existing studies to develop sound, sustainable crucifer IPM strategies that significantly reduce pesticide hazards, and are acceptable to the growers in the east range of the Changjiang River Valley. It has involved five institutes in China, working in close collaboration with two institutes from Australia (Liu *et al.* 1996). The working strategy consists of three overlapping and ongoing phases: problem definition, research and development, and implementation. Structured problem definition workshops, involving all groups of stakeholders and in particular farmers and extension workers, were organised at the start of the project to promote information flow, determine priority issues, address priority needs and propose action plans (Liu *et al.* 1996, Norton & Mumford 1993). Work has since been concentrating on the following five, interacting components: (1) survey and evaluation of natural beneficial insects, (2) rational application of insecticides, in particular promoting use of biological insecticides, (3) development of action thresholds, (4) development of management strategies, through season-long in-field IPM trials and (5) IPM implementation activities.

Survey and evaluation of parasitoids

Regular sampling in both farmers' fields and unsprayed fields in Hangzhou showed that a range of parasitoids attack each of the major pests. For example, the diamondback moth (DBM), *Plutella xylostella* is attacked by at least eight species of parasitoid, of which *Cotesia plutellae, Oomyzus sokolowskii* and *Diadromus collaris* were the major larval, larval-pupal and pupal parasitoids respectively (Liu *et al.* 2000). *Pieris rapae* is attacked by the following 7 species of parasitoids: *Trichogramma chilonis* in the egg stage, *Cotesia glomeratus* and *Hyposoter ebeninus* in the larval stage, and *Pteromalus puparum, Brachymeria lasus, Pimpla disparis* and *Iseropus kuwanae* in the pupal stage. Of the 7 species, *C. glomeratus* and *P. puparum* were most abundant.

Insect parasitoids are active in the fields despite heavy use of chemical insecticides in the crop systems over the years. For example, parasitoids usually achieved 10–60% parasitism of DBM larvae and pupae during June to early July and September-November each year when DBM was most abundant (Liu *et al.* 2000). The biology of the major DBM parasitoids has been studied to provide information essential for understanding and evaluation of these beneficial insects (Wang *et al.* 1999; Liu *et al.* 2001, 2002; Shi *et al.* 2002; Wang & Liu 2002). Liu *et al.* (2004) showed that DBM resistance to an insecticide not only confers some protection to an endo-larval parasitoid, but also helps selection of resistance genes in the latter. This new information may help to gain more understanding of parasitoid survival in the field. The impact of natural enemies on the survival of DBM in the field has been investigated under different management strategies (see below).

Evaluation of biological and selective insecticides

Biological and chemical insecticides were bio-assayed in the laboratory and tested in the field. A number of Bt and NPV products were shown to have high efficacy in killing the target pests without side effects on the

beneficial insects (Liu & Zhang 1997, Shi & Liu 1998). Other insecticides showing desirable selectivity include: abamectin, avermectin, spinosad and fipronil against DBM and *P. rapae,* chlorfluazuron and chlorfenapyr against *S. litura* and *S. exigua,* and imidacloprid against aphids (Liu & Zalucki 2001, Guo *et al.* 2003).

Development of action thresholds

Laboratory and greenhouse trials by artificial defoliation demonstrated that the common cabbage (cv. Jingfeng No. 1) can endure some defoliation without reduction of head weight at harvest. There was substantial evidence of over-compensation for defoliation at the pre-heading stage. However, the plants were sensitive to defoliation at the cupping stage (Table 1). Results of trial by insect defoliation in the field seemed to agree with the findings of artificial defoliation (Figure 2). These data were used to develop action thresholds for practical application (Table 2). Of particular value was the characterisation of crop growth stages sensitive to insect damage. Thus, farmers and extension officers were asked to monitor the insect pests more carefully at both the seedling and cupping stages.

Table 1. Head weight of cabbage at harvest after artificial defoliation of middle and outer leaves at various growth stages

Growth	Head	Proportion of defoliation			
Stage	Measurements	1/8	1/4	1/2	Control
Pre-heading	Head weight (kg)[a]	1.01 ± 0.26 a	0.84 ± 0.22 b	0.82 ± 0.16 b	0.92 ± 0.18 ab
	Increase (%)[b]	9.78	-8.70	-10.86	
Cupping	Head weight (kg)	0.88 ± 0.28 ab	0.81 ± 0.24 b	0.82 ± 0.20 b	0.92 ± 0.18 a
	Increase (%)	-4.34	-11.96	-10.86	
Heading	Head weight (kg)	0.95 ± 0.25 a	0.91 ± 0.23 a	0.86 ± 0.23 a	0.92 ± 0.18 a
	Increase (%)	3.26	-0.84	-5.53	

[a] Mean ± S.D. head weight per plant (n = 45 to 50) , and means in the same row followed by the same letter do not differ significantly (P>0.05, Fisher LSD test).
[b] Percent increase of head weight in comparison with that of the control, i.e. zero defoliation.

Table 2. Action thresholds (mean number of insects/plant) used in IPM treatment

Pests	Cabbage growth stages			
	Transplants	Pre-heading	Cupping to early heading	Heading to mature
Lepidoptera[a]	0.5	1.0	1.0	4.0
Aphids	5	500	500	2000

[a] Numbers of lepidopterous larvae were converted to "standard" insects by the following formula:
1 standard insect = 1 *Pieris rapae* = 1 *Spodoptera exigua* = 0.5 *S. litura* = 5 *Plutella xylostella.*

IPM field trials

Based on the findings of studies of various components and information from literature, management strategies were formulated and tested in the field to develop practical IPM guidelines and protocols. The major components in the IPM strategy included use of action thresholds in decision-making and strategic use of biological and selective insecticides (Tables 2 and 3). Field IPM trials with common cabbage were conducted in Hangzhou from 1996 to 2000 and, in 2000, trials were conducted at five sites at Zhejiang and Shanghai. The results showed that biological and selective insecticides could offer effective control of all the insect pests. The activities by natural enemies were evidently promoted (Table 4). Compared with conventional practice, IPM practice could usually reduce insecticide input by as much as 30–70%, with little risk of crop loss (Table 4, Zhang *et al.* 1999).

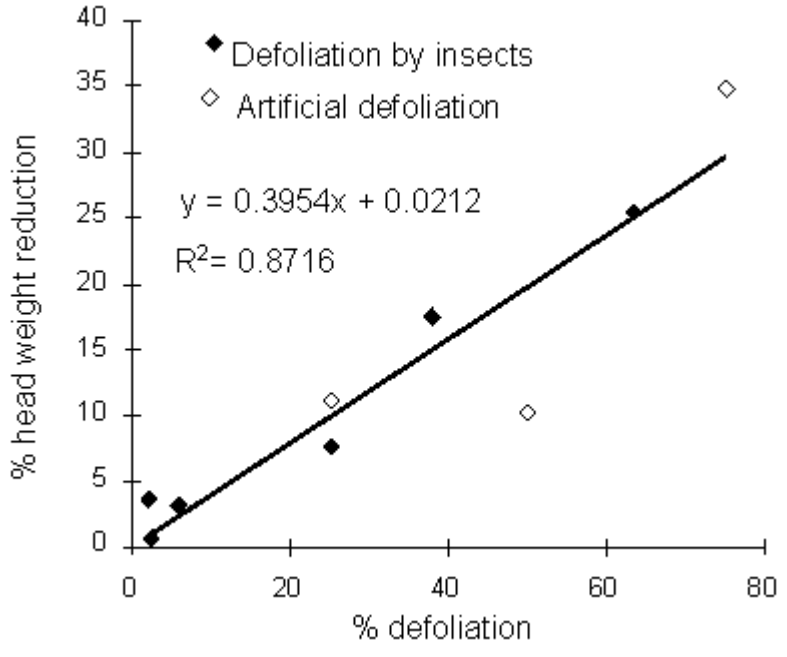

Figure 2. Relationship between defoliation at the cupping stage and reduction of head weight at harvest in common cabbage (cv Jingfeng No.1). Each data point represents the mean value of 50 plants.

Table 3. Summary of designs of field IPM trials

Treatment	Description	Application of insecticides
IPM	Use of action thresholds, apply biological and selective insecticides	Spray Bt for control of DBM and *Pieris rapae,* spray NPV and chlorfluazuron for control of *Spodoptera* spp. and spray imidacloprid for control of aphids
Farmer	Simulation of typical practice by farmers, or recording of farmer's practice	Calendar sprays with mixtures of hard chemical insecticides such as chlorpyrifos, fenvalerate, methomyl, fipronil, and methamidophos

Table 4. Examples of IPM trial results in Hangzhou in autumn 1998 and autumn 2000

	1998		2000	
Assessments [a]	**IPM**	**Farmer**	**IPM**	**Farmer**
Mean head weight (kg)	1.23 a	1.11 a	1.18 a	1.02 a
% marketable heads	94.4 a	88.0 b	95.6 a	91.1 a
% heads without insect damage	52.5 a	16.7 b	76.7 b	96.7 a
Number of sprays	7 (8)	8(23)	3(5)	5(8)
Cost of insecticide application per ha (RMB)	2,700	3,780	680	1,025
% parasitism of DBM larvae	19.4 a	2.0 b	35.2 a	7.1 b
% parasitism of DBM pupae	32.6 a	1.3 b	18.8 a	13.0 a

[a] Figures in the same row of the same year followed by the same letter do not differ (P>0.05, Student-t test).
[b] In the IPM treatment, usually one insecticide and only rarely a mixture of 2 insecticides was used per spray, while in the Farmer treatment, usually a mixture of 2–3 insecticides was used per spray. Figure in brackets indicates the relative amount of insecticide input calculated on the basis of one insecticide in one spray at the recommended rates.

Implementation

Implementation activities included grower involvement in field trials, field days and participatory workshops, frequent dissemination of fact sheets, as well as short training courses for extension officers and growers (Liu *et al.* 1996, Liu & Zalucki 2001). An independent project evaluation in the project areas showed substantial improvement in farmers' knowledge, attitude and approaches towards IPM. For example, 36% of the growers in the project areas do regular monitoring of insect pests on their crops and try to

choose biological or selective insecticides for spray, compared with about 20% in the non-project areas. Growers in the project areas were found to have much more frequent contacts with extension officers than the non-project areas (Liu & Qiu 2001).

Future challenges

Morse and Buhler (1997) analysed the conditions for successful IPM, which include relatively simple agro-ecosystems, strong research and extension capacity, and stable markets, among others. These authors rightly point out that it is much more difficult to develop and adopt IPM in developing countries than in developed countries. Because of the complicated nature of crucifer vegetable ecosystems in the Changjiang River Valley, the development and implementation of crucifer IPM in this region indeed presents serious challenges to all the stakeholders involved.

Research and development

For IPM methods to be widely acceptable, they must be simplified as much as possible, particularly the use of monitoring and action thresholds. With the erratic vegetable cropping systems in the Changjiang River Valley, development of practical and reliable IPM methods for any crop will certainly take well-designed field trials across several seasons.

Implementation

Wearing (1988) lists the obstacles to IPM implementation under five interrelated headings: technical, financial, educational, marketing/social and organisational. These apply to crucifer IPM in the Changjiang River Valley. Liu and Yan (1998) discussed these obstacles in some detail. For example, in regard to the organisational obstacles, they pointed out that the co-ordination among organisations, disciplines and personnel will remain a serious problem. IPM can only be implemented effectively on an area-wide scale (Morse & Buhler 1997). This calls for close co-operation of many farmers in an area, which is difficult to achieve. Lack of trained extension workers will continue to be a major obstacle. In China, many of the state-employed extension workers have been directly involved in marketing chemical pesticides since the late 1980s. Consequently, their advice to farmers is no longer IPM-oriented but biased towards increasing pesticide inputs. This unfortunate situation has been seen to be a major reason for the rapid increase of pesticide application in recent years in China. It seems unlikely that this organisational problem will be overcome quickly.

Future opportunities

Major opportunities for promotion of IPM in crucifer vegetable will come from: (1) consumers' demand of food safety, (2) development of better organised farming, (3) increased support for research and extension, and (4) policy and legislation support (Liu & Yan 1998).

Consumer aversion to pesticide residues and increasing demands for food safety have been major forces driving implementation of IPM in vegetables in many Asian countries. In China, serious poisoning of humans by insecticide residue on crucifer vegetables has frequently been reported since the mid 1980s. These poisoning events initiated the demand by consumers for reducing chemical pesticide use on vegetables. Cosmetic standards of vegetables have become less stringent (Liu *et al.* 1996). Monitoring of pesticide residue has increased in both domestic vegetable supplies and international trade. There is some evidence that many consumers are prepared to pay a slightly higher price for “green and clean” vegetables. As the life style of consumers improves, their demand for eliminating pesticide residue on vegetables will become stronger and provide increasing opportunities for biological methods of pest control.

In China, IPM has been formally promoted as the national plant protection policy since 1975. However, effective policy support and legislation are still under development. National and local policies and legislative measures that ensure reduction of chemical insecticides, and increased food safety and environment protection are essential for successful implementation of IPM on crops, including crucifer vegetables. There is much to be done in this area.

Concluding remarks

Our work in the last several years proves that improvement of crucifer IPM can be achieved through a participatory approach involving growers, extension agents, and researchers. However, the control of insect pests on crucifer vegetable crops in the Changjiang River Valley as a whole still relies heavily on chemical

insecticides. While obstacles to vegetable IPM are many, consumer aversion to pesticide residues, changes in the cropping system, increased support for research and extension, and a more favourable policy and legislation environment will act together to provide opportunities to achieve area-wide implementation of crucifer IPM in the years to come.

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Variability in *Plutella* and its natural enemies: implications for biological control

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Abstract

Since 1996, as part of a cooperative ARS/CIRAD *Plutella* biocontrol program, 115 *Plutella* populations have been collected in 32 countries from cole crops and cruciferous weeds. Twenty-seven primary hymenopterous parasitoid species were found, especially *Diadegma* spp., *Cotesia plutellae* and *Oomyzus sokolowskii*. A number of fungal pathogens, isolates of *Paecilomyces fumosoroseus, Paecilomyces* sp., *Metarhizium* sp. and *Beauveria* sp. were found in Australia, Benin, Romania and Georgia respectively. Biological, biochemical and genetic differences have been shown in DBM and their natural enemy populations from different geographic origins. Marked differences have been found in the behaviour of *C. plutellae* and *O. sokolowskii* towards different populations of DBM and some inter population crossings resulted in failure or only male progeny. *Metarhizium* from Romania and *Paecilomyces* from Australia killed 70–95% of DBM larvae exposed to them; other pathogens were less effective. Biocontrol of DBM is required in the south western USA where another pest, *Bemisia tabaci* biotype B, present in the same habitat, is managed by biocontrol based means. For successful biocontrol of DBM it may be necessary to evaluate and select natural enemies based on their association with the target DBM population.

Keywords

Biocontrol, *Diadegma*, *Cotesia*, pathogens

Introduction

Plutella xylostella (L.) (Lepidoptera: Yponomeutida*e*) the diamondback moth (DBM) is a widely distributed and damaging pest of crucifer crops, resistant to many pesticides and *Bacillus thuringiensis* var. kurstaki and causes about $1 billion losses annually (Talekar & Shelton 1993). More than 90 species of insect parasitoids *(Ichneumonidae*, *Braconidae* and *Eulophidae*) are recorded, but less than ten have biocontrol potential (Noyes 1994). More than ten species of predators are known (Coccinellidae, Syrphidae and Neuroptera) (Alam 1992). Nine species of fungal pathogens: *Zoophthora radicans*, *Beauveria bassiana*, *Paecilomyces fumosoroseus*, *Pandora blunckii*, *Hirsutella* sp., *Erynia* sp., *Conidiobolus* sp., *Scopulariopsis* sp. and *Metarhizium* sp. (Mercadier *pers. comm.*) and one species of nematode: *Steinernema carpocapsae* (Baur *et al*. 1995), have been recorded worldwide. It is generally agreed that larval and pupal parasitoids such as *Diadegma insulare* (Cresson), *D. semiclausum* Hellén, *Diadromus collaris* (Gravenhorst) (Ichneumonidae), *Cotesia plutellae* (Kurdjumov) (Braconidae) and *Oomyzus sokolowskii* (Kurdjumov) (Eulophidae) have the greatest control potential and egg parasitoids contribute little to natural control (Talekar & Shelton 1993).

DBM is one of the key pests in crucifer crops in the USA and any attempt at its control needs to take into account the control of other pests in the crop such as *B. tabaci* biotype *B. Bemisia* and DBM are major, region-wide pests, often occurring together in the southern USA where crucifers are an important component of the agricultural system. Both pests exhibit strong invasive potential, resistance to pesticides and, in the case of DBM, resistance to *Bacillus thuringiensis*. In addition, *Bemisia* has a wide host plant range which makes it a very serious threat to a broad range of crops. Both have spread widely due to the intervention of man in moving infested cuttings and seedlings from one part of the country to another. In the USA, losses due to *Bemisia* were $500–750 million/annum and to diamondback moth sporadically $100s of millions/annum. The ARS European Biological Control Laboratory, Montpellier, France, in cooperation with the Entotrop laboratory of CIRAD, Montpellier, Rhodes University, South Africa and AVRDC Taiwan, in association with ICIPE, Kenya, began foreign exploration for natural enemies of DBM in 1996. One of the aims of this cooperative work is to discover and evaluate natural enemies for biological control of *Plutella xylostella* in the south-western USA where *B. tabaci* biotype B, which is currently successfully managed by

bio control based means, is also present in the same habitat. Related goals are to study the interactions of the pests and their natural enemies in the field and laboratory and contribute to the elucidation of pest and natural enemy biotypes.

Material and methods

Collections were made in many countries often in collaboration with local entomologists. The insects were brought to Montpellier under French Agriculture Department and often country of origin permits, reared through one generation at CIRAD-AMIS and identified using the CIRAD reference collection. *Plutella* populations were conserved in liquid nitrogen for later biotyping. Diseased insects were collected in the field, isolated in Montpellier, identified and deposited in the EBCL and ARS Ithaca NY entomopathogenic fungi collections. Parasitoids were reared through one generation and either conserved or used in experiments.

DBM rearing

The population of DBM used in the experiments originated from Cotonou (Benin). Parasitoids and DBM rearing took place on *Brassica oleracea* L. (cv. Château Renard) in a controlled temperature room under the following conditions, temperature: 26 ± 1°C; relative humidity: 70% ± 5%; photoperiod: 12 L:12 D.

Crossing experiments using *O. sokolowskii*

Populations of *O. sokolowskii* originated from DBM larvae collected at Natitingou Benin (B), Cluj in Romania (R) and near Lahore in Pakistan (P). Rearing took place under the same conditions described previously. Thirty females and six males of each *O. sokolowskii* population were successively introduced into separate boxes (diameter 8 cm x 5 cm high) containing 15 *P. xylostella* IV larvae. These conditions were maintained for 24 h, after which the larvae were withdrawn and fed until pupation.

Pupae of *O. sokolowskii*, recovered from DBM pupae parasitised 15 days previously, were put individually into Petri dishes. At emergence, a female from one population was put together for 24 h with a male from another population. A IV instar larva was added then withdrawn after 24 h and fed until pupation. Each of the six possible crossings was repeated five times. On emergence of the F_1, the egg-adult duration and sex ratios (number of males to females) were noted. Adult F_1 were kept for 24 hours in their respective Petri dishes, before the addition of five IV DBM larvae for a further 24 hours before withdrawal. After emergence of F_2 from those larvae, egg-adult duration and sex ratios were noted. The letters denote the geographic origins of the female (first) and the male.

Oviposition behaviour in the presence of variable numbers of females

Every day, a variable number of IV larvae was exposed to parasitism for 24 h. At the end of this time larvae were withdrawn, placed in individual Petri dishes and fed until their pupation. The three tests carried out were repeated six times. Test 1/1 was (1 female *Oomyzus*/1 DBM larva), test 1/10 (1 female *Oomyzus*/10 DBM larvae) and test 10/10 (10 female *Oomyzus*/10 DBM larvae). The information noted was, the number of parasitised larvae per female, the number of adult parasitoids emerged per pupa, the progeny of a female, the sex ratio of the progeny, the length of the life history and 50% of the progeny observed.

Morphometric study (Ratio of antennal length: body length).

The antennal lengths and body lengths of eighty individuals of each sex from each population were measured.

Crossing of *Cotesia plutellae* populations

All *Cotesia* populations were reared under the same conditions, 25°C, 12h/12h photoperiod and 75% Relative Humidity. The *Cotesia* populations were from South Africa, Benin, Martinique, Réunion and Taiwan and were collected where no exotic *Cotesia* have been released. Control crossings were made between males and females from the same source to confirm compatibility and the appearance of females from F_2 crossings (*C. plutella*e is haploid and the presence of males only, indicates infertility). In addition, incompatibility may only be recorded in the F_2 because sterile females may be produced in the F_1 stage. The partners of each pair come from different populations and were chosen at random. The same procedure was used in the F_2 pairings. Ten replicates per combination were made. Ten all-Martinique pairs were used as a control.

Genetics

Reported elsewhere in these proceedings (Pichon et al. 2004).

Pathology

Aerial conidia from three strains of fungal pathogens isolated from *Plutella* were produced in Petri dishes of saborau/dextrose/yeast agar. Conidia were sprayed at two concentrations onto 20 II instar *Plutella* larvae from each geographic strain, replicated four times/strain.

Results

The results of current exploration are presented below (Table 1).

Table 1. Number of primary DBM parasitoids by region

Area	Ichneumonidae	Braconidae	Chalcididae	Eulophidae	Total
Africa	5	3	1	1	10
N America	1	1	0	0	2
S America	2	2	2	0	6
Asia	3	2	0	1	6
Australia	0	0	0	1	1
Caribbean	1	2	0	1	4
Europe	5	3	0	2	10
Indian Ocean	2	1	0	2	5
Total species	12	7	3	4	27

Twenty seven primary parasitoid species were collected (Appendix 1), *Diadegma* species being the most common, with *D. semiclausum* Hellén present in all samples collected from the Palearctic except North Africa. Other *Diadegma* spp. were found in Réunion, Brazil and the Dominican Republic. *Diadromus collaris* (Gravenhorst) was collected in France. The most common Braconidae species was *Cotesia plutellae* (Kurdjumov). *Dolichogenidea litae* (Nixon) was found locally (North and West Africa) and *Dolichogenidae* sp. commonly in Brazil (Brasilia). The genus *Microplitis* is present in temperate regions: *M. mediator* (Haliday) in Romania and *M. plutellae* Muesebeck in USA. The eulophid, *Oomyzus sokolowskii* (Kurdjumov), which acts mostly as a primary parasitoid (Talekar 1997), was widespread and found in Africa, Asia, Australia and Europe (Table 2).

Table 2. DBM parasitoids (selected countries)

Country	Ichneumonidae	Braconidae	Chalcididae	Eulophidae
France	3*-***	2**	0	1***
Romania	3*-***	2*-***	0	2*-***
Réunion	2***	2*-**	0	3*-**
Brazil	1***	2*-***	1*	1*
Benin	0	1***	1*	1***
Ethiopia	2***	0	0	0
S. Africa	3*-***	2*-**	0	1***
Australia	0	0	0	1**
Pakistan	0	0	0	1***
Uzbekistan	2***	1***	0	0

Common***, Local**, Scarce*

Crossings between *O. sokolowskii* populations

Those from Romania and Pakistan produced either a higher number of males: female or no progeny, all other crossings produced similar sex ratios and viable progeny (Table 3).

Table 3. Results of crossing different populations of *O. sokolowskii,* n = 5

female x male	F_1		F_2	
	Egg-emergence (d)	sex ratio (♂:♀)	Egg-emergence (d)	sex ratio (♂-♀)
B x P	21.8 b	1:7 bc	22.2 a	1:6 b
P x B	20.0 b	1:8 bc	20.0 b	1:7 bc
B x R	23.3 b	1:13 c	21.1 ab	1:10 c
R x B	21.3 b	1:6 b	21.5 ab	1:7 bc
P x R	25.3 a	1:2 a	22.5 a	1:3 a
R x P	*	*	*	*
B x B	22.0	1:5		
R x R	21.3	1:5		
P x P	23.0	1:6		

B: Benin; R: Romania; P: Pakistan; *: no progeny obtained; a, b, c: in a column, significantly different (ANOVA and Newman-Keuls test, at 5 % level)

Oviposition behaviour

Females from the Pakistani and Romanian populations parasitised the same number of larvae regardless of numbers of hosts and number of *O. sokolowskii* females present during oviposition; Benin females by contrast parasitised very few larvae.

Morphometric study (*C. plutellae*)

The ratio antennal length: body length variable in females was significantly different between two groups of populations; South Africa-Martinique and Taiwan-Benin-Réunion. The ratio was > 1 in the South Africa-Martinique group.

Crossing of *Cotesia plutellae*

Two groups of populations were separated out (Figure 1). South Africa-Martinique and Taiwan- Benin-Réunion. They were entirely compatible with each other inside a group, but not with the second group and *vice versa.*

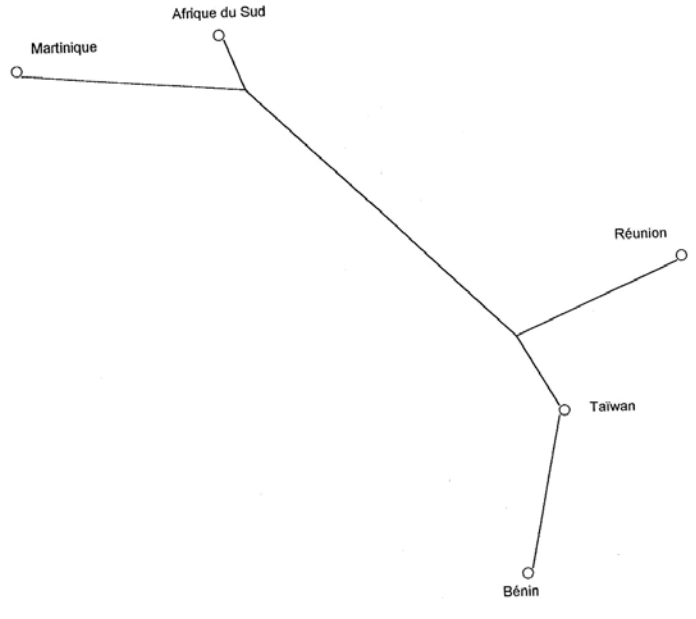

Figure 1. Results of crossing 5 populations of *Cotesia plutellae* represented in tree form (DARwin 3.5).

Pathology

Even at the low concentrations used, *M. anisopliae* from Romania and *P. fumosoroseus* from Australia killed between 70–96% of larvae treated (Table 4).

Table 4. Percentage mortality of *Plutella xylostella* larvae from different geographic origins when treated with Hyphomycetes

Origins of *Plutella xylostella*	*Metarhizium anisopliae* x 10^7	*Paecilomyces fumosoroseus* x 10^7	*Beauveria bassiana* x 10^7
Languedoc	95	71	29
Martinique	90	79	28
South Africa	96	39	32
Benin	80	74	22

Metarhizium anisopliae: Voronetz, Romania.
Paecilomyces fumosoroseus, Atherton Tableland, Queensland, Australia.
Beauveria bassiana: Renée, Republic of Georgia.

Discussion

Both *Bemisia* and *Plutella* are introduced pests into the same crop habitat in the south western US and are vulnerable to classical biocontrol. In the case of *Bemisia*, cole crops act as reservoir plants for overwintering populations and sustain considerable direct damage. After the appearance of *Bemisia* biotype B in the early 1990s, several visible disorders of Cruciferous crops appeared at the same time in California and southern Texas (Perring *et al*. 1991, Elsey & Farnham 1994). Successful biocontrol based management of *Bemisia* in the southern USA has been achieved in 5–8 years using 3 hymenopterous parasitoids chosen after rigorous selection from 36 species/strains of natural enemies (Kirk *et al*. 2001).

Foreign exploration for natural enemies of *Plutella* and the resulting biocontrol organisms discovered, have driven the programme in Montpellier through taxonomy, genetic characterisation, to evaluation. In the past *Plutella* natural enemies have been collected and released without knowledge of possible biological and genetic variability within *Plutella* itself and its natural enemies which may have determined the outcome of introductions.

The main taxonomic problems concern the *Diadegma* (Fitton & Walker 1992). Noyes (1994) has shown that up to 75% of host-parasitoid records are misleading because they are based on misidentifications either of the parasitoid or of the host. They also include wrong host-parasitoid associations. The use of RAPDs PCR technology would elucidate the species and strains present.

Although *Cotesia plutellae* is recorded from a number of hosts, Cameron *et al*. (1997), basing their conclusion on laboratory tests, defined this braconid as a narrowly oligotrophic parasitoid. This situation is probably the same for all the key parasitoids of *Plutella*. This is an important point worthy of reflection as the indirect ecological effects of biological control are increasingly being scrutinised. Specificity of DBM parasitoids, or the lack of it, needs to be shown before releases are made. The Mediterranean region is one of the presumed centres of origin of DBM (Harcourt 1954) and crucifers (Tsunoda 1980) and the maximum diversity of the parasitoid complex may therefore be expected in this region. However we have not found an exceptionally rich natural enemy fauna there. Noteworthy were two new *Diadegma* species found in Ethiopia (which did not attack French DBM populations). South Africa merits special attention also as Kfir (1997) has described a little known, but richly diverse fauna of efficient parasitoids. However Smith (*pers. comm.*, 2001) did not find an exceptional natural enemy fauna in the Eastern Cape, where *Plutella* is not abundant. We did not find an exceptional natural enemy fauna in Romania where Mustata (1992) had recorded a very rich fauna. However we did find many species in great abundance and this fact combined with local cultivation practices and the lack of extensive chemical control made Romania an excellent source of DBM natural enemies.

Species complexes and diverse strains may hide the full potential of parasitoids for biological control based area wide IPM. For example, the behaviour and biology of Pakistani and Romanian *O. sokolowskii* show good potential for their use in biological control despite reduced progeny when large numbers of parasitoids are present; by contrast, in the presence of numerous females, parasitism by Benin females is strongly decreased. In addition to these behavioural differences, the Pakistan/Romania *O. sokolowskii* are incompatible, suggesting that they are different strains of the same species. Equally, five populations of *Cotesia plutellae* divided into two compatible groups suggesting the presence of different strains. The use of RAPDs PCR technology will elucidate the species and strains of DBM and natural enemies present. In addition, strains of DBM may be associated with specific strains of parasitoids.

The results of preliminary phylogenetic and isoenzyme studies show strong polymorphism between DBM populations (Pichon, *pers. comm.*).

EBCL has about 1000 fungal pathogen isolates collected from more than 60 countries from a number of insect sources. The *Metarhizium anisoplae* collected from *Plutella* in eastern Romania was particularly efficacious and its potential for use in biocontrol of DBM is high. Conditions at the time of collection were ideal for fungal pathogens with heavy rain showers and 25–30°C.

In conclusion, the selection and release of natural enemies for biological control based IPM of DBM should take into account specificity of parasitoids to DBM, natural enemy/DBM population associations to ensure successful establishment and, in the south-western USA, the successfully implemented biocontrol based pest management program against *Bemisia* in the same habitats as DBM (Kirk *et al*. 2001).

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Appendix 1. Parasitoid complex of DBM

*** common species; ** fairly common species; * rare species

Trichogrammatidae	
Trichogramma sp. (in course of study)	Réunion
Ichneumonidae	
Diadegma insulare (Cresson)	Canada*, USA*, Dominican Rep.*
Diadegma semiclausum Hellén	Bulgaria***, France***, Romania***, Greece***, Uzbekistan***, Georgia***, Spain***, Italy***
Diadegma leontiniae (Brethes)	Brazil***
Diadegma mollipa (Holmgren)	S. Africa***, Réunion***
Diadromus collaris (Gravenhorst)	France***, S. Africa*, Turkey***, Bulgaria***, Uzbekistan, Greece**
Diadromus subtilis (Grav.)	Georgia***
Diadegma 2 spp.	Ethiopia
Diadromus sp.	France*
Diadromus subtilis (Grav.)	Georgia***
Hyposoter sp.	Romania**
Itoplectis sp.	Hungary*, Romania*, Réunion*, S. Africa*
Braconidae	
Cotesia plutellae (Kurdjumov)	France**, Benin***, Réunion**, Hong Kong***, Guadeloupe**, Martinique***, Brazil*, Japan*, Uzbekistan***, Bulgaria***, Senegal*, Turkey***, S. Africa**, Italy***, Vietnam*, Canada**, Greece*
Apanteles litae (Nixon)	Tunisia**, Senegal**, Ivory Coast***, Benin**, Mali**
Apanteles sp.	France*, Austria*, Romania**
Apanteles piceotrichosus (Blanchard)	Brazil***
Apanteles eriophyes (Nixon)	S. Africa*
Glyptapanteles sp.	Martinique*, Réunion*
Microplitis mediator (Haliday)	Romania**
*Microplitis Plutella*e Muesebeck	Canada,* USA*, Taiwan**, Laos,* Cambodia*
Chalcididae	
Brachymeria sp.	Senegal*, Benin*
Conura sp.	Brazil*
C. pseudofulvovariegata (Becker)	Martinique*, Brazil*
Eulophidae	
Euplectrus sp.	Romania*
Tetrastichus howardi (Olliff)	Réunion*
Tetrastichus sp.	Réunion*
Oomyzus sokolowskii (Kurdjumov) (Occasionally facultative hyperparasitoid)	France***, Romania***, Senegal***, Benin***, Réunion*, India***, Brazil**, Guadeloupe***, Martinique***, Pakistan**, Bulgaria*, Mali**, Turkey***, S. Africa***, Italy***, Greece*

Biological and genetic differences between populations of diamondback moth from different geographic origins

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Abstract

The diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae), is a major pest of *Brassicaceae* and has a worldwide distribution. Biological and genetic differences were studied between populations native to South Africa, Benin, Brazil, France, Japan, United States, Martinique, Réunion Island, Uzbekistan and five localities in Australia. To observe the variability of oviposition behaviour between populations, the eggs laid by 20 females from each population studied were counted daily. Females from Benin and South Africa laid more eggs than the females from Uzbekistan and Martinique. Oviposition duration is longest (30 days) in the Uzbekistan population and varies from 15 to 20 days in the other populations. Females from Benin, Réunion Island and Martinique laid the majority of their eggs in the first four days; those from Uzbekistan and South Africa staggered oviposition over the whole period. Concerning genetic differences, the results of isoenzyme electrophoresis reveal variability between populations for nine loci. Tests of the Hardy-Weinberg equilibrium show heterozygote deficits in many populations. Analysis of allelic frequencies gives an estimate of F_{st} of 0.103 for all the populations studied. The populations from Australia and Japan are the most different from other populations and from each other. Some biological and genetic differences are shown between populations. Genetic differences are not correlated with the geographic distance separating the populations.

Keywords

Plutella xylostella, polymorphism, oviposition, isoenzyme

Introduction

Plutella xylostella (L.) (Lepidoptera: Yponomeutidae) is the most important pest of cultivated brassicas in tropical areas (Talekar & Shelton 1993) and has a global distribution. Its geographic origin is generally considered to be in the eastern Mediterranean area, as are *Brassica* species (Tsunoda 1980). However, Kfir (1998) reports that the origin could be in South Africa. The migratory ability of diamondback moth is in part responsible for this wide distribution (Talekar & Shelton 1993). Massive migrations over long distances (>3000 km) have been reported between the south of Finland and England (Chu 1986) and populations observed in Canada probably migrate from the south of the United States of America (Harcourt 1986). Despite its ability to migrate, important differences in insecticide resistance exist between closed populations; sometimes separated by less than ten kilometres (Cheng 1981, Tabashnik *et al*. 1987). Gene flow between these populations may not be sufficient to overcome the differences.

The habitat of a population (temperature, humidity, environmental factors) and growing conditions (size of cultivated area, use of insecticides) differ from one area to another. Considering populations from various countries where selection pressures are variable, are they different from each other? To elucidate these differences, biological characterisation, oviposition activity and enzyme genotype polymorphism of 14 populations have been studied.

Material and methods

Biological activity

The oviposition activities of populations from South Africa, Benin, Martinique, Réunion Island and Uzbekistan were studied. Each sample collected was reared at 25°C, 75% humidity and photoperiod 12L/12D. Mass rearing was maintained on cultivated brassicas: Chinese mustard (*Brassica juncea*), cabbage (*B. oleracea* cv. Châteaurenard) and cauliflower (*B. oleracea* var. *botrytis*). Pupae were isolated from the first generation obtained during the rearing and 20 pairs of a female and a male were randomly chosen, soon after emergence. Each replicate for a population consisted of five females and five males placed in a plastic box. A Chinese mustard leaf received the eggs laid by females. The adults were fed with honey. Eggs were counted every day, until the death of females and the leaf was changed every day. Results were submitted to

an analysis of variance (ANOVA) and to a Newman & Keuls test (α = 5%) with the software Statitcf (Gouet & Philippeau 1992).

Genetic differences

Enzyme electrophoresis on starch gel (14%) was the technique used to sample populations from South Africa, Benin, Brazil, France, United States of America, Japan, Uzbekistan, Philippines, Réunion Island and five localities in Australia: Adelaide, Brisbane, Mareeba, Melbourne and Sydney. Samples collected in the field (100 individuals) were reared in the laboratory (25°C, 12L:12D, 75% HR). Final sampling was made on individuals of the field generation and of the first generation of the laboratory colony. Adults, deep-frozen in liquid nitrogen, were crushed in 20 µ1 of NADP solution. Buffers used were Tris Citrate pH 8, Histidine pH 6, Tris Maleate EDTA pH 7.4, Tris Citrate pH 8.7. Migration (150 V, 70 mA) was 6 h long. Buffers and staining solutions were prepared following Pasteur *et al*. (1987) and Hillis *et al*. (1996) protocols. Bands were analysed in terms of loci and alleles. GENEPOP version 3.1d (Raymond & Rousset 1995), Fstat version 1.0 (Goudet 1994) and DARwin version 3.6 (Perrier & Jacquemoud-Collet 2000) were used to analyse allelic frequencies.

Results

Oviposition activity

Numbers of eggs laid by 20 females each day, for each population, are shown in Figure 1. Variations were observed between populations. The statistical significance of these differences is given in Table 1. The most fecund females were from South Africa and Benin, followed by those from Réunion Island, Martinique and Uzbekistan. Up to 4 days after emergence, females from Benin, Martinique and Réunion Island lay 60–70% of their eggs, while those from Uzbekistan and South Africa lay only 24 to 36%. Females from the latter populations lay more than 20% of their eggs between the 12th and the 20th day after emergence, whilst populations from Benin, Réunion Island and Martinique lay 1% to 4%. The egg laying period also varies with 13 days for populations from Benin and Martinique to 25 days for those from Uzbekistan and South Africa.

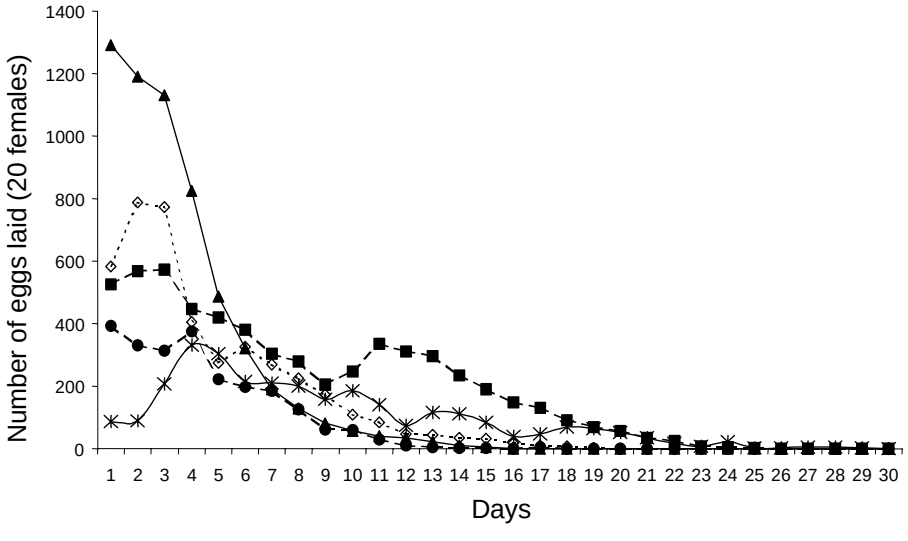

Figure 1. Number of eggs laid each day by 20 females of *Plutella xylostella* with different geographic origins. Black square: South Africa, black triangle: Benin, black circle: Martinique, star: Uzbekistan, diamond: Réunion Island.

Table 1. Differences observed in the oviposition activity of 20 females of *Plutella xylostella* with different geographic origins. a, b, c on the same row: populations significantly different. ANOVA and Newman & Keuls test α = 5%. *: period in days, mean of 4 replicates

Population	South Africa	Benin	Martinique	Uzbekistan	Réunion Island	Fisher test	*P*
Number of eggs	5894[a]	5812[a]	2325[c]	2884[c]	4199[b]	14.19	0.0001
% of eggs laid for day 1 to day 4	35.66[b]	76.11[a]	63.40[a]	24.44[b]	60.49[a]	15.64	0.0000
% of eggs laid for day 12 to day 20	25.95[a]	1.19[b]	1.31[b]	22.01[a]	4.51[b]	8.95	0.0007
Duration*	24[a]	14[bc]	13[c]	25[a]	18[b]	21.28	0.0000

Genetic study

Over 23 enzyme systems were tested in the laboratory, 14 had diffuse bands and two had a monomorphic pattern (glyceraldehydes-3-phosphate dehydrogenase, pyruvate kinase). Seven enzymes had legible and polymorphic loci: isocitrate dehydrogenase (IDH), malate dehydrogenase NADP+ (MDHP), glucose-6-phosphate dehydrogenase (G6PDH), mannose phosphate isomerase (MPI), phosphoglucomutase (PGM), hexokinase (HK), aspartate aminotransferase (AAT). Some isoenzymes had more than one locus: IDH, with IDHs (slow) and IDHf (fast), MDHP, with MDHPs and MDHPf. Hardy-Weinberg equilibrium test revealed that populations have a deficit of heterozygotes concerning loci MDHPs, G6PDH, MPI, PGM, HK and an excess of heterozygotes concerning locus AAT (Table 2).

Table 2. Estimations of Wright's F statistics, for each locus, for all populations of *P. xylostella*

Locus	F_{is}	F_{st}	F_{it}
IDHf	- 0.0098	0.0560	0.0467
IDHs	0.1029	0.0487	0.1466
MDHPf	- 0.0240	0.1401	0.1195
MDHPs	0.1913	0.1730	0.3312
G6PDH	0.4835	0.0199	0.4937
MPI	0.2269	0.0923	0.2983
PGM	0.0084	0.1364	0.1437
HK	0.0256	0.0872	0.1106
AAT	- 0.4165	0.1549	-0.1971
All	0.151	0.103	0.238

Locus PGM is monomorphic in the Japanese population; it is the same for IDHs in populations from Benin, France, Réunion Island and Melbourne. The Fisher test shows that allelic frequencies are significantly different for all pairs of populations, except the pair Benin/Brazil (χ^2=28.029, *P*=0.06162). Those loci having genotypic linkage disequilibria were IDHf with IDHs, MDHPf with G6PDH and MPI. Loci IDHf and MDHPf were not maintained for further analysis. The global fixation index (F_{st}), which defines the level of differences among populations, has a value of 0.103, with $P(F_{st}=0)<0.001$. Populations were very different for loci MDHPs (F_{st}=0.1730), PGM (F_{st}=0.1364) and AAT (F_{st}=0.1549) (Table 2). F_{st} for pairs of populations varies from 0 to 0.2304 (Table 3). Populations from Benin and Brazil are not differentiated. Populations from Australia and Japan are the most different from other populations and from each other. The value of F_{st}, excluding populations from Australia and Japan is 0.047. Differentiation between populations is shown in Figure 2.

Table 3. Fixation index (F_{st}) for pairs of *Plutella xylostella* populations. SA : South Africa, BEN : Benin, BRA : Brazil, FRA : France, USA : United States of America UZB : Uzbekistan, PHI : Philippines, REU : Réunion Island, AUA : Australia (Adelaide), AUB : Brisbane, AUMa : Mareeba, AUMe : Melbourne, AUSy : Sydney

Pop	SA	BEN	BRA	FRA	JAP	USA	UZB	PHI	REU	AUA	AUB	AUMa	AUMe
BEN	0.0168												
BRA	0.0071	0.0002											
FRA	0.0238	0.0639	0.0434										
JAP	0.1236	0.1856	0.1751	0.1846									
USA	0.0317	0.0932	0.0670	0.0670	0.1659								
UZB	0.0262	0.1019	0.0629	0.0360	0.1218	0.0350							
PHI	0.0419	0.0186	0.0132	0.0795	0.1954	0.0809	0.1036						
REU	0.0161	0.0404	0.0132	0.0489	0.1504	0.0676	0.0522	0.0462					
AUA	0.0666	0.1008	0.1070	0.1390	0.0719	0.1168	0.0985	0.1164	0.1267				
AUB	0.1364	0.1797	0.1732	0.2173	0.1664	0.1758	0.1188	0.1958	0.1972	0.1262			
AUMa	0.0858	0.1213	0.1168	0.1689	0.1506	0.1146	0.1281	0.1127	0.1463	0.0973	0.1031		
AUMe	0.1308	0.1756	0.1717	0.2304	0.1749	0.1352	0.1325	0.1799	0.1927	0.1241	0.0387	0.0871	
AUSy	0.0473	0.0440	0.0426	0.0972	0.1590	0.0823	0.0851	0.0445	0.0917	0.0965	0.0878	0.0558	0.0691

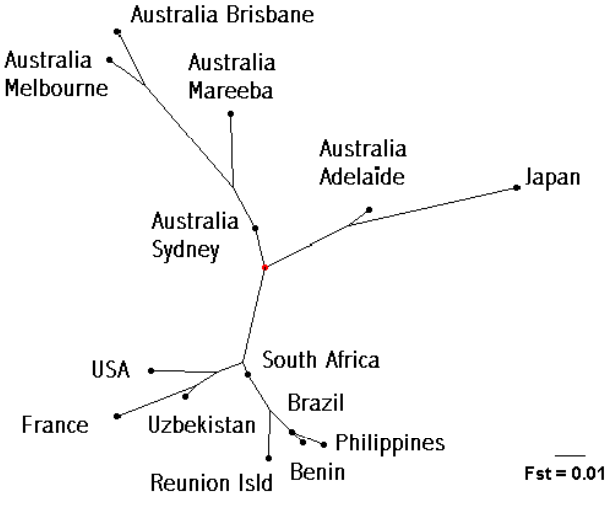

Figure 2. Unrooted tree for fixation index (F_{st}) among populations of *Plutella xylostella*, calculated with the method of Unweighted Neighbour Joining.

Discussion

Biological differences

In this work, the oviposition activity reveals differences among populations concerning fecundity of females, percentages of eggs laid at the start and the end of the oviposition period and duration of oviposition. Results do not reveal a correlation between these variables for the populations analysed. Females from Benin have a short oviposition period and lay a majority of eggs in the first week, whereas those from Uzbekistan show the opposite tendency. But this type of characterisation is not valid for other populations. We observed different characteristic oviposition activities. Females from Uzbekistan have the highest longevity, but the fecundity observed is not maximal. This result is in contradiction with the hypothesis of Poelking (1992) that fecundity and longevity are positively correlated.

Differences are observed among populations placed under the same laboratory conditions. It is conceivable that differences do exist in the field. Variations among females in a population are probably higher and the oviposition may vary according to environmental conditions (temperature, humidity). Whatever their geographic origin, all females do not have the same oviposition pattern. Populations of *P. xylostella* can adapt to their environment.

Those environmental factors affecting the reproduction capacity of females are the host plant (wild or cultivated brassicas) and temperature during biological development. Females lay fewer eggs on wild brassicas (Muhamad *et al*. 1994). The quality and quantity of nutrients of the host plant can influence the

female's reproductive ability (Begum *et al*. 1996). We used F_1 adults from the first generation completely reared in the laboratory on cultivated brassicas; differences observed were not caused by rearing conditions.

Diamondback moth females collected during winter are more fecund than those collected in summer (Yamada & Umeya 1972). A generation of *P. xylostella* reared at 15°C will have bigger adults than a generation reared at 25°C (Shirai 1995). Moreover, fecundity is positively correlated with the size of female adults (Moller 1988, Muhamad *et al*. 1994). Fecundity is also correlated with the photoperiod (Harcourt *et al*. 1966). Numerous factors can influence oviposition and their effects have been analysed. The adaptive ability of *P. xylostella* is important, but still not well known.

Genetic differences

Heterozygote deficits can be due to the sampling method and a Walhund effect may be observed. Moreover, some loci are completely monomorphic in several populations and these heterozygote deficits probably exist in natural populations. F_{st} global value (0.103) is relatively high and above values calculated in previous studies: Caprio & Tabashnik (1992), F_{st}=0.028–0.034; Kim *et al*. (1999), F_{st}=0.0215. For other lepidopteran species, F_{st} among populations are equivalent: F_{st}=0.109 for populations of *Panolis flammea* (Wainhouse & Jukes 1997) or smaller F_{st}=0.080 among populations of *Proclossiana eunomia* (Nève *et al*. 2000) and F_{st}=0.007 among populations of the migrating lepidopteran, *Agrotis ipsilon* (Buès *et al*. 1994). Analysis of data reveals no correlation between geographic distance separating populations and differentiation among them. Samples used in this work have very distant geographic origins (several thousand kilometres for the majority); migrations are reduced among them. Populations from Benin and Brazil have similar allelic frequencies. By contrast, populations from Australia are the most differentiated. Concerning the Benin and Brazil populations, results from enzyme electrophoresis are not informative enough to determine a phylogenic link among them. Further studies with DNA-based markers will elucidate this question. The effects of genetic drift are more important on small populations, as a consequence, a higher number of migrants will produce a gene flow sufficient to overcome the genetic drift (Allendorf & Phelps 1981). The small size of populations from Australia may be an explanation of the differences observed, the genetic drift is important and gene flow therefore reduced.

Concerning loci MDHPS, PGM and AAT, the level of differentiation (F_{st}) is very high. An explanation is a selection of genotypes of allozymes, which confers an advantage to the population. In diamondback moth, the implication of esterase in insecticide resistance (penthoate) has been demonstrated (Miyata *et al*. 1986). Moreover, the function of glutathione-S-transferase in resistance to organophosphorus compounds has been described in previous studies (Cheng *et al*. 1990). In this study, we have frequency analyses of allozymes which have a function in the central metabolic pathways in insects. For example, PGM, G6PDH and HK are implicated in glycolysis pathways or linked metabolic reactions. These enzymes are submitted to selection pressures and their genotypes may vary in function (Carter & Watt 1988). In addition, Herrero (2001) has demonstrated a correlation between the presence of an allele of the locus MPI and resistance to the toxin Cry1A of *Bacillus thuringiensis* in *P. xylostella*. A physiological process does not induce the presence of this allele and it is transmitted to the progeny. Insecticide pressure can select genotypes of enzymes that do not have a direct function in the resistance process.

Conclusions

The analyses of the oviposition activity and of the allozyme genotypes of populations of *P. xylostella* with different geographic origins show differences among them. Concerning the biology, the data obtained in the laboratory show that females from several populations do not lay the same quantity of eggs with the same frequency. In the field, differences probably exist among populations, but the importance of variations within a population over time is to be considered. A better knowledge of the adaptive ability of diamondback moth populations to their environment will permit improvement of the methods to control this pest.

On a genetic level, differences among populations are shown with analysis of allozyme genotypes. However, insecticide pressure and the development of resistance can select these markers. This hypothesis is to be confirmed. In this study, allozyme markers cannot give information on the phylogeny of the populations. Studies based on other molecular markers, independent of insecticide pressure, will elucidate the phylogeographic structure of *P. xylostella* populations.

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Development and assessment of microsatellites and AFLPs for *Plutella xylostella*

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Abstract

If we are to both understand and manipulate diamondback moth populations, a thorough grasp of *P. xylostella* population genetics is essential. However, reliable and informative molecular markers are currently unavailable. We have developed *P. xylostella* microsatellites and report preliminary results on their variability within and between selected populations. We characterised twelve microsatellite loci with 5–16 alleles within a small geographical area (Cameron Highlands, Malaysia) and up to 57 alleles across populations from nine countries. These loci should prove valuable tools for understanding *P. xylostella* gene flow and population biology. The AFLP approach also generated numerous variable markers (up to 156 informative bands per restriction enzyme pair) and offers an apparently quicker and cheaper alternative. However, assigning fractions of AFLP variability to different sources suggests that these anonymous markers are less consistent in performance and may sometimes detect loci in *P. xylostella* symbionts and parasites. The procedures required to remove or control for parasite AFLP reduce the time and cost benefits of AFLPs for population studies and we suggest that microsatellites provide the best immediate prospects for *P. xylostella* population genetics.

Keywords

Wolbachia, microsporidia, genetic diversity

Introduction

The diamondback moth, *Plutella xylostella*, is a major pest of crucifer production on all continents, leading to a strong impetus to control its populations in order to limit economic losses of food production (Talekar & Shelton 1993). Many different approaches have been used, including the introduction of natural enemies, IPM and many different insecticides. However, *P. xylostella* remains a major pest, largely due to the rapid emergence of resistance to a variety of different pesticides (e.g. Shelton *et al*. 1993). As with any pest species, our ability to control *P. xylostella* depends on our understanding of its fundamental biology. There has been considerable recent progress in some research areas, such as our understanding of the genetic basis of resistance to various pesticides, particularly *Bacillus thuringiensis* insecticidal endotoxins (Gahan *et al*. 2001, Ferré & van Rie 2002). However, we still have only a very limited grasp of the population structure and gene flow in this key pest species.

A better understanding of *P. xylostella* population biology would be valuable for a number of reasons. Given the propensity of *P. xylostella* to evolve pesticide resistance, and our increasing understanding of its genetic basis, we need to model the evolution and spread of different forms of resistance to consider and implement management alternatives (Peck *et al*. 1999). However, such models can only be realistic if we can characterise population structure and gene flow in *P. xylostella* populations. In particular, and in the light of recent theory, it will be important to quantify gene flow between selected and unselected sub-populations. In fact, dispersal in general is a very important issue in the management of insect pests, though notoriously difficult to study. At the two extremes there are reports of very long-distance dispersal by *P. xylostella* (Chu 1986, Talekar & Shelton 1993) and, in contrast, of very low dispersal distances from controlled release points (Shirai & Nakamura 1994). Clearly, we do not yet have a good understanding of average dispersal distances and how these vary with environmental factors, such as spatial distribution of oviposition sites. Such issues can be addressed using population genetic studies, but first require the development of reliable, polymorphic, molecular markers.

Many population genetic studies of Lepidoptera (including *P. xylostella*) have utilised allozymes. However, these markers generally have low variability, which limits the detection of population structure. They also require storage of insects at –70ºC prior to analysis, which is frequently impractical in field studies. In contrast, DNA-based techniques offer easier storage (ambient temperature in 95% ethanol), are not

compounded by environmental or developmental post-translational or post-transcriptional changes and often reveal greater variability. The downside is the requirement for considerable effort in development and testing markers.

The advantages of microsatellites (e.g. codominance, species- and locus-specificity) are well documented (Goldstein & Schlötterer 1999, Sunnucks 2000). However, time and cost considerations have led to the popularity of "universal" anonymous locus techniques such as RAPDS and AFLPs (Vos *et al*. 1995, Suazo & Hall 1999). These methods may well be quicker and cheaper to develop, but the consistency of RAPDs is often unacceptably poor, while AFLPs remain relatively untested in insects. Since AFLPs are anonymous and (mostly) dominant markers, they may also be subject to the well-known problems that have plagued RAPDs. However, less attention has been given to the fact that AFLPs and RAPDs may also detect endosymbionts or parasites of the target organisms. For example, *P. xylostella* is host to both *Wolbachia* bacteria and microsporidia.

We have developed *P. xylostella* microsatellites and AFLPs and made preliminary studies of genetic diversity at both a local (Cameron Highlands, Malaysia) and global (populations from nine countries) scale. We also compared the markers within and between isofemale lines over several generations to test for consistency. Finally, we used inbred lines with different parasite infections to test whether some AFLP or microsatellite loci might belong to internal parasites.

Materials and methods

P. xylostella cultures and experimental lines

Stock cultures of *P. xylostella* were established from 300 insects collected from each of Blue, Mensum and Bertram Valleys in the Cameron Highlands (Malaysia) in December 2000 and maintained on Chinese cabbage. Subcultures were established on an artificial medium (details available by request). *P. xylostella* isofemale lines were established from microsporidia-free parents by single pair matings for six generations. Microsporidia-infected (M) sub-lines were then created by addition of 100 microsporidial spores cm^{-2} to the surface of the larval food. All isofemale lines were initially *Wolbachia*-infected (W), so sub-lines were cured of *Wolbachia* by rearing larvae on a tetracyclin-HCl (3 mg ml^{-1}) supplemented diet for three generations and then maintained for a further 3 generations before screening markers. Infection status was confirmed using specific PCR (described below). In this way, three *P. xylostella* isofemale lines (termed A, B and O) of different geographic origin were established, each with sub-lines of different infection status (uninfected, W, M, W+M).

DNA extraction, genomic libraries and microsatellite screening

DNA (>50 Kbp fragments) from 40 freshly killed *P. xylostella* adults (with guts removed) was extracted and digested to completion with either *Sau*-3A, *Alu*-1, *Hae*3 or *Rsa*1. Complete genomic libraries (3.3–17.9 $x10^6$ colonies; mean insert size 300–320 bp) were made using pBluescriptKS and electrocompetent DH10B (>10^{10} cells/µg) *E. coli* cells and screened for microsatellite-containing clones as described by Butcher *et al*. (2000). Probes to all possible mono- (2), di- (2) tri- (14) and tetra- (49) nucleotide microsatellite motifs (excluding self-homologous repeats and the restriction sites used in library constructions) were used to screen duplicate nylon membrane lifts. Positive clones were sequenced utilising Big Dye (Perkin Elmer) chemistry and resolved on an ABI 3700 sequencer.

PCR primers to the consensus flanking sequences of microsatellites were designed manually and loci were tested over three generations to confirm lack of sex linkage, reliable amplification and Mendelian segregation. Each primer set was then evaluated on *P. xylostella* samples from the Cameron Highlands and surrounding lowlands (Malaysia), Sarawak (Malaysia), Sumatra (Indonesia), China, England, Hawaii, France, Greece, Thailand and Japan, to assess allelic diversity. This also permitted detection of null allele prone primer pairs, which we redesigned further away from the core repeat unit and re-evaluated. To confirm species-specificity, primers were also used on DNA from the two principal *P. xylostella* endoparasitoids (*Cotesia plutellae* and *Diadegma semiclausum*), as well as an entomopathogenic fungus (*Zoophthora radicans*).

Microsatellite, AFLP and symbiont PCR

Prior to DNA extraction, all *P. xylostella* samples were cleaned and dissected to remove visible parasites (mites, parasitoid larvae, nematodes or fungal hyphae) and the intact gut was also removed. *Wolbachia* and

microsporidia PCR analysis utilised DNA extracted from the gonad and surrounding fat body tissues, whilst microsatellite and AFLP PCR did not use adult abdomens as a DNA source to avoid false polymorphisms due to sperm. Long PCR with *Taq/pfu* was used to remove ambiguity due to incomplete *Taq*-based 3' adenylation (stutter bands) (Butcher *et al*. in prep.) and involved an initial 60 s denaturation at 96ºC followed by 38 cycles of: 94ºC for 30 s, 55ºC for 30 s and 72ºC for 60 s. Microsatellite and AFLP amplicons were 45% formamide-heat denatured for 5 min at 96ºC and resolved by denaturing gel electrophoresis on 60 cm TBE-7.8 M urea-acrylamide gels (8% for microsatellites and 4–6% gradient for AFLP) at 55–60ºC and visualised by silver staining (Butcher *et al*. 2000). For microsatellite loci, GC and AT cycle sequencing tracts of PKS were resolved on each gel as size standards, while the cloned plasmid was also amplified and resolved on the gel as both a known size standard and stutter band control. For AFLPs, 100 bp and 1 Kbp ladders (Gibco) were used.

Wolbachia and microsporidia were detected by long PCR using primers specific to the *Wolbachia wsp, fts*-Z and *gro*-EL regions (Butcher *et al*. in prep.) with an estimated detection threshold of 10–50 bacteria (based on calibration with plasmid-cloned inserts) and microsporidia 16S rDNA (Weiss & Vossbrinck 1999). Amplicons were resolved using TAE-0.8% (w/v) agarose /2.5 gml^{-1} ethidium bromide gels.

AFLP variation in two Cameron Highlands valleys

Fifteen individual *P. xylostella* larvae from each of the Blue and Bertram valleys (Cameron Highlands, Malaysia), ascertained to be symbiont and parasite free, served as DNA templates for AFLP analysis. Preliminary analysis revealed that single-stage amplification (Suazo & Hall 1999) produced too few bands, so a two-stage process was carried out as described by Vos *et al*. (1995), but with additional restriction enzyme (RE) combinations based upon *Xba*1, *Xh0*1 or *Pst*-1 in place of *Eco*R1, and *Alu*-1 or *Bfa*1 in place of *Mse*1. Secondary amplification used the same primers as the first stage, but as six separate PCR reactions, with AC or CT (*Eco*R1, *Pst*-1, *Xba*1) and AGT, ATA or AGC (*Mse*1 and *Bfa*-1) 3' additions. *Alu*-1 and *Xho*-1 based AFLP was problematical (over 8% inconsistent bands) and was not optimised further.

For each sample, three independent AFLP reactions were scored and non-consensus bands deemed artefacts were excluded. The pooled total number of different bands observed across all 30 samples is shown in Table 2, along with the number of bands that were observed to vary between individuals. AFLP analysis was also performed in the presence of realistic amounts of parasite template DNA (*D. semiclausum*, *C. plutellae* parasitoids or *Z. radicans* fungus) that might be found in a late instar *P. xylostella* larva (from dissection data).

Detecting symbiont loci

Twenty individuals from each of four sub lines (uninfected, W, M, W+M) were genotyped for each isofemale line. For example, the F_1 progeny of an A line male mated to each of the four B sublines (U, W, M, W+M) were assayed with AFLPs and microsatellites. Two-stage AFLP analysis after *Eco*R1/*Mse*1 restriction was performed with three replicates per sample. Differences in banding within a sample, but between replicates, were excluded as artefacts (<1.8%) and the remaining number of reliable bands is displayed as a pooled total from the different secondary amplifications (Table 3). Comparison of the bands observed with the isofemale line A profile to all the other samples revealed the number of informative (different) bands (Table 3) due to both host genetic differences (e.g. A versus B) and symbiont/parasite infection status (e.g. A versus B+W minus (A v. B).

Results

Microsatellite diversity in the Cameron Highlands

Screening of *Sau*-3A and *Alu*-1 genomic libraries, excluding mononucleotide motifs, yielded approximately 8300 putative microsatellite-containing clones. Sequencing of 1537 of these clones revealed 21% with either no (repeat motif size < 6) or a cryptic microsatellite and a 2.3 fold replication of clones, leaving 528 unique microsatellite clones. Of these, 91 loci contained microsatellite repeat unit sizes of over 10 and detect more than six alleles within the Cameron Highlands, while 15 are highly polymorphic with over 35 alleles. Taken together, the data reveal crude but conservative estimates of ~820 loci with >20 alleles and ~95 with >35 alleles and an average inter-locus distance of 12.3 Kbp (Butcher *et al*. 2000).

The first 12 microsatellite loci evaluated with more than 10 repeat units were used to estimate genetic variability. Each locus is polymorphic in the Cameron Highlands and has further alleles at a global scale (Table 1). As expected, the microsatellite primers did not amplify from associated parasites.

Table 1. Evaluation of twelve microsatellite loci for population genetic studies of *Plutella xylostella*

Locus	Core repeat[a]	Size[b]	Primer sequence	PCR[c]	CH Alleles[d]	Global[e]
PxDi1	$(GT)_{15}$	95	F: AGCAATGCACCTCTGCCTA R: GGAAAGTTAATATAACCGAAC	55ºC	8 (89–115)	17 (85–125)
PxDi2	$(GT)_{12}$	182	F: TAGGTATACAAATTAGTTGTATT R: CAGCATAAATAAATTATTAAATG	54ºC	6 (174–200)	15 (170–228)
PxDi3	$(AC)_{18}$	134	F: ATGCTAGTGCGACTTGCC R: TTCCTGATATAGCTGAAAAGC	54ºC	9 (128–144)	17 (114–146)
PxDi18	$(GT)_{26}$	99	F: GCGTACATTAGTACAAGGC R: GATCGATATTAAATTTGTCCTA	54ºC	16 (87–119)	57 (65–165)
PxTri1	$(GCG)_3GTG$ $(GCG)_8(CCGGG)_3$	203	F: AAATCAAACCTGAAATGAGA R: AACAGTCGAGCCTCCGA	55ºC	5 (194–215)	11 (189–226)
PxTri2	$(TCA)_{16}$	118	F: TCCTTAGGAGACGCCTATG R: CGCAAGCCTGTCAACCC	55ºC	6 (88–118)	16 (88–133)
PxTri3	$(GTT)_{14}(GCT)_4GT(TGC)_4$	116	F: CCACATTCAAATCCGGATTC R: GATCGTGTGAGGCAGCAA	54ºC	7 (101–119)	23 (90–137)
PxTri4	$(TGA)_{16}$	107	F: CCTGTGTCTAGCAGTTGAC R: ACTTTAGTAGGATTTTGGATAT	54ºC	8 (95–116)	21 (80–122)
PxTri5	$(ATC)_{19}$	98	F: ACTGCCGCACGAGAAGAC R: GATCAGCGGGATGGGCT	52ºC	10 (77–117)	21 (62–125)
PxTri6	$(CGC)_{12}$	100	F: ATTCAGAAAGTTGGTCCCC R: AAGAAGCGTTTAAGTAATTGC	54ºC	9 (88–121)	19 (81–124)
PxTet1	$(CAGA)_{16}$	135	F: TCCGTTCAGTAGTTTTGG R: GTACTCAGGTGAGTGCTT	54ºC	6 (89–135)	17 (89–143)
PxTet2	$(GTCT)_{11}$	155	F: CCAAATTTCATTCAAATCCGTT R: CACTTGACCATCCTTAATGTCGAA	55ºC	13 (137–167)	41 (137–203)

a - The core microsatellite repeat unit of the most common clone, b - PCR amplicon size (bp) of most common clone, c - Optimal annealing temperature in direct 3 stage long PCR at 2 mM Mg^{2+}, d - Number (and amplicon size range) of resolvable alleles from 450 larvae from 3 valleys in Cameron Highlands, Malaysia, e - As for d but with samples from nine different countries. Note that allelic sizes observed do not always match integer changes in microsatellite repeat numbers, suggesting indels, which have been confirmed in two alleles by sequencing.

AFLP diversity in the Cameron Highlands

In the two-stage amplification AFLP analysis, all paired restriction enzyme combinations tested revealed resolvable and informative bands (Table 2). However, the paired combinations of *Pst*-1 and *Eco*R1 with *Mse*-1 yielded the most informative bands when used in conjunction with three nucleotide selective 3' extensions. Within any sample, ignoring ghost bands, 98% of the bands were reproducible with DNA equivalents of 20–30% of a III instar larva, falling to 94–95% with 5% DNA equivalence. In addition, AFLP analysis revealed many informative bands associated with low levels of endoparasitoid or entomopathogenic fungal contamination (Table 2).

Table 2. AFLP bands resolved with different primers and DNA templates in *Plutella xylostella*

Template DNA	Preamp. primers	Bands	Informative
P. xylostella	EcoR1/Mse-1	421	142 (34%)
	EcoR1/Bfa-1	387	103 (27%)
	Pst-1/Mse-1	465	156 (34%
	Pst-1/Bfa-1	424	121 (29%)
	Xba-1/Mse-1	229	54 (24%)
	Xba-1/Bfa-1	252	58 (23%)
P. xylostella +	EcoR1/Mse-1	458	216 (47%)
D. semiclausum	Pst-1/Mse-1	471	245 (52%)
P. xylostella +	EcoR1/Mse-1	438	265 (61%)
C. plutellae	Pst-1/Mse-1	462	247 (54%)
P. xylostella +	EcoR1/Mse-1	428	208 (49%)
Z. radicans	Pst-1/Mse-1	399	209 (52%)

Comparing microsatellites and AFLPs using isogenic lines with different parasite infections

Within the isogenic A line (expected heterozygosity <0.1%), neither AFLPs nor microsatellites detected variation, as expected. However, addition of endoparasites led to about 5% variable AFLP bands, but no microsatellite variation (Table 3). When comparing uninfected host lines there were major differences in both AFLPs and microsatellites. For example, lines B and O were fixed for different alleles to line A at eight and 12 microsatellite loci, respectively.

However, only the AFLP method revealed increased variability when infected individuals were assayed. In the most extreme case, the double infected B+W+M sub-line, 16/72 = 22% variable bands were potentially attributable to infection status (Table 3).

Table 3. AFLP detection of symbiont genomes in *Plutella xylostella*

Isofemale line A	AFLPs			Microsatellites (12 loci)		
Relative to[a]	Bands	Variable	Symbiont bands[b,c]	Bands	Variable	Symbiont bands
A	370	0	control	12	0	control
A + W	368	17	17	12	0	0
A + M	371	19	19	12	0	0
A + W + M.	373	25	25	12	0	0
B	357	56	control	12	8	control
B + W	361	64	8	12	8	0
B + M	360	69	13	12	8	0
B + W + M	364	72	16	12	8	0
O	378	96	control	12	12	control
O + W	376	103	7	12	12	0
O + M	381	105	9	12	12	0
O + W + M	380	111	15	12	12	0

a - Different isofemale lines are denoted by A, B and O and different infection status by W (*Wolbachia*) and M (microsporidia), b - The uninfected comparison provides the control number of variable host bands (e.g. A relative to B = 56) and subtraction of this number from the total seen in an infected treatment (e.g. A relative to B+W = 64) gives the number attributed to symbionts (e.g. 8), c–In each of the three lines, the number of putative symbiont bands for the (W+M) case is less than the combined total for W and M tested separately (e.g. for B 8+13<16). This may reflect competitive amplification effects when more target genomes are available.

Discussion

Reliable and informative genetic markers are needed to investigate the population genetics of *P. xylostella*. Studies to date have mostly utilised allozymes (e.g. Caprio & Tabashnik 1992, Kim *et al*. 1999, Pichon *et al*. 2004) which have generally failed to detect clear evidence of population differentiation. Despite the advantages of codominance, relative species and locus specificity, and frequently high levels of polymorphism, the cost and time required to develop microsatellites has led to increased popularity of alternative markers such as AFLPs. The situation is compounded in Lepidoptera since this Order has been viewed as deficient in polymorphic microsatellites (Meglécz & Solignac 1998, Neve & Meglécz 2000). However, there is no coherent hypothesis to explain why this should be the case and we suggest that it may not be true. Other DNA-based mutation rates do not appear aberrant in Lepidoptera and Reddy *et al*. (1999) reported (GT) repeats in *Bombyx mori* at frequencies similar to those of other animals. Nevertheless, most lepidopteran studies have found few microsatellite loci, each with relatively few alleles (Meglécz & Solignac 1998, Saccheri *et al*. 1999, Harper *et al*. 2000, Bogdanowicz *et al*. 1997, Tan *et al*. 2001, Keyghobadi *et al*. 1999, Reddy *et al*. 1999). An exception is the nymphalid, *Speyeria nidalia*, which has four very polymorphic loci (Williams *et al*. 2002). Our library screening suggests that there are about 820 very polymorphic microsatellite loci in the diamondback moth genome. We have isolated 81 of these and described here twelve evaluated loci that should prove valuable for population studies.

The great utility of AFLPs for genome and pedigree mapping is unequivocal. However, their (usually) dominant nature, lack of species-specificity and dependence on template-quality and quantity are drawbacks for population genetics studies. Our AFLP analysis certainly revealed a wealth of informative bands, illustrating the potential of the method for population genetic studies. However, in addition to well-known

problems with AFLPs, we have highlighted the possibility that some bands are primed in symbiont or parasite genomes. This calls for some care in using AFLPs for studies between samples with a low genetic diversity, especially when one considers spatial and temporal variation in parasite infections. We must emphasise that the symbiont origin of the extra bands now requires direct testing and has so far only been inferred from the experimental design. Nevertheless, this caveat is important.

It is interesting that so many putative symbiont AFLP bands were detected (Tables 2 and 3). The symbionts in question have genome sizes crudely estimated at around 2.5% (*Wolbachia*) to 7.5% (microsporidia) of the *P. xylostella* genome, perhaps suggesting that around 10% of all bands could be of symbiont origin. The number of informative bands of symbiont origin may be much lower than this because microbial genomes are deficient in satellite DNA, one source of polymorphism resolved by AFLPs. Nevertheless, when comparing infected and uninfected individuals all (even if there are few) symbiont/parasite bands will be "informative" by definition.

This could be considered a significant "nuisance factor" for studies of host population genetics. On the other hand, comparisons of banding patterns of infected and uninfected members of the same host isofemale line could offer a novel method for identifying and cloning new symbiont gene fragments. However, with regard to *P. xylostella* population genetic studies, we currently favour the use of the polymorphic microsatellite loci described here and further loci that are being evaluated by ourselves and others.

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The origins of infestations of diamondback moth, *Plutella xylostella* (L.), in canola in western Canada

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Abstract

Recent evidence that a population of *Plutella xylostella* (L.) overwintered successfully in western Canada prompted studies to evaluate overwintering survival of diamondback moth under field conditions in Alberta and Saskatchewan. Successful overwintering was not demonstrated at either site for any life stage under a variety of tillage and organic matter treatments, using either laboratory-reared or field-acclimated specimens of diamondback moth. Diamondback moth infestations in western Canada evidently originate primarily from southern U.S.A. or Mexico when strong winds carry adults northward in spring. To provide early warning predictions of infestations, air parcel trajectories into western Canada were investigated for monitoring long-range movement of *P. xylostella* early in the season. Using wind fields generated by the Canadian Meteorological Centre's Global Environmental Multiscale model, three-dimensional air parcel trajectories were calculated using time-forward (prognostic) and time-backward (diagnostic) modes for several sites in North America. The model predicted strong northerly airflow in May 2001 which coincided with the occurrence of massive infestations of diamondback moth in canola in western Canada. The model can therefore serve as an important new tool for monitoring the dispersal of this pest to western Canadian canola crops.

Keywords

overwintering, air flow trajectories

Introduction

Populations of diamondback moth routinely infest canola (*Brassica napus* L. and *Brassica rapa* L.) in western Canada. In most years, *P. xylostella* causes minor economic damage, but in some years populations reach outbreak densities and substantial crop losses occur. For example, in 1995 more than 1.25 million ha were sprayed with insecticide to control diamondback moth populations at an estimated cost to producers of $45 to 52 million (Can.) (WCCP 1995). An outbreak on an even greater geographic scale occurred in 2001, with approximately 1.8 million ha treated with insecticide in western Canada (WCCP 2001).

The capability of diamondback moth to overwinter in Canada has been the subject of some controversy. Harcourt (1957) found that in eastern Ontario, *P. xylostella* survived in the field only until mid-December. Butts (1979) observed complete mortality of diamondback moth in field cages in Ontario, but predicted that the species should be able to overwinter there successfully. Putnam (1978) assumed that diamondback moth did not overwinter in Saskatchewan, but found that one of its parasitoids, *Microplitis plutellae* Muesebeck (Hymenoptera: Braconidae) survived under snow cover in the field. Although western Canadian populations were believed to originate from annual migrations from the south (Putnam and Burgess 1977; Philip and Mengersen 1989), Dosdall (1994) reported evidence for overwintering of *P. xylostella* in central Alberta during 1991–1992. Overwintering by diamondback moth in western Canada has important implications for its pest status in canola; consequently several studies were undertaken to investigate overwintering success under field conditions in Alberta and Saskatchewan. In addition, wind trajectories to western Canada from southern U.S.A. were analysed to determine the coincidence of favourable wind patterns with the appearance of *P. xylostella* in canola crops.

Materials and methods

To investigate conditions that favoured overwintering of diamondback moth in western Canada, replicated field trials were conducted from October to July in 1993–1994 and 1994–1995 at Vegreville, AB (112°02'N; 53°05'W). The experiments were randomised complete block designs with five replications conducted on

plots seeded in the preceding season to *B. napus*. The study comprised four treatments: 1) untilled canola stubble; 2) untilled canola stubble covered with 1.7 kg per m^2 of dried, threshed canola plant matter; 3) tilled canola stubble and 4) tilled canola stubble covered with 1.7 kg per m^2 of dried, threshed canola matter. In mid-October 1993 and 1994, one insect cage was placed onto each experimental plot; the cages each enclosed an area of 1 m^2. The cages were described in Dosdall *et al*. (1996) and were dug into the soil to prevent escape of insects from within the traps or their entry from the outside. Approximately 425 adults, 200 pupae, 300 larvae and 500 eggs of *P. xylostella* were placed into each cage. The diamondback moth specimens were F_3 progeny of ca. 100 adults collected in the field in June each year (1993 and 1994) and reared on canola in greenhouse chambers. The field cages were examined three times per week for the presence of living diamondback moths from 1 May to 31 July in 1994 and 1995.

The experiments were repeated in 1995–1996 and 1996–1997 at Vegreville and in 1996–1997 at Saskatoon, SK (106°38'N; 52°07'W). The same experimental design, treatments and diamondback moth numbers were used as described above except that the insects placed into the cages were acclimated, not derived from laboratory colonies. Acclimation of the diamondback moth specimens was accomplished by placing five large screened cages (3.5 m x 3.5 m at the base and 2 m high) onto tilled soil in the spring of 1995 and 1996, digging the cages into the soil and then seeding canola within each cage. Six weeks later, ca. 40 field-collected adults of *P. xylostella* were added to the cages and progeny from these individuals were removed and placed into the overwintering cages in mid-September of 1995 and 1996. The field cages were examined three times weekly for the presence of living diamondback moth specimens from 1 May to 31 July in 1996 and 1997.

A third overwintering study was conducted from mid-October 1997 to late July 1998 at the Vegreville, AB site. The study used the same experimental design, treatments, diamondback moth numbers and acclimated specimens as described above, but sheets of styrofoam (90 x 90 cm, and 5 cm in thickness) were secured over the diamondback moth specimens within the 1 m^2 field cages to simulate snow cover. The styrofoam sheets were placed in the field cages on 20 October 1997 and secured with ropes and metal stakes to the soil surface. The sheets were removed on 20 April 1998, corresponding to the approximate time of snow melt. The cages were examined three times weekly for living diamondback moth specimens from 1 May to 31 July 1998.

To investigate dispersal of diamondback moth to western Canada from source populations to the south, air parcel trajectories were analysed to follow air movement from southern North America to western Canada during 1999–2001. The trajectories were constructed from wind fields at discrete intervals and solved numerically (D'Amours & Pagé 2001). The trajectories utilised wind fields of the Global Environmental Multiscale (GEM) model, which had a horizontal resolution of 24 km and 28 vertical levels over North America. The trajectories have been used successfully as a diagnostic tool for documenting long-range transport of air pollutants (Olson *et al*. 1978), tracking volcanic ash clouds for aircraft advisories (Servranckx *et al*. 1999) and for other applications. In our study, the model was run at three levels corresponding to approximately near surface (950 hPa) and 1500 (850 hPa) and 3000 (750 hPa) m above sea level and followed parcels of air on curves denoting their successive positions in time. By following forward trajectories for the air parcels through time, it was then possible to diagnose advection from possible source locations.

Results

No living specimens of any life stage of diamondback moth were collected in the field cages on any of the tillage or organic cover treatment types at Vegreville, AB from 1 May to 31 July 1994 and 1995. Similarly, no overwintered, living specimens were collected at Vegreville or Saskatoon, SK from May to July 1996 and 1997 in the field studies using acclimated specimens of diamondback moth. No living specimens of *P. xylostella* were recovered from 1 May to 31 July 1998 in field cages after removal of the styrofoam sheets used to simulate snow cover.

From 1 May to 30 June 1999 and 2000, there was no evidence of sustained advection from southern North America to western Canada. However, in 2001 prolonged advection occurred for approximately 10 days in early May involving airflow from Texas, Louisiana, Georgia, and Florida into eastern Saskatchewan, Manitoba, and Ontario. A map of airflow into western Canada is presented in Figure 1 for a selected time during 2–7 May 2001; the map is reasonably representative of the direction of movement of air parcels on several occasions during this period.

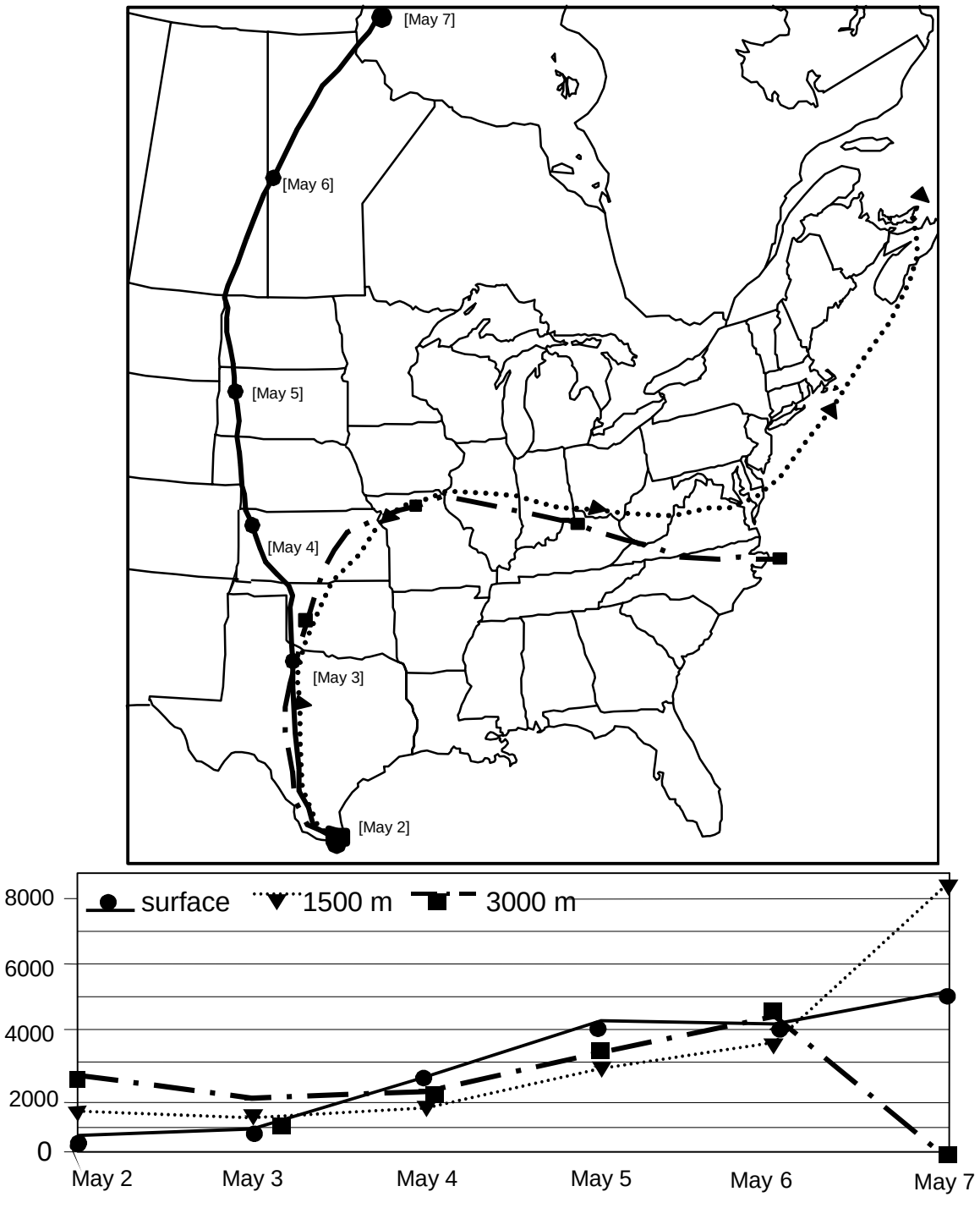

Figure 1. Canadian Meteorological Centre time-forward trajectories starting at Brownsville, TX at 00 UTC, 2 May 2001 and ending at 00 UTC, 7 May 2001. Air parcels were tracked from initial pressure levels of 950, 850 and 750 hPa (approximately near surface, 1500 and 3000 m above ground). The approximate altitude of the air parcels is shown on the vertical cross-section (bottom) and the horizontal position is shown on the top diagram.

Data indicate that air movement at all three levels (near surface, 1500 and 3000 m) was directed northward from the Texas-Mexico border near Brownsville, TX from 2–3 May 2001, but thereafter air parcels that had originated near the surface on 2 May continued on a northward trajectory, but airflow originating at 1500 and 3000 m on 2 May veered northeastward. The airflow that originated at 1500 and 3000 m on 2 May travelled through Oklahoma, Kansas, Missouri, Illinois, Indiana and Ohio before traversing the eastern seaboard of the U.S. and moving over the Atlantic Ocean. The air parcel originating at 1500 m on 2 May eventually veered northward after crossing the U.S. eastern seaboard to traverse Nova Scotia and Prince Edward Island in eastern Canada. Air parcels that originated near the surface on 2 May at Brownsville, TX

climbed to 2000 m on 4 May while traversing the states of Oklahoma and Kansas and then on 5 May were elevated to 4000 m and crossed Nebraska and South Dakota. From 5–6 May, this air stream passed across North Dakota and Saskatchewan at a height of approximately 4000 m. By 7 May, the air parcels had crossed all regions of canola production in western Canada, and moved across Hudson Bay (Figure 1).

Diamondback moth is routinely abundant on cole crops in the Lower Rio Grande Valley of southern Texas (Santa Ana 1999) and in spring 2001 enormous numbers of *P. xylostella* larvae were observed in northwestern Texas feeding on brassicaceous weeds associated with wheat crops (Porter & Leser 2001). A report issued by the Lubbock Research and Extension Center, Lubbock, TX stated that on 30 April 2001 larvae were pupating and would be flying in four to five days (Porter & Leser 2001).

In early May 2001, large numbers of diamondback moth adults were observed in canola crops in Manitoba, Saskatchewan and Alberta (WCCP 2001).

Discussion

Successful overwintering of diamondback moth, as reported by Dosdall (1994), is evidently a rare phenomenon in western Canada because we found no evidence for overwintering survival of *P. xylostella* in six site-years of study in either Alberta or Saskatchewan using laboratory-reared and field-acclimated specimens, with or without simulated snow cover. Overwintering of diamondback moth therefore could not presently contribute substantially to economic damage caused by this species in canola crops in western Canada. Nevertheless, current models of climate change predict that with increased greenhouse gas emissions, conditions in western Canada will become warmer and drier in future years (Yonetani & Gordon 2001) and, should this occur, the pest status of *P. xylostella* in canola in western Canada is certain to increase.

Although adults of *P. xylostella* disperse poorly under their own power, generally travelling less than 1 km in their lifetime (Shirai & Nakamura 1994), long distance passive dispersal by this species has been documented both in Europe and in Asia. French (1967) reported a sudden, vast increase in diamondback moth populations in north-eastern England and Scotland in 1958, where numbers increased over a few days from near zero to approximately 70–140 million adults per ha. According to airflow trajectories, the moths originated in Scandinavia and migrated approximately 3700 km. The invasion of a weather ship in the Atlantic, some 800 km from the coast of Scotland, by thousands of moths in 1958 could only be explained by long range migration on prevailing winds from Norway (French & White 1960). In Asia, diamondback moth specimens have been collected in the Pacific Ocean far from their nearest possible source locations in China and Japan (Chu 1986).

In Canada, invasions by insects arising from advections from the south have been reported previously. For example, long-range migration from Texas to Saskatchewan has been demonstrated for the sunflower moth, *Hoemosoma electellum* (Hulst) (Lepidoptera: Pyralidae) (Rogers *et al*. 1986). Although similar long-range migration has been proposed for diamondback moth, little direct evidence exists from previous studies to document this phenomenon. Smith and Sears (1982) proposed that diamondback moth populations in southern Ontario originated from south-eastern United States because successful overwintering was not observed in Ontario and pheromone trap captures of *P. xylostella* correlated well with periods of airflow from the south. Putnam and Burgess (1977) and Philip and Mengersen (1989) stated that advection from the south was responsible for diamondback moth infestations in canola in western Canada, but provided no corroborative evidence. Our study provides evidence to support the assumptions of these authors. Overwintered diamondback moth populations could not explain the massive outbreaks of this species in canola in western Canada in 1995 and 2001, or the sudden occurrence of enormous population densities of adults observed throughout Alberta, Saskatchewan, and Manitoba in early May 2001. We have documented advection from southern Texas during 2–7 May 2001 that occurred concurrently with great increases of diamondback moth adults in canola fields throughout western Canada. The source location was reported to harbour large numbers of diamondback moths in April 2001 (Porter & Leser 2001).

In the southern United States, cole crops and brassicaceous weeds would form the principal reservoirs of diamondback moth populations that could then be carried northward when strong winds develop. Peak densities of *P. xylostella* usually occur in this region from late April to early May (Reid & Bare 1952) at precisely the time of the northward advections observed in 2001.

Advection from southern U.S.A. to Saskatchewan and Manitoba in the first week of May 2001 cannot, in isolation, explain the enormous increases of *P. xylostella* populations observed in 2001 over some 11 million ha of canola cropland ranging geographically from the Peace River region of northwestern Alberta to fields in southeastern Manitoba near the Canada-U.S.A. border. The diamondback moth outbreak of 2001 in western Canada was probably initiated by a number of disjunct advection events, originating from more than one southern source location.

The outbreak of diamondback moth in canola in western Canada during 2001 was terminated by attack of its natural enemies. The principal agents responsible for biocontrol of *P. xylostella* were parasitism by *Diadegma insulare* (Cresson) (Ichneumonidae), *Microplitis plutellae* Muesebeck (Braconidae) and *Diadromus subtilicornis* (Gravenhorst) (Ichneumonidae) (Mason & Dosdall, unpublished data), and epizootics of fungal pathogens (Keddie & Dosdall, unpublished data).

The origins of diamondback moth parasitoid populations are uncertain: they may either overwinter in western Canada, migrate northward passively with the same or similar air currents that bring their hosts, or they may actively disperse northward after diamondback moth has invaded canola in western Canada. The latter hypothesis is unlikely because parasitoid infestations occurred too early in the season for wasps to have migrated such extensive distances independently. Overwintering of parasitoids is a more likely possibility and this may explain attack by at least one parasitoid species. Putnam (1978) concluded that *M. plutellae* could overwinter in western Canada and was important in regulating diamondback moth populations early in the season. However, not all parasitoids of diamondback moth may overwinter in western Canada. *Diadegma insulare* has greatest importance for reducing *P. xylostella* populations late in the season (Putnam 1978) and because it occurs as far south as Venezuela (Carlson 1979), it may not overwinter in western Canada, but migrate northward along with its host.

Information on the site(s) of origin of diamondback moth populations invading western Canada's canola crops and their genetic background(s), has important implications for devising pest management strategies. Applications of broad-spectrum chemical insecticides are used routinely in canola to control diamondback moth infestations when densities exceed threshold levels (Philip & Mengersen 1989), so information on the origin of western Canadian populations can be important for determining appropriate chemical control measures. Insecticide resistance is common in this species following continual application of the same product over time (Talekar & Shelton 1993), so historical information on the insecticide treatment regime used on the migrants at their site of origin can be a key factor for determining the most appropriate control recommendations for use in canola in western Canada. By using airflow trajectories, it should be possible to determine this important background information and implement a sustainable management strategy for diamondback moth infestations in canola in western Canada.

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Regional outbreaks of diamondback moth due to movement of contaminated plants and favourable climatic conditions

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Abstract

Outbreaks of diamondback moth, *Plutella xylostella* (L.), are being documented more frequently in many regions of the world and the causes for the outbreaks are often associated with insecticide resistance and lack of natural enemies, the latter often due to use of broad-spectrum insecticides. Growers are likely to think of insect management on an individual field basis (i.e. their fields) without sufficient regard to the larger context in which the insect operates on a regional basis, and this may limit their ability to manage the pest. Like any insect, *P. xylostella* populations fluctuate on an intra-regional and inter-regional basis and outbreaks in either case may be due to many factors. In this paper two causes of specific outbreaks of *P. xylostella,* movement of contaminated plants and an unusual climate within a region, are discussed.

Keywords

transplant, contamination, dispersal, insecticide resistance, permethrin, methomyl, seedlings

Introduction

Outbreaks of diamondback moth, *Plutella xylostella*, frequently occur in various parts of the world (Talekar & Shelton 1993) and result in severe losses (Javier 1992, Shelton 2001). Outbreaks are often the result of a population of *P. xylostella* having developed resistance to the available insecticides or lack of sufficient control by natural enemies. In the latter case, populations of natural enemies, which would have provided some level of control, may have been reduced due to the use of the broad spectrum insecticides used against *P. xylostella* and against which *P. xylostella* may have developed resistance. However, outbreaks may also be caused by movement of *P. xylostella* or climatic conditions which favour host development or reduce the occurrence of fungal diseases.

Growers are likely to think of insect management on an individual field basis (i.e. their fields) without sufficient regard to the larger regional context in which the insect operates, and this may limit their ability to manage the pest. Intra-regional and inter-regional approaches are needed for more sustainable management practices and these are being developed through approaches such as investigations of movement studies of *P. xylostella* and its natural enemies and the 'window strategy' for resistance management.

In this paper I will discuss two examples of how an improved understanding of inter-regional movement of contaminated plants and an unusual climate within a region can affect outbreaks of *P. xylostella*. Full reports of both papers have been published, as noted in the section headings.

Movement of contaminated plants and resistant insects (Shelton et al. 1996)

In New York State, ca. 5,000 ha of cabbage, *Brassica oleracea capitata* (L.) are grown annually and beginning in the late 1980s we began to see problems in controlling *P. xylostella* with our commonly-used insecticides. Because of the short growing season in New York and the absence of *P. xylostella* overwintering in an adjacent area (Ontario, Canada) (Harcourt 1986), high levels of resistance would not be expected to have developed in New York. Thus, we suspected insects either flew into our area or were being transported on plants which were grown in another location and shipped to New York for transplanting. It is estimated that nearly 70% of the cabbage grown in New York is grown from transplants and the majority of these are produced in other states.

From 1989 through 1992, cabbage transplants were obtained from growers or brokers in New York who received shipments of transplants from Florida, Georgia and Maryland, the states which supply the majority of transplants to New York. We also obtained locally grown transplants as a check. We sampled cabbage transplants as they arrived in crates or boxes at the growers' fields or at the brokers. From 1989 to 1992 we sampled a total of 49 different shipments of transplants. As soon as a shipment was received, a sample of

transplants was inspected visually for *P. xylostella* larvae. In addition, transplants were inspected for cabbage looper, *Trichoplusia ni* (Hübner), imported cabbageworm, *Pieris rapae* (L.) and cabbage webworm, *Hellula rogatalis* (Hulst). *Plutella xylostella* larvae collected during the first inspection were counted and transferred to rape seedlings, *Brassica napus*, and reared (Shelton *et al*. 1991) for insecticide assays. The inspected transplants were then placed in soil in large pots and kept for two additional weeks in a greenhouse at 20–25°C, at which time a second inspection was performed.

In addition to examining the plants for insect contamination, we also tested some colonies for their susceptibility to commonly used insecticides. In 1989, we were able to establish four colonies of *P. xylostella*: two from Florida and one each from Maryland and Georgia. In 1990 we established one colony of *P. xylostella* from Florida and one from Georgia. All colonies were established from the *P. xylostella* taken from the transplants and then we used the F_2 generations to test for susceptibility to permethrin and methomyl using a leaf dip bioassay (Shelton *et al*. 1993a).

Samples collected from 1989 to 1992 documented that *P. xylostella*, was introduced into New York in early spring on cabbage transplants grown in the southern United States (Table 1). During 1989, transplant shipments from five transplant companies in Florida, Georgia and Maryland had average seasonal infestations ranging from 1.3 to 3.5 *P. xylostella* per 100 transplants. During June, when the majority of transplants arrived in New York, *P. xylostella* infestations were as high as 12.8 insects per 100 transplants on an individual shipment. Infestations by *T. ni*, *P. rapae*, and *H. rogatalis* on an individual shipment were as high as 19.7 insects per 100 transplants. Compared with a standard susceptible field population, the *P. xylostella* which were collected from transplants demonstrated moderate to high (> than 100-fold in one case) levels of resistance to permethrin or methomyl (Table 2). In 1990, average seasonal infestations per transplant company varied from 0.3 to 12.0 *P. xylostella* per 100 plants, but an individual shipment from Florida had 30.4 *P. xylostella* per 100 transplants. A population of *P. xylostella* collected in 1990 from Florida transplants had >200-fold resistance to methomyl. Despite intensive treatments, a New York grower who used the transplants with high contamination of resistant *P. xylostella* was unable to achieve acceptable control in his field. Samples collected from 1989 to 1992 from a transplant grower in Maryland indicate that better management in the field can reduce contamination levels to <0.5%.

As a result of this work, the Cornell Cabbage IPM Program now recommends that growers inspect transplants before putting them in the field and consider rejecting the load if >5% of the plants are contaminated with *P. xylostella*. While New York growers depend on having transplants as part of their production practices, transplant growers in the southern states should realise that their management practices may influence the level of *P. xylostella* control that can be obtained by the growers in other regions who receive their transplants. Since this study was performed and presented to New York cabbage growers, they have become acutely aware of the risk involved in receiving contaminated transplants, especially from regions in which insecticide resistance in *P. xylostella* is known to occur. As a result, some New York growers have constructed their own greenhouses to ensure their plants can be available at the proper time and be free from contamination of resistant *P. xylostella*.

Favourable climatic conditions lead to outbreaks of *P. xylostella* (Shelton et al. 2000)

Climatic conditions, including higher temperatures and decreased rainfall, have been cited as major factors which regulate the population dynamics of *P. xylostella* (Harcourt 1986). Increased temperatures can lead to the production of more generations per season and increased rainfall can lead to increased incidence of fungal diseases (Talekar & Shelton 1993), direct mortality of small larvae, or perhaps even to mating disruption (Tabashnik & Mau 1986). In fact, sprinkler irrigation has been shown to reduce damage by *P. xylostella* in cabbage (McHugh & Foster 1995). However, because of the history of *P. xylostella* resistance, outbreaks are often attributed to insecticide resistance rather than favourable conditions. Beginning in 1997 we had a chance to assess an outbreak of *P. xylostella* in California to determine its cause(s).

California is the leading U.S. producer of broccoli where nearly 50,000 ha are grown annually with a farm gate value of nearly $0.5 billion. The major production areas extend from Monterey Bay to the Imperial Valley (Figure 1). In 1997 there was an outbreak of *P. xylostella*, which resulted in crop losses estimated to be > $6 million (Sances 1997). In the central valley and north coast regions of California, *P. xylostella* is not considered a major pest, although it was in 1997. To help assess the possible causes of the 1997 outbreak we conducted a survey of *P. xylostella* in the principal broccoli growing regions of the state. The survey consisted of evaluating populations for their susceptibility to three commonly used insecticides (methomyl,

permethrin and *Bacillus thuringiensis* subsp. *kurstaki*). A single collection of approximately 300 *P. xylostella* larvae was made from each of nine locations (Figure 1) between October and November of 1997. All insects were transported to the New York State Agricultural Experiment Station where bioassays were performed. An insecticide susceptible population of *P. xylostella*, Geneva 88 (Shelton *et al*. 1993a), was also reared at the same laboratory for comparison. Populations were cultured on rape seedlings (Shelton *et al*. 1991) and toxicity of the insecticides was measured using a cabbage leaf dip bioassay similar to that reported previously (Shelton *et al*. 1993a,b).

Table 1. Insects (*P. xylostella* and others) found on cabbage transplants shipped to New York by companies from other states, 1989–1990[a]

		April		May		June		Season average	
Company	n	P.x	other[b]	P.x	other	P.x	other	P.x	other
1989									
Georgia A	2957	0	0	2.7	0.1	c	c	1.3	0
Georgia B	1059	1.3	0.1	c	c	c	c	1.3	0.1
Georgia C	1892	4.3	0.2	c	c	2.7	0.4	3.4	0.3
Maryland A	8755	c	c	0.2	0.5	7.3	5.6	3.5	2.8
Florida A	5702	0	0	0.3	0.1	8.2	0.1	2.5	0
New York A	3190	c	c	c	c	1.1	0.2	1.1	0.2
New York B	1039	c	c	c	c	1.1	0.2	1.1	0.2
New York C	512	c	c	c	c	0.6	0.2	0.6	0.2
1990									
Florida B	2280	6.7	0	17.4	0	c	c	12.0	0
Georgia B	2022	c	c	3.7	0	c	c	3.7	0
Georgia C	1006	c	c	1.8	0	c	c	1.8	0
Maryland A	3599	c	c	0.3	0	0.3	0	0.3	0

a Values listed are [(# insects/# plants inspected) x 100]
b Other insects included imported cabbageworm, cabbage looper and cabbage webworm
c No transplants intercepted from source during that particular month

Table 2. Susceptibility to permethrin and methomyl of III instar *Plutella xylostella* larvae obtained from US companies producing southern transplants

		Permethrin			Methomyl		
Company	n	Slope±SE	LC_{50} (90% CL) mg (AI)/mg	RR[a]	Slope±SE	LC_{50} (90% CL) mg (AI)/mg	RR
Virginia (standard)	105	1.39±0.27	0.083 (0.035–0.166)	1.0	0.88±0.17	0.26 (0.11–0.52)	1.0
1989							
Florida A (site 1)	105	1.72±0.30	0.687 (0.285–1.814)	8.3	2.11±0.49	28.5 (18.0–47.5)	109.6
Maryland A	105	1.77±0.30	0.326 (0.176–0.863)	3.9	0.90±0.15	3.35[b]	12.9
Georgia C	105	0.74±0.25	0.029[b]	0.3	0.34±0.14	1.24[b]	3.8
Florida A (site 2)	105	2.03±0.30	0.607 (0.381–0.982)	7.3	2.70±0.20	8.46 (5.00–14.2)	32.5
1990							
Florida B	175	1.55±0.22	1.662 [b]	20.0	0.85±0.150	52.76[b]	202.7
Georgia B	175	2.43±0.36	0.349 (0.236–0.528)	4.2	0.76±0.11	2.32 (0.68–9.54)	8.9

[a]RR is the resistance ratio determined by dividing the LC_{50} for a population by the LC_{50} for the standard population (i.e. Virginia)
[b]The 90% CL could not be determined because g > 0.5 (Russell *et al*. 1977)

Figure 1. The major production areas for cole crops in California and the nine collection sites of *Plutella xylostella* in 1997.

In addition to the assays, insecticide records for 1997 were collected for each field and weather data were collected from stations near the sites identified in Figure 1. The latter was done to assess whether climatological data may help explain the population outbreaks observed in 1997. Precipitation and average daily temperatures were summarised for each month for the following locations, which constitute the principal broccoli production areas where outbreaks occurred: Watsonville, Salinas, King City, Coalinga, Santa Maria and Oxnard. To provide a comparison of the climatic conditions in 1997 with previous years, we averaged the monthly temperature and precipitation data of these five sites and compared these averages to the 30-year average (1961–1990). All data were compiled using information from weather observing sites supervised by the National Oceanic and Atmospheric Administration/National Weather Service and received at the National Climatic Data Center (Ashville, NC).

Elevated levels of resistance were seen only with permethrin and seven of the nine populations had tolerance ratios (RR) >100 (Table 3). Only the two populations collected from the Imperial Valley area had RR values similar to Geneva 88, indicating that these populations had not been intensively selected for resistance and also that the Geneva 88 population was a realistic standard for these assays. Traditionally, cole crops are grown for only 3–4 months in the Imperial Valley, compared to year-round in the rest of California, so selection pressure would be reduced. A significant difference, based on non-overlapping of the 95% FL of the LC_{50} values, was observed between Imperial Valley #1 and Geneva 88 populations, but this translated to a RR value of only 1.6. Based on previous reports, this difference would not result in control failures in the field, although RR values for permethrin of >100 would (Shelton *et al*. 1993a). For methomyl, the three most susceptible populations (Imperial Valley #1, Imperial Valley #2 and Geneva 88) had LC_{50} values not significantly different from each other, but these three were significantly different from the other populations. However, all populations had RRs <10 and, based on previous reports (Shelton *et al*. 1993a), these levels would probably not result in control failures in the field. For *B. thuringiensis* there were significant differences between some populations, but all populations except one had RR values <10. The Santa Maria population had a RR value of 11.1 and this value is probably borderline for control problems in the field (Perez & Shelton 1996).

Table 3. Resistance ratios (RR) of commonly used insecticides against populations of *Plutella xylostella* collected from California in 1997. For complete table including slopes, LC_{50} values and 95% CL see Shelton *et al.* 2000

Population	Btk[a]	Methomyl	Permethrin
G88	1.0	1.0	1.0
Imperial Valley #2	5.1	0.7	1.3
Soledad	6.7	3.9	110.7
Guadalupe	6.0	3.9	121.7
Oxnard	4.8	6.5	206.3
Santa Maria	11.1	6.6	110.3
Sprecklers	5.3	4.5	145.3
Coalinga	1.4	7.0	154.3
Ocean Cliff	3.9	7.1	126.0
Imperial Valley #1	3.6	0.8	1.7

[a]*Bacillus thuringiensis* subsp. *kurstaki*

Weather data provide some insight into possible causes for the outbreak in 1997. Hot, dry conditions are known to favour outbreaks of *P. xylostella* and the mild winter of 1996/7 and the below normal rainfall and warmer conditions in the growing season of 1997 met these criteria. Temperatures during the winter of 1996/7 were above normal and this continued through until the normal harvest time of late August of 1997. During the month of May when much of the broccoli was in the ground, daily mean temperatures were 110% of normal. Rainfall during the main production period, February–August, was well below normal. In fact, only in August did the precipitation exceed 50% of the 30-year average and then it only reached 67% of the norm.

Our surveys indicated that growers used an array of insecticides in each of the fields from which we obtained populations. At one time or another, however, most growers used either methomyl or a *B. thuringiensis* product. Based on our assays, these materials should have provided adequate control under normal circumstances, if applied correctly. However, because of favourable climatic conditions for *P. xylostella* that resulted in much higher than normal populations throughout the year, it appears that the insects could not be controlled to the level required, regardless of which insecticides were used unless, perhaps, they were used more frequently than they were in 1997.

In 1998 there were no reported significant outbreaks of *P. xylostella* in California cole crops. Most likely this was primarily due to the considerably higher rainfall (300% in some areas) which reduced populations especially during the winter and spring. However, it could also be due to the fact that spinosad became registered in the fall of 1997 and was used in 1998, or that growers were able to get control with other materials such as methomyl or *Bacillus thuringiensis*. Most likely it was a combination of reduced insect pressure and the use of effective insecticides. In years in which populations will not be so suppressed by environmental conditions, it will be important to know the geographic distribution of resistance to specific classes of insecticides so they can be avoided.

Discussion

The two examples of outbreaks of *P. xylostella* mentioned above help illustrate the importance of regional management. The transplant growers whose practices led to insecticide resistance within their greenhouses or fields also contributed to a regional problem when they shipped contaminated plants. It is unlikely that federal and state legislation could have prevented this situation with existing resources, although interstate movement of plants is subject to certification. The size of *P. xylostella* eggs as well as the vast numbers of plants moved makes detection difficult. In the New York example, the 'solution' for New York growers was to take their business elsewhere, and many of them have done this by purchasing greenhouse plants from Canada or constructing their own greenhouses. Growers in other regions of the world have become sensitised to the problem of moving contaminated transplants because of the New York example, so perhaps this will decrease the likelihood of movement of contaminated plants being a source of outbreaks of *P. xylostella*.

Even if contaminated plants are the source of an initial infestation and even if this is exacerbated by the insects being resistant to available insecticides, outbreaks will occur only if favourable weather conditions

exist. Outbreaks of *P. xylostella* are far less likely to occur in cool, wet conditions (Harcourt 1986, Talekar & Shelton 1993), but besides overhead irrigation (often unavailable to many growers and deleterious to disease management programs) there is little that can be done to manipulate the weather. Perhaps the best advice that can be provided to growers is to advise them of the need for enhanced awareness of the potential for regional outbreaks before and during times of favourable climatic conditions and to stress the need to begin their field season with transplants not already contaminated with *P. xylostella*, especially if they are resistant to the insecticides they intend to use.

Acknowledgements

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Estimation of some characteristic dispersal ranges of diamondback moth *(Plutella xylostella) (Lepidoptera: Plutellidae)*

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Abstract

The dispersal ranges of the diamondback moth (*Plutella xylostella*) were estimated with mark-recapture collected during 1998-2000 in commercial cauliflower and broccoli crops in the northern Adelaide plains. Moths were marked with fluorescent powder and released at one or more points within the experimental fields. Recaptures of the marked moths were made with pheromone traps and yellow sticky buckets (YSB), the latter were used to trap both sexes of the moth. Four indices of dispersal ranges were estimated, the average dispersal distances and the distances within which 95, 99 and 99.9% of the released moths were expected to remain. The average dispersal distances estimated from the recapture data with pheromone traps were 21-35 m and those from the YSB recapture data were 14-18 m for the males and 13-24 m for the females. The 95, 99 and 99.9% distances estimated from YSB recapture data were 41-54 m, 69-90 m and 117-164 m respectively for the males and 40-72 m, 63-120 m and 113 –203 m respectively for the females. The corresponding distances estimated from the recapture data with pheromone traps were 62-106 m, 104-177 m and 176-300 m respectively. Implications of the results in the designing and implementation of insecticide resistance management strategies and in a number of alternative control strategies are discussed.

Keywords

mark-recapture, sticky traps

Introduction

Diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is known to be able to migrate over long distances (Mackenzie 1958, Lorimer 1981, Chu 1986). However, little is known about its dispersal ranges within active host crops or local dispersal ranges. Such information is crucial in the designing and/or implementation of a number of new management strategies for DBM. These include rotations of insecticides (Liu & Tabashnik 1997, Mo *et al*. 1999), provision of susceptible populations (Shelton *et al*. 2000), mating disruption (McLaughlin *et al*. 1994, Mitchell *et al*. 1997, Schroeder *et al*. 2000), crop-break (Heisswolf *et al*. 1996), pathogen auto-dissemination (Pell *et al*. 1993, Furlong 1995) and trap crops (Srinivasan & Moorthy 1991, Pawar & Lawande 1995). For insecticide rotations, ideally the same rotation plan should be uniformly implemented across an entire region. Where uniform implementation is not feasible, areas under different rotation plans should be separated by large enough distances so that exchange of individuals among local populations is below a predefined level. Similarly, dispersal information is needed to determine the minimal separation distance between host crop areas managed and not managed by the crop-break strategy or the mating disruption strategy. Another IRM strategy is the provision of refuges for insecticide-susceptible populations. For this strategy to be effective, the refuges should be set up within certain distances from the target populations to ensure significant exchanges of individuals between susceptible and resistant populations. Similarly, dispersal information is needed to determine the maximal separation distances between the trap crop and target crop, and the maximal trap intervals in the pathogen auto-dissemination strategy.

As part of a national project on the integrated management of DBM, a local dispersal study of DBM was conducted in South Australia between during 1998-2000. The objectives of this study were to estimate the dispersal ranges of male and female DBM moths within active host crops, in particular the likelihood of the moths dispersing beyond given distances.

Materials and methods

Test insects

Moths used in this study were from laboratory colonies reared with potted canola, cabbage, or Chinese cabbage. Rearing conditions were 25 – 28°C and 12L:12D light cycle. Each spring, a new colony was started with wild DBM larvae/adults collected from *Brassica* weeds/canola crops. The colony was reinvigorated every 2-3 months by introducing wild individuals.

Marking and release

Fluorescent powder (Magruder Color Co., Elizabeth, NJ) was chosen as the marking agent. Marking was done in flat Décor® containers (26 x 19 x 6 cm). Moths were introduced into the marking container through a mesh sleave opening to a circular hole cut in the centre of the cover. About 500 moths were placed in each container. The marking containers were transported to the experiment sites in an Esky® (40 L) cooler box with ice packs inside. Just before the release, 0.05–1.00 g of the fluorescent powder was placed into each container and shaken for a few seconds. The moths were then released by opening the caps of the marking containers.

To determine the effect of marking on the mortality of marked moths, healthy pupae from the culture were sorted into males and females and placed in individual glass tubes. Emergence of the pupae was checked daily. Moths emerged on the same day from each sex were randomly divided into two groups with equal numbers in each group. Moths from one group in each sex were marked with the fluorescent powder according the method described above and transferred back into clean glass tubes. The test moths were checked daily for life-death status until the last moth had died. The test condition was the same as in rearing. Significant differences in the average life span between marked and unmarked individuals were checked with two-sample t-tests.

The effect of marking on the response of marked males to the pheromone sources was studied in a wind tunnel (temperature: 20°C; wind speed: 0.1 m/s; light period: 12L:12D). A pheromone trap (25 cm x 35 cm) baited with a 3-component DBM sex pheromone (R. Vickers, CSIRO Long Pocket Laboratories, Brisbane, Australia) was placed in the centre of the upwind end (10 cm away) at a height of 30 cm. Marked and un-marked virgin males were released in the downwind end of the wind tunnel and allowed to respond to the pheromone source for 24 h. The number of moths caught in each category (marked or un-marked) was recorded at the end of the test and the differences were analysed with chi-squared tests.

To determine the effect of irrigation and rain on the persistence of the marking on the moth bodies, marked moths were placed in a cylindrical bag made of coarse mesh and showered with a constant flow of water from a sprinkler for either 0.5 h or 1.0 h. The diameter of the bag was made about the same as that of the sprinkler to ensure full coverage of the bag with the shower. After the draining of excess water, the moths were checked for fluorescent marking with an UV spotlight (TrAc Pack Pro, Labino AB, Sweden).

Experiment sites and host crops

All mark-recapture experiments were conducted on a commercial broccoli and cauliflower farm in the northern Adelaide plains (34°43'S, 138°33'E). The crops were planted continuously throughout the year, resulting in a mixture of different ages of the crops at any time. Each planting was made in adjacent rectangular blocks (10 m wide, 110 – 230 m long) separated by 5-m wide tractor paths. The distance between adjacent plants within a block was about 0.5 m. The crops were regularly irrigated with overhead sprinklers. All experiments were conducted when the crops were about 6-10 weeks old.

Recapture of released moths was made with delta pheromone traps and yellow sticky buckets (YSB). The pheromone traps were made out of 2 L blank milk cartons. The side length of the triangular opening of the trap was 25 cm and the length of the trap 35 cm. A cardboard piece (25 x 35 cm) coated with Tanglefoot® was inserted on the base of the pheromone trap. A rubber tube impregnated with the 2-component sex pheromone of the DBM (Long Pocket Laboratories of the CSIRO Division of Entomology) was placed in the centre of the sticky base. The YSB consisted of an inverted plastic bucket (base diameter: 25 cm, opening diameter: 35 cm, height: 35 cm) and a 5 cm wide ring made from particleboard (Figure 1). The outer surface of the bucket and the upper surface of the ring were coated with Tanglefoot®. A square hole was cut in the centre of the bucket base to allow a wooden stake to poke through. The height of the trap was controlled with a bull clip placed on the wooden stake. The ring of the YSB was designed to increase the

trapping efficiency as observations showed that a considerable number of moths that bumped into the bucket wall did not get stuck, but fell to the ground. Pheromone traps were placed at a height of *ca*. 50 cm and YSB *ca*. 30 cm (from the ring to the ground).

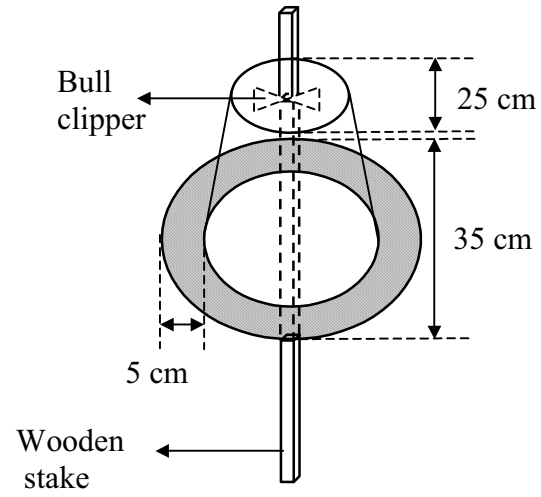

Figure 1. An illustration of the yellow sticky bucket (YSB) trap. The exterior surface of the plastic bucket and the upper surface of the ring were painted with Tanglefoot®.

Experimental design

Traps were placed in grid patterns across continuous patches of host fields. The inter-trap distance was fixed at 10 m. The dimensions of grids in terms of the number of rows of traps and the number of traps per row varied from 5 x 21 to 7 x 21, depending on the dimensions of the crop fields available for experiments. Marked moths were released in the centre of the grids. To minimise possible artefacts arising from moths bumping into the traps immediately following the releases, the centre traps were not placed until 1 h after the releases. The total number of moths released in each experiment varied from 1000 to 3000. Six grid-based experiments were conducted, three with pheromone traps and three with YSB. In most experiments, more than one release was made. With multiple releases, separations of recaptures from different releases were made possible by the use of different colours of the fluorescent powder. Recapture data from different releases were treated as different data sets. In experiments using pheromone traps, the sticky bases of the pheromone traps were replaced every 1-2 days. All sticky bases and the traps were checked under UV light for marked moths at the end of each experiment. In experiments using YSB, the buckets were checked daily at the site with a portable UV light. Duration of the experiments ranged from five to nine days.

Meteorological data

Temperature, relative humidity, wind direction and wind speed were recorded hourly during the experiments with a data logger (STARLOG, Model 6004, UNIDATA, Western Australia) with a wind speed and direction sensor (Model 6504-FS, UNIDATA, Western Australia). The data logger and the temperature and relative humidity probes were placed inside a Stevenson's Screen placed at the experimental sites.

Data analyses

Recaptures from individual traps were grouped according to the distances of the traps to the release point. The average number of recaptures per trap (y) was then calculated for each distance (x). The relationship between Y and X was modelled with the empirical dispersal equation of Hawkes (1972):

$$y = \exp(a - b\sqrt{x}) \qquad (1)$$

Parameters a and b were estimated with non-linear regression. Equation (1) gives the density of moths at a given point. Since dispersal occurs in all directions, the density of moths at a given distance should be the point density multiplied by $2\pi x$, ie. $2\pi xy$. The number of moths remaining within a distance of x_c is estimated by:

$$\int_0^{x_c} 2\pi xy \qquad (2)$$

The distance within which the probability of moths remains is p, $x_{p,}$ is therefore given by the following equation:

$$\int_0^{xp} 2\pi xy = p \int_0^{\infty} 2\pi xy \qquad (3)$$

For each experiment, x_p was estimated for p = 0.95, 0.99, and 0.999. These were the estimated distances from the release point within which 95%, 99%, and 99.9% of the moths would remain. The estimates were obtained with numerical integrations. The average dispersal distances were estimated as $20/b^2$ (Hawkes 1972), where b is the parameter in equation (1).

Results

No significant differences were detected in the longevity between marked and un-marked moths (*P*>0.05, Student-t tests), irrespective of the sexes (Figure 2). Wind tunnel experiments revealed no significant differences in the percentages of moths caught by the pheromone trap between marked and unmarked males (*P*>0.05, Chi-squared tests) (Figure 3). All marked moths could be clearly detected under UV light after having been subjected to a constant flow of water from a sprinkler for 0.5 h or 1.0 h.

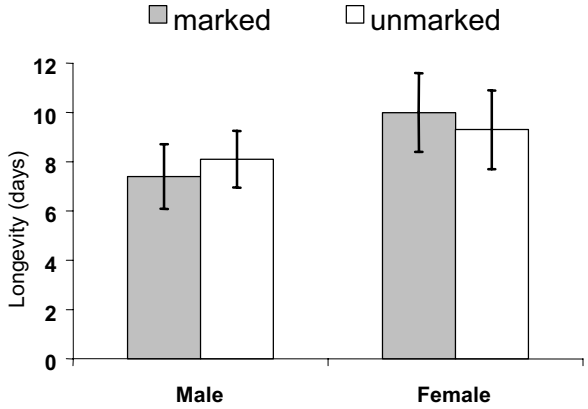

Figure 2. Comparisons of longevity between diamondback moths marked with fluorescent powder and un-marked moths. The number of moths tested in each category and each sex was 10. The bars show the 95% confidence intervals.

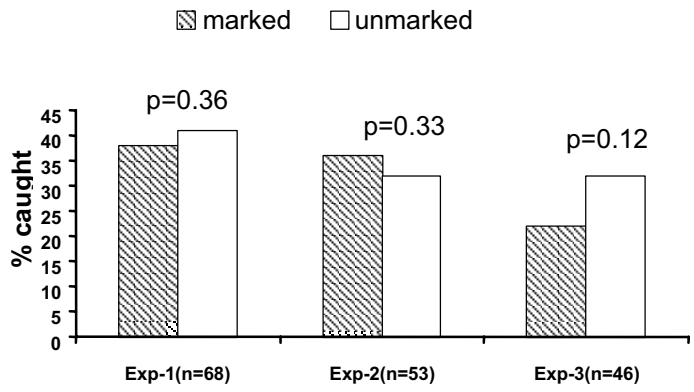

Figure 3. Percentages of marked and unmarked diamondback moths caught by a delta pheromone trap inside a wind tunnel. The *P*-Values were obtained with Chi-squared tests.

A total of 11 data sets had non-zero recaptures at more than five different distances, five from pheromone trap based experiments and six from YSB based experiments. All were satisfactorily fitted with the dispersal model, as indicated by the high r^2 values (Table 1). The recapture-distance relationships in all data sets were characterised by a rapid decline of the number of recaptures as the traps moved away from the release point (Figure 4).

Table 1. Fitted parameters of the dispersal model $y = exp(a - b \cdot \sqrt{x})$ and the estimated dispersal ranges based on the model. The 95, 99 and 99.9% distances are distances from the release point within which 95, 99 and 99.9% of the released moths are expected to remain respectively.

	Model fitting			Estimated Dispersal ranges (m)			
Data	**a**	**b**	**Variance explained (%)**	**Average distance**	**95% distance**	**95% distance**	**99.9% distance**
Males from pheromone traps							
P1A	4.6821	0.7547	0.9983	35	106	177	300
P1B	3.6636	0.7905	0.9978	32	96	162	273
P2	3.5836	0.9511	0.9994	22	67	112	189
P3-A	4.9053	0.9856	0.9995	21	62	104	176
P3-B	4.8829	0.9617	0.9997	22	65	109	184
Males from yellow sticky buckets							
Y1A	5.9839	1.1685	0.9999	15	44	74	125
Y1B	5.1375	1.1637	0.9997	15	44	75	126
Y2A	4.2627	1.0670	0.9990	18	53	89	150
Y2B	4.7707	1.1037	0.9997	16	49	83	140
Y2C	5.3706	1.2060	0.9998	14	41	69	117
Y3	5.8290	1.0572	0.9998	18	54	90	164
Females from yellow sticky buckets							
Y1A	5.3376	0.9169	0.9996	24	72	120	203
Y1B	4.8903	0.9988	0.9998	20	60	101	171
Y2A	4.0775	1.0435	0.9995	18	55	93	157
Y2B	4.6348	0.9980	0.9997	20	60	101	172
Y2C	5.2311	1.2284	0.9999	13	40	67	113
Y3	5.1761	1.0621	0.9997	18	53	89	151

The estimated dispersal ranges based on the relationships are given in Table 1. The average dispersal distances were 21-35 m for males from pheromone recapture data, 14-18 m for males from YSB recapture data and 13-24 m for females from YSB recapture data. The estimated distances within which 95, 99 and 99.9% of the males remained were 62-106, 104-177 and 176-300 m respectively for pheromone recapture data and 41-54, 69-90 and 117-164 m for YSB recapture data. The corresponding distances for females were 40-72, 67-120 and 113-203 m respectively.

Data from the YSB provided direct comparisons of the dispersal ranges of the males and the females. The estimated dispersal ranges were similar in males and females in three of the six data sets and slightly higher in females in the other data sets (Tables 2-3). Comparisons of the mean recapture distances revealed no significant differences between males and females in four data sets ($P>0.05$, t-test). In the remaining two data sets, the mean recapture distance was significantly higher in females in one data set and significantly higher in males in the other data set ($P<0.01$, t-test) (Figure 5).

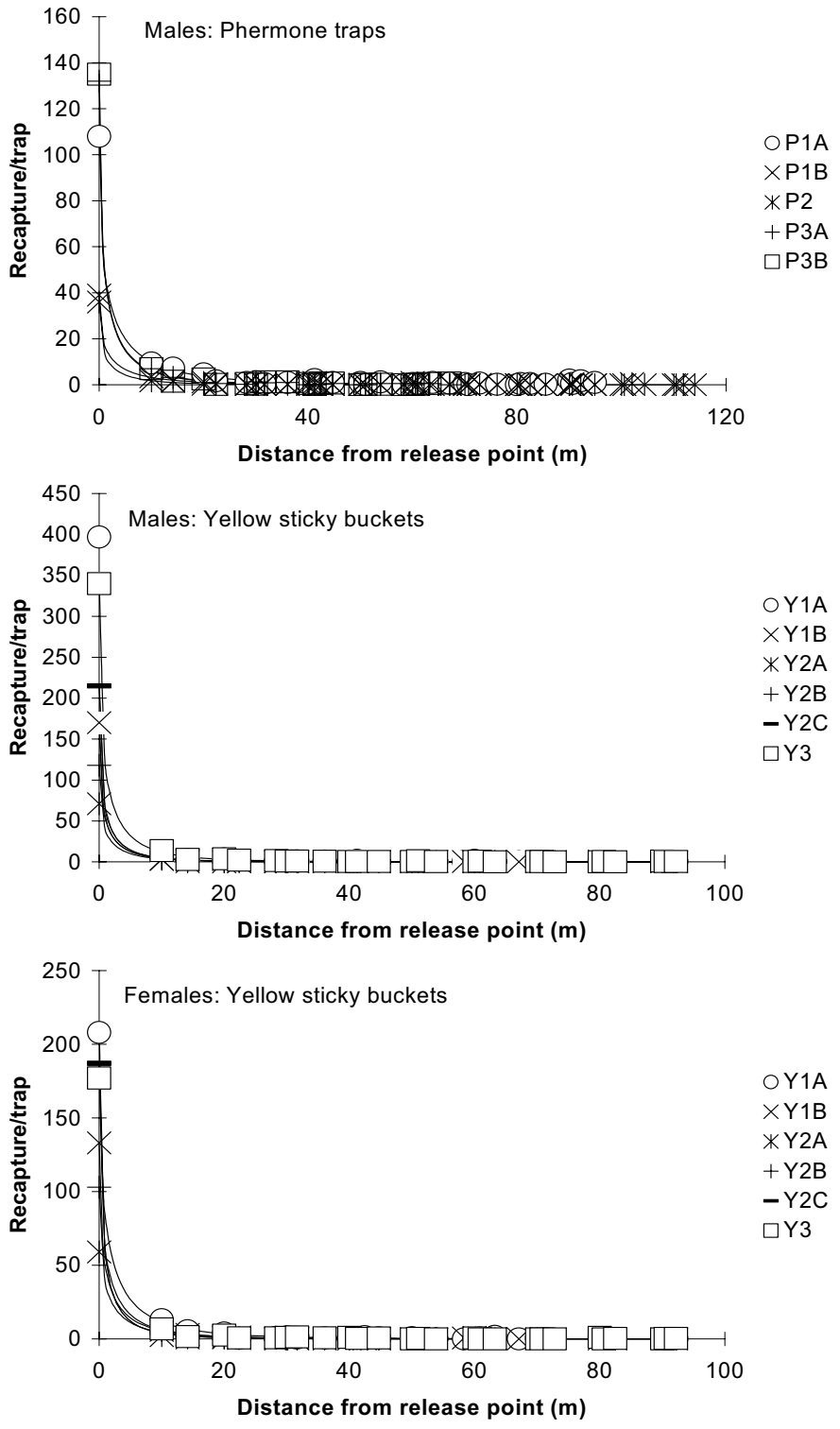

Figure 4. Fitted relationships between recaptures of diamondback moth and distance from release point.

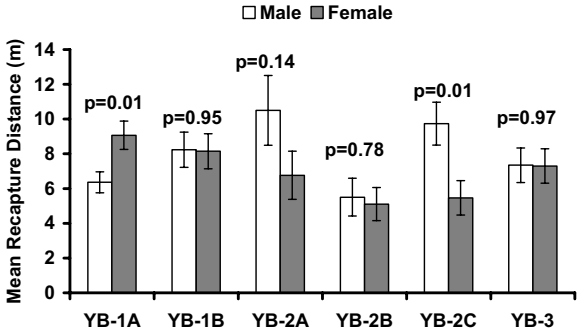

Figure 5. Comparisons of the mean recapture distances between male and female diamondback moths caught on yellow sticky buckets. The error bars show the standard deviations. The *P*-values were obtained from t-tests.

Discussion

This paper reports, for the first time, estimates of some characteristic dispersal ranges of male and female diamondback moths based on quantitative relationships between distance and moth density. The results suggest very limited movement of both sexes of the adults within healthy host crops. Less than 5% are expected to disperse over 110 m and less than 1% are expected to disperse over 200 m. Dispersal ranges estimated from pheromone traps were higher than those from YSB. This was probably because of different trapping capacities of the two types of traps. The trapping surface of the YSB is over 10 times larger than that of the pheromone trap. As a result, recaptures by pheromone traps close to the release point may have been artificially limited and hence a more gentle decline of recaptures over the distances. There was no compelling evidence suggesting different dispersal distances of the two sexes of DBM adults.

Shirai and Nakamura (1994) reported much higher average dispersal ranges for the males (286 – 615 m). However, their estimates were based on a few individuals caught in the non-release fields. The majority of the moths (80 – 94%), which were caught in the release fields, were not included in their calculations. When calculated over all recaptured moths, the average dispersal range was as low as 17 m, similar to the average dispersal distances ranges obtained in this study. Elsewhere, Caprio and Tabashnik (1992) noted that over 92% of the marked moths were caught by traps located within 10 m of the release point in their small-scale and non-replicated mark-recapture experiment. Some indirect data such as seasonal patterns of pheromone trap catches and spatial patterns of resistance levels also suggested short dispersal distances by residential DBM populations (Shirai & Nakamura 1994). Observations with night vision goggles by the authors showed that most DBM moths flew close to the ground and below the plant canopy, again suggesting mostly trivial movements and hence limited dispersal ranges.

Results from this study can be used in the designing and/or implementation of a number of IPM/IRM strategies against DBM. The success of resistance dilution with the provision of susceptible refuge populations relies on frequent gene flow between the target populations and the refuge populations. Hence the refuge populations should not be placed too far away from the target populations. Using the average dispersal distances as a guideline, the suggested maximal separation of refuge and crop is 35 m, the latter being the highest average dispersal distance obtained from this study. This distance may also be considered as the maximal trap interval for the alternative control strategy of pathogen auto-dissemination. However, for maximal effect, the trap interval may have to be <20 m, the distance around which the average dispersal distances from most experiments were centred.

Some strategies require that target populations be isolated from non-target populations, such as mating disruption, rotations of insecticides and crop-break. These strategies should ideally be implemented uniformly across the whole crop production area to minimise migrants from non-target populations. However, when uniform implementation is not possible because of some practical difficulties (e.g. disagreements between farmers), the target populations should be separated from the non-target populations by some minimal distances. Results from this study showed that 99% of the moths would not disperse in excess of 300 m. Multiplied by a safety factor of two, this gives a minimal separation distance of 600 m between target and non-target populations.

Dispersal within healthy host crops represents only one aspect of DBM movement. The movement patterns of the insect from harvested crops are likely to be quite different from those in healthy host crops, due to the need to find suitable host patches. Depending on the location of the closest host patches, moths from harvested crops may have to travel hundreds of metres to kilometres. Since growers normally plant their crops sequentially during the growing season, it is likely that crops of all ages will be present at any given time. Hence dispersal from harvested crops should be considered as common events. Long-distance migration is another movement process that needs to be addressed. The insect is known to engage in long distance migrations (Mackenzie 1958, Lorimer 1981, Chu 1986). In the southern states of Australia, there is an annual influx of DBM in the spring (Goodwin & Danthanarayana 1984). The likely sources of migrants are local *Brassica* weeds or canola crops or populations from warmer areas. Unlike local dispersal, migrations of moths are normally wind-assisted and can cover hundreds of kilometres. To better understand the dispersal process and use the information to fine tune relevant management strategies, it is important that dispersal from harvested crops, weeds and canola crops be studied.

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DBM development: are we measuring the right temperatures?

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Abstract

We modelled the population phenology of the diamondback moth (DBM), *Plutella xylostella* (L.) (Yponomeutidae) with a simple degree day model implemented using DYMEX, a population dynamics modelling program. Predicted and observed developmental periods of DBM reared under field conditions during the months of October 1999 to February 2000 at Forthside Research Station, Tasmania (which is in the temperate zone) were compared. Threshold temperature values of the second and third instar larvae were modified using values from previously published data to obtain a marginally closer fit to the observed developmental periods. The model overestimated the developmental periods when standard daily Stevenson's screen temperatures were used. Better predictions were obtained using daily temperatures recorded at 20 cm above ground level. We test this further by comparing observed and predicted development for DBM cohorts reared on potted Chinese cabbage under open, semi-shade, fully shaded and glasshouse conditions during August to November 2000 in Brisbane (which is in the sub-tropics). Temperatures recorded on the plant surface gave good predictions of development period from egg to adult emergence for all cohorts. The development model needs to be tested under a wider range of conditions, but our results suggest DBM development can be readily modelled. This work can form the basis of attempts to forecast DBM population dynamics and interpret population phenology.

Keywords

population phenology modelling, DYMEX, developmental rate, degree days, micro-habitat temperatures

Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) is a serious pest of cruciferous crops around the world. A plethora of research has been initiated on the management of DBM. Various pest management strategies have been developed and central to these is an understanding of the species' population phenology and dynamics.

Fundamental to modelling a species' phenology is an understanding of the insects' development rate in relation to temperature. From such relations the species phenology in an area can be predicted. We use DYMEX, an interactive population dynamics modelling program which predicts the relative abundance of an organism over a given period and allows rapid evaluation of management options (Maywald *et al.* 1999), to model DBM development.

Stanaway and Houlding (2000) built a DYMEX model for DBM on *Brassica* crops under field conditions. The model employed temperature driven cohort development based on studies of Goodwin (1976) and development was described by a linear above threshold function. Model predictions were compared with DBM larval counts obtained from Gatton Research Station Queensland (27° 33' S, 152° 16' E) over a three year period (1992-1995) and its overall fit was reported as good, despite problems encountered with over estimations of relative abundance (Stanaway & Houlding 2000). To further validate the model we compared the development periods of DBM obtained from field rearing at Forthside Research Station in Tasmania (41° 21'S, 146° 26' E) and a range of conditions in Brisbane, Queensland (27°28' S, 153° 02' E), with the predictions of the model. We show that with appropriate development rate functions and associated temperatures DBM development can be readily modelled.

Methods

Experiment 1

Development data obtained from rearing DBM on thirteen potted broccoli plants at Forthside Research Station during the months of October 1999 to February 2000 were used to test the model (Hill *et al.* unpublished). Locally reared adults were allowed to lay eggs on leaves of potted broccoli plants (except where wild eggs collected from turnip cotyledons were placed on two plants) and development period from egg to adult emergence was recorded. In two cases developmental period from neonate larvae to adult

emergence was measured. The experimental plants were caged around the onset of the pre-pupal stage and day of emergence of adult moths inside the cage recorded.

Minimum, maximum and average temperatures for the period of 1 October 1999 to 8 March 2000, recorded at Forthside Research Station were used to drive development in the model. Best fitting predictions were obtained in three steps. Firstly, predictions yielded by using unchanged, default development parameter values were compared with the observed development periods. In step two, the default parameter values affecting development in II and III instar larvae were modified based on previously published data to obtain a marginally close fit. Thirdly, using the changed development parameters, better predictions were obtained when temperatures recorded 20 cm above the ground level during the experimental period were used to drive the model.

Experiment 2

Cohorts of DBM were reared on potted Chinese cabbage under glasshouse, open, semi-shade and fully shaded conditions thrice from August to November 2000 in Brisbane. We used DBM adults from the Gatton Research Station culture caged over potted plants. Newly laid eggs were thinned to 8 eggs per plant. There were 6 plants per treatment. Development of immatures and temperatures were recorded twice daily; once early in the morning (0500-0700) and again after mid-day (1300-1500). Temperatures were recorded using an optical infra-red thermometer (RayTek-Pmplus ™, Santa Cruz, USA) at a number of locations where immature stages were found on each plant. By keeping plants under a range of cage conditions we generated a number of distinct temperature regimes at the one time. Time of adult emergence was recorded. We used a simple degree-day model based on thresholds for the entire immature period and compare this prediction with the observed number of degree-days required for development.

Results

Experiment 1

Predictions of developmental periods, yielded when default parameter values were used, over-estimated the observed developmental periods in the range of 4 to 87% (Table 1). After changing the threshold temperature values to 8°C from 13°C and 11.5°C in II and III instar larvae respectively and the temperature slope values to 0.05 from 0.065 in both the above instars, marginally close predictions were obtained (Prediction II, Table 1). The difference of this prediction with the observed was in the range of -13% to 71%. When simulations were run using adjusted parameter values and temperatures recorded 20 cm above ground level the predictions differed from the observed in the range of –27 to 26%, with a mean difference of only 3% (Prediction III, Table 1). In this case, four predictions of development periods matched exactly with the observed and three differed from the observed in the range of 4 to 8%.

Experiment 2

By placing cohorts in a range of microclimatic conditions, we varied both the temperatures experienced and the range of development times recorded at the one time of year (Figure 1, Table 2). Using a simple degree-day model for the entire immature period (egg to adult) with a developmental threshold of 7°C and the temperatures recorded for each cohort, we could readily predict the mean development period of DBM (Table 2).

Table 1. Comparison of mean development time of DBM in days observed on 13 plants at Forthside, Tasmania and that predicted using a simple degree day model implemented using DYMEX. Prediction I **is based on development parameters taken from Goodwin (1976) and Stevenson screen temperatures. Prediction** II **is as in** I, **but has adjustments to some thresholds and rates (see text for details). Prediction** III **is as in** II, **but uses 20 cm temperatures. % differences are calculated as: (predicted-observed)/observed.**

Plant sample	Observed	Prediction I	Prediction II	Prediction III
4	44	69	58	46
6	40	63	47	38
10	31	58	53	39
11	41	64	60	43
12	43	64	60	43
13	43	64	60	43
14	39	60	53	38
16	55	57	48	40
17	35	50	42	35
18	35	50	42	35
19	32	49	45	35
20	29	48	45	34
21	29	48	45	34
% difference				
Smallest		4	-13	-27
Largest		87	71	26
Average		53	35	3

Table 2. Mean plant maximum and minimum temperatures (standard deviation) recorded for each treatment run and the degree days (DD) elapsed to the maximum emergence of DBM adults. Values in brackets are the minimum and maximum DD for adult emergence. The predicted emergence time is 285DD.

Treatment	Run	Max	Min	DD
Glasshouse	1	30.4 (3.3)	10.5 (8.4)	271 (256-313)
	2	31.0 (4.8)	17.4 (1.0)	302 (252-333)
	3	29.3 (3.7)	19.9 (1.5)	272 (272-325)
Open	1	31.2 (10.9)	8.9 (6.7)	247 (233-317)
	2	33.6 (3.0)	14.1 (4.8)	281 (250-311)
	3	32.5 (1.6)	17.7 (1.9)	280 (246-317)
Semi-shade	1	28.0 (8.6)	8.6 (7.5)	287 (262-316)
	2	31.5 (4.1)	13.9 (4.8)	278 (249-320)
	3	31.3 (4.0)	18.3 (1.8)	257 (222-311)
Shade	1	26.7 (8.0)	8.2 (8.9)	271 (235-328)
	2	29.1 (5.9)	14.2 (6.2)	282 (258-312)
	3	29.0 (5.6)	17.8 (1.2)	289 (256-318)

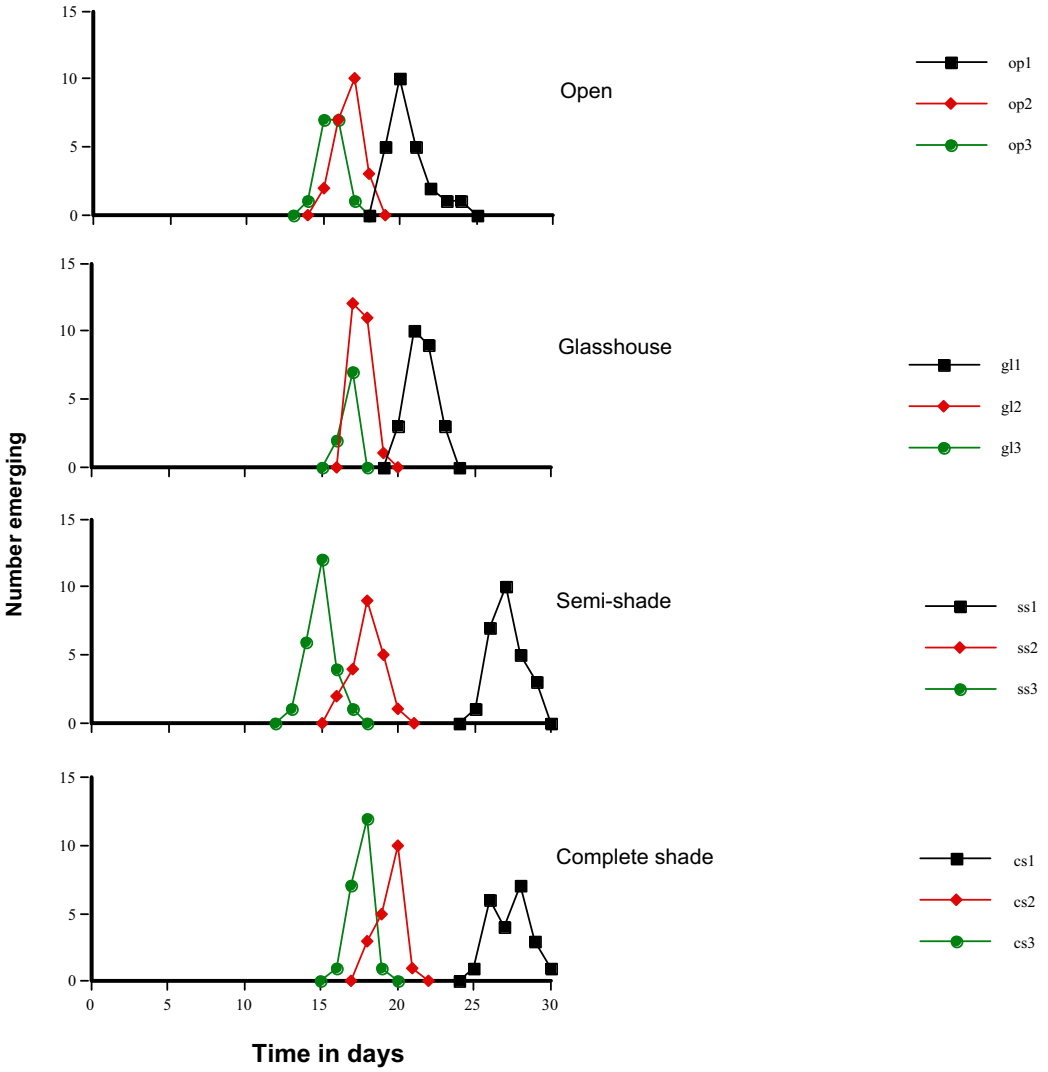

Figure 1. Daily emergence pattern of DBM adults in each of three trials under four microclimatic conditions (as indicated) conducted from August to November 2000 in Brisbane

Discussion

It is well known that the rate of development of an insect is temperature dependent and the relationship is linear above a threshold temperature for a wide range of temperatures (e.g. Allsopp *et al*. 1991). Having appropriate threshold temperatures, slopes and using the temperatures experienced are important when modelling development time. Predictions were up to 87% higher than the observed development time when default parameter values were used. Default values of threshold temperatures across all immature and adult stages were based on Goodwin (1976). Compared with previously published reports on development of DBM these default threshold temperatures were found to be high. Adjustment brought the predicted development period closer to the observed (Table 1). Use of precise lower threshold temperature values for immature stages of DBM is important to obtain reliable predictions especially when temperatures are fluctuating in this range.

Temperatures recorded by standard Stevenson's screen are often not the same as temperatures experienced by animals. Considering the average height attained by *Brassica* crops in the field, best predictions were obtained when daily temperatures recorded at 20 cm above the ground level were used to drive development in the model for Forthside. Use of plant temperatures gave very good predictions of development time for Brisbane conditions as well.

The adjustment of threshold temperatures for each of the life stages of DBM was obtained from previously published reports using linear regression analysis. Development occurring above 30°C, which was above the linear range was neglected from these studies and only that occurring along the linear range was analysed

(Figure 2). We selectively removed data that were based on low sample sizes, where temperatures were not well recorded or where animals were checked too infrequently to give good estimates of development time.

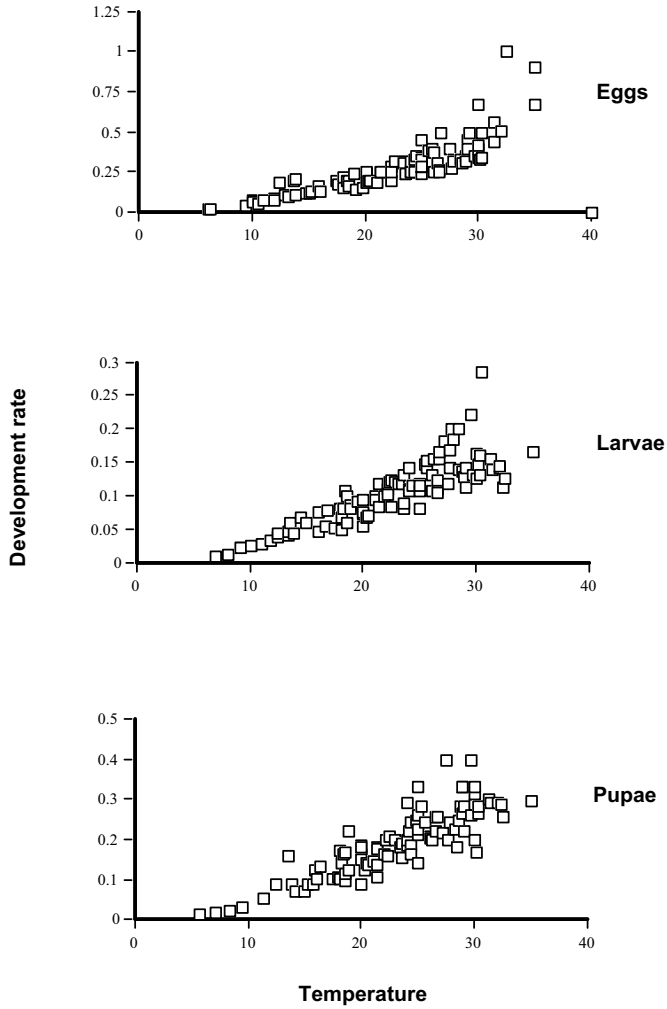

Figure 2. Simple scatter plot of all the development rate data for DBM eggs, larvae and pupae we could find in the literature indicating the large variation recorded among studies. No attempt has been made to distinguish studies or to selectively remove points.

The scatter in development values among the studies (Figure 2) can be attributed to the influences of multiple factors, including: differences in temperature and humidity conditions and control (constant versus fluctuating temperatures), host plants used in the experiments, source of DBM and frequency of checking. Published records show the development rate to vary when insects were reared on different cruciferous plants including wild hosts of DBM (Wakisaka *et al.* 1992). Development time when reared on wild host plants were longer than when reared on cultivated varieties.

Umeya and Yamada (1973) reported differences in development rate between three geographically isolated strains of DBM within Japan. When development rates of Japanese and Thailand strains of DBM were compared they showed no significant difference in development time (Sarnthoy *et al.* 1989). This view is confirmed by comparing the development performance of nine geographically isolated strains from nine different locations within Asia (Shirai 2000). In the later study, the strains were kept under laboratory conditions for a long time and acclimatization to local conditions by the strains was not checked. In both studies, least minimum temperatures used to test development rates were only 17°C (Sarnthoy *et al.* 1989) and 15°C (Shirai 2000). Inclusion of more low temperature regimes in such experiments would give an indication of the differences in development rate among strains from temperate zones against those from tropical and sub tropical zones.

Recently, Liu *et al*. (2002) provided a comprehensive study of DBM development under a range of constant and fluctuating conditions, particularly at high and low temperatures. Assuming there are no major geographic variations in development rate parameters, by using the non-linear functions from this study and appropriate temperatures we will be able to model DBM development anywhere. This model needs to be tested.

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Elevated atmospheric CO_2 may affect the performance of specialist (*Plutella xylostella*) and generalist (*Spodoptera littoralis*) on *Brassica* plants*

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Abstract

The general hypothesis is that herbivores are adversely affected by changes in plant chemical quality at elevated atmospheric CO_2 and that they are likely to become less abundant. Most of the chewing insects generally perform less well at elevated CO_2 than at ambient conditions. However, specialist and generalist insect herbivores show differential response to changes in secondary metabolite concentration of host plant. We studied how elevated CO_2 affects the performance of the generalist *Spodoptera littoralis* on two oilseed rape (*Brassica rapa* subsp. *oleifera* DC) cultivars, Tuli and Valo, and the glucosinolate concentrations in the leaves. Presently we are studying the impact of elevated CO_2 on crucifer specialist, diamondback moth (*Plutella xylostella*) on the two cultivars of oilseed rape and cabbage (*Brassica oleracea* L. subsp. *capitata*) plants.

Our results indicated that the generalist *S. littoralis* thrived less on oilseed rape plants grown under elevated CO_2 according to our hypothesis. As a result, the relative growth rate and the total food consumption of the larvae were reduced on elevated CO_2 plants compared with that on the plants grown under ambient conditions. The results of chemical analysis indicated that the leaves of cultivar Valo oilseed rape, grown under CO_2 exposure had low concentrations of 4-hydroxyglucobrassicin and neoglucobrassicin. However, the leaves of the cultivar Tuli had considerably high concentrations of two individual glucosinolates, gluconasturtin and neoglucobrassicin, and also the total amount of glucosinolate was increased in CO_2 exposed Tuli plants. Observed changes in glucosinolates after CO_2 exposure may be associated with the performance of the generalist herbivore and may have effects also on diamondback moth. We elucidate on the effects of elevated CO_2 on the composition of phenolic compounds and glucosinolates in leaves of oilseed rape and cabbage that may explain the differences in performance observed.

*Full paper to be published elsewhere

Interactions between transgenic plants, the diamondback moth and natural enemies

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Abstract

Several *Brassica* crops have been transformed with δ-endotoxin genes from *Bacillus thuringiensis* (Bt) to provide resistance to the diamondback moth (*Plutella xylostella*) and other lepidopteran pests. This paper describes studies of the direct and indirect effects of Bt plants on a parasitoid of *P. xylostella* using Cry1Ac-expressing transgenic oilseed rape (canola) as an example. The braconid wasp *Cotesia plutellae* is a solitary endoparasitoid of *P. xylostella* larvae. We present evidence that, although *C. plutellae* larvae that were forced to develop in Bt-treated susceptible hosts inevitably died with their hosts, behavioural factors are likely to limit the impact of this effect on field populations. *Cotesia plutellae* mortality in susceptible hosts was not due to a direct toxic effect of Cry1Ac, but due to premature host mortality since *C. plutellae* larvae developed normally in Bt-resistant hosts on Bt plants. Adult *C. plutellae* females were highly attracted to Bt plants damaged by Bt-resistant hosts. Any tactic aimed at suppressing pest populations risks affecting the dynamics of some natural enemies, simply due to a reduction in host or prey densities. The extent of these effects will depend on the ecological specialisation of the different natural enemy species present in the crop ecosystem. They are likely to be most pronounced for species, such as hymenopteran parasitoids, that tend towards greater host or prey specificity. However, the apparent lack of direct effects of Bt plants on survival and host-seeking ability of *C. plutellae* implies an environmental advantage compared with the broad-spectrum insecticides used for diamondback moth management in many areas.

Keywords

Cotesia plutellae, *Brassica napus*, *Bacillus thuringiensis*, resistance, *Plutella xylostella*, transgenic crops, tritrophic interactions

Introduction

Brassica crops are grown worldwide and form an important part of the human diet, particularly in Asia (Sivapragasam *et al*. 1997). Pests such as the diamondback moth (*Plutella xylostella* (L.), Lepidoptera, Plutellidae) remain a major problem despite advances in pest control (Gujar 1999, Sivapragasam *et al*. 1997, Talekar 1992, Talekar & Shelton 1993). A number of *Brassica* crops, including cabbage (*Brassica oleracea* L. subsp. *capitata*), broccoli (*B. oleracea* subsp. *italica*) and oilseed rape (*Brassica napus* L.) have been transformed with lepidopteran-active δ-endotoxin genes derived from *Bacillus thuringiensis* Berliner (Bt) (Jin *et al*. 2000, Kuvshinov *et al*. 2001, Metz *et al*. 1995, Stewart *et al*. 1996, Zhao *et al*. 2000), but so far none of these Bt plants has been commercially released.

Parasitoids are very important natural enemies of *Brassica* pests (Billqvist & Ekbom 2001, Fournet *et al*. 2000, Murchie *et al*. 1997, Sato *et al*. 1999, Sivapragasam *et al*. 1997). They are insects that complete their larval development on a single host insect and their survival and fitness are therefore intrinsically linked to the quality and fate of their host (Quicke 1997). Parasitoids represent one of the groups of non-target insects at risk from GM crops since they may come into direct contact with the transgene product when the larva feeds on host tissues or when the adult feeds on hosts. In addition, Bt plants have the potential to affect parasitoids indirectly by reducing host availability, quality and survival.

Microbial Bt formulations have been used for some time against *P. xylostella* and play a major part in integrated pest management (IPM) programmes since Bt δ-endotoxins, in contrast to many synthetic insecticides, have no contact toxicity against natural enemies (Shelton *et al*. 1993, Sivapragasam *et al*. 1997, Talekar & Shelton 1993). Microbial Bt formulations applied orally or to the host, are generally considered non-toxic against parasitoids. However, some laboratory studies have reported negative effects (reviewed by Glare and O'Callaghan (2000)), but these early studies were mostly conducted with a mixture of toxin crystals and bacterial spores and it is only relatively recently that the effects of purified Bt toxins have been

investigated. In addition, most wildtype Bt δ-endotoxins are protoxins, which require activation by gut proteases. In contrast, most Bt plants express already partially activated Bt toxins and this could potentially broaden their spectrum of activity.

Plutella xylostella has developed resistance to all major classes of insecticide and is considered the most damaging pest of brassicas on a worldwide scale (Ooi 1992, Talekar & Shelton 1993). Insecticide resistance in *P. xylostella* has led to control failures and overuse of insecticides (Gujar 1999). The susceptibility of *P. xylostella* to a number of Bt toxins has therefore made this pest the main target for the development of insect resistant transgenic brassicas. However, in some countries extensive use of microbial Bt formulations has already led to *P. xylostella* populations with resistance to Cry1A toxins (Ferré *et al.* 1991, Shelton *et al.* 1993, Tabashnik 1994, Wright *et al.* 1995). While Bt brassicas are considered by some a welcome new tool for the management of lepidopteran pests they are considered by others a serious threat to integrated pest management of *P. xylostella*. To provide factual and reliable information for regulators, growers and extension staff, further research is required into the effects of Bt brassicas on biocontrol agents, the potential role of Bt plants as a component of IPM programmes and appropriate resistance management strategies.

This paper describes the effect of transgenic oilseed rape (*Brassica napus*) on the solitary larval endoparasitoid *Cotesia plutellae* Kurdjumov (Hymenoptera, Braconidae), which is one of the major natural enemies of *P. xylostella* (Ooi 1992, Talekar & Shelton 1993). The interactions between Bt oilseed rape, *P. xylostella* and *C. plutellae* were studied using small-scale bioassays representing a worst-case scenario as well as behavioural and population scale choice experiments.

Transgenic plants

The Bt oilseed rape line used in this study (cv. Oscar, line O52) expressed a truncated synthetic Bt cry1Ac gene under the control of the cauliflower mosaic virus 35S promoter (Stewart *et al.* 1996). Untransformed wildtype plants of the parent cultivar were used as controls.

Effect of Bt oilseed rape on development and survival of *Cotesia plutellae* in small scale bioassays

The effects of Bt oilseed rape on development and survival of *C. plutellae* in susceptible and Bt-resistant *P. xylostella* larvae were compared in no-choice Petri dish bioassays.

Susceptible *P. xylostella* larvae fed Bt leaves caused very little feeding damage, did not increase in size and all larvae died within five days. No parasitoids emerged from hosts fed with Bt leaves while 63% of susceptible hosts fed wildtype leaves produced viable parasitoid offspring. The remaining susceptible hosts in the wildtype treatment were not parasitised and pupated normally. When susceptible hosts were dissected within two days following exposure to parasitoid females, parasitoid eggs or I instar parasitoid larvae were found indicating that the parasitoids attacked susceptible *P. xylostella* larvae on Bt leaves in this no-choice situation and that eggs hatched if the host lived long enough.

Larvae of the highly Bt-resistant NO-QA *P. xylostella* strain (Tabashnik *et al.* 1997) developed normally on Bt oilseed rape and no increase in mortality compared with the control was observed (Schuler *et al.* 1999). The mean time from egg to parasitoid emergence from hosts ranged between seven to nine days both on Bt leaves and wildtype leaves and there was no statistically significant difference in the distribution of parasitoid emergence from hosts between plant types. The level of parasitism of resistant larvae on Bt leaves was also not decreased compared with the wildtype treatment. After emergence from hosts, *C. plutellae* larvae spin a cocoon in which they pupate. Successful adult parasitoid emergence from cocoons ranged between 76-100% and there was no consistent effect of the plant line. There was also no evidence for a significant effect of the Bt line on the sex ratio of the parasitoid progeny.

Windtunnel choice tests with *Cotesia plutellae*

Parasitoid females use volatiles released from damaged plants to locate their hosts (Turlings *et al.* 1990, Vinson 1991). The responses of adult female *C. plutellae* to Bt oilseed rape leaves were, therefore, investigated in a series of dual choice tests in a wind tunnel (Potting *et al.* 1999, Schuler *et al.* 1999) to investigate if the females distinguish between (a) Bt and wildtype oilseed rape plants and (b) plants damaged by either susceptible or Bt-resistant *P. xylostella* larvae. *Cotesia plutellae* predominantly uses plant-derived stimuli in its in-flight host searching behaviour and the presence of hosts is not essential (Potting *et al.* 1999). However, host-damaged leaves are more attractive to *C. plutellae* females than

undamaged leaves while artificially-damaged leaves are as attractive as host-damaged leaves (Potting *et al*. 1999). Host-damaged leaves were obtained by allowing two *P. xylostella* larvae, either Bt-susceptible or Bt-resistant, to feed on each leaf overnight. Flights were recorded as a choice if they ended in landings on one of the leaves, within five minutes of take off. The amount of feeding damage inflicted by the hosts was measured after each bioassay.

Susceptible *P. xylostella* larvae caused significantly less feeding damage to the Bt leaves than to wildtype leaves and very few *C. plutellae* females (11%) chose to land on a Bt leaf over a wildtype leaf when these leaves were presented as a choice in the wind tunnel (Schuler *et al*. 1999). No significant difference was found between the feeding damage of resistant larvae on Bt compared to wildtype leaves and the parasitoid females did not distinguish between these two treatments. Similarly, parasitoids did not prefer one plant type to the other if leaves were artificially damaged to the same degree. When the parasitoids were given a choice of two Bt leaves damaged by either Bt-resistant hosts or Bt-susceptible hosts the majority (79%) of *C. plutellae* flew to the Bt leaves damaged by resistant hosts (Schuler *et al*. 1999).

Population scale studies with *Cotesia plutellae*

Further experiments were conducted in large cages in the laboratory to compare the level of parasitism on Bt and wildtype plants under conditions in which female parasitoids could freely choose between host populations on either plant type. The experiments were conducted with Bt-resistant *P. xylostella* larvae since Bt-susceptible *P. xylostella* larvae do not survive on Bt oilseed rape plants.

A mixture of wildtype and Bt plants were placed together in each cage as described previously (Schuler *et al*. 2001) and each plant was infested with *P. xylostella* eggs. Female *C. plutellae* were released into half the cages once most *P. xylostella* larvae had reached the III larval instar. The remaining cages served as controls. Numbers of parasitised and unparasitised hosts were assessed at the end of one parasitoid generation.

The results from one experiment showed a significantly higher level of parasitism on Bt plants but this result could not be reproduced and two further experiments showed no significant difference in parasitism between Bt and wildtype plants.

Discussion

The Bt oilseed rape plants caused 100% mortality of susceptible *P. xylostella* larvae and no *C. plutellae* larvae were able to complete their development in such hosts. However, the mortality of the parasitoid larvae was due to the premature death of the host and not due to a direct effect of Cry1Ac, since *C. plutellae* larvae developed normally in Bt-resistant *P. xylostella* feeding on Bt plants.

Parasitoid females use plant volatiles from damaged plants to guide them to hosts (Potting *et al*. 1999). Bt oilseed rape leaves damaged by Bt-resistant *P. xylostella* were highly attractive to *C. plutellae* females and it is possible that the ability of *C. plutellae* to locate and parasitise Bt-resistant hosts on transgenic crops, or crops sprayed with microbial Bt formulations, might assist with constraining the spread of genes for Bt resistance in the field. In contrast, Bt oilseed rape leaves damaged by susceptible *P. xylostella* were not very attractive to the parasitoid because the susceptible larvae caused only very limited feeding damage (Schuler *et al*. 1999). Any *C. plutellae* eggs deposited inside susceptible *P. xylostella* larvae on Bt plants would represent a waste of parasitoid resources, but the lack of attraction to this plant-host complex is likely to limit attacks of Bt-susceptible hosts in the field.

Many synthetic insecticides used for control of *P. xylostella* have broad spectrum contact action (Sivapragasam *et al*. 1997, Talekar & Shelton 1993) and cause not only parasitoid mortality indirectly through premature host mortality, but also have acute contact toxicity for adult parasitoids. In this respect, Bt plants offer an environmental advantage over broad spectrum synthetic insecticides.

In some areas *P. xylostella* has already developed resistance to microbial Bt sprays (Shelton *et al*. 2000, Tabashnik 1998, Verkerk & Wright 1997) and any commercial use of Bt brassicas has to be approached with great caution. The ability of parasitoids to detect hosts through plant-volatiles at long range, coupled with the observation that *C. plutellae* develops normally in Bt-resistant hosts, may contribute to resistance management. The conservation of specialist natural enemies such as *C. plutellae* in or near Bt crops will partly depend on the presence of weedy hosts and/ or untreated crops in the vicinity and may require active

management. Further research is necessary to determine the effect of parasitoids on the development of resistant pest populations and to investigate the potential of non-Bt refuge areas for conserving parasitoids as well as Bt-susceptible pests.

The present study provides an example of how realism can be introduced into laboratory studies investigating the effect of transgenic plants on non-target organisms. Experimental methodology presented here is recommended as part of a three-tiered scheme for assessing risks of GM plants on non-target organisms (Schuler *et al*. 2000).

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Diamondback moth, *Plutella xylostella* (L.), on peas in Kenya: impact of the host shift on the pest and its parasitoid

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Abstract

A population of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (DBM), was detected in 1999 on sugar snap peas (*Pisum sativum*) in the export vegetable growing area south of Lake Naivasha in the Rift Valley Province of Kenya. The performance of this new pea strain (DBM-P) was compared with the normal crucifer strain (DBM-C) in laboratory studies. DBM-P survived equally well on kale and on pea, whereas DBM-C survival on pea was only 2.4%. Larval development of DBM-C took five days longer on pea than on kale and larval growth was severely reduced. Pupal weights of DBM-C on pea (3.8 mg) were significantly lower than those of DBM-P (4.6 mg) and those of both strains on kale (5.7 and 5.3 mg, respectively). Crosses between the strains in both directions produced fertile offspring.

The influence of the host shift on *Diadegma mollipla* (Holmgren), the most abundant indigenous parasitoid of DBM in the Kenyan highlands, was also investigated. The number of larvae parasitised in a no-choice situation by two strains of *D. mollipla,* one collected on pea and the other from cabbage, was higher on pea than on cabbage. In a choice situation with entire plants, parasitism on pea offered alone was four times higher than on cabbage. When both host plants were offered simultaneously, the level of parasitism dropped to the level of cabbage offered alone. In conclusion, it is suggested that *D. mollipla* is a non-specialist parasitoid in the crucifer/DBM system and the significance of this observation for biological control of DBM is discussed.

Keywords

crucifers, *Pisum sativum*, *Diadegma mollipla*, tritrophic interactions

Introduction

Most herbivorous insect species are specialised feeders on just one plant family (Bernays & Chapman 1994). Still, a few abrupt host shifts to new plant families have been reported (Strong 1979, Bush 1994). The most serious pest of crucifer crops worldwide, the diamondback moth, *Plutella xylostella* (L.) was thought to be restricted to the family Cruciferae (Talekar & Shelton 1993). However, in 1999, DBM was first collected from a heavily infested sugar snap pea (*Pisum sativum* L.) field in Naivasha in central Kenya (Löhr 2001). This localised population seemed to have overcome two major hurdles for its adoption of peas as host plants: females are attracted to peas for egg laying and larval survival is sufficient to allow a viable population to develop (Löhr & Gathu, in preparation).

Apart from the obvious relationship with their primary pest species, plants are known to strongly influence the evolutionary and behavioural ecology of host-parasitoid associations (Godfray 1994). Therefore, the effect of this host shift on *Diadegma mollipla* (Holmgren), the most abundant indigenous parasitoid of DBM in the east African highlands, was considered worth studying. Very little information is available about the biology of *D. mollipla* in association with DBM. The species was first described by Holmgren in 1868 as *Limneria mollipla*, a parasitoid of the potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) (Gelechiidae). It is reported to be indigenous to southern and eastern Africa, but the original host is unknown (Broodryk 1971, Gupta 1974). Because of its effectiveness on PTM, this species was introduced for biological control into various countries, e.g. Peru (Smit *et al.* 1998) and Yemen (Kroeschel 1993). In a recent revision of the genus *Diadegma* as DBM parasitoids, Azidah *et al.* (2000) grouped all African specimens under *D. mollipla.* In Kenya, *D. mollipla* is frequently found on DBM on *Brassica* crops, but parasitism rates are not particularly high. Field parasitism in Kenya, with *D. mollipla* being the most abundant species, was less than 20% (Oduor *et al.* 1996).

In this paper, the performance of the novel DBM pea strain is compared with the common crucifer strain of DBM on both the original and acquired host. In addition, the effect of the host shift by DBM on its most important indigenous parasitoid is assessed.

Materials and methods

DBM laboratory cultures

The cabbage strain (DBM-C) was originally obtained from a cabbage field in Limuru, a peri-urban vegetable growing area about 25 km north-west of Nairobi. The culture had been maintained for about one year, first in the laboratory and then in a purpose-built insectary, as described by Löhr and Gathu (in preparation).

The pea strain (DBM-P) originated from an export vegetable farm along South Lake Road, Naivasha. After initial difficulties, a self-sustaining culture was established in August 2000 and repeatedly replenished with field-collected material from the original collection site. Snowpea cultivar "Snowgreen" and sugar snap pea cultivar "Sugar Pod" were used to sustain the culture.

Comparison of performance of DBM strains on peas and cabbage

Small tissue paper strips (approx. 15 x 30 mm) were placed inside transparent acrylic 30 ml vials and moistened with a drop of tap water. A piece of kale leaf, (*Brassica oleracea* var. *acephala* L. cv. Thousandheaded), of approximately the same size was placed on top of the tissue paper. In the pea treatment, an entire pea leaflet (*Pisum sativum* cv. Oregon sugar pod) was used. Neonate larvae were transferred with a fine brush into the prepared vials. Because of their delicate nature, the larvae were not touched, but lifted on their own thread and gently lowered onto the leaf surface. The procedure eliminated larval mortality due to handling. Pea leaflets were changed whenever the leaflet showed signs of wilting, while kale was changed when it started to turn yellowish. Care was taken to have enough food at any time. The vials were closed with a plastic cap without ventilation holes. Balancing the amount of water to avoid condensation inside the vial was delicate, but unventilated vials had proven the best option in preliminary trials.

The treatments consisted of a) DBM-C larva on kale; b) DBM-C on pea; c) DBM-P on kale and d) DBM-P on pea, all were evaluated concurrently in any one replication of 50 vials of each treatment. Five replications were run between July and November 2000. The vials of each treatment were placed on a metal grid and the whole set kept on a laboratory bench at room temperature (23 ± 3ºC during the day, 19± 2ºC at night).

The vials were checked every morning between 09:00 and 11:00. Parameters recorded were larval position during feeding (mining or on the leaf surface), survival, day of pupation and day of adult emergence and sex. Pupae were removed with the cocoon from the vial, weighed on a Mettler analytical balance and returned for adult emergence.

Rearing of parasitoids

(a) Cabbage strain of Diadegma mollipla

Cultures were established from cocoons collected from cabbage fields at Kapsabet in Nandi District of western Kenya and Limuru, Kiambu District of central Kenya. Parasitoids were reared on diamondback moth larvae on excised cabbage leaves in small Perspex cages. DBM larvae were renewed every two to three days until the parasitoids died. Parasitised DBM larvae were maintained separately. Pupae were collected into a vial and newly emerged parasitoid adults of both sexes were then kept together for at least one day to ensure mating.

(b) Pea strain of D. mollipla

The culture was established with a single pair collected with DBM larvae from pea fields at Naivasha. The culture was maintained on DBM-P larvae as previously described for the DBM-C culture.

Parasitism

All experiments were conducted under laboratory conditions (23 ± 2°C). To compare parasitism of the two *D. mollipla* strains on DBM-C and DBM-P, single mated 2–3 day old female *D. mollipla* were used. Preliminary tests with *D. mollipla* showed peak searching activity beyond this period. Females were kept for 24 h in small plastic containers (5 x 8 x 17 cm) with 25 II instar DBM larvae, 4 days old, on leaves. Fully expanded leaves from 4 to 6 week old plants of both species were used. Parasitism experiments were carried out with both c-*D. mollipla* on DBM-C and on DBM-P, p-*D. mollipla* on DBM-C and DBM-P.

After removing the parasitoid, the DBM larvae were reared individually in vials until reaching adult stage on leaves or leaf discs of their respective food plants. Dead DBM larvae were dissected in order to search for parasitoid eggs. In this experimental setup, naïve and experienced parasitoids were tested. To gain experience, females were allowed to parasitise larvae of the DBM strain they emerged from 24 h before the trial.

Effect of host plants on parasitism

In an exploratory test, single excised cabbage and pea leaves of similar size were kept in vials with water. A day before exposure, the leaves were infested with ten II instar DBM larvae each (DBM-C on cabbage and DBM-P on pea). Both vials were placed at a distance of approximately 30 cm in a Perspex cage (43 x 23 x 22 cm). Three 3–4 day old mated and experienced (on DBM-C) females of c-*D. mollipla* were then released into the cage. Larvae were removed after 24 h and reared in separate containers. The number of parasitoid pupae on both plants was recorded. The treatment was replicated five times.

Further tests described in this chapter were conducted with whole plants in a screened metal-framed cage measuring 60 x 45 x 45 cm. Only the experienced c-*D. mollipla* strain was used. To reduce the influence of variability of performance for individual females, three parasitoids were released in the cage. All treatments were replicated three times.

Single host plant and mixed host plant exposure

Four cabbage plants (4–6 weeks after transplanting; 6–8 leaves) were placed in the cage at a distance of approximately 20 cm. Each plant was infested a day before exposure with ten II instar DBM-C larvae. They were then exposed for 48 h to three 3–4 day old parasitoids. The larvae were subsequently reared in plastic containers on cabbage leaves. Larvae of the same plant were kept together. The number of parasitised larvae was recorded. A similar experiment was conducted with DBM-P larvae on four pea plants offered as single host and in a mixed host plant situation with two pea and two cabbage plants with their respective DBM larvae.

Statistical analysis

Analysis of variance was performed on the larval development of DBM in order to determine significant differences among treatments. A mixed model was assumed with the trial effect as random. Treatment means were separated using the Tukey's HSD test at 5% significance level. For the performance of the parasitoid, the Student-Newman-Keuls test at 5% significance level was used additionally.

Results

Effect of host plant and DBM strain on development and survival

(a) Larval development

Neonate larvae of DBM usually pass the first larval stage mining within the leaf parenchyma. DBM-C larvae mined for an average of 2.1 days in kale leaves, but only one of the 250 tested larvae managed to mine a pea leaflet for one day. In contrast, mining time of DBM-P larvae on kale was even longer than that of DBM-C (3 days). Pea strain larvae also managed to mine pea leaves, though for a shorter period than they mined on kale (Table 1). Differences in mining time were significant between both strains and on both crops.

Table 1. Performance of a cabbage and a pea strain of diamondback moth on kale and pea

DBM strain	Host plant	Mining days[1]	Larval period	Pupal period	Pupal weight	% survival	Sex ratio[2]
cabbage							
	kale	2.1 ± 0.05 b	8.5 ± 0.07 d	5.6 ± 0.07 ab	5.7 ± 0.06 a	87.6	1.33
	pea	0.01 ± 0.01 d	13.6 ± 0.55 a	5.8 ± 0.17 a	3.8 ± 0.13 d	2.4	2.00
pea							
	kale	3.0 ± 0.06 a	9.0 ± 0.10 c	5.3 ± 0.06 b	5.3 ± 0.06 b	85.2	0.94
	pea	0.6 ± 0.04 c	10.6 ± 0.13 b	5.5 ± 0.07 ab	4.6 ± 0.06 c	82.8	0.93

[1] average ± SE, [2] Females:males. (Means in the same column having same letter are not significantly different at 5% level using Tukey's HSD test).

Larval development showed a similar pattern for both DBM strains: faster development on kale and slower development on peas. However, whereas on kale DBM-C developed significantly faster (8.5 days) than DBM-P (9.0 days), its development on peas (13.6 days) was greatly retarded, also in comparison to DBM-P (10.6 days, Table 1). Development of both strains on kale and of DBM-P on pea was also more homogeneous than for DBM-C on peas, where some larvae remained in the larval stage for 22 days.

Heavy larval mortality occurred only among DBM-C on peas and was concentrated during the first three days. After the second day, 55.6% of the larvae had died (Figure 1). In total, 92% of DBM-C completed the larval stage on kale compared with only 7.2% on peas, while 88.4 and 90.0% of the pea strain completed larval development on kale and peas, respectively.

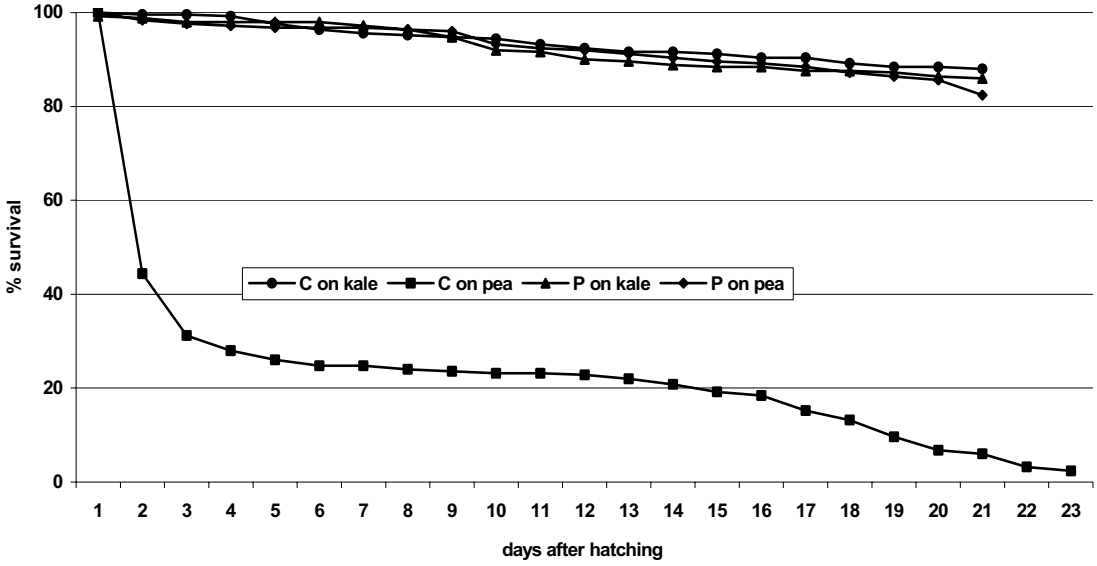

Figure 1. Survival of a cabbage (C) and a pea (P) strain of diamondback moth on kale and pea

(b) Pupal development
Differences in the duration of the pupal stage were small and only significant between those of DBM-C on peas (5.8 days) and DBM-P on kale (5.3 days, Table 1). There were differences, however, in the rates of survival during pupal stage: 95.6% of pupated DBM-C raised on kale emerged as adults as compared with 33.3% on pea. Survival of the DBM-P was similar on both plants, kale and pea (97.3% and 92% respectively, Figure 1).

(c) Pupal weight
Significant differences were also recorded in pupal weight between all treatments and strains (Table 1). Cabbage strain pupae on kale were heaviest (5.7 mg), followed by DBM-P on kale (5.3 mg) and peas (4.6 mg). The lightest (3.8 mg) were the pupae of the DBM-C that had survived on peas. Their weight was so low that many of them failed to emerge as adults.

The sex ratio of emerging adults was female-biased for DBM-C, irrespective of the host plant, while the ratio for DBM-P was slightly male-biased.

Effect of host shift on the parasitoid

In Table 2, the number of parasitised larvae is shown for the two *D. mollipla* strains, c-*D. mollipla* and p-*D. mollipla* on both DBM strains. Generally parasitism was low, but parasitoid performance was better on peas than on cabbage. The number of parasitised larvae per naïve female ranged from 0 to 11 for c-*D. mollipla* on DBM-C, 0 to 17 on DBM-P and from 0 to 20 for p-*D. mollipla* on DBM-P.

With experienced *D. mollipla,* the number of parasitised larvae was higher and ranged between 1 to 15 for p-*D. mollipla* on DBM-P and 10 to 14 for c-*D. mollipla* on DBM-P. In both experiments, parasitism on DBM-P was higher than on DBM-C. The range of parasitised larvae per female given in Table 2 illustrates the high variation in performance of individual females. Therefore, these figures are not statistically different, but

can be taken as tendencies. Missing data are due to microsporidian infection problems in the DBM culture and the collapse of the p-*D. mollipla* culture during the experiments.

Table 2. Parasitism of naïve and experienced *Diadegma mollipla* on diamondback moth larvae reared on cabbage and on pea

Parasitoid strain	Host strain	No. parasitoids tested	No. larvae exposed	Range of larvae parasitised	Mean no. larvae parasitised[1]
Naïve *D. mollipla*					
cabbage	cabbage	12	23	0 - 11	3.0 ± 1.15 ns
	pea	12	23	0 - 17	3.8 ± 1.62 ns
pea	cabbage	-	-	-	-
	pea	7	22	0 - 20	5.8 ± 2.19 ns
Experienced *D. mollipla*					
cabbage	cabbage	3	25	6 - 11	8.3 ± 1.44 ns
	pea	3	23	10 - 14	12.6 ± 1.33 ns
pea	cabbage	5	23	1 - 15	7.3 ± 3.49 ns
	pea	-	-	-	-

Effect of host plants on parasitism

When larvae were exposed on excised leaves kept in vials, parasitism was low with 24 DBM larvae out of 100 parasitised. However, with 16 larvae parasitised on peas, preference was more biased towards peas than towards cabbage (eight parasitised larvae).

When DBM was exposed on cabbage plants only, the parasitism rate of c-*D. mollipla* was 6.1%, thus being significantly lower than on pea plants alone (26.5%) (Table 3). When both host plants were offered simultaneously, parasitism was comparable to cabbage offered alone (3.5%). However, a higher proportion of larvae was parasitised on peas (2.6%) than on cabbage (0.9%).

Table 3. Influence of host plant on parasitism of diamondback moth by *Diadegma mollipla* reared on cabbage

Host plant	**No. DBM larvae exposed**	**Average no. of larvae recovered**	**Average no. of larvae parasitised[1]**	**Parasitism rate [%]**
cabbage alone	40	38	2.3 ± 0.88 a	6.1 a
pea alone	40	38	10.0 ± 2.52 b	26.5 b
pea/cabbage	40	39	1.3 ± 0.33 a[2]	3.5 a [*) 2.6, **) 0.9]

[1] average ± SE, [2] average number parasitised on pea =1.0, on cabbage = 0.3
*) parasitised on peas, **) parasitised on cabbage. Means from 3 replicates.
Means in the same column having same letter are not significantly different at 5% level using Tukey's HSD test

Discussion

Diamondback moth has been recorded in the field from host plants outside its "natural" host plant range before. Reichart (1919, in Talekar *et al.* 1985) reported DBM on chickpea and a chenopodiaceous vegetable in Russia. More recently, DBM was found on okra in Ghana (Anonymous 1971) and on faba beans in Egypt (Badr *et al.* 1986). These occurrences seem to have been either sporadic and were not given due attention, or were the result of misidentifications and, therefore, never heard of again. In the case reported here, crosses between DBM-C and DBM-P in both directions produced fertile offspring (Löhr, unpublished data), so the identity of the species is not in question.

To date, only few studies have looked into the host plant range of DBM, all under laboratory conditions. Gupta and Thorsteinson (1960a) observed that DBM fed on nine non-cruciferous species under no-choice

situation in the laboratory. Pea was one of the six species in the Leguminosae family found suitable for DBM feeding in these studies. An additional 12 species were accepted only when sinigrin was added as phagostimulant on the leaf discs. The mentioned authors did not observe any differences between the survivors on peas and the ones raised on mustard. However, in our experiments, pupal weights and in consequence, adult size of the surviving normal strain DBM on pea was significantly lower than on kale. Gupta and Thorsteinson (1960b) attributed the non-host character of peas in the field to the egg-laying behaviour of the female moths, implying that those may not be attracted to the crop. In Kenya, profuse egg-laying has been observed in the field and Löhr and Gathu (in preparation) investigated the ability of DBM to adapt to new hosts. They found that in only four generations, a DBM strain could be selected that survived equally well on kale and pea.

The data presented here also show that *D. mollipla,* the major DBM parasitoid in the Kenyan highlands, has managed to follow its host onto the new host plant. Preference experiments revealed significantly higher parasitism of DBM larvae on peas compared with cabbage. Two conclusions can be derived from these observations: *D. mollipla* is only loosely associated with DBM and its original host plant range and there must be a factor that renders DBM on crucifers less attractive than DBM on peas. The first observation is supported by reports of *D. mollipla* as an important parasitoid of the potato tuber moth (PTM) on potato and tobacco in southern Africa (Broodryk 1971) and on potato in Yemen (Kroeschel 1993). However, as PTM is an introduced species to Africa and *D. mollipla* seems to be indigenous, PTM cannot be the original host of this species. It is therefore reasonable to assume that *D. mollipla* is a parasitoid with considerable host plasticity and that it might be found to parasitise more free-living or leaf mining species of microlepidoptera. This lack of specialisation may also explain the generally low parasitism rates of DBM observed in the laboratory (Akol 2001) and thus its irrelevance for the control of DBM field populations (Oduor *et al*. 1996, Löhr, unpublished survey data). As for the second observation, concerning factors for the higher attractivity of peas, a few published papers relate to the influence of DBM host plants on parasitism, but they all refer to DBM specialist parasitoids on crucifers (Talekar & Yang 1993, Beck & Cameron 1990, Idris & Grafius 1996). However, the preference of *D. mollipla* for DBM feeding on peas may be explained by cues used for host location. The use of infochemicals by hymenopterous parasitoids to locate their hosts is well documented (e.g. reviewed in Vet & Dicke 1992). Parasitoids associated with crucifer specialist herbivores were shown to be attracted by volatile isothiocyanates (mustard oils) typically released by crucifers when injured (Pivnick 1993, Murchie *et al.* 1997). For *D. mollipla,* crucifer volatiles are unlikely to be used for host location. We assume that these substances, known to be toxic for many herbivores, even reduce attractiveness of cruciferous plants for *D. mollipla.* We therefore assume that the crucifer growing system is not a preferred habitat for *D. mollipla.* However, it is commonly accepted because of its wide availability and therefore easy accessibility in Kenya.

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Trap crops for diamondback moth and other crucifer pests in Guam

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Abstract

The major pests of cabbage, *Brassica oleracea* var. *capitata* on Guam are diamondback moth, *Plutella xylostella;* cabbage webworm, *Hellula undalis;* cutworm, *Spodoptera litura;* cabbage cluster caterpillar, *Crocidolomia pavonana;* the fleahopper, *Halticus tibialis* and aphids. In the past, Chinese cabbage, *Brassica chinensis* cv. Tempest for *C. pavonana;* radish, *Raphanus sativus* cv. Minowase Summer Cross 3 and mustard, *Brassica juncea* cv. Indian for *H. undalis; B. chinensis* cv. Tempest, and *R. sativus* cv. Minowase Summer Cross 3 for *H. tibialis;* and *B. juncea* cv. Indian for aphids were found to be effective as trap crops in cabbage fields. Indian mustard has proven to be an effective trap crop for diamondback moth in India and South Africa, however, it was not attractive for the population in the Pacific. Collards, *Brassica oleracea acephala* cv. Vates, have been noted as an effective trap crop for *P. xylostella* in cabbage fields in Guam. The population of diamondback moth in the continental United States was also attracted to collards. It is possible that the populations of diamondback moth in the New World and the Pacific are different from those of South Asia and Africa. Of the six major pests of cabbage on Guam, trap crops have been identified for five of them. A trap crop for *S. litura* is yet to be identified.

Introduction

Cabbage, *Brasssica oleracea* var. *capitata,* is one of the common crucifer crops grown throughout the world. It is also a common vegetable crop grown on Guam throughout the year, mostly the varieties that are heat tolerant and resistant to tip burn. It is attacked by over a dozen insect species of which about half are major pests. The major pests of cabbage on Guam are diamondback moth, *Plutella xylostella* (L). (Lepidoptera: Plutellidae); cabbage webworm, *Hellula undalis* (F.) (Lepidoptera: Pyralidae); cutworm, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae); cabbage cluster caterpillar, *Crocidolomia pavonana* (F.) (Lepidoptera: Pyralidae); the flea hopper, *Halticus tibialis* (Reuter) (Hemiptera: Miridae) and aphids. In the past, trap crops, Chinese cabbage, *Brassica chinensis* cv. Tempest for *C. pavonana;* radish, *Raphanus sativus* cv. Minowase Summer Cross 3 and mustard, *Brassica juncea* cv. Indian for *H. undalis; B. chinensis* cv. Tempest, and *R. sativus* cv. Minowase Summer Cross 3 for *H. tibialis* and *B. juncea* cv. Indian for aphids were found to be effective in the head cabbage fields. We have been working on biological and chemical control methods for the past three decades without much success primarily due to multiple pests attacking this crop, low threshold level required, short duration of the crop, chemical control countering the effectiveness of biological control and insecticide resistance development.

Trap cropping separates individual species of insect from the group by concentrating them in some parts of the field and permits use of biological control, selective use of pesticides, reduction in use of pesticides and other control strategies without countering one another. Hokkanen (1991) has reviewed trap cropping in pest management. In Finland, trap crops of Chinese cabbage, oilseed and turnip rape, sunflower and marigold in a mixture were used to suppress the damage by the blossom beetle, *Meligethes aeneus* F. (Coleoptera: Nitidulidae), to cauliflower (Hokkanen *et al.* 1986, Hokkanen 1989). Buechi (1990), in Switzerland, found that turnip rape (*Brassica rapa* var. *silvestris*) sown as a perimeter strip of 6 to 12 m within the rape (*Brassica napus* L.) attracted cabbage stem flea beetle, *Psylliodes chrysocephala* L. (Coleoptera: Halticidae), rape stem weevil, *Ceutorhynchus napi* Gyll. (Coleoptera: Curculionidae); cabbage stem weevil, *Ceutorhynchus quadridens* (Coleoptera: Curculionidae) and blossom beetle, *M. aeneus.* Srinivasan and Krishna Moorthy (1991) reported the use of Indian mustard as a trap crop for *P. xylostella* in cabbage fields in Bangalore, India. Silva-Krott *et al.* (1995) found Indian mustard was not attractive to diamondback moth in Guam. However, Charleston and Kfir (2000) found Indian mustard to be attractive to diamondback moth in South Africa and recommended its use as a trap crop in cabbage.

Muniappan and Marutani (1992), Silva-Krott *et al.* (1995) and Muniappan *et al.* (1997) reported the use of Chinese cabbage, radish and Indian mustard as trap crops in cabbage fields for control of cabbage webworm, *H. undalis,* cabbage cluster caterpillar, *C. pavonana,* fleahopper, *H. tibialis* and mustard aphid, *Liphaphis erysimi* (Davis) (Homoptera: Aphididae) in Guam. Smyth (1999) reported Chinese cabbage to be

attractive to *C. pavonana* in laboratory studies conducted at Cornell University with an insect culture obtained from Indonesia. Mitchell *et al.* (2000) found collards to be attractive to diamondback moth in cabbage fields in Florida. In this paper, we report further work on trap cropping in cabbage fields on Guam.

Materials and methods

The plants used in the tests were cabbage, *B. oleracea* var. *capitata* cv. Scorpio; Chinese cabbage, *B. chinensis* cv. Tempest; radish, *R. sativus* cv. Minowase Summer Cross 3; mustard, *B. juncea* cv. Indian and collards, *B. oleracea* var. *acephala* cv. Vates. The first trial was conducted from November 2000 to February 2001 and the second trial from March to May 2001.

Seeds of these plants were sowed in a tray filled with peat moss for germination. Seedlings were kept in the tray for four weeks and fertilised weekly. Seeds of trap crops radish, mustard, Chinese cabbage and collards were sown a month earlier than cabbage. Seedlings were transplanted in a field at the Yigo Agricultural Research Station in Guam. The soil was classified as clayey, gibbsitic, isohyperthermic, very shallow, highly calcareous with limited moisture holding capacity (Young 1988). The field was ploughed, fertilised, tilled and laid with drip irrigation lines before transplanting. Trap crops were planted four weeks ahead of cabbage. The field was laid out with one row each of Chinese cabbage, radish and mustard, nine rows of cabbage in the middle and six rows of collards on the other side. Periodically the field was examined for the incidence of pests. Weekly observations were made and the number of different pests was recorded once the pest incidence was noted. Larvae of *H. undalis, C. pavonana, S. litura* and *P. xylostella* and adults and nymphs of *H. tibialis* were counted on ten plants at random in each row. Whenever a heavy incidence of pests was observed, Naled (Dibrom 8 emulsion) was applied at 10 ml/L to save the plants from total damage and for further observations during the succeeding weeks.

The data were analysed by averaging insect counts to a total of ten plants per crop. The data collected during the length of experiment were analysed using ANOVA (SYSTAT 1992).

Results and discussion

The larval counts of *P. xylostella, H. undalis* and *S. litura* for two seasons and *C. pavonana* and *H. tibialis* for one season were analysed. The incidence of *P. xylostella* larvae on the crops tested in both the trials are shown in Figures 1 and 2.

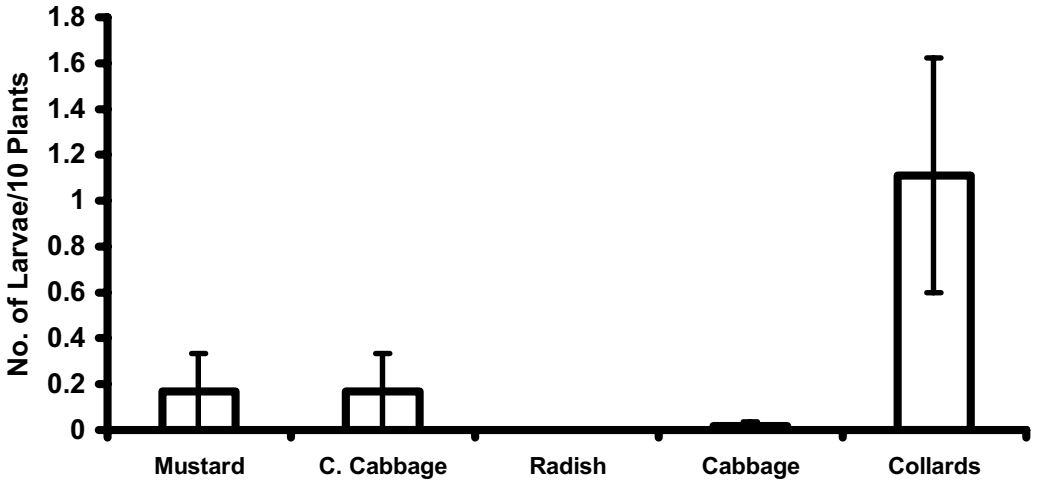

Figure 1. Incidence of *Plutella xylostella* on crucifer crops in Guam (trial 1).

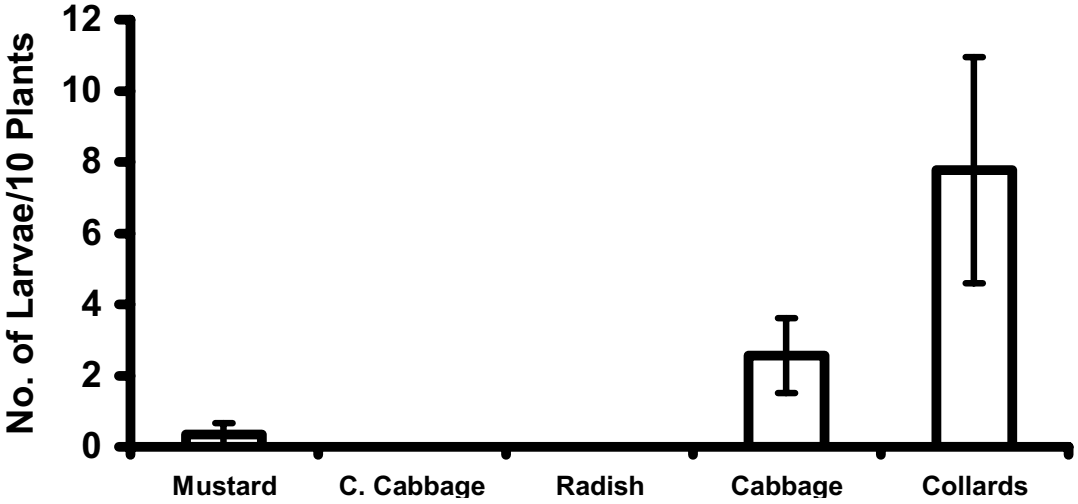

Figure 2. Incidence of *Plutella xylostella* on crucifer crops in Guam (trial 2).

The collards consistently had more larvae than the cabbage in both the trials, (P=0.0052, trial 1, P=0.0002, trial 2). Srinivasan and Krishna Moorthy (1992) reported that Indian mustard attracted *P. xylostella* in the cabbage fields. Silva-Krott *et al.* (1995) did not find any difference in incidence of the larvae of *P. xylostella* in cabbage and Indian mustard plots in Guam. However, Charleston and Kfir (2000) found more egg laying, but low survival rate of the larvae on Indian mustard. In our trials, collards had more larvae than mustard (P=0.0139, trial 1; P=0.0001, trial 2). In Florida, Mitchell *et al.* (2000) also found that collards attracted more larvae in the cabbage fields. Now, trap cropping with collards in cabbage fields has become a popular practice in the United States of America. Since the *P. xylostella* populations in India and South Africa are attracted to the Indian mustard and the populations in Guam and the continental U.S.A are attracted to collards, it is possible that the populations in India (South Asia) and South Africa (Africa) may be different from the ones in the Pacific and the U.S.A. Larval counts of *H. undalis* in the two trials are shown in Figures 3 and 4. Mustard (P=0.0001) and radish (P=0.0001) had more larvae in the first trial and, in the second trial, mustard (P=0.0232) had more larvae than cabbage. Even though there were more larvae on Chinese cabbage and radish than on cabbage, the difference was not statistically significant. These results conform with previous reports of Muniappan and Marutani (1992), Silva-Krott *et al.* (1995) and Muniappan *et al.* (1997).

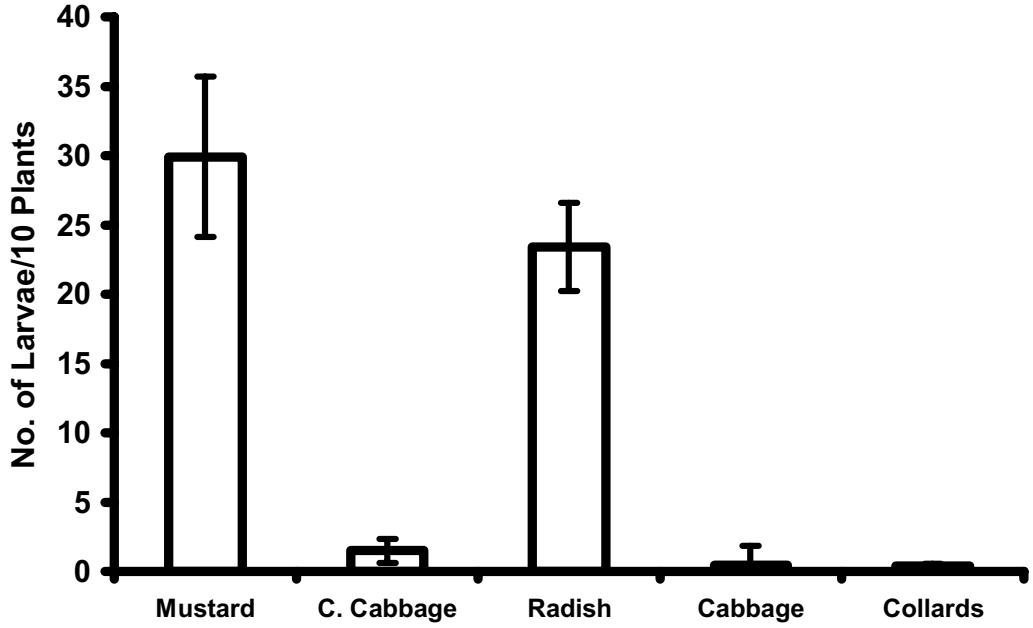

Figure 3. Incidence of *Hellula undalis* on crucifer crops in Guam (trial 1).

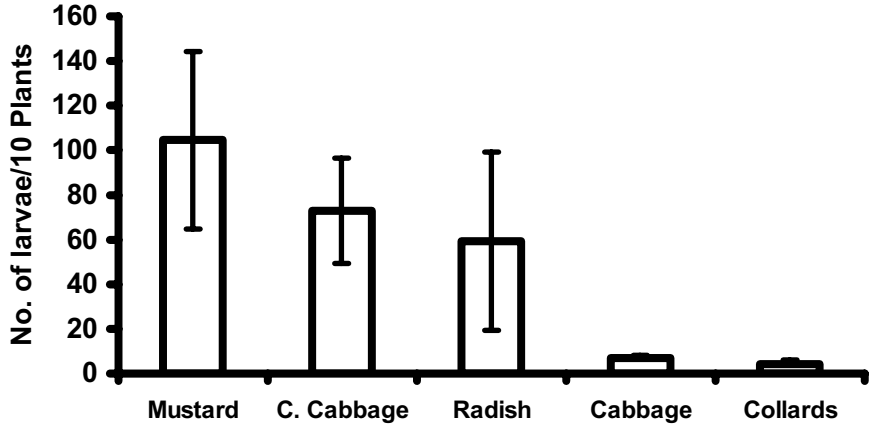

Figure 4. Incidence of *Hellula undalis* on crucifer crops in Guam (trial 2).

There were more larvae of *S. litura* on collards than on Chinese cabbage (P=0.0275), radish (P=0.0077) and mustard (P=0.0147), but numbers were not significantly different from those on cabbage in trial 1. In the second trial there were more larvae on cabbage than on other crops (P=0.0001) (Figures 5 and 6). These results also conform with previous reports. The thick and leathery leaved crucifers like cabbage and collards seem to attract more *S. litura* than the thin leaved radish, mustard and Chinese cabbage. We are yet to find a trap crop for *S. litura* in cabbage fields.

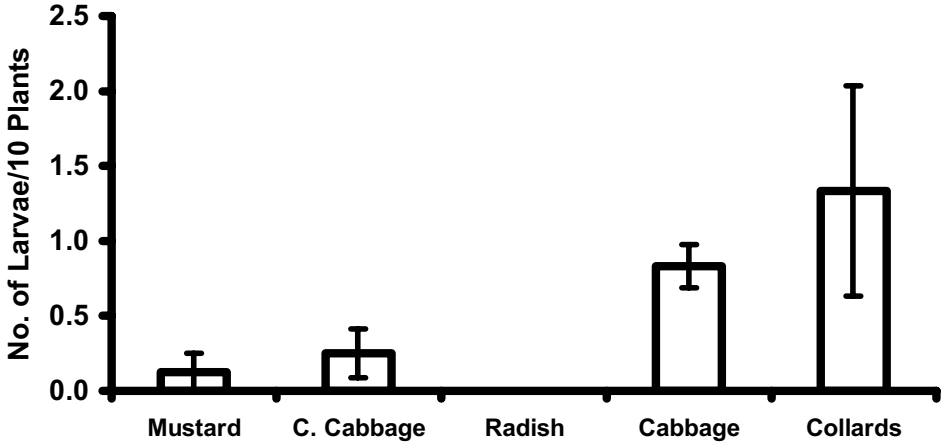

Figure 5. Incidence of *Spodoptera litura* on crucifer crops in Guam (trial 1).

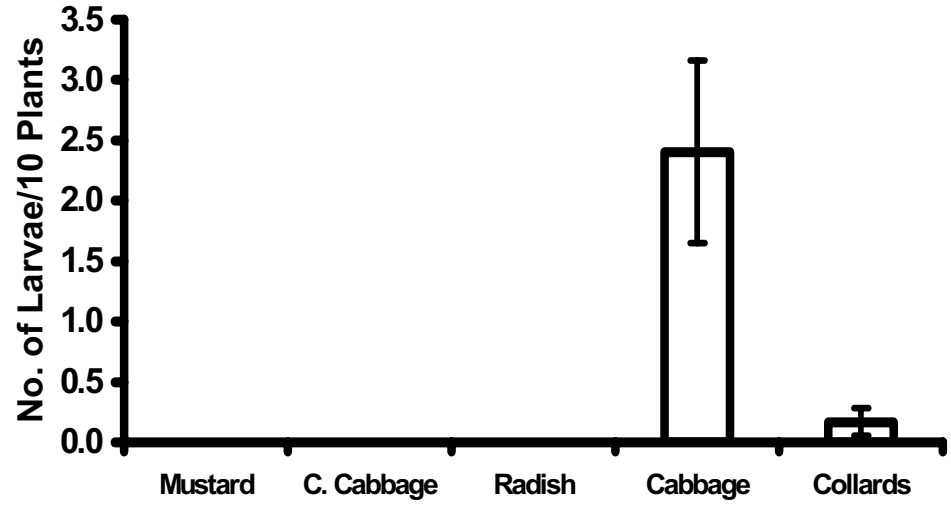

Figure 6. Incidence of *Spodoptera litura* on crucifer crops in Guam (trial 2).

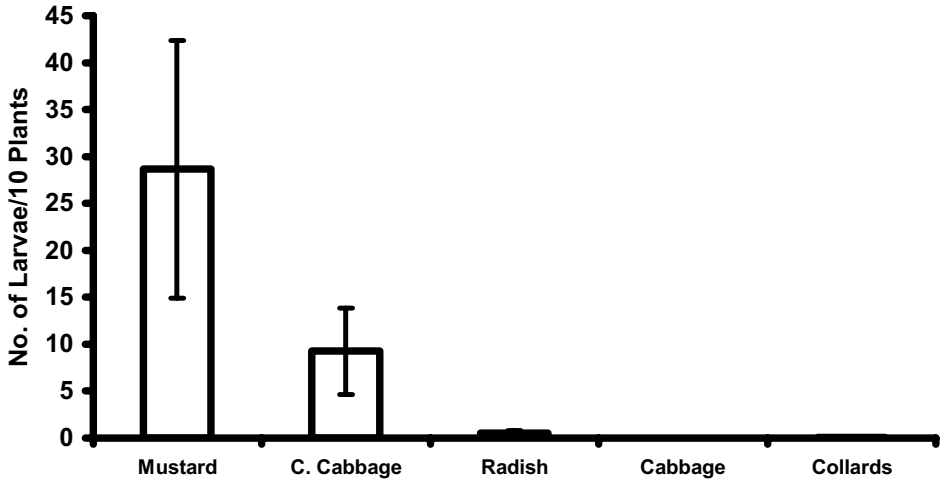

Figure 7. Incidence of *Crocidolomia pavonana* on crucifer crops in Guam (trial 1).

The incidence of *C. pavonana* populations in both the trials was low. Only in the first trial were we able to analyse the data. It was found that mustard attracted significantly more larvae than did other crops tested (Figure 7). There was more *H. tibialis* in Chinese cabbage and radish in trial 1 (Figure 8).

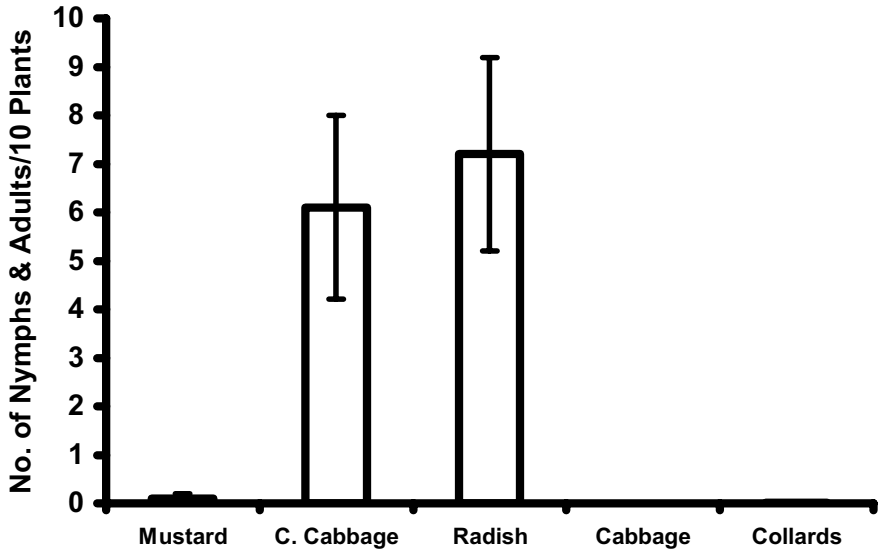

Figure 8. Incidence of *Halticus tibialis* on crucifer crops in Guam (trial 1).

Growing Chinese cabbage, radish, mustard and collards as trap crops in cabbage fields could effectively reduce the incidence of *P. xylostella, H. undalis, C. pavonana* and *H. tibialis.* In earlier trials, mustard was also found to attract aphids. This method could be incorporated in integrated pest management programs as it reduces pesticide use, harbours natural enemies thus enhancing biological control and is amenable for other methods of control without interference.

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Arabidopsis thaliana as a model host plant for *Plutella xylostella*

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Abstract

Investigations of host plant-pest interactions have rarely used plant material in which the phenotype could be linked directly to a known genotype. However, in recent years, *Arabidopsis thaliana* has been intensively studied by molecular biologists and, as a result, there are a large number of genetically characterised *A. thaliana* mutants available to the scientific community. Our preliminary laboratory investigations have shown that *A. thaliana* can be used as a host plant by *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) and have demonstrated that larval period, pupal period and pupal weight measurements of *P. xylostella* developing on *A. thaliana* are comparable to those on *Brassica rapa.* These factors, combined with its relatively small size and easy growth, make *A. thaliana* a suitable model host plant for investigation of both genotypic and phenotypic factors which influence the biology of *P. xylostella,* the aim of our research.

Our initial investigations have used the *A. thaliana* lines Col-0 and Col-5. These lines are genetically very similar except that Col-5 is homozygous for a gl1–1 mutant allele that is not present in Col-0. This mutant allele results in an absence of trichomes on the leaves and stem of the plant, which are normally present in Col-0. In our investigations, it was found that female larvae took significantly longer to develop and produced lighter pupae on Col-0 compared with Col-5. However, this effect was not seen in male insects. In both males and females, there was no significant effect on pupal period. It was also found that adults that had developed from larvae on Col-0 produced significantly fewer eggs than those that had developed on Col-5.

Keywords

trichome, development, IPM

Introduction

Successful integrated pest management (IPM) is, almost without exception, based on a large number of investigations of the biology of the pest and its interactions with its environment. One of the most important relationships for study is undoubtedly that between the pest and its host plant, but studies have often been limited by the inherent complexity of these interactions. Model systems using plant material that is genetically well characterised can allow investigation of chosen aspects of plant-pest relationships by limitation of the number of variables during experimentation.

Arabidopsis thaliana, a member of the Brassicaceae, is one species for which there is a wide range of genetically characterised mutants available. Its relatively small genome (25,498 genes) with almost no repetitive DNA, combined with a compact size and easy growth have resulted in its intensive study by molecular biologists and sequencing of the entire genome (The *Arabidopsis* Initiative 2000). While *A. thaliana* is not directly of agricultural significance, it may be suitable as a model system, as many aspects of the plant-pest relationship are conserved in a broad range of species (Mitchell-Olds 1999).

Arabidopsis thaliana is known to be susceptible to attack by a significant number of commercially important pests of the Brassicaceae including *Plutella xylostella*. This pest is a global economic problem due to its large geographical distribution, voracious eating habits and the wide choice of commercial plants which are suitable hosts, including those of agricultural and ornamental importance. The aim of our research is to study the effects of *A. thaliana* phenotype and genotype on *P. xylostella*. The first objective was to assess the suitability of *A. thaliana* as a host plant for studying Brassicaceae-pest interactions, of which there has been some question, due to its comparatively small size and ephemeral nature. Therefore, initial

investigations compared *P. xylostella* growth and development on *A. thaliana* with *Brassica rapa* var. *pekinensis,* a typical host plant species which is also commonly used as a laboratory host for *P. xylostella* rearing.

Our second objective was to examine the effect of host plant trichomes, or hairs, on the development of *P. xylostella*, using *A. thaliana.* A presence or abundance of trichomes has often been linked to increased resistance to phytophagous insects. Non-glandular trichomes, such as those found in many members of the Brassicaceae (Metcalfe & Chalk 1950), can affect pest activity in a variety of ways; from restriction of movement and prevention of settling for feeding (Palaniswamy & Bodnaryk 1994) to provision of support during oviposition (AVRDC 1987).

Morphology of trichomes varies considerably (Metcalfe & Chalk 1950). On *A. thaliana* leaves, trichomes usually consist of three branches radiating from a stem, however, on stems and sepals, unbranched spikes are more often found. Amongst brassicas, when present, the trichomes also usually have a relatively simple form (Gomez-Campo 1980).

Many different genes are known to affect trichome growth and development in *A. thaliana* (Marks 1997); one of the most significant of these is *GLABROUS1 (GL1).* This gene encodes a myb transcription factor whose function is believed to be restricted to controlling trichome development (Koornneef *et al.* 1982, Oppenheimer *et al.* 1991). The gl1–1 mutant allele contains a deletion that removes the entire coding region of *GL1* and flanking promoter elements (Oppenheimer *et al.* 1991)*;* phenotypically this results in lack of trichomes in areas other than the leaf margins.

Our studies to investigate the effect of trichomes on *P. xylostella* development have used the *A. thaliana* lines Col-0 and Col-5. These lines are genetically very similar except that Col-5 is homozygous for the gl1–1 mutant allele and therefore lacks trichomes that are present in Col-0 plants.

Materials and methods

Plutella xylostella were kept as a continuous culture on *Brassica rapa* var. *pekinensis* at approximately 25°C, 16:8 light:dark cycle. The culture has been the University of Reading since 1994*,* when insects were provided from a twenty-six year old culture at IACR-Rothamsted. The first instar larvae used experimentally were from eggs oviposited on the inside of plastic containers.

Brassica rapa var. *pekinensis* were grown in glasshouse facilities, using John Innes No. 2 compost, at 22 ±5 °C with 16:8h light:dark cycle. Plants used experimentally were approximately 4 weeks old and at the 4–6 true leaf stage. *Arabidopsis thaliana* plants were grown at 19°C*,* 16:8 h light:dark cycle in controlled environment conditions using a 6 parts John Innes No.2 compost: 6 parts John Innes Multipurpose: 1 part Perlite soil mix. Col-0 and Col-5 seed material was provided by Dr E. Holub at HRI-Wellesbourne, UK. Plants used experimentally had a rosette diameter of 5-8 cm and were 6-8 weeks old.

Plutella xylostella larvae were reared individually, from first instar, on whole plants (type varies according to treatment) these were arranged randomly and kept at 23-25°C 16:8 h light:dark cycle. The apparatus consisted of individual plants in 9 cm pots, enclosed on the top with inverted plastic tubs (approx. 9.5 cm diameter x 4.6 cm tall) from which the central bases had been removed and replaced with fine netting.

Observations made at 12 h intervals recorded length of larval and pupal period. Approximately 36 h after the onset of pupation, when larval segmentation was no longer visible, pupal weight was also measured. Following emergence, adults were sexed (Kwapong 1997). Males and females from the same treatment, which had emerged within 12 h of each other, were paired in clear plastic containers and the number of eggs laid on the sides counted after 48 h.

Following preliminary investigations to assess the normality of the data and therefore the assumptions held, a two-way analysis of variance procedure (Genstat 5 1998) was used to compare pupal weights statistically. Larval duration from the start of the experiment and pupal duration from time point first observed as a pupa, were analysed using a Mann-Whitney U test (Genstat 5 1998). For these data, which were measured at 12 h intervals, midpoint values of these categories are recorded, e.g. 6.75 represents 6.5–7. Data concerning male and female insects were analysed separately. Data from individuals that did not reach adulthood were not

included in the analyses. Analysis of eggs laid by insect pairs was executed by two-sample unpaired t-tests, including assessment of variance between data sets (Genstat 5 1998).

Development of *P. xylostella* on *Brassica rapa* var. *pekinensis* was compared with that on *A. thaliana* line Col-0. Forty of each type of plant were used and, using the procedure outlined above, larval period, pupal period, pupal weight and number of eggs laid were recorded. The development of *P. xylostella on* Col-0 and Col-5 *A. thaliana* was compared using the procedure outlined above. Twenty-one plants of each treatment were used and larval period, pupal period, pupal weight and number of eggs laid recorded.

Results

Of the forty larvae set up on either *B. rapa* or *A. thaliana* plants, thirty-seven and thirty-four, respectively, survived to adulthood.

Pupal weight and larval and pupal periods were not significantly different between treatments ($P>0.05$) for either male or female insects (Table 1). There was no significant interaction between insect sex and treatment for pupal weight.

Treatment had no significant ($P>0.05$) effect on the number of eggs laid by insect pairs over the 48 h period (variance between the populations was not significantly unequal, $P>0.05$). Insect pairs which had developed on *B. rapa* and *A. thaliana* laid on average 87.7 ±8.1 standard error (SE) and 84.5 ±5.3 SE eggs respectively.

Of the twenty-one larvae set up on either Col-0 or Col-5 plants, seventeen and eighteen, respectively, survived to adulthood. When the duration of the larval period for individual sexes was compared there was a significant treatment effect ($P<0.01$) on females, but not males (Table 2). However, the duration of the pupal period did not differ significantly, in either male or female insects, between the treatments (Table 2).

Table 1. Summary statistics for development of *Plutella xylostella* reared on *Arabidopsis thaliana* versus *Brassica rapa* var. *pekinensis*

	Number of replicates	Larval period (days)[a]	Pupal period (days)[a]	Pupal weight (mg)[b]
Males				
B. rapa	20	6.75 (6.25, 7.25)	4.75 (4.75, 5.25)	4.709 ± 0.092
A. thaliana	14	6.75 (6.75, 7.25)	4.75 (4.75, 4.75)	4.871 ± 0.102
Females				
A. thaliana	20	7.50 (6.75, 7.75)	4.25 (4.25, 4.75)	5.626 ± 0.151
B. rapa	17	7.25 (6.75, 7.75)	4.25 (4.25, 4.75)	5.861 ± 0.186

*For insects of the same sex, significant difference in values between treatments at $P= 0.05$ level
**For insects of the same sex, significant difference in values between treatments at $P= 0.01$ level
[a] Median (lower quartile, upper quartile)
[b] Mean ± standard error

Pupae of females which had developed on Col-0 were significantly lighter ($P<0.05$), on average by 0.599 mg (0.298, 0.900 ± 2 standard error of difference (s.e.d.)), than those on Col-5. However, no significant difference was found between the two groups in males (Table 2) and no significant interaction was observed between insect sex and treatment.

The treatment was found to have a highly significant ($P<0.01$) effect on the number of eggs laid by insect pairs over the 48 h period (variance between the populations was not significantly unequal, $P>0.05$). Pairs, which had developed on Col-0, produced on average 23.8 eggs less (10.11, 37.56 ± 2 s.e.d.) during the period than those that had developed on Col-5. The insect pairs developing on Col-0 and Col-5 laying 112.5 ±4.4 SE and 136.3 ±4.4 SE eggs, respectively.

Table 2. Summary statistics for development of *Plutella xylostella* reared on *Arabidopsis thaliana: Col-0 vs. Col-5*

	Number of replicates	Larval period (days)[a]	Pupal period (days)[a]	Pupal weight (mg)[b]
Males				
Col-0 (+trichomes)	8	7.50 (7.0, 7.75)	5.25 (5.25, 5.25)	5.229 ± 0.089
Col-5 (-trichomes)	10	7.25 (6.75, 7.25)	5.25 (5.25, 5.75)	5.424 ± 0.104
Females				
Col-0 (+trichomes)	9	7.75 (7.75, 7.75)**	4.75 (4.25, 4.75)	6.687 ± 0.113**
Col-5 (-trichomes)	8	7.25 (7.25, 7.5)	4.75 (4.5, 4.75)	7.286 ± 0.097

*For insects of the same sex, significant difference in values between treatments at P=0.05 level
**For insects of the same sex, significant difference in values between treatments at P=0.01 level
[a] Median (lower quartile, upper quartile)
[b] Mean ± standard error

Discussion

These studies have demonstrated that several key developmental measures of *P. xylostella* raised on *A. thaliana* are comparable to those on *B. rapa,* a common host crop. This confirms a certain degree of suitability of *A. thaliana* as a model host plant for *P. xylostella*, in our future research. Under these conditions, larval duration is less than ten days, a time period where there is minimal physiological change in the plant. In different conditions, or should an alternate pest species be used however, the ephemeral nature of *A. thaliana* may become a significant issue.

The preliminary results to compare *P. xylostella* development on Col-0 and Col-5 *A. thaliana* suggest that the presence of trichomes has a negative effect on the development of the female larvae. If this is the case, the trichomes may be acting as a physical barrier decreasing the quantity of food that can be consumed in a given time period. This would result in an increase in the time needed to reach adequate nutrition for pupation and may lead to achievement of a lower overall nutritional status, reflected in the lower pupal weight and longer larval period observed.

Although studies have indicated no phenotype other than loss of trichomes associated with the gl1–1 mutant allele (Oppenheimer *et al*. 1991), it is possible that the results are actually due to a pleiotropic effect of the GL1 gene, which is affected by the gl1–1 mutant allele. A difference in the genetic background of Col-0 and Col-5 could also have had a significant effect. Current research is therefore using previously unavailable lines that do not differ genetically apart from the gl1–1 mutant allele, and hopes to address these queries.

These initial results do not indicate that either the gl1–1 mutant allele or the presence of trichomes affect the development time of male insects. However, further studies in both male and female insects will be necessary to confirm the results obtained.

The results show that the *A. thaliana* lines used affect the egg laying capacity of the insects within the 48 h period subsequent to pairing. Although oviposition can vary over time between treatments (Hillyer & Thorsteinson 1971), our studies (unpublished) have shown that, under these conditions, *P. xylostella* oviposit more than 99.99% of eggs in this period. These results may therefore reflect an overall difference in reproductive fitness between the test groups, but further investigation to compare egg viability and an increased sample size would be needed to validate this.

Differences in food quality and availability can result in a decrease of fecundity. Therefore, a lower fecundity in insects reared on Col-0 suggests that these plants are either of lower nutritional value than Col-5, possibly as a result of an undefined genetic difference between the two, or that the availability of the Col-0 as a food source was less than Col-5. Although in both treatments plant material was provided in excess of larval needs, if the trichomes on Col-0 acted as a physical barrier to food acquisition by the larvae, the actual amount of food available per unit time would be decreased. An interesting follow-up study might therefore investigate the quantity of leaf consumed and assimilated over time per treatment.

When experimental measures of *P. xylostella* raised on Col-0 in the two studies are compared, it is apparent that the results differ substantially. The cause is likely-to be the high sensitivity of *P. xylostella* development

to temperature fluctuations. However, this emphasises the importance of randomization of treatments and standardisation if comparisons are to be made between experiments.

In summary, our investigations have shown a degree of suitability of *A. thaliana* as a model host plant for *P. xylostella* and preliminary investigations suggest that the presence of trichomes could have a significant negative effect on their development. However, further investigations are necessary to elucidate and confirm the exact targets of this treatment effect and link these indisputably with the gl1–1 mutant allele.

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Resistance mechanisms of cabbage cultivar "Shinsei" against infestation of the diamondback moth - effect of leaf angle and hardness of outer leaf

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Abstract

Various studies have been conducted on the mechanisms of host plant resistance (HPR) of cabbage against the diamondback moth (DBM). Understanding the role of wax-bloom has been a major focus of these studies. Our study distinguishes itself from previous research by focusing specifically on outer leaf angle and leaf hardness. The resistance mechanism of cabbage cultivar "Shinsei" against DBM was examined. Generally outer leaves of "Shinsei" cabbage have an erect position when compared with a common cultivar, "Kinkei 201." Lethal effects of rainfall on II instar larvae on the outer leaves of "Shinsei" and "Kinkei 201" were investigated with an artificial rainfall system. The results showed that mortality of II instar larvae on the outer leaves of "Shinsei" was higher than that on outer leaves of "Kinkei 201." Compared with II instar larvae on prone outer leaves, II instar larvae on erect outer leaves were killed more frequently by artificial rainfall. Tests showed that experimental results between erect and prone leaves were similar in both cultivars. First instar larvae took longer to mine into the outer leaves of "Shinsei" than into those of "Kinkei 201" and the probability of success in mining into the leaf was lower on "Shinsei" than on "Kinkei 201." Artificial diets mixed with methanol extracts from outer leaves of each cultivar were fed to I instar larvae. However, there were no significant differences between mining rates on both artificial diets. On the other hand, outer leaves of "Shinsei" were harder than outer leaves of "Kinkei 201." These results showed that the difference of leaf hardness was the cause of the difference in mining time and success rate. We concluded that the major HPR mechanisms of "Shinsei" were outer leaf angle and hardness.

Keywords

host plant resistance, HPR, leaf hardness, mining rate, rainfall

Introduction

Nemoto *et al.* (unpublished) have researched DBM densities in two common cultivars and four potentially resistant cultivars of cabbage within Saitama prefecture. When compared with the two common cultivars, "Shinsei," a resistant cultivar, proved to have significantly lower densities of DBM. The current research has indicated the potential use of DBM resistant cultivars for control of DBM density.

Various studies have been conducted on the mechanisms of host plant resistance (HPR) of cabbage against the diamondback moth (DBM) and understanding the role of wax-bloom has been a major focus (e.g. Dickson & Eckenrode 1980, Lin *et al.* 1983, Eckenrode *et al.* 1986, Shelton *et al.* 1988, Dickson *et al.* 1990, Eigenbrode *et al.* 1990, Stoner 1990, Eigenbrode & Shelton 1990, Dickson *et al.* 1992, Verkerk & Wright 1994). The wax-bloom present on the cabbage leaves contains material which apparently repels DBM larvae from feeding. The wax-bloom has also been found to inhibit successful oviposition by DBM (Eigenbrode & Shelton 1992, Uematsu & Sakanoshita 1989).

Our study distinguishes itself from the previous research by focusing specifically on outer leaf angle and leaf hardness.

Materials and methods

The DBM used in the experiment were brought in from the Saitama Agriculture and Forestry Research Center in 1997 and reared in 16L:8D incubator conditions at 28ºC. The rearing methods used followed the protocol established by Nemoto (1991). The cabbage was cultivated by common methods and every 2–3 days the pests were removed manually by brush. No chemical pesticides were used.

Distribution (%) of eggs on different parts of cabbage plant

At the heading stage, 30 plants from each cultivar, "Shinsei" and "Kinkei 201," were randomly selected and an egg count was done for each. Egg counts for the head and the whorl of each plant were kept separate. The different whorl structures were also used as a base for comparison.

Number of DBM II larvae dropped after the artificial rain treatment from leaves with different angles

The leaves for "Shinsei" and "Kinkei 201" were artificially set at 10° and 70° angles and had ten larvae placed on them before the artificial rain treatment. The experiments were repeated ten times for each cultivar and angle. A waiting period of one hour was used between inoculation and treatment. The artificial rainfall machine was set for 17.3 mm/h at a droplet size of 2.5 mm. After treatments, the remaining larvae were observed and counted.

Age-specific survival curves of DBM immatures (egg, larva, pupa) on heads of two cultivars

Ten eggs were placed on each of ten randomly selected leaves of both "Shinsei" and "Kinkei 201" cultivars at the heading stage. Survival rates were observed at 24 h intervals until pupation of the larvae. There were occasionally eggs that did not hatch and one week after egg placement, these eggs were removed and survival rates were then calculated based on the remaining number of eggs.

Mining rate of I instar larvae of DBM on whorl leaves of "Shinsei" and "Kinkei 201"

Ten whorl leaves from both "Shinsei" and "Kinkei 201" were selected and used to raise 30 I instar larvae per leaf. From the time the larvae were released on the leaves, mining rate observations were taken at 0.5, 1, 2, 4, 8, 12 and 24 h intervals.

Percentage of larval mining with artificial diet containing "Shinsei" and "Kinkei 201" MtOH extract

Methanol extracts from "Shinsei" and "Kinkei 201" whorl leaves were added to an artificial diet created by Miyasono *et al.* (1992). 30 larvae were released on each diet and after 24 hours' mining, rates for the "Shinsei" and "Kinkei 201" extract diets were calculated.

Leaf hardness for the "Shinsei" and "Kinkei 201" whorl leaves was calculated with a Williams (1954) type penetrometer (Figure 1). Various parts of the "Shinsei" whorl leaf were tested for hardness in the penetrometer. Different leaf sections were cut from the "Shinsei" whorl according to their different hardness. These sections were then fed to I instar larvae 30 minutes after hatching. Ten larvae were added to each 2 cm × 2 cm sample. Mining rates were observed after 3 hours.

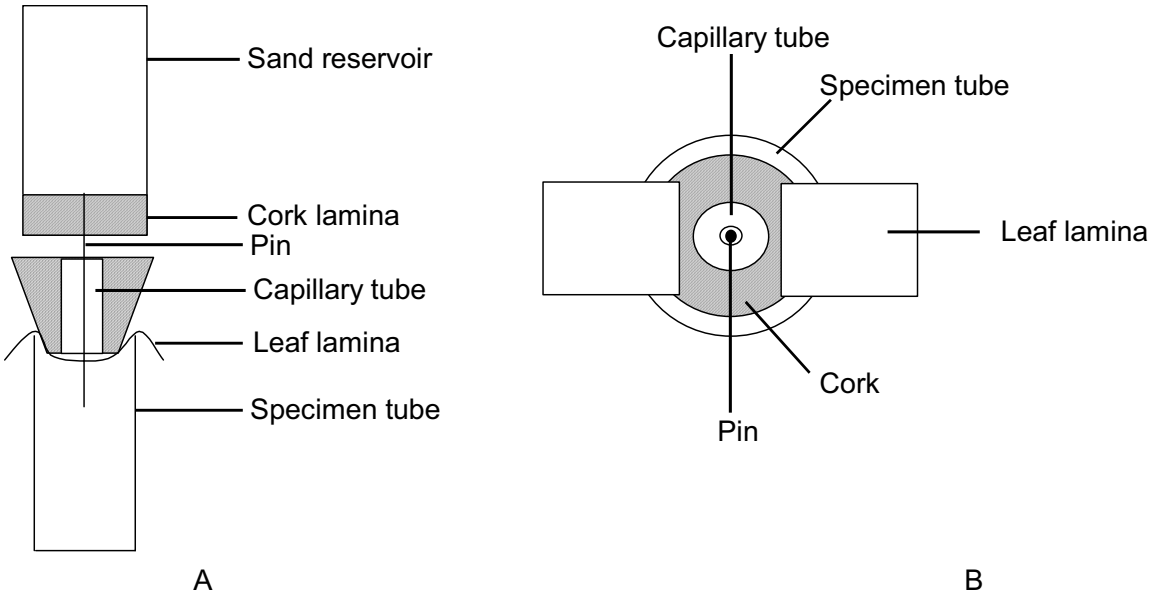

Figure 1. Williams (1954) type penetrometer. A: Lateral view, B. Cross section

Results and discussion

Distribution (%) of eggs on different parts of cabbage plant

For the cultivar "Kinkei 201," DBM egg distribution in the whorl was found to be 76.3%, while egg distribution in the whorl for "Shinsei" was determined at 91.0% (Table 1). There was a clear bias for egg distribution in the whorl of "Shinsei." The whorl leaves of "Shinsei" are significantly more erect than those of "Kinkei 201." The primary reason for whorl bias in larvae distribution was the degree of erectness of the whorl leaves.

Table 1. Distribution (%) of DBM eggs on different parts of heading stage cabbage plant

	Part of cabbage plant		
Cultivar	**Stem**	**Head**	**Whorl**
Shinsei	0	9.0	91.0
Kinkei 201	0	23.7	76.3

Number of DBM II larvae dropped after the artificial rain treatment from leaves with different angles

The rate of fallen larvae in the erect (70°) whorl leaves was significantly higher than in the more prostrate (10°) leaves, however, the difference in rate of larvae fallen between the different cultivars was not found to be significant (Table 2). Whorl erectness decreased the ability of the larvae to hold on to the leaf surface.

Table 2. Number of DBM II instar larvae dropped after artificial rain treatment from leaves with different angles

		Whorl angle	
Cultivar	**n**	**70°**	**10°**
Shinsei	10	8.8 ± 0.33a	5.7 ± 0.51b
Kinkei 201	10	8.8 ± 0.29a	6.2 ± 0.65b

Mean ± SE, Means followed by the same letter in a column are not significantly different (P<0.01, two-way ANOVA using √transformation value).

Age-specific survival curves of DBM immatures (egg, larva, pupa) on heads of two cultivarsWhen the whorl leaves of "Shinsei" were used as feeding material for larvae, there was a sharp drop in survival rate (Figure 2). This dramatic change in larval survival occurred between 3–5 days after egg placement at the I instar stage. Because of this result, it was realised there were characteristics of the "Shinsei" whorl, when used as feeding material for larvae, that were unfavourable to their survival and consequently the following research was focused on determining the reasons for this.

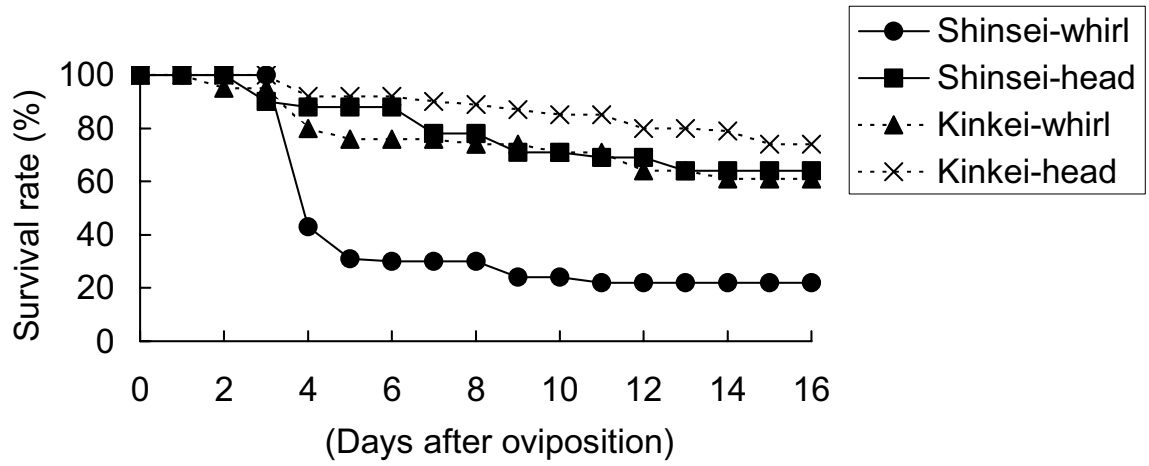

Figure 2. Age-specific survival curves of DBM immatures (egg, larva, pupa) on heading stage of two cabbage cultivars.

Mining rate of I instar larvae of DBM on whorl leaves of "Shinsei" and "Kinkei 201"

At the end of 24 h, the mining rate observed on the "Shinsei" whorl leaves was 43.6% and 60.3% for the "Kinkei 201" whorl leaves (Figure 3). It was also evident that the time elapsed before mining in the "Shinsei" whorl leaves took place was longer than for "Kinkei 201." Evidently some component of the "Shinsei" whorl leaves caused a large number of I instar larvae to be unsuccessful in their mining attempts and thus produced a high mortality rate for larvae placed on the "Shinsei" whorl leaves. Two possible reasons for unsuccessful mining on "Shinsei" whorl leaves were suggested and experiments were conducted to confirm their validity.

1. Chemical components of the "Shinsei" leaf material
2. Physical aspects of the "Shinsei" leaf material

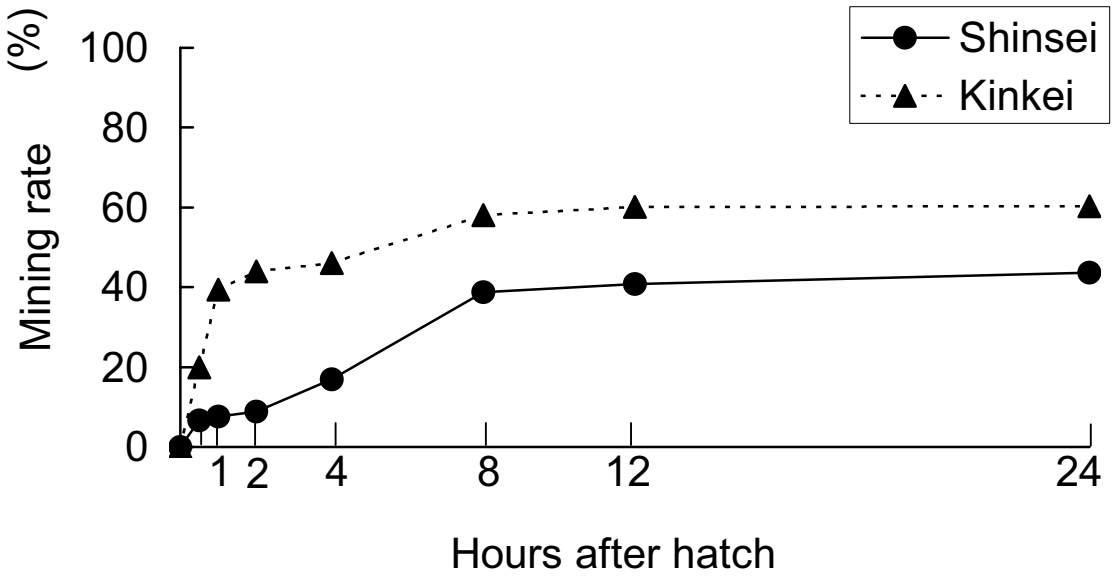

Figure 3. Mining rate of I instar larvae of DBM on whorl leaves of "Shinsei" and "Kinkei 201".

Percentage of mining larval with artificial diet containing "Shinsei" and "Kinkei 201" MtOH extract

The mining rate for the "Shinsei" artificial diet was determined to be 52.0% and for the "Kinkei 201" artificial diet, 50.3% (Table 3). These differences were insignificant. In this experiment we were unable to find a chemical reason for decreased mining in the "Shinsei" whorl leaves. The used of MtOH to create the "Shinsei" and "Kinkei 201" diet extracts may have destroyed certain enzymatic components of the leaf material. Also, other chemical components may have evaporated with the MtOH, so chemical deterrence to larval mining can in no way yet be discounted.

Table 3. Percentage of mining DBM larvae with artificial diet containing "Shinsei" and "Kinkei 201" MtOH extract

Cultivar	Mining larvae (%)
Shinsei	52.0 ± 1.9 a
Kinkei 201	50.3 ± 2.0 a

Mean ± SE, Means followed by the same letter in a column are not significantly different (P>0.05, t-test)

Whorl leaf hardness of "Shinsei" and "Kinkei 201" measured by Williams type penetrometer

The average hardness of the "Shinsei" whorl leaves was 18.33 g and that of the "Kinkei 201" leaves was 14.38 g (Table 4). There was a distinctly higher degree of hardness in the "Shinsei" leaves. In the "Shinsei" whorl, as the leaf sections increased in hardness, the number of non-mining larvae increased proportionally (Figure 4). From these experiments we were able to conclude that leaf hardness plays a major role in larval leaf mining activity.

Table 4. Whorl leaf hardness of "Shinsei" and "Kinkei 201" measured by Williams type penetrometer

Cultivar	Threshold weight for piercing (g)
Shinsei	18.33 ± 0.70 a
Kinkei 201	14.38 ± 0.42 b

Mean ± SE, Means followed by the same letter in a column are not significantly different (P>0.05, t-test).

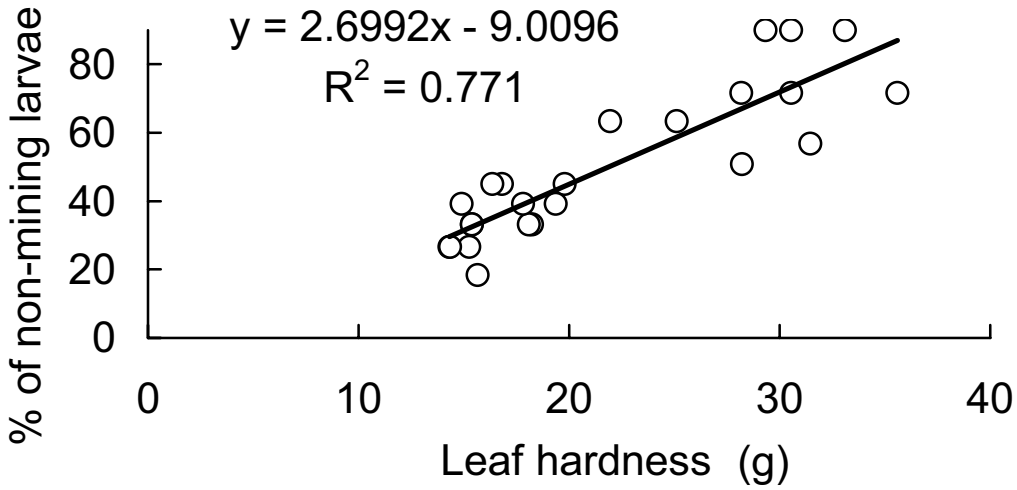

Figure 4. Regression of leaf hardness to mining rate of DBM. For regression analysis, data were transformed into angular values before calculation.

Conclusion

Due to the erect nature of the "Shinsei" whorl, the cabbage head is less exposed to DBM infestation. Because of this, a large proportion of DBM eggs is laid on the whorl of "Shinsei," rather than the head. Comparing "Shinsei" whorl leaves to "Kinkei 201" whorl leaves, the erectness of the "Shinsei" whorl causes the larvae to be more susceptible to being physically removed from the leaf surface by rain. Also, due to the hardness of the "Shinsei" whorl leaves, the overall result is a higher mortality rate in "Shinsei" than in "Kinkei 201" cabbage. From the experimental evidence, the probability of a number of physical factors contributing to DBM resistance becomes high. While considering the influence of various physical factors such as leaf hardness and erectness, we would like to further ideas on how they might be used in pest management.

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Coping with glucosinolates: disarming the mustard oil bomb*

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Abstract

Glucosinolates are plant secondary metabolites found in crucifers. Upon tissue rupture, e.g. caused by a chewing insect, glucosinolates are hydrolysed by an endogenous plant enzyme, myrosinase. This leads to the formation of a variety of breakdown products (isothiocyanates, nitrile and others) that are toxic to many insect species, especially generalist herbivores. However, insects specialized on crucifers survive despite this glucosinolate-myrosinase defence system. In many cases, glucosinolates and their breakdown products are even utilized to locate suitable host plants. Little is known about the metabolic processes that allow specialist and some generalist insects to survive on glucosinolate containing plants. To elucidate which properties enable herbivorous insects (especially diamondback moth) to overcome their hosts' defences, we use a variety of molecular biological and genetic approaches; e.g. EST sequencing and transcript profiling. These allowed the identification of a so-called “glucosinolate sulfatase” gene, whose gene product prevents the formation of toxic glucosinolate breakdown products.

*More detailed information may be found in:
Ratzka A, Vogel H, Kliebenstein DJ, Mitchell-Olds T & Kroymann J. 2002. Disarming the mustard oil bomb. *Proceedings of the National Academy of Sciences of the USA* 99: 11223–11228.

Forecasting attacks by pest insects of cruciferous crops

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Abstract

The timing of pest insect attacks can vary greatly from region to region and from year to year. A simulation method, based on rates of insect development, has been developed for forecasting the timing of insect attacks on cruciferous crops. The method is based on using a fixed number of individuals from one generation to the next and simulates the timing of events in the life cycle of the pests rather than their population dynamics. Forecasts produced for the cabbage root fly, the bronzed-blossom beetle and various species of Lepidoptera have been validated using pest monitoring data. Forecasts can be generated on either a regional basis from standard meteorological data, or on a local basis from air and soil temperatures collected by participating growers.

Introduction

Pest insects of horticultural crops are often controlled by spraying insecticide onto established crops. Such sprays are used against root-feeding insects because the insecticides applied at drilling or planting have usually degraded by the time the later generations of the pest become active. Sprays are also the simplest way of controlling foliar pests such as aphids and caterpillars. As the majority of insecticides recommended currently are of relatively short persistence, treatments are most effective if they are targeted to coincide with periods of peak pest activity. Unfortunately, the timing of such peaks can vary considerably from region to region and from year to year. Although it is possible to monitor the activity of many pest species using insect traps, routine monitoring is laborious and often requires specialist knowledge.

An alternative is to use weather data to forecast the timing of pest attacks. Forecasting systems have been developed for many insects. Many of these forecasts have been based on accumulated day-degrees (e.g. Eckenrode & Chapman 1972, Butts & McEwen 1981). However, day-degree forecasts have severe limitations, as their accuracy is based on the assumption that the relationship between the rate of insect development and temperature is strictly linear (Baker 1980). In addition, day-degree forecasts can be used only to predict the start and/or the peak of activity of the population. They cannot readily predict the spread of activity nor can they cope easily with insect populations that have polymodal patterns of activity. For example, the cabbage root fly (*Delia radicum*) can occur as one of two developmental biotypes, that emerge either `early' (April-May) or `late' (June-July) in the season (Finch & Collier 1983; Finch *et al.* 1988). As a result, the population of cabbage root flies within a particular locality, may consist primarily of one biotype or be a mixture of the two. Further problems in using day-degree models occur when attempts are made to include periods of diapause or aestivation, the major phases of insect dormancy that are induced by changes in temperature or photoperiod. As it is usual for only a proportion of the insect population to respond at any one time to a particular environmental cue, there is always variation between individuals in their rates of development. This is true of cabbage root fly populations during both aestivation and diapause (Collier & Finch 1983, Finch & Collier 1985).

At Horticulture Research International, a simulation method, based on rates of insect development and which allows for variation within the individuals in the insect population, has been produced for forecasting the timing of attack by a number of pest insects (Phelps *et al.* 1993). The simulation method has been used to develop forecasts for the cabbage root fly, bronzed-blossom beetles (*Meligethes* spp.) and more recently for some of the pest Lepidoptera of *Brassica* crops. The cabbage root fly and bronzed-blossom beetle forecasts are now used by growers. Similar forecasts for caterpillars are being validated currently. The biological basis, validation and practical uses of such forecasts are discussed in this paper.

The model

The forecasts were developed using a Monte Carlo simulation method (Phelps *et al.* 1993). The method uses a fixed number of individuals (usually 500, to obtain repeatable simulations) from one generation to the next and simulates the timing of events rather than the population dynamics of the insects. To develop each model, individuals at each stage of development (egg, larva, pupa, adult) were reared in cooling incubators

at a range of constant temperatures between 6 and 30°C. The data recorded were used to determine the relationship between the rate of insect development and temperature. Linear or non-linear (Gompertz) curves were fitted to these data to provide equations, which could be incorporated into the model. In addition, variability was incorporated using the 'same-shape property' (Sharpe *et al.* 1977, Shaffer 1983). This implies that the coefficient of variation of the rate of insect development is constant at all temperatures. Account was taken also of periods of dormancy (aestivation and diapause) and of activity thresholds which might affect the outcome of the forecasts.

Ideally, the forecasts should be run using daily maximum and minimum air temperatures and maximum and minimum soil temperatures at a depth of 6-10 cm. However, maximum and minimum soil temperatures are not available from standard agro-meteorological stations in the UK. Therefore, the program uses several equations to estimate soil maximum and minimum temperatures from the air maximum, air minimum and 10 cm soil temperatures recorded daily at 09.00 h GMT (Phelps *et al.* 1993). If data for soil maximum and minimum temperatures are available, then the forecasts could be run equally well using these.

In general, the forecasts are run using accumulated temperatures for which the accumulation is started usually on 1 February each year. This is because late January is the natural break in insect development as it is usually the period of the year when temperatures are at their lowest. When temperatures start to rise again in the spring most of the species that overwinter in diapause have completed their diapause development and are ready to start post-diapause development. The diamondback moth (*Plutella xylostella*) is an exception. This species does not appear to overwinter successfully in large numbers in the UK (R. Collier, unpublished data) and the majority of insects are migrants from Europe and North Africa. Thus the forecast model has to be triggered by the arrival of migrant moths.

Forecast validation

The forecasts are validated using insect monitoring data. Cabbage root flies are monitored using either yellow water traps (Finch & Skinner 1974) or by sampling for fly eggs (Finch *et al.* 1975); bronzed-blossom beetles by using yellow sticky traps (Finch *et al.* 1990) and pest Lepidoptera by using yellow water traps, pheromone traps (for moth species) and by sampling plants for eggs and larvae.

Cabbage root fly

The cabbage root fly forecast (Collier *et al*. 1991) was developed originally for timing the application of mid-season insecticide treatments to control the fly on long-season *Brassica* crops such as swedes. Other uses of the forecast include warnings of the likely onset of third generation attack to Brussels sprout buttons and to autumn-sown crops of oilseed rape. At present, most insecticide treatments to leafy brassicas are applied prophylactically, before or soon after transplanting, and treatment against subsequent generations of this fly is usually unnecessary. However, the forecast could be used to indicate 'windows' where treatments would not be required. Figure 1 shows a comparison of the observed and forecast cabbage root fly activity at Kirton, Lincolnshire in 1999.

Local variations in cabbage root fly activity include the co-existence in certain regions of the two developmental biotypes, with diapause of different durations (Finch & Collier 1983; Collier *et al.* 1989). Late-emerging flies emerge several weeks later than early-emerging flies so that, in effect, the generations of the two biotypes alternate. Similar damage to brassicas is caused by the closely-related turnip fly (*Delia floralis*) in Scotland and in some areas of south-west Lancashire (Finch *et al.* 1986). The presence of the two cabbage root fly biotypes and turnip fly in areas of south-west Lancashire means that in that specific locality there is continuous root fly pressure to *Brassica* crops throughout the summer. The cabbage root fly model produces forecasts for populations that contain specified proportions of the two biotypes. A turnip fly forecast has not yet been developed.

The cabbage root fly model has also been used to predict the changes that might occur as a result of global warming (Collier *et al.* 1990) and to predict cabbage root fly phenology in Spain, an EU member country in which calabrese production for the UK market can be affected severely by damage done to the crop florets by fly larvae.

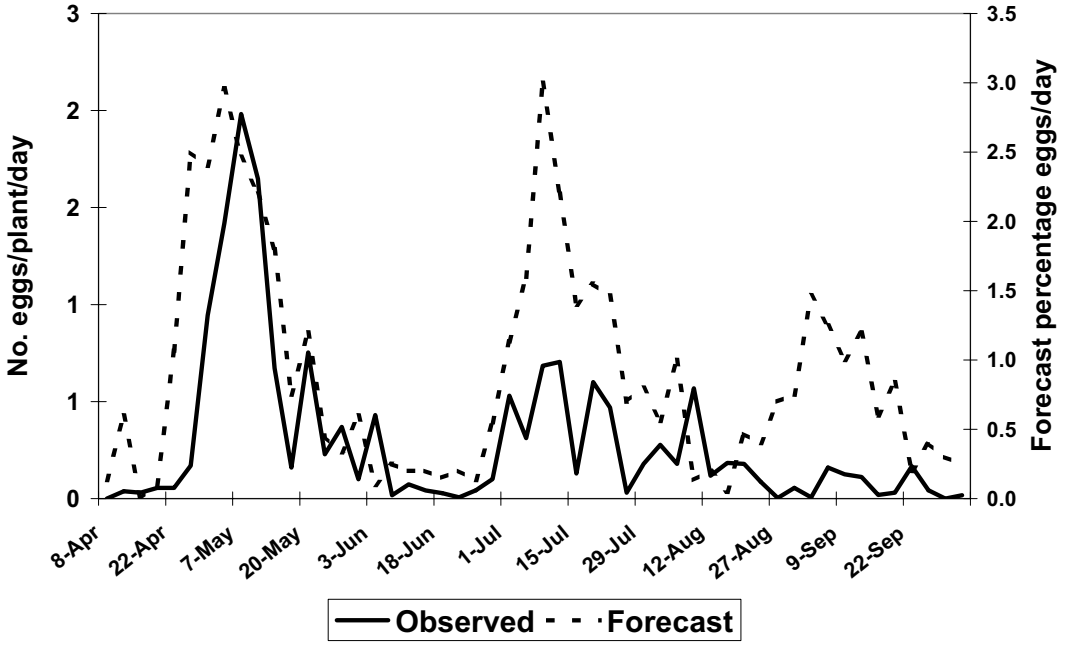

Figure 1. Comparison of the numbers of cabbage root fly eggs laid at Kirton, Lincolnshire in 1999 with a forecast of egg hatch generated using data from the nearby weather station.

Bronzed-blossom beetle

Feeding by adult bronzed-blossom beetles in mid-summer damages the curds or florets of cauliflower and calabrese (Finch *et al*. 1990) so that spray treatments are sometimes necessary. The forecast is used to predict the emergence of adult beetles from pupae within the previous host crop; usually oilseed rape. However, beetle infestations are not inevitable and seem to depend both on the proximity of oilseed rape crops and on the occurrence of warm, humid conditions during the main period of beetle migration. Figure 2 shows comparisons of observed and forecast bronzed-blossom beetle activity at Kirton in Lincolnshire in 1996.

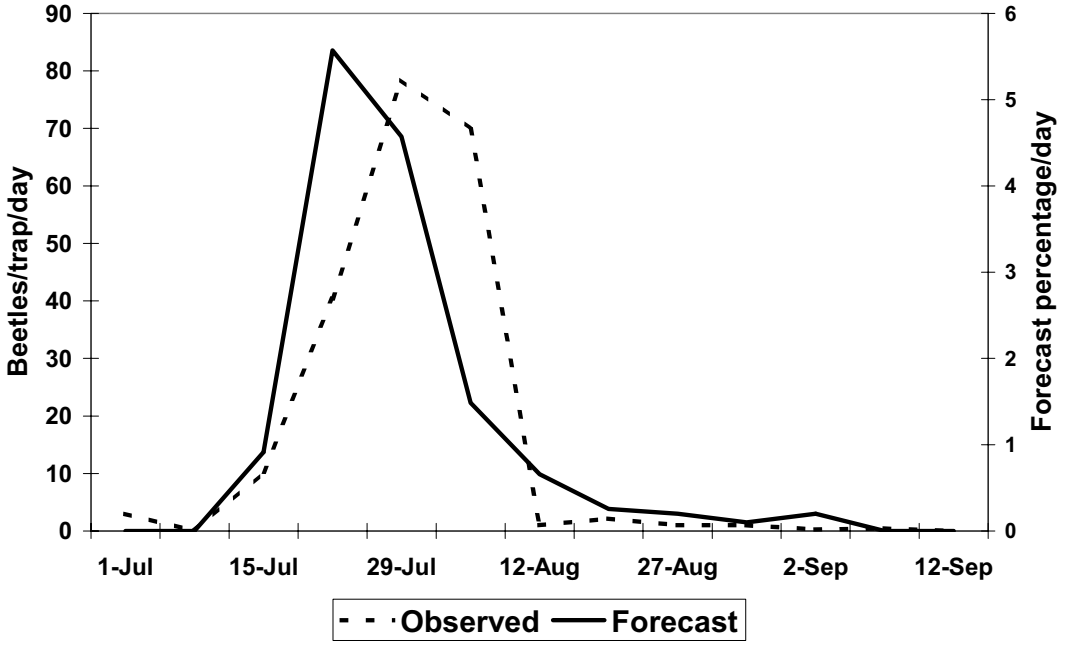

Figure 2. Comparison of the numbers of bronzed-blossom beetles (*Meligethes* spp.) captured on sticky traps at Kirton, Lincolnshire in 1996 with a forecast of beetle emergence generated using data from the nearby weather station.

Caterpillars

In the UK, the caterpillars of several species of butterfly and moth can damage cruciferous crops. However, attacks by caterpillars tend to be sporadic and so do not occur in every crop every year. Considerable savings can be made in applications of insecticides for caterpillar control by applying sprays only when there are sufficient insects in the crop to warrant treatment. Forecasts of the development of the diamondback moth, small white butterfly (*Pieris rapae* L.), cabbage moth (*Mamestra brassicae* L.) and garden pebble moth (*Evergestis forficalis* L.) have been developed. In the UK, the diamondback moth and small white butterfly are the most important and widespread pest species. Because the diamondback moth is a migrant, pheromone traps are the most effective way of determining when infestations will occur, and the current diamondback moth model uses moth counts to trigger a forecast of subsequent population development. The other species overwinter well in the UK and forecasts for the small white butterfly and garden pebble moth gave a good indication of the periods when caterpillars were likely to be found in *Brassica* crops. It was not easy to validate the cabbage moth forecast, because caterpillar numbers were so low. Figures 3-5 show comparisons of the observed and forecast activity of the diamondback moth, the small white butterfly and the garden pebble moth at sites in Lincolnshire.

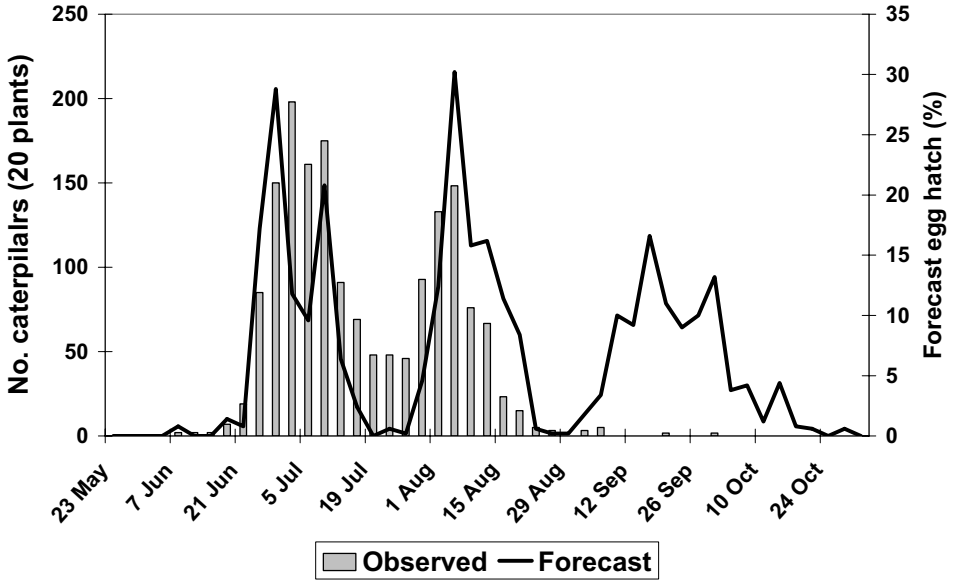

Figure 3. Comparison of the numbers of diamondback moth caterpillars found on insecticide-free Brussels sprout plants at Holbeach, Lincolnshire with forecasts of egg hatch generated using data from the nearby weather station. The forecasts were triggered using pheromone trap captures during the 'first' generation.

Forecast validation

The current forecasts have been validated against as many sets of insect monitoring data as possible. The timing of pest activity may vary by 3-5 weeks between years and the numbers of generations may also vary. For example, the cabbage root fly may have a 'partial' third generation in warm locations, whilst completing only two generations in cooler areas such as Scotland. Use of the models has indicated that monitoring data must consist of >100 insects per generation if estimates of the timing of pest attacks are to be accurate to within one week (Collier & Phelps 1994). As the forecasts provide an indication only of the timing of pest attack and not of its severity, forecast data are generally expressed as percentages. When the times to 10% and 50% activity have been predicted and compared with the monitoring data, the majority of the pest forecasts have been accurate to within one week.

To date, forecasts for commercial growers have been produced using a network of rather widely-dispersed weather stations. However, there are obviously local differences in climate and in the degree of shelter, which might affect the timing of pest activity in a particular field. With very mobile insects such as the cabbage root fly, there may be little point in recording temperatures in individual fields, as the infesting population will have experienced the climate of the previous weeks, or months, in a different, unknown location. An intensive study in the Vale of Evesham (Finch & Skinner, unpublished data) indicated that there was very little difference in the timing of cabbage root fly activity from crop to crop. Although timing of

activity may vary little within a region, intensive sampling in south-west Lancashire showed that the relative proportions of the two cabbage root fly biotypes varied considerably over relatively short distances (Finch *et al.* 1986).

At present, pest forecasts are based on Meteorological Office data collected from the network of weather stations, some of which are not particularly close to areas of commercial vegetable production. The actual forecasts are projected forwards using weather data from a previous, warm year. Since 1991, forecasts of cabbage root fly and bronzed-blossom beetle activity, based on weather data obtained from approximately 40 weather stations throughout the UK, have been made available to growers and advisers. Forecasts have been sent to growers each week for several weeks before and during the period of pest activity. The forecast models are now also available as part of a software package (MORPH), so that forecasts can be generated using growers' own weather data.

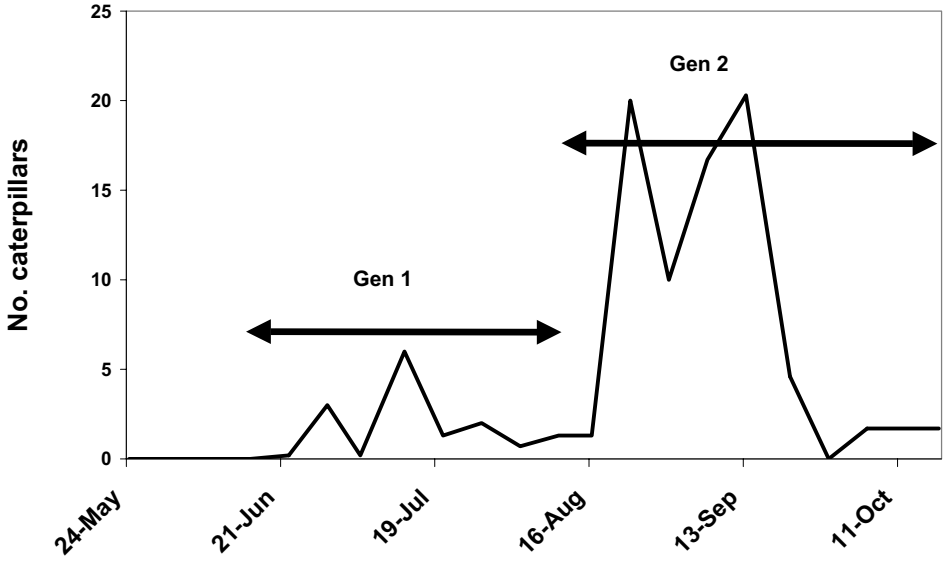

Figure 4. Comparisons between observed and forecast small white butterfly generations (total for three sites in south Lincolnshire). The forecasts were generated using data from the nearby weather station at Kirton. The horizontal lines with arrows indicate the periods when caterpillars would be expected from the forecast.

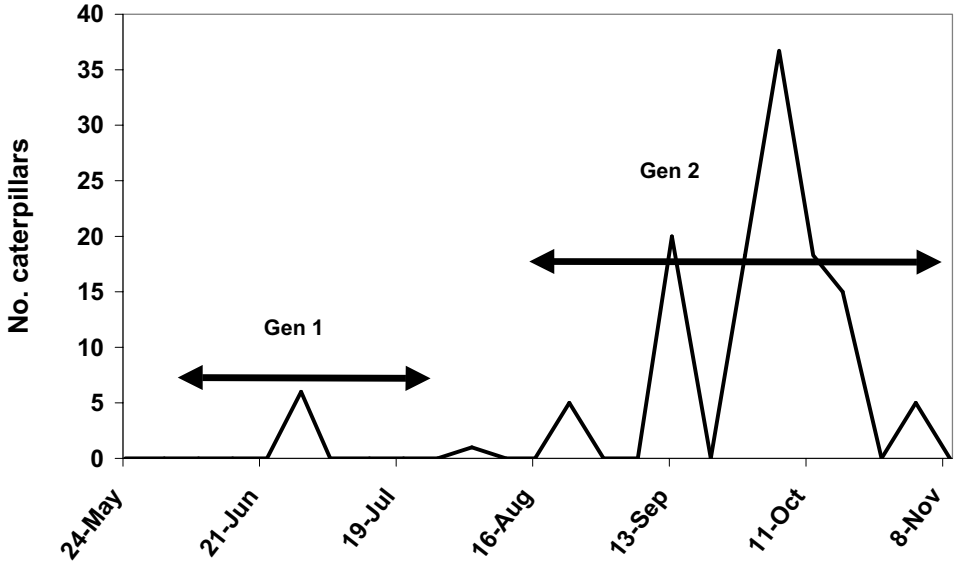

Figure 5. Comparisons between observed and forecast garden pebble moth generations at Holbeach, Lincolnshire in 2000. The forecasts were generated using data from the nearby weather station. The horizontal lines with arrows indicate the periods when caterpillars would be expected from the forecast.

Future developments

The use of less-persistent insecticides, together with pressure from consumers and retailers to reduce the number of insecticide treatments applied to crops, means that growers need to target insecticide treatments more accurately. The next logical step, after determining the timing of pest activity, is to develop treatment thresholds to determine which of the various treatments are actually necessary. However, a considerable amount of further basic research will be required if we are ever to produce robust systems that will allow final crop damage to be forecast accurately from the numbers of insects monitored during the early stages of crop infestation.

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Evaluation of presence-absence sampling plans for the diamondback moth (*Plutella xylostella*) (*Lepidoptera: Plutellidae*)

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Abstract

Two sets of sequential presence-absence sampling plans for the management of diamondback moth (DBM) were developed and evaluated, one for the classification of levels of the proportions of plants infested with larvae and the other for the classification of levels of larval density. The action thresholds investigated were 0.15, 0.25, 0.35 and 0.45 for proportion-based sampling plans and 0.2, 0.4, 0.6 and 0.8 larvae/plant for density-based sampling plans. Under the proportion-based sampling plans, the expected correct decision rates were ≥95% for 86-87% of all possible population levels and the expected average sample size was ≤50 plants for 73-87% of all possible population levels. Re-sampling analyses showed average sample sizes of <40 plants in reaching the ≥95% accuracy. For density-based sampling plans, an empirical proportion-density model was first established. The resulting model was highly significant (P<0.001) and explained 97% of the total variation in the independent variable. Satisfactory performance (≥95% accuracy at ≤50 plants sample size) of the density-based sampling plans can be expected when the true population density does not lie in the vicinity of the action threshold. In conclusion, the sequential binomial sampling plans presented here can be used effectively in the monitoring of DBM populations for decision making.

Introduction

Diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a key pest of the important vegetable group that includes cabbage, broccoli, cauliflower, collards, rapeseed, mustard and Chinese cabbage. DBM is notorious for its rapid development of resistance to insecticides. In recent years, concerns over the resistance problem, human health and environment have forced the *Brassica* vegetable industry in many countries to implement some form of integrated pest management (IPM) and insecticide resistance management (IRM) (Talekar & Shelton 1993). However, grower adoption of IPM/IRM strategies has been slow. In Australia, most growers still spray their crops largely on a calendar basis. To encourage more growers to adopt threshold-based spray programs, which is the cornerstone of IPM and IRM, easy-to-use and time-efficient sampling plans are needed, as well as extension effort to convey the benefits of IPM and IRM.

One easy-to-use sampling method is presence-absence sampling. Recording for each sampling unit only the presence/absence of the target pest, presence-absence sampling provides an attractive alternative to enumerative sampling, in which the number of pests in each sampling unit has to be recorded. The advantage is obvious for pest species forming aggregation clusters or those that are easily overlooked because of small size or cryptic behaviour of the pests and when the density of the pest population is high (Jones 1994). Presence-absence sampling is also the logical choice for IPM programs using proportion-based action thresholds, which are commonly used in horticulture crops. Sequential presence-absence sampling, or presence-absence sampling implemented under a sequential rule, provides the additional attractiveness of being potentially time-efficient as it enables decisions regarding pest population levels being made at minimal sample sizes.

Although DBM does not form clusters, its earlier instars are quite small (<5mm) and easily overlooked on their leafy host plants. The larvae also tend to feed in hidden locations and wriggle away or drop down when disturbed. As a result, accurate recording of the number of larvae on a plant can be very difficult under field conditions. This paper investigates the efficiency and reliability of presence-absence sampling in the sequential classifications of DBM population levels under Wald's (1947) sequential probability ratio test (SPRT). Sequential sampling plans for both proportion-based and density-based action thresholds were developed and evaluated. The action thresholds used were based on those practised by *Brassica* vegetable growers in Australia. Practical applications of the sampling plans are discussed.

Methods

Data description

Sampling data from four host crops, Brussels sprouts, cabbage, cauliflower and broccoli, collected in three states, South Australia (SA), Victoria (Vic) and Queensland (Qld), were used in this study. Sample sizes ranged from 35 to 300 plants. Data sets with a sample size of less than 100 plants were used for setting up the sampling plans and the rest for validating the sampling plans.

Sequential sampling plans

Under SPRT, a population is classified as below or above a prescribed action threshold (the AT) according to the positions of sample points relative to two parallel stop lines, the lower stop line and the upper stop line. The position of a sample point (n, T_n) is determined by the total number of plants sampled (n) and the number of infested plants found (T_n). The two stop lines are determined by the value of the AT and the distribution of the number of infested plants in the target population. In presence-absence sampling, the subject of interest is the proportion of infested plants in the target population. Since the real proportion of infested plants in a population at any given time is a fixed value, the number of infested plants found during a random sampling process observes binomial distribution, regardless of the patchiness of the distribution of the target organism causing the infestation. Stop lines under binomial distribution are calculated according to Fowler and Lynch (1987):

$$T_{lower} = \frac{\ln \frac{\beta}{1-\alpha}}{\ln \frac{p1(1-p0)}{p0(1-p1)}} + \frac{\ln \frac{1-p0}{1-p1}}{\ln \frac{p1(1-p0)}{p0(1-p1)}} n \qquad (1)$$

$$T_{upper} = \frac{\ln \frac{1-\beta}{\alpha}}{\ln \frac{p1(1-p0)}{p0(1-p1)}} + \frac{\ln \frac{1-p0}{1-p1}}{\ln \frac{p1(1-p0)}{p0(1-p1)}} n$$

where p0 and p1 are the nominal proportions of infested plants around the AT (p0 <the AT <p1), α the error rate for recommending control when in fact the infested proportion is below the AT, β the error rate for recommending no control when in fact the infested proportion is above the AT. If $T_n < T_{lower}$, the population level is considered below the AT. If $T_n > T_{upper,}$ the population level is considered below the AT. If $T_{lower} \leq T_n \leq T_{upper}$, the population level relative to the AT cannot be determined and more plants need to be sampled.

In this study, α was set to 0.1 and β to 0.05. The lower β value was chosen to guard against the error of recommending no-control decision when control is needed. Based on personal survey of local *Brassica* growers, four ATs each were investigated for the classification of the proportions of infested plants (0.15, 0.25, 0.35 and 0.45) and larval density (0.2, 0.4, 0.6 and 0.8 larvae/plant). For the classification of the proportions of infested plants, p0 and p1 were set to AT-0.05 and AT+0.05, respectively. For the classification of larval density, p0 and p1 were set to AT-0.1 and AT+0.1, respectively.

Conversion between proportions of infested plants and densities

Classification of the mean density with presence-absence sampling requires the conversion of density-based ATs into proportion-based ATs. This was done with the inverse of the empirical equation of Gerrard and Chiang (1970):

$$\ln(-\ln[1-p]) = \gamma + \delta \ln(m) \qquad (2)$$

where p is the proportion of plants infested with larvae, m is the larval density, γ and δ are parameters to be estimated. This equation was used because of its independence from underlying distributions.

Evaluation

Performances of the sampling plans were evaluated with the operational characteristics (OC) and the average sample number (ASN) curves. The OC curve is a plot of the probability of "no intervention" (or no

spray) versus the true population level (larval density or proportions of plants with larvae in this study). For each sampling plan, the range of population levels for which OC≤0.05 or OC≥0.95 (OC95) was determined. The OC95 ranges correspond to population levels for which the expected rate of correct classification is ≥95%. The ASN curve is a plot of the average sample size over the population level. As growers do not normally sample more than 50 plants in their monitoring of DBM populations, ranges of population levels for which ASN≤50 plants (ASN50) were determined for each sampling plan. Calculations of the OC and ASN values were done with the algorithms of Nyrop and Binns (1992).

Validation

Validation of the sampling plans was performed by simulated re-sampling of 20 independent data sets. The sample sizes of these data sets ranged from 100 plants to 600 plants. The proportion of infested plants was 0.02-0.56 and the larval density was 0.02-1.46 larvae/plant. Individual plants within a data set were randomly selected with replacement. The initial sample size was set to ten plants and the increment to one plant. Sampling was terminated when a decision could be made with regard to the population level relative to the AT. For each AT, 1000 simulations were run, at the end of which the percentages of simulation runs which correctly classified the population level relative to the AT (Correct%) and the average sample size (ASN_{sim}) were calculated.

Results

Classification of proportions of infested plants

Sampling plans for the four proportional ATs are given in Table 1. The OC curves under these sampling plans were parallel curves centred on their respective ATs (Figure 1). Depending on the action thresholds, the OC95 ranges were found at a minimum of 0.07 proportion units below the AT (proportion of plants with larvae <AT + 0.07) and a minimum of 0.05~0.06 proportion units above the AT (proportion of plants with larvae <AT + 0.05~0.06). These OC95 ranges represented 87~88% of all possible levels of the proportions of infested plants (0-1).

Table 1. Sequential stop lines at 4 proportion-based action thresholds and 4 density-based action thresholds

Action Threshold	Upper stop line (T_{upper})	Lower stop line (T_{lower})
15% plants infested (0.15)	2.7762 + 0.1452 n	-3.5643 + 0.1452 n
25% plants infested (0.25)	4.1768 + 0.2477 n	-5.3625 + 0.2477 n
35% plants infested (0.35)	5.0953 + 0.3489 n	-6.5418 + 0.3489 n
45% plants infested (0.45)	5.5524 + 0.4497 n	-7.1285 + 0.4497 n
0.2 larvae/plant	2.0580 + 0.1441 n	-2.6422 + 0.1441 n
0.4 larvae/plant	4.0978 + 0.2709 n	-5.2610 + 0.2709 n
0.6 larvae/plant	5.8037 + 0.3716 n	-7.4512 + 0.3716 n
0.8 larvae/plant	7.2786 + 0.4556 n	-9.3448 + 0.4556 n

As the AT increased from 0.15 to 0.45, peak ASN values increased from 81 plants to 160 plants (Figure 1). The ASN50 ranges were found at a minimum of 0.08~0.15 proportion units below the AT and a minimum of 0.05~0.12 proportion units above the AT (Table 2). The larger separation distances of the two ranges were associated with the higher ATs. Overall, these ASN50 ranges represented 73-87% of all possible population levels, with higher representation shown by sampling plans for the lower ATs (Table 2).

Results of re-sampling analyses of these sampling plans were shown in Table 3. For AT=0.15, the percentage of correct decisions was 100% for all data sets in which the proportions of infested plants differed from the AT by at least 0.05 proportion units. For AT=0.25, 0.35, and 0.45, the percentages of correct decisions were slightly lower (95-99%). The average numbers of plants sampled to reach these decisions were 17, 36, 30, and 35 under the sampling plans for the AT=0.15, 0.25, 0.35, and 0.45, respectively. Over 70% of the data tested within these population ranges had an average sample size of less than 50 plants, irrespective of the ATs. As expected, the rates of correct decisions were much lower and the average sample numbers were much higher for data sets in which the proportions of infested plants were very close to the ATs (| p - AT | <0.05) (Table 3).

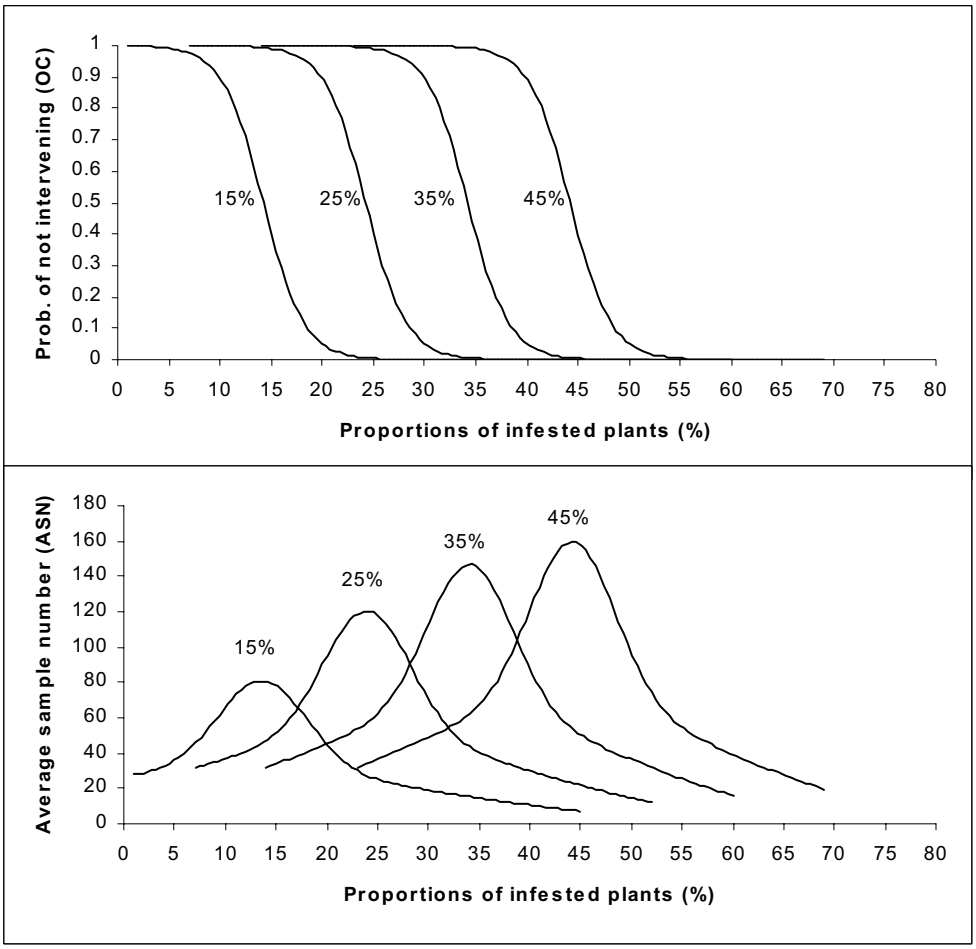

Figure 1. Operating characteristic (OC, =probability of not intervening) and average sample number (ASN) functions for the classification of proportions of plants infested with DBM larvae at 4 action thresholds (15%, 25%, 35%, and 45% infested plants) using sequential presence-absence sampling.

Table 2. Ranges of population levels (prop. of infested plants or larval density) over which the expected correct classification rate was at least 95% (OC95) (corresponding to OC≤0.95 or OC≤0.05) and those over which the expected average sample size is ≤50 plants (ASN50). The matching maximal ASN value or maximal error rate for each range was given for cross-references. Numbers in brackets are percentages of the widths of the specified ranges over the width of the entire population level range (0-1)

	Action threshold	OC95 range	Maximal ASN	ASN50 range	Maximal error rate
Proportion of	0.15	p<0.08 or p>0.20 (88%)	52	p<0.07 or p>0.20 (87%)	0.05
infested plants (p)	0.25	p<0.18 or p>0.31 (87%)	73	p<0.14 or p>0.33 (81%)	0.01
	0.35	p<0.28 or p>0.41 (87%)	88	p<0.21 or p>0.46 (75%)	0.00
	0.45	p<0.38 or p>0.51 (87%)	94	p<0.30 or p>0.57 (73%)	0.00
Number of	0.2	m<0.05 or m>0.31	28	Any m values	1
larvae/plant (m)	0.4	m<0.18 or m>0.58	50	m<0.20 or m>0.58	0.07
	0.6	m<0.31 or m>0.87	70	m<0.28 or m>1.00	0.01
	0.8	m<0.42 or m>1.16	85	m<0.33 or m>1.40	0.01

Table 3. Percentages of simulation runs that correctly classified the proportion of infested plants in each of 20 independent data sets relative to each of the four proportional action thresholds (0.15, 0.25, 0.35 and 0.45) (Correct%) and the corresponding average sample sizes (ASN_{sim}). One thousand simulation runs were performed for each data set. Within each data set, individual plants were randomly sampled with replacement. Initial sample size was set to 10 and the sample increment to 1. N=number of plants in the data set, p=proportion of plants with larvae in the data set.

			Correct%				ASN_{sim}			
Data	**N**	**p**	**0.15**	**0.25**	**0.35**	**0.45**	**0.15**	**0.25**	**0.35**	**0.45**
S-cabb	600	0.17	83.3	98.4	100.0	100.0	71	70	39	26
PCB98	420	0.55	100.0	100.0	100.0	100.0	11	15	28	58
PCB99	360	0.63	100.0	100.0	100.0	100.0	10	13	20	34
Bshrt11	100	0.73	100.0	100.0	100.0	100.0	10	11	14	21
Bshrt12	100	0.71	100.0	100.0	100.0	100.0	10	11	15	22
Bshrt29	100	0.2	100.0	100.0	100.0	100.0	29	24	20	17
CabIHD1	100	0.34	100.0	99.5	56.2	99.5	18	49	163	65
CabIHD2	100	0.63	100.0	100.0	100.0	100.0	10	12	20	33
Bshrt2-68	100	0.26	100.0	70.7	98.6	100.0	27	122	73	38
Bshrt5-32	100	0.16	73.7	99.0	100.0	100.0	82	63	35	25
Bshrt3-10	100	0.12	79.3	100.0	100.0	100.0	87	43	30	22
Bshrt26-50	100	0.12	75.9	100.0	100.0	100.0	84	43	29	22
Bshrt37-63	100	0.17	82.5	98.7	100.0	100.0	78	68	37	26
Bshrt61-69	100	0.13	67.3	99.8	100.0	100.0	92	47	31	23
Bshrt44-48	100	0.71	100.0	100.0	100.0	100.0	10	11	15	23
Bshrt18-40	100	0.38	100.0	100.0	88.1	95.8	15	35	121	98
Bshrt13-56	100	0.35	100.0	99.4	56.1	99.0	16	43	158	75
Bshrt19-21	100	0.4	100.0	100.0	100.0	100.0	34	27	22	18
Bshrt27-34	100	0.17	80.7	98.6	100.0	100.0	75	68	38	26
Bshrt46-57	100	0.19	92.0	94.8	100.0	100.0	59	91	43	28

Relationships between p and m

The 108 data sets used in the construction of p-m relationship can be grouped into 5 groups according to the crop variety and the states from which the data were collected: cabbage, cauliflower and broccoli in Victoria, cabbage in Queensland and Brussels sprouts in South Australia. Linear regressions of ln[-ln(1-p)] over ln(m) were performed for each of the 5 groups of data sets (Figure 2). These regression lines did not differ significantly in either the slopes ($F=0.9129$, df=4, 98, $P>0.05$) or the intercepts ($F=2.2437$, df=4, 102, $P>0.05$). Hence a common regression line was fitted to represent the relationship for all data sets. The resulting regression line was highly significant ($F=3104.6903$, df=1, 106, $P<0.001$), explaining 97% of total variation in the dependent variable (Figure 2).

Classification of larval density

Sampling plans for the four density-based ATs are given in Table 1. The OC curves of these sampling plans (Figure 3) were not as steep as the OC curves of the sampling plans for the classification of the proportions of infested plants (Figure 2). As a result, the OC95 ranges were located further away from the ATs (Table 2). To have a ≥0.95 probability of making a no-spray decision, the larval density had to be lower than the AT by at least 0.15 larvae/plant at AT=0.2 larvae/plant and by at least 0.38 larvae/plant at AT=0.8 larvae/plant. Conversely, to have a ≥0.95 probability of making a spray decision, the larval density had to be higher than the AT by at least 0.11 larvae/plant at AT=0.2 larvae/plant and by at least 0.36 larvae/plant at AT=0.8 larvae/plant.

The peak ASN values increased from 41 plants at the AT=0.2 larvae/plant to 160 plants at the AT=0.8 larvae/plant (Figure 3). The ASN50 range at AT=0.2 larvae/plant covered the entire range of possible population levels. For the other three ATs, the ASN50 ranges were found 0.2~0.47 larvae/plant below the AT and 0.18~0.6 larvae/plant above the AT (Table 2). The higher the AT, the further away the ASN50 range was from the AT.

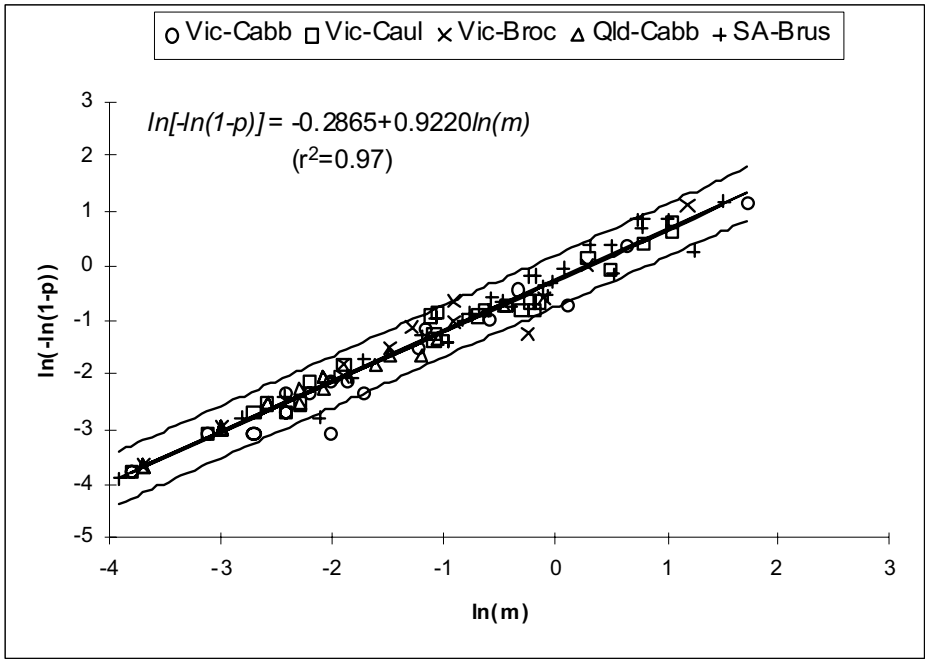

Figure 2. Plot of ln[-ln(1-p)] on ln(m) from 5 groups of data sets and the common regression line, where m is the larval density and p the proportion of plants with larvae present. The two parallel lines on either side of the common regression line show the 95% confidence interval of the regression.

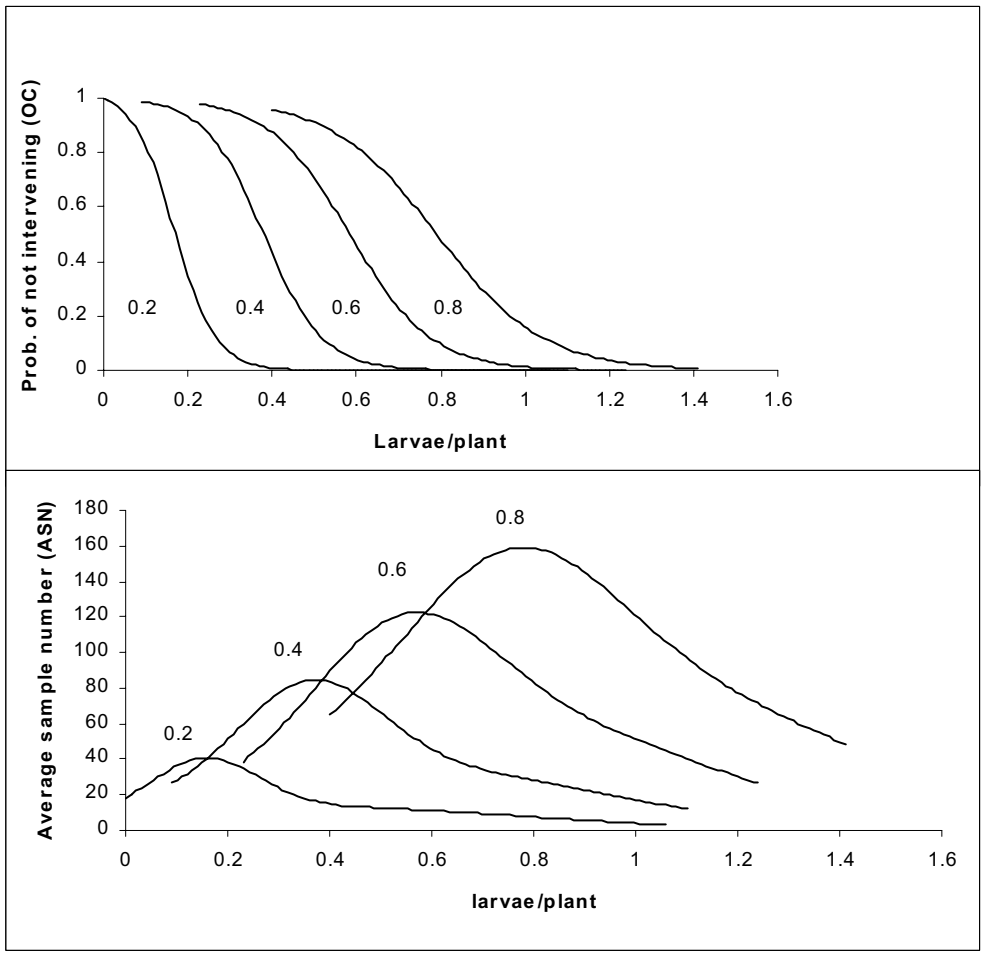

Figure 3. Operating characteristic (OC =probability of not intervening) and average sample number (ASN) functions for the classification of DBM larval density at 4 action thresholds (0.2, 0.4, 0.6, and 0.8 larvae/plant) using sequential presence-absence sampling.

Results of the re-sampling analyses showed that the percentage of correct decisions was >95% for all data sets in which the larval density differed from the AT by at least 0.08 larvae/plant when AT=0.2 larvae/plant and by at least 0.16 larvae/plant when AT=0.6 larvae/plant. (Table 4). At the AT=0.8 larvae/plant, the percentages of correct classifications were consistently high (>99%). The average sample sizes taken to reach these decisions were 18 plants at AT=0.2 larvae/plant, 35 plants at AT=0.4 larvae/plant, 31 plants at AT=0.6 larvae/plant, and 45 plants at AT=0.8 larvae/plant (Table 4). As expected, simulations for those data sets with larval density close to the AT yielded less accurate results and used larger sample sizes.

Table 4. Percentages of simulation runs that correctly classified the larval density in each of 20 independent data sets relative to each of the four proportional action thresholds (0.2, 0.4, 0.6 and 0.8 larvae/plant) (Correct%) and the corresponding average sample sizes (ASN_{sim}). One thousand simulations were run for each data set. Within each data set, individual plants were randomly sampled with replacement. Initial sample size was set to 10 and the sample increment to 1. N=number of plants in the data set, m=number of larvae per plant in the data set.

			Correct%				ASN_{sim}			
Data	**N**	**m**	**0.2**	**0.4**	**0.6**	**0.8**	**0.2**	**0.4**	**0.6**	**0.8**
S-cabb	600	0.26	80.6	99.1	100.0	100.0	45	55	39	34
PCB98	420	1.18	100.0	100.0	100.0	99.9	10	16	34	79
PCB99	360	0.92	100.0	100.0	100.0	100.0	10	13	24	44
Bshrt11	100	1.49	100.0	100.0	100.0	100.0	10	11	17	27
Bshrt12	100	1.31	100.0	100.0	100.0	100.0	10	11	18	29
Bshrt29	100	0.02	100.0	100.0	100.0	100.0	22	22	22	22
CabIHD1	100	0.53	99.8	98.2	82.0	100.0	15	58	166	82
CabIHD2	100	1.28	100.0	100.0	100.0	100.0	10	13	24	44
Bshrt2-68	100	0.28	99.0	98.0	99.7	100.0	22	65	54	48
Bshrt5-32	100	0.18	32.5	99.6	100.0	100.0	48	49	36	32
Bshrt3-10	100	0.12	69.4	100.0	100.0	100.0	52	35	31	28
Bshrt26-50	100	0.12	70.2	100.0	100.0	100.0	52	36	30	28
Bshrt37-63	100	0.2	75.0	99.5	100.0	100.0	46	53	38	33
Bshrt61-69	100	0.2	61.8	100.0	100.0	100.0	52	38	32	29
Bshrt44-48	100	1.46	100.0	100.0	100.0	100.0	10	11	18	30
Bshrt18-40	100	0.52	100.0	99.8	32.0	99.3	13	39	197	123
Bshrt13-56	100	0.44	100.0	86.0	74.0	99.9	14	54	183	92
Bshrt19-21	100	0.04	99.7	100.0	100.0	100.0	26	24	23	23
Bshrt27-34	100	0.22	76.0	99.6	100.0	100.0	47	52	38	34
Bshrt46-57	100	0.24	87.0	97.8	100.0	100.0	37	63	42	36

Discussion

There have been no published reports of binomial sampling for DBM. A sequential sampling plan based on enumerative data was developed for DBM monitoring in Malaysia (Hing & Sivapragasam 1997). Application of this sampling plan elsewhere is limited because of its use of relatively high action thresholds (7-14 larvae/plant) and involvement of a distribution-specific parameter (k of the negative-normal distribution).

Two sets of sequential binomial sampling plans were developed in this study, one targeting the proportion of plants infested with DBM larvae and the other larval density, with the objective of providing simple and efficient monitoring methods for DBM populations. The results showed that all four proportion-based sampling plans performed well in classifying the proportions of infested plants relative to the action thresholds. The expected rate of correct classification was ≥95% for most possible population levels (87-88%). This was confirmed in the simulated re-sampling of 20 independent data sets, which showed a 100% correct classification rate for all data sets in which the proportion of infested plants was not in the immediate vicinity of the action threshold (separated by >0.05 proportion units). Although the expected sample size to achieve the ≥95% accuracy was up to 94 plants, a sample size of 50 plants was sufficient when the true population level differed from the action thresholds by a minimum of 0.07-0.15 proportion

units. In fact, simulated re-sampling of actual data showed average sample sizes of <40 plants in reaching ≥95% accuracy.

Classification of larval densities relative to action thresholds using sequential binomial sampling plans involves the additional step of converting the density-based action thresholds into proportion-based action thresholds. This inevitably introduces some errors to the sampling plans due to the uncertainty of any proportion-density models (Schaalje *et al*. 1991). As a result, the expected performance of the density-based sampling plans was not as satisfactory as the proportion-based sampling plans. However, satisfactory performance (≥95% accuracy at ≤50 plants sample size) can be expected from the density-based sampling plans if the true population density is some distances away from the action threshold (>0.38 larvae/plant below the action threshold and >0.57 larvae/plant above the action threshold). The required separation distances were much smaller for the lower action thresholds. This was again confirmed in the re-sampling analyses of 20 independent data sets. A correct decision rate of >95% was achieved at an average samples sizes of ≤ 45 plants for all data sets in which the true population density differed from the action thresholds by a minimum of 0.16 larvae/plant. Presence-absence sampling is a special form of binomial sampling with a cut-off point of 1. Many studies have shown that the precision of classifying population densities with sequential binomial sampling can be increased by using higher cut-off points (Binns 1990, Boeve & Weiss 1997, Naranjo *et al*. 1996, Nyrop & Binns 1991). Other cut-off points were not investigated in this study as the action thresholds practised by most *Brassica* vegetable growers in Australia are mostly below one larva/plant.

As in any sequential sampling plan, when the true population level was close to the action threshold, the probability of making correct decisions were relatively low (<0.95) and the average sample sizes were relatively high (up to 160 plants). To avoid excessive sample sizes with no promise of significant improvement on sampling precisions, it is suggested that the maximum sample size be limited to 50 plants. If no decision is reached by that sample size, growers are advised to make their own decisions according to other relevant information such as historical pest level data, crop development stage and pheromone trapping data. Termination of a sequential sampling plan in this way introduces errors (Fowler & Lynch 1987). However, the likelihood of taking such an action is not high, as seen from results of this study.

Selection between the two sets of sampling plans depends on grower's primary concern about DBM infestation and crop development stage. If the primary concern is the proportion of plants infested with DBM larvae, or the crop is in a development stage sensitive to DBM damage and one larva is sufficient to cause significant damage to the host plant, then the use of proportion-based sampling plans would be the better choice. If the primary concern is the abundance of DBM larvae in the crop, or the crop is in a development stage not particularly sensitive to DBM damage and the total damage to a host plant is the accumulative work of all larvae, then density-based sampling plans may be considered.

The proportion-based sequential presence-absence sampling plans developed here can also be used in multi-pests situations. In these situations, infestations by individual pest species can be combined and treated as the infestation by a single pest species. The total number of plants infested with any one of the pest species still follows the binomial distribution. Since the development of a proportion-based sequential presence-absence sampling plan is affected only by the nominal action thresholds and allowed error rates and not by any species-specific parameters, the same sampling plans can be used. However, there are two things that need to be considered when applying the sampling plans to multi-pests situations. Firstly, the action threshold may need to be adjusted. Theoretically, the new action threshold can be calculated as $1 - \Pi [1-at(i)]$, where Π denotes the product and at(i) is the action threshold of species i. In practice, if the action thresholds for individual pest species are somewhat arbitrary in the first place, it is just as acceptable for the grower to nominate the new action threshold based on his/her tolerance level to the combined infestation of the pest species concerned. Secondly, it only makes sense to combine those pest species for which the management actions are similar. Ideally the pest species to be combined should also have similar feeding habits and growers have similar tolerance levels to them, such as lepidopteran pests in *Brassica* crops.

Acknowledgements

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Yellow sticky traps as a monitoring tool for *Plutella xylostella* in *Brassica* vegetable crops

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Abstract

Yellow sticky traps may be an alternative monitoring tool to monitoring plants for assessing *Plutella xylostella* (Lepidoptera: Plutellidae) in *Brassica* vegetable crops (Sivapragasam & Saito 1986, Hallett *et al*. 1993). Previous research has shown a significant relationship between the number of moths on sticky traps and total larvae or pupae in the crop two weeks after the trap catch. A trial was designed to test if commercial yellow sticky traps could be used as an indicator for the early instars of *P. xylostella* in the *Brassica* vegetable crops in Queensland. Predicting hatching eggs and small larvae enables effective targeting of pesticide applications. Results indicate some relationships exist, however practical use may be considered costly.

Material and methods

Successive crops of broccoli were planted monthly, for 12 months. Each planting was 600 m^2 and divided into six plots. A yellow sticky trap was placed in the middle of each plot, making a trapping density of one trap/100 m^2. Traps were positioned just above crop height and adjusted as the crop grew.

The number of *P. xylostella* moths per trap was recorded weekly. Twice weekly, five plants were randomly chosen from within each plot and the number of eggs, each larval instar and pupae were recorded.

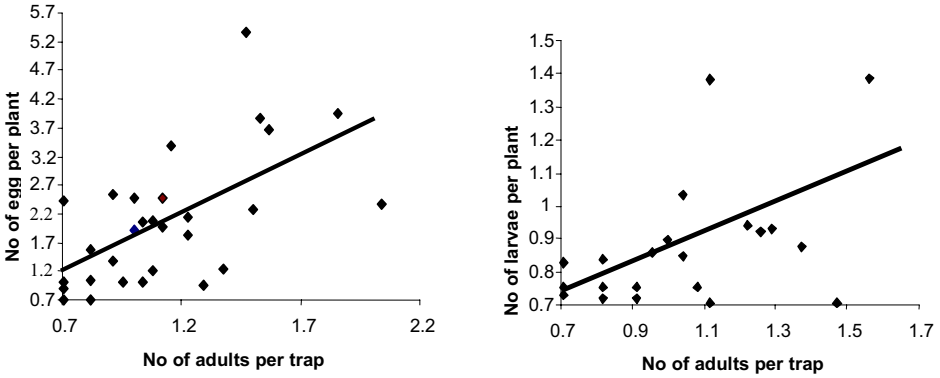
For analyses, trap and plant monitoring data were bulked across the plant ages thereby treating the area as a single population sample for any one date. The relationship between same day trap catches and plant monitoring events were investigated, as well as with subsequent plant monitoring events. Data were transformed using sqrt(x+0.5). Spearman's ranked correlation test was used for the analysis.

For comparing the costs of each style of monitoring, trap monitoring was based on 30 seconds to count moths on traps and replace the trap and the commercial cost of $1 per trap was used. For plant monitoring commercial consultant rates of $45/hour were used.

Results and discussion

Similar to Hallett *et al*. (1993), a significant relationship existed between the number of moths on traps and larvae in the crop, as well as eggs. However by comparison, the strongest relationship with eggs and small larvae was found 0-3 days after the trap count and up to 6-8 days for larger larvae ($P<0.05$) (Figures 1 and 2). These relationships were found at a lower trapping density of 100 traps/ha (1/100 m^2), compared with 400 traps/ha (1/25 m^2) used by Hallett *et al*. (1993). Further analyses showed no significant relationship at a trap density lower than 100 traps/ha.

This relationship can be taken a step further to consider the implications of using traps and their effect on correct pest management decisions. It assumes that current practices based on using an action threshold of 1.1 larvae per plant (Figure 2) for plant monitoring, provide acceptable yields. Back calculating, this gives a moth count of 1.3 moths per trap as the trap action threshold. Using these thresholds, sticky trap and plant monitoring result in the same spray decision 57% of the time. On 11% of monitoring occasions however, the sticky traps suggest no spray action while plant monitoring suggests spraying; whilst 14% of occasions the reverse was true (Figure 2).



a) b)

Figure 1. Significant relationships between *P. xylostella* adults on traps and a) eggs and b) II instar larvae 0-3 days after trap catches. Data transformed using sqrt(x+0.5).

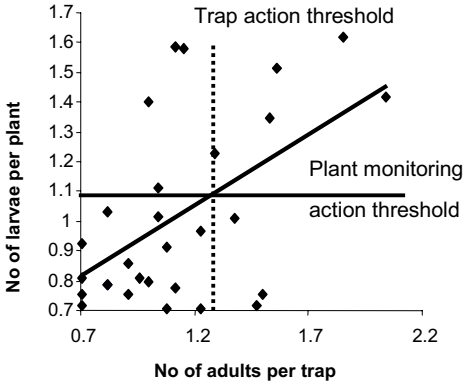


Figure 2. Significant relationships between *P. xylostella* adults on traps and III to IV instar larvae 6-8 days after trap catches. Data transformed using sqrt(x+0.5).

The other practical consideration for growers is the cost of sticky trap monitoring compared with plant monitoring. Current commercial cost of traps and plant monitoring in Australia (Table 1), mean traps may only be considered for use at certain times in the cropping period or be more suitable for small cropping units. However, using the same trap for two consecutive weeks can create savings. Commercial sticky traps have two sticky sides. This means one side can be uncovered in the first week and then in the second week, after the number of moths is recorded on the first side, the trap can be turned over and the other side be uncovered and used to catch moths in the second week. By using this method, the cost comes down closer to the price of not monitoring at all, $90/ha. Given the level of accuracy in the trap action thresholds discussed previously, this cost saving may not be significant for a grower choosing to establish traps rather than not monitoring at all.

Table 1. Cost per monitoring event of different monitoring methods for DBM

Monitoring method	Pest management cost ($/ha)
Trap 1/100 m^2 including labour	140
Plant monitoring by scout	10[a]
No monitoring	80[b]

a Current commercial practice of 10-20 plants inspected per planting
b Assumes 1-2 extra sprays/ha used

At this stage, a relationship between sticky trap catches and actual plant counts has only been investigated for *P. xylostella*. This means in locations where there is a pest complex, for example where other Lepidopteran pests or aphids and thrips need to be considered, it is more cost effective for a grower to use plant monitoring than trapping.

Despite the apparent shortfalls of yellow sticky traps, they are still used at a low trapping density by crop consultants. They serve as a tool that provides complementary information to plant monitoring, indicating the pattern of pest abundance relative to the previous week as well as a measure of some beneficial insect activity.

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Quantitative evaluation of the biotic mortality factors affecting diamondback moth in south-east Queensland, Australia

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Abstract

The impact of natural enemies on immature stages of the diamondback moth was quantitatively assessed in commercial cabbage crops in southeast Queensland, Australia, in 2000 and 2001. The survivorship of *Plutella xylostella* (L.) eggs, larvae and pupae on cabbage plants caged to exclude predators and larval and pupal parasitoids was compared with survivorship on cabbage plants which were caged to simulate the ambient conditions created by the exclusion cages, but which allowed natural enemy access. In 2000, six cohorts of *P. xylostella* were studied on three commercial *Brassica* farms which adopted an integrated approach to pest management and six cohorts were studied on three farms which adopted a calendar spray approach to pest management; a further two cohorts were followed in cabbage plots at Gatton Research Station. In 2001, two cohorts were followed on a farm adopting IPM and two cohorts were followed on a farm practising calendar spraying. Estimated losses due to predation ranged from 2-85% in 2000 and from 22-77% in 2001. When losses and mortality due to parasitism were combined, total estimated losses ranged from 2-98% in 2000 and from 22-90% in 2001. Larval and pupal parasitism rates were low in both years of the study but, in order of abundance, the hymenopteran parasitoids detected could be ranked *Diadegma semiclausum* > *Diadromus collaris* > *Apanteles ippeus* > *Oomyzus sokolowskii*. This study represents the first record of *O. sokolowskii* attacking *P. xylostella* in Australia. Pitfall trapping indicated that Araneae (Lycosidae and Oxyopidae) were the most abundant insectivorous predators but that Coleoptera (Carabidae, Staphylinidae and Coccinellidae) and Hemiptera were also relatively abundant on commercial *Brassica* farms in south-east Queensland.

Keywords

predators, parasitoids, exclusion

Introduction

Naturally occurring arthropod predators and parasitoids are known to significantly affect populations of pest Lepidoptera in various *Brassica* crops (Jones 1987, Schmaedick & Shelton 1999). In order to maximise the contribution which natural enemies can make to integrated pest management programs it is imperative that their potential value is recognised and that the factors underpinning their activity are elucidated. Although the parasitoid fauna associated with the diamondback moth, *Plutella xylostella* (L.), is well documented (Talekar & Griggs 1986, Talekar 1992, Sivapragasam *et al*. 1997), quantitative assessment of its impact, and that of generalist predators, on populations of the pest is much less well understood. In order to assess the impact of predators as well as parasitoids, an exclusion cage approach was chosen over the more simple life table studies which identify agents which cause mortality, but do not allow its quantification (Luck *et al.* 1988). This study summarises an area wide approach that was taken towards understanding the importance of arthropod parasitoids and predators of the diamondback moth on commercial farms in the Lockyer Valley, southeast Queensland, Australia.

Materials and methods

Experimental sites

Following a series of interviews and farm visits conducted in the Lockyer Valley in February 2000, six commercial *Brassica* farms were identified as suitable experimental sites. Consideration of the types of insecticide used, the frequency of application, scouting procedures, initial planting and final harvest dates were used to broadly categorise farm management practice. Three farms that were considered to adopt an integrated approach to insect pest management ("IPM" sites) and three farms that were considered to practise an approach tending towards calendar insecticide application ("CAL" sites) were selected. Although it was not possible to select adjacent farms practising different methods of insect pest management for

inclusion in the study, suitable sites were selected on the basis of location as well as proposed management practice. The first experimental site (IPM1) was located in the south-east of the Lockyer Valley and the second site (CAL1) was located in the north of the valley, thereafter experimental sites were selected so that site categories alternated with each other (Figure 1). The second set of experiments was performed in the same order as the first set, these experiments are denoted by the suffix "a" in site labels in all subsequent tables and figures. Experiments performed in 2001 are followed by the suffix "(01)".

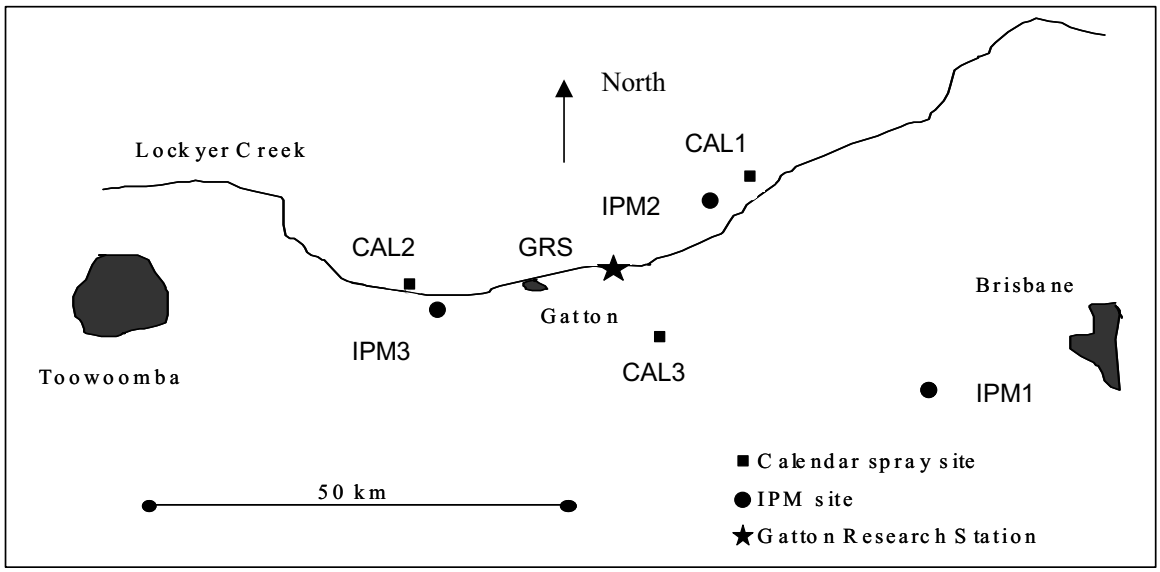

Figure 1. The location of experimental sites within the Lockyer Valley, Queensland.

Plants and insects

Cabbage was chosen as the study crop as it is grown over a wide area and a long period (multiple sequential plantings February-August) and it is relatively easy to sample. Three cabbage cultivars, "Warrior", "Neptune" and "Sugarloaf" were grown on the experimental sites. The cultivar to be grown at a particular site was determined by consultation with the relevant grower approximately 1 week prior to field transplantation. One-two weeks after field transplantation, 50 seedlings of that cultivar were transplanted in 20 cm diameter pots containing a 1:1 mixture of Gatton Research Station soil and potting mix and grown in a glasshouse for two weeks.

Plants were exposed to diamondback moth for oviposition by placing 40 potted seedlings on the floor of a nylon net cage (2 m x 1.5 m x 0.4 m) in a controlled environment room (22 ±2°C; 12:12 (L:D) h; 60% RH) and releasing approximately 1500 recently emerged (1-2 days post eclosion) and mated diamondback moth adults into the cage. After 12-18h exposure, plants were removed from the cage and examined. Excess eggs were removed from leaves so that all plants supported 25 eggs that were distributed as evenly as possible between the plant's six leaves. The location of each egg on each plant was marked with a permanent marker. Eggs that were laid on the plant pot were removed by washing the pot with a scourer and warm soapy water and eggs that were laid on the soil surface were destroyed by turning over the soil with a fork.

Exclusion of predators and larval and pupal parasitoids

Survivorship of *P. xylostella* on plants caged to exclude predators and larval and pupal parasitoids was compared with survivorship on plants caged to allow total or restricted predator access. All cages consisted of a central cylindrical frame (45 cm high x 45 cm diameter) made from wire (2 cm diameter mesh). Modifications to the fine nylon-netting sleeve (mesh 0.5 mm) covering the central frame allowed construction of cages to effect:

a) Total natural enemy exclusion. The nylon mesh sleeve was buried under the plant pot and completely covered the central wire frame.

b) Total natural enemy access. The cage consisted of only the central wire frame.

c) Natural enemy access and ambient environmental conditions similar to those within total exclusion cages. One half of the central wire frame and the top of the cage were covered with the nylon mesh.

d) Natural enemy access and ambient environmental conditions similar to those within total exclusion cages, but ground predators excluded. As in c) above but the top 4 cm of the plant pot was treated with Tanglefoot®.

The bottom of all cages, except those providing total exclusion of natural enemies, was buried approximately 10 cm into the ground and a single egg-laden potted plant was placed into each cage. In all treatments, the pots were buried so that the top of the pot, or the bottom of the sticky barrier, was flush with the soil and the cages anchored to the ground by 3 bamboo canes. The tops of the nylon sleeves of the total exclusion cages were tied and the cages fixed in place by 4 bamboo canes placed around the edge and fastened with string (Figure 2).

At each field site, cages were arranged in four blocks within two adjacent beds of cabbage plants; the two blocks in the same bed were placed 20 m apart. Cages within a block were spaced 5 m apart and their position within the block randomly assigned. Each treatment was replicated twice within a block to give a total of 8 replicates per treatment.

On the day that the experimental plants were transferred to the field, 30 plants within the field were randomly selected and sampled for diamondback moth, other cabbage pests and natural enemies. Sites were visited regularly during the course of the experiments (2-3 times each week); all experimental plants were examined and field-laid *P. xylostella* eggs removed. Regular liaison with growers determined when insecticides were to be applied to the crops and all experimental cages were covered with large garbage bags before insecticide application and removed approximately 1 h later. The experiment was terminated when the majority of insects on the plants in the cages had reached the pupal stage.

Plants were cut at their base and transferred individually to labelled plastic bags. Any diamondback moth larvae or pupae on the cage or rim of the pot were transferred to the same bag as the plant. In the laboratory, individual plants were carefully examined and all larvae and pupae recorded and then reared (22 ±2°C; 12:12 (L:D) h; 60% RH) until moths or parasitoids emerged or it was certain that individuals had died.

In 2000, the experiment was performed twice within cabbage crops at each field site between March and August and in experimental plots at Gatton Research Station from August-September. In 2001, the experiment was performed twice within cabbage crops at two of the field sites between June and August (Figure 1, Table 1). Insecticide input at each field site prior to and during each experiment was recorded (Table 1).

Sampling for ground dwelling predators

In the third week of each experiment set up after June 7, 2000 (Sites IPM1a, IPM2a, IPM3a, CAL1a, CAL2a, GRSA and GRSB) and in all experiments performed in 2001, pitfall traps were used to sample ground dwelling predators within the immediate vicinity of the exclusion cages. Traps were constructed by burying plastic cups (200 ml; 6.5 cm diameter) so that soil was level with the rim, adding approximately 100 ml of detergent solution (1% (vol./vol.)) to each and then covering each cup with plastic disc (15 cm diameter) supported 3 cm above ground level by 3 steel nails (10 cm). Sixteen pitfall traps were placed within each experimental site and each trap was positioned a minimum distance of 10 m from its nearest neighbour. Traps remained in the field for 7 days before they were collected and returned to the laboratory for analysis.

Statistical analysis

The number of *P. xylostella* recovered from each of the cage treatments at each of the experimental sites was compared by ANOVA. Similarly, the number of parasitised *P. xylostella* recovered from each of the cage treatments at each of the experimental sites was also compared by ANOVA. In order to estimate the impact of predators on each *P. xylostella* cohort at each of the experimental sites, the number of pupae and final instar larvae recovered from the open and half cage treatments was expressed as a proportion of the original number of eggs on each plant and corrected by the proportion of individuals recovered from the total exclusion treatments. To increase the reliability of corrected estimates of predation and to ensure that the confidence intervals calculated were centred around the appropriate mean, a modified version of Abbott's formula (Abbott 1925) incorporating an estimate of the variance of the control mean (total exclusion recovery rate) was used (Rosenheim & Hoy 1989). Within years, total predator numbers sampled at each experimental site were compared by ANOVA.

Table 1. Insecticide input at experimental sites prior to and during natural enemy impact assessment trials, south-east Queensland

			Insecticides applied[2]								
Site[1]	**Year**	**Duration[1]**	**Bt**	**S'sad**	**P'zole**	**E'ctin**	**P'roid**	**OP**	**OC**	**C'ate**	**Total**
IPM1	2000	15/3 - 30/3	3	-	-	-	-	-	-	-	3
CAL1		8/4 - 28/4	2	-	-	1	3	1	-	1	8
IPM2		19/4 - 15/5	3	-	-	-	-	1	1	-	5
CAL2		8/5 - 4/6	1	-	4	-	-	-	-	-	5
IPM3		15/5 - 18/6	6	-	1	1	-	-	-	-	8
CAL3		1/6 - 11/7	4	-	4	-	-	-	-	-	8
IPM1a		8/6 - 16/7	4	-	-	-	-	-	-	-	4
CAL1a		18/6 - 22/7	6	-	1	1	5	-	-	-	13
IPM2a		8/7 - 9/8	1	-	-	-	-	-	1	-	2
CAL2a		21/7 - 25/8	1	1	2	-	-	-	-	-	4
IPM3a		23/8 - 15/9	6	-	1	-	-	-	-	-	7
GRSA		27/8 - 18/9	-	-	-	-	-	-	-	-	0
GRSB		27/8 - 18/9	-	-	3	-	-	1	-	-	4
IPM2	2001	19/6 - 24/7	3	-	-	-	-	1	1	-	5
CAL1		23/6 - 24/7	2	-	-	1	3	1	-	1	8
IPM2a		28/7 - 28/8	1	-	-	-	-	-	1	-	2

[1]The date on which the experiment commenced to the date on which *P. xylostella* were recovered from the cages in the field.
[2] Abbreviations for insecticides applied during the course of the experiments; *Bt= Bacillus thuringiensis*, S'sad= Spinosad, P'zole= Pyrazoles, E'ctin= Emamectin, P'roid= Pyrethroids, OP= Organophosphates, OC= Organochlorines, C'ate= Carbamates

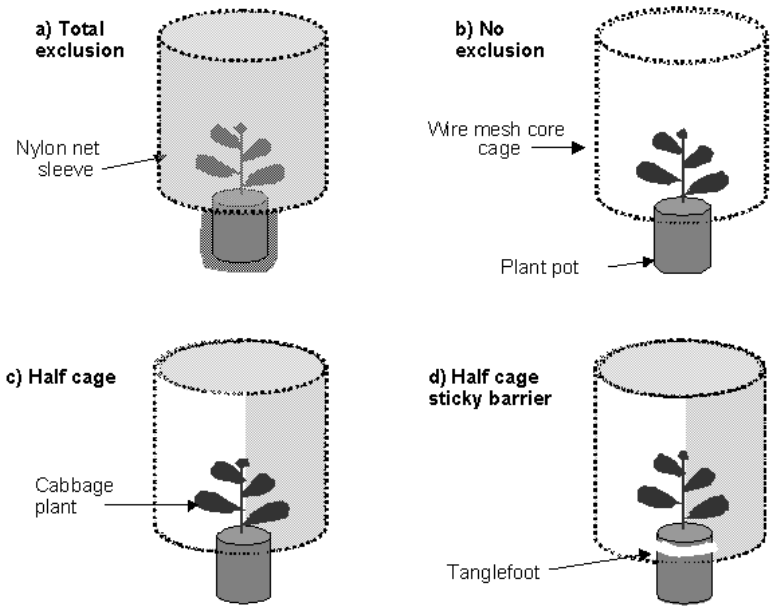

Figure 2. Design of the various field cages used in the experiments to permit natural enemy access or to effect total natural enemy exclusion.

Results

Exclusion of predators and larval and pupal parasitoids

Application of insecticide to the cabbage crop before experimental cages could be covered rendered data from sites CAL1 and CAL3a useless (Table 2). Recovery of *P. xylostella* varied enormously between the remaining experimental sites (Table 2). Initial analysis of variance showed that Tanglefoot® around the top of the plant pot in the nylon mesh covered cages to which predators and parasitoids had access had no significant impact on the number of *P. xylostella* recovered (LSD $P>0.05$) at any of the experimental sites. These data were combined for further analysis and the Tanglefoot® treatment was subsequently excluded from experiments performed at Gatton Research Station in 2000 and all the experiments performed in 2001.

Table 2. The recovery and parasitism rates of *Plutella xylostella* from each of the cage treatments at each of the field sites, exclusion cage experiment, south-east Queensland

	Mean no. *P. xylostella* recovered (±SE)						Mean no. *P. xylostella* parasitised (±SE) [2]		
Field Site	Total exclusion	Half cage	Open cage	F[1]	*P*[1]	$LSD_{0.05}$[1]	Total exclusion	Half cage	Open cage
IPM1	21.4 (±4.4)	21.8 (±2.5)	7.9 (±1.8)	6.15	0.006	4.75	0	2.4 (±1.5)	2.1 (±1.3)
CAL1	-	-	-	-	-	-	-	-	-
IPM2	19.1 (±2.2)	19.8 (±1.4)	12.9 (±2.3)	4.27	0.024	2.83	0	0.4 (±0.3)	1.9 (±0.9)
CAL2	12.0 (±1.4)	11.8 (±1.0)	8.0 (±1.0)	2.87	0.073	1.98	0	0	0
IPM3	9.8 (±1.9)	7.7 (±1.2)	8.9 (±1.5)	0.57	0.574	2.30	0	0	0
CAL3	10.6 (±1.9)	5.3 (±1.0)	2.6 (±0.8)	9.38	<0.001	1.89	0	0	0
IPM1a	22.1 (±1.8)	13.3 (±1.7)	12.9 (±2.6)	4.52	0.020	3.60	0	2.3 (±1.0)	2.8 (±0.8)
CAL1a	20.8 (±1.4)	20.4 (±1.3)	13.6 (±1.4)	4.93	0.015	2.87	0	0.1 (±0.1)	0
IPM2a	21.3 (±2.3)	12.9 (±1.3)	10.6 (±1.9)	9.23	<0.001	2.67	0	3.1 (±1.0)	3.8 (±1.3)
CAL2a	20.1 (±2.1)	11.3 (±1.2)	7.4 (±1.4)	13.91	<0.001	2.48	0	4.5 (±1.2)	3.9 (±1.5)
IPM3a	16.3 (±1.8)	12.4 (±1.3)	11.3 (±2.2)	1.82	0.181	2.77	0	0.1 (±0.1)	0.3 (±0.2)
CAL3a	-	-	-	-	-	-	-	-	-
GRSA	23.8 (±2.7)	8.4 (±0.8)	4.1 (±0.8)	37.36	<0.001	5.02	0	3.5 (±1.4)	3.6 (±1.1)
GRSB	21.3 (±2.8)	10.0 (±1.7)	3.4 (±0.7)	21.27	<0.001	5.83	0	2.9 (±0.9)	2.8 (±1.1)
IPM2(01)	17.3 (±1.9)	8.6 (±1.7)	4.0 (±0.6)	16.66	<0.001	4.80	0	3.6 (±1.2)	2.3 (±0.9)
CAL2(01)	7.4 (±0.9)	5.8 (±2.2)	3.4 (±0.6)	1.76	0.196	4.45	0	0	0
IPM2a(01)	19.0 (±1.5)	7.9 (±1.6)	7.3 (±1.3)	17.89	<0.001	5.60	0	6.6 (±1.9)	5.4 (±1.2)

[1]The number of *P. xylostella* recovered from each type of cage was analysed by ANOVA. For field sites IPM1, CAL1, IPM2, CAL2, IPM3, CAL3, IPM1a, CAL1a, IPM2a, CAL2a, IPM3a degrees of freedom =2, 30 and for field sites GRSA, GRSB, IPM2(01), CAL2(01) and PM2a(01) degrees of freedom = 2, 22.
[2] The number of *P. xylostella* recovered from each type of cage subsequently found to be parasitised. Within an experiment, there was no significant difference between the numbers of parasitised *P. xylostella* recovered from either type of cage to which parasitoids had access (LSD, $P>0.05$). Parasitoids found attacking *P. xylostella* larvae or pupae were *Diadegma semiclausum*, *Diadromus collaris*, *Apanteles ippeus* and *Oomyzus sokolowskii* (Table 3).

The different cage designs significantly affected the number of *P. xylostella* recovered at all but four (CAL2, IPM3, IPM3a and CAL2(01)) of the experimental sites (Table 2). At all remaining sites the number of *P. xylostella* recovered from completely open cages was significantly lower than the number recovered from cages that totally excluded natural enemies (LSD, $P<0.05$; Table 2). At sites IPM1, IPM2 and CAL1a there was no significant difference between the number of *P. xylostella* recovered from cages which totally excluded all natural enemies and cages which were partially covered with nylon netting but which permitted natural enemy access (LSD, $P>0.05$). At all other experimental sites (CAL3, IPM1a, CAL1a, IPM2a, CAL2a, IPM3a, GRSA, GRSB, IPM2(01) and IPM2a(01)) the number of *P. xylostella* recovered from cages which totally excluded all natural enemies was significantly greater than the number recovered from cages which were partially covered with nylon netting, but which permitted natural enemy access (LSD, $P<0.05$; Table 2).

Table 3. Species of hymenopteran parasitoids attacking immature stages of *Plutella xylostella* at field sites in the Lockyer Valley, March-September 2000 and June-August 2001

		Total number of *P. xylostella* parasitised by each parasitoid at each site			
Field site[1]	***Year***	***D. semiclausum***	***D. collaris***	***A. ippeus***	***O. sokolowskii***
IPM1	2000	2	34	0	17
CAL1		-	-	-	*
IPM2		1	20	0	0
CAL2		0	0	0	0
IPM3		0	0	0	0
CAL3		0	0	0	0
IPM1a		47	11	0	1
CAL1a		1	0	0	0
IPM2a		77	1	3	0
CAL2a		103	0	0	0
IPM3a		2	0	0	0
GRSA		30	0	27	1
GRSB		26	0	18	0
IPM2	2001	47	0	0	0
CAL1		0	0	0	0
IPM2a		93	0	0	0

[1] Quantitative assessment of data from field sites CAL1and CAL3a was impossible. However, some larvae recovered from CAL1 were parasitised by *O. sokolowskii*.

No parasitised larvae were recovered from cages that totally excluded *P. xylostella* natural enemies (Table 2). There was no significant difference between the overall parasitism rates in larvae recovered from cages which were partially covered with nylon netting but which permitted natural enemy access and completely open cages at any of the experimental sites ($P>0.05$; Table 2). The major parasitoids attacking the immature stages of *P. xylostella* were *Diadegma semiclausum*, *Diadromus collaris*, *Apanteles ippeus* and *Oomyzus sokolowskii* and their relative abundance varied both between and within experimental sites over time (Table 3). In 2000, the rates of parasitism were generally low and no parasitism was detected in experiments that were initiated between the first week of May and the first week of June. Rates of parasitism increased in experiments performed subsequently, but never exceeded 17% of available larvae at a given experimental site (Table 2). In 2001, only parasitism by *D. semiclausum* was detected and, although parasitism rates were slightly higher than in 2000, they never exceeded 26.5% of available larvae (Table 2).

When *P. xylostella* recovery rates from cages that were partially covered with nylon netting but which permitted natural enemy access and completely open cages were corrected by recovery rates from cages from which natural enemies were totally excluded (Rosenheim & Hoy 1989) estimated rates of predation ranged from 2-85% in 2000 and from 22-77% in 2001 (Figure 3). Similar statistical treatment of data that incorporated *P. xylostella* mortality due to parasitism into overall recovery data estimated that the overall combined mortality due to predation and parasitism ranged from 2-98% in 2000 and from 22-90% in 2001 (Figure 4).

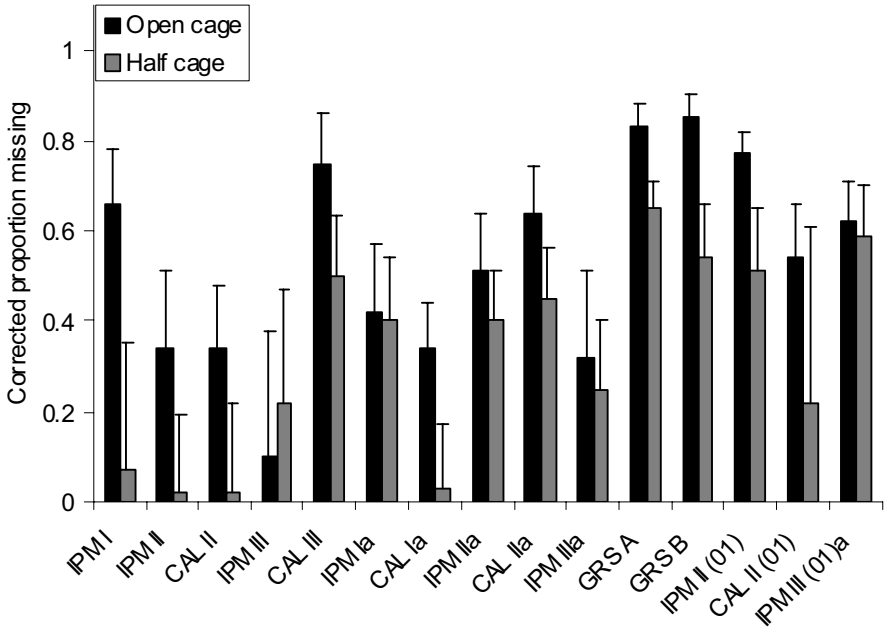

Figure 3. The corrected proportion of *Plutella xylostella* "missing" from totally open cages and cages which were partially covered with nylon netting, but which permitted natural enemy access.

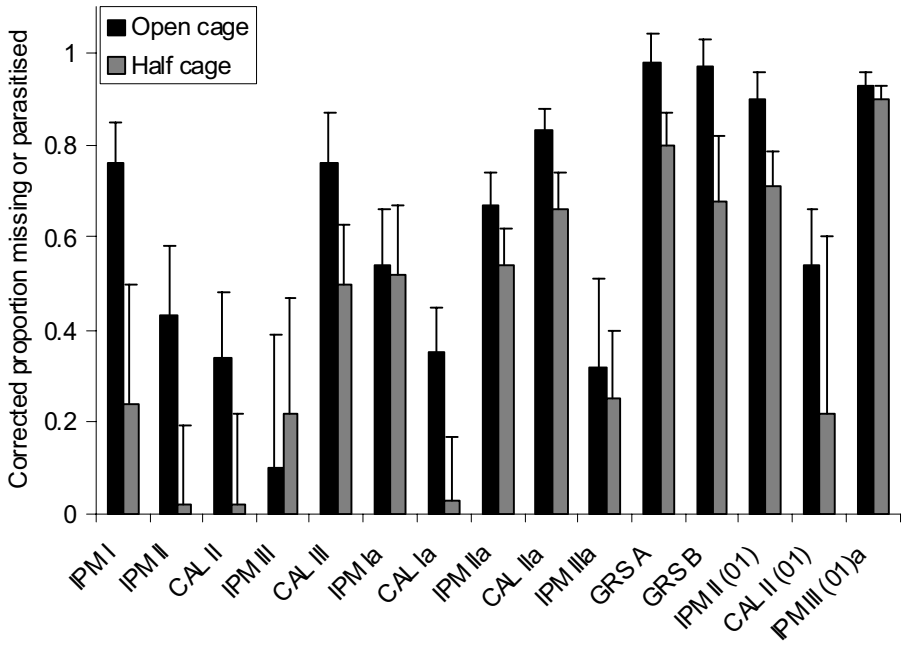

Figure 4. The corrected proportion of *Plutella xylostella* "missing" or parasitised in totally open cages and cages which were partially covered with nylon netting, but which permitted natural enemy access.

Sampling for ground dwelling predators

The number of potential predators of *P. xylostella* caught in pitfall traps varied significantly between experimental sites in both 2000 ($F_{6,97}$=7.8, P<0.001; Figure 5a) and 2001 ($F_{2,41}$=12.2, P<0.001; Figure 5b). In 2000 the total numbers of potential predators caught at CAL2a, IPM3a and GRSB were significantly lower than those caught at the remaining sites (LSD, P<0.05). In 2001 the total number of potential predators caught at IPM2a was significantly greater than at the other two sites (LSD, P<0.05). Of the potential *P. xylostella* predators caught in pitfall traps, spiders (Lycosidae and Oxyopidae) were the most abundant, followed by Coleoptera (Carabidae, Staphylinidae and Coccinellidae) and Hemiptera.

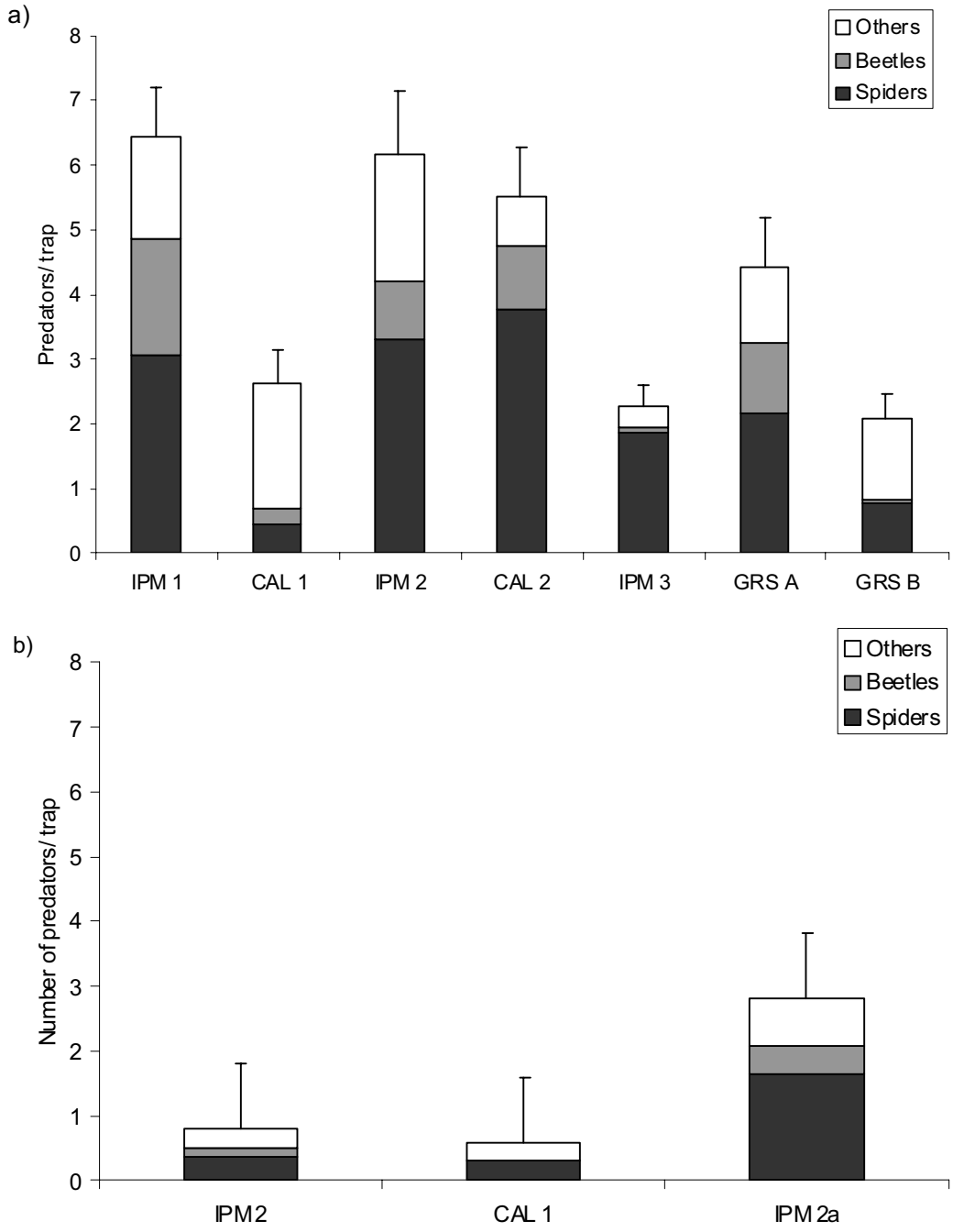

Figure 5. a) Predaceous arthropods sampled at experimental sites in 2000, b) Predaceous arthropods sampled at experimental sites in 2001.

Discussion

The great variability in the rates of recovery of insects throughout the series of experiments indicated that biotic and abiotic mortality factors varied both over time and between experimental sites. Comparison of survival rates of test insects between cages that totally excluded natural enemy access and those that allowed natural enemy access shows that both parasitoids and predators can cause significant mortality of *P. xylostella* infesting cabbage on commercial properties (Table 2, Figure 3).

The low levels of parasitism by hymenopteran parasitoids detected during the experiments were probably due to the low population pressure of *P. xylostella* (populations on commercial properties never exceeded 1.5 larvae per plant). Over both years, *D. semiclausum* was the most abundant parasitoid of *P. xylostella* followed by *D. collaris*, *A. ippeus* and *O. sokolowskii*. *O. sokolowskii* is an important parasitoid of *P. xylostella* throughout Asia and much of Africa and samples collected in these experiments represent the first record of *O. sokolowskii* in Australia (it has since been recorded in Tasmania and Western Australia, M.

Keller, F. Berlandier, pers. comm.). It appears that this species of parasitoid is widespread throughout the Lockyer Valley with specimens being reared from *P. xylostella* collected at central, northern and south-eastern experimental sites as well as from larvae feeding on a wild host (*Rapistrum* sp.) in the western region of the valley.

Pitfall trapping indicated that spiders (Lycosidae and Oxyopidae) were the most abundant insectivorous predators present on commercial *Brassica* farms in the Lockyer Valley, but Coleoptera (Carabidae, Staphylinidae and Coccinellidae) and Hemiptera were also relatively abundant.

At the beginning of the study, experimental sites were chosen depending on the farmer's planned approach to pest management. Farmers at three sites (IPM1, IPM2 and IPM3) planned to take an integrated approach to insect pest management (e.g. use of insecticides which are less harmful to parasitoids and predators, observation of a break in *Brassica* production, utilisation of a crop scout to collect information for informed decision making, application of techniques to encourage natural enemies) while farmers at the remaining three sites (CAL1, CAL2 and CAL3) planned a less flexible approach to insect pest management, intending to apply insecticides on a calendar basis. In the event, only site CAL1 was managed by prophylactic application of insecticides and frequent use of chemicals known to have a detrimental effect on predators and parasitoids (Table 1). The management practices adopted by farmers at sites CAL2 and CAL3 were more similar to those at designated IPM sites than predicted while application of *B. thuringiensis* to site IPM3 was far more frequent than expected. Consequently, direct comparison between management practices was not possible. However, significant natural enemy activity was recorded at sites IPM1, IPM2, CAL2, IPM3 and CAL3 with combined predator and parasitoid diversity being particularly great at sites IPM1 and IPM2; farms where an integrated approach to insect pest management has been taken over several years. Estimated rates of predation and monitored natural enemy activity were consistently low at CAL1 over both years of the study indicating that pest management practices at this field site may have negatively impacted on natural enemy populations.

The great variation in the estimated rates of predation and the measured rates of parasitism illustrates the unpredictability of the impact of natural enemies on *P. xylostella* populations. However, at times the effect of natural enemies was extremely significant and further research is required to understand the processes underlying natural enemy activity so that they can be effectively incorporated into integrated pest management programs for the diamondback moth.

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Effect of parasitoid elimination on populations of diamondback moth in cabbage

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Abstract

An insecticidal exclusion method was used to assess the effect of parasitoids on level of infestation by the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), in cabbage. In field trials conducted at Rietondale, Pretoria, Gauteng Province and Brits, North-West Province, South Africa, a selective organophosphate pesticide, with systemic and contact action, dimethoate, was applied twice weekly to three plots in cabbage fields that had been divided into six plots. The three remaining plots served as controls. At weekly intervals, ten plants were randomly selected from each plot and thoroughly scouted for DBM infestation. Infestation levels were recorded and larvae, pupae and parasitoid cocoons were collected and taken to the laboratory. To determine parasitism levels all collected larvae were kept individually in Petri dishes with fresh cabbage leaves, and pupae and parasitoid cocoons were kept individually in glass vials until either parasitoids or moths emerged. Incidences of parasitism were high in the control plots, peaking above 90% on several occasions. The fauna of DBM parasitoids was rich; during the study period 23 species of parasitoids and hyperparasitoids were identified. The most abundant parasitoids were the larval parasitoid *Cotesia plutellae* (Kurdjumov), the larval-pupal parasitoid *Oomyzus sokolowskii* (Kurdjumov), the pupal parasitoid *Diadromus collaris* Gravenhorst, and the hyperparasitoids *Mesochorus* sp. and *Pteromalus* sp. Egg parasitoids were not recorded. At both sites, infestation levels in the sprayed plots were significantly higher than those in the control plots. On the other hand, parasitism levels of DBM in the control plots were significantly higher than in the treated plots. It was concluded that the higher infestation level of cabbage by DBM in the sprayed plots was because of partial elimination of parasitoids by the pesticide.

Keywords

Plutella xylostella, Cotesia plutellae

Introduction

Diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is the most damaging insect pest of cole crops in the world (Talekar & Shelton 1993). It has developed resistance to all major classes of chemical pesticides (Talekar *et al*. 1985) and to the bacterial insecticide *Bacillus thuringiensis* (Tabashnik *et al*. 1990). Early in the past century, DBM was studied in South Africa by Gunn (1917) where it was considered to be an important pest of cole crops (Annecke & Moran 1982). However, its pest status in South Africa is much lower than in other countries with similar climates (Kfir 1996). Ullyett (1947) studied the natural enemies of DBM in the Pretoria area in the 1930s and recorded eleven parasitoids, several predators and the fungus, *Zoophthora radicans* Brefeld (Zygomycetes: Entomophthorales) (recorded as *Entomophthora sphaerosperma* Fres.). He concluded that DBM is well controlled by its natural enemies in South Africa. Because of the low pest status of DBM in South Africa, almost no research has been conducted on the pest for almost 60 years since Ullyett's work. A renewed interest started after farmers reported outbreaks of DBM in cabbage fields and difficulties controlling the pest with insecticides. It was shown that because of indiscriminate use of insecticides by farmers in South Africa, local field populations of DBM started to show signs of resistance to synthetic pyrethroids, organophosphates and carbamates (Sereda *et al*. 1997). In further studies, Kfir (1996, 1997a) recorded 22 species of parasitoids and hyperparasitoids of DBM larvae and pupae in South Africa. Because of the large number of indigenous plants from the Brassicaceae in South Africa, on which DBM can develop, and the large number of parasitoids of DBM in the region, Kfir (1998) speculated that DBM might have originated in southern Africa. This is in contradiction with the widely accepted theory that DBM had originated in the Mediterranean region of Europe, and spread around the world with the cultivated brassicas (Hardi 1938, Harcourt 1954).

The only published study on the effect of eliminating parasitoids on DBM populations is by Lim *et al.* (1986) in Malaysia. The results showed that *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) the only important parasitoid at the time of the study, could contribute significantly to the control of DBM. The

parasitoid fauna of DBM in South Africa is richer, more diverse and normally a higher proportion of the pest population is parasitised, compared with the situation in Malaysia (Kfir 1997b, Lim 1986). However, no published studies are yet available on the effect of parasitoids on DBM populations in South Africa or in other parts of Africa. High parasitism levels of larvae and pupae of DBM have been recorded in unsprayed cabbage crops in various parts of South Africa (Kfir 1997b, Waladde *et al.* 2001), but the effectiveness of parasitoids in reducing damage to crops by reducing DBM populations remained unknown.

The aim of this study was to examine the effects of removing parasitoids from a cabbage crop on DBM populations in South Africa. Experimental data on the effect of parasitoids are important for their conservation as resident natural enemies for the control of DBM.

Materials and methods

A plot of 1100 m^2 was transplanted on 29 July 1998 with cabbage, *Brassica oleracea* var. *capitata* L. of the cultivar Green Star at Hartebeespoort Agricultural Research Farm near Brits (25°38'S; 27°47'E; elevation 1102 m), North-West Province, South Africa. An identical plot was transplanted on 8 August 1998 at Rietondale Experimental Farm in Pretoria (25°44'S, 28°13'E, elevation 1333 m), in Gauteng Province, South Africa.

Previous studies at Hartebeespoort near Brits indicated that the number of diamondback moths caught in pheromone traps and DBM larval infestations in cabbage were low from January to August and much higher during September to December, peaking during the spring months of September-October (Kfir 1997b). The planting dates in this study were chosen to coincide with high populations of DBM in the field to ensure maximum natural infestations. Each plot was divided into six subplots of 160 m^2 each. The remaining planted area served as buffer between the treated and untreated plots.

To suppress natural enemies, a selective insecticide, dimethoate, an organophosphate compound with both systemic and contact action (emulsifiable concentrate 400 g/L active ingredient) at a concentration of 4 mL per 10 litres water, was used. Agral® was added as a wetting agent. In California, dimethoate was used to suppress predators in cotton plots, which in turn caused an increase in abundance of *Spodoptera exigua* Hübner (Eveleens *et al*. 1973) and *Trichoplusia ni* Hübner (Ehler *et al*. 1973). This was an indication that dimethoate suppresses insect natural enemies, but causes no harm to Lepidoptera. Dimethoate was sprayed twice weekly with a knapsack sprayer on three subplots, whereas the remaining three subplots served as controls. Spraying started two weeks after transplanting the cabbage seedlings in the field and lasted until the trials were terminated.

Ten plants were selected randomly from each subplot and thoroughly scouted for DBM larvae, pupae and parasitoid cocoons. Samples were taken to the laboratory where all live larvae were kept individually in Petri dishes. Pupae and parasitoid cocoons were kept individually in glass vials (2.5 x 10 cm) stoppered with cotton wool. Larvae were provided with fresh cabbage leaves, which were replaced every third day, until larvae pupated or parasitoid cocoons formed. Collected pupae were kept until either parasitoids or moths emerged. Insects were held in the laboratory at 23°C ± 2°C, 60 ± 5% RH. All emergent parasitoids were identified and their incidence calculated. Larvae that escaped or died of unknown causes were disregarded for calculating rates of parasitism. For presentation of results, the data from the three subplots of each treatment were pooled.

For the duration of the trials, three delta-shaped sex-pheromone traps were deployed in each site to monitor the flight pattern of male moths. In the traps, sticky floors coated with a layer of polybutene adhesive were used to trap the moths. The sticky floors were replaced weekly when the traps were examined and moth catches recorded. The pheromone dispensers were placed in the middle of the sticky floor within the metal trap (26 x 9.5 x 13 cm high). The dispensers were replaced once a month.

All voucher specimens have been deposited in the National Collection of Insects, Biosystematics Division, ARC-Plant Protection Research institute, Pretoria.

Statistical analysis

The data were analysed using the statistical program GenStat (GenStat Committee 2000).

A t-test between two independent samples (Snedecor & Cochran 1967) was used to indicate significant differences between DBM population levels (larvae and pupae) on sprayed and control plots at Brits and Rietondale.

The difference between the proportions of infested plants in the sprayed and the unsprayed plots, and the difference between the proportions of parasitised DBM in the sprayed and the unsprayed plots was tested by using the Generalized Linear Model (GLM) (Dobson 1990) with the binomial distribution. The GLM with the binomial distribution was used because the proportions of infested plants and the proportions of parasitised DBM were not normally distributed.

Results

At Brits, the number of male moths caught in the pheromone traps increased gradually from around 4 moths/trap/week in July, peaked during the second half of October at 45 moths/trap/week and then declined to very low levels during December (Figure 1a). At Rietondale, the number of moths caught was very low during September, increased sharply from the end of October to reach a peak of 60 moths/trap/week in the middle of December, and declined sharply to low levels during February (Figure 1b).

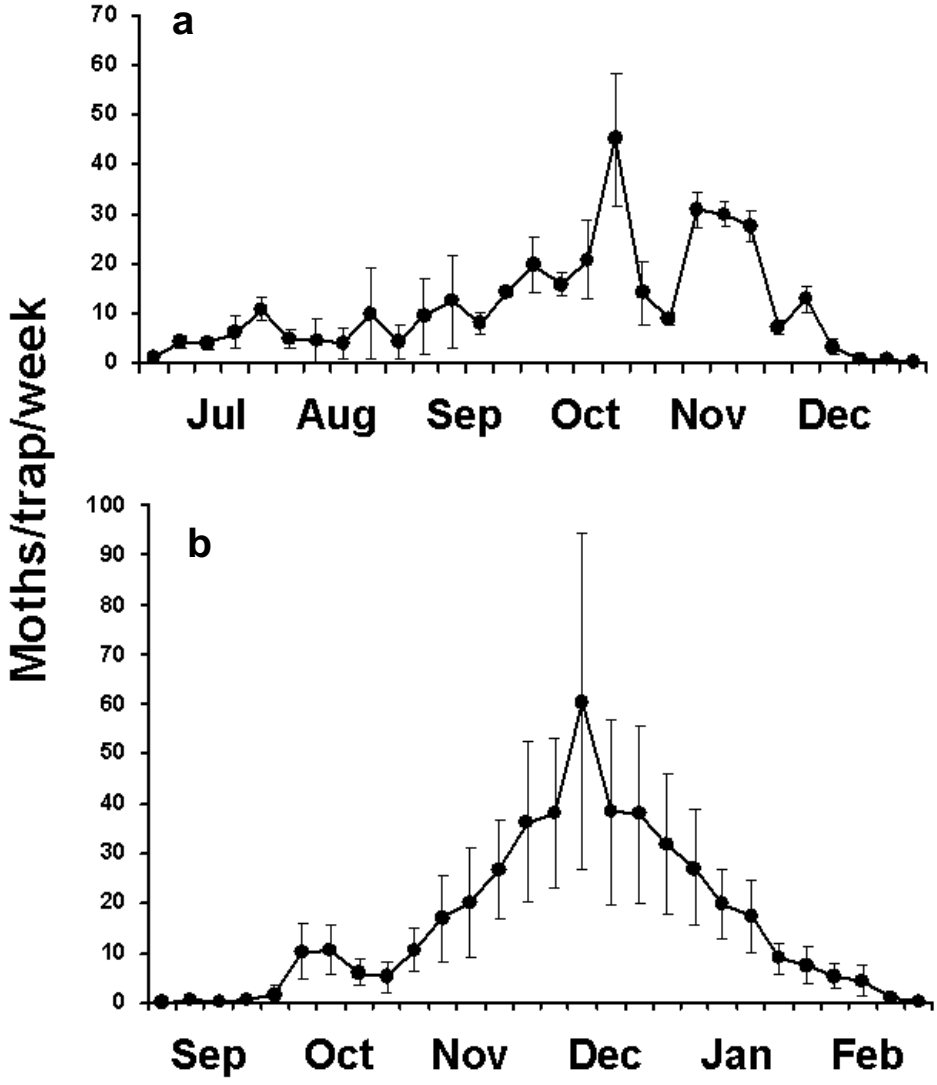

Figure 1. Sex pheromone trap catches of diamondback moth, *Plutella xylostella,* male moths at a. Brits and b. Rietondale, South Africa. Bars represent standard errors (SE) when larger than symbol size.

The peaks of moth flights (Figure 1) coincided with the peaks of larval infestations on the crops (Figure 2). At Brits, populations were relatively low during August and the first half of September, around 1 DBM/plant, and then increased rapidly from the second half of September, peaking during the second half of October at 40.7 and 12.4 DBM/plant in the sprayed and control plots, respectively (Figure 2a). At

Rietondale, populations were low during September and the first half of October increasing rapidly in the sprayed plots to peak at 27.7 DBM/plant during the second half of December. In the control plots, populations fluctuated around two DBM/plant peaking at 4.7 DBM/plant at the same time as the treated plots (Figure 2b).

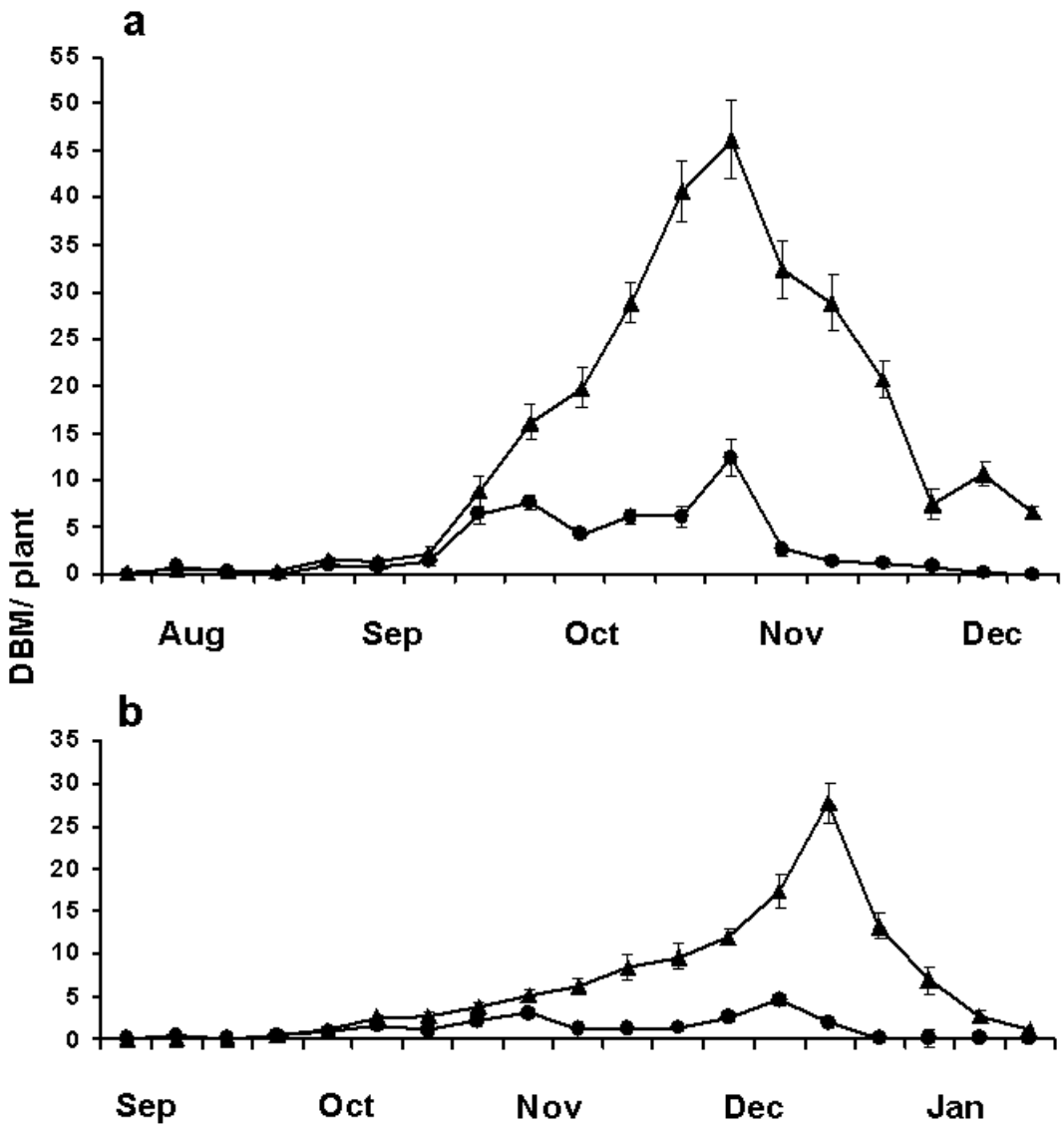

Figure 2. Abundance of diamondback moth, *Plutella xylostella*, larvae and pupae on sprayed (triangles) and control (circles) cabbage at a. Brits and b. Rietondale, South Africa. Bars represent standard errors (SE) when larger than symbol size.

At the two study sites, population levels of DBM on the sprayed plants were significantly higher than on the control plants (Figure 2). At Brits, a total of 8,205 DBM larvae and pupae were collected from the sprayed plants and 1607 from the control plants (t=-16.59, 4 df, $P<0.001$). At Rietondale, 3,648 DBM were collected from the sprayed plants compared with 734 DBM from the control plants (t=-16.28, 4 df, $P<0.001$).

The proportion of infested plants in the sprayed plots at the two sites was higher than in the unsprayed plots (Figure 3). At Brits, 100% of infested plants was recorded in the sprayed and the control plots. However, in the sprayed plots (seasonal mean of 81.8%) this was reached by the second half of September and lasted for the remaining of the season, a period of 12 weeks. In the control plots (seasonal mean of 56.7%), however, 100% infestation was also recorded by the second half of September, but it lasted only to the second half of October, a period of five weeks, and then declined rapidly (Figure 3a). At Rietondale, proportion of infested plants in the sprayed plots (seasonal mean of 73.5%) peaked at 100% from the middle of November to early in January whereas in the control plots (seasonal mean of 52.4%) proportion of infested plants peaked at about 93% in the middle of December (Figure 3b).

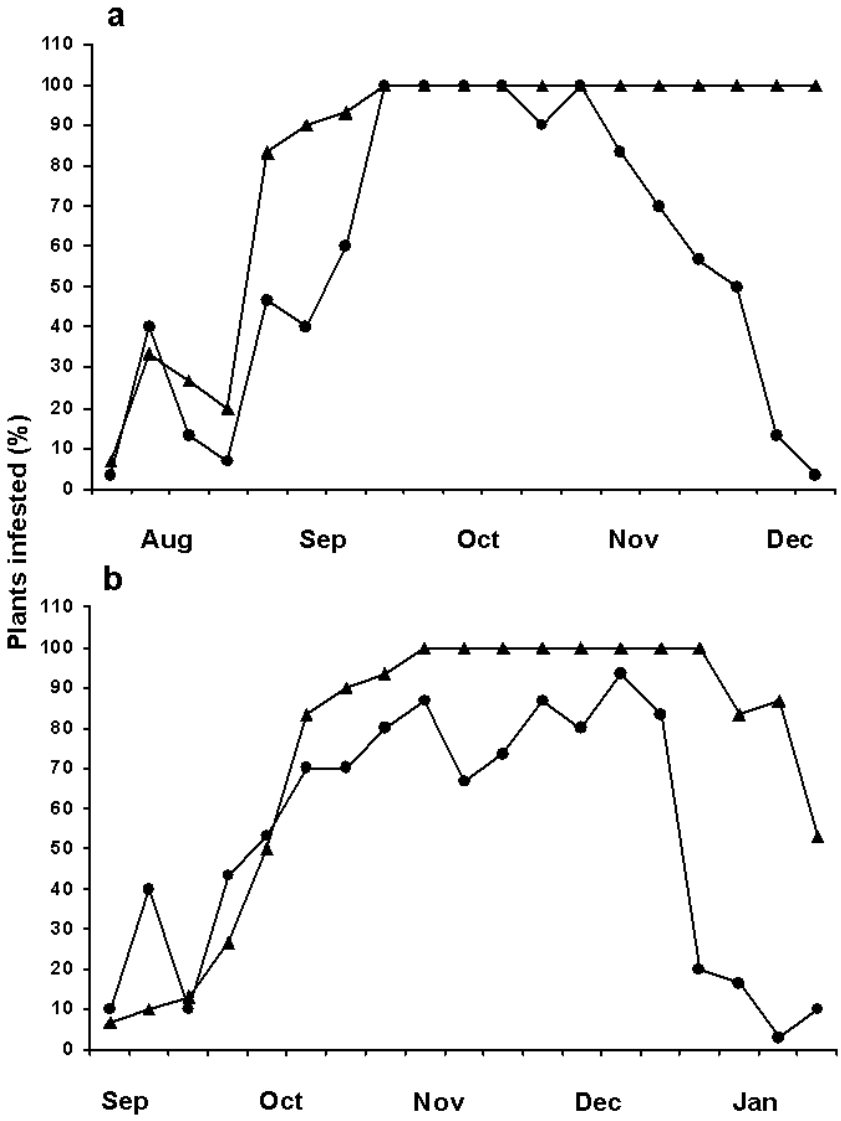

Figure 3. Proportion of sprayed (triangles) and control (circles) cabbage plants infested by diamondback moth, *Plutella xylostella,* larvae and pupae at a. Brits and b. Rietondale, South Africa.

The GLM analysis indicated that at Brits, the sprayed cabbage plants were about three and a half times (3.426) more likely to be infested with DBM than the unsprayed plants, whereas at Rietondale the sprayed plants were two and a half times (2.515) more likely to be infested than the unsprayed plants. At both sites the sprayed and control plots were highly significantly different (P<0.001) (Table 1).

Table 1. Mean proportions and standard errors of the mean (SEM) of cabbage plants infested by diamondback moth, *Plutella xylostella*, in control and sprayed plots at Brits and Rietondale, South Africa

	Brits		Rietondale	
	Mean proportion	**SEM**	**Mean Proportion**	**SEM**
Control plots	0.5667	0.0208	0.5246	0.0209
Sprayed plots	0.8175	0.0162	0.7351	0.0185

Percent parasitism of DBM at both sites throughout the season was higher on the unsprayed plots (Figure 4). At Brits, in the sprayed plots, percent parasitism fluctuated around 5% throughout the season (seasonal mean of 4.9%). In the control plots, however, parasitism levels increased rapidly to above 90% towards the end of the season (seasonal mean of 65.9%) (Figure 4a). At Rietondale, parasitism in the sprayed plots fluctuated around 10% with a peak of 17.9% in middle of December (seasonal mean of 12.8%) (Figure 4b)

coinciding with peak of larval infestation (Figure 1b). In the control plots, parasitism rose quickly and remained high (70-95%) from the middle of November to the middle of January (seasonal mean of 64.9) (Figure 4b).

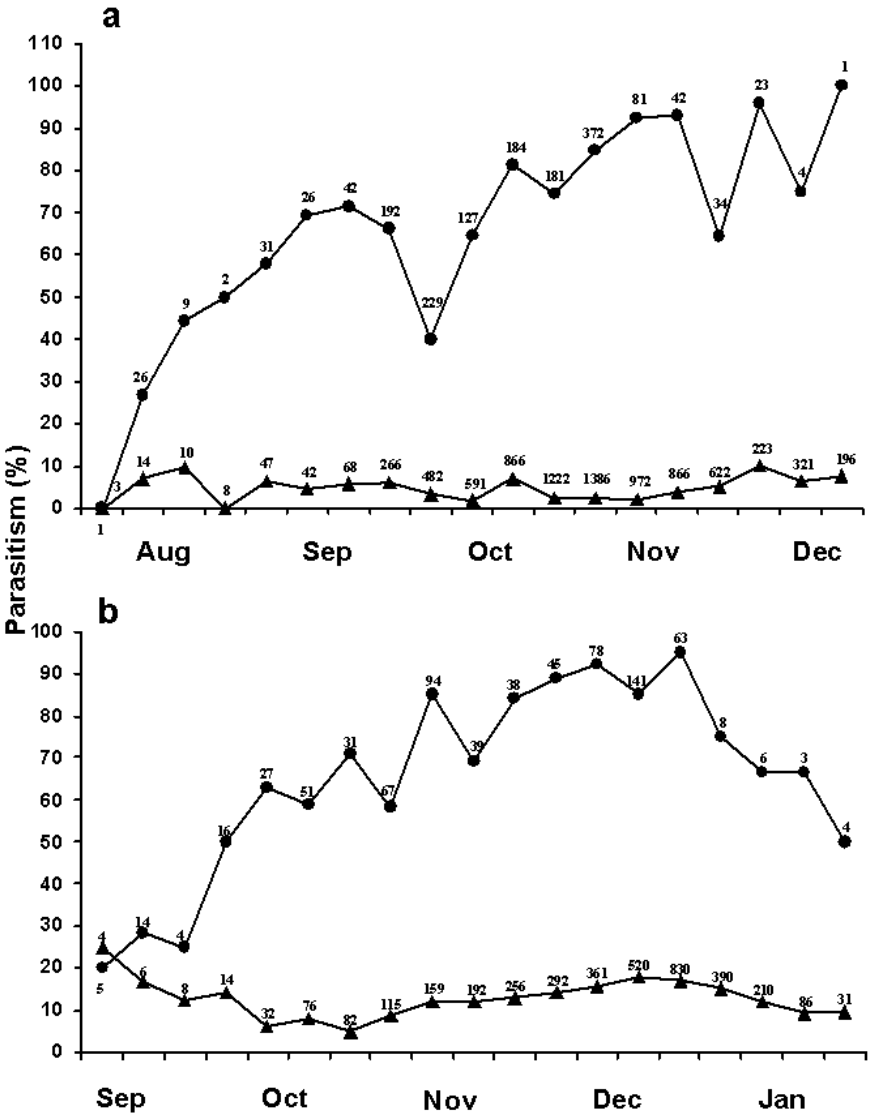

Figure 4. Percentage parasitism of diamondback moth, *Plutella xylostella,* larvae and pupae on sprayed (triangles) and control (circles) cabbage at a. Brits and b. Rietondale, South Africa. Numbers represent sample size.

At Brits, the GLM analysis indicated that DBM infesting the sprayed plants were about 59 times (1/0.01704) less likely to be parasitised than DBM infesting control plants. At Rietondale, insects on sprayed plants were 20 times (1/0.0497) less likely to be parasitised than on control plants. At both sites the proportions of parasitised DBM in the sprayed and unsprayed plots were significantly different (P<0.001) (Table 2).

Table 2. Mean proportions and standard errors of the mean (SEM) of parasitised diamondback moth, *Plutella xylostella*, infesting cabbage plants in control and sprayed plots at Brits and Rietondale, South Africa

	Brits		Rietondale	
	Mean Proportion	**SEM**	**Mean Proportion**	**SEM**
Control plots	0.7096	0.0113	0.7725	0.0155
Sprayed plots	0.0400	0.0022	0.1444	0.0058

No egg parasitoids were recorded in the current study. Two egg-larval parasitoids were recorded; *Chelonus curvimaculatus* Cameron and *Chelonus* sp. (Hymenoptera: Braconidae). The most abundant larval parasitoid was the solitary endoparasitoid, *Cotesia plutellae*. Other larval parasitoids were *Apanteles halfordi* Ullyett (Hymenoptera: Braconidae), *Cotesia* sp., *Habrobracon brevicornis* (Wesmael) (Hymenoptera: Braconidae) and *Peribaea* sp. (Diptera: Tachinidae). Recent taxonomic studies suggest that *A. halfordi* is a senior synonym of *Apanteles eriophyes* Nixon, a matter that will be dealt with in the taxonomic literature (G.L. Prinsloo, personal communication). This species is specific to *P. xylostella* and is known only from South Africa (Walker & Fitton 1992). Three larval-pupal parasitoids were recorded. The most abundant was *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae), which is the only gregarious primary parasitoid *of P. xylostella*. *Oomyzus sokolowskii* occasionally acted also as a hyperparasite and emerged from cocoons of *C. plutellae.* The other larval-pupal parasitoids were *Diadegma mollipla* (Holmgren) (Hymenoptera: Ichneumonidae) and *Itoplectis* sp. (Hymenoptera: Ichneumonidae). Recently, Azidah *et al*. (2000) taxonomically revised the species of *Diadegma* attacking *P. xylostella* and reported that *D. mollipla* is an Afrotropical species occurring also on some Indian and South Atlantic islands. It is also a parasitoid of the potato tuber moth, *Phthorimaea operculella* (Zeller). Broodryk (1971) studied the biology of *D. mollipla* in South Africa. The most abundant pupal parasitoid was *Diadromus collaris* Gravenhorst (Hymenoptera: Ichneumonidae). Other pupal parasitoids were *Brachymeria* sp. and *Hockeria* sp. (Hymenoptera: Chalcididae), *Tetrastichus howardi* (Olliff) (Hymenoptera: Eulophidae) and an unidentified ichneumonid. *Tetrastichus howardi* is an introduced species in South Africa (Kfir *et al*. 1993). Hyperparasitoids were active when *P. xylostella* populations were high and primary parasitoids abundant. The most abundant hyperparasitoids were *Mesochorus* sp. (Hymenoptera: Ichneumonidae) and *Pteromalus* sp. (Hymenoptera: Pteromalidae). Both emerged from cocoons of their primary parasitoid hosts. Other hyperparasitoids were *Aphanogmus fijiensis* (Ferrière) (Hymenoptera: Ceraphronidae), *Eurytoma* sp. (Hymenoptera: Eurytomidae), *Tetrastichus* sp. (Hymenoptera: Eulophidae), *Hockeria* sp., *Brachymeria* sp. and *Proconura* sp. (Hymenoptera: Chalcididae). All these hyperparasitoids are solitary except *A. fijiensis.* Between four to seven *A. fijiensis* emerged from each cocoon of *C. plutellae* or *A. halfordi*.

Discussion

Ullyett (1947) and Kfir (1997a, 1997b) have studied the parasitoids of DBM in South Africa. Le Pelley (1959), Kibata (1997) and Oduor *et al*. (1997) have studied them in Kenya. A very rich fauna of indigenous parasitoids was recorded from DBM larvae and pupae in South Africa (Kfir 1997a). High parasitism levels, often above 90%, were recorded on unsprayed cabbage crops in North-West Province (Kfir 1997a) and in the Eastern Cape Province (Waladde *et al*. 2001) of South Africa.

During this study it was revealed that although dimethoate had substantial adverse effects on the parasitoids' populations it could not completely eliminate them. Similar observations were reported from the eastern Cape Province of South Africa where parasitism by *C. plutellae* was observed in cabbage plots treated regularly with chemical insecticides such as methamidophos, mercaptothion, cypermethrin and others (Waladde *et al*. 2001). This might indicate some level of tolerance or resistance by the local populations of *C. plutellae* in South Africa to chemical pesticides. In Malaysia, Lim *et al*. (1986) recorded similar results when Sevithion (carbaryl + malathion) was sprayed on a cabbage field and did not completely eliminate the *C. plutellae* population.

Methods for evaluation of natural enemies have been developed, i.e. introduction and augmentation, cages or other barriers, removal of natural enemies, prey enrichment, direct observations and evidence of feeding (Luck *et al*. 1988). However, these methods are unique to interactions of particular parasitoid-host or predator-prey species groups (Luck *et al*. 1988). These methods are more suitable for the assessment of the effects of natural enemies of insects that form large colonies such as scale insects, aphids, whiteflies and mealybugs. Evaluation of parasitoids of Lepidoptera on the other hand is more complicated because of the relative low densities and the mobility of the pests and their parasitoids. Removal of natural enemies with insecticides, first described as the insecticidal check method by DeBach (1946), is a good experimental technique for evaluating the efficacy of natural enemies (Luck *et al*. 1999). It can be used to determine the level of control provided by parasitoids (Jones 1982, Kenmore *et al.* 1984, DeBach & Rosen 1991). The technique was used successfully in West Africa to evaluate the efficacy of the introduced parasitoid, *Apoanagyrus lopezi* (De Santis), in controlling the cassava mealybug, *Phenacoccus manihoti* Matile-Ferero without affecting the mealybug (Neuenschwander *et al*. 1986).

Insecticidal applications can stimulate Lepidoptera populations by altering the plant physiology. This could result from affecting the photosynthetic rate of plants (Jones *et al*. 1983) making them more attractive oviposition sites for Lepidoptera such as *Helicoverpa zea* Boddie and *Heliothis virescens* Fabricius. Although this aspect cannot be ruled out completely, there are no indications that the sprayed plots in this study attracted more oviposition by DBM than the control plots.

The five-fold increase in the DBM population levels in the treated plots after the partial removal of the parasitoids indicates that parasitoids play an important role in curtailing DBM populations in South Africa and without their activities, annual yield losses would be much higher.

Conclusion

The findings from the current study clearly demonstrate that the parasitoid complex in South Africa contributes significantly to the control of DBM. The higher infestation level of cabbage by DBM in the insecticide-treated plots was caused by partial removal of parasitoids.

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Integration of biological control and botanical pesticides - evaluation in a tritrophic context

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Abstract

The plant kingdom is by far the most efficient producer of chemical compounds, synthesising many products that are used in defence against herbivores. Extracts made from some plants, particularly extracts from plants within the Meliaceae (mahogany) family, have been shown to have insecticidal properties. We investigated the potential of these extracts and the possibility of integrating botanical pesticides with biological control of the diamondback moth, *Plutella xylostella*. Sub-lethal doses of botanical extracts were prepared from leaves of the syringa tree (*Melia azedarach*) and commercial preparations (Neemix 4.5®) from the neem tree (*Azadirachta indica*). In "no-choice" tests, bioassay trays were used to test the impact of three different doses on first- instar larvae. In "choice" tests, half a leaf was treated with extract and the other half left untreated. The impact that these extracts had on natural enemies was investigated using two parasitoid species, *Cotesia plutellae* and *Diadromus collaris*. Results indicated that these extracts had a significantly negative impact on first-instar larvae of *P. xylostella*. However, the extracts had no direct negative impact on their parasitoids. Therefore, it appears that biological control and botanical pesticides can be combined to control *P. xylostella*.

Keywords

tritrophic interactions, *Melia azedarach*, *Azadirachta indica, Plutella xylostella*, *Diadromus collaris*, *Cotesia plutellae*

Introduction

The plant kingdom is by far the most efficient producer of chemical compounds, synthesising many products that are used in defence against herbivores. Extracts prepared from plants have a variety of properties including insecticidal activity, repellence to pests, antifeedant effects, insect growth regulation, toxicity to nematodes, mites and other agricultural pests, also antifungal, antiviral and antibacterial properties against pathogens (Prakash & Rao 1986, 1997).

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), continues to present one of the greatest threats to crucifer production in many parts of the world, sometimes causing more than 90% crop loss (Verkerk & Wright 1996). Pesticides have dominated attempts to control *P. xylostella* for more than 40 years (Syed 1992, Shelton *et al.* 1997). Largely because of the negative impact of pesticides and the increasing difficulty encountered in controlling diamondback moth populations, much effort has been devoted to finding alternative control measures for this pest.

Biological control is widely recognised as a major component of *P. xylostella* management strategies particularly where control with chemicals has failed. A wide range of parasitoids has been associated with *P. xylostella*. Over 50 egg, larval and pupal parasitoids have been recorded in the literature. However, as a sole method of pest control in a specific target crop, biological control is seldom sufficient (Hokkanen 1997). Therefore the requirements of biological control must be integrated with the needs and uses of other control tactics such that a synergistic outcome is obtained.

Investigations into alternative control mechanisms for *P. xylostella* have led to the testing of plant extracts. Of the 1,800 plant species reported by Grainge *et al.* (1984) to possess pest control properties, only 82 species have been reported to be active against *P. xylostella* (Morallo-Rejesus 1986). Plants within the Meliaceae (the mahogany family), Asteraceae, Fabaceae and Euphorbiaceae contain most of the insecticidal plant species reported. Extracts from the neem tree, *Azadirachta indica* A. Juss. (Meliaceae) have been made from seeds and kernels and have been found to give good control of *P. xylostella* (Schmutterer 1997, Verkerk & Wright 1993, Prijono & Hassan 1993). A closely related species, the syringa tree, *Melia*

azedarach L. (Meliaceae) also has insecticidal properties (Ascher *et al*. 1995) and has been tested against a number of insect species including *P. xylostella*. These botanical pesticides are thought to be compatible with biological control as they have little or no impact on natural enemy species.

Plutella xylostella was recorded as a pest on cabbage in South Africa as early as 1917 (Gunn 1917). Twenty-three species of parasitoids and hyperparasitoids have been reported to attack *P. xylostella* in the field (Ullyett 1947, Kfir 1998). However, control of crucifer pests in South Africa is heavily dependent on insecticides, despite the abundance of natural enemies in the country. Cabbage is an important subsistence crop in South Africa (Bell & McGeoch 1996), and it is estimated that 80% of small-scale rural farmers that have access to water are growing cabbage. Although *A. indica* does not grow in South Africa, the closely related exotic species, *M. azedarach* is common in this country.

Many farmers in developing countries do not have the resources to buy and apply chemical pesticides. Biological control in the form of locally abundant natural enemies and botanical pesticides that can be easily prepared from local trees are free to the farmer and, therefore, uniquely suited to low-input integrated pest management systems. We investigated the impact of extracts prepared from *M. azedarach* and commercial extracts from *A. indica* on *P. xylostella,* with the aim of integrating these botanical pesticides with biological control.

Materials and methods

The plant extracts

Leaves were collected from *M. azedarach* at Rietondale in Pretoria, South Africa (28°15'S; 25°44'E). The leaves were placed in a glasshouse (30°C ± 5°C) and left to dry. The leaves were then crushed into a fine powder and stored in an air-tight container until use. Three different extracts were prepared by using different weights of crushed leaves, 1 g, 3 g and 5 g. Each extract was made with 100 mL of distilled water. The water was heated to 48°C, and the leaves were added to the water and shaken for approximately one minute. The extract was left in a refrigerator overnight. The following morning the extract was filtered using Advantec® filter paper no. 2. Three drops of liquid detergent were added to the final extract to act as a wetting agent.

A commercial preparation of *A. indica,* Neemix 4.5®, was provided by Thermo Trilogy Corporation, Columbia, USA. Three different sub-lethal doses were prepared. 10.7 µl (low), 16 µl (medium) and 32 µl (high) per 100 mL of distilled water. These doses are thirty, twenty and ten times below the recommended doses respectively. The control used consisted of 100 mL of distilled water mixed with three drops of liquid detergent.

Experimental plants and insects

Cabbages, *Brassica oleracea* var. *capitata* L. (Cruciferae) were bought as seedlings and planted in black plastic bags in a glasshouse at 30°C ± 5°C. *Plutella xylostella* larvae were taken from a culture started in 1993, in which several hundred larvae were collected from *B. oleracea* var. *capitata* in the field in Rietondale and an experimental farm near Brits (25°38'S; 27°47'E), South Africa. The laboratory culture is maintained on canola seedlings, *Brassica napus* L. (Cruciferae), however, for these experiments, hatchlings were removed from the canola and placed on cabbage for 24 hours before being exposed to the experimental plants. The two parasitoid species most common in the field were *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae), and *Diadromus collaris* Gravenhorst (Hymenoptera: Ichneumonidae). These two species were chosen for the experiments and maintained in culture in the laboratory. Insect rearing and all laboratory experiments were maintained in a controlled environment (24 ± 2°C; 65% r.h., 16:8 L:D).

No choice test

Leaf discs (30 mm x 35 mm) were cut from the cabbage plants. The leaf discs were placed in a randomised block design in bioassay trays (440 mm x 210 mm). The bioassay trays had 32 cells (4 x 8 cells). The leaf discs were cut to fit into these cells. Four blocks were used and each block had eight treatments. The treatments consisted of the three extracts prepared from *M. azedarach*, the three extracts prepared from *A. indica*, and two control treatments. The leaves were dipped into the treatment and left to dry for approximately 60 minutes. First instar larvae were used in the experiment. One larva was exposed in each cell. The bioassay tray was sealed with "Bio CV 4" vented covers to prevent the larvae from escaping. Six trays were placed randomly on the laboratory bench for each test. The test was repeated six times. The trays

were checked every two days and the leaves replaced. The number of dead larvae or pupae and the number of moths emerging were recorded. Larvae that survived were weighed at the pupal stage.

Choice test

One half of the leaf was treated with plant extract or the control treatment and one half was left untreated. The treated side was marked with a felt tip pen. Seven treatments were used: the three extracts from *M. azedarach*, the three extracts prepared from *A. indica* and one control. The treated half of the leaf was dipped into the extract solution and left to dry for approximately 60 minutes. Entire leaves approximately the same size (± 6 cm diameter) were used. Each leaf was placed into a Petri dish with a lid (9 cm diameter). For each treatment 10 leaves were used. The treatments were arranged randomly on the laboratory bench. The test was repeated six times. One first-instar larva was placed in the Petri dish, the dish was sealed and the position of the larva was recorded every hour during daylight.

Parasitoids

A single parasitoid was placed in a test tube with some honey and a strip of filter paper (6 cm x 1 cm). Seven treatments were used: the three extracts prepared from *M. azedarach*, the three extracts prepared from *A. indica*, and one control treatment. The filter paper was dipped in the treatments and was replaced every two days. For each test, ten parasitoids were exposed to each treatment and the tests were repeated six times for *C. plutellae* and five times for *D. collaris*.

Statistical methods

Data were analysed using the Genstat 5 statistical package, version 4.2 (Genstat 5 Committee 2000).

For the no-choice test, mortality was calculated as the proportion of larvae that died out of the total that survived to become moths. Proportions usually follow a binomial distribution, therefore differences between mortality for the different treatments were tested using the generalised linear model (GLM) with binomial distribution (Dobson 1990). Fisher's protected t-test of least significant differences (LSD) was applied to separate the mean proportions at the 5% level of significance. An unbalanced analysis of variance was carried out for the pupal weights, and once again Fisher's protected t-test of least significant differences (LSD) was applied to separate the treatment means at the 5% level of significance. For the choice test, a one sample chi-square test was used and comparisons were made between treatments. An unbalanced analysis of variance was used to analyse the impact of the extracts on the parasitoids. Each parasitoid species was analysed separately.

Results

No choice test

When the overall proportion of dead larvae was compared for the different treatments, results showed that there were significant differences between the treatments ($F_{6,35}$= 48.58, P<0.001). It is not the intention to compare the *A. indica* and the *M. azedarach* treatments as the doses are not comparable. Therefore we compared differences within the treatments.

Survival of larvae feeding on *M. azedarach*.

Results from Fisher's protected t-test showed that there were significant differences between the three doses. Survival of larvae was highest on the control treatment. Approximately 64% of the larvae feeding on the control treatment survived to the moth stage, followed by 58% of the larvae feeding on the 1 g treatment. These differences were not significant (P=0.252). Approximately 25% of the larvae feeding on the 3 g survived to the moth stage and only 17% of the larvae feeding on the 5 g treatment survived to the moth stage, these differences were also not significant (P=0.125). However, survival on the control and the 1 g treatment was significantly higher (P<0.001) than survival on the 3 g and 5 g treatments (Figure 1).

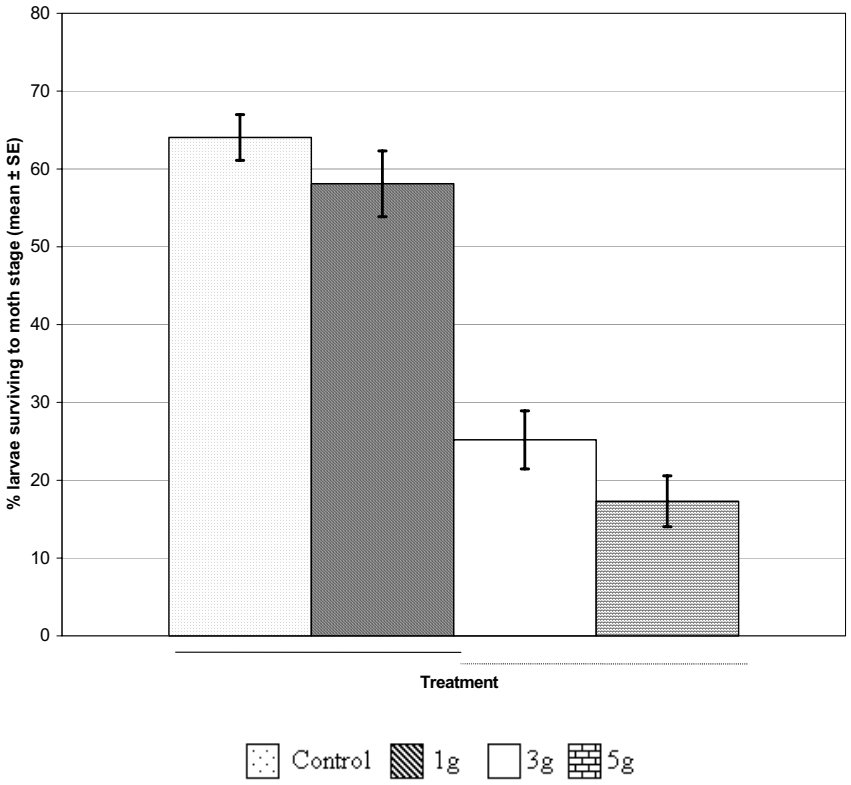

Figure 1. Percentage of larvae surviving to the moth stage after feeding on leaves treated with *Melia azadarach*. Treatments underlined are not significantly different.

Survival of larvae feeding on *A. indica*

Results from Fisher's protected t-test showed that there were significant differences between the treatments and the control and also between the different doses. Survival of larvae feeding on the control treatment was significantly higher (P<0.001) than on the other treatments. Approximately 64% of the larvae feeding on the control survived to the moth stage. Approximately 17% of the larvae feeding on the medium treatment survived to the moth stage and 13% feeding on the low treatment made it to adulthood, these two treatments were not significantly different (P=0.353). Only 3% of the larvae feeding on the high treatment made it to the moth stage, this was significantly lower than all the other treatments (low P=0.009, medium P=0.002) (Figure 2).

Average weight of the pupae found on different treatments

The statistical analysis indicated that there were significant differences ($F_{6,178}$=6.48, P<0.001) between the different treatments.

Weight of pupae found on *M. azedarach*

Results from Fisher's protected t-test showed that the larvae which had been feeding on the control treatment were significantly (P<0.001) heavier as pupae than those larvae, which had been feeding on the treated plants. The pupae that were found on the 1 g treatment were also significantly heavier than those found on the 5 g treatment (P=0.015). There were no significant differences between the pupal weights of those larvae that had been feeding on the 3 g or 1 g treatment (P=0.183), nor between those feeding on the 5 g and 3 g treatments (P=0.273) (Figure 3).

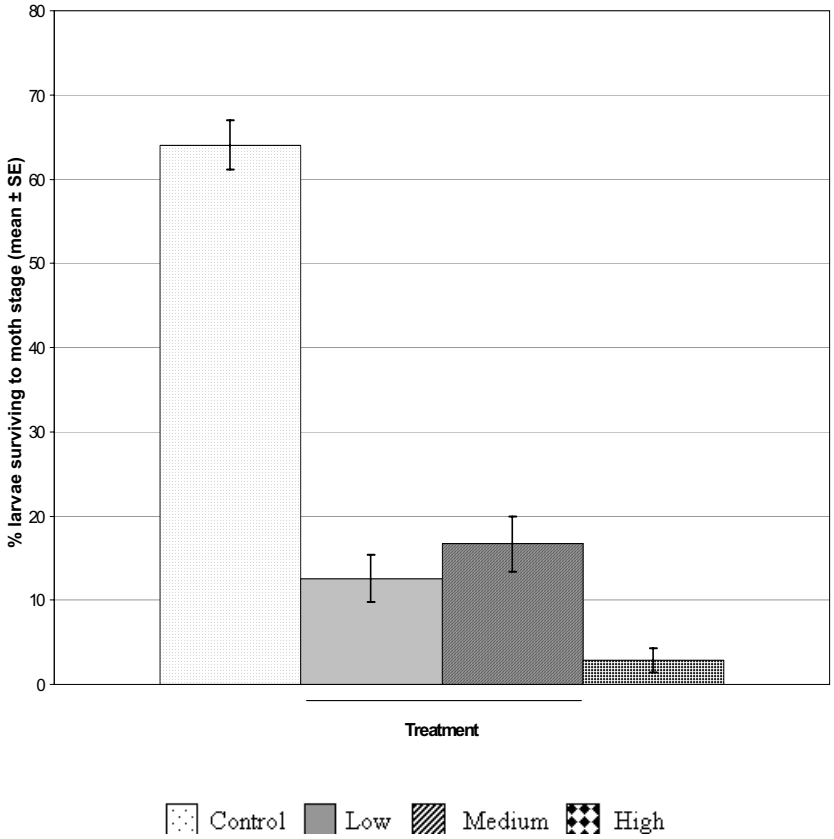

Figure 2. Percentage of larvae surviving to the moth stage after feeding on leaves treated with *Azadirachta indica*. Treatments underlined are not significantly different.

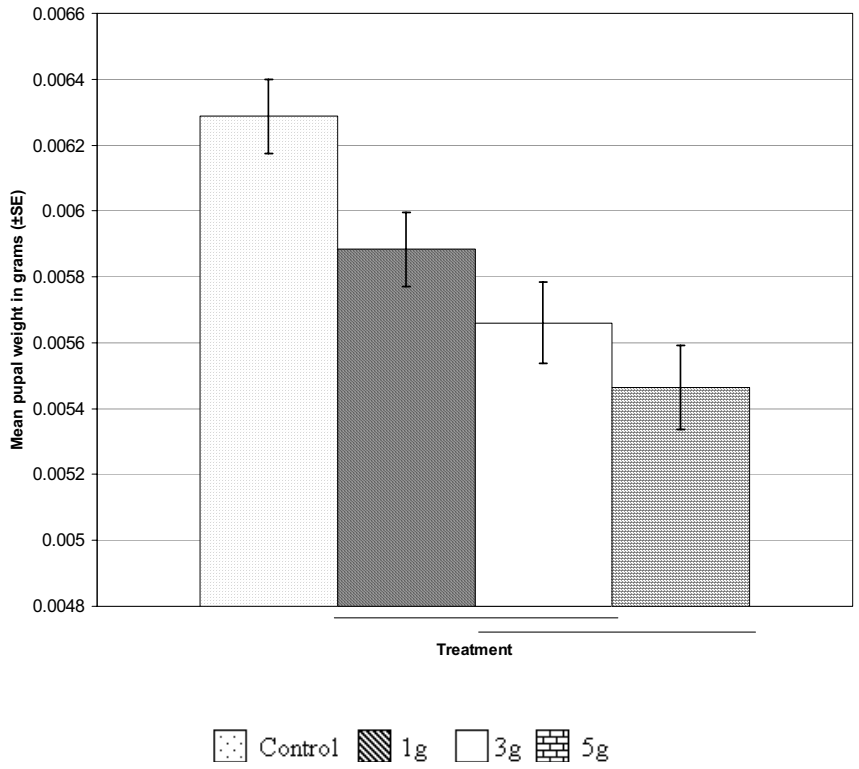

Figure 3. Average weight of pupae found on leaves treated with *Melia azedarach*. Treatments underlined are not significantly different.

Weight of pupae found on *A. indica*

Results from Fisher's protected t-test showed that there were significant differences between the pupal weights of larvae that had been feeding on the different treatments. Those larvae that had been feeding on the control treatment were significantly (P<0.001) heavier as pupae than those that had been feeding on the treated plants (Figure 4).

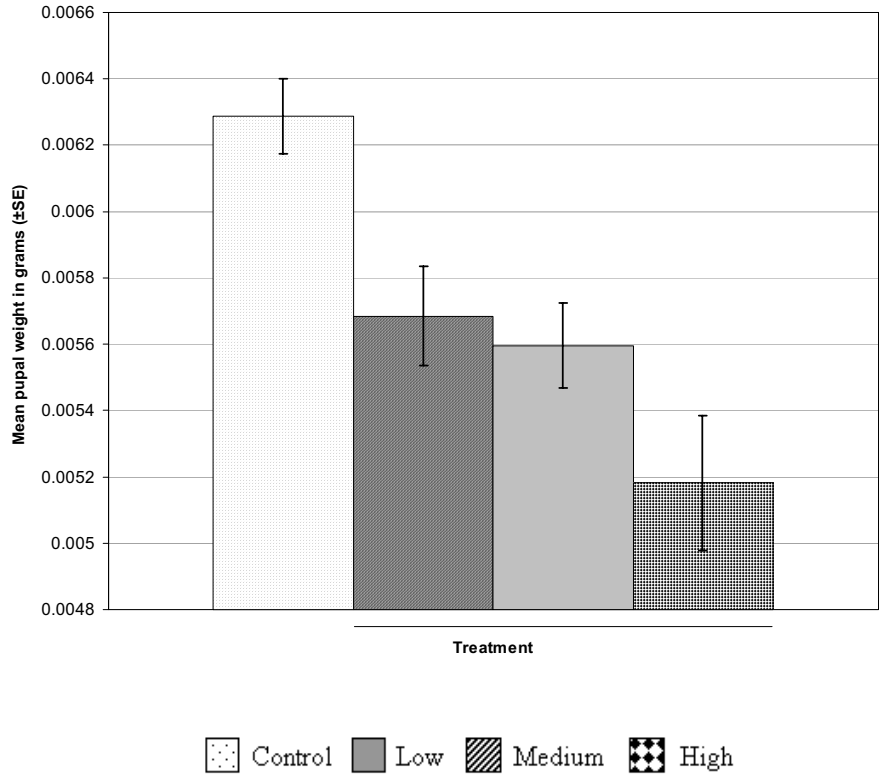

Figure 4. Average weight of pupae found on leaves treated with *Azadirachta indica*. Treatments underlined are not significantly different.

Choice tests

A one sample Chi-squared test was done comparing the number of times the larva was found on the treated side of the leaf with the untreated side of the leaf. The larvae spent significantly more time on the untreated side of the leaf if the plants had been treated with the extract (Figures 5 & 6). However, for the control leaf, which had been treated with the distilled water mixed with the liquid detergent, the differences between the number of times that the larvae spent on either the treated side or the untreated side of the leaf was not significant (χ^2=0.251, P=0.6230). On occasion, the larvae were found on the Petri dish, or died before the trial could be completed.

Parasitoids

Results from the analysis of variance indicated that there were no significant differences between the survival of the parasitoids exposed to the different extracts ($F_{6,383}$=1.14, P=0.341 - *C. plutellae*; $F_{6,290}$=0.44, P=0.852 - *D. collaris*), and in fact they lived for a slightly longer period on the treated strips of filter paper.

Mortality was high at the beginning of the period, with a few individuals surviving for longer periods. Among *C. plutellae*, mortality had reached approximately 50% by the fifth day, with a maximum survival of 34 days for one individual. *Diadromus collaris* is a much longer-lived parasitoid. Once again the plant extracts did not appear to have any significantly negative impact on the parasitoid, with mortality reaching approximately 50% by the 18th day, with a maximum survival of 184 days for one individual.

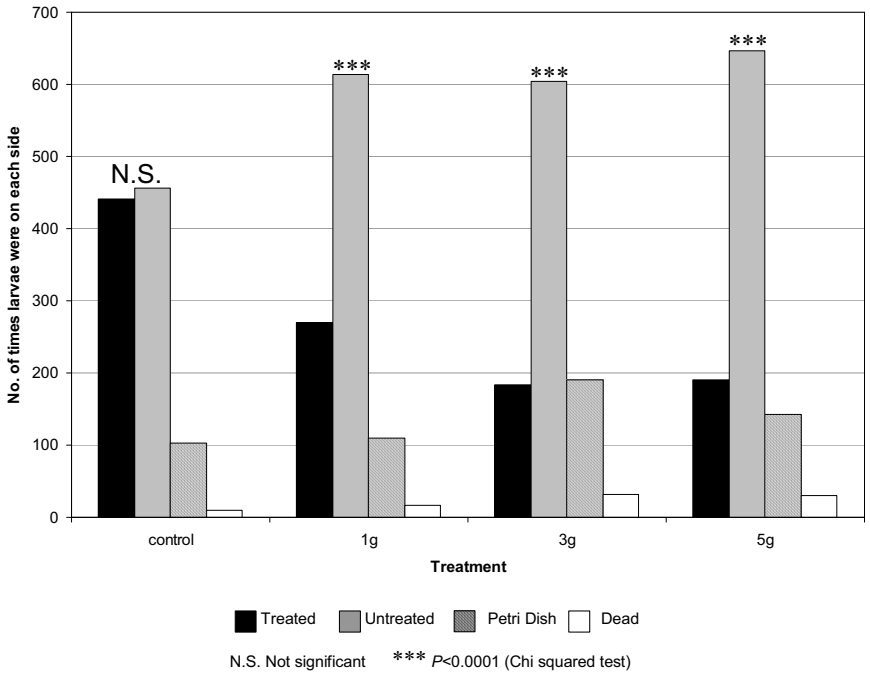

Figure 5. Number of times larvae were found on each side of leaves treated with *Melia azedarach,* or found on the Petri dish, or dead.

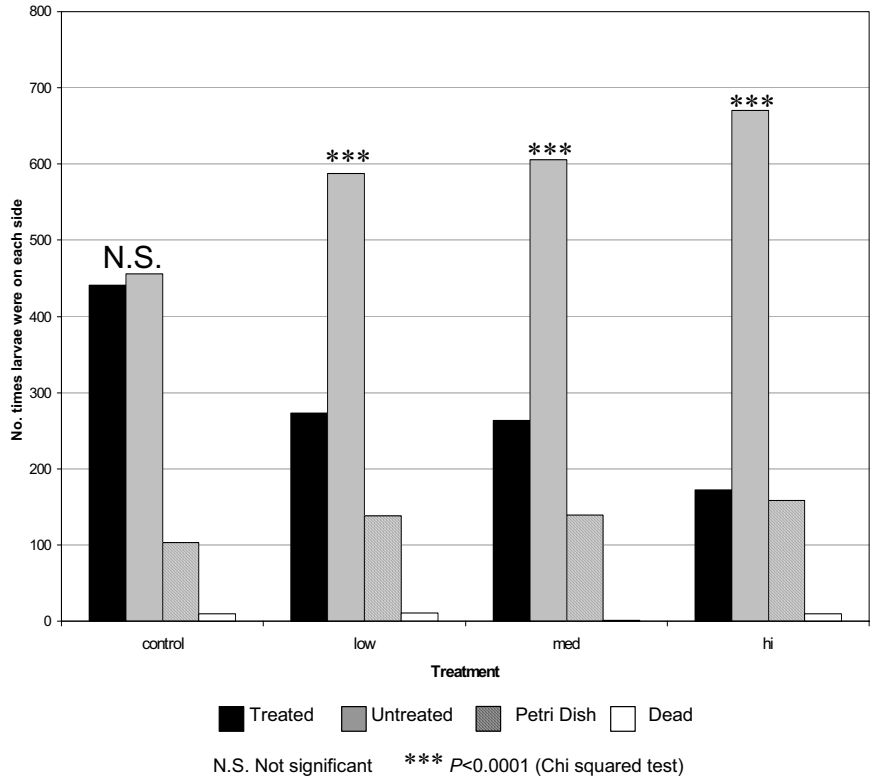

Figure 6. Number of times larvae were found on each side of leaves treated with *Azadirachta indica,* or found on the Petri dish, or dead.

Discussion

Plants have evolved a variety of ways to defend themselves from invading organisms. From a phytocentric perspective, the chemical options available to a plant could simply be described as repel, deter or kill (Renwick 1996). Members of the plant family Cruciferae are chemically linked by the almost universal presence of glucosinolates, a class of sulfur-containing glycosides, also called mustard oil glycosides or thioglycosides. These compounds are considered the first line of defence of crucifers against insects and other organisms (Renwick 1996). However, some insects, such as *P. xylostella*, have adapted to this line of defence and are crucifer specialists that make use of the glucosinolates and their volatile hydrolysis products

to recognise or locate suitable host plants. The presence of secondary chemical substances such as glucosides and volatile mustard oils make crucifers a highly phagostimulant food substrate (Fagoonee 1981), causing increased feeding of the crucifer specialist.

In contrast, the complex tetranortriterpenoids found within plants from the Meliaceae family are thought to be feeding deterrents (Jacobson 1981). Deterrent chemicals play an important, if not major role, in host plant selection by phytophagous insects (Morgan 1981). In terms of secondary plant chemistry, the Meliaceae is best characterised by the production of limonoids, a group of modified triterpenes. The neem tree contains upwards of 100 different limonoids in its different tissues (Isman *et al*. 1996). Many of these are biologically active against insects as anti-feedants. There has been ample testimony, of a semi-scientific or folklore form, as to the insecticidal, repellent or deterrent qualities of the neem tree, *A. indica*, and the closely related syringa tree, *M. azedarach* (Morgan 1981). In this study we investigated the possibility of using extracts from these trees against the crucifer specialist *P. xylostella*.

Results indicated that neem and syringa extracts were effective against *P. xylostella*, significantly reducing the survival of larvae feeding on cabbage leaves treated with these extracts. When larvae were given a choice they preferred to remain on the untreated side of a leaf. Once the cabbage is treated with neem and syringa extracts, it no longer acts as a phagostimulant. The neem and syringa extracts appear to mask the inherent attractive property of the cabbage plant to the larvae. Similar results have been found for other crucifer specialists. *Crocidolomia binotalis* Zell. (Lepidoptera: Pyralidae) no longer fed on leaves that had been treated with neem extracts (Fagoonee 1981). Zhu (1991) investigated biological effects of syringa extracts on four species of lepidopteran cabbage pests, *Pieris brassicae* L., *Pieris rapae* L. (Lepidoptera: Pieridae), *P. xylostella* and *Mamestra brassicae* (L.) (Lepidoptera: Noctuidae). At low concentrations, the extract caused a disturbance of metamorphosis of IV instars of *P. xylostella* and of various larval instars of the other three species at higher concentrations. *Pieris brassicae* and *P. xylostella* were more susceptible than the other two pests to these extracts. Zhang and Chiu (1983) tested extracts from seed kernels of syringa and found that a 2% extract gave an anti-feeding rate of 74% in choice tests and 76% in no-choice tests for *P. rapae*. When sprayed on the leaves of Chinese cabbage exposed to I instar larvae of *P. rapae*, the extract caused 75% mortality at a concentration of 5 000 ppm and 20% mortality at a concentration of 1 000 ppm. The authors consider the mortality to be due to both feeding inhibition leading to starvation and to stomach poisoning. The results from our current study appear to support this.

Biologically active substances show effects on target organisms but also side effects on non-targets. Consequently neem and syringa products being medium to broad spectrum biochemicals for pest control are not free of side effects on non-targets, but at the same time, these effects are as a rule relatively slight and therefore tolerable, especially in IPM (Schmutterer 1995). Parasitoids are in general less sensitive to neem products than are predators and sometimes even favoured by neem application, for example, when their hosts become more easily accessible for parasitism (Schmutterer 1995). In this study we did some initial trials looking at the direct impact of neem and syringa extracts on two parasitoid species, *C. plutellae* and *D. collaris*. Results indicated that these extracts do not have any direct negative impacts on these two species. However, some negative effects have been observed on growth of parasitoid larvae, weight of pupae and adults and longevity (McCloskey *et al.* 1993). In small species, negative influences have been recorded on emergence rate, walking and searching ability, longevity and fecundity (Feldhege & Schmutterer 1993). Further studies are currently underway to investigate these aspects within *C. plutellae* and *D. collaris*.

Repellents and attractants modify the behavioural response of insects. This is the basis for the principle of behavioural insect control, whereby a given species is either attracted to a bait, or pheromone; or repelled from a host plant by a repulsive agent (Fagoonee 1981). Any factor that selectively influences the production of either deterrent or stimulant could directly influence the direction in which the balance is tipped. We hope to use botanical pesticides to modify the behaviour of both the pest *P. xylostella*, and the parasitoids, *C. plutellae* and *D. collaris*. Results from this study are the first step in this direction, and indicate that syringa and neem extracts may play a role in altering the attractive properties of crucifer plants to *P. xylostella*. At the same time, these extracts appear to have no direct negative influence on *C. plutellae* and *D. collaris*, two important parasitoid species abundant in the field in South Africa. Further experiments are underway to investigate the impact that these extracts have on the semiochemical properties of cabbage plants and how these extracts may influence the behaviour of the pest and its parasitoid species.

Results from this study will help in understanding the tritrophic relationships that are important in the integrated pest management of *P. xylostella*. It is hoped that the results will be used to aid the small-scale

rural farmers in South Africa, and elsewhere, through the enhancement of biological control and a reduction in the use of chemical pesticides.

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Evaluation of two neem insecticides for non-target effects on the larval parasitoids of the diamondback moth, *Plutella xylostella* (L.)

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Abstract

The effects of two neem insecticide formulations (an oil-free powder formulation and an oil formulation) on field parasitism of diamondback moth, *Plutella xylostella* (L.), larvae and, on the longevity and foraging behaviour of *Diadegma mollipla* (Holmgren), were evaluated in field and laboratory tests. Overall, larval parasitism in plots sprayed with the oil-free neem formulation was not significantly different from that in the water-sprayed plots over the entire observational period, but parasitism in plots receiving the oil formulation was significantly lower during two weeks. Field parasitism by two individual parasitoid species, *D. mollipla* and *Oomyzus sokolowskii* (Kurdjumov) showed substantial differences in their relative response to the neem treatments. In laboratory tests, longevity and foraging behaviour of *D. mollipla* was not affected by contact with the neem insecticide sprays. The results indicate that the neem products may be relatively safe to larval parasitoids of the diamondback moth.

Keywords

Diadegma mollipla, *Oomyzus sokolowskii*

Introduction

Crucifers are important vegetables in Kenya, providing necessary micronutrients as well as income to small-scale farmers, the main producers. The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is the most serious pest on Crucifers worldwide and also the most difficult to control (Talekar & Shelton 1993). Unilateral reliance on chemical insecticide control is no longer viable because of the pest's capacity to develop insecticide resistance and spiralling costs of newer, effective insecticides. Current control strategies for DBM place greater emphasis on the integrated use of biorational products such as neem-based insecticides (Schmutterer 1992) and larval parasitoids (Talekar 1992, Verkerk & Wright 1996). Several studies have demonstrated the efficacy of neem insecticides against DBM (Dreyer 1987, Schmutterer 1992, Javaid *et al*. 2000). Neem insecticides are also reported to be safer to natural enemies (Schmutterer 1990, 1995, 1997). In Kenya, Neemros® (0.5% azadirachtin) and Neemroc EC® (0.03% azadirachtin) are two neem insecticide formulations derived from the neem tree (*Azadirachta indica* A. Juss) that have been registered for use against pests in horticulture, including the DBM. Neemros® is an oil-free neem kernel cake powder (NKCP) formulation while Neemroc EC® is a neem seed oil (NSO) formulation. Formulation, among other factors, determines the extent to which a neem-based insecticide will affect organisms (Schmutterer 1995) and thus there is a need to test new formulations, both for their efficacy against the pest as well as for their side effects on natural enemies. The objective of this study was to evaluate the effect of the two neem products on field parasitism rates of DBM. Bioassays were also conducted to assess longevity and foraging behaviour in *Diadegma mollipla* (Holmgren) (Hymenoptera: Ichneumonidae), a common larval parasitoid of DBM in Kenya, following direct sprays with the neem products.

Materials and methods

Treatments

Neemroc EC® (=NSO) and Neemros® (=NKCP) were applied as foliar sprays at the recommended field dose rate of 15ml/L and 25 g/L of water, respectively (Varela 1998, Waiganjo 1998). Water alone was used as a check.

Insects

Diadegma mollipla adults were obtained from an insectary colony that had been reared for no more than four generations on a DBM-cabbage (*Brassica oleracea* var. *capitata* cv. Copenhagen Market) system, at 22-

25ºC and 14L:10D photoperiod. Parasitoid adults were maintained on a 20% honey solution and distilled water.

Effects of the neem insecticides on field parasitism rates

A field trial was conducted in May to July 1999 at Juja (Kenya), which lies at longitude 37°00' E, latitude 1°05' S and approximately 1525 m above sea level, with a bimodal pattern of rainfall (856 mm per year). Four-week old cabbage seedlings were transplanted to plots measuring 4.2 x 4.4 m, at a spacing of 60 cm between and 40 cm within rows. The treatments were compared in a randomised complete block design replicated six times. Weekly applications of the treatments were made by high volume (hand-operated knapsack calibrated to deliver 100 L spray/ha) spraying, starting from the second week after transplanting (WAT) when the DBM population established naturally until the ninth WAT. Furrow irrigation was adopted to minimise interference to DBM establishment. Sampling and counting of DBM was done prior to the neem applications each week. To assess larval parasitism, at least thirty IV instar or prepupal DBM were collected each week from the remaining plants in each plot, for recording DBM infestation. The samples were reared in the laboratory until DBM or parasitoid adults emerged. Unemerged pupae were dissected to determine whether or not they had been parasitised. Percent parasitism for each week was determined as a proportion of the hosts sampled that were parasitised.

Longevity of neem-sprayed *D. mollipla*

Two-day old parasitoids were sprayed with 4-6 ml of aqueous solutions of NKCP, NSO or water. Daily records were taken of the number of wasps dying in each treatment.

Foraging behaviour of neem-sprayed *D. mollipla*

Two-day old naïve, mated parasitoid females were given the same treatment as in the previous experiment. After 24-36 h, a single parasitoid was introduced into a Perspex® cage containing a DBM-infested cabbage plant and its foraging behaviour on the plant continuously observed for 25 min using The Observer® software (Noldus 1995). Records were made of the number of hosts parasitised and duration of bouts of searching, oviposition and grooming during the 25 min period.

Data analysis

Parasitism data were arcsine transformed prior to analysis using Repeated Measures Analysis of Variance (PROC GLM, SAS Institute 1990). One-way ANOVA was used to test for differences in mean longevity, number of successful attacks and mean duration of bouts of searching, oviposition and grooming among the treatments. Where significance was indicated, means were separated at $P<0.05$ using the Student-Newman-Keuls (SNK) multiple range test.

Results

Effects of the neem insecticides on field parasitism rates

Overall (= all parasitoid species combined) parasitism in the treatments varied significantly over the periods (WAT) ($F= 3.09$, $df= 8, 36$, $P<0.05$). Overall larval parasitism did not differ significantly between NKCP and control plots in any individual period, but significantly lower parasitism levels were recorded in the NSO plots during the fifth and eighth WAT (Table 1).

Larval parasitism of DBM during the season was contributed by three parasitoid species: *D. mollipla, Oomyzus sokolowskii* Kurdjumov (Hymenoptera: Eulophidae) and *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae). Analysis of percent parasitism by the individual parasitoid species revealed no significant three-way interaction for periods, species and treatments ($F=1.63$, $df=16, 74$, $P>0.05$) or between periods and treatment ($F=1.58$, $df=8, 48$, $P>0.05$). However, a significant interaction was observed between periods and species ($F=14.44$, $df=8, 48$, $P<0.001$). Furthermore, parasitism levels were also significantly different between the species ($F=60.03$, $df=2, 27$, $P<0.001$) (Figure 1). Parasitism by *C. plutellae* was very low throughout the period, accounting for less than 2% of the parasitised hosts and so is not discussed further in this text. *D. mollipla* was the predominant parasitoid in the first week of observation in all treatments, but its rate of parasitism decreased thereafter to less than 5% from the fifth WAT. It did not show any significant differences between the treatments in any period. Parasitism by *O. sokolowskii* was initially low, but increased in subsequent periods. Parasitism level of this species did not differ significantly between

NKCP and control plots. However it was significantly lower in NSO plots when compared with control plots after the fourth WAT.

Table 1. Field parasitism of diamondback moth on cabbage receiving foliar sprays of two neem insecticide formulations at successive weeks after transplanting (WAT), Juja, Kenya, 1999

WAT	Percent larval parasitism (mean ± s.e)		
	NKCP	NSO	Water
2	-	-	-
3	27.1 ±2.8 a (n=41)	37.2 ±6.6 a (n=54)	32.7 ±4.9 a (n=53)
4	16.1 ±2.2 a (n=23)	12.7 ±3.4 a (n=18)	20.2 ±4.6 a (n=41)
5	24.5 ±2.4 ab (n=57)	6.7 ±6.7 b (n=4*)	22.0 ±3.4 a (n=73)
6	-	-	-
7	33.8 ±5.6 a (n=98)	24.0 ±4.5 a (n=26)	37.1 ±4.7 a (n=107)
8	31.3 ±3.1 a (n=83)	16.3 ±7.7 b (n=15)	33.6 ±1.9 a (n=65)

Within rows, means compare treatments and means with the same letter are not significantly different at $P<0.05$, SNK test. N=number of parasitised DBM collected. *some plots excluded in the analysis of data because no observations were made.

Longevity of neem-sprayed *D. mollipla*

The mean longevity of *D. mollipla* was not significantly different among the treatments (F=0.74, d.f.=2, 272, $P>0.05$) (Table 2).

Table 2. Mean longevity of *Diadegma mollipla* adults sprayed with a neem insecticide formulation or water

Treatment	N	Longevity (days) (mean ± s.e.)
NKCP	82	13.4 ± 1.04[a]
NSO	91	12.6 ± 0.96[a]
Water	102	14.2 ± 0.92[a]

N=number of parasitoids tested

Foraging behaviour of neem-sprayed *D. mollipla*

The number of hosts parasitised within the observation period was not significantly different among the treatments (F=0.44, d.f.=2, 25, $P>0.05$) (Table 3). Similarly, mean duration of a bout of searching, oviposition or grooming was not significantly different among the treatments ($P>0.05$).

Table 3. Number of hosts parasitised and duration of a bout for three components of foraging behaviour by neem- and water-sprayed *Diadegma mollipla* during a 25 min period on an infested cabbage plant

Treatment	# hosts parasitised (mean ± s.e.)	Duration (sec) of bout (mean ± s.e.)		
		Searching	Oviposition	Grooming
NKCP (N = 22)	6.3 ± 1.0 a	6.8 ± 0.65 a	11.8 ± 1.6 a	12.5 ± 1.59 a
NSO (N = 20)	8.0 ± 1.3 a	7.7 ± 0.50 a	10.4 ± 1.0 a	9.8 ± 1.31 a
Water (N = 26)	7.5 ± 1.4 a	6.8 ± 0.83 a	9.8 ± 1.0 a	9.5 ± 0.74 a

N=number of parasitoids individually observed. Means within a column compare the same parameter among the treatments

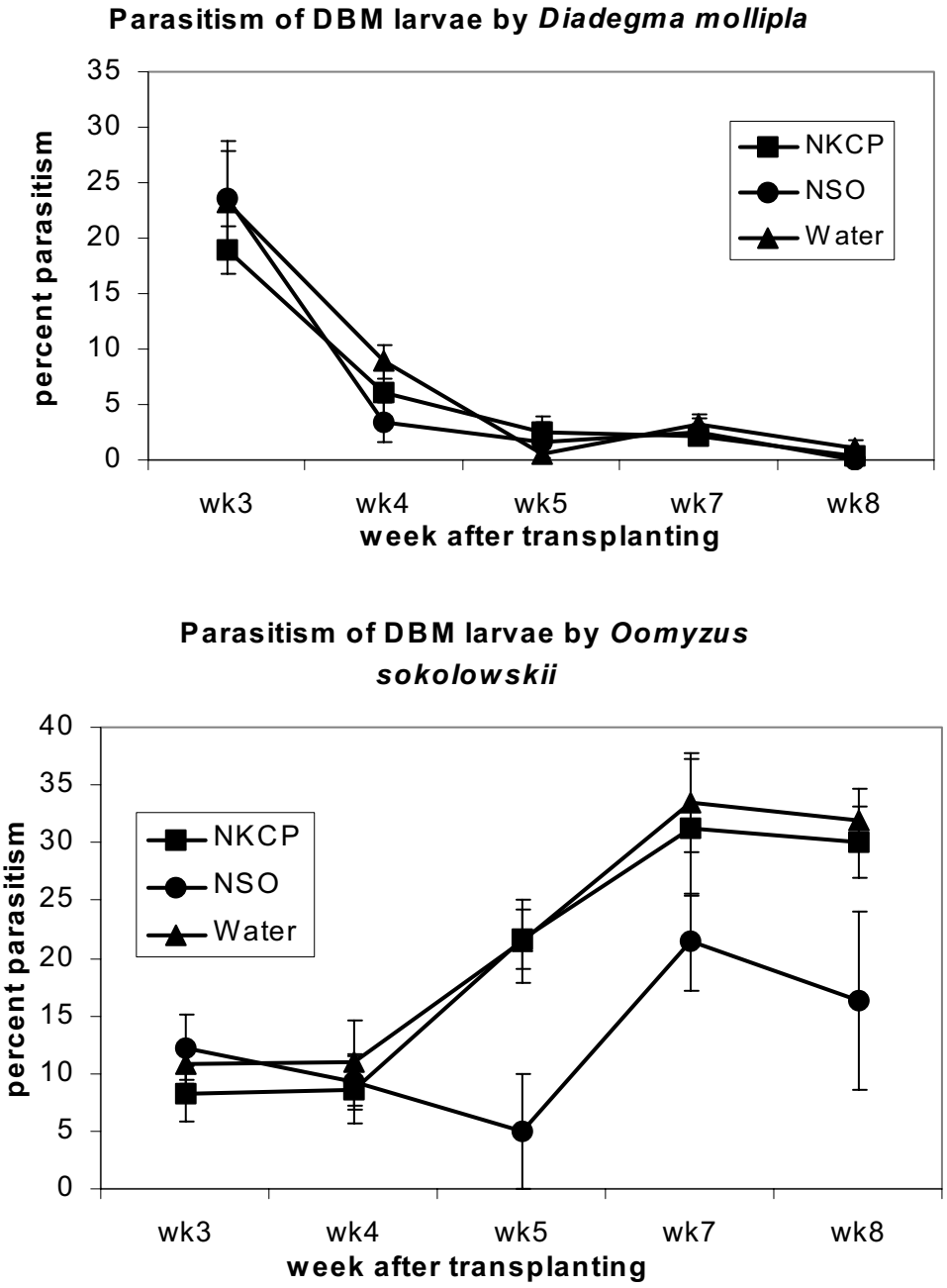

Figure 1. Field parasitism of diamondback moth larvae by two parasitoid species in cabbage receiving foliar sprays of two neem insecticide formulations. Error bars represent standard errors.

Discussion

This is perhaps the first report on the non-target effects of the two neem insecticide formulations on DBM larval parasitoids. It was evident that the oil-free NKCP formulation did not have any significant effects on overall field parasitism or parasitism by individual species. In contrast, NSO had a more profound negative impact on overall field parasitism and parasitism by *O. sokolowskii* in some periods. This finding is consistent with Schmutterer's (1995) observation that neem products containing a high percentage of neem oil tend to show stronger adverse effects on non-target organisms than oil-free products. The laboratory bioassays showed no adverse effect of the neem products on the longevity or foraging behaviour of *D. mollipla* at the concentrations tested. These results indicate that at the recommended field doses, the two neem insecticide products may be relatively safe to *D. mollipla*. The differential response of the two predominant larval parasitoid species (*D. mollipla* and *O. sokolowskii*) to the individual neem formulations indicates the need for monitoring individual key parasitoid species when assessing the non-target safety of neem insecticides, rather than depending on overall larval parasitism as the parameter.

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Effects of several insecticides on the larval parasitoid, *Cotesia plutellae* Kurdjumov, of diamondback moth, *Plutella xylostella* (L.)

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Abstract

Insecticide resistance in diamondback moth (DBM), *Plutella xylostella* (L.) is one of the most important problems in the management of DBM. To retard or avoid the development of insecticide resistance in DBM, it is becoming more important to preserve natural enemies. We evaluated the toxicity of several insecticides on cocoons and adults of larval parasitoid, *Cotesia plutellae* Kurdjumov and effects of insecticides on parasitism. Under the experimental conditions, most insecticides applied at recommended concentrations showed high insecticidal activity against adults, but were less toxic to cocoons. However, parasitism by surviving adults was seriously affected.

Keywords

parasitism, selectivity

Introduction

Diamondback moth (DBM), *Plutella xylostella* (L.), is one of the most notorious pests in the world because it can easily develop resistance to various types of insecticides (Miyata *et al*. 1986, Saito *et al*. 1995). To retard or avoid the development of insecticide resistance in DBM, the selection pressure of insecticides to DBM should be reduced by the rotational use of insecticides which do not show cross resistance. This strategy should be accompanied by monitoring of DBM resistance to insecticides and use of other management techniques such as the introduction of sex pheromone (Miyata 1989, Saito *et al*. 1996). It is well known that DBM populations can be controlled by natural enemies. Goodwin (1979) stated that there were more than 90 species of DBM parasitoids. In Thailand, Keinmeesuke *et al.* (1995) reported that the egg parasitoid, *Trichogramma confusum* Viggiani, the larval parasitoid *Cotesia plutellae* Kurdjumov and the pupal parasitoid, *Diadromus collaris* (Gravenhorst) were found in highland areas while the egg parasitoid *Trichogrammatoidea bactrae* Nagaraja and the larval parasitoid, *C. plutellae*, were found in lowland areas.

To preserve natural enemies by introducing selective insecticides is one of the most important strategies to retard or avoid the development of resistance of insect pests to insecticides (Saito *et al*. 1991). Kao and Tzeng (1992) evaluated toxicity of 17 commonly used insecticides to *C. plutellae*. Among them, seven insecticides were harmful (mortality >99%) to adults of *C. plutellae*, while the remaining 10 insecticides proved to be harmless (mortality <50%). In this paper, we examine the effects of several insecticides which have been recently introduced to Thailand, against the larval parasitoid, *C. plutellae*.

Materials and methods

Insecticides

Candidate insecticides used for toxicity tests on *C. plutellae* were Ascend® 5% SC (fipronil), Rampage® 10% SC (chlorfenapyr), Vertimec® 1.8% EC (abamectin), Polo® 25% EC (diafenthiuron) and Ripcord® 15% EC (cypermethrin). Recommended rates of application were 10 ml/L for fipronil, chlorfenapyr and abamectin, 30 ml/L for diafenthiuron and 15 ml/L for cypermethrin, respectively.

Rearing of *C. plutellae*

Potted common cabbage plants were used for mass rearing DBM larvae. Only the II instar larvae were used as the host larvae for parasitoid rearing. DBM eggs were collected on aluminium foil strips. Ten aluminium foil sheets were prepared at a time. Each sheet was crinkled to form parallel lines. Aluminium foil sheets were dipped into autoclaved cabbage juice (blend 130 g of cabbage leaf material in 1000 ml water and autoclaved at 120ºC, 2-3 atmospheric pressure for 20 minutes) and were allowed to air dry. Each sheet was

folded and cut into 30 strips 2.5 cm wide. These strips were stored in the refrigerator at 4ºC until used. Three strips were hung in the oviposition chamber from the lid. About 200 DBM moths were introduced into the chamber and fed with 10% sugar-water solution with yellow food colouring contained in a 50 ml flask. A cotton dental wick was inserted through the flask. Eggs were collected consecutively every 48 hours. The egg sheets were sterilized with 10% formalin for 30 minutes, rinsed under running tap water for 10 minutes and then allowed to air dry.

Egg mass strips were placed onto the cabbage plants for subsequent hatching of larvae. Cabbage plants were placed into a wooden cage covered with fine mesh screen (1 mm^2). The potted cabbage plants were covered with aluminium foil sheet just above soil surface level. Plants were watered daily. The II instar larvae of DBM were used for rearing and multiplying *C. plutellae*.

A potted cabbage plant with about 1000 II instar larvae of DBM was placed into the stock culture cage of *C. plutellae*. Freshly detached cabbage leaves were placed around the cabbage plant pot on the floor of the cage. This served as food for those larvae of DBM which fell from the plant. The larvae of DBM were withdrawn from the parasitoid cage at 24 hours after introduction to the parasitoid cage. The parasitised larvae of DBM were observed and transferred to parasitoid free cabbage plants for further rearing until pupation, from 8 to 10 days later. The cocoons of *C. plutellae* were collected using forceps. Meanwhile any surplus cocoons were stored in the refrigerator for delayed emergence without loss of cocoon viability and fecundity of emerging wasps.

Toxicity test of insecticides to adults of *C. plutellae*

The dry film method was employed as a test method (Kao & Tzeng 1992, Keinmeesuke *et al*. 1994). Five insecticides were applied at recommended dosage concentrations and a water-treated test tube was provided as a control. Each insecticide solution of 0.05 ml was added to a test tube of 3.5 cm diameter and 15 cm in height. All tests were replicated five times. The insecticide solution was distributed evenly around the inner wall of the test tubes and allowed to air dry. One adult *C. plutellae* (1-2 day old) was released into each test tube and fed with honey soaked cotton wool. Ten adults of unsexed *C. plutellae* were used for one treatment. Mortality was determined 24 and 48 hours after treatment. The effect of insecticides on *C. plutellae* and their selectivity was classified and categorized according to the IOBC/WPRS Working Group method (Hassan *et al*. 1985). The data obtained were statistically analysed by applying analysis of variance (ANOVA) and Duncan's multiple range tests (DMRT).

Effects of insecticidal toxicity on cocoons and parasitism by emerged adults of *C. plutellae*

Five candidate insecticides were diluted with water based on recommended dosage concentrations and 2 ml of each insecticide solution was sprayed onto a cocoon of *C. plutellae*. Cocoons treated with water were provided as a control. Each treatment was replicated five times. Adult emergence and dead adults at 24 hours after emergence were recorded daily. The mortality data were recorded and calculated by Abbott's formula. The obtained data were statistically analysed by applying ANOVA and DMRT.

The surviving adults were transferred to a cylindrical cage (30 cm height and 15 cm in diameter) and were fed with 10% honey-solution. One hundred II instar larvae of DBM were provided on the 2^{nd}, 4^{th}, 6^{th} and 8^{th} day after emergence. The number of DBM larvae parasitised was counted and calculated for each cage. The reduction in parasitism was measured by the following formula:

% reduction in parasitism = total % parasitism in control - total % parasitism in treated parasitoids) * 100

The experiments were conducted at 25˚C and 80% RH with 16L:8D photoperiod.

Results and discussion

Mortality of *C. plutellae* as a result of exposure to different insecticides is presented in Table 1. Fipronil and chlorfenapyr caused 100% mortality at 24 hours after treatment. Diafenthiuron caused 92% mortality at 24 hours and 100% mortality at 48 hours after treatment. Abamectin caused 68% mortality at 24 hours and 88% mortality at 48 hours. Cypermethrin caused 34% mortality at 24 hours and 58% at 48 hours after treatment.

Table 1. Toxicity of various insecticides to adults of *Cotesia plutellae* assessed by dry film method

Treatment	% Mortality after treatment		Rating
	24 hours	48 hours	
Ascend® 5% SC (fipronil)	100	100	4
Rampage® 10% SC (chlorfenapyr)	100	100	4
Vertimec® 1.8% EC (abamectin)	68	88	3
Polo® 25% EC (diafenthiuron)	92	100	4
Ripcord® 15% EC (cypermethrin)	34	58	2
Control	0	0	-

1 = harmless (< 50%), 2 = slightly harmful (50 – 79%), 3 = moderately harmful (80 – 99%), 4 = harmful (> 99%) (Hassan *et al*. 1985).

Based on the criteria suggested by Hassan *et al*. (1985), fipronil, chlorfenapyr and diafenthiuron were rated as harmful, whereas, abamectin and cypermethrin were rated as moderately harmful and slightly harmful to adults of *C. plutellae*, respectively. These results showed that adults of *C. plutellae* were extremely affected by fipronil, chlorfenapyr, diafenthiuron and abamectin. Therefore, these four insecticides will not be recommended for use in IPM programs that involve mass release of *C. plutellae* adults for biological control.

Our toxicity data for abamectin, which showed high mortality to adults of *C. plutellae*, were different from the report of Keinmeesuke *et al*. (1994). They demonstrated that at the rate of 2000 dilution times, abamectin (Agrimec® 1.8% EC) caused 0 and 20% mortality at 24 and 48 hours after treatment. On the other hand, they demonstrated at the rate of 2000 dilution times, cypermethrin (Sherpa® 25% EC) caused 53.5 and 76.7% mortality at 24 and 48 hours after treatment. The cause in the difference in mortality is not clear, but may be derived from the difference in formulations or differences in the *C. plutellae* populations used.

Mortality of emerged adults of *C. plutellae* when cocoons were treated with insecticides was shown in Table 2. Emergence of adults was not affected. However, emerged adults were partially affected within 48 hours after emergence. Based on the criteria suggested by Hassan *et al*. (1985), five candidate insecticides were considered as harmless to pupae of *C. plutellae*.

Table 2. Toxicity of various insecticides to cocoons of *Cotesia plutellae* by the spraying method

Treatment	% Adult emergence	% Mortality of adults after emergence[a]		Rating[b]
		24 hours	48 hours	
Ascend®	96	45 d	48b	1
Rampage®	100	44 d	44 b	1
Vertimec®	100	29c	30 ab	1
Polo®	100	14 b	16 a	1
Ripcord®	96	1 a	7 a	1
Control	100	0	0	-

[a]Means followed by a common letter are not significantly different at the 5% level by DMRT.
[b]1=harmless (<50%), 2=slightly harmful (50–79%), 3=moderately harmful (80–99%), 4=harmful (>99%) (Hassan *et al*. 1985).

The effect of insecticides on parasitism by *C. plutellae* at different intervals was shown in Table 3. Generally, at two days after emergence, *C. plutellae* showed the highest parasitism activity followed by 4, 6 and 8 days. Fipronil and chlorfenapyr were moderately harmful, abamectin was slightly harmful and diafenthiuron and cypermethrin were harmless.

Table 3. Effects of various insecticides on parasitism of DBM larvae by *Cotesia plutellae*

Treatment	% Parasitism after emergence[a]					% Reduction in parasitism
	2 days	4 days	6 days	8 days	Total	
Ascend®	5.2 d	1.4 d	0.6 d	0 d	7.2	92
Rampage®	14.2 c	7.2 cd	2.6 d	1.2 d	25.2	88
Vertimec®	18.6 c	14.2 c	9.2 c	5.2 d	47.2	78
Polo®	40.2 b	40.4 b	29.4 b	21.4 c	131.4	39
Ripcord®	47.4 b	47.0 b	33.2 b	28.0 b	155.6	28
Control	64.4 a	59.8 a	50.0 a	41.2 a	215.4	-

[a]Means followed by a common letter are not significantly different at the 5% level by DMRT

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Host resistance to an insecticide and selection at larval stage favour development of resistance in the parasitoid, *Cotesia plutellae*

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Abstract

Laboratory selection for fenvalerate resistance was conducted in two colonies of the parasitoid, *Cotesia plutellae,* reared on two colonies of its host, *Plutella xylostella,* which differed in resistance to the insecticide. In the selection regime, the insecticide was applied to the host larvae that harboured the parasitoid larvae. Compared with the unselected parasitoid colony, the parasitoid colony selected with the susceptible hosts acquired 4.5 fold resistance after 14 selection cycles, while the colony selected with the more resistant hosts acquired 13.6 fold resistance after 13 selection cycles. These results demonstrate that parasitoid larvae could be exposed to insecticide selection via the hosts, selection with more resistant hosts could accelerate development of resistance in the parasitoid and resistance genes selected during larval development could be expressed at the adult stage. Comparison of detoxifying enzymes between the insect colonies revealed that fenvalerate resistance was positively related to increased monooxygenase activity, but was unrelated to carboxylesterases and general esterase in both the host and the parasitoid, indicating that the two insects shared a major metabolic mechanism for resistance to fenvalerate. This information can help improve selection procedures for the development of insecticide resistance in parasitoids.

Keywords

insecticide resistance, selection method, fenvalerate

Introduction

The use of naturally or artificially selected insecticide-resistant strains of natural enemies has been advocated to enhance the compatibility of biological and chemical controls (Croft 1990). Some natural enemy populations have developed high levels of resistance to insecticides in the field and can survive field application rates (Rathman *et al.* 1990, Baker & Weaver 1993). Many attempts have been made to achieve genetic improvement of natural enemies for insecticide resistance through laboratory selection (Johnson & Tabashnik 1994). The most successful cases are strains of predatory phytoseiid mites that can survive insecticide applications in the field (Hoy *et al.* 1983, Whitten & Hoy 1999). Using laboratory selection, azinphosmethyl resistance was increased 7.5 fold in the aphid parasitoid, *Trioxys pallidus* Haliday (Hoy & Cave 1989).

While examples exist to illustrate the potential of natural enemies to develop insecticide resistance in the laboratory and field, documented cases of insecticide resistance in field populations of natural enemies are relatively rare (Croft 1990). Likewise, laboratory selections for insecticide resistance in natural enemies have had limited success, particularly with insect parasitoids (Johnson & Tabashnik 1994). Many authors have recognised the need to improve selection methods for more successes (Rosenheim & Hoy 1988, Whitten & Hoy 1999).

To date, in all selection programs with parasitoids, the insects were exposed at the adult stage to insecticides, usually as residue inside glass vials or as a toxicant mixed in sugar solutions (Johnson & Tabashnik 1994, Li & Liu 2001). While this method provides a simpler procedure than selection of immature stages, it has an obvious disadvantage of exerting selection on, in most cases, only female phenotypes because mating usually precedes selection and male genomes are transmitted randomly with respect to insecticide resistance throughout a selection regime. This random transmission of male genomes prior to each selection cycle may delay the selection response. It is also possible that immature stages have different responses to selection from adults. A possible advantage for selection with immature stages is to explore the use of host resistance in the process, as parasitoid eggs and larvae develop inside the hosts.

In this study, we investigated the selection responses of the larval stage of the parasitoid, *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae), to fenvalerate, using two host colonies that differed in

susceptibility to the insecticide. *Cotesia plutellae* is a major parasitoid of the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (Talekar & Shelton 1993, Liu *et al.* 2000). Our purposes were to determine the feasibility of selection at the larval stage of the parasitoid and whether host resistance to an insecticide would favour selection of resistance in the parasitoid.

Materials and methods

Host and parasitoid colonies: rearing methods

A fenvalerate-susceptible stock culture of DBM, originally collected from Wuhan, Hubei, China, has been maintained on rapeseed seedlings since 1995. In March 1999, approximately 1600 pupae were collected from this culture and randomly divided into two groups to start a non-selected and a selected colony of DBM on potted cabbage respectively (Table 1). For convenience, these founding pupae are referred to as Generation 0 and their progenies as Generation 1, etc.

A laboratory stock culture of *C. plutellae* was initiated with approximately 200 cocoons collected from a cabbage farm in Hangzhou, China in 1995. Every year 100–200 newly field-collected cocoons were added to the culture. Fenvalerate has been one of the major insecticides used in the collection area since the mid 1980s. In February 1999, approximately 2100 cocoons were collected from this stock culture and divided randomly into three groups of approximately 100, 1000 and 1000 respectively. The first 100 were used to initiate a non-selected colony of the parasitoid using the non-selected colony of DBM as hosts. The next two groups were each provided with approximately 9000 II and III instar larvae from the non-selected host colony to initiate the selected with non-selected host ("Selected-NH") and the selected with selected host ("Selected-SH") colonies respectively (Table 1). As for the host colonies, these founding wasps are referred to as Generation 0 and their progenies as Generation 1, etc.

Table 1. Establishment and maintenance of selected and non-selected colonies of *Plutella xylostella* and *Cotesia plutellae* for artificial selection of resistance to fenvalerate

Colony	Details
A. *Plutella xylostella*	
Non-selected	Initiated with 800 pupae from a fenvalerate-susceptible colony and maintained on cabbage plants without exposure to insecticides
Selected	Initiated with 800 pupae from the same fenvalerate-susceptible colony as for the Non-selected colony, maintained on cabbage plants and also used as the host for maintaining the Selected-SH colony of *C. plutellae,* selected with fenvalerate at the IV instar (together with the Selected-SH colony of *C. plutellae*) every generation for 13 generations
B. *Cotesia plutellae*	
Non-selected	Initiated with 100 cocoons collected from a laboratory culture of *C. plutellae* and maintained using larvae from the Non-selected colony of *P. xylostella* as hosts without exposure to insecticides
Selected-NH	Initiated with 1000 wasps from the same laboratory colony as for the Non-selected colony, provided with II and III instar larvae of the Non-selected colony of *P. xylostella* as hosts, and selected at larval stage with fenvalerate every generation for 14 generations
Selected-SH	Initiated with 1000 wasps from the same laboratory colony as for the Non-selected colony, provided with II and III instar larvae of the Selected colony of *P. xylostella* as hosts, and selected at larval stage with fenvalerate every generation for 13 generations

All of the colonies were maintained on potted cabbage plants in stainless steel rearing cages in separate cubicles at 25±1°C, 14L:10D and 50–80%RH.

Insecticide

Fenvalerate, technical grade (85%, Hangzhou Pesticide Factory) was diluted with acetone for topical application to DBM larvae in bioassays. Fenvalerate, 20%EC (Jiangsu Red Sun Group Ltd) was diluted with distilled water to the desired concentrations when used in the resistance selection sprays and for the bioassays with the parasitoid adults.

Artificial selection

Selection was carried out when the parasitoids were in the larval stage inside host larvae. When host larvae developed to the II and early III instars, they were exposed to adult parasitoids for parasitism. Six days after exposure, all host larvae, either parasitised or not, reached the mid-late IV instar, and the parasitoid immatures inside were in the early to mid stages of larval development. The plants bearing the exposed hosts were then sprayed with a fenvalerate solution to run-off using a hand-sprayer. Host pupae and parasitoid cocoons were collected 4–5 days later to start the next generation cycle.

The selections for both the Selected-NH and Selected-SH colonies of the parasitoid were started with a spray of 200 mg ai/L fenvalerate, an approximate LC_{50} for the non-selected colony of DBM. From the second generation onward, the parasitoid adults of the Selected-NH colony were always provided with host larvae collected from the Non-selected DBM colony for subsequent selection. For the selection of the Selected-SH colony, the moths that survived the previous selection were provided with host plants for oviposition to continue the Selected DBM colony (Table 1). Part or all of the host larvae, depending on the number available, were then exposed to the parasitoids that survived the previous selection. The fenvalerate concentrations were adjusted at each selection cycle so as to ensure some survivors to continue the colonies. Figure 1 shows the concentrations of fenvalerate used for the selections.

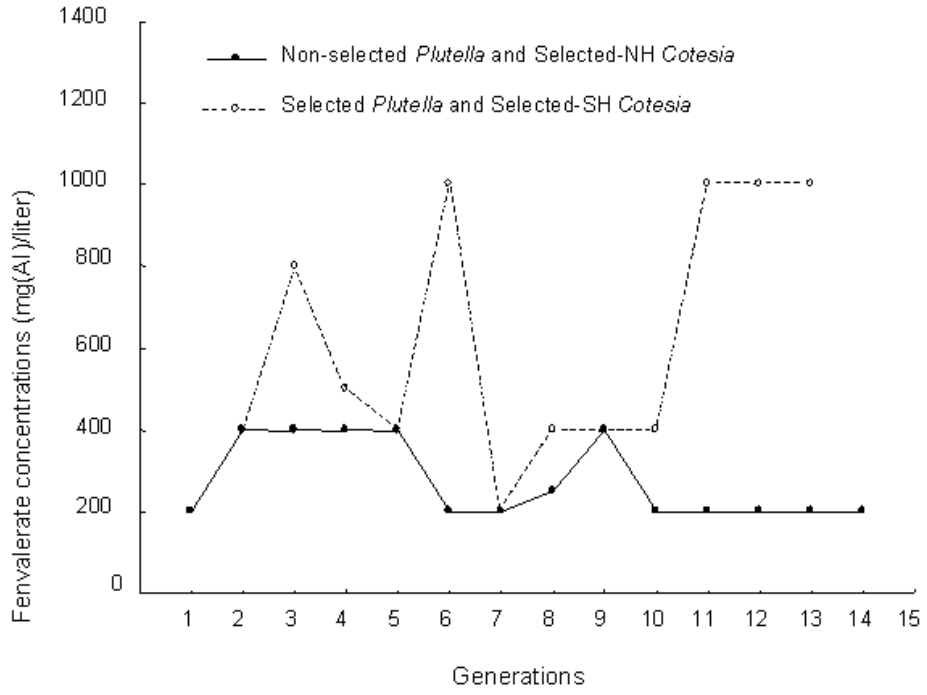

Figure 1. Concentrations of fenvalerate used in the selection regime for two colonies of *Cotesia plutellae* reared respectively in two colonies of *Plutella xylostella* that differed in resistance to the insecticide as the selection proceeded.

Bioassay of Plutella xylostella *larvae*

A series of five concentrations of fenvalerate diluted in acetone (10–90% mortality range) was made for the bioassay of each colony, in which five doses plus a control were each replicated three times with 10 larvae per replicate. One droplet of 0.5 µl fenvalerate solution was applied to the dorsal region of each larva using a Hamilton microsyringe. The larvae were reared in groups of 10 on cabbage leaf discs in 8 cm Petri dishes at 25°C, 14L:10D and mortality was assessed after 48 h.

Bioassay of Cotesia plutellae *adults*

For each *C. plutellae* colony, the susceptibility of adults to fenvalerate was assessed using a direct contact residual method with glass vials. For each bioassay, six concentrations (10–90% mortality range) of fenvalerate plus a control were prepared in distilled water. A fenvalerate solution of a given concentration was poured into each vial to its full capacity. After 10 s, the solution was poured off and the residue was air-dried at 20°C for 12 h, and three vials were treated with each concentration. Ten male and 10 female adults (12–24 h post emergence and fed with 10% honey solution) were introduced into each of the treated vials. The vials were capped with clean, untreated nylon gauze. No food was provided in the vials. The test vials were placed at 25°C, 14L:10D and mortality was assessed after 24 h.

Measurement of detoxifying enzyme activities

Activities of monooxygenase, carboxylesterases and general esterase in the various colonies of both the host and parasitoid were measured at the end of the selection regimes following the methods of van Asperen (1962) and Shang and Soderlund (1984). For the biochemical analysis of enzymes with non-parasitised DBM, IV instar larvae were used. For measuring enzyme activities in parasitised DBM and parasitoid larvae, parasitised DBM IV instar larvae harbouring mid-stage parasitoid larvae were dissected to remove parasitoid larvae, and the host larvae and the parasitoid larvae were then tested separately in the biochemical analysis. For enzyme analysis with parasitoid adults, male and female wasps (0–24 h after emergence) were used. Three samples, consisting of five individuals each, were analysed for each colony of the host and parasitoid.

Statistical analysis

Fenvalerate doses for bioassays with DBM larvae were translated to µg ai/mg body weight of test larvae for probit analysis. Dose or concentration-mortality data were analysed by probit analysis using POLO (LeOra Software 1997). Differences in susceptibility were considered significant when 95% confidence limits of LD_{50}s or LC_{50}s did not overlap.

Results

Selection of resistance

Selection was carried out over twelve months (March 1999 to March 2000). During this period, 14 generations of the Selected-NH colony and 13 generations of the Selected-SH colony were maintained. Selection was successfully applied to each generation of both colonies. The fenvalerate concentration used in selection for the Selected-NH colony was doubled from 200 mg ai/L to 400 mg ai/L from 2nd to 5th generation, but was reduced to 200 mg ai/L at the 6th generation because of the low number of survivors. Thereafter, we were able to increase the selection pressure only at the 8th and 9th generation, and used 200 mg ai/L for most of the generations to the end of 14 selection cycles (Figure 1). By contrast, we were able to apply much higher selection pressure to several generations of the Selected-SH colony (Figure 1).

Bioassays for fenvalerate susceptibility for the host and parasitoid colonies were undertaken at the end of the selection. Differences between LD_{50} values of the Non-selected and Selected DBM colonies were significant. Compared to the Non-selected colony, the resistance factor to fenvalerate of the Selected colony increased to 60 fold (Table 2). Differences between LC_{50} values for adults of the three colonies of *C. plutellae* were also significant. Compared to the Non-selected colony, the resistance factors to fenvalerate of the Selected-NH and Selected-SH colonies increased 4.5 and 13.6 fold respectively (Table 3).

Table 2. Susceptibility of *Plutella xylostella* larvae to fenvalerate

Colony	N	Slope (SE)	LD_{50}(95%CL) (µg/mg)[a]	χ^2	df	RF[b]
Non-selected	150	0.790 (0.139)	0.103 (0.054–0.216)	1.24	3	1
Selected	150	0.998 (0.150)	6.244 (3.645–10.936)	1.98	3	60.3

[a] µg ai/mg of body weight of test larvae. [b] RF, Resistance factor calculated by dividing the LD_{50} of selected colony by LD_{50} of the Non-selected colony.

Table 3. Susceptibility of *Cotesia plutellae* adults to fenvalerate

Colony	n	Slope (SE)	LC_{50} (95% CL) (mg ai/Litre)	χ^2	df	RF[b]
Non-selected	240	0.865 (0.140)	4.1 (2.0–6.7)	0.63	2	1
Selected-NH[a]	300	0.833 (0.103)	18.4 (11.2–28.9)	1.49	3	4.5
Selected-SH[a]	300	0.673 (0.094)	55.7 (31.3–107.2)	2.78	3	13.6

[a] Selected-NH = Selected with the Non-selected host colony; Selected-SH = Selected with the Selected host colony. [b] RF, Resistance factor calculated by dividing the LC_{50} of the selected colonies by LC_{50} of the Non-selected colony.

Detoxifying enzyme activities

In DBM larvae, the activities of monooxygenase in the Selected colony were higher than those in the Non-selected colony, whether the larvae were healthy or parasitised (Table 4). In the parasitoid, the activities of monooxygenase of larvae from the Selected-NH and Selected-SH colonies were 1.11 and 1.50 times that of the Non-selected colony (Table 4). Likewise, the activities of monooxygenase of parasitoid adults from the Selected-NH and Selected-SH colonies were 1.18 and 1.57 times that of the Non-selected colony (Table 4). In contrast, no increases in the activity of either carboxylesterases or general esterase were detected with increasing host or parasitoid resistance (Table 4).

Table 4. Detoxifying enzyme activities (µg product/mg protein/min, mean±SD) of healthy and parasitised larvae of *Plutella xylostella,* and larvae and adults of *Cotesia plutellae*

Insects	Monooxygenase	Carboxylesterases	General esterase
A. *Plutella xylostella*			
Non-Selected, larvae, healthy	4.2 ± 0.46	19.4 ± 2.17	23.4 ± 2.13
Selected, larvae, healthy	4.8 ± 0.75	18.2 ± 0.79	28.7 ± 1.07
Non-Selected, larvae, parasitised	3.3 ± 0.90	14.1 ± 3.48	18.0 ± 4.03
Selected, larvae, parasitised	3.9 ± 0.90	14.8 ± 3.10	17.9 ± 1.45
B. *Cotesia plutellae*			
Non-selected, larvae	5.4 ± 0.57	22.2 ± 0.33	25.7 ± 2.37
Selected-NH, larvae	6.0 ± 0.99	15.4 ± 2.87	18.8 ± 3.70
Selected-SH, larvae	8.1 ± 0.94	18.9 ± 6.65	26.5 ± 4.78
Non-selected, adults	4.9 ± 0.95	23.2 ± 7.36	29.7 ± 3.53
Selected-NH, adults	5.8 ± 0.86	19.1 ± 5.10	26.4 ± 3.76
Selected-SH, adults	7.7 ± 0.88	20.4 ± 3.74	27.3 ± 6.18

Discussion

In this study, no special effort was made to increase the frequency of the resistance allele in the initial parasitoid colony for selection by means of prior field selection or collections of material from different sites. Yet, the Selected-SH colony increased its resistance to fenvalerate 13.6 fold after only 13 selection cycles. This increase is impressive in view of the general slow and moderate increases in tolerance to insecticides recorded in past selection programs with parasitoids (Rosenheim & Hoy 1988, Croft 1990, Ke *et al.* 1991, Tabashnik & Johnson 1999). Equally impressive was the significantly higher level of resistance acquired by the Selected-SH colony than that acquired by the Selected-NH colony (Table 3). The results of this study thus provide new information for improving the methods for laboratory selection of insecticide resistance in parasitoids in several ways. First, the data demonstrated that parasitoids could be exposed to selection pressure at the larval stage, probably both within and outside the hosts prior to cocoon formation. It has been shown that an insecticide applied to a host larva can reach the parasitoid larva even when the primary resistance mechanism results in a lower rate of accumulation of toxicant in the host, e.g. enhanced degradation, and many metabolic breakdown products may also be toxic (Furlong & Wright 1993). However, direct evidence for the contact with, and/or consumption of, the insecticide by the parasitoid larvae in our case is yet to be obtained. It was likely that the parasitoid mature larvae were also exposed to the insecticide residue on the host larval surface and plant substratum during the period between egression from the hosts and pupation. Second, selection with resistant hosts could speed up the response by the

parasitoids. The two host colonies were derived from the same source and their difference in susceptibility to the insecticide occurred in the selection process. Third, resistance genes selected during larval development could be expressed at the adult stage.

Compared to selection with parasitoid adults, application of an insecticide to the larvae enabled selection pressure to be exerted on the parasitoids before mating and thus would speed up concentrating resistance genes in the colony. However, direct comparison between selections with larvae and adults are needed to quantify the difference and to discern whether the parasitoid response to selection differs physiologically between the stages, in addition to the differences concerned with mating.

The use of host resistance in promoting response to selection by a parasitoid has a prerequisite that the host must have much higher tolerance to the insecticide than the parasitoid. This is feasible in most cases because it is usually practical to select highly resistant insect pests in the laboratory, which may then be used as hosts to select for a resistance level in the parasitoid sufficiently high to survive field application rates.

The mechanisms of host resistance to fenvalerate in promoting response to selection by *C. plutellae* are not entirely clear. One apparent factor seemed to be the higher selection pressure applied to parasitoids via more resistant hosts (Figure 1). Oxidative degradation has been shown to be a major metabolic mechanism in pyrethroid resistance in DBM (Hung *et al.* 1990). Comparison of detoxifying enzymes between the host or parasitoid colonies in this study demonstrated that fenvalerate resistance was positively related to increased monooxygenase activities, but was unrelated to activities of carboxylesterases and general esterase in both insects (Table 4), indicating that the two species shared a major metabolic mechanism for resistance to fenvalerate. It remains to be shown whether the acceleration of resistance selection in a parasitoid by the resistance in its host depends on the nature of resistance mechanisms.

Since a more resistant host harbouring a parasitoid larva would survive higher rates of the insecticide than a susceptible host and the parasitoid could develop to adulthood, host resistance can confer protection to endo-larval parasitoids such as *C. plutellae* (Furlong & Wright 1993, Iqbal & Wright 1996). Our results suggest that host resistance to an insecticide not only confers protection to the parasitoids, but also helps selection of resistance genes in the parasitoid. This information may offer new insights in understanding the development of resistance to insecticides by parasitoids in the field (Tabashnik & Johnson 1999).

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Plant volatiles and adult experience affect selection by *Cotesia plutellae* of host larvae on different plants

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Abstract

Laboratory experiments were conducted to examine selection by *Cotesia plutellae* between host larvae feeding on two plant species: Chinese cabbage, *Brassica campestris* ssp. *pekinensis* and common cabbage, *Brassica oleracea* var. *capitata*. When *C. plutellae* wasps were provided with equal numbers of *Plutella xylostella* larvae on both species of plants in one arena, the parasitoid parasitised 4-15 fold more host larvae on the Chinese cabbage than on the common cabbage and this preference did not change with host density. However, an experience of oviposition or searching on a leaf of the less-preferred plant, the common cabbage, significantly increased the preference for parasitising host larvae on this plant. Plant volatiles from Chinese cabbage were more attractive to *C. plutellae* adult females than those emanating from common cabbage. Feeding by *P. xylostella* larvae increased the attraction of both plant species to *C. plutellae*, but the infestation and the presence of *P. xylostella* larvae on the plants did not affect their relative levels of attraction to the parasitoid. In parallel to the increased parasitism on common cabbage following experience, an oviposition in host larvae on this less-preferred plant significantly increased the response to volatiles emanating from the plant. These results indicate that host plants may strongly influence the foraging behaviour of *C. plutellae,* but their differential attractions to the parasitoid may be significantly offset by the learning behaviour of the insect.

Keywords

Plutella xylostella, host plants, host foraging, parasitoid learning

Introduction

Plants may mediate many of the interactions between herbivores and their insect parasitoids and thereby increase or decrease the effectiveness of natural enemies (Cortesero *et al*. 2000). Understanding these multitrophic effects may help explore the potential for manipulating crop-pest-parasitoid interactions for improved pest management (Bottrell *et al*. 1998, Verkerk *et al*. 1998).

Insect parasitoids are important biological control agents of the diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), yet their performance has not been examined in a multitrophic context until very recently (Talekar & Shelton 1993, Verkerk & Wright 1996). Field observations suggest that parasitoids may show different rates of parasitism of DBM on different crops (Verkerk & Wright 1997, Liu *et al*. 2000). Bogahawatte and van Emden (1996) showed that the endo-larval parasitoid, *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae) preferred the odour of the host plant on which it had developed. Potting *et al*. (1999) showed that *C. plutellae* used plant volatiles in its in-flight searching behaviour, which was modified by its experience with plant material. Shiojiri *et al*. (2000) demonstrated that *C. plutellae*, being a host-specific parasitoid, showed very specific responses to volatile stimuli and exhibited differential responses between host- and nonhost-plant complexes.

Here, we investigate the relative foraging success for DBM larvae by *C. plutellae* on two species of host plants. We report the response of the parasitoid to volatiles emitted by the two host plants, and the role of experience in determining parasitism success on the two plants and the response to volatiles. Our objectives were to determine the effects of host plants and in particular their volatiles on the foraging behaviour and success of this parasitoid, and to determine whether these effects are modified by the parasitoid's experience.

Materials and methods

Insects and plants

Three species of plants were used in this study: (1) Chinese cabbage, *Brassica campestris* L. ssp. *pekinensis*, cv. Zaoshou No. 5; (2) common cabbage, *Brassica oleracea* L. var. *capitata*, cv. Jingfeng No. 1; and (3)

radish, *Raphanus sativus* L. cv. Yidianhong. They were grown from seed in potting mix in small pots (11 cm diameter) in screen houses to the 6-7 leaf growth stage when used.

Three cultures of DBM were started from a sample collected from a cabbage field in a suburb of Hangzhou, China and maintained on the three plant species using the procedures as described in Wang *et al*. (1999). A culture of *C. plutellae* was started from a sample collected from a radish field in a suburb of Hangzhou. The culture was maintained by exposing approximately 500 II and III instar DBM larvae on radish plants to 10 mated female parasitoids (3-4 days after emergence) in a ventilated cage (55 × 55 × 55 cm, with a glass top, screen-covered opening on three sides and a clear plastic front door) for two days. The exposed larvae were reared until parasitoid cocoons developed. The cocoons were transferred to ventilated plastic containers for adult emergence and mating. The adults were fed with 10% honey solution upon emergence. To obtain parasitoids for the experiments, II or III instar DBM larvae were exposed individually to single mated female parasitoids and each larva was observed to receive oviposition only once to avoid superparasitism. The exposed DBM larvae were then reared until parasitoid cocoons developed. The cocoons were collected and placed in clean containers (i.e., without any plant or host material) for adult emergence and mating (provided with honey solution). Mated female wasps were collected 3-4 days after emergence for use in the observations. All insect cultures were maintained in constant temperature rooms at 25 ± 1°C, 14L:10D and 60-80% RH.

Parasitism of host larvae on two plant species

The day before the parasitism was measured, II and early III instar DBM larvae from the culture maintained on Chinese cabbage were collected and placed on the test Chinese cabbage plants, and DBM larvae from the culture maintained on common cabbage were collected and placed on the test common cabbage plants. Two Chinese cabbage and two common cabbage plants each bearing the same number of DBM larvae were placed in alternate position at the four corners of a cage (size and structure as described above), the top of which was covered with tracing paper to defuse the light from above. Wooden boards were placed near the four lateral sides of each cage to avoid interference from lateral light. The cages were placed in a constant temperature room at 25 ±1°C with minimum or no airflow. Two naïve parasitoid females (with no experience of oviposition or searching on a plant) were introduced into each cage for oviposition for 6 h and then discarded. The exposed DBM larvae on each of the test plants were collected and dissected to determine parasitism. The trial was conducted with four levels of host density (5, 10, 20 or 30 larvae per plant) with 10 replicates for each level of density.

In a separate trial, one Chinese cabbage and one common cabbage plant each bearing the same number of DBM larvae were placed 25 cm apart near the back of a cage. One naïve female parasitoid was released from a point near the front door of the cage (with equal distance to the two plants) and its behaviour was observed continuously for 30 minutes to record time spent on each of the two plants (with a stop watch) and the number of ovipositions in larvae on each of the two plants. The trial was conducted with four levels of host density (5, 10, 20 or 30 larvae per plant) with 30 females observed for each host density. The positions of the two plants in a cage were alternated between observations.

Effect of adult experience on host foraging

The experimental set-up was similar to that used for parasitism of host larvae in a cage, except that the female parasitoids were given various experiences immediately prior to their introduction into the cage. Six pre-treatments of parasitoid females were conducted (see Table 3): the females were provided with a DBM-damaged leaf of either the Chinese cabbage (1) or common cabbage (2) to search for 10 minutes; or each female was allowed to oviposit once in a DBM larva, which had been reared on either the Chinese cabbage (3) or common cabbage (4), in a test tube without plant material; or each female was allowed to search for 10 minutes and oviposit once in a DBM larva on a host-damaged leaf of either the Chinese cabbage (5) or common cabbage (6). Two Chinese cabbage and two common cabbage plants each bearing 20 DBM larvae were placed in alternate position at the four corners of a cage. Two pre-treated females were introduced into each cage for 6 h and 10 replicates were carried out for each pre-treatment.

Response to volatiles of different plants

The response of parasitoid females to volatile chemicals emitted by different odour sources was investigated in a Y-tube olfactometer. The olfactometer consisted of a Y-shaped glass tube 2.8 cm in internal diameter. The stem and the two arms (at a 75° angle) of the Y-tube were 16 and 28 cm in length, respectively. Each

arm was connected via a Teflon hose to an odour source chamber consisting of a glass box measuring 25 × 25 × 35 cm, large enough to hold a whole test plant plunged in a small water bottle. Air was drawn by the negative pressure of an electric pump, filtered through an activated-charcoal filter and humidified by bubbling through distilled water before being pulled into the odour source chamber at approximately 200 ml/min. The observations were made at 25±1°C in a box with defused light above the Y-tube.

Female parasitoids were released individually into the base of the stem of the Y-tube, and each of them was given 5 minutes to move upwind towards the ends of the arms of the tube. When a female penetrated more than 10 cm into one of the two arms and remained there for more than 30 seconds, it was recorded as a choice for that arm. The connections of the odour sources to the olfactometer arms were exchanged after testing five parasitoids to remove any asymmetrical bias in the set-up. The olfactometer tube was washed with alcohol after testing 10 females.

For each of the two species of plants, four types of odour sources were tested: (1) intact plants; (2) mechanically damaged plants: the plants were each punched with 20 5-mm holes in the leaves 24 h before the test and were punched again in the wounds 10 minute prior to the test; (3) infested plants: each plant was infested with 20 II instar DBM larvae 24 h prior to the test and the larvae were left on the plant during the test and (4) previously infested plants: the plants were infested as in (3) but the larvae were removed 10-20 minutes prior to the test.

Four pairs of odour sources were compared between the two plant species and one pair was compared within each of the two plant species (Figure 1, Results section). For each pair of odour sources, about 40 females were individually tested. A goodness-of-fit *G*-test, with the application of Williams's correction, was applied to analyse the numbers of females that made a choice in each pair of odour sources with the null hypothesis of no preference (Sokal & Rohlf 1995).

Effect of adult experience on response to plant volatiles

The test materials and methods were the same as above, except that, 5-10 minutes prior to introduction into the Y-tube, each test female was allowed to search and oviposit once in a III instar DBM larva feeding on a host-damaged leaf of either Chinese cabbage or common cabbage.

Results

Parasitism of host larvae on two plants species

When provided with equal numbers of DBM larvae feeding on the two plant species, the female parasitoids parasitised 5-16 times more larvae on the Chinese cabbage than on the common cabbage (Table 1). The relative proportion of DBM larvae parasitised on Chinese cabbage increased with host density in the range of 5-20 larvae per plant and then declined as host density further increased to 30 per plant (Table 1). The results of direct observations showed that female parasitoids spent a much longer time and had many more ovipositions in DBM larvae on Chinese cabbage than on common cabbage (Table 2). Note that the number of ovipositions does not equal the number of DBM larvae parasitised, because superparasitism was common under these conditions especially at higher host density.

Table 1. Number of *Plutella xylostella* larvae parasitised by *Cotesia plutellae* on plants of two species with the same host density in choice tests for 6 h at 25°C

Host density (Larvae/plant)	No. of replicates	Mean ± SE number of larvae parasitised per plant		
		***B. campestris* (a)**	***B. oleracea* (b)**	**a/b**
5	10	2.0 ± 0.2	0.3 ± 0.2	6.7
10	10	6.8 ± 0.4	0.7 ± 0.3	9.7
20	10	10.9 ± 0.9	0.7 ± 0.3	15.6
30	10	11.9 ± 1.2	2.5 ± 0.5	4.8

Note: At each of the four levels of host density, the two mean numbers of DBM larvae parasitised on the two species of plants differ significantly ($P<0.01$ in all cases, Student-*t* test).

Table 2. Time spent and number of ovipositions in host larvae by *Cotesia plutellae* on two plant species with the same host density in choice tests for 30 minutes at 25°C

Host density (larvae/plant)	n	Mean ± SD duration (s) spent on		Mean ± SE number of ovipositions on	
		B. campestris	*B. oleracea*	*B. campestris*	*B. oleracea*
5	30	216 ± 27	9 ± 3	0.97 ±0.14	0.23 ± 0.08
10	30	564 ± 44	24 ± 4	4.10 ± 0.35	0.57 ± 0.12
20	30	842 ± 16	29 ± 4	8.90 ± 0.13	0.77 ± 0.16
30	30	1073 ± 18	41 ± 5	12.20 ± 0.39	1.40 ± 0.20

Note: At each level of host density, both the mean durations between the two species of plants and the mean numbers of ovipositions between the two plants differ significantly ($p<0.01$ in all cases, Student-*t* test).

Effect of adult experience on host foraging

Compared with the much higher number of DBM larvae parasitised on Chinese cabbage than on common cabbage by naïve females (Table 1), females that had searched on a leaf of common cabbage parasitised similar numbers of larvae on the two plants. Females that had searched and oviposited in a larva feeding on a leaf of common cabbage, parasitised twice as many larvae on common cabbage than that on Chinese cabbage (Table 3). Similarly, females that had experience of searching on a leaf of Chinese cabbage or search coupled with an oviposition in a larva feeding on the leaf increased their preference for hosts on this plant further, resulting in parasitism on Chinese cabbage only and no parasitism on common cabbage. In contrast, females that had oviposited in a DBM larva without access to the plant, regardless of the plant species from which the larva had been reared, did not change their preference for host larvae between the two plants (Table 3).

Table 3. Number of *Plutella xylostella* larvae parasitised by *Cotesia plutellae* on plants of two species in choice tests for 6 h at 25°C when the parasitoid female adults had different prior search experiences

Prior search experience	Mean ± SE number of larvae parasitised/plant[a]		
	B. campestris (a)	*B. oleracea* (b)	a/b
(1) Search on a *B. campestris* leaf for 10 min	15.2 ± 0.3	0	-
(2) Search on a *B. oleracea* leaf for 10 min	11.5 ± 0.5a	10.2 ± 0.8a	1.1
(3) Oviposition in a larva previously reared from *B. campestris* in a tube without plant material	11.9 ± 0.7a	1.1 ± 0.3b	10.8
(4) Oviposition in a larva previously reared from *B. oleracea* in a tube without plant material	11.7 ± 0.7a	0.9 ± 0.3b	13.0
(5) Oviposition in a larva feeding on a *B. campestris* leaf	15.9 ± 0.5	0	-
(6) Oviposition in a larva feeding on a *B. oleracea* leaf	4.6 ± 0.8b	11.5 ± 0.7a	0.4

[a] Ten replicates in each treatment, means in the same row followed by the same letters do not differ significantly ($P>0.05$, Student-*t* test).

Response to volatiles of different plants

When female parasitoids were offered a choice between airflows carrying volatiles from the two plant species, about twice as many of them moved towards the Chinese cabbage as towards the common cabbage, although the preference in the test with intact plants did not reach statistical significance due to the low number of females that responded (Figure 1). Within each of the two plant species, the females showed high preference for infested plants compared with intact ones (Figure 1).

Effect of adult experience on response to plant volatiles

Compared with naïve female parasitoids, those with a prior experience of ovipositing in a DBM larva feeding on a leaf of the common cabbage increased their preference for volatiles emitted from this plant to those emitted from Chinese cabbage, when the plants were infested, previously infested or mechanically damaged (Figure 2B). Interestingly, the response of the parasitoid to intact plants of the two species was not affected by prior experience (Figure 2B). When female parasitoids had an experience of ovipositing in a DBM larva feeding on a leaf of the Chinese cabbage, their preference for Chinese cabbage was further

increased when the plants were infested with DBM larvae, but was unaffected when the plants were intact, mechanically damaged or previously infested (Figure 2A).

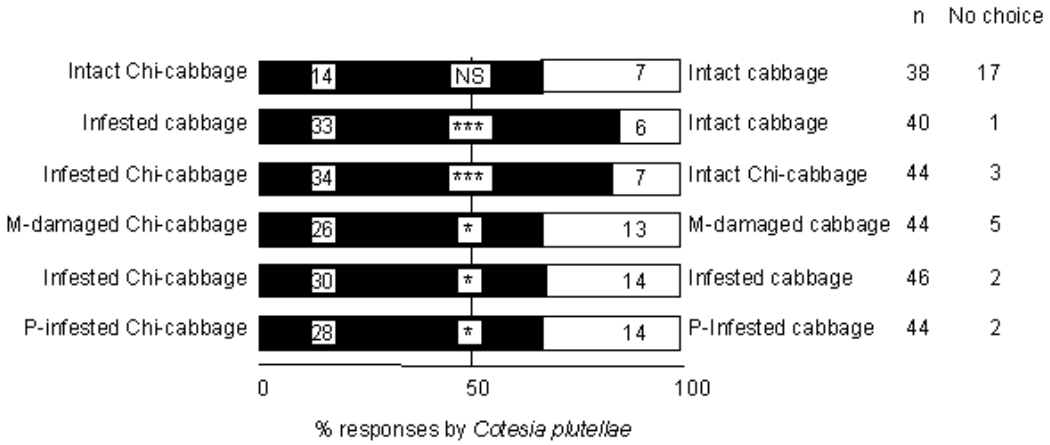

Figure 1. Numbers of *Cotesia plutellae* female adults showing a response to plant volatiles of *Brassica campestris* (Chi-cabbage) and *B. oleracea* (cabbage). Asterisks indicate statistically significant preferences in a choice test (*: *P*<0.05, **: *P*<0.01, *: *P*<0.001). NS: not significant. n: number of parasitoid wasps tested. The number of wasps that did not choose either of the odour sources is listed under "No choice". M-damaged: mechanically damaged. P-infested: previously infested.**

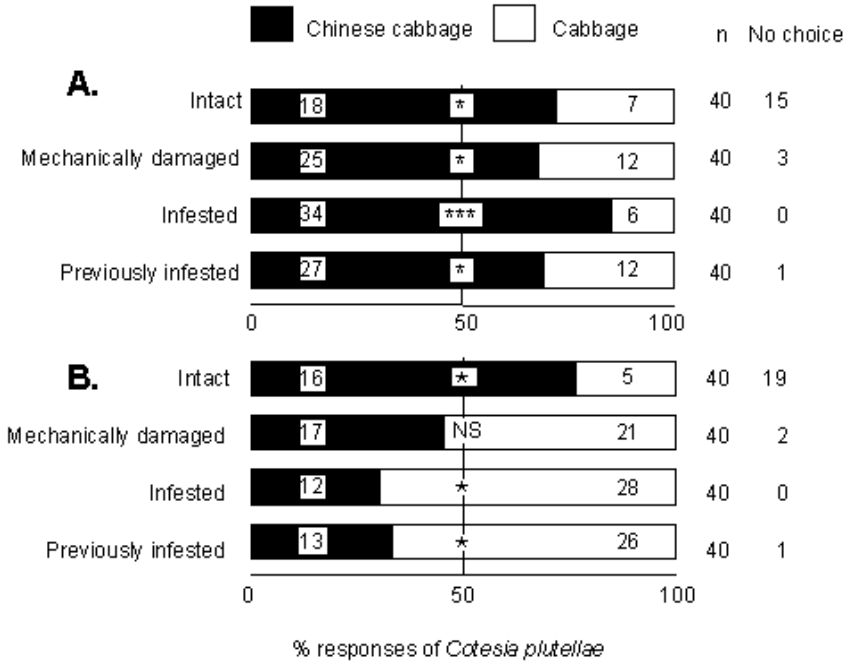

Figure 2. Numbers of *Cotesia plutellae* female adults showing a response to plant volatiles of *Brassica campestris* (Chinese cabbage) and *B. oleracea* (cabbage) after the adults had oviposited either in a host larva feeding on a Chinese cabbage leaf (A) or in a host larva feeding on a cabbage leaf (B). Asterisks indicate statistically significant preferences in a choice test (*: *P*<0.05, **: *P*<0.01, *: *P*<0.001). NS: not significant. n: number of parasitoid wasps tested. The number of wasps that did not choose either of the odour sources is listed under "No choice".**

Discussion

When offered a choice between Chinese cabbage and common cabbage, *C. plutellae* showed a strong preference for searching and parasitising DBM larvae on the former (Tables 1 and 2). This innate preference, exhibited by naïve female parasitoids, is apparently mediated, at least in part, by the different levels of attraction from volatiles emitted by the two plants (Figure 1). However, this innate preference could be immediately and significantly modified by experience of searching and oviposition by the female parasitoids, to the extent that a ten-minute search coupled with a single oviposition experience on a host-

damaged leaf of the common cabbage resulted in higher parasitism on this innately less-preferred plant than that on the innately-preferred plant (Table 3). This modification of preference by experience was evidently associated with learning of plant volatiles and to a much less extent with learning of host-derived stimuli (Figure 2).

Cotesia plutellae is a specific larval parasitoid of DBM, the latter can feed and reproduce on plants of at least 28 genera of the family Brassicaceae (Talekar & Shelton 1983). In fact, DBM has been observed to occur in high numbers on crops of many *Brassica* species in many parts of the world in the last 30 years (Talekar & Shelton 1993, other reports in this volume). Such an association between the three trophic levels, i.e., specific connection between the parasitoid and herbivore, but diverse connections between the herbivore and its host plants, has been speculated to favour the evolution of learning by the parasitoid to deal with the variability of plant cues (Vet *et al*. 1995). Our results offer support for this theory. In several parasitoids the learned responses were shown to wane and disappear in a short time relative to the adult's longevity as a consequence of another experience (Vet *et al*. 1995, Fukushima *et al*. 2001). If this happens in *C. plutellae*, one may expect that the parasitoid could be more efficient in parasitising DBM on Chinese cabbage than on common cabbage when small plots of the two plants are grown in close proximity. Direct evidence for such differential levels of parasitism between these two plant species in the field is lacking. Liu *et al*. (2000) showed that rates of parasitism of DBM larvae by *C. plutellae* on mustard, *B. juncea*, a species more closely related to Chinese cabbage than to common cabbage, were usually higher than on common cabbage, when crop growth periods and levels of DBM density were similar between fields of the two plant species.

Our observation of the effects of plant volatiles and experience on the foraging behaviour of *C. plutellae* agrees with the earlier reports by Bogahawatte and van Emden (1996) and Potting *et al*. (1999), in that this parasitoid uses mainly plant volatiles in its orientation towards infested plants and experience of plant volatiles increases the preference to these cues. While the study by Bogahawatte and van Emden (1996) showed the influence of the host plant on which the parasitoid had developed and searched after emergence, our study and the study by Potting *et al*. (1999) demonstrate the dramatic effects of a brief experience by the adults on their foraging behaviour. Jiang (2001) further showed that experience acquired during the development of immature stages had limited influence on the preference for plants by the adults. This rapid learning ability by the adult females of *C. plutellae* may be utilised to increase its effectiveness for biological control. For example, mass-reared parasitoids pre-treated with odour of a target crop may search more efficiently and remain longer in the field of release.

Acknowledgements

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Interspecific competition between *Diadegma semiclausum* and *Oomyzus sokolowskii*, parasitoids of diamondback moth, *Plutella xylostella*

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Abstract

Interspecific competition between *Diadegma semiclausum* (Hellén) (Hymenoptera: Ichneumonidae) and *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae) was investigated at 25°C in the laboratory, by exposing III instar larvae of diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) to either species alone or exposing the host larvae already exposed to one species to the other. Both *D. semiclausum* and *O. sokolowskii* could lay eggs into the host larvae that had previously been parasitised by the other species, leading to occurrence of multiparasitised hosts. When host larvae were exposed first to *D. semiclausum* and then to *O. sokolowskii*, and the time intervals between the two exposures were shorter than one day, ensuing parasitoid adults from the multiparasitised host larvae were nearly always *D. semiclausum*. When host larvae that were first exposed to *D. semiclausum* and then exposed to *O. sokolowskii* either immediately or 3-4 days later, immature stages of both parasitoids were found inside the host larvae by dissection. However, at 6-7 days after parasitism by *D. semiclausum*, only one larva of *D. semiclausum* remained in each host, suggesting that *D. semiclausum* physically removed the *O. sokolowskii* immatures through feeding. When host larvae were first exposed to *O. sokolowskii* and then exposed to *D. semiclausum*, the percentage of *O. sokolowskii* adult emergence increased as the time intervals between the two exposures increased. When host larvae exposed to *O. sokolowskii* three days previously were exposed to *D. semiclausum*, no *D. semiclausum* could survive to adulthood.

Introduction

Interspecific competition is one of the most predominant interspecific interactions and can influence the size and structure as well as the stability of communities (Mackauer 1990). Interspecific competition between parasitoids can be of great importance in the application of biological control (van Alebeek *et al*. 1993) because it could reduce the potential of parasitoids against target pests (Leveque *et al*. 1993) and sometimes lead to failure of an introduction of a natural enemy (Pijls *et al*. 1995).

Diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a major pest of crucifer crops worldwide and has developed high resistance to almost all types of insecticides (Talekar & Shelton 1993, Noda *et al*. 2000). *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae) and *Diadegma semiclausum* (Hellén) (Hymenoptera: Ichneumonidae) are two major parasitoids of DBM (Ooi 1988, Wang *et al*. 1999). They have been introduced into many countries or regions to enhance biological control of DBM (Ooi 1988, Yang *et al*. 1993, Talekar & Hu 1996, Noda *et al*. 2000). Because both parasitoids oviposit into DBM larvae, they may compete for hosts. But, as yet, no information is available on their competition. In this study, we observed the oviposition of these species into host larvae already parasitised by the other and determined the development of the two parasitoids in multiparasitised hosts.

Materials and methods

Insects and plants

All insect stock cultures were maintained in temperature-controlled rooms at 25±2˚C, 60-80% RH with a photoperiod of 14:10 (L:D). DBM was originally collected from a cabbage field in a suburb of Hangzhou, China in June 1999. The stock culture of DBM was reared on potted plants of cabbage, *Brassica oleracea* var *capitata*, using the method described by Wang *et al*. (1999).

The stock culture of *O. sokolowskii* was established from parasitised DBM pupae collected from a cabbage field in a suburb of Hangzhou in July 2000. The culture was maintained using DBM as hosts reared on cabbage plants and had been reared for 5-6 generations prior to the experiments.

The stock culture of *D. semiclausum* was started from 100 cocoons of the parasitoid from a stock culture at the Institute of Plant Protection, the Academy of Agricultural Sciences of Yunnan Province, China. The

institute at Yunnan introduced *D. semiclausum* from Taiwan in 1997. The culture was maintained using DBM as hosts reared on cabbage plants and had been reared for 4-7 generations prior to experiments.

All parasitoids of both species used in experiments were randomly chosen, mated adult females, at 1-2 days post-emergence. They were provided with 10% honey solution as food. Host larvae used in experiments were in early III instar.

To obtain larvae parasitised by *D. semiclausum*, we exposed DBM larvae individually to a female parasitoid in a test tube, and observed each of them to be attacked once. With this method, 20-30 attacked larvae could be obtained in half an hour. However, it was not possible to obtain the parasitised larvae by *O. sokolowskii* with this method because this parasitoid attacked only a few DBM larvae per female per day (Wang *et al*. 1999). Therefore, we obtained *O. sokolowskii* parasitised larvae by exposing 30 host larvae to 20 wasps for 24 h.

Oomyzus sokolowskii parasitism of larvae already parasitised by *Diadegma semiclausum*

Thirty larvae attacked by *D. semiclausum* in early III instar and 30 healthy host larvae of the corresponding age were exposed either 0 h (immediately after), 48 h, 72 h or 96 h later to 10 female wasps of *O. sokolowskii* for 24 h. At the end of exposure, 10-15 larvae in each of the replicates were dissected and the numbers of host larvae containing eggs or larvae of the two parasitoid species were recorded. The remaining15-20 larvae were reared until emergence of parasitoids. The numbers of hosts that produced parasitoid adults of either species were recorded. All dead DBM larvae and the pupae that did not produce either moth or parasitoid were dissected to determine whether they had been parasitised by either of the two species.

In a separate test, 30 host larvae attacked by *D. semiclausum* were exposed either 0 h, 72 h or 96 h later to 10 *O. sokolowskii* female adults for 24 h. After the exposure, the host larvae were sampled and dissected at 24 h intervals, and the numbers of host larvae containing eggs or larvae of the two parasitoid species were recorded.

Diadegma semiclausum parasitism of host larvae already parasitised by *Oomyzus sokolowskii*

The experiment design was similar to that described above, but DBM larvae were exposed first to 20 female wasps of *Oomyzus sokolowskii* for 24 h, then to two *D. semiclausum* females for 6 h either 0 h, 24 h, 48 h or 72 h later, and series sample dissections at 24 h intervals were made.

Results

Oomyzus sokolowskii parasitism of larvae already parasitised by *Diadegma semiclausum*

Oomyzus sokolowskii oviposited in the host larvae that had previously been parasitised by *D. semiclausum*, leading to the occurrence of multiparasitised hosts. Oviposition was not deterred by the presence of eggs or larvae of *D. semiclausum* in the host larvae (Table 1). Those multiparasitised host larvae had lower survival, and almost all of them failed to produce wasps of *O. sokolowskii* except when oviposition of *O. sokolowskii* occurred 48 h after parasitism by *D. semiclausum* (Table 2). As the parasitised host larvae continued to develop, the percentage of host larvae with *O. sokolowskii* decreased while that with *D. semiclausum* remained unchanged. In all three treatments, no *O. sokolowskii* individuals were found in the multiparasitised larvae on the 6-7th day after parasitism by *D. semiclausum* (Figure 1 and Figure 2).

Diadegma semiclausum parasitism of larvae already parasitised by *Oomyzus sokolowskii*

Although attacks by *D. semiclausum* on DBM larvae were hasty, almost all attacks (*ca.* 95%) resulted in parasitism (Table 1). No significant differences were found in percentage of parasitism by *D. semiclausum* between the host larvae previously exposed to *O. sokolowskii* and the healthy ones. However, in both treatments and controls, percentage of parasitism by *D. semiclausum* decreased with the increase of larval age (Table 3).

When oviposition by *D. semiclausum* occurred after that by *O. sokolowskii*, the percentage of hosts that produced wasps of *D. semiclausum* decreased with increase of the intervals between the two oviposition events. When the interval reached 72 h, no adults of *D. semiclausum* were produced (Table 2).

When host larvae were exposed immediately to *D. semiclausum* after the exposure to *O. sokolowskii*, the percentage of hosts with *D. semiclausum* remained high, while that with *O. sokolowskii* decreased as the parasitised host larvae developed (Figure 3). However, when the hosts were attacked by *D. semiclausum* after *O. sokolowskii* had developed for 48 h or 72 h, the percentage of hosts with *O. sokolowskii* remained virtually unchanged as that with *D. semiclausum* (Figure 4).

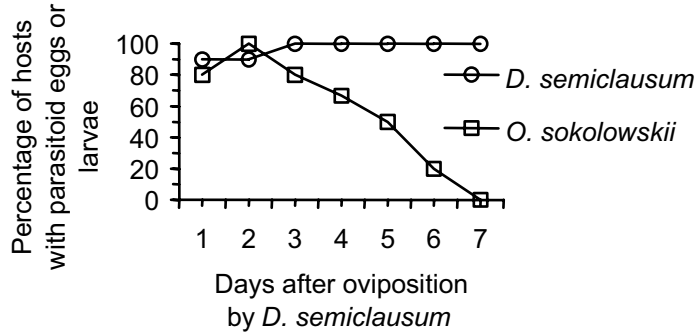

Figure 1. Percentage of *Plutella xylostella* host larvae with parasitoid eggs or larvae when the host larvae were first attacked by *Diadegma semiclausum* and then immediately exposed to *Oomyzus sokolowskii*.

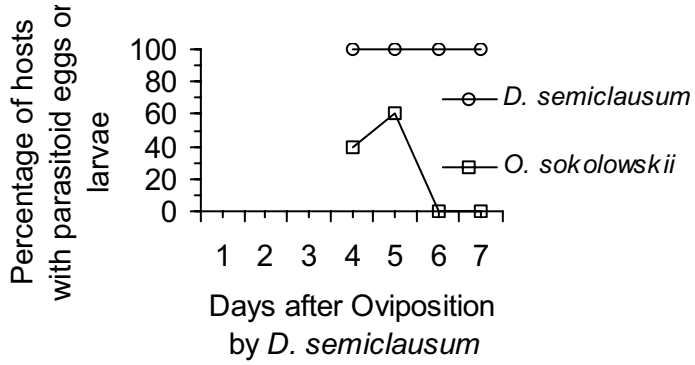

Figure 2. Percentage of *Plutella xylostella* host larvae with parasitoid eggs or larvae when the host larvae were first attacked by *Diadegma semiclausum* and then exposed to *Oomyzus sokolowskii* 72 h later.

Table 1. Percentage parasitism by *Oomyzus sokolowskii* of *Plutella xylostella* larvae that were already parasitised by *Diadegma semiclausum* 0-96 h previously

Time in h[a]	% parasitism of hosts exposed to both parasitoids by		% parasitism of host exposed to
	D. semiclausum	O. sokolowskii	O. sokolowskii *only*
0	95.0 ±2.4 (10)[b]	71.0 ±5.3	71.3 ±7.3 (5)
48	93.3 ±3.7 (5)	52.0 ±9.5	56.7 ±5.6 (5)
72	98.2 ±1.8 (5)	53.0 ±7.2	45.3 ±4.9 (5)
96	98.5 ±1.5 (5)	52.3 ±4.3	54.7 ±5.7 (5)

[a]Time in hours between exposure to *Diadegma semiclausum* and exposure to *Oomyzus sokolowskii.*
[b]Mean ±standard error; data in parentheses are the number of replicates.

Table 2. Successful parasitism of *Plutella xylostella* and adult emergence of *Diadegma semiclausum* and *Oomyzus sokolowskii* in relation to oviposition sequence and interval

Host exposure sequence to parasitoids[a]	% of host survival to prepupa	Number (%) hosts that produced adults of	
		D. semiclausum	*O. sokolowskii*
D-0-O	31.5	52 (96.3)	2 (3.7)
D-48-O	9.6	3 (60.0)	2 (40.0)
D-72-O	22.9	6 (85.7)	1 (14.3)
D-96-O	54.4	30 (96.8)	1 (3.2)
O-0-D	31.6	79 (96.3)	3 (3.7)
O-24-D	62.5	18 (75.0)	6 (25.0)
O-48-D	32.8	2 (13.3)	13 (86.7)
O-72-D	35.2	0 (0.0)	10 (100)

[a]D=*Diadegma semiclausum,* O=*Oomyzus sokolowskii*, 0, 24, 48, 72, 96 are hours between exposures to two parasitoids, thus D-0-O and D-48-O stand for host exposures to *O. sokolowskii* 0 or 48 h after exposure to *D. semiclausum* respectively.

Table 3. Percentage parasitism by *Diadegma semiclausum* of *Plutella xylostella* larvae that were already exposed to *Oomyzus sokolowskii* 0-72 h previously

Time in h[a]	% parasitism of hosts exposed to both parasitoids by		% parasitism of host exposed to
	O. sokolowskii	D. semiclausum	D. semiclausum *only*
0	84.0 ±7.8 (5)[b]	97.3 ±1.6	96.7 ±3.3 (5)[b]
24	75.6 ±2.2 (5)	68.9 ±5.9	66.7 ±5.4 (5)
48	86.7 ±3.0 (5)	57.8 ±11.5	54.7 ±8.5 (5)
72	72.5 ±5.6 (5)	51.7 ±4.4	49.3 ±8.1 (5)

[a]Time in hours between exposure to *Oomyzus sokolowskii* and exposure to *Diadegma semiclausum.*
[b]Mean ±standard error; data in parentheses are the number of replicates

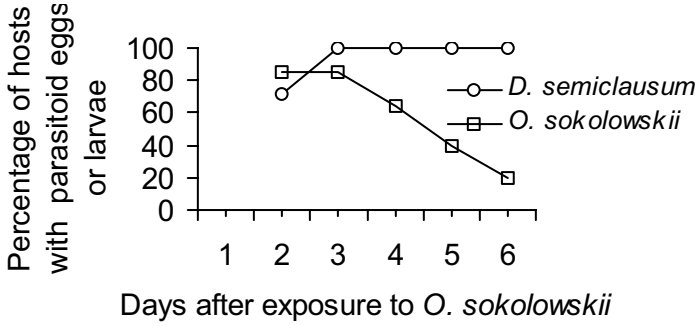

Figure 3. Percentage of *Plutella xylostella* hosts with parasitoid eggs or larvae when the host larvae were first exposed to *Oomyzus sokolowskii* and then exposed to *Diadegma semiclausum* immediately.

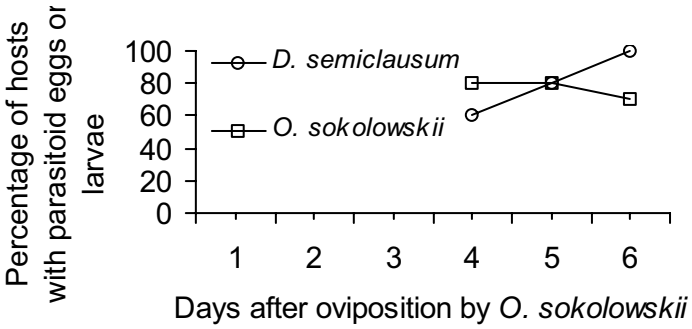

Figure 4. Percentages of *Plutella xylostella* hosts with parasitoid eggs or larvae when the host larvae were first exposed to *Oomyzus sokolowskii* and then exposed to *Diadegma semiclausum* 48 h later.

Discussion

Many parasitoids can discriminate between hosts parasitised by conspecific females and those from non-parasitised hosts, but interspecific host discrimination has been rarely reported, particularly for larval parasitoids. As larval parasitoids usually incur strong physical defence from hosts that they attack (Brodeur *et al*. 1996), host larvae may be too active to be held by parasitoids for sufficient duration to judge whether they had been parasitised. The results obtained in this study demonstrated that both parasitoid species could parasitise host larvae previously parasitised by the other species, no matter whether the first parasitoid was in the egg or the larval stage. Both parasitoid species showed little discrimination and seemed to take the strategy to deposit all their eggs into hosts as soon as possible, to increase their fitness.

Yang *et al*. (1994) reported that only one parasitoid could survive to adult in one *D. semiclausum* superparasitised larva. Eggs of *D. semiclausum* began to hatch 1-2 days after oviposition at 25˚C (Yang *et al*. 1993). Dissections showed that newly hatched *D. semiclausum* larvae were swimming in the host haemocoele, suggesting that the early larval stage lived on the materials in the haemolymph. In all treatments where ovipositions by *D. semiclausum* were followed by *O. sokolowskii*, and where ovipositions by *O. sokolowskii* were immediately followed by *D. semiclausum*, the proportion of hosts with *O. sokolowskii* reduced sharply after *D. semiclausum* had developed for 4-5 days. It could be inferred that *D. semiclausum* killed and swallowed the *O. sokolowskii* immatures during its late larval stages. On the contrary, when ovipositions by *O. sokolowskii* were followed by *D. semiclausum* with 2 or 3 days delay, the proportions of hosts with *O. sokolowskii* did not change as the host developed. After the host had pupated, they remained suitable for the pupation of *O. sokolowskii*, but became unfavourable to larval development and survival of *D. semiclausum*. Therefore, hosts from such treatments produced more *O. sokolowskii* than *D. semiclausum*.

Endoparasitoids require sufficient nutrients from their hosts to develop to maturity. One DBM larvae usually support the complete development of 8-10 *O. sokolowskii* (Ooi 1988, Wang *et al*. 1999), or one *D. semiclausum* (Yang *et al*. 1994). Over use of host material by both parasitoids may be responsible to the low survival rates of multiparasitised host larvae. Although multiparasitism by the two species reduces their fitness, it may not necessarily reduce their effectiveness against DBM because their differential requirements for temperature (Yang *et al*. 1993, Wang *et al*. 1999) and possibly other environmental factors may result in niche separation between the two species. However, as *O. sokolowskii* has a wide range of hosts and is a facultative hyperparasitoid as well as a primary parasitoid, caution must be taken when introduction of this parasitoid into new areas is considered.

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Parasitoids associated with the diamondback moth, *Plutella xylostella* (L.), in the Eastern Cape, South Africa

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Abstract

Seasonal fluctuations of diamondback moth and its hymenopteran parasitoids were recorded weekly from April 1997 to November 1999 at four cabbage sites in the Grahamstown area of the Eastern Cape, South Africa. Two sites were commercial farms with active spraying programmes; the others were unsprayed. Infestation levels were highest during spring (September to November) and autumn (March to May), where 100% infestation of plants was reached at times. The highest infestation was found during the spring months, where 12 larvae/plant were found at the unsprayed sites and between 6 and 10 larvae at the sprayed sites. At the unsprayed sites abundance of diamondback moth larvae and parasitoids was high during 1997, but much lower during 1998 and 1999, indicating possible control by the parasitoids.

Nine species of parasitoid were recorded from diamondback moth during this period and four (*Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae), *Diadegma mollipla* (Holmgren) (Hymenoptera: Ichneumonidae), *Diadromus collaris* Gravenhorst (Hymenoptera: Ichneumonidae) and *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae)) showed potential as biological control agents. The highest rate of parasitism was found from mid-autumn to the beginning of winter (April to June) and from mid-spring to the beginning of summer (October to December). Percent parasitism varied throughout the year, ranging between 10% and 80%. Parasitism of 100% was observed when moth numbers were low. Different species of parasitoids were found to be dominant at different times of the year.

Keywords

Cotesia plutellae, Diadegma mollipla, *Diadromus collaris, Oomyzus sokolowskii*

Introduction

Crucifiers, especially cabbage, *Brassica oleracea* var. *capitata*, are important crops, forming the staple diet for many South Africans and are grown on both a small and large scale. 80% of small-scale rural farmers grow cabbage as a subsistence crop and commercially, 160,000 tons/annum are harvested (Charleston 1998). Control of the cosmopolitan cabbage pest, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) diamondback moth is difficult as it has become resistant to nearly all insecticides used against it (Talekar & Shelton 1993). For this reason the use of biological control agents, particularly parasitoids has become important.

South Africa is fortunate to have a large number of parasitoids, many of them indigenous, that are associated with the diamondback moth and that can provide suitable control in particular circumstances. Ullyett (1947) recorded 14 species of primary parasitoids in the Pretoria region and more recently Kfir (1998) recorded 22 species. The pest status of diamondback moth is much lower in South Africa than in other parts of the world with similar climates (Kfir 1998), but the pest still causes serious damage.

Materials and methods

Four study sites were selected. Site 1 (33°19'S; 26°36'45" E) and Site 2 (33°19'45"S; 26°38'30"E) were large-scale commercial farms, growing cabbage as a cash crop. At the other two sites, Site 3 (33°29'30"S; 26°09'15"S) and Site 4 (33°18'30"S; 26°33'S) cabbage was grown on a smaller scale and solely for the purpose of the study. All sites were in the vicinity of Grahamstown, Eastern Cape. Site 1 was sampled during 1997 and January 1998, after which cabbages were no longer grown at this site. Sampling continued at the second commercial farm (Site 2) from February 1998 until the end of the study in November 1999. Spraying programs, mainly using synthetic pyrethroids, were in effect at both Site 1 and Site 2. At Site 3, sampling was carried out from April 1997 until November 1999, but rearing of larvae and pupae to determine parasitoid emergence only started in August 1997. Cabbages had not been grown in this area before. Sampling at Site 4 was carried out from August 1997, but rearing of larvae and parasitoids only started in January 1998. Sampling was inconsistent at this site, as there were periods when cabbages were not grown. Site 3 and 4 were not treated with insecticide.

Once a week, 30 randomly selected cabbages were sampled at each site. Diamondback moth larvae and pupae and parasitoid cocoons were recorded. Larvae and pupae were collected and reared out in the laboratory, at 24°C and 16:8 hours (L:D), to determine moth emergence and parasitism levels in the field. The dates of pupation and emergence of either moth or parasitoid were recorded. Parasitism was related to the sampling date and not the emergence date.

Results

Infestation levels of *Plutella xylostella*

The percentage of cabbage plants infested by *P. xylostella* follows a similar pattern at each site with the infestation levels being highest during spring, from September to November. Infestation levels of the plants (Figure 1) at all sites were low until the beginning of August 1997. From then until the end of October 1997 infestation was high, reaching 100% at all sites. These levels decreased over November and December 1997 and remained below 40% at the two unsprayed sites (Site 3 and 4) until the beginning of September 1998. Infestation levels at Site 3 and 4 increased during spring1998, reaching 60% and 97% infestation, respectively. In 1999 infestation levels remained below 40% until August where it increased, reaching 68% and 86%, at Site 3 and 4 respectively. At Site 2, infestation levels remained high during 1998, generally above 50%. In 1999, infestation levels dropped slightly, but by October 1999 infestation reached 100%.

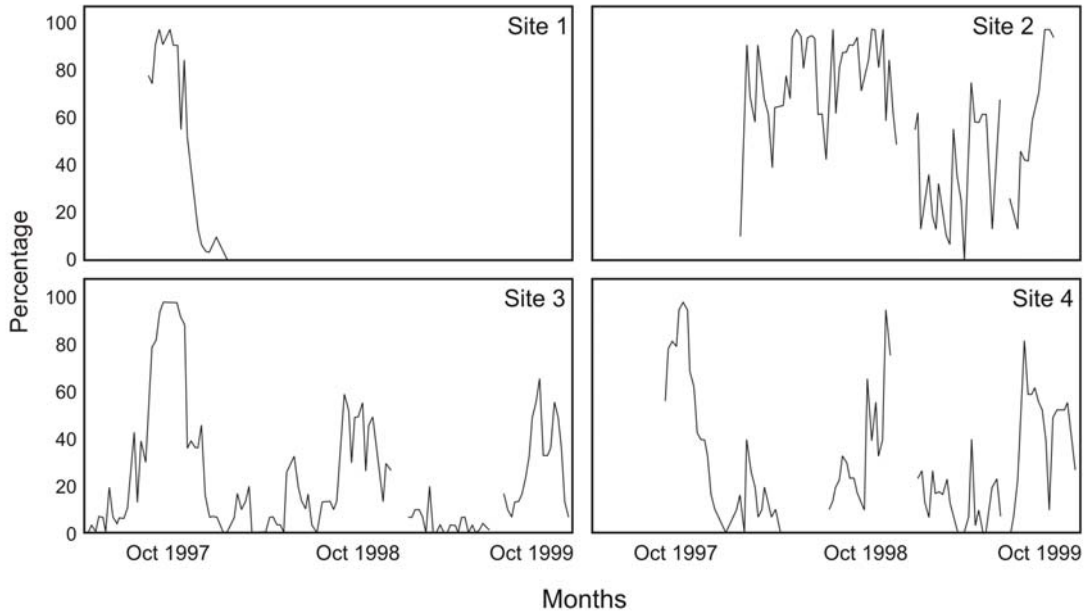

Figure 1. Infestation levels of *P. xylostella* at four sites in Grahamstown, Eastern Cape, South Africa, over the study period April 1997 to November 1999.

The highest numbers of larvae per plant were recorded during spring (September to November) (Figure 2). At Site 1 and 2, the sprayed sites, 7 larvae/plant were recorded in 1997 and 10 larvae/plant in 1998, respectively. At Site 3, the unsprayed site, 12 larvae/plant were found in 1997. In 1998 and 1999 it dropped to below 2 larvae/plant. At the second unsprayed site (Site 4) numbers remained below 4 larvae/plant.

Abundance of *Plutella xylostella*

The abundance of *P. xylostella* larvae and pupae varied between sites and between years. Figure 3 shows the abundance of the larvae and pupae at Site 2, a sprayed site and at Site 3, an unsprayed site. Abundance increased during August, reaching a peak over September to November. There were additional peaks in abundance over the autumn months, from March to May at some of the sites. At the sprayed site (Site 2) abundance of larvae and pupae were generally always high. At the unsprayed site (Site 3) abundance of larvae and pupae was high in 1997, but decreased drastically in 1998 and 1999.

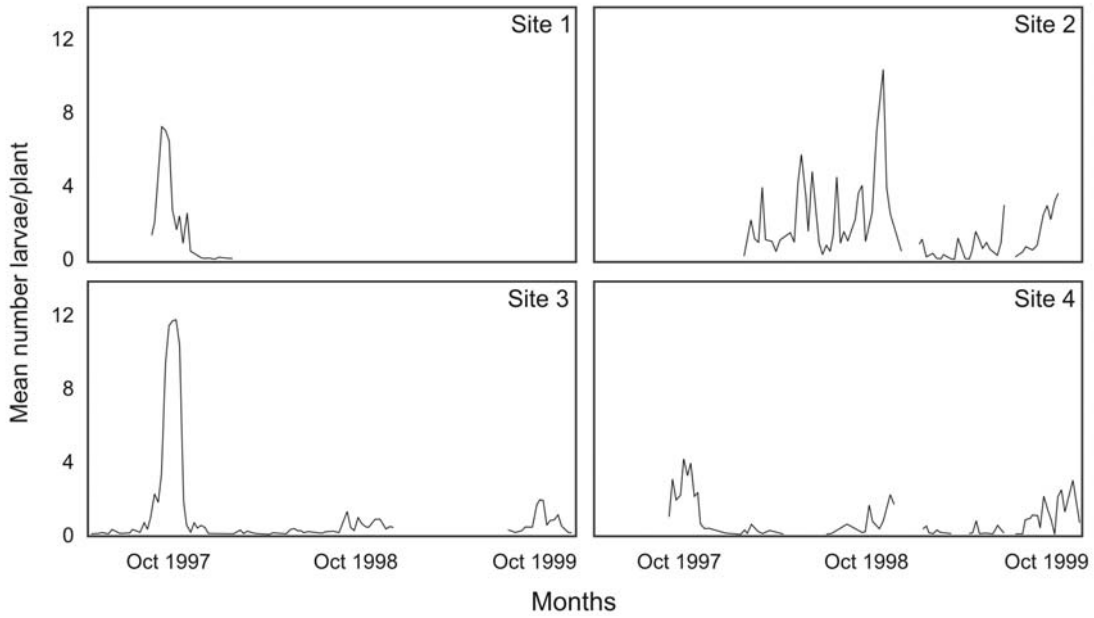

Figure 2. Mean number of *P. xylostella* larvae/plant at four sites in Grahamstown, Eastern Cape, South Africa, over the study period April 1997 to November 1999.

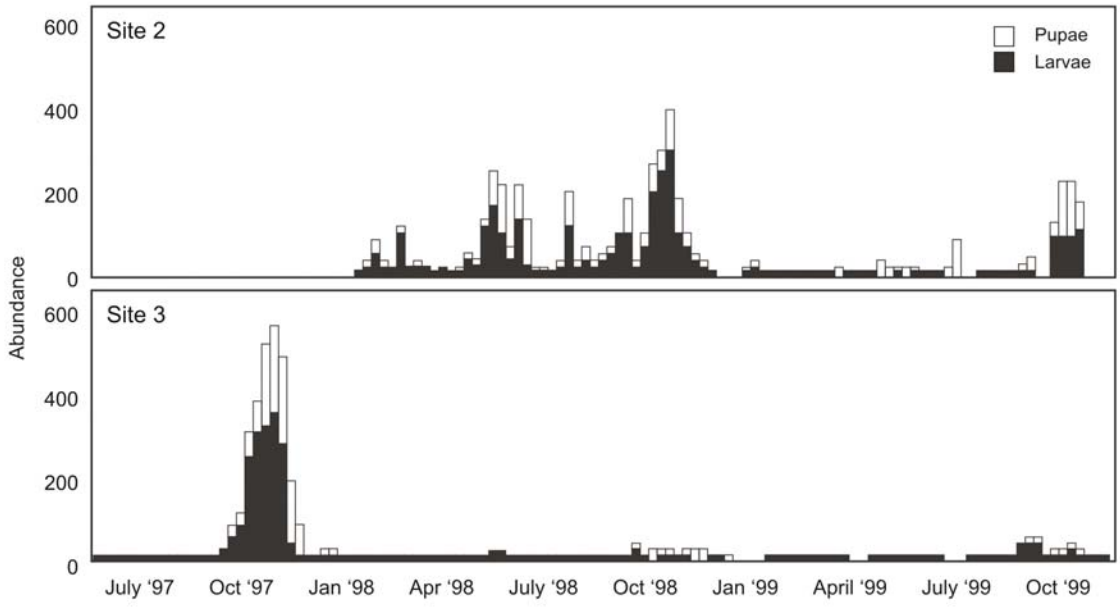

Figure 3. Abundance of *P. xylostella* larvae and pupae at Site 2 and 3 in Grahamstown, Eastern Cape, South Africa, over the study period April 1997 to November 1999.

Parasitoid species present

Nine species of parasitoid wasp were recorded from diamondback moth larvae and pupae (Table 1), including primary and hyperparasitoids. The different species attacked different stages of the *P. xylostella* life cycle. They differed in abundance throughout the year and between sites. *Apanteles eriophyes* Nixon, *Itoplectis* sp. and hyperparasitoids were found in very low numbers.

Abundance of parasitoids

The abundance of the different parasitoid species at two sites, Site 2 and 3, are shown in Figure 4. *Cotesia plutellae* (Kurdjumov) was most abundant at Site 2, a sprayed site, throughout the study period. At Site 3, an unsprayed site, *C. plutellae*, *Diadegma mollipla* (Holmgren) and *Diadromus collaris* Gravenhorst and *Oomyzus sokolowskii* (Kurdjumov) were abundant during spring of 1997, but their abundance dropped severely in 1998 and 1999 as a result of the low abundance of *P. xylostella* larvae and pupae in the field.

Table 1. Parasitoids and hyperparasitoids associated with the diamondback moth in the Eastern Cape, South Africa

Species	Family	Stage of Attack
Cotesia plutellae (Kurdjumov)	Braconidae	Larval
Apanteles eriophyes Nixon	Braconidae	Larval
Diadegma mollipla (Holmgren)	Ichneumonidae	Larval - pupal
Diadromus collaris Gravenhorst	Ichneumonidae	Pupal
Oomyzus sokolowskii (Kurdjumov)	Eulophidae	Larval - pupal
Itoplectis sp.	Ichneumonidae	Larval - pupal
Mesochorus sp.	Ichneumonidae	Hyperparasitoid
Pteromalus sp.	Pteromalidae	Hyperparsitoid
Proconura sp.	Chalcididae	Hyperparasitoid

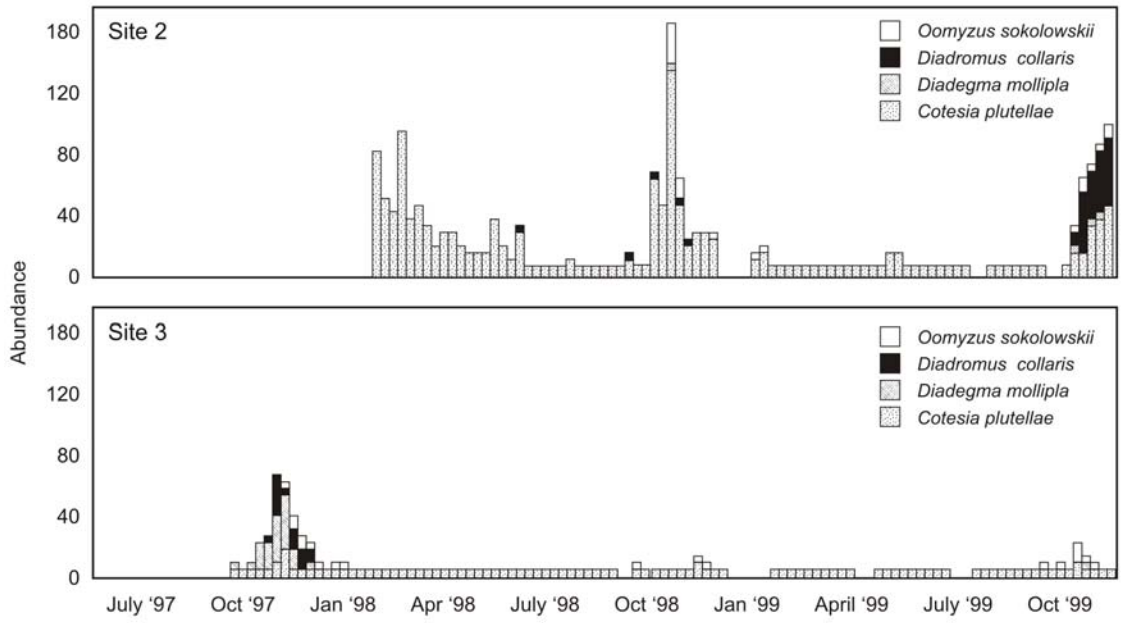

Figure 4. Abundance of the four common parasitoids of *P. xylostella* at Site 2 and 3 in Grahamstown, Eastern Cape, South Africa, over the study period April 1997 to November 1999.

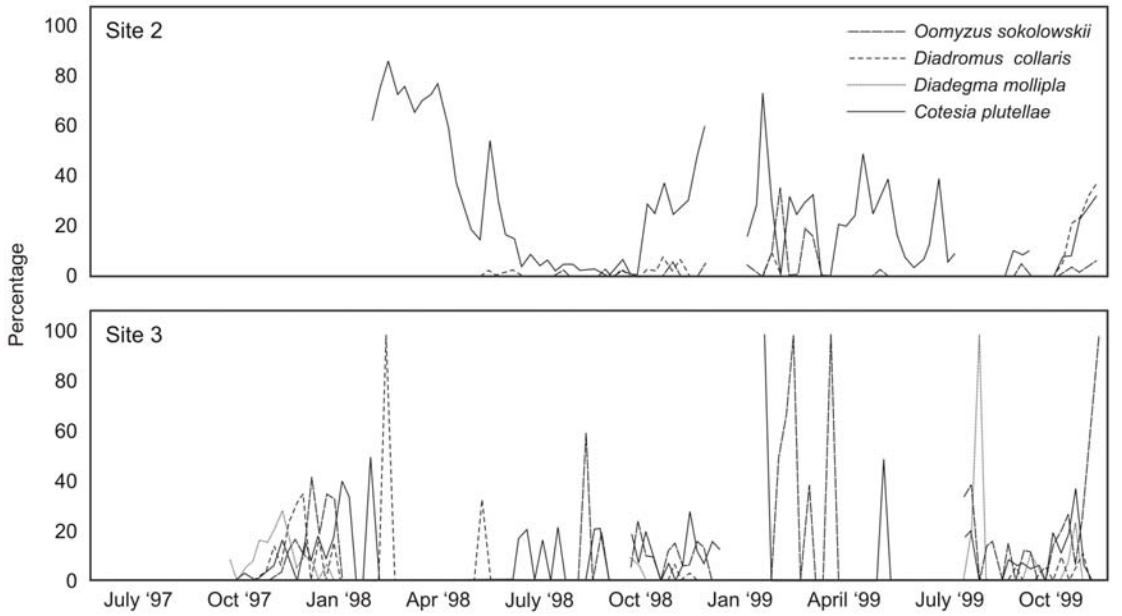

Figure 5. Percentage parasitism of the four common parasitoids at Site 2 and 3 in Grahamstown, Eastern Cape, South Africa, over the study period April 1997 to November 1999.

Percentage parasitism

The percentage parasitism by the different parasitoid species varied between the sites. Figure 5 shows the percentage parasitism at Site 2, a sprayed site, and at Site 3, an unsprayed site. The highest rates of parasitism by *C. plutellae* (88%) were recorded at the sprayed site, Site 2. The rates of parasitism by other species remained below 25%. At the unsprayed site, Site 3, parasitism by *C. plutellae* was generally below 60%, but parasitism by other species increased. At Site 3 parasitism rates reached 100% at times, probably due to the low infestation levels of diamondback moth larvae and pupae in the field.

Discussion

The infestation level of diamondback moth at all the sites increased almost simultaneously at the end of the winter period and remained high, in some cases 100% of plant infestation was recorded for the first two months of the spring period in 1997. At Site 2, a commercial farm, infestation remained high over the entire sampling period even with the weekly use of pesticides. Cypermethrin was the common pesticide used and resistance to this pesticide has been found in other parts of South Africa (Sereda *et al*. 1997). The high infestation levels at Site 2 could indicate pesticide resistance. Ullyett (1947) found that in the Pretoria region of South Africa the numbers of *P. xylostella* were highest in early summer and reached 11.6 larvae/plant, but this did not result in serious economic damage. The mean number of larvae per cabbage in the Eastern Cape increased at the end of the winter period and numbers remained at their highest (12 larvae/plant) until late spring.

Cabbage is grown all year round in the Eastern Cape. Because of this continuous resource and the mild climatic conditions, *P. xylostella* is present all year, but seasonal variation occurs and low numbers are found in winter months. Both moth and parasitoid abundance is highest in spring, from September to November, with additional peaks in abundance in autumn (March to May). The different parasitoid species show seasonal variation between years and between sites. *C. plutellae* was the most abundant parasitoid at sprayed sites, Sites 1 and 2, and was found in much lower abundance at unsprayed sites, Site 3 and 4. However, *D. mollipla*, *D. collaris* and *O. sokolowskii* were more abundant at unsprayed sites than at sprayed sites. This suggests that *C. plutellae* may have developed resistance to pyrethroids used at the sprayed sites.

At Site 3, the unsprayed site, all four major parasitoids were present and showed the highest abundance in the spring of 1997, in 1998 and 1999 the abundance of the moth and its parasitoids decreased drastically. This suggests that the parasitoids were able to establish and reduce the population of diamondback moth to a very low level after the first year. It also suggests that it is important that there is a complex of parasitoids, working together to reduce the diamondback moth population. A similar pattern was found at the second unsprayed site, Site 4, with diamondback moth larvae and pupae decreasing after the first year.

There is a complex of parasitoids in the Eastern Cape region of South Africa that are capable of providing suitable control against *P. xylostella* if they are allowed to establish in a pesticide-free environment. Hyperparasitoids are present in very low numbers and as a result did not reduce the effectiveness of the primary parasitoids.

Acknowledgements

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Impact of parasitoid wasps on *Plutella xylostella* in Perth, Western Australia

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Abstract

This study reports a field trial to measure the impact of beneficial parasitoid wasps on diamondback moth, *Plutella xylostella,* in Perth, Western Australia. Cabbage seedlings each inoculated with a set number of *P. xylostella* eggs were planted amongst a commercial cabbage crop in April 2001. Inoculated plants were enclosed using one of four different cages designed to give naturally-occurring wasps varying levels of access to the test plants, ranging from restricted to full access. Test plants were protected from insecticidal sprays applied by the farmer. Three weeks later when most of the *P. xylostella* had pupated, numbers on test plants were counted and collected. These remaining *P. xylostella* were reared in the laboratory on canola to determine levels of parasitism within the different cages. *Diadegma rapi* and *Diadegma semiclausum* made up 47% and 39% respectively of the parasitoids found in addition to low numbers of *Apanteles ippeus* and *Diadromus collaris*. *Plutella xylostella* survival was highest (42%) in cages where wasps had no access to test plants and lowest (17%) where wasps had the greatest access to plants. In the treatment where wasps had the greatest access to test plants, 100% of remaining *P. xylostella* were parasitised after three weeks. High rates of parasitism by wasps, particularly by *D. semiclausum* and *D. rapi,* were encouraging despite regular applications of insecticide sprays to plants surrounding the test plants.

Keywords

Diadegma semiclausum, Diadegma rapi, *Apanteles ippeus*, *Diadromus collaris*

Introduction

The caterpillar of the diamondback moth, *Plutella xylostella*, is the most destructive pest of crucifer vegetables in Australia and worldwide (Talekar & Shelton 1993). To improve control of this pest, growers are utilising Integrated Pest Management (IPM) techniques, which includes the tactic of encouraging natural enemies.

The natural enemies of *P. xylostella* include a range of parasitoid wasp species (Hassell & Waage 1984). Six species of parasitoid wasp, of which *Diadegma semiclausum* and *Apanteles ippeus* were the most common, were recorded in an earlier survey of farms surrounding the Perth Metropolitan area. Although these beneficial insects are often mentioned in the context of IPM, few studies have quantified their impact on pest populations. This report outlines a preliminary experiment conducted on a farm near the Perth Metropolitan Area which aimed to measure the impact of naturally occurring parasitoid wasps on *P. xylostella* populations.

Materials and methods

The trial site was situated in a commercial cabbage crop located in Mandogalup, 50 km south of the Perth CBD, Western Australia.

Plants and insects

We used the same cabbage seedling variety (Green Coronet) as that planted in the test crop. Four-week-old seedlings were exposed to egg lay by a laboratory colony of *P. xylostella* adults for a 48 h period. Each plant was then carefully examined and surplus eggs were removed to leave 20 eggs per seedling. Seedlings were then transplanted into 12.5 cm pots and planted amongst cabbage rows at the farmer's property (5 April 2001), spaced 1.5 m apart. Each inoculated plant was caged using exclusion cages designed to allow one of four different levels of natural enemy access to the *P. xylostella* on the test plants. All cages consisted of a central frame (45 cm high x 45 cm diameter) made from trellis. Modifications to the fine nylon netting sleeve covering the central frame allowed construction of cages with the following treatments:

a) *Full cage covered*. The central trellis frame covered the plant and was totally covered with a nylon mesh sleeve. This treatment totally excluded natural enemies.

b) *Frame only*. The cage consisted of only the central trellis frame and gave total natural enemy access

c) *Partial cage + sticky barrier*. Nylon netting sleeve was used to partially cover the central trellis frame, and the rim of the pot was treated with a sticky barrier (Tac-gel®). This limited natural enemy access to flying insects.
d) *Partial cage only*. Same as c) above, but pot rims without sticky barrier.

The treatments were arranged in a randomised block design, replicated 10 times. Treatment (c), where the central trellis frame was partially covered by the nylon netting, was designed to allow natural enemy access to the cage while creating ambient environmental conditions similar to that within the completely sealed cages. The sticky barrier prevented access to the plants by ground-dwelling natural enemies.

Counts of field insect populations were taken every seven days. The surrounding crop was treated for pests at the farmer's discretion. Sprays were applied weekly during the trial, starting on 30 March 2001 and were (in order of application) *Bacillus thuringiensis* (Delfin®, Novartis) + alpha-cypermethrin (Dominex®, Cropcare), emamectin benzoate (Proclaim®, Novartis), fipronil (Regent®, Rhône-Poulenc) + methomyl (Electra®, Farmoz) and Delfin® + Dominex®.

All cages were covered with plastic bags before spray application to protect test plants and insects from insecticide sprays. We terminated the experiment after three weeks (26 April, 2001) when most of the insects had pupated. At this time the appearance of each test plant was ranked as follows: 1 = no damage; then depending on per cent of leaf area skeletonised, 2 = 1 - 10%; 3 = 11 - 30%; 4 = 31 - 60%; 5 = 61 - 90%; and 6 = 91 - 100%.

All *P. xylostella* larvae collected at the end of the experiment were reared on cabbage leaves in the laboratory at 21°C. Any III instar or smaller larvae originating from wild *P. xylostella* eggs that were found on plants on 26 April 2001 were also recorded, but not retained. Mature wasps and *P. xylostella* moths that emerged were preserved and identified. Differences in *P. xylostella* survival and plant damage ratings amongst treatments were analysed using analysis of variance (ANOVA) tests.

Results

More *P. xylostella* survived in the treatment where natural enemies were excluded (a) ($P = 0.002$) and, of these, only 1% were parasitised (Table 1). Ground-dwelling natural enemies observed included carabid beetles and predatory mites (not identified to species level) which presumably also had some effect on the *P. xylostella*.

Table 1. Numbers of *Plutella xylostella* recovered 3 weeks after the trial commenced, % survival of original infestation and *P. xylostella* damage to leaves from different exclusion treatments using field cages

Treatment	No. surviving (%)	Damage rank
Full cage covered (a)	83 (42%)	4.8
Frame only (b)	34 (17%)	3.3
Partial cage + sticky barrier (c)	61 (31%)	4.0
Partial cage only (d)	37 (19%)	3.4

The plants in the cages where the parasitic wasps could freely access the *P. xylostella* (treatment b) suffered significantly less damage ($P<0.005$) than plants in the cages where the parasitic wasps had been excluded (treatment a) (Table 1).

Fewer *P. xylostella* survived in cages where both ground-dwelling and aerial natural enemies had access to test plants: frame only cage (b) or partial cage only (d) (17-19% survival). The sticky barrier of treatment c prevented the ground-dwelling natural enemies from accessing the eggs or larvae and *P. xylostella* survival was higher at 31% after three weeks. Of these, 40.9% and 13.1% respectively were parasitised by *Diadegma rapi* and *D. semiclausum* (Table 2). *Diadegma semiclausum* and *D. rapi* were the dominant species found across the trial and occurred in similar proportions.

Table 2. Species of parasitoid wasps recovered and % parasitism of surviving *Plutella xylostella* from different exclusion treatments using field cages.

Treatment	*Diadegma rapi*	*Diadegma semiclausum*	*Apanteles ippeus*	*Diadromus collaris*	Total parasitoids	% parasitised
Full cage covered (a)	1	0	0	0	1	1.2%
Frame only (b)	13	20	0	0	35	103.0%*
Partial cage + sticky barrier (c)	25	8	1	1	39	64.0%
Partial cage only	10	13	0	1	29	78%
Total	41	49	1	2	104	

*parasitism of >100% could be due to more than one wasp egg being laid developing in a single *P. xylostella.*

Discussion

Although the barrier cage (full cage treatment "a") protected *P. xylostella* from parasitoids, 58% of the *P. xylostella* were not recovered and were assumed to have been killed by unknown factors. Of the survivors, only 1.2% were parasitised. Fully enclosing plants resulted in low levels of parasitism as wasps either had no access or very limited access (on the occasions when cages were briefly opened to examined test plants) to *P. xylostella*. In contrast, *P. xylostella* survivorship (17%) was lowest with the highest levels of parasitism (100%) in the frame treatment (b) because of constant exposure to natural enemies. Increased exposure to wind and rain may also have contributed to the lower survivorship of the DBM in the frame treatment (b).

It was encouraging that all the *P. xylostella* in one treatment were parasitised, demonstrating the effectiveness of the parasitoids. In addition, the two dominant wasp species, *D. rapi* and *D. semiclausum*, which attack all larval stages, particularly II instar larvae, can reduce the amount of damage the larvae can cause. In contrast, *Diadromus collaris* which attacks prepupae and pupae, gives the pest more time to chew and damage the plant leaves. *Diadegma rapi* was more common in this study than in 1998-1999, reflecting seasonal population variation.

Plants with the greatest exposure to parasitic wasps (treatment b) were least damaged by *P. xylostella* compared with the three other treatments. However, this level of damage was greater than that currently accepted by fresh produce markets. As smaller wild *P. xylostella* larvae were only found in low numbers (0 – 3/ plant), we assumed they had a negligible effect on plant damage rankings.

Further work is required to determine how to best utilise natural enemies of *P. xylostella* in commercial crops sprayed with insecticides. High rates of parasitism by wasps, particularly by *D. semiclausum* and *D. rapi* were recorded, in spite of regular applications of insecticide sprays to the surrounding cabbage crop. Perhaps the species of wasps found may have developed some tolerance to insecticides used, or the wasps moved into the trial from unsprayed areas. Neither of these possibilities was tested. Nonetheless, beneficial insects such as parasitic wasps should be encouraged by using less harmful insecticides. Parasitic wasps should be used in conjunction with "soft" insecticides to develop an IPM program for *P. xylostella.*

Further work is also needed to measure the effects of seasonal differences on parasitoid wasp activity and their impact on *P. xylostella* in other *Brassica* vegetables. This study complements other studies being conducted in Queensland and Tasmania.

Acknowledgements

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Guild structure of aphid parasitoids in broccoli: influence of host and neighbouring crops

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Abstract

Naturally occurring parasitoids may provide sufficient control of aphid pests in fields undisturbed by insecticides. Yet the efficacy of these parasitoids may be strongly influenced by characteristics of their hosts and by the availability of alternative hosts in neighbouring crops. In the present study, parasitoid guild structure was compared in two aphid species, *Brevicoryne brassicae* and *Lipaphis erysimi*, in broccoli and in adjacent wheat and cotton fields. *Diaeretiella rapae* constituted 81 and 75% of all parasitoids (including hyperparasitoids) recovered from *B. brassicae* and *L. erysimi*, respectively. *Aphidius* species were also represented at low levels. *Diaeretiella rapae* does not appear to attack other aphids in neighbouring non-cruciferous crops; Aphids in wheat and cotton are attacked primarily by *Aphidius* and *Lysiphlebus* species, respectively. Another factor that hampered parasitoid population build-up in broccoli was the action of hyperparasitoids, which were active throughout the broccoli-growing season (November-March). To enhance biological control, our data suggest that it is important to keep specific non-crop vegetation as a parasitoid source near broccoli fields. This vegetation must not harbour aphid species that may move onto broccoli but instead be infested with other aphids that are readily attacked by *D. rapae*.

Keywords

parasitoid dispersal, cabbage aphid, turnip aphid, landscape structure

Introduction

Many natural enemies attack a range of species on an assortment of crop and non-crop plants. The impact of such natural enemies on pest populations in a particular crop is likely to depend on their dynamics on nearby crop and non-crop vegetation (Stary 1972, Andow 1991, Hopper 1989, Landis & Haas 1992). Changes in landscape design (size, shape, proximity of areas of various crops and non-crop plants) could greatly modify colonization of fields by natural enemies and thus the suppression of pest populations (Marino & Landis 1996, Coll 1998).

For biological control to work, either natural enemies must reduce regional abundance of target pests to levels that prevent damaging colonization of crops or natural enemies must colonize crops rapidly enough and in sufficient numbers to prevent colonizing pest populations from reaching damaging levels. Whether natural enemies can do either of these depends on the spatial distribution of habitats suitable for pests and natural enemies and on the relative dispersal abilities of pests and natural enemies (Marino & Cornell 1992, Coll *et al*. 1994, Corbett & Rosenheim 1996, Coll & Bottrell 1996, Baur & Yeargan 1996). Yet our understanding of the dynamics of parasitoid populations on a landscape level is extremely limited.

Aphids (Homoptera: Aphididae) are important pests in many vegetable and field crops worldwide. High mobility, parthenogenetic reproduction, high rate of population increase and sheltered feeding sites make them difficult to control. Yet aphids are often attacked by a large number of natural enemy species, and particularly by parasitic wasps. Increasing the efficacy of naturally occurring parasitoids through habitat management promises to provide a cost-effective, safe and sustainable method for aphid control. This method could be the central component of an integrated aphid control program.

In the study reported here, we draw inferences about the ability of wasps to move between neighbouring habitats and attack aphid pests in broccoli by comparing the species composition of aphid parasitoids in such habitats with that found in broccoli. Specifically, we compared the species composition of parasitoids that attack aphids in wheat (i.e. oat-birdcherry aphid, *Rhopalosiphum padi* (L.), corn leaf aphid, *R. maidis* (Fitch) and green bug, *Schizaphis graminum* (Rondani)); cotton (i.e. cotton aphid, *Aphis gossypii* Glover) and broccoli (i.e. cabbage aphid, *Brevicoryne brassicae* (L.) and turnip aphid, *Lipaphis erysimi* (Kaltenbach).

At our study sites in Israel, these crops are grown in adjacent fields with an overlap in growing season (Figure 1). Cruciferous crops are grown between November and March, when winter wheat is infested by several aphid species. Cotton is planted from late-March to mid-May and harvested in October. Aphid infestation in cotton occurs in April through June and again in September. Thus, periods of aphid infestation in wheat and cotton usually overlap with aphid infestations in cruciferous crops. Therefore, we expected parasitoids to move between crops and attack aphid pests in each crop. Our working hypothesis was that some parasitoid species are shared by aphid pests in adjacent broccoli, wheat and cotton fields.

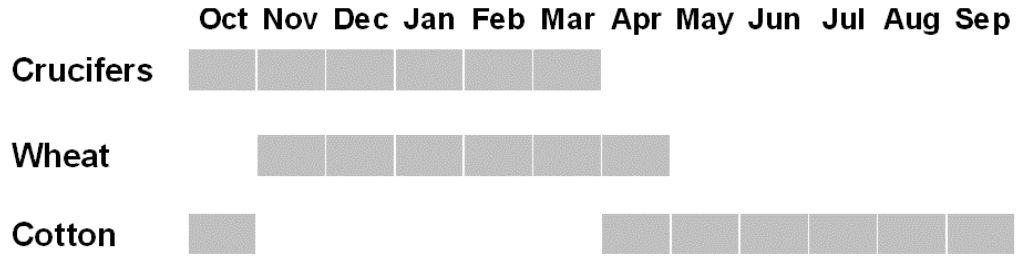

Figure 1. A schematic representation of the growing seasons of the studied crops in Israel.

Material and methods

Aphids were collected weekly between March 1998 and June 2001 in two regions in Israel about 120 km apart. The sampled fields were in an area of about 90 and 120 km^2 in the Judea plain and the Ysrael valley, respectively. Overall, about 100,000 aphids were collected in broccoli, more than 100,000 in wheat and about 335,000 in cotton. The collected aphids were sorted to species, counted and transferred to appropriate potted host plants. These potted plants were caged and monitored daily for mummified aphids and wasp emergence.

Wasps that emerged from field-collected aphids were placed in absolute ethanol then classified as Aphidiinae (Braconidae), primary parasitic Aphelinidae (genus *Aphelinus*), or hyperparasitic Aphelinidae. The aphidiine braconids were further identified to the genus.

Results

The aphidiid parasitoids reared from field-collected aphids belong to three genera: *Diaeretiella*, *Aphidius* and *Lysiphlebus*. Species composition of parasitoids within a crop type did not differ between the regions so we pooled data from the two regions. *D. rapae* (McIntosh) was the dominant primary parasitoid in broccoli, constituting 93% of all primary parasitoids. The cabbage aphid was also attacked by *Aphidius* spp., whereas the turnip aphid was also attacked by *Aphelinus* spp. (Figure 2). Nevertheless, the overall species composition was similar on the two aphid hosts in broccoli. Likewise, a similar parasitoid guild attacks the three aphid species in wheat. However, the dominant primary parasitoids in wheat were *Aphidius* spp. (96%, Figure 3). Finally, *Lysiphlebus* spp. dominated the guild of primary parasitoids that attack *Aphis gossypii* in cotton (83%, Figure 4).

The results suggest that the primary parasitoids that attack aphids in the three study crops are differentially exposed to attacks by hyperparasitoids. Overall, 16% and 8% of the parasitised aphids yielded hyperparasitoids in broccoli and wheat, respectively. In cotton, this value was 34% of the parasitised aphids. In broccoli, hyperparasitoids were active throughout the growing season (Figure 5) whereas in wheat and cotton they were important toward the end of the aphid infestation periods (in April and May in wheat and in June and July in cotton).

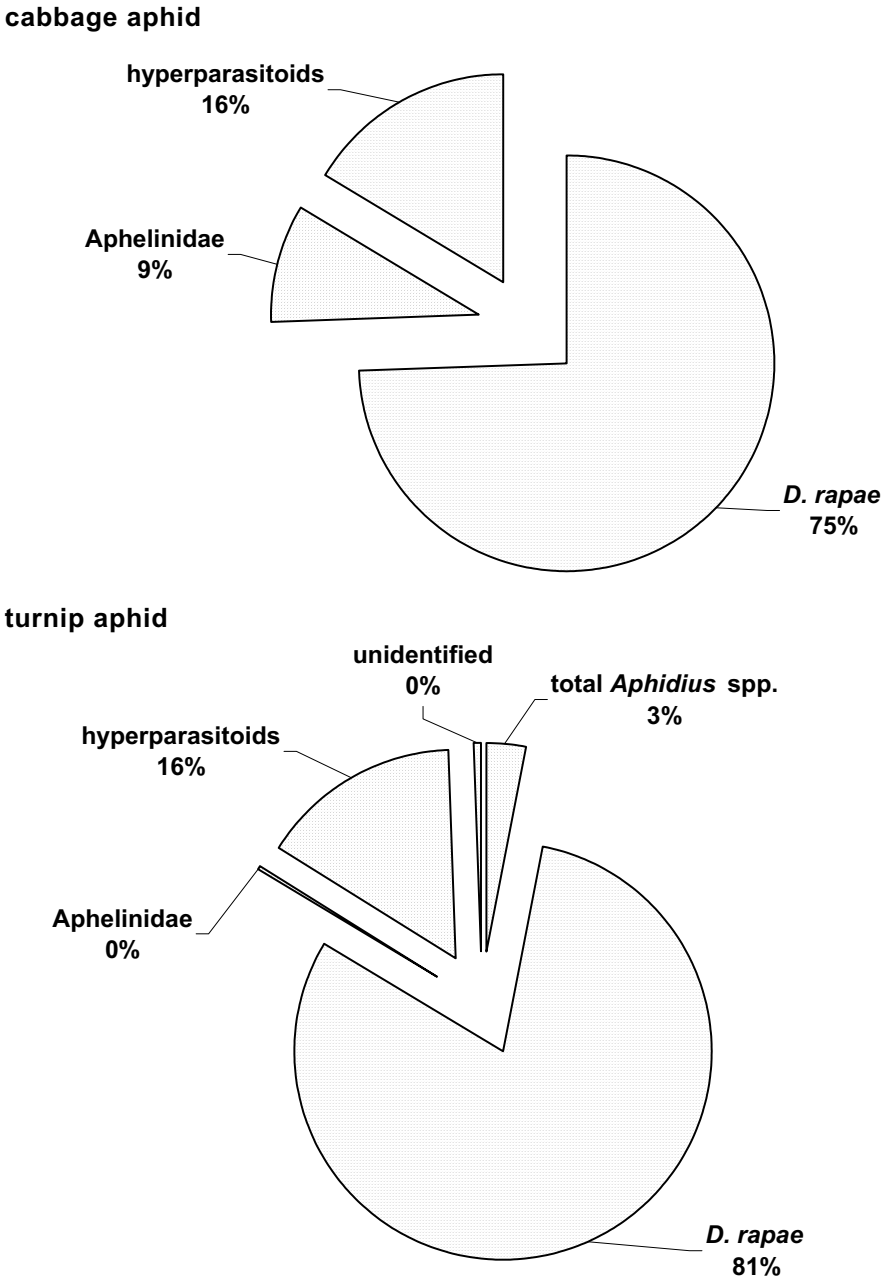

Figure 2. Species composition of parasitoids that emerged from cabbage aphid (*Brevicoryne brassicae*) and turnip aphid (*Lipaphis erysimi*) collected on broccoli in Israel.

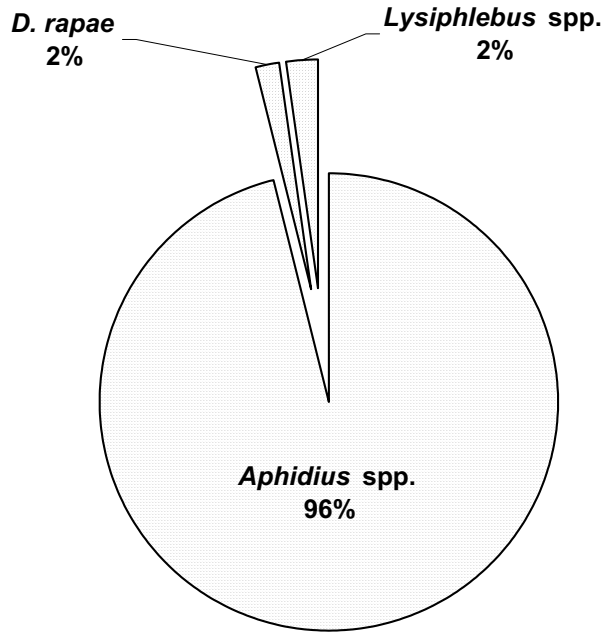

Figure 3. Species composition of primary parasitoids that attack aphids in wheat in Israel.

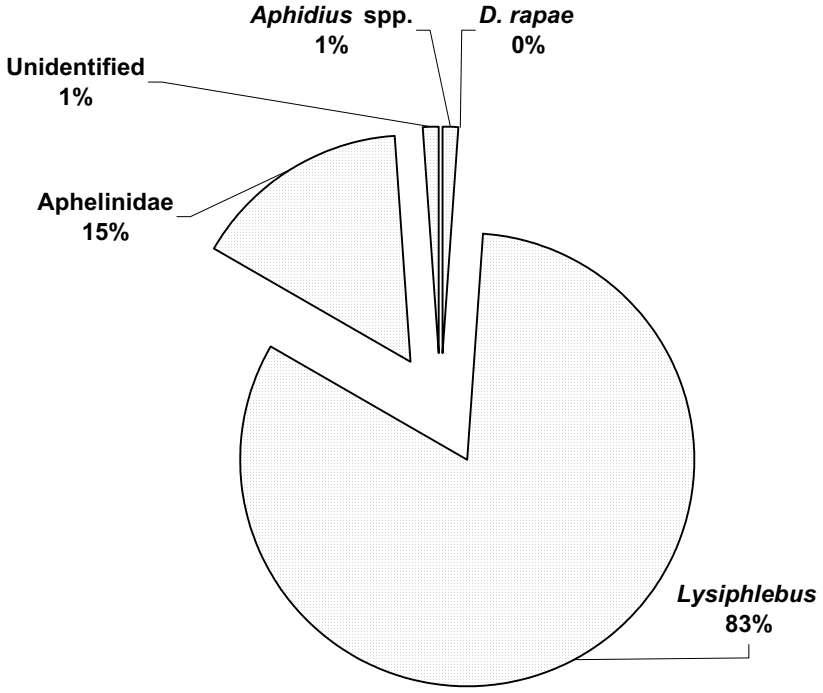

Figure 4. Species composition of primary parasitoids that attack aphids in cotton in Israel.

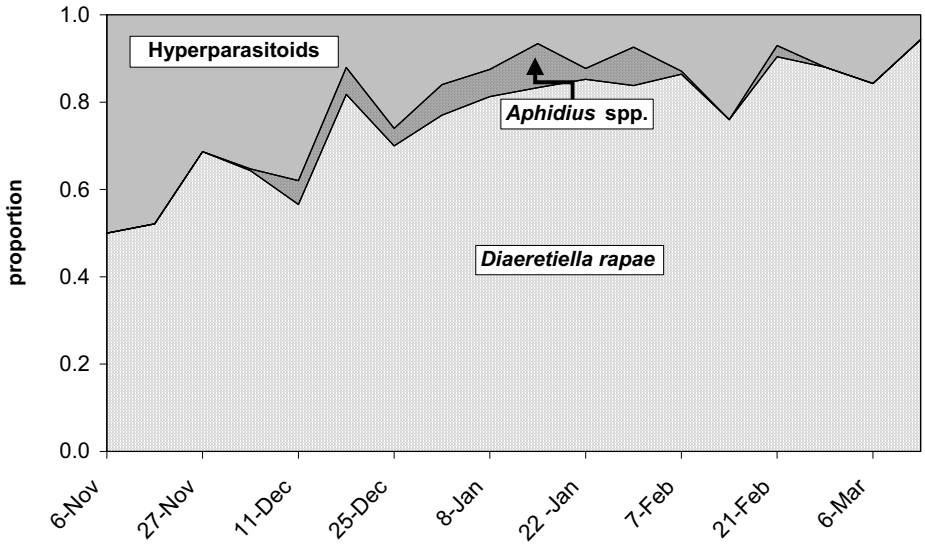

Figure 5. Seasonal dynamics of primary and hyperparasitoids that emerge from cabbage aphid (*Brevicoryne brassicae*) collected in broccoli fields in Israel.

Discussion

To enhance the activity of natural enemies against pest populations, we should encourage their movement into infested fields and discourage their departure. The movement pattern of parasitoids depends primarily on the availability of suitable hosts in vegetation surrounding the target fields. Our results show that different guilds of parasitoids attack aphid pests in different crops. These guilds were dominated by *D. rapae, Aphidius* spp. and *Lysiphlebus* spp. in broccoli, wheat and cotton, respectively. *Diaeretiella rapae* was also dominant in aphids collected from several non-crop plant species, primarily crucifers such as *Sinapis arvensis*, but also *Amaranthus* sp*., Hordeum vulgare, Malva sylvestris, Silybum marianum, Sonchus* sp. and *Sorghum halepense*. It seems, therefore, that the proximity to wheat and cotton has only a small effect, if any, on parasitoids that attack aphids in broccoli. Instead, *D. rapae* probably moves into broccoli from non-crop plants nearby.

Results from a companion study of *Aphidius colemani* Viereck, a major parasitoid in wheat, show that female preference and offspring performance both differ among aphid and host plant species. In addition, female *A. colemani* appear to be conditioned to oviposit in the host species in which they develop. Although this parasitoid is reported from a wide range of host species (Stary 1975, 1983), our results indicate that it is unlikely that an *A. colemani* population would attack many different host species at the same time and place. Instead, *A. colemani* may be composed of distinct host races that rarely switch between host species in the field, like some other polyphagous parasitoid species (Nemec & Stary 1983, Stary 1983, Powell & Wright 1988, Atanassova *et al*. 1998, Takada & Tada 2000). This would be a major obstacle for the enhancement of this parasitoid through habitat management.

Another barrier to this approach is the need to avoid growing, in close proximity, plants that share herbivore species; movement of pests from neighbouring vegetation would aggravate infestation levels in the target crop. Yet, host plant characteristics also influence the structure of parasitoid guilds. Data show that different guilds of parasitoids attack aphids on taxonomically distant plant species. This is apparently the case not only for species with a narrow host range, such as *D. rapae*, but also for species that are known to attack a wide range of hosts in diverse vegetation, such as *A. colemani* (Stary 1975, 1983). Our need to separate related plant species in space or in time (e.g. through crop rotation) reduces the rate at which parasitoids could colonize infested fields. Also, the creation of croplands that consists of weed-free, patchily arranged monocultural fields of unrelated plants does not allow parasitoids to build-up populations as they move between hosts and host plants as they become available at different times and places.

To take advantage of naturally occurring parasitoids we need to design favourable systems based on a deep understanding of the life history, behaviour and population ecology of key species. These systems are likely to be tailored to enhance the activity of a specific parasitoid species (or a few closely related species) by matching, for example, their host and host-plant preference, dispersal ability and seasonal dynamics. In

cases where it is necessary to control more than one pest species in a particular crop, the prevailing approach is to design agroecosystems that enhance the activity of either generalist predators (through the provisioning of prey, shelter, etc.) or of both predators and several parasitoid species (by providing them with nectar and pollen food sources) (Pickett & Bugg 1998). However, results presented here suggest that similar parasitoid species attack different aphid species in a given crop. This was the case for the cabbage and turnip aphids in broccoli and for the oat-birdcherry and corn leaf aphids in wheat. Therefore, a single landscape design may favour a whole suite of parasitoids that would attack several pests in a particular crop.

Acknowledgements

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Parasitism of *Nyctemera amica* (White) (Lepidoptera: Arctiidae) and *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) by *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae)

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Abstract

The parasitoid, *Cotesia plutellae*, is used as a biological control agent for diamondback moth, *Plutella xylostella*, in many countries and has been evaluated as a candidate for release in New Zealand. *C. plutellae* was originally released in Australia in 1951, but is rarely found. A glasshouse host specificity trial was conducted in Australia to assess whether *C. plutellae* would parasitise *Nyctemera amica*, magpie moth, on the noxious weed ragwort (*Senecio jacobæa* L.) in the presence of *P. xylostella* on cabbage. Although *P. xylostella* was expected to be the preferred host of *C. plutellae*, a greater proportion of the *N. amica* larvae was parasitised. It is likely, therefore, that *C. plutellae* would parasitise *N. amica* on ragwort in the vicinity of *Brassica* plants in the field. The closely related moth, *Nyctemera annulata* (Boisduval), is valued as a native species in New Zealand and significant parasitism of this insect would not be acceptable. The proposal to release *C. plutellae* will not be pursued until further information on host specificity is obtained.

Keywords

biological control, host specificity, *Senecio jacobæa*

Introduction

Cotesia plutellae (Hymenoptera: Braconidae) is being assessed as a potential biological control agent for diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), in New Zealand. This parasitoid has been released in most *Brassica* growing regions worldwide to augment natural populations or as a new introduction (Talekar & Shelton 1993). *P. xylostella* is the major pest of vegetable brassicas in New Zealand and *Diadegma semiclausum* (Hellén), the only larval parasitoid present, is not well synchronised with pest populations (Cameron *et al*. 1998). *C. plutellae* has been released in many countries for biological control of *P. xylostella* (Fitton & Walker 1990) and was imported from Italy and released in Australia for this purpose in the 1950s (Wilson 1960). Michael Keller of the University of Adelaide made a further introduction from Taiwan in the 1990s.

In the laboratory, host specificity assessments using no-choice tests (Cameron & Walker 1997) demonstrated that *C. plutellae* is capable of developing in several species of Lepidoptera other than *P. xylostella*, including a naturally occurring hybrid of *Nyctemera amica* (White) and *N. annulata* (Boisduval) (Lepidoptera: Arctiidae). However, in a flight tunnel, *C. plutellae* was equally attracted to species that were unsuitable for development (Cameron & Walker 1997). These observations showed that laboratory arenas produced results that are difficult to relate to the field host range of *C. plutellae*. A summary of laboratory and field records (Fitton & Walker 1990) noted that *C. plutellae* has been reared from 20 species of Lepidoptera, but most of these are rare occurrences. These records included two from the Family Arctiidae, based on five specimens of *Diacrisia urticae* (Esp.) (Wilkinson 1939) and <0.01% parasitism of *Hyphantria cunea* Drury (Bogavic 1953). The present study was initiated to provide further test data on the host status of *Nyctemera* spp.

The magpie moth, *N. amica*, occurs from central Queensland to Tasmania and into south-western Australia (Common 1990). Larvae of *N. amica* feed on the introduced weed, ragwort, *Senecio jacobæa* L., as well as on native species of *Senecio*. There has been no assessment of the impact of feeding by larvae of native moths, such as *N. amica*, on introduced weeds, but it is presumed to have some benefit for weed control (Common 1990). Biological control programs for ragwort, in Australia, are based on the use of exotic insects (Field 1990).

The presence of *N. amica* and the availability of cultures of *C. plutellae* provided a useful test situation for the New Zealand biological control programme, as well as providing an assessment of the potential interactions between an exotic parasitoid and an endemic Australian species of Lepidoptera. This investigation was conducted using semi-field conditions to verify previous records of host specificity of *C. plutellae*. The trial was designed to determine whether *C. plutellae* would locate and parasitise *N. amica* in the glasshouse and the proportion of parasitism of *N. amica* and *P. xylostella* was compared.

Materials and methods

P. xylostella larvae were reared on cabbage seedlings (*Brassica oleracea* cv. Green Coronet) seedling leaves in the laboratory. First to III instar larvae of *P. xylostella* were placed on cabbage and I to II instar larvae of *N. amica* were placed on ragwort seedlings (*S. jacobæa*) before each release, with the exception of the release on 22.vi.99 in which host larvae were carried over from the release on 17.vi.99 (Table 1). *Cotesia plutellae*, obtained from the University of Adelaide colony that originated in Taiwan, was reared on *P. xylostella* larvae at 22°C. Three glasshouse releases of *C. plutellae* were made between 28.v.99 and 22.vi.99 at the Institute for Horticultural Development, Knoxfield, Victoria, Australia. Adult *C. plutellae* (released on 28.v.99, 11.vi.99 and 22.vi.99) were released from plastic vials opened in the vicinity of the plants. Cocoons (released on 17.vi.99) in plastic vials were placed between the pots until wasps emerged. Wasps were free to move throughout the full glasshouse space.

Table 1. Numbers of potted plants, potential host larvae and wasps used in three glasshouse releases for host specificity testing of *Cotesia plutellae*

		Release 1 28.v.99	**Release 2** 11.vi.99	**Release 3a** 17.vi.99	**Release 3b** 22.vi.99
Plants	Ragwort	20	50	50	50
	Cabbage	20	50	50	50
Insects	*Nyctemera amica*	103	350	170	
	Plutella xylostella	140	350	170	
	Cotesia plutellae	180	340	200 cocoons	100

Cabbage seedlings were planted into PVC pots, 140 mm in diameter, and allowed to establish. At the time of Release 1 (28.v.99), cabbage seedlings had at least ten leaves. Ragwort plants were grown from seed or transplanted from infested sites into PVC pots of 120 mm diameter. All potting mix contained pine bark and sand (3:1). At the time of Release 1, each ragwort plant had at least five leaves. For later releases, plants were correspondingly larger.

Releases were confined to one section of a glasshouse with dimensions of 7.5 x 5.9 m. Cabbage and ragwort plants were arranged in a chequerboard pattern of alternating species on a bench measuring 2.3 x 1.4 m. Automatic sprinkler irrigation occurred twice per day. Glasshouse temperature was measured with a Hobo® data logger. The average temperature recorded was 15.3°C. Temperature extremes of 6.0°C and 22.6°C were recorded during the study period.

From six days onward after release of *C. plutellae*, larvae were collected and placed into individual Solo® cups for rearing on fresh leaves in the laboratory at 22°C. The fate of all larvae was noted and parasitoids were retained for identification.

Results

Larvae of *N. amica* tended to move away from the plants to sheltered areas (e.g. under the rims of the plastic pots) between feeding sessions. Larvae of *P. xylostella* rarely moved from the cabbage seedlings and, consequently, more *P. xylostella* larvae were re-collected than *N. amica* larvae (χ^2=186.6, df=1, P<0.05) (Table 2).

Table 2. Numbers of potential host larvae (*Nyctemera amica* and *Plutella xylostella*) placed on plants before release of *Cotesia plutellae* and numbers collected after parasitoid release

	Nyctemera amica	*Plutella xylostella*
Total released	623	660
Total collected	340	586
% collected	55	89

χ^2 test for association between species and potential to be recollected: χ^2=186.6, df=1, P<0.05

Both *N. amica* and *P. xylostella* were parasitised by *C. plutellae* in the glasshouse (Table 3). Parasitism of *N. amica* was significantly higher than parasitism of *P. xylostella* (χ^2=22.1, df=1, P<0.05). There was a low background level of parasitism of *P. xylostella* by *D. semiclausum*.

Table 3. Total parasitism of *Nyctemera amica* and *Plutella xylostella* by *Cotesia plutellae* in three glasshouse releases

	Nyctemera amica	*Plutella xylostella*
Total collected from 3 releases	340	586
Total parasitised by *Cotesia*	73	60
% parasitised by *Cotesia* (of those collected)	21.5	10.2
% parasitised by *Diadegma* (of those collected)	0	4.5

χ^2 test for association between species and susceptibility to parasitism: χ^2=22.1, df=1, P<0.05

Some predators were present in the glasshouse throughout the experiment. Spiders (Salticidae) were common and one was observed taking a larva of *P. xylostella*. Low numbers of ants were present and one was observed taking an adult *C. plutellae*.

Discussion

Cotesia plutellae had a choice of *P. xylostella* and *N. amica* as potential hosts in the glasshouse. *Nyctemera amica* was the preferred host for *C. plutellae* under these experimental conditions and this result augments laboratory host records from a *Nyctemera* hybrid in New Zealand (Cameron & Walker 1997). This hybrid of the Australian species *N. amica* and the New Zealand species *N. annulata* occurs naturally in the field in the Auckland region (Kay 1980). The combined results of the semi-field tests and the previous laboratory tests, suggest that *C. plutellae* would parasitise *Nyctemera* spp. that were near *Brassica* plants in the field.

Cotesia plutellae parasitised several other species of Lepidoptera in no-choice tests in the laboratory (Cameron & Walker 1997), which suggests that it may have a broader host range than the two species tested in the glasshouse. It was also attracted to several species of Lepidoptera in flight tunnel tests, including species that were unsuitable for development of the parasitoid (Cameron & Walker 1997). The latter results suggest that *C. plutellae* is attracted to insect damaged foliage rather species-specific stimuli. Similar attraction has been reported for *Cotesia rubecula,* a specialist parasitoid of *Pieris rapae* (Agelopoulos & Keller 1994), indicating that there is still difficulty in designing and interpreting laboratory tests that will predict host specificity in the field. As host location may be disrupted in small-scale laboratory experiments due to confinement (Sands 1993), field or semi-field experiments such as those used here could be expected to give a better prediction of host range in the field.

Field data on the host range of *C. plutellae* indicate that it rarely parasitises Lepidoptera other than *P. xylostella*. Extensive field collections in Malaysia and Fiji where *C. plutellae* is abundant (Cameron *et al.* 1998) detected only rare instances of alternative hosts. In addition, the pyralid, *Crocidolomia binotalis* Zeller, which is a known laboratory host for *C. plutellae* (Lim 1982), was not detected as a field host in these regions. These results are consistent with those of Fitton and Walker (1990), who suggested that variable information on host specificity may be explained by the existence of different parasitoid strains.

To resolve these issues and provide information for the potential introduction of *C. plutellae* from Australia to New Zealand, we considered that the semi-field experiment provided the best prediction for the host status of *Nyctemera* spp. As *N. annulata* is valued as a native species in New Zealand and significant

parasitism of this insect is not acceptable, the proposal will not be pursued until further information is obtained.

In Victoria, the significance of any interaction between *N. amica* and *C. plutellae* is restricted by the virtual absence of the parasitoid in this state. *Cotesia plutellae* was released in Australia in the 1950s and was subsequently recovered from *P. xylostella* in the ACT, New South Wales and Queensland (Wilson 1960). It is now rarely collected in any state. Surveys for *C. plutellae* have mostly been based on collections of *P. xylostella*, but collections of other Lepidoptera from brassicas in South Australia have not revealed *C. plutellae* (Keller & Cameron unpublished data). Parasitoids of *N. amica* identified by Clarke (1996) in Tasmania also did not include *C. plutellae*.

Acknowledgements

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The development of endemic baculoviruses of *Plutella xylostella* (diamondback moth, DBM) for control of DBM in East Africa

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Abstract

A project to develop non-chemical methods of DBM control on *Brassica* crops in Kenya has been exploring the use of endemic pathogens as potential control agents. Initial surveys for endemic pathogens identified *P. xylostella* granulovirus (*Plxy*GV) on farms in Kenya. Subsequently 14 genetically distinguishable isolates were identified from field collected material. These were purified and ranging bioassays showed these isolates were pathogenic to Kenyan strains of DBM with LC_{50s} varying from 2.36 x 10^6 to 3.95 x 10^7 occlusion bodies (OB) per ml for II instar DBM. One isolate (Nya-01) was selected and subsequently used for field trials in Kenya. The trials showed that unformulated *Plxy*GV applied at weekly intervals at a rate of 3.0 x 10^{13} OB/ha could control DBM on kale more effectively than available chemical insecticides. After application, infection rates in DBM can reach 90%. Further field trials are currently underway to determine the lowest effective dose rate for this virus when applied as a formulation. Initial virus production studies using *in vivo* propagation in II instar DBM reared on cabbage showed an initial productivity of 4.0 ± 0.44 x 10^{10} OB/larva.

Keywords

Brassica, granulovirus, biocontrol, Kenya

Introduction

Larvae of the diamondback moth (DBM) *Plutella xylostella*, feed only on plants from the family Brassicaceae and are a major pest of *Brassica* vegetables (kale, cabbage, rapeseed, etc.) throughout Kenya (Michalik 1994). Presently, conventional chemical insecticides are heavily relied upon to control them (Kibata 1997). It is well known that DBM has become resistant to chemical insecticides in many countries throughout the world (Roush 1997) and current programs underway in Kenya have indicated that chemical resistance in DBM is also occurring there (Kibata 1997). The chemical insecticides currently recommended for control are expensive, damaging to the environment and in some areas simply not available to the small-scale farmers who account for a high percentage of the *Brassica* vegetable production of Kenya (Kibata 1997).

To address this issue, a collaborative project between the Natural Resources Institute (NRI), the Kenya Agricultural Research Institute (KARI) and CAB International, Africa Regional Centre (CABI-ARC) was set up to investigate alternative methods of DBM control. One component of the project investigated the possible use of endemic baculoviruses.

Before this study GVs of *P. xylostella* had been reported from Japan (Asayama & Osaki 1970), Taiwan (Wang & Rose 1978, Abdul Kadir 1986), China (Abdul Kadir *et al.* 1999) and India (Rabindra *et al.*1997), but there were no previous published records from Africa. A number of other NPVs, some uncharacterised (Padamvathamma & Veeresh 1989), have been reported as infecting DBM, but a review of the potential of DBM pathogens concluded that only the GV showed promising levels of pathogenicity (Wilding 1986). More recently an NPV has been identified from *P. xylostella* in China. This was characterised as being genetically similar to, though genetically distinct from, *Autographa californica* MNPV and *A. falcifera* MNPV (Kariuki & McIntosh 1999).

Materials and methods

Pathogen survey and identification

To collect baculoviruses, a survey of *Brassica* farms was conducted around Nairobi. In total, 27 farms were surveyed within a 170 km radius of Nairobi. In field sampling, larvae showing signs of baculovirus infection, puffy appearance and pale-yellow to white colouration (Asayama & Osaki 1970) were collected and individually stored for later examination. Standard, unstained wet mounts of infected larvae were examined using a microscope and dark-field contrast at x400 magnification to detect the presence of baculoviruses. Each candidate GV isolate was propagated *in vivo* in 15 II instar DBM following methods described by Parnell (1999). Restriction endonuclease analysis (REN) of the baculovirus isolates was performed on each of the GV isolates individually following the protocol of Smith and Summers (1978) as modified by Rabindra *et al.* (1997).

Bioassay of pathogen strains

The pathogenicity of the different isolates was determined by means of two bioassay methods. Comparative bioassays using single discriminate doses were performed on nine GV isolates displaying different DNA profiles. Subsequently, in order to obtain LC_{50} values, dose series bioassays were carried out on three of those eight isolates and the *Plxy*GV-Tw isolate. The concentration of GV was determined by counting using a 0.02 mm depth bacterial spore-counting chamber viewed under dark phase illumination at x200 magnification. Discriminate dose bioassays and dose response bioassays were carried out as per Parnell (1999). Bioassay data were corrected using Abbot's correction for control mortality and dose series data analysed using a probit analysis with the SPSS data analysis package.

Field trials

To evaluate the potential of the Kenyan *Plxy*GV to control crop loss caused by DBM, isolate Nya-01 was selected for mass production and use in small-plot field trials. This isolate was selected because it had been indicated as the most pathogenic strain in the laboratory bioassays. The virus was applied as a simple unformulated suspension using standard farmer equipment. Volume application rate for all treatments was 800 litres/ha. The first field trial was carried out on the research farm at Jomo Kenyatta University of Agricultural Technology (JKUAT) 25 km outside Nairobi and ran for 12 weeks in late1998. This was a randomised-block design trial carried out on small plots of 5 m x 5 m with a one metre gap between plots and a plant/row spacing of 60 cm. The test crop was kale (var. Thousand headed). This trial compared two virus treatments, a weekly application of high application rate of 3.0×10^{14} (occlusion bodies {OB}) and a medium rate of 3.0×10^{13} OB/ha. There was a no treatment control and a standard farmer insecticide treatment schedule based upon weekly application of the local standard pyrethroid insecticide (Karate®- lambda-cyhalothrin).

A second field trial was carried out at the National Agricultural Research Laboratory (NARL) farm on the outskirts of Nairobi in 2000. In this trial there were five treatments arranged in randomised replicated plot design. The treatments were three virus application rates (3×10^{14}, 3×10^{13} and 3×10^{12} OB/ha) a no treatment control and a standard insecticide treatment with Karate® as before. The plots were 5 x 5 m with a one metre gap between plots and a plant spacing of 60 x 60 cm.

In both trials, 10 random plants in the central area of the plot were sampled weekly for numbers of DBM larvae, numbers showing symptoms of GV infection and damage caused by DBM. In addition, in the second trial yield data were also collected. To assess more precisely the disease incidence in the plots, after three weeks of the trial 45 larvae of each instar were collected from each treatment and reared individually in the laboratory and the disease occurrence recorded. The yield data were analysed using 2 way ANOVA on the SigmaStat statistical package (SPSS Inc., USA).

*Plxy*GV productivity

In order to estimate the productivity of the *Plxy*GV when produced *in vivo*, two hundred II and III instar larvae were inoculated with a range of concentrations of the strain Nya-01 and reared under standard conditions until death. Progeny virus was collected, counted and its identity confirmed using REN.

Results

During the field survey, 127 larvae with disease symptoms were collected from eight of the 27 farms included in the survey. Microscopic examination confirmed that 95 larvae collected from four of the eight

farms were suffering from GV infection. The areas in which GV-infected larvae were found were Nyathuna (84 larvae-two farms), South Kinangop (9 larvae) and Naivasha (2 larvae).

The REN analysis of the 95 *Plxy*GV isolates showed that 14 had fragment profiles that could be distinguished from any other with both *Eco*R1 and *Pst*1 cuts (Figure 1). Comparison of these 14 Kenyan *Plxy*GV isolates to an isolate of *Plxy*GV from Taiwan (*Plxy*GV-Tw) revealed that, although the profiles had many similarities, there were major band differences between all isolates. Both the *Pst*1 and *Eco*R1 digests revealed between 2 and 6 major band differences between isolates, even in those collected from the same location (Figure 1).

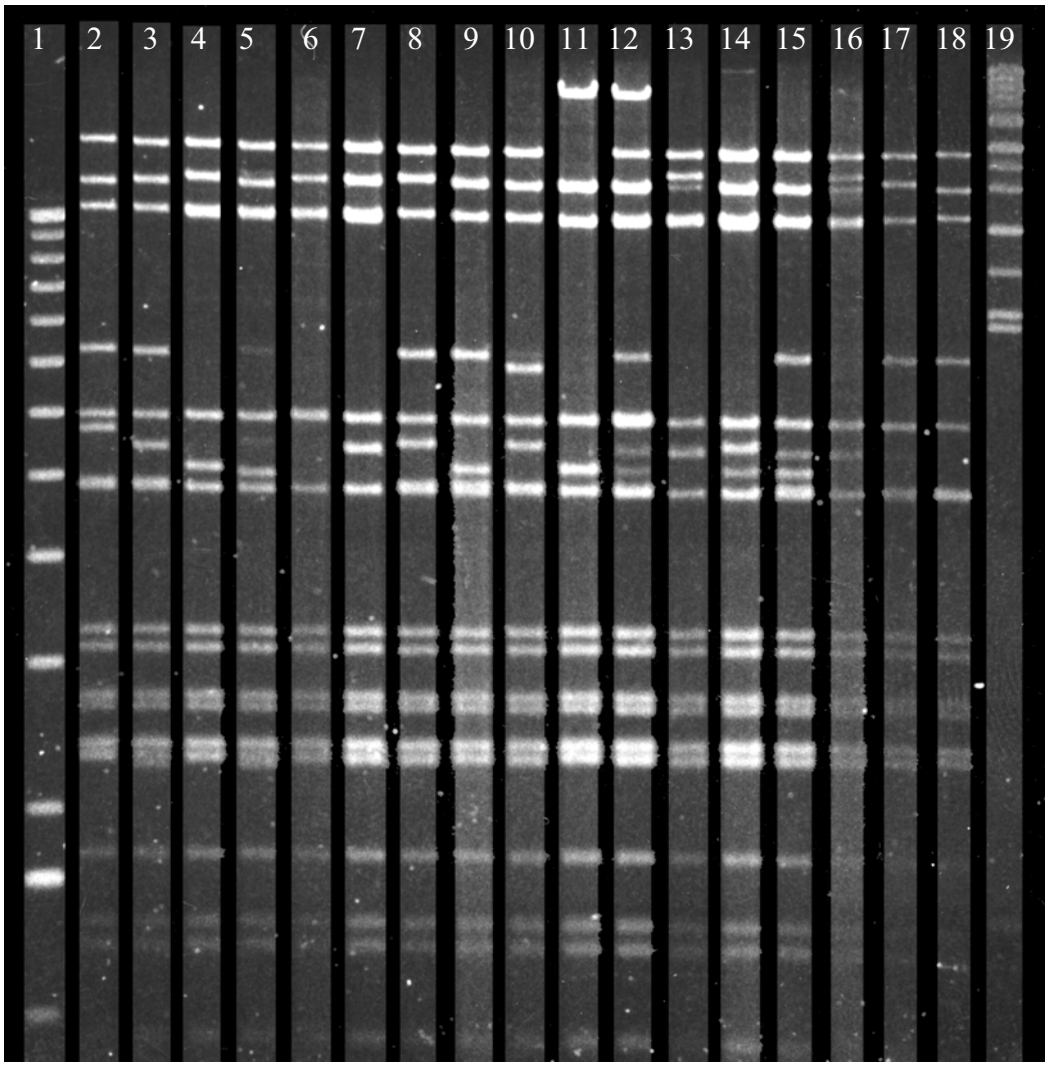

Figure 1. Comparison of *Plxy*GV isolates. DNA of each isolate was digested with *Pst*1 restriction endonuclease, fragments were separated on 0.6% agarose gel. Track 1 (far left of page), 1kb molecular size standard; tracks 2-16, Kenyan *Plxy*GV isolates from Nyathuna (Nya-01, Nya-02, Nya-03, Nya-06, Nya-07, Nya-14, Nya-15, Nya-25, Nya-27, Ny-29, Nya-35, Nya-37, Nya-40, Nya-42, Nya-52 respectively), track 17, *Plxy*GV isolate from South Kinangop (SK-01); track 18, Taiwanese *Plxy*GV; Track 19, λ 19-Mix molecular size standard.

Results from the discriminate dose assay showed every Kenyan isolate to be significantly more potent than the *Plxy*GV-Tw with average % mortality ranging from 26.2% to 40.3% compared with 5.2% for the *Plxy*GV-Tw (Figure 2). However in the dose response bioassays, no significant differences in LC_{50} values between Kenyan isolates and the *Plxy*GV-Tw isolate were observed. Average LC_{50} values for II instar DBM larvae varied from 2.36×10^6 OB/ml for Nya-01 *Plxy*GV to 3.95×10^7 OB/ml for Nya-40 *Plxy*GV. In comparison, the LC_{50} for the *Plxy*GV-Tw was 1.55×10^7 OB/ml.

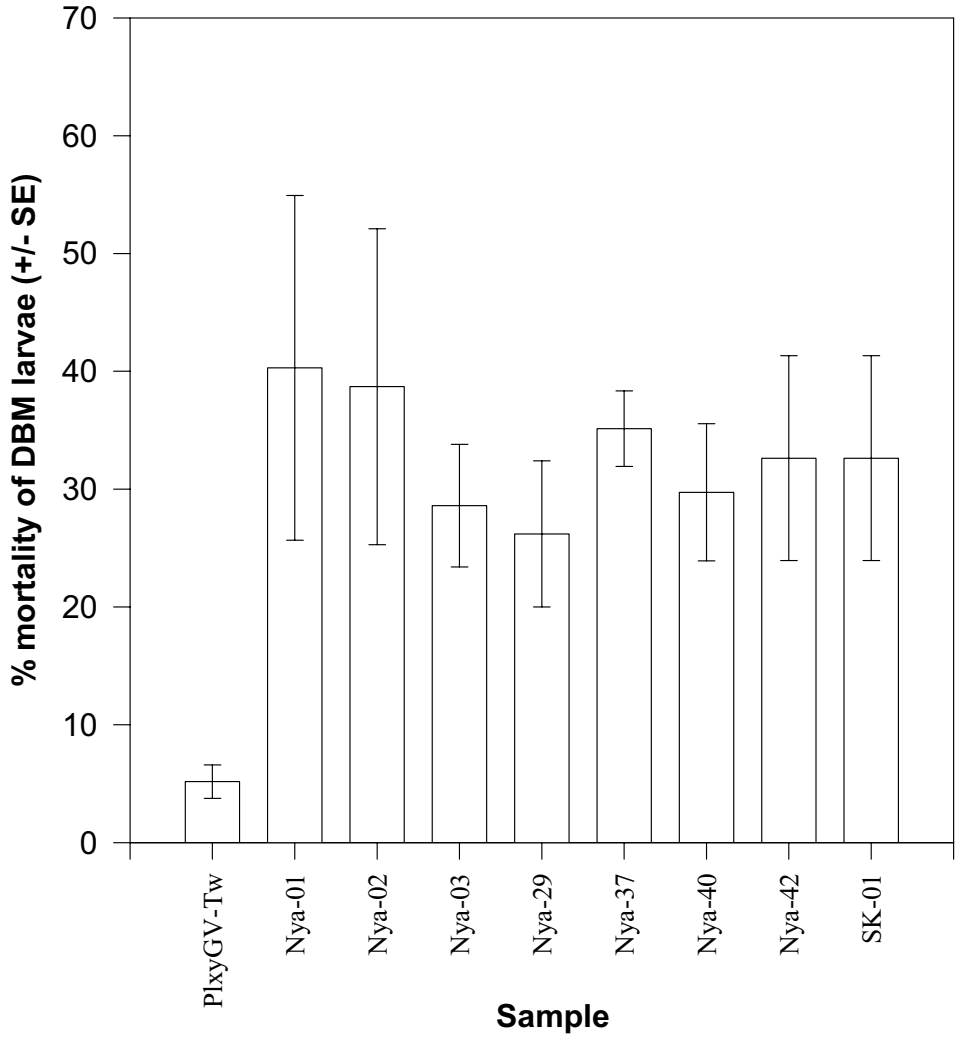

Figure 2. Average percent mortality in discriminate dose bioassays of Kenyan *Plxy*GV and Taiwanese *Plxy*GV.

The field trials carried out at JKUAT showed that the *Plxy*GV when sprayed using standard farmer application equipment was highly infectious to DBM, spreading rapidly in trial plots and infecting 80-90% of larvae within two to three weeks of application (Figure 3). Very little occurrence of infected insects was recorded from the control or insecticide treated plots. Both the high dose rate of 3.0×10^{14} OB/ha and the lower dose of 3.0×10^{13} OB/ha reduced DBM damage to crops to below that seen in either unsprayed controls or insecticide treated plots (Figure 4).

In the second trial at NARL, the yield data (Figure 5) showed that the highest application rate dose gave significantly higher yield than the no treatment control (37% higher, $P<0.001$ df=4 and 28, F=6.25) or the insecticide treatment (17% higher, $P<0.001$ df=4 and 28, F=6.25). The average DBM numbers in each treatment showed an application-rate effect with the lowest numbers occurring in the highest virus rate treatment (Figure 6). In the second trial, average observed DBM infection rates in virus treated plots also showed a clear application-rate trend with the highest dose producing an average of 40% (Figure 7). In this trial there was some infection observed in the control and insecticide plots. From insects sampled from the *Plxy*GV application-rate plots, the true infection rate was much higher than that observed in the field and Table 1 shows the percent virus mortality recorded from insects taken from the plot treated at 3×10^{13} OB/ha.

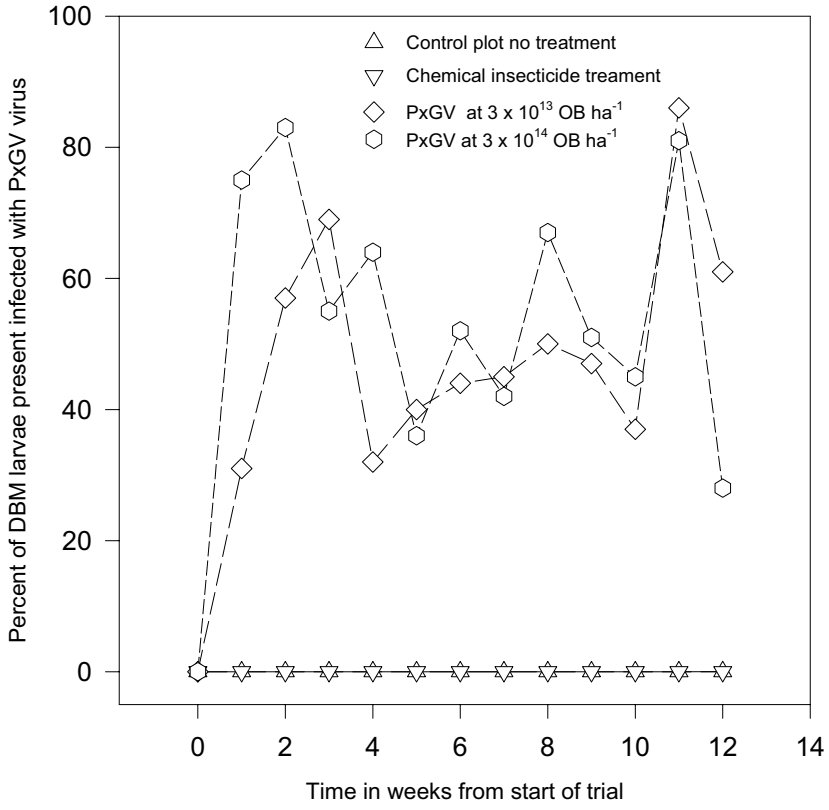

Figure 3. The level of *Plxy*GV infection observed in treatments from the first field trial in Kenya.

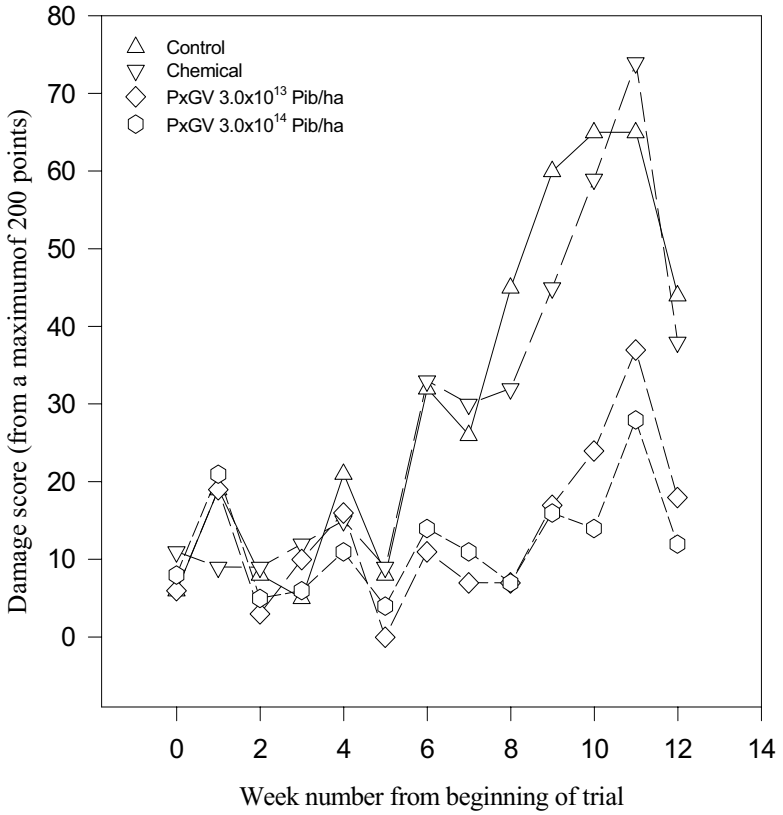

Figure 4. The level of crop damage observed in treatments from the first field trial in Kenya.

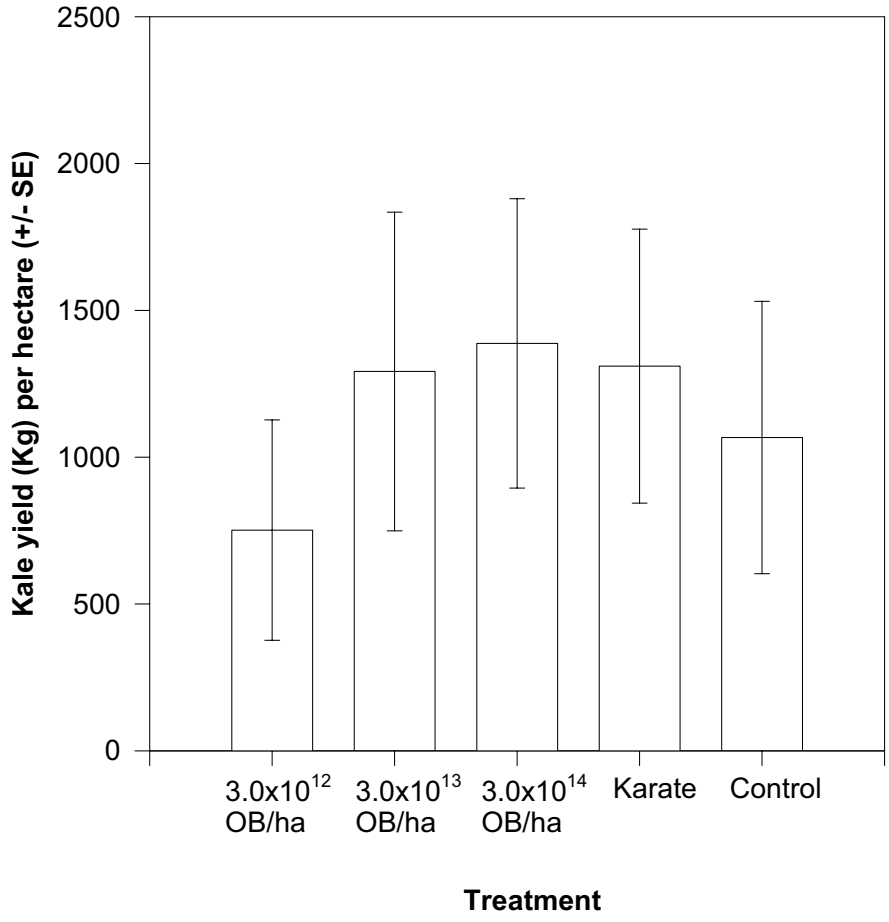

Figure 5. Average kale yield per hectare for each of the treatments from the NARL field site.

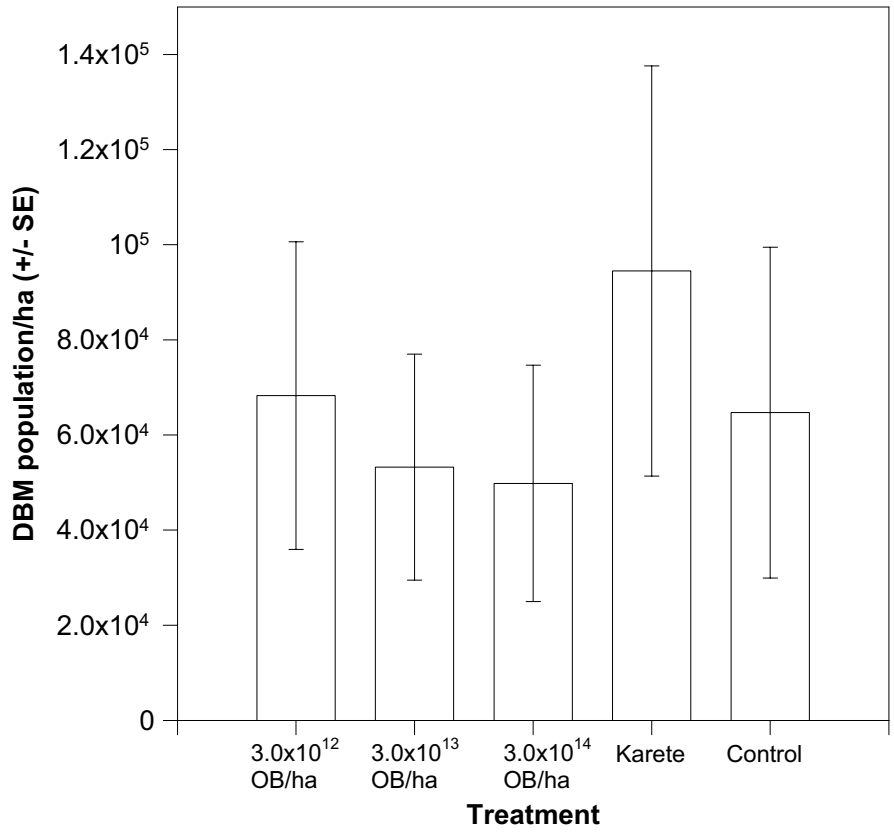

Figure 6. Average DBM population per hectare for each of the treatments at the NARL field site.

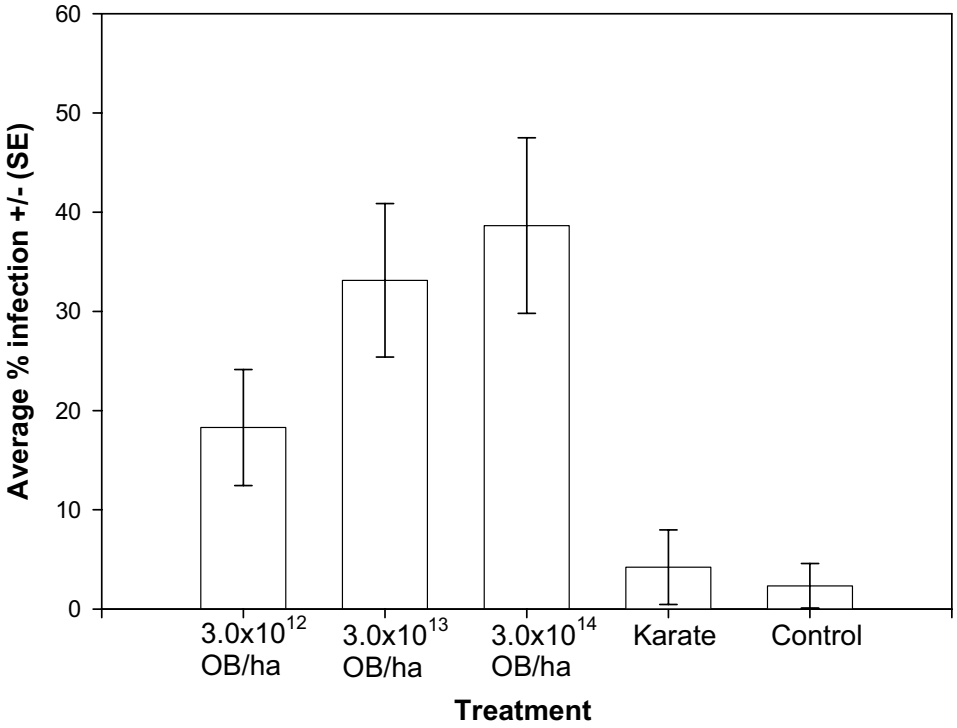

Figure 7. Average *Plxy*GV infection-rate observed in DBM larvae for each of the treatments at the NARL site.

Table 1. Percentage GV infections that developed in laboratory-reared larvae sampled from virus plots sprayed with 3 x 10^{13} OB/ha

Larval instar	DBM Infection Rate
I	90
II	82
III	64
IV	60

The maximum productivity of the *Plxy*GV was found to be 4.0 ± 0.44 x 10^{10} OB/larva obtained from II instars inoculated with 2.0 x 10^{8} OB/ml.

Discussion

The pathogen survey revealed that the GV of DBM occurred on 50% of the farms surveyed, though, in all cases, with a relatively low incidence. On no farm were widespread epizootics observed or reported by local farmers questioned. The discovery of so many different genetic isolates (14) in the small number of infected larvae collected is therefore striking. Previously reported work (Abdul Kadir *et al.* 1999) has characterised only two genetically distinct isolates one from China and one from Taiwan. Other studies of DBM pathogens have also only reported finding a single genetically distinct isolate from India (Rabindra *et al.* 1997) and Japan (Yamada & Yamaguchi 1985).

The GV isolates from Kenya are genetically similar to, though genetically distinct from, the previously reported Taiwanese isolate. This isolate we now know is itself similar to and closely related to the Chinese isolate (Abdul Kadir *et al.* 1999). The two isolates studied differed from each other by one to three major bands in the *Eco*R1, *Bam*H1 and *Hind*III profiles. The differences in the Kenyan isolates studied here were greater at two to six bands with only two profiles *Eco*R1 and *Pst*1, even amongst isolates collected from the same farm. This genetic diversity amongst isolates of *Plxy*GV from Kenya could be extremely useful as a diverse genetic resource that could be exploited in the development of a GV for DBM control. The high level of variation in the *Plxy*GV isolates could indicate a long association between *Plxy*GV and DBM in the region and could have a bearing on the debate concerning the origin of DBM. This was generally considered to be somewhere in Mediterranean Europe having evolved on cultivated brassicas also believed to have

European origin (Hardy 1938). Recently however, the Mediterranean origin of DBM has been brought into question by Kfir (1998) who hypothesised a southern African origin for DBM on the basis of the diversity of wild hosts and endemic parasitoids found in South Africa. The genetic variation in *Plxy*GV isolates discovered in Kenya during the present study and apparent lack of diversity in isolates from other regions of the world might be interpreted as providing additional support to the theory that the origin of DBM lies in Sub-Saharan Africa.

The initial discriminate single dose bioassay results showed all the Kenyan isolates to be significantly more pathogenic than the Taiwanese isolate. However the LC_{50} data from the subsequent dose response assays showed no significant differences, even though the mean LC_{50} for Taiwanese isolate was 6.5 times higher that of the most active Kenyan isolate (Nya-01). This result reflects the high variability in response seen with some Kenyan isolates including Nya-01. These were originally *in vivo* propagated, but not cloned, which might have reduced this variability. These isolates have since been cloned and the assays are currently being repeated on these cloned isolates.

The productivity of the Kenyan isolates is high at $4.0 \pm 0.44 \times 10^{10}$ OB/larva, equivalent to 8.0×10^{9} OB/mg. This may be compared with between 1.9×10^{10} and 4.5×10^{9}/larva reported with other GVs produced in Lepidoptera (Evans 1986). High productivity is a valuable asset in a potential biopesticide as it reduces the number of insects needed to produce the desired application rate. At this rate of production, the highest application rate used in these trials, 3.0×10^{14} OB/ha would be equivalent to 7,500 infected larvae/ha. In comparison, most existing commercial baculovirus products are applied at rates of between 50-500 larval equivalents/ha (Moscardi 1999).

The first field trial showed that application of *Plxy*GV at 3×10^{13} OB/ha could reduce DBM damage much better than either the use of the standard chemical insecticide or the no treatment control. The very limited effectiveness of the standard insecticide, lambda-cyhalothrin, suggests that significant resistance has developed in DBM. Resistance has since been confirmed by other work in Kenya (Cooper 2001) and lambda-cyhalothrin is now no longer recommended for DBM control.

The speed with which weekly sprays of *Plxy*GV initiated infection rates of 90% could indicate that one or two applications of *Plxy*GV at the start of the season might be sufficient to start an epizootic infection in resident DBM populations. However whether augmentative approach alone would be sufficient to produce control of DBM numbers and damage, would need testing under field conditions. While collection of a high percentage of infected insects in virus treated plots suggests that recycling of *Plxy*GV is very important, its precise contribution to control remains to be quantified.

In the second trial, the yield results showed that again the *Plxy*GV performed significantly better than the chemical insecticide at the highest application rate used 3×10^{14} OB/ha. A similar result in terms of controlling DBM numbers has been reported by Su (1989) using a Taiwanese isolate applied as here at seven day intervals. However direct comparisons are difficult, as in Su's (1989) trial, the *Plxy*GV was quantified in terms of larval equivalents per litre and no direct enumeration of the GV was carried out.

Glasshouse trials again have showed that application of the Taiwanese isolate can reduce DBM numbers and that there is a dose response over the range 9×10^{11} to 9×10^{13} and at the highest dose the *Plxy*GV reduced damage as effectively as application of *Bacillus thuringiensis* (Abdul Kadir 1992). In addition it was shown that the addition of molasses to a formulation could increase the viruses efficacy by a factor of ten and allow for a consequent reduction in the application rate of *Plxy*GV. This finding closely mirrors that of *Ballard et al.* (2000) who found that addition of 10% molasses produced a similar 10 fold increase in efficacy with the codling moth (*Cydia pomonella*) granulovirus (*Cp*GV) on apples.

The two granuloviruses that have been commercialised to date, *Cp*GV and *Adoxophyes orana* granulovirus, are both sold for application at rates of 1×10^{13} OB/ha. In comparison, the rate of *Plxy*GV used here which produced a significant increase in yield is $3 \times 1\ 0^{14}$ OB/ha. Even given that the Kenyan *Plxy*GV seems to be more productive than other GVs, this suggests a need to reduce the application rate by a factor of ten if its use is to be commercially attractive.

The trials reported here did not include formulation ingredients and field trials of such a formulation are underway now in Kenya to evaluate the efficacy of reduced rate formulated *Plxy*GV. Formulation might also address the short persistence time on field crops seen with GVs. Abdul Kadir (1986) reported that with

*Plxy*GV-Tw exposure of unformulated virus to seven hours of sunlight in Malaysia was sufficient to reduce virus efficacy by 50%. Although the persistence of the Kenya *Plxy*GV has yet to be quantified it is unlikely to be longer.

In conclusion, while the results of these trials of *Plxy*GV are promising it has yet to be determined that *Plxy*GV can be effective or reliable enough for consistent control of DBM.

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Impact of a granulosis virus on larval food consumption and development duration of the diamondback moth, *Plutella xylostella* (L.)

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Abstract

Food consumption and developmental duration of virus-infected DBM larvae were measured in the laboratory to determine whether *Plutella xylostella* granulosis virus (PxGV) can reduce DBM damage to cruciferous vegetables. The newly moulted larvae of each instar fed on vegetable leaves treated with a suspension of PxGV. The developmental duration of treated larvae was lengthened and the food consumption of virus-treated larvae of each instar increased slightly compared with the healthy larvae. However, the total leaf consumption of each instar was significantly reduced compared with untreated larvae. The proportion of decreasing food consumption from the I to IV instar larva was 93%, 77%, 53% and 46% compared with the corresponding healthy larval stage. The change of food consumption depends on two factors: feeding rates and time, either of which can result in change of food consumption of larval feeding.

Keywords

Feeding, development time, PxGV

Introduction

The diamondback moth, *Plutella xylostella* (L.), is one of the key insect pests of cruciferous vegetables and its feeding can seriously reduce quality and yield of vegetables. Feeding area and hole size by lepidopteran caterpillars have been taken as criteria for feeding consumption evaluation (Greene *et al.* 1969, Sears *et al.* 1983). Several researchers have developed action thresholds for control of *Pieris rapae, Trichoplusia ni* and *P. xylostella* with insect viruses in cabbage fields (Webb & Shelton 1991, Mailloux & Belloncik 1995). In this study, the relationship between numbers of DBM larvae and leaf loss or yield loss of flowering Chinese cabbage and Chinese cabbage was examined and the effects of food plants on feeding activity were investigated to provide basis for determining damage criteria.

Materials and methods

DBM and food resources

Test insects originated from pupae collected in the field. After emergence, the moths laid eggs on filter papers treated with extracted liquid of vegetable leaf. Newly hatched I larvae were obtained from eggs laid on filter paper, while the II, III and IV instar larvae tested in the experiments were raised on potted crucifer plants. Leaves of Chinese kale were provided as food for larvae.

Test procedures

The newly moulted larvae of each instar fed for 24 hours on leaves of Chinese kale, which had been dipped in a suspension of PxGV with one larval equivalent per litre (LE/L). They were then transferred to an uncontaminated leaf held in a clear Petri dish. Each of the 40 larvae of each instar per treatment was put into a Petri dish with a fresh leaf disc. The glass dishes were sealed with Parafilm® and kept at 25±1˚C, 16L: 8D. The food consumption of each larva was evaluated every half-day by estimating leaf area consumed and development age was recorded until the larvae either died or pupated.

Statistical analysis

Data from the experiments were subjected to analysis of variance and means were separated by Duncan's Multiple Range Test (P=0.05 level). The calculation was conducted using DPS software (Tang & Feng 1997).

Results and discussion

Effects of PxGV on larval developmental duration

First instar larvae treated with virus died before reaching III instar and the development time for these larvae was significantly longer than for the untreated group at both I and II instar. Second instar larvae treated with virus had an extended period of development at III instar and died before reaching IV instar (Table 1). Larvae fed virus at either III or IV instar had a significantly longer development period than the untreated larvae.

Table 1. Development duration and total feeding time of PxGV-treated DBM larvae

	Development duration of larva (days)				
Treatment time	**I instar**	**II instar**	**III instar**	**IV instar**	**Total development duration**
Early I instar	2.54±1.221[a]	2.41±0.340[a]	—	—	n.a.
Early II instar		1.10±0.384[c]	4.16±1.175[a]	—	n.a.
Early III instar			2.21±0.418[b]	4.61±1.572[a]	10.45[a]
Early IV instar				3.82±0.863[b]	8.83[b]
Control	2.15±0.754[b]	1.47±0.507[b]	1.38±0.652[c]	2.82±0.758[c]	7.83[b]

Means in the same column followed by the same letter are not significantly different (P=0.05), Duncan's Multiple Range Test.

Variation in food consumption of virus-fed larvae

Significant reduction in initial food consumption was observed only for the IV instar larvae fed virus (Table 2). However, total food consumption was reduced by 93%, 76%, 53% and 47% respectively after treatment of I, II, II and IV instar larvae. This reflects the reduced longevity of virus-infected larvae. Older larvae (III and IV instar) were less sensitive to disease after inoculation with virus. Thus, their food consumption was higher than those of younger (I and II instar) larvae, but still much less than the untreated larvae.

Table 2. Food consumption of PxGV-fed larvae

Treatment time	**Average food consumption**				**Total food consumption (mm^2 /larva)**	**Reduction percentage (%)**
	I instar	**II instar**	**III instar**	**IV instar**		
Early I instar	0.019±0.014[a]	0.092±0.0466[a]	—	—	0.111[d]	93.2
Early II instar		0.035±0.0215[b]	0.340±0.1437[a]	—	0.375[c]	76.8
Early III instar			0.121±0.0439[b]	0.448±0.4346[b]	0.757[b]	53.2
Early IV instar				0.636±0.3213[b]	0.872[b]	46.7
Control	0.02±0.01[a]	0.050±0.0222[b]	0.166±0.1054[b]	1.339±0.7387[a]	1.636[a]	n.a.

Means in the same column followed by the same letter are not significantly different (P=0.05), Duncan's Multiple Range Test.

Effects of granulosis virus on larval feeding rates

After virus feeding, feeding rates of all stages of larvae were significantly lower than those of the larvae in the control group (Table 3). This reflects the increased time of development of each larval stage post-infection.

Table 3. Feeding rates of PxGV-treated DBM larvae

Treatment time	Mean daily feeding rates (± s.d.) (mm^2/d)			
	I instar	II instar	III instar	IV instar
Early I instar	0.0087±0.0009[b]	0.0367±0.0190[a]	—	—
Early II instar		0.0313±0.0113[a]	0.0832±0.0302[b]	—
Early III instar			0.0568±0.0226[ba]	0.1048±0.1041[b]
Early IV instar				0.173±0.1008[b]
Control	0.0398±0.179[a]	0.0355±0.0151[a]	0.1283±0.0866[a]	0.4981±0.3096[a]

Means in the same column followed by the same letter are not significantly different (P=0.05), Duncan's Multiple Range Test.

Larval food consumption was determined by the two factors of feeding rate and duration. Larvae infected at the stage of II and IV instar initially developed at the same rate as the untreated larvae, but their rate of leaf consumption was also reduced. The feeding time of larvae infected at III instar was 2.6 days longer than that of the untreated control. The area eaten by a larva infected at either the III and IV instar was 0.072 and 0.325 mm^2 per day, respectively, less than the control group. Therefore, their total leaf consumption was decreased. These results are similar to reports in *Pieris rapae*, *Ectropis oblique hypulina* and *Trichoplusia ni* (Wang & Hu 1986, Hu *et al.* 1990, Harper 1973).

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Insect pathogens for biological control of the diamondback moth with particular emphasis on the fungus *Zoophthora radicans* in New Zealand

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Abstract

The diamondback moth (DBM), *Plutella xylostella*, is a serious world-wide pest of *Brassica* crops which has developed resistance to all categories of chemical insecticides and to toxins of the bacterium, *Bacillus thuringiensis*. Potential sources of novel control options for DBM include the use of microbial agents such as entomopathogenic fungi. Infective fungal spores, or conidia, are fragile and short-lived. Therefore, the identification of robust alternative inoculum sources and the development of novel formulation and application techniques can contribute to realising their potential. Under certain environmental conditions, some entomopathogenic fungi, including the common DBM pathogen *Zoophthora radicans*, produce specialised resting spores which could have potential as an alternative commercial inoculum as they are robust and long-lived. Research is underway to determine the mechanisms of resting spore production and germination to underpin their effective exploitation.

Keywords

Plutella xylostella, entomopathogenic fungi, microbial control, resting spores

Introduction

The diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Plutellidae), occurs throughout the world on cruciferous plants. In some areas of the world, DBM populations are effectively regulated by natural enemies and do not cause economic damage (Wilding 1986). However, in many regions its high reproductive rate coupled with heavy selection pressure for insecticide resistance make it a very severe pest. Rapid development of resistance to all categories of insecticides including toxins of the biological agent *Bacillus thuringiensis* (*Bt)* and increased awareness of the environmental consequences of excessive pesticide use has led to the investigation of novel, non-chemical methods for DBM control (Shelton *et al.* 1997).

Microbial agents for control of DBM

Microbial control agents with potential against DBM include bacteria (such as *Bacillus thuringiensis)*, viruses and entomopathogenic fungi. Some of these organisms contribute to the natural regulation of DBM populations in the field and their exploitation has focussed on augmentation and conservation strategies to improve their natural efficacy. Others are not commonly found in DBM populations, but are highly pathogenic to DBM and, therefore, if they can be produced, formulated and applied, also have potential as microbial control agents.

Bacteria

Of all the microbial control agents developed, *Bacillus thuringiensis* has been the most commercially successful (Lacey & Goettel 1995). This is partly because it can be used in a very similar way to conventional chemical insecticides; it is fast acting, can be produced on inexpensive media, applied inundatively by conventional equipment, has a long shelf life and minimal effects on non-target organisms (Schacter 1999, Lacey & Goettel 1995). Potentially this has also contributed to the limitations in its use associated with resistance. *Bacillus thuringiensis* does not recycle in insect populations and kills through the action of a crystalline protein toxin (Cry proteins) and not via invasion and growth within the insect tissue. As such resistance can, and has, evolved to the toxin in the same way as resistance develops to chemical insecticides when selection pressure is high. Integration of *B. thuringiensis* with other biologicals/microbials

can contribute to resistance management strategies in the same way as integration of conventional insecticides with other control strategies.

Viruses

In the field, DBM can be infected by two types of virus, nuclear polyhedrosis viruses (NPV) and granulosis viruses (GV). Unlike *Bt*, viruses can recycle in DBM populations developing epizootics (epidemics) and are relatively persistent in the environment. They can be slower to kill than *Bt* and some have a wide host range and/or are difficult to mass produce (Granados & Williams 1986, Shapiro 1986). Like *Bt* they can be applied by conventional means as inundative sprays and have been used successfully in this way to control DBM. Unformulated DBM granulosis virus (*Plxy* GV), applied at weekly intervals at a rate of 3.0×10^{13} occlusion bodies/ha, controlled DBM on kale in Kenya more effectively than available chemical insecticides (Grzywacz *et al*. 2004).

Fungi

There are approximately 750 species of fungi from 56 genera that infect arthropods (Hawkesworth *et al.* 1995). Insect pathogenic fungi are mostly found in the orders Moniliales (Deuteromycotina: Hyphomycetes syn. Deuteromycetes) and the Entomophthorales (Zygomycotina: Zygomycetes) (Flexner & Belnavis 1998). DBM populations are commonly regulated by two entomophthoralean species, *Zoophthora radicans* and *Erynia blunckii*, but are also susceptible to several species of Hyphomycetes which are not usually found in DBM populations. These include *Beauveria bassiana*, *Paecilomyces fumosoroseus* and *Metarhizium anisopliae* (Wilding 1986).

Like viruses, entomopathogenic fungi are ubiquitous and in appropriate hosts are capable of natural recycling. Unlike the other microbials discussed, they cause infection by direct penetration through the host cuticle without the requirement for ingestion (Lacey & Goettel 1995). This is advantageous as it limits the potential for the target to avoid consuming a lethal dose, but it also means that fungi are reliant on appropriate environmental conditions to infect and multiply. Like viruses, speed of kill can be variable and host specificity varies between species and even among isolates of a single species (Pell *et al.* 1993, Yeo *et al.* 2001). Hyphomycetes can have broad host ranges in contrast to Entomophthorales which are usually highly host specific (Pell *et al. 2*001).

The development of fungal entomopathogens as biological control agents has been the subject of considerable research, particularly since the 1970s. However, there are only limited examples of currently available marketed products (Shah & Goettel 1999). Exploitation of fungi, like other microbial agents, has focussed on using them in a similar way to conventional insecticides, i.e. as an inundative spray application or 'mycoinsecticide' with no requirement for secondary cycling. For example, *Beauveria bassiana* (Mycotrol®) applied to seedlings grown in a nursery was effective at controlling DBM before they were transplanted into the field (Shelton *et al*. 1998). In open field trials in the USA, *B. bassiana* significantly reduced the numbers of DBM larvae when used alone (Vandenberg *et al.* 1998) and when integrated with *Bt* could control three lepidopteran pests on brassicas (Vandenberg *et al.* 1999). This approach reduces the number of applications of *Bt* and therefore contributes to resistance management.

Entomophthoralean fungi such as *Z. radicans* are common natural enemies of DBM and contribute to the natural regulation of DBM populations worldwide (examples in Pell *et al*. 2001). The high natural pathogenicity to DBM, strain specificity, relatively fast speed of action, pre-mortality impacts on DBM biology and potentially positive interactions with insect natural enemies means that *Z. radicans* has excellent potential as a biological control agent of DBM when exploited in an appropriate integrated strategy (Pell *et al*. 1993, Furlong *et al*. 1997, Yeo *et al*. 2001). They develop epizootics which can eliminate DBM populations at a local level clearly demonstrating its potential as a microbial control agents. However, epizootics are unpredictable and can be too late to prevent crop damage, so augmentation has been attempted. Mass production of mycelial material for augmentation is possible (McCabe & Soper 1985), but is currently limited by production and economic constraints.

An early attempt at inundative release was made by Kelsey (1965) in New Zealand. A spray comprised of macerated and diluted *Z. radicans*-infected larvae was applied to two DBM-infested *Brassica* fields. The time taken to give adequate control was not reduced by such sprays when the fungus was already present, but Kelsey suggested that there was merit to introducing the fungus into uninfected caterpillar populations.

Kelsey (1965) also observed that only one spray was necessary per season, indicating evidence of natural recycling within the population, thereby suggesting that an inoculative approach could be appropriate.

Inoculative augmentation strategies for *Z. radicans* include the development of auto-dissemination where DBM behaviour is manipulated using pheromones to encourage transmission and the establishment of population regulating epizootics in DBM populations before they reach damaging levels (Pell *et al.* 1993, Furlong *et al*. 1995, Vickers *et al*. 2004). This approach exploits the ability of *Z. radicans* to transmit in DBM larval populations even at low host densities (Furlong & Pell 2001).

Underpinning ecological studies on the fungus are essential to identify appropriate strategies (inoculation, inundation and/or conservation) for the effective exploitation of the selected approaches. More imaginative control opportunities are only possible when the positive and negative attributes of the organism are considered and addressed. The potential of fungi as insect control agents is affected by the many biotic and abiotic constraints on the ability of fungi to infect their target hosts (Lacey & Goettel 1995), but all these constraints could ultimately be overcome if the right propagule, formulated in an optimum fashion is introduced into the population at the right time and in the right manner. One challenge to the development of *Z. radicans* as a microbial control agent is to identify and produce alternative propagules for release.

Future research on *Z. radicans* in New Zealand

Zoophthora radicans produces two spore types: conidia for dispersal and resting spores (azygospores) for persistence. In addition, conidia may either produce secondary conidia, or capilliconidia (Pell *et al.* 1993). Conidia, which are the dominant infective propagule in nature, are fragile, short lived and subject to environmental desiccation (Furlong & Pell 1997, Uziel & Shtienberg 1993). The potential of conidia as the basis of a commercial product is therefore limited by their rapid environmental desiccation. In contrast, resting spores, which are the long term survival structure of the fungus, are thick-walled and robust, long-lived, environmentally stable, and have a period of dormancy. Resting spores, therefore have potential as an alternative commercial inoculum for use in augmentation (inoculative and mycoinsecticide) and conservation approaches.

Individual isolates of *Z. radicans* differ in their ability to form resting spores in infected cadavers; some form resting spores in few or no cadavers, whereas others form resting spores in many, under similar conditions (Glare 1988, Pell *et al*. 1993, Yeo *et al*. 2001). Resting spore production rates increase at low temperature and high humidity, high inoculum density, differing host age or physiological condition and when hosts are infected with more than one isolate (Perry *et al*. 1982, Glare *et al*. 1989), but the conditions for resting spore production are not fully understood. Perry & Fleming (1989), found resting spores of *Z. radicans* (= *Erynia radicans*) germinated after storage for >2 months at 4°C or by natural overwintering.

Research is, therefore, ongoing to determine the mechanisms of production and germination of resting spores by *Z. radicans* in DBM to facilitate the use of resting spores as an alternative commercial inoculum for use in microbial control of DBM.

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Proof-of-concept trials for control of DBM by autodissemination

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Abstract

On two occasions over a nine-day period, a total of 1100 diamondback moths (DBM), infected with the fungus *Zoophthora radicans* were released within a 16.4 m x 16.4 m field cage containing 542 DBM-infested potted broccoli plants. Larval and pupal populations on a sub-sample of 25 plants were examined on five occasions over the following 48 days for evidence of *Z. radicans* infection. Ten DBM-infested plants were placed individually in poly-organza covered cages so that they could not be contacted by infected adults and larvae within the larger field cage. DBM on these plants served as controls.

Infected larvae were first detected on treated plants five days after the initial release of infected adults. After 14 days, 20% of all larvae and pupae sampled were infected and at the final survey, 48 days after initial release, the infection rate was 79% (93% amongst III and IV instar larvae). The detection of infected larvae and pupae on the control plants 35 days after initial release, together with the presence of large numbers of *Z. radicans* conidia (spores) on microscope slides exposed within the field cage suggest that aerially-borne conidia were a major factor in transmission of the fungus.

There was no evidence to suggest that losses of infected adults and larvae due to predation were significantly higher than those suffered by uninfected adults and larvae. The proportion of uninfected males recaptured at pheromone traps was twice that of infected males, although the difference in terms of cumulative catch only became significant three days after release of the males. The significance of these findings for the development of autodissemination as a practical control technique is discussed.

Keywords

Zoophthora radicans, epizootic

Introduction

Diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), is the most significant and widespread insect pest of crucifers, with an estimated annual management cost of US$1 billion (Shelton *et al*. 1997). Its pest status is related to the high reproductive potential of the moth, the disruption or lack of natural enemies caused by excessive insecticide use against co-occuring pests and its ability to develop resistance to all currently available insecticides, including toxins of the microbial agent, *Bacillus thuringiensis*. More imaginative integrated pest management (IPM) strategies are urgently required (Talekar & Shelton 1993, Shelton *et al*. 1997, Lim *et al*. 1997). In this regard, the combined use of fungal natural enemies and synthetic female sex pheromones (autodissemination) has the potential to contribute to control of diamondback moth.

The entomopathogenic fungus, *Zoophthora radicans* Brefeld (Zygomycetes: Entomophthorales) is an important member of the natural enemy complex attacking the diamondback moth. Epizootics commonly develop in larval populations and can eradicate them at a local scale (e.g. Ooi 1981, Yamamoto & Aoki 1983, Riethmacher *et al*. 1992). However, epizootics are unpredictable and often only occur in large host populations too late in the crop season to maintain damage below the economic threshold. To maximise the potential of *Z. radicans* for diamondback moth management, epizootics must be initiated early in the season and in low density pest populations. It may be possible to achieve this by manipulating moth behaviour to facilitate autodissemination of fungus to susceptible conspecifics in the crop.

Autodissemination involves the attraction of male moths into specially designed inoculation traps in response to synthetic female sex pheromone. Once inside the trap they become contaminated with infective conidia from a sporulating source of *Z. radicans* and, on leaving the trap, return to the crop, disseminating disease amongst their own populations (= autodissemination). The benefits of this system over the

conventional use of mycoinsecticides are threefold. Use of a specific sex pheromone targets the inoculum to the diamondback moth as this is the only species entering the trap. Only small quantities of fungal inoculum and pheromone are required, thereby limiting production costs. Whilst inside the trap the fungus can be protected from the damaging effects of UV radiation and an abiotic environment that favours sporulation and infection can be provided.

Laboratory and preliminary field studies have been carried out to test this hypothesis (Pell *et al*. 1993, Furlong *et al*. 1995, Vega *et al*. 2000), but until now, no large scale 'proof of concept trials' have been made. Here we describe trials in Australia designed to quantify the potential to establish early season epizootics of *Z. radicans* in *P. xylostella* populations using autodissemination in which dispersal, transmission, epizootic establishment and susceptibility of *Z. radicans* infected cadavers to predation were measured.

Materials and methods

Cultures

A laboratory culture of *P. xylostella* was established in April 1998 from larvae collected in an infested crop of broccoli in the Lockyer Valley of south-eastern Queensland. The insects were reared on potted broccoli seedlings under a 14:10 L:D cycle at 18-25°C. The *Z. radicans* culture was derived from infected *P. xylostella* larvae collected in a commercial broccoli crop at Gatton in April 1999 and was maintained on a medium of Sabouraud dextrose agar supplemented with egg yolk and milk (SEMA) (Wilding & Brobyn 1980). Isolate virulence was maintained by sub-culturing a maximum of three times at *ca.* monthly intervals before it was again passaged through the host and re-isolated.

Experiment 1: Initiating an epizootic

The trials were conducted within a field cage measuring 16.4 x 16.4 x 2.3 m (l x w x h) covered with knitted shadecloth that allowed 50% light transmission. Over a period of *ca.* 5 days during March 2000, 552 broccoli seedlings were planted individually in 200 mm plastic pots and set out 0.6 m apart within and between rows in a grid pattern. Twenty-five plants evenly distributed throughout the cage were selected for regular examination to monitor development of the fungus. A further ten plants, also evenly distributed throughout the cage, were selected as controls. They were placed within individual poly-organza covered cages (450 x 470 x 920 mm (l x b x h) to prevent direct contact with infected adults and larvae from the treated plants. An overhead sprinkler irrigation system provided water as needed.

After *ca.* 3 weeks the plants were deliberately infested with DBM, both by introducing laboratory-reared larvae, pupae and adults to the cage and by temporarily removing some plants and exposing them overnight to ovipositing females in the laboratory. These procedures ensured the presence of all DBM stages when attempts to initiate an epizootic of *Z. radicans* commenced. Temperature and humidity were recorded every 30 min within a Stevenson screen in the centre of the cage. Daily temperature and humidity levels at 9:00 am outside the cage were derived from a meteorological station situated 300 m from the trial site.

Release of infected adults

Two hundred and twenty 2-4 day old male moths were released on 3rd May 2000 (day 0) and a second release of 740 males and females was made on 12th May (day 9). A sub-sample of five moths (first release) was retained in individual containers in the field cage to allow determination of mortality due to *Z. radicans* infection.

Monitoring of sentinel plants

Comprehensive surveys of healthy and infected DBM were made on days 14, 19, 23, 35 and 48. The trial was terminated on day 51. All leaves and stems on the selected plants were examined and larvae and pre-pupae recorded as infected if there was evidence of rhizoids or sporulation. On day 15, a survey was conducted of ten plants selected at 6 m intervals along a 60 m transect in a 1,800 m^2 (60 m x 30m) open field of broccoli whose closest boundary was 10 m from the field cage. The purpose of this survey was to determine whether or not *Z. radicans* was present naturally in the vicinity of the field cage.

Detecting aerially-borne *Z. radicans* conidia

Pairs of glass microscope slides measuring 75 mm x 25 mm were attached horizontally *ca.* 50 cm apart with bulldog clips to a bar so that their broad surfaces were parallel with and *ca.* 30 cm above the ground. The

pairs of slides were evenly distributed throughout the large field cage and were exposed on day 36 for 12 days, after which they were stained with lactophenol cotton blue and examined under a compound microscope for evidence of *Z. radicans* conidia. Counts were made of the number of conidia appearing in the field of view during a single transect of each slide from one end to the other. It is estimated that 1% of the surface was examined using this technique.

Experiment 2: Loss of DBM by predation

Predation upon infected DBM may adversely influence efforts to initiate an epizootic if a significant proportion of infected individuals is removed before sporulation occurs. Trials were conducted to determine whether there was any difference in levels of predation amongst healthy and infected larvae and adults.

Fourth instar larvae and adults recently killed by the fungus were produced under laboratory conditions. A second group of uninfected larvae and adults, killed by freezing them for 15 minutes at -20° C, served as controls. All cadavers were individually secured with double-sided adhesive tape to 50 mm-square pieces of clear acetate sheet. Protection from crawling predators was provided by encircling half the available larvae and adults with a narrow band of Tanglefoot®. These were designated 'protected' and the remainder 'exposed'.

The acetate sheets were attached with a paper clip to the underside of a leaf within an unsprayed 60 m x 30 m plot of broccoli. Treatments were examined daily for the duration of the trials and scored according to whether or not the larva or adult was present. Trials were conducted on three occasions over a 12 month period. Analyses of variance were performed on the number of adults and larvae lost, weighted by number put out and on proportion lost, with adjustments to allow for the high proportion of zeros.

Experiment 3: Effect of *Z. radicans* infection on male dispersal

Three hundred and ninety *Z. radicans*-infected (i.e. inoculated with conidia and maintained under humid conditions for 24 hours to ensure infection) and 361 healthy 2-3 d old laboratory-reared males were released from a central point within a 30 m x 60 m field of broccoli about 1 h before dusk. The treated and untreated males were differentiated by dusting them with yellow and blue Dayglo® powder respectively. Over each of the following three days, pheromone traps, evenly spaced around the circumferences of concentric circles with radii of 5 m (4 traps), 10 m (8 traps) and 15 m (16 traps) centred on the release point, were examined for the presence of marked moths. Contingency tables were constructed to show cumulative numbers of trapped and untrapped untreated and treated moths on the first, second and third days after their release. Chi-squared analyses were used to determine if there were any significant differences between treatments in terms of cumulative catch, irrespective of trap position.

Results

Experiment 1: Initiating an epizootic

Infected larvae were first detected on treated plants four days after the initial release of infected adults. By day 14, 20% of all larvae and pupae sampled were infected and at the final survey on day 48 the overall infection rate had reached 79%. Amongst III and IV instar larvae it was 93%. A single infected I - II instar larva was recorded on day 19 in the controls, representing an infection rate of 0.5%. None were recorded during the subsequent survey conducted on day 23, but over the following 12 days, infections developed very rapidly, reaching 40% by day 35 and 49% by day 48. Figure 1 depicts progress of the epizootic on the treated and control plants over the survey period. No infected larvae were detected during the survey conducted in the open field of broccoli on day 15. Of the five laboratory-infected adults retained to provide a measure of mortality in those released within the field cage, two were dead as a result of *Z. radicans* infection by the time of the first inspection 72 hours after release. The remaining three died, again as a result of *Z. radicans* infection, over the following 24 hours.

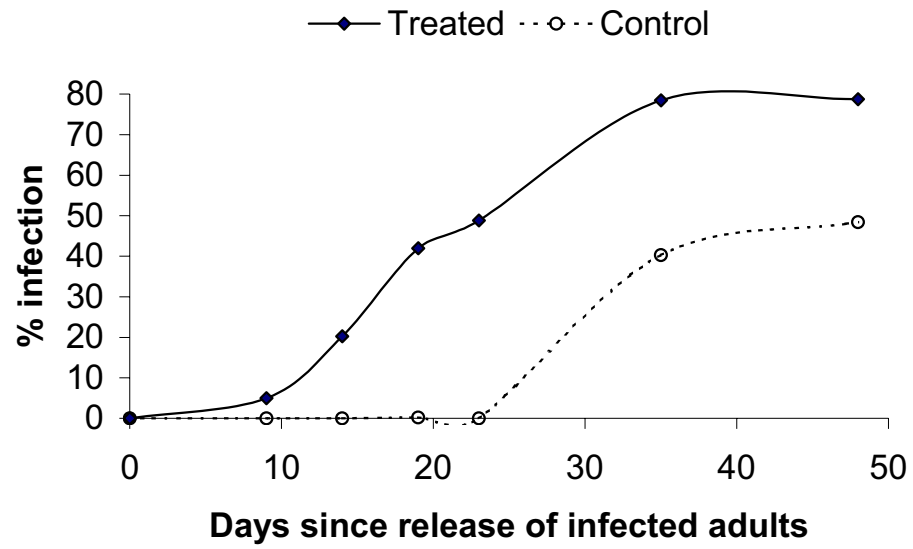

Figure 1. Rate of infection of DBM larvae and pupae with *Zoophthora radicans* within a 16.4 m x 16.4 m field cage. (Percent infection estimated for day 9 on treated plants. A second release of infected adults was made on day 9. See text).

Detection of aerially-borne conidia

Counts were very variable. In some instances there was more than a three-fold difference between counts on slides within a pair. The mean number of conidia/slide was 1085 ± 710 (s.d.). There was no evidence of any pattern in their distribution throughout the cage.

Meteorological conditions

Over the period of the trial, temperature and humidity within the cage averaged 14.4°C (range 0.7–30.1°C) and 78% (range 26.3–100%) respectively. Diurnal fluctuations in temperature and humidity within the cage for the period 3rd May – 20th June (days 0–48) are depicted in Figure 2. Over the same period, but at 9:00 am, mean temperature and humidity within the cage was 13.0°C and 88.1%, compared with 14.9°C and 80.2% outside the cage.

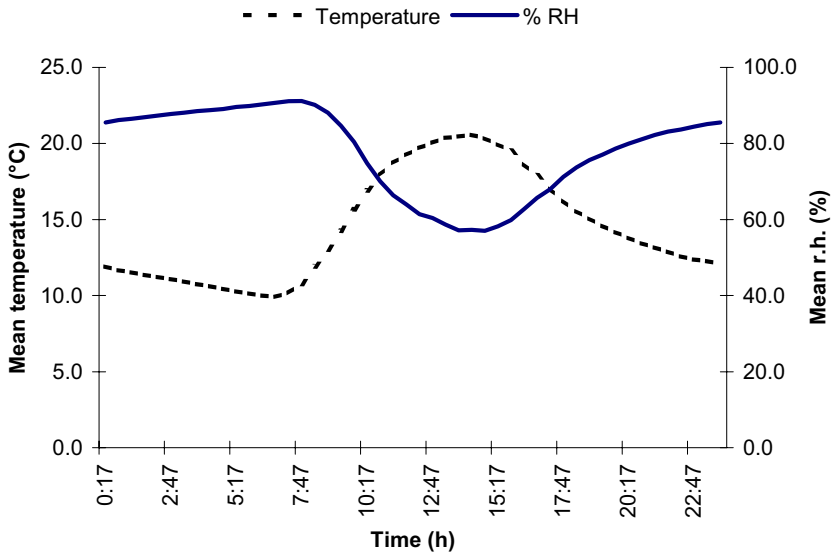

Figure 2. Mean temperature and relative humidity recorded at 30-minute intervals within the large field cage for the period 3 May – 20 June 2000.

Experiment 2: Loss of DBM by predation

There was no evidence to suggest that *Z. radicans*-infected DBM were more susceptible to predation than healthy individuals. Of 339 DBM (166 adults and 173 larvae) placed in the field, only 45 (13.3%) were missing, presumed lost to predation, after three days. They comprised 16 infected and 16 healthy adults and

five infected and eight healthy larvae. After allowing for the high proportion of zeros (i.e. no loss) and for trial and block differences, an analysis of variance revealed no significant difference between losses of infected and healthy individuals ($P<0.05$).

Experiment 3: Dispersal of infected males

Sixteen (4.1%) and 29 (8.1%) of the treated and control moths respectively were recaptured and although more control than treated moths were caught on each of the three days following release, differences in terms of cumulative catch irrespective of trap position were not significant until the third day (day 1: $\chi^2=0.16$, n.s.; day 2: $\chi^2=2.99$, n.s.; day 3: $\chi^2=5.14$, $P<0.05$). Total catches over the three days following release were, at 5 m from release point: 13 infected and 18 uninfected moths; 10 m: 1 infected and 3 uninfected and at 15 m: 2 infected and 8 uninfected.

Discussion

The results clearly demonstrate that it is possible to initiate an epizootic of *Z. radicans* by releasing inoculated/infected adults into a healthy population of DBM, albeit under conditions favourable to fungal development (reduced incidence of ultra-violet light and an average humidity of almost 78%). However in less humid conditions it may be possible to manipulate the microclimate with strategic irrigations to enhance development of epizootics. Our observation after one particularly dry period, that the majority of *Z. radicans*-infected adults placed in the field during the scavenging trials sporulated immediately after the crop was irrigated, suggests that this may be possible.

The influence of DBM population density on the rate of development of the epizootic is not known, although intuitively one would expect the relationship to be direct because of the increased probability of conidia produced by infected individuals contacting healthy ones with increasing pest density. *Z. radicans* may be a suitable choice of pathogen in this regard, given the substantial contribution made by aerial transmission of the conidia. Under these circumstances physical contact between infected and healthy individuals is less critical for development of epizootics than is the case for *Beauveria* spp., for example, thus facilitating transmission in low population densities (Furlong & Pell 2001).

The next step in evaluating autodissemination as a means of controlling DBM will be to demonstrate that an epizootic can be initiated via inoculation traps and when DBM population densities are at or below the economic threshold. This will require the development of inexpensive traps that provide an environment in which the fungus can continue to sporulate over several days and that provide suitable access to males attracted to them by pheromone. Research is also needed to determine appropriate trap densities and for how long it will be necessary to maintain them in order to generate an epizootic. Given that the strategy would be to initiate epizootics whilst the crop is young and when DBM population densities are low, epizootics may take some time to develop and it may be necessary to maintain traps for several weeks initially. However, where crops are planted sequentially, as is often the case in Australia, it may only be necessary to initiate the epizootic in the first crop of the season. Conidia generated from within the initial crop and distributed aerially may be sufficient to prevent DBM from developing into a threat to subsequent crops.

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Some studies on *Nosema* infecting DBM in Malaysia

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Abstract

Field and laboratory studies on *Nosema bombycis* infecting diamondback moth (DBM), *Plutella xylostella* (L.), were conducted. Percent *N. bombycis* infection and mean infection intensity were significantly different (P<0.05) among DBM larvae or pupae collected from either highland (CH) or lowland (SG) areas. Percent infection was significantly higher (P<0.05) for both larvae and pupae (71.3 and 66.8%) in the CH than in the SG (10.0 and 2.4%). It was also noted that the dead DBM larvae and pupae collected from the field had an abundance of *Nosema* spores especially DBM from the highlands. Percent mortality was significantly (P<0.05) higher in smaller instars (I and II) than larger instars (III and IV) even at lower spore concentration (4,260 spore/µl) and 24 h after treatment. There was also evidence that *Nosema* had developed resistance to the antibiotic (Fumidil-B) commonly used in making artificial diet for DBM. The recommended Fumidil-B seems to be ineffective in stopping disease development even at 400 ppm (220 ppm – current recommendation). Other antibiotics showed no better effect in controlling the disease infection than Fumidil-B. Temperature treatment was also unable to check the disease development even at 50°C. There was evidence that *Diadegma semiclausum* was involved in horizontal transmission of *Nosema* spores among DBM larvae. *Nosema* was observed to have a negative effect on the diurnal behaviour of DBM and *D. semiclausum*. Because the time spent by severely infected parasitoids was less than that of less or uninfected parasitoids, the prevalence of disease in the field might have a negative impact on the role of the parasitoid as an effective biological control agent of DBM.

Keywords

Nosema bombycis, diamondback moth, *Diadegma semiclausum*, antibiotic, temperature

Introduction

Diamondback moth (DBM), *Plutella xylostella* (L.), is a major insect pest of crucifer crops worldwide. In Malaysia, DBM was reported to cause serious damage on crucifers in 1925 (Ho 1965) and, since the 1940s, the main method of control has been the use of insecticides. The demand for these synthetic chemicals has been substantial and seems endless. Such over-dependence on insecticides has led to several pesticide-related problems such as resistance development to almost all insecticides including *Bacillus thuringiensis* (Bt) by DBM, hazards to non-target organisms, environmental pollution, poisoning and residues in the harvested produce (Ooi 1986, Loke *et al*. 1997).

An integrated DBM management (IPM) package stressing the use of DBM biological control agents in combination with other suitable methods such as insect growth regulators and the microbial insecticide (*Bacillus thuringiensis*, Bt), taking crop phenology into consideration and applying need-based treatments of pesticides has been used since the 1980s (Ooi 1986, Lim 1986, Ooi 1992, Loke *et al*. 1997). However, the problem of crop damage due to DBM seems to persist, since outbreaks of this pest occur at least every 2–3 years (Syed 1992). We noted that only a few farmers have used chemical and microbial insecticides alternately to control DBM.

DBM field populations in Malaysia have been naturally infected by several insect pathogens such as viruses (nuclear polyhedrosis virus, NPV and granulosis virus, GV), fungi (*Zoophthora radicans* and *Paecilomyces fumosoroseus*) and protozoa (*Nosema* spp. and *Vairimorpha* spp.) (Canning *et al*. 1999, Hussan 1992, Lim 1986, Ibrahim & Low 1993, Idris & Sajap 2001). However, their impact on the DBM population does not seem to reduce chemical insecticide use for controlling DBM.

As with most microsporidia infecting other insects (Steinhaus 1949), the impact of *Nosema bombycis* on DBM field populations has so far not been considered important. This is probably because of its slow killing action on the host. To date there are few scientific papers about microsporidia infecting DBM (Idris *et al*. 1997, Idris & Grafius 1999, Canning *et al*. 1999). However, the impact of *Nosema* on DBM and its parasitoids in laboratory culture is very severe (AK Hussan, AM Shelton and D Dough, personal communication). For example, laboratory cultures of DBM have to be renewed after two or three generations. *Nosema* infection of DBM reared in the laboratory may contribute to a compound effect if used for experiments. In the field, *Nosema*-infected DBM may have a negative impact on the parasitoid population of DBM, as was reported for the impact of *N. pyrasuta* on *Lydella thompsoni* (Diptera: Tachindae), a parasitoid of the European corn borer, *Ostrinia nubilalis* (Lewis 1982). This may be one of the factors that indirectly hamper the role of DBM parasitoids and IPM programs for controlling DBM, especially in Malaysia.

This study was conducted to investigate (1) the prevalence of *N. bombycis* infection in field populations of DBM in Malaysia, (2) behaviour of *Nosema*-infected DBM and *D. semiclausum*, (3) effect of antibiotics and temperature on *Nosema* infection, (4) percent mortality of DBM larvae infected with *N. bombycis* and (5) the possibility of *D. semiclausum* being involved in horizontal transmission of *N. bombycis*.

Materials and methods

Prevalence of *Nosema* infection in DBM field populations

Two sites were selected, the Cameron Highlands (CH) in Pahang and Serdang-Gombak (SG) in Selangor, Malaysia, which are 400 km apart. The CH and SG sites represent highland and lowland cabbage growing areas, respectively. The altitudes from which samples were taken were 1700 - 1800 m (Kea Farm) and 3 - 10 m above sea level at CH and SG, respectively. The average daily temperature was 15 – 25°C for CH and 29 – 35°C for SG. Sampling was carried out over a period of two days at each site in the month of October 1998 (season I) and April 1999 (season II). DBM larvae and pupae were collected from 10% (≈ 90 plants) of the cabbage plants (selected randomly prior to sampling) per respective fields per site. At least 50 larvae or pupae were collected from each site per sampling per occasion.

The percentage of infection in the larvae or pupae was determined by examining impression smears of individuals using phase-contrast microscopy at 400x. The intensity of infection was determined from ten randomly selected individuals. These individuals were homogenized in 1 ml distilled water using a tissue-homogeniser. A drop of homogenate was pipetted onto a haemocytometer and *Nosema* spores were counted. Chi-squared tests were used to test for differences in percentage of infection and mean infection intensity among locations and seasons.

Mortality of DBM larvae infected with *Nosema bombycis*

DBM eggs and artificial diet used were obtained from MARDI (Malaysian Agriculture Research and Development Institute). The *Nosema*-free DBM eggs were placed in 15 cm diameter Petri dishes for hatching. Spore solutions were prepared by crushing 50 infected DBM larvae in centrifuge tubes, adding 20 ml distilled water and centrifuging three times at 2,500 rpm for 10 minutes at 10°C. The spore pellet collected at the bottom of the tube was diluted to a 10^{-3} spore concentration (407150, 41420, 4260 and 420 spores/µl) following a serial dilution method. A slice of artificial diet (5 x 5 x 2 mm) was wet with 50 µl spore solution of the required spore concentration for 3 minutes and placed in a multi-well plate. Disease-free DBM larvae of known instars were placed in the wells (one larva per well) for one day under laboratory conditions. Larvae were transferred to another multi-well plate, fed on a spore free-diet and mortality was recorded at 24, 48 and 72 h after treatment. Dead larvae were crushed in a tissue grinder tube to which 1 ml of distilled water was added and then shaken. Ten µl of the larval solution was pipetted and placed onto a haemocytometer for spore counting. Data were analysed using two-way ANOVA and probit analysis. Distilled water was used to treat the diet in the control.

Effect of antibiotics on disease-infected DBM

The *Nosema*-infected DBM eggs and untreated artificial diet (provided by MARDI) were put in hatching cups placed in a growth chamber. Four concentrations of Fumidil-B (100, 200, 300 and 400 ppm) were prepared. A slice of artificial diet 4 cm^2 and 0.1 cm thick (without Fumidil-B) was soaked in a solution of Fumidil-B for 15 minutes (to ensure diet was impregnated by the antibiotic), air dried for 2 h and placed in a 15-cm diameter Petri dish. Five I instar DBM larvae (3 h after hatching) were randomly selected and placed

in Petri dishes with diet (25 larvae per treatment). Diet was changed daily. Mortality of larvae was recorded every other day, starting at two days after hatching and continuing until the tenth day when most surviving larvae had started to pupate. The untreated diet (treated with distilled water) was used as a control. Each treatment was replicated four times. In another experiment, Fumidil-B, Suprim, Albendazole and Tetracycline at 50, 100, 200, 300 and 400 ppm were tested as above. Percent mortality was calculated as the total number of larvae per replicate minus the accumulated dead larvae and divided by the total larvae x 100. Data were analysed by one-way ANOVA and the treatment means were separated by Fisher's Protected LSD test.

Effect of temperature on disease-infected DBM

One-day-old *Nosema*-infected DBM eggs were dipped into a hot water bath at various temperatures (20, 25, 30, 35, 40, 45, 50 or 55°C) for 2, 3 or 6 h. In another study, the eggs were subjected to different temperatures (by putting them into a growth chamber) as above for 1 or 2 h. Treated eggs were placed in 15 cm diameter Petri dishes (100 eggs per dish) with artificial diet for 3 days (when all eggs had hatched). Larvae were transferred to other Petri dishes (10 larvae per dish), fed the same diet (changed every two days) until pupation. Number of IV instar larvae surviving was recorded. Data were analysed using two-way analysis of variance (ANOVA).

Involvement of *Diadegma semiclausum* in horizontal transmission of disease

Pupae of *D. semiclausum* were collected from cabbage fields in the Cameron Highlands, Pahang, Malaysia and temporarily kept in a refrigerator at 4°C. Twenty *D. semiclausum* pupae of a similar age were selected and placed in emergence cages consisting of clear 300 ml plastic containers with 2.0 cm and 1.5 cm diameter lids on the top and sides. Cages were placed 50 cm below a white fluorescent light. Cotton wool with diluted honey was placed on the bottom of the cage as food for the newly eclosed parasitoid adults. The parasitoid adults were allowed to mate for five days before being used in the experiment.

Thirty DBM II instar larvae were placed in a modified clear plastic container (see above) as a parasitism arena with four slices (0.2 x 2.0 x 2.0 cm) of artificial diet for 24 h. A 5 day old mated female *D. semiclausum* was randomly selected from the container using an aspirator and was released into the parasitism arena via the hole in the top lid. A paper tissue moistened with diluted honey was inserted through the side hole to provide food for the parasitoid adult. The food was replaced every day. Each parasitoid was allowed to parasitise DBM larvae for 4 hours and was then taken out and kept in the freezer for use in the next study. The presumed parasitised DBM larvae were reared individually in 14.5 cm diameter Petri dishes, fed artificial diet as above and kept under laboratory conditions until pupation. The experiment was replicated eight times. For a control, DBM larvae were not exposed to the parasitoid adult. Numbers of larvae that died before pupation, pupae formed, adult parasitoids and DBM emerging were recorded.

Eighty DBM II instar larvae were exposed for parasitism (four replicates, 20 larvae per replicate per parasitoid female) and then reared as above until pupation. The one-day-old parasitoid pupae were taken out of their cocoons using forceps and placed in a test tube filled with 70% alcohol. The test tubes were shaken on an electric shaker for one minute to dislodge any possible microsporidian spores adhering to the body of the parasitoid pupae, after which the pupae were placed into different test tubes and shaken. This process was repeated four times. Pupae were then placed onto a glass slide with a drop of distilled water and crushed using a cover slip.

Ten adult females from the laboratory study were killed immediately after emergence, while 20 adult females collected from the field were killed by placing the test tube containing each parasitoid in sunlight in the field for 15 minutes. The dead parasitoid adults were kept temporarily in the freezer. Each individual female was put into a centrifuge tube filled with 50 ml of 70% alcohol, shaken as above for 10 min after which the parasitoid was taken out and kept for use in the next experiment. The supernatant was centrifuged for 10 min at 13,000 rpm at 10°C. The supernatant was poured out, leaving the pellet at the bottom of the tube. Five ml of distilled water was added to the tube and shaken as before. One ml of spore suspension was pipetted onto a glass slide and covered with a cover slip after which the presence of spores was observed as above. Similar female adults were again subjected to inspection for the presence of spores in the sexual organs and within the abdomen. The abdomen of similar females was dissected using dissecting scissors and knives to take out just the internal body parts which were examined for the presence of spores. A total of 20

males (10 from the above study and another 10 collected from the field) were also treated as for the females, to examine the presence of spores on the body and in the internal organs within the abdomen.

The presence of spores was observed under a compound microscope at 400x magnification. The number of DBM larvae that died before pupation, percent parasitism and adult emergence (parasitoid and DBM) were analysed using paired t-tests.

Diurnal behaviour of *Nosema*-infected DBM and *D. semiclausum*

Pupae of DBM and *D. semiclausum* were collected from a cabbage field near Kea Farm, Cameron Highlands. The pupae were individually kept in a transparent plastic container (11 x 12 x 10 cm) that was placed in a growth chamber environment (20°C, 12 h light: 12 h dark, installed 20 cm above the container, and 50-70% relative humidity) until adult emergence. Adults were maintained in the growth chamber and fed honey water on cotton wool placed on the floor of the container. The flying, feeding, moving, grooming and resting behaviour of DBM or *D. semiclausum* was observed for 2 h (0900 – 1100 h) from outside the chamber on the 5^{th} day after emergence. After the behaviours were observed, each insect was crushed in a centrifuge tube filled with 50 ml distilled water and centrifuged three times (10 minutes each) at 2,500 rpm at 10°C. Ten ml of distilled water was added to the spore pellet which was then shaken and diluted to 10^{-2} spore solution. One ml of this spore solution was pipetted onto a haemocytometer for counting of spores. For parasitism behaviour (approaching and attacking the host larvae exposed to individual *D. semiclausum* female in parasitism arena – similar clear plastic container as above), eleven 5 day old parasitoid females were tested in a similar environment as before. Data were analysed using either chi-square or regression analysis.

Results and discussion

Prevalence of *N. bombycis* in DBM field populations

There was a significant difference in the percentage of *N. bombycis* infection ($P<0.05$) and mean infection intensity ($P<0.05$) among DBM larvae or pupae collected from different sites (CH and SG) and seasons. The percentage of infection of DBM larvae and pupae in CH was much higher (71.3 and 66.8%) than in SG (10.0 and 2.4%). The mean intensities of *Nosema* infection (number of spores per larva) of DBM larvae were 259.3×10^5 and 150.2×10^5 in CH while in SG the mean intensity was 14.0×10^5 and 2.0×10^5. However, the range of infection intensity on DBM larvae in CH (0.02×10^7 - 8.0×10^7 and 0.01×10^7 - 9.7×10^7) was wider than that of SG (0.03×10^7 – 0.27×10^7 and 0.01×10^7 – 0.02×10^7). The mean intensity of infection and range of infection intensity per pupa showed similar trends as for DBM larvae. The mean infection intensity of DBM pupae in CH was much higher (67.2×10^5 and 22.3×10^5) than that of SG (26.5×10^5 and 7.0×10^5).

The microsporidian infection was comparatively more severe in DBM collected from the highlands than in the lowland areas. Temperature may have influenced the severity of infection. Low temperatures in the highlands (CH) would have prolonged larval developmental periods (Ooi 1986). This may have increased the success of infection as DBM larvae were exposed for a longer time to *Nosema* spores. High temperature in the lowland area may cause the insect growth rate to outpace disease development in DBM leading to less infection compared with the highland DBM population. Low sunlight intensity in the CH probably inactivated fewer *Nosema* spores that contaminate plant leaf surfaces (Sikorowski & Lashomb 1977). Consequently, DBM larvae in this area may have ingested more spores while feeding on cabbage leaves than DBM larvae at SG. Unlike DBM larvae, the difference in percentage of *Nosema* infection on DBM pupae between the populations from CH and SG in both seasons was smaller (13.3% and 12.51 for CH and 8.1 and 7.0% for SG respectively). This was probably due to the fact that many infected DBM larvae in CH failed to develop to the pupal stage.

Mortality of DBM larvae infected with *Nosema bombycis*

Percent mortality was significantly ($P<0.05$) higher in smaller instars (I and II) than larger instars (III and IV) even at low spore concentration (4,260 spore/µl) and 24 h after treatments. The mortality reached 100% when smaller instars were infected with a higher spore concentration (407,150 spore/µl) whereas mortality of larger larvae reached 40% at 72 h after treatment. No mortality was observed in the control. Similar results were also reported for *Bombyx mori* larvae infected by *N. bombycis* (Lian 1991). The LC_{50} (number of spores per µl) of smaller larvae was also significantly lower than the LC_{50} value of larger larvae at 48 and

72 h after treatment. At 24 h after treatment, LC_{50} values for instars I and II was 15,955 and there was no mortality of instars III and IV. However, at 72 h after treatment, the LC_{50} values were 3206 for instars I and II, and 7,955 and 11,516 for instars III and IV. This indicates that more spores are needed to kill larger larvae. The sudden increase in numbers of spores in smaller larvae may have damaged the host cell membrane to a greater degree than in larger larvae (Jurand *et al*. 1967).

Effect of antibiotics on disease-infected DBM

The highest accumulated percent larval mortality (92.5% at day 10) was observed when larvae were fed untreated diet (Table 1). Mortality was significantly lower when larvae were fed diet treated with 200 ppm Fumidil-B solutions on day 8 and 10 than those treated with 100 ppm Fumidil-B. However, mortality was significantly lower when larvae were fed diet treated with 300 ppm of Fumidil-B compared with those fed with diet treated with 200 ppm. This indicates that the recommended rate of 220 ppm Fumidil-B used in preparation of DBM artificial diet is unable to contain development of the disease. Further increase in Fumidil-B concentration to 400 ppm significantly increased the percent mortality, indicating that there was a deleterious effect of Fumidil-B on DBM larvae because there were no *Nosema* spores observed from the dead larvae. Results of this study showed that the *Nosema*-infected colony of DBM reared at MARDI might have developed resistance to Fumidil-B.

Table 1. Mean percent mortality (accumulative) of diamondback moth larvae fed artificial diet treated with various concentrations of Fumidil-B

Concentration (ppm)	Days after treatment				
	2	4	6	8	10
Untreated	45.4 ± 5.6 a	60.5 ± 7.8 a	70.3 ± 8.5 a	85.4 ± 10.2 a	92.5 ± 10.2 a
50	20.5 ± 10.5 b	25.7 ± 7.3 b	38.5 ± 5.6 b	52.6 ± 6.3 b	72.5 ± 7.8 b
100	23.3 ± 11.3 b	24.4 ± 6.7 b	35.6 ± 4.7 b	50.5 ± 7.3 b	69.5 ± 8.2 b
200	15.5 ± 10.2 c	18.3 ± 5.6 c	22.4 ± 5.3 b	30.5 ± 5.4 c	36.3 ± 4.2 c
300	1.5 ± 2.4 d	2.6 ± 4.2 d	3.5 ± 5.6 c	6.3 ± 3.3 d	10.9 ± 3.8 d
400	0d	10.5 ± 1.5 d	15.5 ± 1.5 c	17.5 ± 1.5 d	21.5 ± 1.5 d

Means in column with same letters are not significantly different (Fisher's Protected LSD, $P>0.05$)

Percent mortality of DBM larvae was significantly lower when fed on diet treated with Fumidil-B at all concentrations than when fed on diets treated with other antibiotics or untreated (Table 2). This indicates that Fumidil-B is comparatively a better antibiotic for treating a *N. bombycis*-infected DBM culture or preventing disease development. Since mortality of DBM was still 25.4% at 400 ppm of Fumidil-B, it is suggested that its concentration could be increased for total control of disease development.

Table 2. Percent mortality (mean) of disease-infected DBM larvae fed artificial diet treated with antibiotics of different concentrations

Antibiotics	Concentration (ppm)			
	50	100	200	400
Suprim	75.3 ± 7.4 b	76.2 ± 10.2 b	72.1 ± 11.3 b	56.5 ± 8.4 c
Albendazole	85.3 ± 6.2 a	80.4 ± 11.3 b	75.6 ± 10.2 b	70.4 ± 8.5 b
Fumidil-B	55.5 ± 7.4 c	45.3 ± 6.7 c	40.2 ± 6.5 c	25.4 ± 4.5 d
Tetracycline	85.7 ± 10.8 a	85.5 ± 9.5 a	85.2 ± 8.9 a	80.4 ± 9.3 b
Untreated	100a	100a	100a	100a

Means in column with same letters are not significantly different (Fisher's Protected LSD, $P>0.05$).

Effect of temperature on disease-infected DBM

The percentage of DBM larvae surviving to IV instar (when *Nosema*-infected eggs were treated at 45°C for 3 h) was significantly higher (75.3%)($P<0.05$) than when eggs were treated with other temperatures (Figure 1). The rate of survival was lower than 20% when eggs were dipped in hot water at temperatures below 35°C or higher than 50°C (6 h exposure). Eggs treated with various temperatures in the growth chamber

showed similar trends. This indicates that disease development is high at low temperatures, while high temperatures of hot water (>50°C) might have deleterious effects on development of larvae within eggs which resulted in a low hatching rate. Schulz-Langner (1957) reported that temperatures of 37°C and higher, suppressed *Nosema apis* in the honeybee, whose body temperature may reach 44°C. The number of hours the bees spent at 37°C in the hive proportionately retarded the development of the *Nosema* infection.

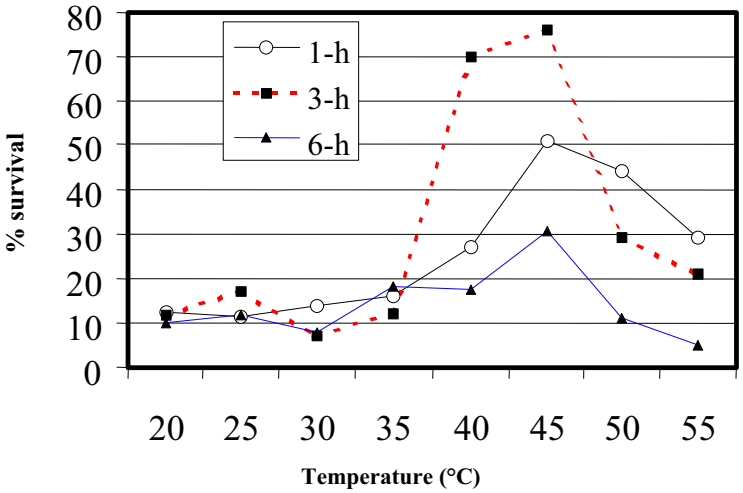

Figure 1. Percent of DBM larvae surviving to IV instar after eggs were dipped in hot water of various temperatures for 1, 3 or 6 hours.

Involvement of *D. semiclausum* in horizontal transmission of the disease

The number of dead larvae was significantly (P<0.05) higher for the treated larvae than for the control (Table 3). The number of *D. semiclausum* adults that emerged was significantly (P<0.05) higher than that of DBM adults. However, there was no significant (P>0.05) difference between the number of *D. semiclausum* and DBM pupae formed in this experiment. All the dead larvae in the treatment had microsporidian spores, indicating that *D. semiclausum* is involved in horizontal transmission of spores to its host. Microsporidian diseases transmitted by parasitoids in other host-pathogen systems were reported by Brown (1987), Geden *et al*. (1995) and Sajap and Lewis (1988).

Table 3. Percent mortality of parasitised diamondback moth (DBM) larvae, mean number of pupae formed, adult DBM and parasitoids emerging and percent of larvae containing microsporidian spores

Treatment	% mortality before pupation	% *D. semiclausum* adults emerged	% of dead larvae with spores
Exposed	41.3 ± 4.6 a	22 ± 4.3 a	100 a
Unexposed	0.1 ± 0.2 b	0 b	0 b

Eight *D. semiclausum* individuals were used. Each had access to 30 DBM larvae for 3 hours
Means in column with same letters are not significantly different (Fisher's Protected LSD, P > 0.05).

85.5% of parasitoid pupae contained *Nosema* spores (Table 4). The microsporidian spores were observed on and within the body of both sexes of the parasitoid. On the body, the females had more spores than the males. Within the body of both sexes of a parasitoid, however, there was no difference in the percentage of individual parasitoid adults having spores. Most (90.4%) parasitoid female sex organs had spores.

Table 4. Microsporidian spores detected within parasitoid pupae, on and within the body of parasitoid females or males

Parameters	Percentage of sample with spores
Within parasitoid pupae	85.5 ± 10.31
On the body of adult	
Females (n=30)	85.7 ± 12.9
Males (n=20)	50.3 ± 7.8
Within the body (abdomen)	
Females (n=30)	65.4 ± 9.6
Males (n=20)	58.8 ± 5.4
Sex organs (females, n=30)	90.4 ± 10.3

Results of this study also indicate that *Nosema* infection had a negative impact on the parasitoid populations in the field. However, our field observations (unrecorded data) indicated that this parasitoid was abundant despite high disease incidence and pesticide usage. Percent parasitism cannot be estimated in this study due to the death of parasitised and nonparasitised DBM larvae. The parasitism rate of DBM larvae by *D. semiclausum* and the closely related species, *D. insulare*, ranged from 20 to over 80% in the field (Ooi 1992, Idris & Grafius 1993). It is hypothesized that the parasitoid is capable of avoiding the negative impact of the microsporidian disease. Both the disease and the parasitoid could be synergists to each other in using DBM as a host. However, further research needs to be done to test these hypotheses. Nevertheless, Siegel *et al*. (1986) reported that the level of *N. pyrausta* infection in the European corn borer (ECB) corresponded to the level of infection in its parasitoid, *Macrocentrus grandii* (Hymenoptera: Braconidae) and that infection had reduced the number of parasitoids exiting the host (ECB) as well as the emergence of the parasitoid adults. Results of another study conducted by Geden *et al*. (1995) found that the *Muscidifurax raptor* (Hymenoptera: Pteromalidae) parasitoid of filth-breeding flies (Diptera: Muscidae), infected by *Nosema* disease had serious loss of fitness, with infected females taking longer to develop, having shorter lives and producing only 12–50% offspring of the uninfected ones. Although we did not specifically monitor the parasitoid egg for spores, the results of this preliminary observation indicated that *D. semiclausum* is one of the possible factors involved in horizontal transmission of microsporidian disease of DBM.

Diurnal behaviour of *Nosema*-infected DBM and *D. semiclausum*

It was observed that DBM adults spent significantly more time (75%) resting than moving (walking) (15%), grooming (5%), feeding (3%) or flying (2%). Although the observation time occurred at the time they are actively flying in the field (personal observation), the space (cage) and lack of external cues (host plant volatiles and pheromones) may have caused DBM to be less active. There was no significant relationship (P>0.05) between the total time spent resting and the spore concentration (number of spores/µl) per individual DBM adult. However, moving (r=0.67, F=27.7, df=1 & 18, P=0.001) and grooming (r=0.54, F=9.8, df=1 & 18, P=0.006) behaviours positively correlated with the spore concentration per insect. This indicates that DBM movement rate increased with the severity of disease infection. An increase in movement rate of disease-infected DBM helps the epizootics of the disease (Maddox 1987).

Diadegma semiclausum adult females were observed spending significantly (P<0.05) more time moving (65%) than resting (25%), flying (5%), feeding (4%) or grooming (1%). It was not certain whether the space factor influenced the parasitoid diurnal behaviour in this study. However, in the field, the parasitoid was observed actively flying between 0800 and 1100 h on a clear or partly sunny day. Idris and Grafius (1998) also reported that diurnal flight behaviour of *D. insulare* is optimal around 1000 h under favourable weather conditions (15-23°C, at least partly sunny day, calm wind). Moving (r=0.90, F=15.2, df=1 & 13, P<0.05), flying (r=0.45, F=9.54, df=1 & 13, P<0.05) and grooming (r=0.75, F=34.4, df=1 & 13, P<0.05) behaviours were positively correlated with the spore concentration in the parasitoid body. This indicates that infected individual parasitoids spend more time on activities that are not related to parasitism. This was also shown by the negative relationship between the amount of parasitism behaviour in 2 h observation time and spore concentration per insect (r=-0.78, F=10.3, df=1 & 13, P=0.001) (Figure 2).

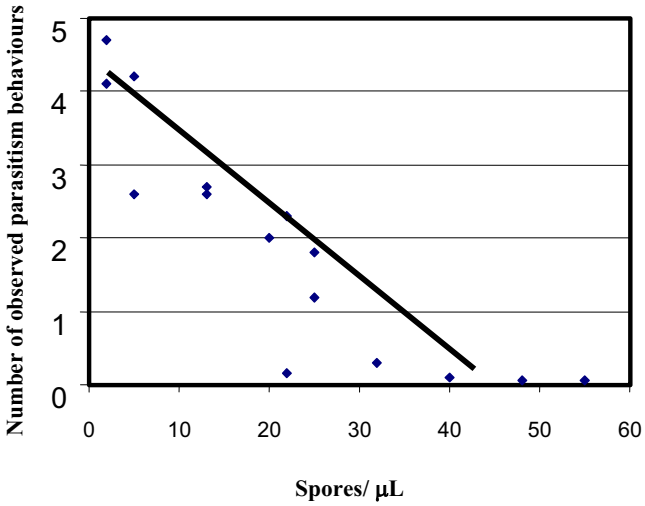

Figure 2. Relationship between the number of parasitism behaviours in 2 h of *Diadegma semiclausum* and *Nosema bombycis* spore concentration per insect.

Conclusion

The prevalence of *N. bombycis* infection in field populations of DBM in the Cameron Highlands of Malaysia and high mortality rate of DBM larvae indicates that this microsporidian disease is one of the important mortality factors of DBM. Could it be a potential candidate for a biopesticide of DBM? Interestingly, *N. bombycis* also infects other insects including *Spodoptera litura*, *S. exigua*, *Delia radicum*, *Pieris rapae*, *Chrysodeixis eriosoma* and *Vallaga nigricornis* (Idris *et al*., unpublished data). *Nosema bombycis* causes serious problems in laboratory cultures of DBM. Our results showed that hot water or high temperature treatment could not prevent disease development in a DBM culture. Antibiotic treatment (especially Fumidil-B) may be able to control the disease development at high concentrations (>400 ppm). However, further study of the antibiotic's deleterious effect on DBM at high concentrations is required. The influence of *N. bombycis* on the behaviour of DBM seems to have a negative impact on the spread of the disease. Nevertheless, the heavily disease-infected *D. semiclausum* spent less time on parasitism activities than did those less or uninfected individuals. This would have a negative impact on its role as a major mortality factor of DBM and its impact in integrated DBM management. The evidence of *D. semiclausum* being involved in the horizontal transmission of this disease among its host could increase disease prevalence. Whether or not this would eventually lead to its population becoming extinct is an area for further study.

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Diamondback moth, *Plutella xylostella* (L.), resistance management in Hawaii

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Abstract

Resistance to spinosad insecticide in field populations of diamondback moth was discovered at Kunia, Hawaii, in November 2000, about two and a half years after its introduction. Leaf-dip assays of field populations from the islands of Oahu, Maui and Hawaii confirmed moderate to high levels of resistance compared with pre-introduction baselines. Resistance occurred despite label restrictions that were designed to prevent overuse. A major contributing factor was the lack of suitable alternatives and the unsynchronised use of pesticide classes that led to continuous population exposure. Region-focused resistance management plans were implemented by growers and University of Hawaii extension advisers in an IRAC sponsored program. The two goals were to mitigate resistance to spinosad and avoid resistance to emamectin benzoate and indoxacarb. The use of emamectin benzoate and indoxacarb was limited to month-long windows that were rotated. There was a ban on spinosad use in crucifer production until regional DBM LC_{50} decreased to a 5 ppm level.

Keywords

IRM, insecticide resistance, spinosad

Introduction

The Hawaiian Islands have a wide range of climatic conditions that allow crucifers to be grown year round on most islands. The majority of cabbage, broccoli and cauliflower production occurs at elevations below 100 metres on the islands of Hawaii, Maui and Oahu. Leafy crucifer varieties (choy sum, bok choy, gai lon, gai choy) are commonly grown in lowland farms below that elevation. The majority of farms are clustered in regions and range in size from 8 to 20 hectares. There are a few that are about 400 hectares in size. To meet annual fresh-market needs, more than 650 hectares of cruciferous crops are produced. Each grower routinely establishes plantings of 0.13 to 2 hectares every week in sequential, adjacent plots.

Diamondback moth (DBM) is the key pest of crucifers, but the crops can be affected by occasional caterpillar pests that include the imported cabbage webworm (*Hellula undalis*), imported cabbageworm (*Pieris rapae*), and cabbage looper (*Trichoplusia ni*). There are 12 to 17 DBM generations per year. DBM populations in the major production regions are resistant to organophosphate, carbamate, pyrethroid and organochlorine insecticides (Tabashnik *et al.* 1987). Although resistance to *Bacillus thuringiensis* (Bt) has been documented, growers report that Bt. products are effective after periods of non-use.
Products from new insecticide classes (chlorfenapyr, emamectin benzoate, fipronil, indoxacarb and spinosad) were highly effective in laboratory and field tests against Hawaiian populations of diamondback moth (Mau *et al.* 1997, Mau & Gusukuma-Minuto 1999). Unfortunately, regulatory labelling of the products for crucifers has been slow and incremental. To date, only emamectin benzoate, indoxacarb and spinosad received national labels for use on cruciferous crops.

Emamectin benzoate (Proclaim®, Syngenta Crop Protection, Inc.) was intensively used in Hawaii under emergency provisions from June 1996 to May 1998. Naturalyte Insect Control (spinosad, Dow AgroSciences) became available for use by Hawaii's growers in April 1998 under the product name Success. During June 1998 through May 2000, spinosad was intensively used throughout the state. It was the only labelled insecticide that was highly effective for DBM management. The farm-focused resistance management restrictions for spinosad were included in the manufacturer's product label. Use was limited to three times per 30 days followed by at least a 30-days of non-use. There was a maximum of 6 applications allowed per crop cycle. *B. thuringiensis* insecticides were only partially effective, but were used when spinosad could not be used. Emamectin benzoate was subsequently labelled for national use in June 1999. Indoxacarb (Avaunt®, E.I. duPont de Nemours & Co., Inc) became labelled for use on some cruciferous crops in December 2000.

In September 2000, University of Hawaii extension entomology workers investigated complaints of non-performance of spinosad against DBM. At the time, spinosad was being rotated with emamectin benzoate. This paper discusses discovery and confirmation of resistance to spinosad at several locations in Hawaii. We outline the establishment of region-focused DBM resistance management programs that were created to conserve effective insecticides and mitigate resistance to spinosad.

Materials and methods

Bioassay procedures were as follows. The leaf residue bioassay method described by Tabashnik and Cushing (1987) was used for the tests with the following modifications. Pesticide free cabbage seedlings were used. Discs (4.5 cm diameter) were cut from leaves using a cookie cutter. Each disc was attached to a paper clip, immersed in the appropriate pesticide solution and allowed to air dry for 1–2 hours. The assays were conducted in 50 x 9 mm sterile Petri dishes. III instar larvae were used in the laboratory bioassays. Where possible, F_1 larvae from each of the colonies were used in the assays. Eight to 12 larvae were placed on each disc. Larvae were allowed to feed for three days before final mortality data were taken. There were at least ten replicate cohorts for each treatment dose. Mean lethal concentrations (LC_{50}) for field populations were calculated using probit analysis (SAS).

The original assays for resistance to spinosad was first performed on a field-collected colony from a farm at Kunia, Oahu. Subsequent assays were performed on colonies established from other geographic regions throughout the State of Hawaii (Figure 1). Late stage DBM larvae and pupae were used in establishing the colonies. All colonies were reared on pesticide-free cabbage or rape. A susceptible colony that was collected in 1994 at Kamuela, Hawaii was used for the toxicity ratio (TR) calculations. Toxicity ratios were calculated by dividing the LC_{50} of the assayed population by that for the susceptible laboratory population (Kamuela 94).

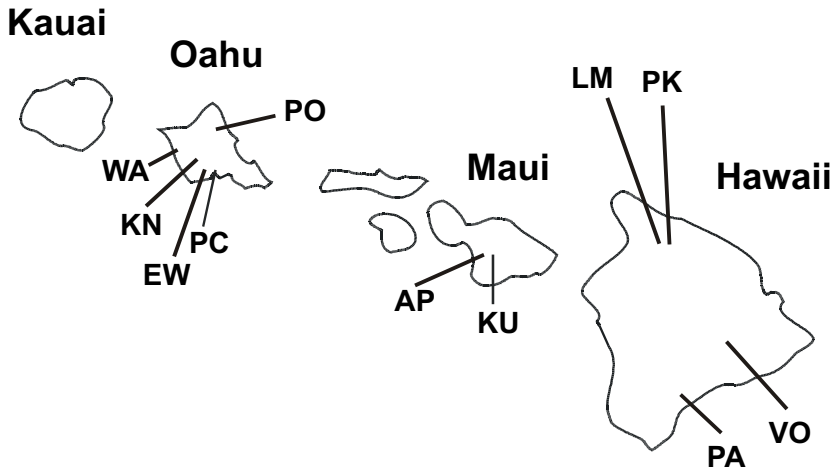

Figure 1. Major production regions for cruciferous crops in Hawaii and the specific localities where field-collections of diamondback moth were made (AP = Lower Kula, EW = Ewa, KN = Kunia, KU = Middle Kula, LM = Lalamilo, PA = Pahala, PC = Pearl City, PK = Puukapu, PO = Poamoho, WA = Waianae, VO = Volcano).

Results

Moderate to high levels of resistance to spinosad were found in several DBM populations (Mau & Gusukuma-Minuto 2001; Zhao *et al.* 2002). Toxicity ratios of resistant populations ranged from 204 at Puukapu, Hawaii to 1,340 at Ewa, Oahu (Table 1). Resistant field populations were found on all islands, but resistance was greatest on the island of Oahu where crops were grown at lower elevations (Table 1). Moderate resistance levels were found at Lalamilo and Puukapu, Hawaii. DBM populations at Volcano and Pahala, Hawaii, where spinosad had not been used, were very susceptible to spinosad.

Table 1. Toxicity Ratios (TR) for spinosad (Success 2 SC) for Hawaiian field-collected diamondback moth populations

Population	Generation in laboratory	Number Tested	Toxicity Ratio[1]
1998 Ewa (pre-spinosad introduction)	F_1	669	1
Susceptible Kamuela-94 strain	F_{119}	120	1
OAHU			
Ewa	F_2	244	1340
Kunia	F_4	476	642
Pearl City	F_5	484	1080
Poamoho	F_2	400	23
Waianae	F_2	420	248
MAUI			
Kula, lower	F_3	153	35
Kula, middle	F_6	291	592
HAWAII			
Lalamilo	F_1	176	25
Puukapu	F_1	249	204
Pahala	F_2	450	3
Volcano	F_2	420	2

[1]Toxicity ratio = LC_{50} of tested population divided by the LC_{50} of the susceptible laboratory strain
Leaf dip assays were read at 72 hours.

There was adequate proof that susceptibility to spinosad had changed. A susceptibility baseline for the Ewa population had been performed just prior to registration of spinosad in 1998. That toxicity ratio was calculated as 1.0. Comparing the TR values, we found that there was a 1,340-fold decrease in susceptibility for the DBM population at Ewa.

Establishment of regional resistance management programs

Extension educators and Dow AgroSciences officials presented a series of regional seminars about development and management of resistance using information from the Insecticide Resistance Action Committee (IRAC), Dow AgroSciences and University of Hawaii. Alternative tactics for resistance management were discussed. Grower committees and extension advisers devised regional integrated resistance management (IRM) programs for DBM. Improved crop hygiene, conservation of naturally occurring biological controls, pest monitoring and rotation of insecticide classes were tactics that were included. A month-long crucifer-free fallow was considered, but it was not adopted because a majority of the farmers would lose income and market relationships for at least three months.

The voluntary program was based on education and peer pressure, not regulatory mandate. There were isolated cases of non-compliance with the insecticide rotation program during the first few months, but this was not widespread. Compliance and susceptibility monitoring helped to encourage growers to maintain the program. Random checks of grower records showed widespread compliance.

The regional programs were approved by growers and implemented in February 2001. There were similar plans for the three growing regions—Central Oahu, Kula, Maui, and Kamuela, Hawaii. Therapeutic insecticide use for DBM was based on grower determined larval thresholds and was subject to rotation of insecticide classes using month-long windows. Emamectin benzoate and indoxacarb were used in the programs. During the first rotation window, one could make a maximum of four applications of indoxacarb. A maximum of three applications of emamectin benzoate was allowed during the subsequent month-long window. If it was needed, a *B. thuringiensis* product was used for DBM control after two sequential weekly applications of emamectin benzoate. Use of organophosphate, carbamate and pyrethroid insecticides for control of occasional pests was greatly reduced to conserve DBM parasitoids.

Mitigation of resistance to spinosad

All of the regional groups voluntarily removed spinosad from their pest management programs to mitigate resistance levels. Many growers ceased using the insecticide after the first educational meeting. A monitoring plan was created to assure compliance. Random collections of crucifer leaves were taken at intervals during the year. Antibody assays were performed to detect the presence of spinosad residues. The assays of initial field samples were negative leading us to believe that growers fully participated in the mitigation program.

The susceptibility of DBM populations from three islands was measured at 3–6 month intervals after the start of the mitigation program. Dow AgroSciences and the University of Hawaii set a reintroduction LC_{50} threshold for spinosad at 5 ppm. Susceptibility to spinosad increased more rapidly than we anticipated (Table 2). The spinosad thresholds for reintroduction of spinosad were met for Kula, Maui and Kamuela, Hawaii populations within 6–8 months after spinosad use was suspended. Growers in these regions will be allowed to use spinosad in 2002.

Table 2. Changes in field susceptibility of diamondback moth to spinosad during the resistance mitigation period

Population	Original Toxicity Ratio (Date)	Recent Toxicity Ratio (Date)
OAHU		
Ewa	1,340 (26/10/00)	219 (26/1/01), 11 (2/10/01)
Kunia	642 (14/9/00)	494 (25/2/01), 45 (5/9/01)
Pearl City	1,080 (17/10/00)	2 (4/6/01)
MAUI		
Kula, lower	5 (2/10/00)	2 (6/6/01)
Kula, middle	8,000 (2/10/00)	5 (6/6/01)
HAWAII		
Lalamilo	4 (15/11/00)	4 (22/2/01), 3 (23/8/01)
Puukapu	29 (11/15/00)	3 (8/23/01)

Discussion

How did resistance to spinosad evolve in such a short time (30 months)? A combination of factors created excessive selection pressure on DBM populations. Obviously, genes that conferred spinosad resistance were common throughout many regional populations. Spinosad was the only effective product available for managing DBM. It was used extensively because therapeutic treatments are usually necessary practically every week of the year. Emamectin benzoate became available 15 months after the introduction of spinosad and was used as a rotation partner. The greater cost per treated-acre of emamectin benzoate was an impediment to its use by some growers. It was impossible to do an effective farm-focused resistance management program given the small plot sizes and continuous weekly planting schedules. In essence, DBM populations were being exposed to spinosad every week of the year.

Can we prevent a similar fate for emamectin benzoate and indoxacarb? We now believe that adoption of the region-focused program will be helpful in prolonging the use of these products. Mitigation of resistance to spinosad has been demonstrated. This makes a three-insecticide class rotation program. Overall selection to each insecticide will be limited to four month-long windows.

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Challenges in implementing spinosad diamondback moth resistance management strategies in intensive vegetable growing areas in Asia

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Abstract

Success® contains the active ingredient spinosad and was registered in 1998 for the control of diamondback moth (DBM) *Plutella xylostella* (L.), in Asia. It has a unique chemistry and mode of action, no known cross-resistance to other compounds, a high level of activity against DBM, a short residual period and large margins of safety for mammals, birds, fish and most beneficial insects. These characteristics make it a highly desirable compound to be used in vegetable crops and other IPM friendly programs.

Besides DBM, field and laboratory testing of spinosad has proven that it is effective on almost all other Lepidoptera, Thysanoptera, some Coleoptera and Diptera. Dow AgroSciences developed an Insect Resistance Management program prior to product launch to prolong the product's life and help growers cope with the resistance issue. Steps taken included labelling and/or governmental recommendations that limit applications, establishing insect susceptibility baselines on key pests prior to introduction, post launch monitoring for shifts in sensitivity and need for corrective measures, controlling supply, pricing appropriately and providing training for customers and centres of influence to promote the importance of IRM.

Our experience so far has been mixed. In areas with good educational systems and cost effective rotational products, we have good acceptance. In areas that lack effective products for DBM control and with less effective educational networks, farmers tend to use Success® repeatedly without alternating with other compounds. The pricing appeared to deter frequent use, but also encouraged cross border trading and use of sub-optimum rates. Economic returns and continuous crucifer production are the main factors in determining the use of the product. Areas with continuous crucifer production by many small farmers with inadequate grower education networks and high economic returns for pest free vegetables, will develop resistance problems. Delaying or not introducing product had limited effect since cross border smuggling was occurring. State sponsored education and development of more sustainable programs that have crop rotations are the long-term answer, however, manufacturers can assist and have an impact with the education efforts and progress is being made.

Introduction

Spinosad or Success®, a newly introduced product by Dow AgroSciences (DAS), is the first member of the naturalyte class of products. It was first introduced for control of diamondback moth (DBM), *Plutella xylostella,* in Asia. It belongs to the family of naturally derived compounds called spinosyns. Spinosad has a novel mode of action. It works by affecting the nicotinic acetylcholine receptors on the postsynaptic nerve cell (Sparks *et al.* 1996).

The diamondback moth is one of the most destructive pests of cruciferous vegetables on a global scale. The larvae feed on the foliage from seedling stage to nearly harvest. Yield loss due to the pest is a common phenomenon faced by vegetable farmers. DBM develop fast and lay eggs in early stages of the adult phase and the life cycle is only about 3 weeks in the tropical climate (Khoo *et al.* 1991). Commercial vegetable farmers have resorted to using insecticides to protect their crops from the highly prolific and voracious DBM since effective compounds could provide quick and highly effective control. Other viable methods of control are considered slow or deemed ineffective by the farmers since damaged plants bring considerably less value in the market.

DBM has been well documented in its ability to develop resistance to insecticides due, in part, to the intensive use of the control agents in vegetable growing areas and an inherent adaptability. Our surveys in

the major centres of vegetable growing areas of Malaysia, Indonesia, Thailand and the Philippines found that the cabbage farmers commonly apply insecticides every 3–5 days. It is not uncommon for 12–16 sprays to be applied in a crop season of 80–85 days, to manage the pest so that the crops produced are minimally damaged and are acceptable to the market. It is also not uncommon for four or more sequential crucifer crops to be produced, resulting in 60 or more selections on the DBM population with insecticides in a calendar year.

DAS has developed regional pest specific Insect Resistance Management (IRM) plans for spinosad and the company has set an ambitious target to protect spinosad market viability for 20 years. This paper serves to document the plans and recommendations that have been implemented by DAS and also to solicit other interested groups to collaborate with the organisation in the IRM programs.

Dow AgroSciences insect resistance management plans

The strategic direction within DAS comes from a team called the Global Insect Resistance Management Team (IRMT) with representatives from the major DAS Business Geographies throughout the world. The team was first established in 1994 as the North American Team and was gradually expanded to include all the geographies in 1998. Under the direction of this team, all country commercial teams have to develop a Resistance Management plan prior to market launch of spinosad. The plan has a comprehensive list of recommendations, but it is focused on four major components:

1. Labelling clearly product use direction and/or with governmental recommendations
2. Establishing insect susceptibility (LC_{50}) baselines for key pests and monitoring plans for future review and corrective measures
3. Pricing appropriately with optimum supply strategy
4. Transferring technology and training customers and centres of influence to promote the importance of resistance management

The Country teams had their resistance management plan reviewed and approved by the IRMT prior to launch. Accountability for initiating reviews of the resistance management plan is clearly identified with a technical expert designated by the company. The technical expert initiates audits of the life cycle of the product from a technical point of view and together with inputs from the functional experts within Research and Development, recommends technical strategies (e.g. registration, formulation, biology) to the Business Management Team.

Countries involved in the IRM plan in Asia business unit

The countries in Asia participating in the IRM plan are shown in Table 1. Japan and Korea are in a different business unit and thus are not shown here. The table also lists the pests and registered recommended rates to control the pests in the countries concerned (Samsudin *et al.* 2001). The main target pest in all of the countries is DBM in the crop *Brassica. Spodoptera,* other Lepidoptera and thrips in vegetables and cotton are secondary targets. Recommended rates for the control of DBM vary from country to country ranging from 12 g to 50 g per ha of active ingredient (Samsudin 1998, Samsudin *et al.* 1999, Downard *et al.* 2000). The variation in rate is highly linked to the intensity of crop cultivation, pest population density and local agronomic practices. In areas of intensive all year round crop cultivation, the pest populations tend to be high and require a single high rate application or a multiple spray of low rates to control the pest. For example, intensive vegetable cultivation areas like Dalat, Central Vietnam, recommended a higher rate of 30 g ai/ha, whereas a less intensive area like Hanoi, North Vietnam, a lower dose of 20 g ai/ha was recommended. Hanoi area required a lower dose since the pest population is generally low and the crop cycle is usually disrupted by the cold winter (Samsudin & Nguyen 1997). The rate in Thailand is unusually high and explanations are provided in the "LC_{50} monitoring programs" section below.

Insect resistance management labelling and/or governmental recommendations

The main point emphasised here is to treat only when the pest population exceeds a threshold level. It is also stressed to use not less than label rates of any insect control product whether it is applied alone or in tank mixtures. It is recommended to treat most susceptible life stages and target applications against eggs and small larvae. Rotations with other suitable insecticide classes are encouraged to take advantage of beneficial insects. More importantly, the product should not be used on crops or pests other than those stated on the label.

Table 1. Regulatory status and recommended field rates for Success® in Asia

Country	Brand name	Registration date	Conc. (g/L)	Crop	Pest	Use rate (g a.i./ha)
Malaysia	Success 25 SC	01-Sep-98	25	*Brassica chinensis*, *B. rapa*, *B. oleracea*, *B. alboglabra*	*Plutella xylostella*, *Spodoptera* sp.	25
Indonesia	Success 25 SC	06-Jan-98	25	Cabbage	*P. xylostella*, *Crocidolomia binotalis*	12.5-25
Philippines	Success 25 SC	12-Nov-98	25	Cabbage	*P. xylostella*	25
Thailand	Success 12 SC	08-Apr-99	120	*Brassica chinensis*	*P. xylostella*	120-240
Vietnam	Success 2.5 SC	18-Feb-99	25	Cabbage	*P. xylostella*	20-30
Taiwan	Success 25 SC	26-Feb-99	25	Cabbage	*P. xylostella*	18.5-38
Taiwan	Success (Conserve) 120 SC	21-May-99	120	Cabbage	*P. xylostella*	18.5-39
PRC	Tracer 48 SC	23-Dec-98	480	Cotton	*Helicoverpa armigera*	30-40
PRC	Success 2.5 SC	23-Dec-98	25	Cabbage	*P. xylostella*	12.5-25

The second point emphasised on the label is to use spinosad not more than two consecutive times per season to avoid treating successive generations. Only two applications to reduce a single insect generation below the economic threshold are permitted. Farmers are advised that if they are uncertain on the generation cycle, they are not to make more than two consecutive applications of an insect control product from the same product class. They are advised to rotate to a different class of insect control product, or use other treatments for the next 30 days.

DAS is linking up with some government and non-government organisations to support IPM and crop rotation efforts. In the long run, IPM will reduce the dependency of the farmers on use of chemical product alone to control the pest. Multiple tactics include cultural or biological controls within an Integrated Pest Management program where available and appropriate. In countries where there are Agricultural Chemical Companies that are interested to jointly promote their product with DAS, we will promote rotations of their product with ours.

LC_{50} monitoring programs

As part of the IRM program, the LC_{50} is established prior to launch to serve as a baseline for future monitoring. A summary of the LC_{50} values is shown in Table 2. The test method employed is the IRAC No. 7 leaf dip method and the figures are taken at 72 hours after exposure to the chemical.

The LC_{50} values vary widely, but are within the normal biological variation seen in other geographies. Higher than normal values were observed in Taiwan in 1999, but they did not repeat or continue upward and no explanation is available. The numbers fluctuate between 1998, 1999 and 2000 within the countries, despite the fact that the insect population was gathered from the same district and this is a laboratory test where all the external factors have been excluded. There also seems to be no trend saying that higher recommended field rates (Table 1) will produce higher LC_{50} values in Table 2. This is evident in Thailand as, despite the high field rate, the LC_{50} is comparable to the other countries. The high field rate could be attributed to the agronomic factor of heavy mechanised watering of the crops twice a day that reduces the residue on the treated plant.

A typical analysis used to compare the sensitivities across strains by dividing the more tolerant field strains by the most sensitive (usually an inbred lab strain). This number is then reported as a tolerance or resistance ratio with the latter terminology being a more common, but debatable use of the term ‘resistance.’ Differences in responses between populations are expected from normal biological variability, particularly with the very sensitive laboratory colonies. Some of the papers coming out will be reporting 10 to 100-fold differences in toxicity or resistance ratios. The most tolerant strains are still very sensitive to our field rates, however, the results demonstrate substantial biological variability as expected in the field.

Table 2. LC_{50} values of *Plutella xylostella* for spinosad at 72 h

Country	Compounds	Mean LC_{50} range		
		1998	1999	2000
Malaysia	spinosad	5.84	0.31-0.96	
	susceptible	0.04	NA	
Thailand	spinosad	NA	0.82-4.45	3.82-7.54
	susceptible	NA	0.02	
Indonesia	spinosad	0.24-0.85	NA	0.13-0.15
Vietnam	spinosad			0.0019-0.87
Philippines	spinosad		<1	0.70-1.711
PRC	spinosad		0.05-0.1	0.039-0.117
	susceptible		0.18	
Taiwan	spinosad	3.4-4.1	4.9-20.5	0.6-0.8

There have been no performance problems related to resistance in the field in Asia. Overall, performance has been excellent. Expectation setting has been the most common problem. A few growers had heard such good things that they had over-optimistic expectations, such as two weeks control or 100% control rather that 95% or so. We have had a few of the standard issues such as spray coverage and some tank mix issues with four and five products in the tank, however, these issues affect other products as well.

Several studies involve the development of susceptibility baselines by surveying the sensitivity of various populations. Some of the studies are sponsored by DAS, but many are independent efforts by university or government researchers. These studies are finding differences in sensitivity, but all are well below the field rates. The natural biological variability does indicate that one could select for resistant strains, but this is true for all compounds.

Pricing and supply

Pricing is one of the commercial tools utilised in the IRM strategy to discourage frequent use of spinosad. It is noted that high cost will not discourage repeated application using the same material as long as the vegetable prices are still profitable for the farmers. In theory, however, if the product is priced at a premium level, the end users will find it uneconomical to use the product too often and will be encouraged to use economic thresholds rather than maintenance sprays. A potential disadvantage is that farmers may only resort to spinosad when less expensive products and potential rotation products have developed high levels of resistance and are, therefore, no longer available for rotation.

Secondarily, premium pricing will also make the product uneconomical to be used in other low value crops to control pests. In effect, this tactic will automatically confine the product to the high value crucifers/DBM segment in the ASEAN countries.

On the supply side, control is mainly accorded through the dealer network. The dealers had to undergo the DAS IRM training program to get certification to sell the product. They are trained on the importance of IRM, especially for DBM control. They are one of our partners to impart IRM knowledge to the farmers and they have to abide by the recommendations from DAS or the privileges to sell may be rescinded. Dealers in a particular district are given a limited amount of product to ensure that amounts to a certain area are not oversupplied. An area is given a specific allotment of product based on the detailed calculation of area, crop seasons per year, estimated number of insecticide applications and expected DAS market share according to the IRM target. We have discovered that there are limits to our ability to control supply and that some product will find its way to high value markets if it is available anywhere in the world which is much smaller today in the age of the internet.

Technology transfer to customers

Many types of tools were utilised to promote our resistance management program to the farmers and dealers. These included seminars, mini workshops, leaflets, posters and recruiting extension workers. Label description about the resistance management program such as the maximum number of applications per

season and rotation with other products are highly emphasised to the trainers. Tie-ups with some programs promoted by local government are always encouraged whenever the chance presents itself.

DAS sales teams were trained in the aspects of IRM and they, in turn, will train the farmers and dealers whenever they promote the product in the field. Each sales person is given a set of slides for their sales promotion to include IRM. In the promotion efforts the sales team will also link up with the government extension program and participate whenever they can to promote IRM. Planned joint promotions with other companies that sell suitable DBM control compounds are also underway. DAS is promoting the products of some other companies in rotation with spinosad.

Summary and conclusions

The most pressing issue currently is cross border trading where the product sourced in a country where the purchase price is low is sold across the border to a country with a higher purchase price by dealers for fast profit. This practice is on the rise and has led to certain areas exceeding the allocated limit. As a result of the additional quantity, the farmers are using Success® more frequently than the recommended "not more than two applications per crop season" as stated on the label. If the trend continues, overuse may lead to resistance. In this aspect we are seeking the help of the local regulatory authority to enforce and stem the cross border movement of the product.

Overuse by farmers can also happen due to lack of alternative effective compounds in a given location. Success® has been proven to be highly effective thus the farmers depend on it to protect their crops. High pricing deters frequent use somewhat, but farmers who are growing their products for export purposes are using Success® anyway. The economic return is high and it exceeds the production costs and they do not have to worry about the residue since spinosad can be used right up to the day of harvest.

Another form of off label usage on the rise is a farmer using the product on pests that are not stated on our label. This will lead to false claims of efficacy failures and unusually high or low use rate to control the unlisted pests. Steps have been taken to communicate to farmers directly on this issue, but logistics problems continue to plague the DAS IRMS communications. We are limited most of the time to dealer level meetings because of the large geography and scattered nature of farms throughout an area.

Interviewing selected farmers occasionally is one of the steps we have taken to establish direct link to them. Survey results indicated that the majority of the farmers interviewed were aware of the history of rapid DBM resistance to pesticides, but on the other hand their main concern is to protect their crop investment. Most of the farmers are not educated enough about selecting pesticides with different modes of action. Rotating pesticides with different MOA to prolong the product's effectiveness is not common either since farmers are afraid to lose their crops to an unproven product. Enforcement of label recommendation is not easy across large areas and often numerous small farms.

The above points detailed some of the technical and as well as commercial recommendations that have been carried out by Dow AgroSciences to implement Insect Resistance Management strategies. They can be wrapped up under three main categories: identifying and recommending an acceptable use pattern; implementing the use pattern and influencing market share with price and supply and education and the logistics of information dissemination. Our experience so far has been mixed. In areas with good educational systems and cost effective rotational products we have good acceptance. In areas that lack effective products for DBM control and with less effective educational networks, farmers tend to use Success® repeatedly without alternating with other compounds. Fortunately the latter is limited to a few areas close to metropolitan areas such as Bangkok that have a history of resistance development. If the growers in these areas maintain continuous crucifer production, it is only a matter of time before spinosad joins the long line of products that have been lost. However, in the majority of the areas we are making progress in assisting regional experts implement IRM programs that include spinosad as one tool. We are hopeful that the areas affected by severe DBM resistance will be minimal in the future and area wide programs will be adopted in the problem areas.

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Reduced susceptibility to permethrin in diamondback moth populations from vegetable and non-vegetable hosts in southern Australia

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Abstract

Diamondback moth (DBM), *Plutella xylostella* (L.), has attained major pest status in *Brassica* vegetable crops around the world. In many cases, use of synthetic pyrethroid insecticides for control of other pests, such as *Pieris rapae (*L.), has disrupted natural enemies and selected for insecticide resistance in DBM, changing the pest status of the moth from minor to major. We estimated levels of resistance to the synthetic pyrethroid, permethrin, using a leaf dip bioassay, for 28 DBM populations collected from brassicaceous weeds, canola, forage turnips and seed turnips and for five DBM populations from *Brassica* vegetables. Populations were collected in Victoria, Tasmania, southern New South Wales, ACT, South Australia and Western Australia between September 1999 and January 2000. Nineteen of 28 populations from non-vegetable hosts were significantly more tolerant than a susceptible laboratory population (resistance ratios ranged from 2.1 to 6.9). All five populations from vegetable hosts were significantly tolerant (resistance ratios from 3.6 to 13.0). These results indicate that populations of DBM with reduced susceptibility to permethrin may be found in areas that are remote from intensive vegetable growing districts. *Brassica* vegetables account for only a small proportion of the host plants available for DBM in southern Australia. Large areas of non-vegetable hosts have, in the past, received few applications of insecticides. Reports that growers of forage brassicas and canola are finding it necessary to apply synthetic pyrethroids to their crops with increasing frequency (1–4 applications per crop) suggest that resistant DBM populations are being generated. Alternatively, DBM populations may be moving from the more intensively sprayed vegetable crops onto non-vegetable hosts. Further studies on the insecticide resistance status of DBM populations from a range of host plants in different locations in conjunction with use of molecular markers to study population structure of DBM, may provide evidence of isolation or mixing of populations which will have important consequences for insecticide resistance management.

Keywords

Plutella, insecticide resistance, pyrethroid, canola

Introduction

Diamondback moth (DBM), *Plutella xylostella*, has damaged *Brassica* vegetable crops throughout the world and is renowned for developing resistance to insecticides (Talekar & Shelton 1993). In Australia, resistance to synthetic pyrethroid insecticides has been identified in DBM populations from vegetable growing areas in all states (Wilcox 1986, Altmann 1988, Hargreaves 1996, Endersby & Ridland 1997, Baker & Kovaliski 1999).

Canola, brassicaceous weeds and forage brassicas are all hosts for DBM (Talekar & Shelton 1993). *Brassica* vegetables account for a very small proportion of the host plants available for DBM in southern Australia. 12,226 hectares were planted to *Brassica* vegetables in 1999 (ABS 2000), whereas the area planted to canola in Australia increased by 79% from 697,000 hectares in 1997–98 to an estimated 1.2 million hectares in 1998–99 (ABS 2000). The biggest increase was in Western Australia, which saw plantings increase by 116% to 536,000 hectares (ABS 2000). There are also vast areas of brassicaceous weeds, particularly in Western Australia where wild radish, *Raphanus raphanistrum*, has developed resistance to acetolactate synthase (ALS)-inhibiting herbicides (Rieger *et al.* 1999). In southern Victoria, dairy farmers often grow large areas of forage brassicas such as turnips in late spring to early summer.

In contrast to the intensively sprayed vegetable crops, non-vegetable host plants have received few, if any, insecticide applications until recent times. If we assume there is no regular long-range movement of DBM from vegetable production areas to remote areas of canola and weeds, we would expect DBM found on such non-vegetable host plants to be susceptible to synthetic pyrethroids. The current study, however, documents low levels of resistance to permethrin in DBM populations from canola, forage brassicas and brassicaceous weeds in southern Australia.

Materials and methods

DBM eggs, larvae and pupae were collected from 33 locations in southern Australia from September 1999 to January 2000. Populations were reared on cabbage seedling leaves (*Brassica oleracea* var. *capitata* cv. Green Coronet) in the laboratory at 25 °C (16h:8h, L:D) for one to three generations. A susceptible laboratory population of DBM was used as a reference in each assay. The susceptible population has been maintained at Knoxfield since it was obtained from the University of Adelaide, Department of Crop Protection, Waite Campus, SA, in 1994. For each DBM population we estimated levels of resistance to permethrin. In all, we tested 28 DBM populations from non-vegetable host plants and five populations from vegetables.

A leaf dip bioassay (after Tabashnik & Cushing 1987) was adopted for testing susceptibility to permethrin. Cabbage leaf discs of 4.5 cm diameter were dipped for 5 s in distilled water solutions of formulated insecticide (Ambush® Emulsifiable Concentrate Insecticide–Crop Care Australasia Pty Ltd) and hung vertically to dry in a fume hood for 2 h. Control discs were dipped in distilled water. No wetting agents were used. Discs were placed into Gelman® 50 mm diameter x 9 mm plastic Petri dishes. Ten larvae were placed on each disc and four replicates of seven concentrations were set up. Mortality was assessed after 48 h at 28ºC. Larvae were considered dead if they did not move when touched with a paintbrush.

Analysis

Concentration-mortality data for each population were analysed using the probit analysis program, POLO-PC (LeOra Software) (Russell *et al.* 1977). We used the program to estimate the lethal concentration expected to cause 50% mortality (LC_{50}) of each insecticide for each DBM population and the 95% confidence intervals for these concentrations. The slope (+ standard error) of the probit line was also estimated.

The program ran χ^2 tests for goodness-of-fit of the data to the probit model. If the model fits, the calculated value of χ^2 is less than the χ^2 table value for the appropriate degrees of freedom. If the model does not fit (i.e. the χ^2 value exceeds the table value), the LC_{50} value for the particular population may not be reliably estimated and is adjusted with the heterogeneity factor (χ^2/df). The index of significance for potency estimation (g) was used to calculate 95% confidence intervals for potency (relative potency is equivalent to tolerance ratio) (Robertson & Preisler 1992).

Parallelism of the probit regression lines implies a constant relative potency at all levels of response (Finney 1971). POLO-PC was used to test equality and parallelism of the slopes of the probit lines for the field population and the laboratory susceptible population. If the slopes are parallel, then overlap of the 95% confidence intervals for the two populations indicates that no significant difference exists between the LC_{50} values.

Results

All five populations from vegetable hosts were significantly resistant (resistance ratios from 3.6 to 13.0) (Table 2) and 19 of 28 populations from non-vegetable hosts showed a low level of resistance to permethrin (resistance ratios ranged from 2.1 to 6.9) (Table 1).

The resistance ratios of the DBM populations from vegetable crops at Bairnsdale and Nairne (Table 2) are approaching the level at which growers were experiencing control failures in 1993–5 (Endersby & Ridland 1997). Since the first round of tests in 1993–6, a decrease in resistance levels of DBM from vegetable brassicas in Werribee is indicated. This may reflect the reduction in use of synthetic pyrethroid insecticides that occurred after the control failures and/or movement of susceptible moths into the area.

The two highest resistance ratios to permethrin estimated in populations from canola are from Western Australia (Table 3). Three other populations from canola were susceptible to permethrin (Balliang and Balliang East from Victoria and Yeelanna from South Australia).

Table 1. LC_{50} and LC_{95} for permethrin tested on DBM populations from southern Australia compared with the standard laboratory population (Waite), 1999–2000 (Het.=heterogeneity, g= index of significance for potency estimation, s.e.=standard error, df=degrees of freedom)

Population	Host	Slope ± s. e.	Het.	g	χ^2 df=26	LC_{50}	95% confidence intervals	LC_{95}	95% confidence intervals
Waite	Lab	2.35 ± 0.34	0.84	0.08	21.9	8.4	5.7–11.1	42.2	31.1–67.4
Werribee VIC	Cabbage	1.68 ± 0.20	1.49	0.09	38.8	25.5	17.3–34.6	244.3	148.1–580.2
Waite	Lab	2.16 ± 0.38	0.75	0.12	19.4	10.5	5.7–15.2	60.8	42.9–108.3
Lindenow VIC	Cabbage	1.57 ± 0.18	1.08	0.06	28.0	52.5	39.9–68.5	590.4	348.7–1364.8
Bairnsdale VIC	Cabbage	1.58 ± 0.19	0.93	0.06	24.3	125.9	97.7–172.7	1378.2	755.0–3570.6
Waite	Lab	2.23 ± 0.28	1.01	0.07	27.2	12.5	8.9–16.2	68.4	49.5–111.9
Arthurton SA	Canola	1.90 ± 0.22	1.05	0.06	26.3	26.1	19.7–32.9	191.6	131.3–341.4
Urania SA	Canola	1.84 ± 0.20	1.19	0.06	30.9	34.7	26.3–44.3	271.5	178.4–520.8
Waite	Lab	1.82 ± 0.24	0.94	0.06	24.4	6.2	4.1–8.3	49.7	34.8–84.2
Minlaton SA	Canola	1.84 ± 0.21	1.53	0.08	39.8	26.7	18.6–35.5	208.4	132.8–442.3
Balliang VIC	Canola	1.19 ± 0.21	0.82	0.12	48.3	7.8	3.2–12.8	189.9	111.6–509.2
Waite	Lab	1.82 ± 0.14	1.66	0.10	43.2	8.8	5.3–12.6	70.9	45.1–150.9
Wauraltee SA	Canola	1.44 ± 0.19	1.93	0.14	50.1	27.1	14.9–41.1	376.2	191.7–1484.6
Yeelanna SA	Canola	1.53 ± 0.27	1.09	0.14	28.3	7.2	2.9–11.4	85.8	55.6–194.4
Waite	Lab	2.26 ± 0.31	0.96	0.07	25.1	7.7	5.4–10.0	41.1	30.1–65.8
Wongan Hills WA	Canola	1.58 ± 0.19	1.58	0.09	41.0	39.1	27.2–53.7	433.1	241.9–1214.7
Burabadji WA	Canola	1.99 ± 0.26	1.24	0.09	32.1	49.2	33.0–67.1	328.8	212.5–682.8
Waite	Lab	2.52 ± 0.27	1.21	0.06	31.4	13.4	10.5–16.6	60.0	43.8–96.5
Balliang East VIC	Canola	0.92 ± 0.19	1.93	0.35	50.3	7.3	0.4–17.3	438.5	157.7–15038.9
Waite	Lab	2.26 ± 0.28	1.49	0.10	34.0	21.0	14.3–28.0	112.4	76.2–216.9
Manjimup WA	Cauliflower	1.25 ± 0.19	1.31	0.12	38.8	108.5	72.7–177.7	2245.3	880.3–14538.9
Waite	Lab	3.51 ± 1.03	0.73	0.33	19.1	11.6	5.3–15.1	34.2	26.3–76.2
Nairne SA	Sprouts	1.30 ± 0.17	1.45	0.11	37.8	124.3	82.1–177.6	2267.9	1092.2–9035.5
Waite	Lab	2.26 ± 0.28	1.49	0.10	34.0	21.0	14.3–28.0	112.4	76.2–216.9
Clunes VIC	Weeds	2.36 ± 0.41	3.10	0.39	80.7	9.7	2.3–15.8	48.4	29.1–234.5
Waite	Lab	2.37 ± 0.25	1.05	0.05	27.4	16.8	13.4–20.6	83.3	60.9–131.1
Bunbury WA	Weeds	1.99 ± 0.22	0.60	0.05	15.7	111.4	90.6–141.2	746.2	482.6–1435.3
Waite	Lab	3.46 ± 0.48	1.16	0.09	30.1	8.1	6.1–10.0	24.2	18.8–36.3
Balingup WA	Weeds	1.12 ± 0.16	1.71	0.16	44.5	55.8	34.2–89.8	1710.4	605.3–17517.9
Waite	Lab	3.42 ± 0.43	0.56	0.06	14.5	12.1	10.0–14.2	36.6	29.2–51.3
Deadman's Gully	Weeds	1.49 ± 0.18	0.71	0.06	18.5	82.9	64.4–110.1	1061.7	590.3–2679.7
Bridgetown SA	Weeds	1.58 ± 0.18	1.49	0.09	38.7	57.5	42.0–79.4	632.7	343.2–1831.3
Waite	Lab	3.43 ± 0.51	1.65	0.15	43.0	19.8	14.0–25.1	59.6	43.7–109.0
Stratford VIC	Weeds	1.21 ± 0.17	1.13	0.10	29.5	25.3	15.5–35.9	584.8	297.3–2010.2
Springhurst VIC	Weeds	1.23 ± 0.17	1.69	0.13	44.0	36.0	22.1–53.2	685.2	317.2–3231.3
Waite	Lab	3.19 ± 0.38	1.81	0.11	39.8	9.3	6.8–11.9	30.4	22.0–52.6
Canberra ACT	Weeds	0.79 ± 0.16	1.75	0.29	45.6	45.2	19.7–87.2	5400.4	1001.7–1324537.9
Finley NSW	Weeds	1.07 ± 0.17	1.57	0.16	40.8	37.0	20.9–57.5	1278.8	475.7–1.1665.0
Jugiong NSW	Weeds	1.18 ± 0.17	1.14	0.10	29.6	45.2	30.7–63.9	1120.0	515.3–4661.7
Waite	Lab	3.59 ± 0.47	0.53	0.07	13.8	5.9	4.8–7.1	16.9	13.3–24.2
Derrinallum VIC	Weeds	1.46 ± 0.20	1.27	0.11	33.0	17.8	10.2–25.7	238.0	140.0–615.5
Waite	Lab	2.47 ± 0.27	3.28	0.17	85.3	9.8	5.8–14.3	45.5	28.3–118.0
Cranbourne VIC	Weeds	1.63 ± 0.21	1.64	0.11	42.7	20.3	11.9–29.3	207.0	123.1–531.4
Waite	Lab	2.46 ± 0.28	1.94	0.11	28.7	8.5	5.6–11.5	39.5	27.0–75.6
Thomastown VIC	Weeds	1.44 ± 0.30	1.10	0.20	50.4	4.1	0.9–7.9	56.6	36.5–131.5
Horsham VIC	Weeds	1.17 ± 0.19	1.02	0.12	26.4	11.0	5.0–17.3	278.0	153.4–852.0
Waite	Lab	2.38 ± 0.26	1.35	0.07	35.0	11.6	8.7–14.8	57.0	40.7–95.8
Loch VIC	Weed	1.21 ± 0.36	1.20	0.46	31.3	1.5	0.004–4.9	33.2	17.7–108.5
Waite	Lab	2.56 ± 0.28	4.14	0.20	107.7	11.8	6.7–17.8	52.3	31.6–157.6
Werribee VIC	Weeds	1.25 ± 0.17	1.01	0.08	26.2	48.1	34.6–65.6	996.2	497.9–3279.8
Ayrford VIC	Turnip	2.23 ± 0.27	0.79	0.06	20.7	50.4	38.9–62.9	274.9	197.6–450.3
Waite	Lab	3.37 ± 0.41	0.46	0.06	12.0	8.6	7.0–10.2	26.5	21.0–37.3
Woolnorth TAS	Turnip	1.25 ± 0.18	1.17	0.10	30.3	43.6	28.1–63.1	895.0	429.7–3474.8
Waite	Lab	2.87 ± 0.36	0.86	0.06	22.3	6.5	5.1–8.0	24.5	18.8–36.0
Curdie Vale VIC	Turnip	1.17 ± 0.17	1.37	0.12	35.6	39.3	25.1–57.5	991.6	432.4–5105.5

Table 2. Resistance ratios to permethrin for DBM populations from vegetable crops in southern Australia, 1999

DBM population	Host plant	Date collected	Resistance Ratio	95% confidence intervals		Generation tested
				Lower	Upper	
Werribee VIC	cabbage	15-Sep-1999	3.6	2.5	5.4	F_1
Manjimup WA	cauliflower	7-Oct-1999	5.2*	3.4	8.0	F_1
Lindenow VIC	cabbage	8-Sep-1999	5.8	4.0	8.8	F_1
Nairne SA	Brussels sprouts	15-Nov-1999	10.7*	6.6	17.2	F_1
Bairnsdale VIC	cabbage	7-Sep-1999	13.0	8.8	20.8	F_1

*calculated at $LC_{50.}$ Resistance ratios assume parallel slopes for each test. If parallel slopes could not be fitted for a particular assay, then ratio was calculated at $LC_{50.}$ A resistance ratio of 1 indicates that a field population is equivalent in susceptibility to the susceptible laboratory population (Waite).

Table 3. Resistance ratios to permethrin for DBM populations from canola crops in southern Australia, 1999

DBM population	Date collected	Resistance Ratio	95% confidence intervals		Generation tested
			Lower	Upper	
Balliang East VIC	12-Oct-1999	0.5*	0.2	1.3	F_1
Yeelanna SA	07-Oct-1999	1.0	0.6	1.5	F_1
Balliang VIC	27-Sep-1999	1.3*	0.6	2.6	F_1
Arthurton SA	07-Oct-1999	2.2	1.6	3.1	F_1
Urania SA	07-Oct-1999	3.0	2.1	4.2	F_1
Wauraltee SA	07-Oct-1999	3.5	2.1	6.0	F_1
Minlaton SA	07-Oct-1999	4.3	2.9	6.5	F_1
Wongan Hills WA	07-Oct-1999	5.1*	3.4	7.5	F_1
Burabadji WA	07-Oct-1999	6.7	4.8	9.6	F_1

*calculated at LC_{50}

Three populations from weeds were collected close to vegetable production areas: Deadman's Gully WA, Werribee VIC and Cranbourne VIC (Table 4) and are likely to have a history of exposure to insecticides. Some extremely susceptible populations were collected in Victoria away from production areas (Thomastown, Clunes and Loch), but there were many other DBM populations showing low levels of resistance which were collected from weeds in areas remote from vegetable growing regions.

Populations from forage and seed turnips in Victoria and Tasmania also showed low levels of resistance to permethrin (Table 5).

The lowest resistance ratio estimated was for a population of DBM from weeds remote from vegetable production areas and the two highest resistance ratios were from DBM collected in commercial vegetable crops (Table 6).

Table 4. Resistance ratios to permethrin for DBM populations from weeds in southern Australia, 1999

DBM population	Date collected	Resistance Ratio	95% confidence intervals		Generation tested
			Lower	Upper	
Loch VIC	07-Sep-1999	0.1*	0.02	0.7	F_1
Clunes VIC	06-Oct-1999	0.5	0.3	0.7	F_1
Thomastown VIC	19-Oct-1999	0.5*	0.2	1.2	F_4
Stratford VIC	12-Oct-1999	1.3*	0.3	6.2	F_2
Horsham VIC	03-Nov-1999	1.3*	0.7	2.3	F_3
Springhurst VIC	24-Oct-1999	1.8*	0.4	8.7	F_1
Cranbourne VIC	28-Oct-1999	2.1*	1.4	3.1	F_2
Derrinallum VIC	08-Dec-1999	3.0*	2.0	4.6	F_1
Finley NSW	27-Oct-1999	4.0*	2.7	6.0	F_1
Werribee VIC	16-Nov-1999	4.1*	2.8	5.8	F_2
Bridgetown WA	07-Oct-1999	4.8*	3.5	6.4	F_1
Canberra ACT	25-Oct-1999	4.9*	3.0	8.0	F_1
Jugiong NSW	26-Oct-1999	4.9*	3.4	7.1	F_1
Bunbury WA	07-Oct-1999	6.6	5.0	8.9	F_1
Balingup WA	07-Oct-1999	6.9*	4.6	10.2	F_1
Deadman's Gully WA	07-Oct-1999	6.9*	5.0	9.4	F_1

*calculated at LC_{50}

Table 5. Resistance ratios to permethrin for DBM populations from turnips in southern Australia, 1999–2000

DBM population	Date collected	Resistance Ratio	95% confidence intervals		Generation tested
			Lower	Upper	
Ayrford VIC forage	08-Dec-1999	4.4	3.0	6.6	F_2
Woolnorth TAS seed	05-Jan-2000	5.1*	3.4	7.5	F_1
Curdie Vale VIC forage	08-Dec-1999	6.0*	4.1	8.9	F_1

*calculated at LC_{50}

Table 6. Permethrin resistance ratio categories of DBM populations from vegetable and non-vegetable host plants in southern Australia, 1999–2000

Host plant	Number of populations in resistance ratio category			
	A	B	C	D
Weeds (Brassicaceae)	2	4	10	0
Canola (*Brassica* spp.)	0	3	6	0
Brassica vegetables	0	0	3	2
Forage turnips (*Brassica rapa*)	0	0	2	0
Seed turnips (*Brassica rapa*)	0	0	1	0
Total	2	7	22	2

A = significantly lower than standard laboratory population (Waite)–susceptible, **B** = no significant difference from Waite population–susceptible, **C** = significantly higher than Waite population–low level of resistance, **D** = significantly higher than Waite population–level approaching control failure

Discussion

In Australia, insecticides are applied to canola and forage brassicas with increasing frequency. Some growers use synthetic pyrethroids early in the crop for control of redlegged earth mite, *Halotydeus destructor* (Tucker), a practice which could inadvertently select for resistance in DBM. Canola growers in

northern Western Australia have started to apply synthetic pyrethroids for control of DBM, particularly in response to very high pest pressure in spring 1999 and winter 2000. Many forage *Brassica* growers in Victoria applied synthetic pyrethroids to their crops in spring 1999, with a frequency of one to four applications per crop.

Many factors may be responsible for increased severity of DBM outbreaks in vegetable crops, elevation of levels of resistance to synthetic pyrethroids and detection of resistance in populations of DBM remote from areas of intensive insecticide use. In some regions, particularly Western Australia, the increase in area of host plants must be generating higher numbers of moths. Weather conditions favourable to DBM such as a dry winter in 1999 and 2000 could explain the massive numbers of DBM in spring canola and forage brassicas. Spraying for other pests may be inducing insecticide resistance in DBM as well as destroying natural enemies of the pest. We observed high levels of biological control by the ichneumonid parasitoid, *Diadegma semiclausum* (Hellén), in unsprayed forage crops around Warrnambool, Victoria, in December 1999, but in crops sprayed with synthetic pyrethroids within the same district, the level of biological control was decreased. The low cost of synthetic pyrethroid insecticides make them a viable option for use in broadacre crops, but a continued increase in their use will exacerbate problems with DBM. Enhanced biological control and other non-insecticide control methods will be the only way to reduce DBM to minor pest status in these crops.

There is little published information about long range movement of DBM in southern Australia between different types of host plant, but resistance levels in remote weed crops suggest that moths are moving away from vegetable and canola growing regions. Management of resistance to insecticides based on knowledge of gene flow and mixing or isolation of Australian moth populations is of major importance to the Australian vegetable and canola industries, but few studies of population structure and movement of DBM in Australia have been made.

The current study will be expanded to gain a better understanding of long range moth movement using microsatellite DNA markers. Such hypervariable genetic markers can provide information about population movement at different spatial scales and may reveal source populations for geographic invasions, founder effects and population bottlenecks (Loxdale 2001). For example, microsatellites are being used to study origins of *Helicoverpa armigera* (Hübner) and *H. punctigera* (Wallengren) populations in south western Queensland (Graham 2000). Microsatellite studies of the Queensland fruit fly, *Bactrocera tryoni*, a major quarantine pest in Australia, were able to identify genetically isolated subpopulations that would be suitable targets for an eradication program (Yu *et al.* 2001). These examples demonstrate that both local and regional information about population movement has important implications for population management and similar information about DBM will have fundamental importance in development of insecticide resistance management strategies.

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Diamondback moth resistance to insecticides in Guangdong Province

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Abstract

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) is one of the most serious and important pests of crucifers in many parts of China, particularly in South China. DBM has developed resistance to all of chemical insecticides commonly used in vegetable growing regions of Guangdong Province. The development of resistance to avermectin and a microbial insecticide, *Bacillus thuringiensis* Berliner has been observed in the laboratory and field populations of DBM for at least eight years since 1992. The field study indicated that DBM resistance of field populations in both Shenzhen and Guangzhou regions to abamectin, *Bacillus thuringiensis* and chlorfluazuron increased greatly in the past decade and that the tolerance of the Shenzhen DBM population was higher for all three agents compared with the Guangzhou DBM population. However, there has been no significant change in resistance to dichlorvos, methomyl, fenvalerate and cartap in DBM since 1992. Laboratory studies indicated that susceptible DBM strain (SS) to abamectin had gradually developed resistance when DBM was treated regularly with sub lethal dosage of the preparation. After 20 generations, the induced resistance level was as high as 11.55-fold compared with susceptible. This case is in accordance with the situation in which field population gave rise to high level of resistance (2-, 12-, and 20-fold, in 1992, 1994 and 1996) to abamectin preparation. There was no cross-resistance between abamectin and Bt (*Bacillus thuringiensis*), dichlorvos, fenvalerate or cartap, but some cross-resistance was found between abamectin and chlorfluazuron. Synergists like SV1 and TPP enhanced the toxicity of abamectin to DBM field strains. Therefore, based on both field and laboratory studies, insecticide rotation has been recommended to vegetable growers and at present, the problem of DBM resistance has been alleviated. Devastation by DBM has been controlled in South China after an insecticide resistance management program was conducted to guide insecticidal application.

Keywords

abamectin, *Bacillus thuringiensis*

Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), is a cosmopolitan pest of cruciferous crops and an important pest of cruciferous vegetables in the Pearl Delta of Guangdong Province in South China. This pest has developed the most severe resistance to insecticides all over the world. It is reported that DBM had resistance to DDT (Ankersmit 1953) and then developed high tolerance to a range of pesticides in the Philippines, Japan, Malaysia, the United States and China. Insecticide resistance in DBM has held the attention of researchers worldwide and three international workshops related to DBM resistance and management have been held in several regions since 1986.

In the turning point of the 1980s and 1990s, DBM in vegetable farms in Pearl Delta, producing for Hong Kong, had built up resistance to almost all chemical insecticides and vegetable production was reduced greatly. In 1989, chlorfluazuron, one of the insect growth regulators, was registered by a Japanese company and used widely in Guangdong Province. However, after six months, a field population of DBM developed high resistance to this new insecticide. Soon afterwards, the Plant Protection Research Institute and Yangzhou Biological experimentation factory collaborated to produce a preparation of *Bacillus thuringiensis* and chemicals which was used widely in the vegetable production region. Until now, Bt products are still the primary insecticides used in the early stages of cruciferous crops. Avermectins, a novel class of macrocyclic lactones that have demonstrated nematicidal, acaricidal and insecticidal activity, were introduced and applied extensively in the Pearl Delta and DBM damage to crucifers was efficiently controlled.

Based on previous work concerning DBM resistance to biological formulations (Feng *et al*. 1996), the aims of this experiment were to monitor resistance variation of avermectin and other chemicals, to select for avermectin resistance in DBM and to study the cross resistance spectrum.

Materials and methods

Test insects

Three strains of DBM were studied: a laboratory, insecticide-susceptible strain from the insect toxicity laboratory, South China Agricultural University, maintained in culture in the pesticide laboratory, PPRI, GAAS; a field strain (resistant DBM I) collected in a suburb of Guangzhou city, Guangdong Province, China and subsequently maintained in the pesticide laboratory; a field strain (resistant DBM II) collected in Shenzhen where most Hong Kong Vegetable Farms are located. Frequency of pesticidal application is higher in the area around Shenzhen compared with that around Guangzhou.

The strains were cultured and tested on flowering Chinese cabbage at 24±2°C and 60±5% RH under natural light conditions at the Plant Protection Research Institute, GAAS. Larval instars of DBM were identified by the width of the head capsule.

Chemical insecticides

The following insecticides were used in this study: abamectin (concentrated fermentation liquid), efficient ingredient (B1a); Dynamec® 1.8% abamectin emulsified concentration (EC), DiPel® (*Bacillus thuringiensis* var. *kurstaki*, 16,000 IU/mg Wettable powder (WP) (Abbott), dichlorvos 80% EC, fenvalerate 20% EC, cartap 98% WP, dimehypo 18% liquid formulation (L), isoprocarb 20% EC and methomyl 24% L.

Testing technique

Bioassays of bio-preparations of abamectin and Bt products were conducted by dipping flowering Chinese cabbage leaves into an aqueous solution of chemicals for 2-3 min. Five concentrations of each preparation with two replications were tested. After being air-dried, the leaves were put into Petri dishes (diameter 9 cm) and 15 III instar DBM larvae were released per dish. The larvae were cultured in a chamber controlled at 25±2ºC. Mortality of DBM larvae was recorded two days after treatment commenced.

Analysis

Concentration-mortality response data were subjected to probit analysis (Finney 1971) where applicable. All concentration-mortality response data were corrected for control mortality (Abbot 1925) unless indicated otherwise. Data analysis was conducted using DPS (Data processing system) (Tang & Feng 1997). Resistance ratios (RR) were calculated by dividing the LC_{50} of each field population by the LC_{50} of the SS population. Synergistic ratios (SR) were calculated by dividing the LC_{50} of one insecticide with synergist by the LC_{50} of the insecticide alone.

Results and discussion

Monitoring insecticidal resistance in field populations

As a great deal of avermectin insecticides had been applied in successive years in the Shenzhen vegetable region from 1992 to 1999, DBM resistance to both concentrated preparations by the Chinese pesticide factory and Dynamec® were bioassayed to trace the resistance tendency of field DBM populations. The resistance level of DBM was evaluated according to resistance ratio (Table 1). The bioassay indicated that in 1992, when avermectin began to be used, the value of the resistance ratio was 2 (sensitive level). After one year of numerous and successive use of avermectin, the resistance ratio increased to 5-10 (low level). In 1994, DBM populations developed resistance as high as 12.00 and 14.52 (medium level) separately to the concentrated preparation of avermectin and to Dynamec®. Since then and up to 1999, DBM resistance remained at a medium level. During this period, the value of the resistance ratio reached as high as 22.75 or 25.19. There were slight differences in resistance ratio or lethal dosage between the avermectin concentrated preparation and Dynamec®, although their tendency is the same in general. The variation may result from purity of products with the concentrated preparation containing more active ingredients and Dynamec® containing 80% B1a and 20% B1b.

Table 1. Diamondback moth (DBM) resistance to a concentrated preparation of abamectin and Dynamec® from 1992 to 1999

Year	Population source	LC_{50} (mg/L)		Resistance ratio	
		abamectin	Dynamec®	abamectin	Dynamec®
1992	Susceptible strain	0.04	0.21		
1992	Field	0.08	0.50	2.00	2.38
1993	Field	0.30	1.73	7.50	8.24
1994	Field	0.48	3.05	12.00	14.52
1995	Field	0.64	3.57	16.00	17.00
1996	Field	0.81	5.17	20.25	24.62
1997	Field	0.91	5.29	22.75	25.19
1998	Field	0.85	5.10	21.25	24.29
1999	Field	0.82	5.05	20.50	24.05

Evolution of resistance to several insecticides

DBM populations in vegetable farms of both Shenzhen and Guangzhou rapidly developed resistance to the microbial insecticides Dynamec® and DiPel® after numerous and frequent applications. In 1992, resistance ratios of DBM to Dynamec® at both places were 2.18 and 2.38 respectively (Table 2). In 1999, ratios had increased to 9.82 and 24.05. Products of *Bacillus thuringiensis* were registered and applied earlier than avermectin and so high Bt resistance occurred in DBM populations. In 1992, resistance ratio of DBM to DiPel® at both Shenzhen and Guangzhou were 17.97 and 5.55 and, in 1999, had increased to 44.82 and 12.23. There was little variation in resistance to dichlorvos, fenvalerate, cartap and methomyl throughout the monitoring period. DBM resistance to fenvalerate decreased slightly because of less use in the field. DBM resistance to insect growth regulators (IGRs) increased.

Table 2. Bioassay results of resistance to insecticides in DBM field populations I - Guangzhou and II - Shenzhen, China

Treatment	LC_{50} of susceptible strain (mg/L)	Resistant DBM I		Resistant DBM II	
		1992	1999	1992	1999
abamectin concentrated	0.04	1.89	14.75	2.00	20.50
Dynamec®	0.21	2.18	9.82	2.38	24.05
DiPel®	30.33	5.55	12.23	17.97	44.82
dichlorvos	68.39	13.78	13.39	14.95	16.04
methomyl	151.29	7.62	6.57	11.03	10.01
fenvalerate	68.22	43.73	38.69	78.98	61.83
chlorfluazuron	2.74	24.67	39.19	28.20	45.36
cartap	140.09	3.65	3.22	4.47	5.20

Cross resistance

In the experiment to study cross resistance, an abamectin resistant strain was selected from a Bt resistant strain through continuous treatment with a sub-lethal dose of abamectin. The Bt resistant strain had been raised in the laboratory for more than 100 generations. Although its resistance level to Bt products was as high as about 60.82-fold, for abamectin resistance it was only less than 3x the susceptible level (Table 3). When the Bt resistant strain was selected with abamectin instead of Bt, the DBM strain at generation F_5 developed abamectin resistance as same as F_0 and Bt resistance was 58.02-fold. At generation F_{10} after selection, abamectin resistance increased to 7.55-fold and Bt resistance decreased to 49.32. At generation F_{20}, abamectin resistance increased to 11.5-fold and Bt resistance decreased to 31.34-fold. It was concluded that there was no cross resistance between abamectin and Bt because Bt resistance declined with enhanced abamectin resistance when high pressure of abamectin was put on the DBM strain.

Table 3. Resistance selection of DBM to abamectin in a Bt resistant strain

Population source	Generation	LC_{50} (mg/L)		Resistance ratio	
		abamectin	Bt	abamectin	Bt
Susceptible strain		0.04	30.29		
Bt resistant strain	F_0	0.09	1842.20	2.29	60.82
Bt resistant strain	F_5	0.12	1757.40	3.11	58.02
Bt resistant strain	F_{10}	0.29	1493.90	7.55	49.32
Bt resistant strain	F_{15}	0.34	1137.69	8.95	37.56
Bt resistant strain	F_{20}	0.44	949.36	11.55	31.34

The abamectin resistant strain was used to test for cross resistance between abamectin and several chemical insecticides. There was no cross-resistance between abamectin and dichlorvos, fenvalerate or cartap, but some cross-resistance of DBM populations was found between abamectin and chlorfluazuron (Table 4).

Table 4. Resistance to commonly used insecticides in an abamectin-induced resistant DBM strain

Treatment	LC_{50} (mg/L)		Resistance ratio
	Susceptible strain	Resistant strain	
chlorfluazuron	2.74	55.56	20.28
abamectin	0.04	0.44	11.55
dichlorvos	68.39	253.32	3.70
methomyl	151.29	344.83	2.28
fenvalerate	68.22	127.63	1.87

Synergistic effect

Some of the synergists tested enhanced the toxicity of abamectin towards DBM (Table 5). Among them, SV1 (O, O-diethyl O-phenyl phosphorothioate) was highest with an 8.44-fold increase. TPP caused a 3.70-fold increase in toxicity. S2 and POB (piperonyl butoxide), one of the main synergists for fenvalerate, had little effect on increasing toxicity of abamectin. SV1 is an inhibitor of microsomal oxidases and carboxylesterase in insects. Synergism with SV1 and TPP indicates that these enzymes play a major role in the mechanism of avermectin resistance. These results are similar to other reports (Li *et al*. 1998, Abro *et al*. 1988).

Table 5. Effects of synergists on abamectin toxicity to DBM

Treatment	LC_{50} (mg/L) at 48 h	Synergist ratio
abamectin (ab)	0.73	
ab + SV1	0.09	8.44
ab +TPP	0.20	3.70
ab + S2	0.22	3.30
ab + PB	0.38	1.92

Discussion

There are many vegetable farms at Shenzhen, Dongguang, Huizhou, Bolo and Zengcheng in Guangdong Province, which are run by investors from Hong Kong. The farms cover a large area of cultivated land on which cruciferous vegetables are grown continuously throughout the year. Pest management personnel have limited plant protection knowledge, insecticides are applied with knapsack equipment and pest control is not effective. In recent years, the products of abamectin were produced and applied more frequently than before and pest managers used them aimlessly without reasonable mixture and rotation. These conditions of use have resulted in high selection pressure of abamectin on DBM field populations and consequently, in the main region of vegetable production, resistance to abamectin in DBM has increased rapidly in the past ten years.

In the Shenzhen region, DBM populations developed resistance to abamectin as much as 10-fold in 1999 compared with resistance levels in 1992. The same situation happened with Bt products. DBM resistance to commonly used chemicals remained stable perhaps because they were used rarely due to low efficacy. In experiments on the abamectin resistance mechanism and selection, SV1 and TPP inhibited activities of carboxylesterase. DBM could develop resistance to abamectin under selection pressure of regular abamectin usage, but its rate of development of resistance was less than that of Bt. An abamectin-induced resistant strain had obvious cross resistance with chlorfluazuron, some with dichlorvos, but none with methomyl and fenvalerate.

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Variation in carboxylesterase frequency and insecticide resistance of *Plutella xylostella* (L.) as a response to environmental gradients

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Abstract

A simple observational approach for studying the response of the diamondback moth (DBM), *Plutella xylostella* (L.), to environmental gradients including latitude, insecticide application and climate change, was developed in this study. Insecticide resistance and carboxylesterase frequency in DBM collected in 1979/1980 and 1986/1987 were selected as two ecological variables for analysing and defining the response. Variation in the correlation between larval carboxylesterase 9b and malathion resistance of this insect was also investigated during the period of 1987 to 1997. A clustering dendrogram based on frequencies of seven carboxylesterase isozymes in 16 Taiwan populations showed a decreasingly latitude-dependent distribution of three major clades orientated from north to south. A multidimensional scaling configuration based on the same data had a stress value of 0.18 indicating that these three clades are barely separated. Data on insecticide resistance of 18 populations to five insecticides in a study by Cheng (1981) were used for clustering and multidimensional scaling analysis. The cluster of insecticide resistance in DBM also showed a latitude-dependent distribution of three clades. Multidimensional scaling configuration of insecticide resistance had a stress value of 0.03 indicating that the whole operative taxonomic unit was unable to be separated except for the Tou-Cheng population. Nevertheless, these two clustering dendrograms were similar to each other in three aspects: 1. susceptible populations of Tou-Cheng and Jeo-Feng diamondback moth in northern Taiwan stood as an outgroup from the two different operative taxonomic units; 2. both clusters and both configurations showed that the Sheh-Tzu population in northern Taiwan was grouped together with three southern populations, indicating autopomorphy; 3. geographically related populations adjacent to one another were grouped together as three latitude-dependent clades. However, discordance of the morphometric gradient of the isozyme of DBM populations among adjacent regions was observed. Of seven carboxylesterase isozymes, frequency of EST 9b in DBM was found to be positively correlated with resistance to mevinphos, malathion, fenvalerate and permethrin in this insect. These findings suggest that: 1. the increasing frequency of EST 9b in these populations is roughly associated with decreasing latitude and the distribution of EST 9b in these populations is not random; 2. populations of higher insecticide-resistance have a higher frequency of EST 9b in the zymogram of DBM; 3. both frequencies of EST 9b and insecticide-resistance in DBM are temporarily sustainable and may have varied little during the period of 1981-1997. However, a series of studies on the correlation between EST 9b frequency in DBM and the susceptibility of DBM to a discriminating dose of malathion (0.12 mg per larva) during 1989-1997 revealed that the slopes of the linear regression lines increased in a tangent angle from 16.7°, 20.4° to 29.0°. All three slopes intercepted approximately at zero frequency of EST 9b. This suggested that the association between the mechanism of malathion-resistance and EST 9b in 1989, 1991 and 1997 increased during this period of time. Frequency of EST 9b in 2001 DBM reached its highest titre of 78% suggesting that the association between EST 9b and the resistance mechanism may reach its maximum. Two factors may be involved in the rapid spread of EST 9b in Taiwan populations: 1. dispersal of EST 9b increased in susceptible populations due to migration of the gene coded for EST 9b; 2. a warm winter season due to *El Niño* in 1998/1999 enhanced the dispersal of EST 9b in all populations. Significance of variation in the correlation between frequency of EST 9b and malathion resistance of DBM is discussed.

Keywords

esterase 9b

Introduction

Synthetic pesticides are invaluable in suppressing damage to agricultural products. However, the side effects of these chemicals to organisms in the environment include the rapid development of resistance, especially to insecticides, in target insect pests. Investigation of the origin of a resistance gene acquired by insect pests and the way by which the resistance gene is dispersed are thus interesting topics for entomologists (Devonshire 1977, Georghiou & Taylor 1977, Campbell *et al.* 1997).

Studies on the resistance mechanism of diamondback moth to diazinon and methomyl were reported in Taiwan (Sun *et al.* 1978). Accordingly, a continuous gradient in increasing level of diazinon resistance in DBM populations was found from north to south. The level is low in the north, intermediate in the north-west and high in the west (Chi 1975). Nevertheless, levels of insecticide resistance in DBM populations which are adjacent to one another in southern, western and eastern counties of Taiwan have been occasionally found to be discordant (Cheng 1981). Population-dependent variations in insecticide resistance of DBM have also been reported in other countries (Miyata *et al.* 1986, Shelton *et al.* 1993, Yu 1993). Usually, a population of DBM is thought to be characterised by a particular geographical range or ecological range of tolerance to insecticides. These characteristics are more or less conservative and minor changes of insecticide-resistance in the moth occur in time (Tabashnik *et al.* 1992). However, how the combined temporary effects of latitude of habitat, weather conditions and insecticide applications affect the susceptibility of DBM to insecticide has not yet been explored in Taiwan.

A previous study (Maa & Liao 2000) revealed that all resistant cultures of Sheh-Tzu (ST) DBM which had slow-moving esterase isozymes (EST 8b or 9b) were associated with malathion resistance. On the other hand, susceptible cultures which had the fast-moving, paraoxon-tolerant, esterase isozyme (EST 4b) were positively related to a low level of malathion resistance. These esterases are likely coded by recessive genes and were suggested to be monitoring proteins, especially EST 9b, for malathion susceptibility in DBM.

Tabashnik *et al.* (1992) indicated that DBM is highly mobile. Thus, differentiation between individual populations in larger regional population units is a matter of time. Carboxylesterase isozymes which are related to malathion resistance in DBM should be used as morphological traits for quantitative gradation in resistance in this study. Variation of esterase isozymes, especially esterase 9b, of DBM populations found in different counties of Taiwan was thus examined during 1988, 1991 and 1997 in order to determine how this insect was affected by the complex effects of insecticide application, weather conditions and time.

Taiwan DBM populations were sampled along a 300 km north to south transect and assayed for malathion susceptibility in 1988/1989. The data were compared with those of insecticide resistant populations assayed by Cheng (1981) in order to determine the temporal variations in insecticide resistance and variation in EST 9b frequency in DBM.

Materials and methods

Insects

Individual populations of DBM were collected from vegetable farms in different counties of Taiwan and were reared in the laboratory according to the method of Maa and Liao (2000). In this study, 11, 24, 18, 20 and 18 field populations were sampled respectively in the years 1987, 1989, 1991, 1997 and 2001. The IV instar 84 h old larvae were used for the susceptibility test, synergistic test and esterase zymogram study. The stocks were initiated by mating 30 pairs of DBM; the offspring of the first generation were used.

Chemicals

All of the chemicals and reagents were of analytical grade or reagent grade. Diazoblue, lauryl sulfate, Fast blue RR, 1-naphthyl acetate (1-NA) and eserine were purchased from Sigma Chem. Co., MO, USA. All of the reagents for electrophoresis were purchased from Bio-Rad Lab., CA, USA. Paraoxon; O, O-diethyl-o-p-nitro-phenylphosphate; malathion; O, O-dimethyl-S-(1,2-dicarboethoxtethyl) phosphorodithioate; piperonyl butoxide (PB); diethyl maleate (DEM); and S, S, S-tributylphosphoro trithioate (TBPT) were purchased from Chemical Service, PA, USA.

Zymogram study and frequency of esterase isozyme

Larvae of 16 populations from 1986/1987 were used. Samples for the zymogram study were prepared and run through PAGE according to Davis (1964). Esterase isozymes were stained with 1-NA, and 10^{-5} M paraoxon was used to characterise the isozyme in the gel according to Ogita and Kasai (1965). The migrating distance (Rf) and the frequency of seven carboxylesterase isozymes (Fq) were used as two measurements for morphometric analysis by clustering and multidimensional scaling. Isozymes in which staining was dim or presence of paraoxon was feint were not explored in this study.

Bioassay and discriminating dose

Batches of 30 larvae were treated topically with malathion in two series of concentrations: 0.5, 1.6, 4.0, 8.3, 16.5 and 33.0 µg/larva for the susceptible larvae and 16.5, 33, 66, 132 and 176 µg/larva for the resistant larvae. The larvae were checked every 12 h until they emerged as adults. Results were calculated using Abbott's formula. Dosage-mortality curves of LD_{50} were calculated using the probit analysis method proposed by Finney (1971). Data of LD_{50} were transformed into log values.

Correlation between the log LD_{50} and the frequency of esterase isozyme in DBM was tested for linear regression. A discriminating dose of 120 µg malathion per larva was also chosen for testing the correlation between frequency of EST 9b and malathion resistance of DBM collected in 1986/1987, 1988/1989, 1990/1991 and 1996/1997. It tested how long the correlation between EST 9b and resistance of DBM can last. Data of log LD_{50} and that of frequency of EST 9b obtained by our laboratory was tested for correlation with the log LC_{50} of DBM resistance to five insecticides obtained by Cheng (1981). It determined if there is cross resistance between malathion and the other five insecticides. It also determined how far the correlation between EST 9b and resistance of DBM can be extended backwards. The purpose of both of the above-mentioned tests was to determine whether EST 9b frequency can be used as a constant parameter to monitor insecticide resistance in DBM.

Effect of temperature

Frequency of EST 9b in DBM collected in 2000/2001 was compared with that of DBM collected in previous years in order to determine the influence of temperature on DBM when the global climate phenomenon of *EI Niño* affected Taiwan during 1998 to 1999. The correlation between survival rate of DBM and temperature for bioassay was determined.

Data analysis

Frequencies of seven carboxylesterases in 18 populations were analysed by clustering dendrogram and multidimensional scaling (MDS) configuration using the PRIME 5 software developed by Clarke and Gorley (2001). Lethal concentrations of mevinphos, carbofuran, fenvalerate, cartap and permethrin administered to the larvae of the DBM populations from Cheng (1981) were used for clustering dendrogram, MDS configuration and linear assay with either a frequency of 9b or LD_{50} of malathion. These two sets of clustering dendrograms and MDS configurations were used as two different ecological variables for determining if there is a common ground to determine the response of DBM to the influence of environmental states during 1980 to 1987.

Results and discussion

Geographical features of collection sites

Taiwan is located to the south of mainland China. The physical map of Taiwan (Figure 1) shows mountainous regions (natural area in white), boundaries of agricultural areas (in shadow), urban areas (in dark), counties and collection sites. Collection sites were located in a 300 km section, mainly in west and north-west Taiwan. Distance between sites was approximately 15 to 35 km.

Clustering and MDS analysis on carboxylesterase isozymes

Taiwan DBM are thought to be descended from a common ancestry. DBM populations that departed from each other geographically retained their own differentiated isozyme pattern. Minor variations in the composition of esterase isozymes of DBM in the same region is to be expected. However, the clustering analysis dendrogram of frequency of the isozyme (Figure 2) shows that Jeo-Feng (JF) is independently branched off as an outgroup at the similarity distance of 0.65 from the main operating taxonomic unit. This population has a higher frequency of EST 3b. Although JF and I-Lan (IL) are adjacent to one another geographically, DBM in these sites showed different isozyme patterns. Lu-Chu (LC) and IL, separated from the south clade and north clade respectively, are counted as secondary outgroups at the distance of 0.85. The rest of the DBM populations are clustered together as a monophyletic group with three clades separated off at a distance of 0.85 (Figure 2). The southern clade (clade A) includes three southern populations, Lin-Yuan (LY), His-Lo (HL) and Pu-Tzu (PT), and a northern population, Sheh-Tzu (ST). The central clade (clade B) includes four western populations: Yung-Jin (YJ), Miao-Li (ML), Ta-Chia (TC) and Chu-Pei (CP). The northern clade (clade C) includes two northern populations, Ta-Yuan (TY) and Shan-Hsia (SH); one west-

central population, Tan-Tzu (TT); and two eastern populations, Chi-An (CA) and Pi-Nan (PN). CA and PN are geographically far away from the rest of the major unit.

The cluster shows that distribution of these taxa follows an increasing latitude gradient, accompanied by a decreasing frequency of EST 9b, or an increasing frequency of EST 3b in the zymogram of the DBM, in orientation from south to north.

It is interesting to note that ST, a northern population, was clustered with three southern populations. These four populations all have a high frequency of EST 9b (Table 1) and retain high resistance to insecticides (Table 2). This suggests that heavy insecticide application in the local cultivated land like ST (Cheng 1981) may result in quantitative or qualitative differentiation of esterase isozymes in this insect. Discordance of the morphometric gradient of the isozyme among adjacent DBM populations is possibly related to two environmental gradients: selective pressure of insecticide and latitude/altitude. In other words, zymogram patterns of esterase isozymes of any two adjacent DBM populations may be alike or unlike if one of the populations was exposed to an extreme selection pressure such as insecticide application.

MDS configuration of esterase isozymes in DBM (Figure 3) shows that most of the populations lie on the left part of the scale. Populations with higher frequencies of EST 9b are on the left side; those with lower frequencies of EST 9b are in the middle zone; JF, with the lowest frequency of EST 9b, is on the right side of the scale. JF and TCh, with higher frequencies of EST 3b, are in the upper right of the scale. The wide dispersal of taxa in the left and middle parts of the model may suggest that JF is different from all other populations. In fact, analysis of variance (ANOVA) in Tukey Studentized Range test revealed that all other DBM populations in Taiwan were somehow significantly differentiated from the JF population in their frequency of EST 9b.

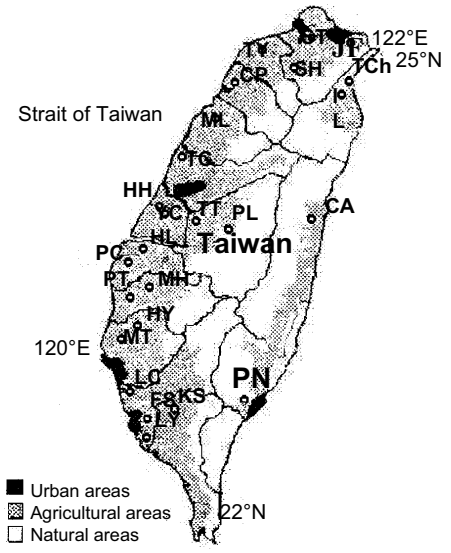

Figure 1. Geographical distribution of 25 DBM populations in Taiwan.

Name Index of Sample Locations:

JF:	Jeo-Fen	ML:	Miao-Li	PT:	Pu-Tzu	IL:	I-Lan	MH:	Min-Hsiung
ST:	Sheh-Tzu	TC:	Ta-Chia	LC:	Lu-Chu	FS:	Feng-Shan	MT:	Ma-Tou
SH:	Shan-Hsia	TT:	Tsao-Tun	LY:	Lin-Yuan	HH:	His-Hu	PC:	Pao-Chung
TY:	Ta-Yuan	YC:	Yuan-Ching	PN:	Pi-Nan	HY:	Hsin-Ying	PL:	Pu-Li
CP:	Chu-Pei	HL:	His-Lo	CA:	Chi-An	KS:	Kao-Shu	TCh:	Tou-Cheng

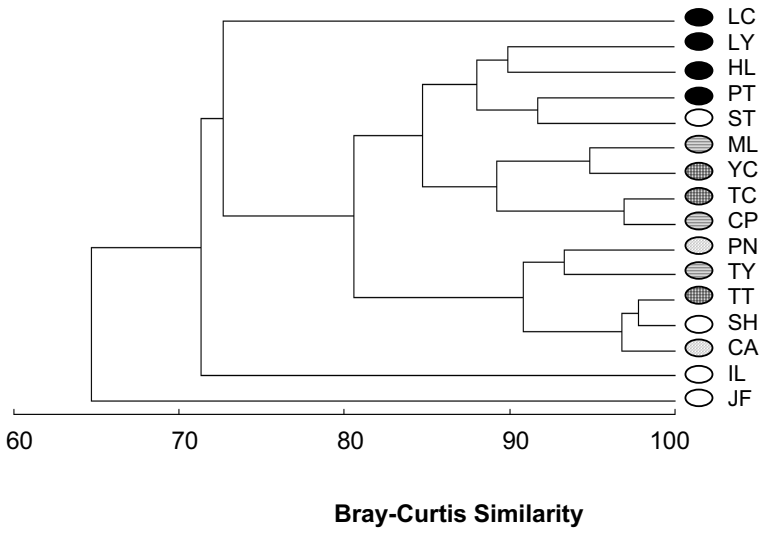

Figure 2. Dendrogram for hierarchical clustering (using group-average) showing relationships among 16 diamondback moth populations based on the percentage of seven carboxylesterase isozymes. Similarity matrix was calculated using the Bray-Curtis similarity index.

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Table 1. Distribution of carboxylesterase isozymes of the larval homogenate of 16 diamondback moth populations in Taiwan (1987) (28 larvae were used for the zymogram analysis)

Population Rf of isozymes	JF	ST	SH	TY	CP	ML	TC	TT	YC	HL	PT	LC	LY	PN	CA	IL
	Frequency of isozyme in percentage															
0.13 (EST 12b)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
0.39 (EST 10b)	0	4	0	7	36	14	29	0	0	0	14	0	0	7	7	29
0.41 (EST 9b)	14	97	50	36	86	93	86	50	100	79	83	86	64	36	50	86
0.43 (EST 8b)	0	7	7	7	7	0	0	7	0	21	7	57	7	4	7	0
0.47 (EST 7b)	0	0	7	7	7	0	7	0	0	14	15	50	7	0	0	0
0.66 (EST 4b)	0	22	0	0	0	0	0	0	0	14	22	0	14	4	0	29
0.72 (EST 3b)	43	0	0	0	0	0	0	0	0	0	0	7	7	0	0	64

However, the difference in EST 9b frequency between JF and other populations of DBM disappeared gradually as time elapsed (Table 3). Meanwhile, DBM populations with high EST 9b frequencies remained, regardless of whether the populations were resistant or not. In fact, the average EST 9b frequency of Taiwan populations was maintained at a level below 75% throughout the years of 1987 to 1997 and the frequency climbed to a high peak of 78% in the year 2000, although the frequency of EST 9b in most populations fluctuated from year to year (Table 3). It seems that dispersal of EST 9b, possibly associated with a resistant gene, is not avoidable in time in any population found in Taiwan.

Table 2. Correlation between the frequency of Est 9b of DBM larvae and LC_{50} or LD_{50} of the larvae from different DBM populations in Taiwan

DBM population	Est 9b freqency	Log values of LC_{50} of five insecticides			Log LD_{50}		
		fenvalerate	carbofuran	mevinphos	permethrin	cartap	malathion
ST	0.92	3.88	3.14	2.52	3.67	2.73	2.29
SH	0.70	2.72	2.45	1.95	2.52	2.73	2.24
TY	0.25	3.07	2.33	2.04	2.75	2.66	1.71
CP	0.92	3.52	2.70	2.41	2.96	2.61	1.94
ML	0.65	3.28	2.37	2.45	2.88	2.72	2.20
TC	0.54	3.66	2.64	2.48	3.10	2.58	2.31
TT	0.86	3.39	2.40	2.43	3.06	2.83	2.31
HL	0.71	3.72	2.67	2.61	3.60	2.75	2.32
PT	0.96	3.99	2.53	2.52	3.41	2.72	2.34
LC	1.00	3.76	2.92	2.84	3.58	2.86	2.42
LY	0.83	4.00	2.87	2.68	3.67	2.84	2.60
PC	0.70	3.64	2.21	2.40	3.13	2.68	2.47
HH	0.83	3.58	2.41	2.51	3.70	2.73	2.40
HY	0.79	3.98	2.55	2.29	3.35	2.81	2.34
KS	0.71	3.50	2.54	2.35	3.09	2.62	2.31
PL	0.85	3.45	2.45	2.42	2.95	2.57	2.27
MH	0.88	3.66	2.32	2.28	3.13	2.73	2.23
IL	0.79	-	-	-	-	-	2.23
TCh	-	2.13	1.62	1.81	2.02	2.40	-

LC data were transcribed from Cheng (1981). Linear regression assay:

1. between insecticides and Est 9b:

malath./Est 9b: slope, 0.577; r, 0.5225 (<*P*.05);
mevinp./Est 9b: slope, 0.651; r, 0.5546 (<*P*.05);
fenval./Est 9b: slope, 0.966; r, 0.5184 (<*P*.05);
permet./Est 9b: slope, 0.981; r, 0.5017 (<*P*.05);
carbof./Est 9b: slope, 0.566; r, 0.4211;
cartap/Est 9b: slope, 0.183; r, 0.3743;

2. between malathion and other insecticides:

malath./mevinp.: slope, 0.629; r, 0.5904 (<*P*.05)
malath./fenval.: slope, 0.928; r, 0.5487 (<*P*.05)
malath./permet.: slope, 1.070; r, 0.6029 (<*P*.05)
malath./carbof.: slope, 0.293; r, 0.2404
malath./cartap: slope, 0.199; r, 0.4492

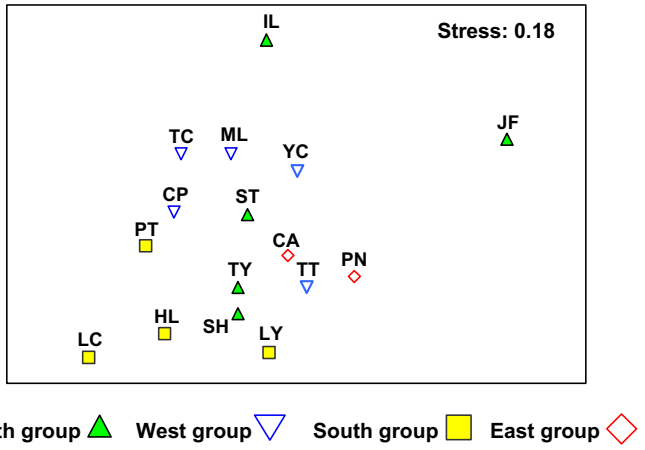

Figure 3. Multidimensional scale model of 16 diamondback moth populations based on the percentage of seven carboxylesterase isozymes. Similarity matrix was calculated using the Bray-Curtis similarity index.

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Table 3. Frequency of EST 9b and malathion resistance of the diamondback moth collected in Taiwan during 1987–2001

DBM population	1987	1989		1991		1997		2001
	EST 9b frequency	EST 9b frequency	Survival rate	EST 9b frequency	Survival rate	EST 9b frequency	Survival rate	EST 9b frequency
IL	0.86	0.79	0.36	0.77	0.78	-	-	0.81
JF	0.14	0.25	0.30	0.22	0.10	0.54	0.28	0.69
ST	0.97	0.92	0.40	0.77	0.55	1.00	0.58	0.80
SH	0.50	0.70	0.50	0.65	0.36	0.54	0.36	0.57
TY	0.36	0.25	0.20	-	0.20	0.39	0.57	0.77
CP	0.86	0.92	-	0.59	0.68	0.81	0.89	0.73
ML	0.93	0.65	0.39	0.76	0.63	0.46	0.51	0.68
TC	0.86	0.54	0.54	0.87	0.41	0.77	-	0.66
TT	0.50	0.86	0.47	-	0.40	-	-	0.87
PL	-	0.85	0.24	-	-	-	-	-
HH	-	0.83	0.55	1.00	0.41	-	-	-
YC	1.00	-	-	-	-	0.69	0.48	0.93
HL	0.79	0.71	0.55	0.88	0.56	0.83	0.64	0.92
MT	-	-	-	0.53	-	-	-	-
PT	0.83	0.96	0.49	0.77	0.56	0.85	0.71	0.87
HY	-	0.79	0.57	-	-	-	-	0.68
KS	-	0.71	0.38	0.59	-	0.58	0.61	0.73
LC	0.86	1.00	0.64	0.53	0.43	0.94	0.75	0.93
FS	-	-	-	-	-	0.77	0.90	0.68
LY	0.64	0.83	0.62	0.76	0.40	0.50	0.31	0.93
M±S.D.	0.72±0.26	0.74±0.22	0.45±0.13	0.69±0.19	0.46±0.18	0.69±0.19	0.58±0.20	0.78±0.11

Twenty-five (25) larvae were used for each treatment; each larva was treated topically with 120 μg malathion.

Clustering and MDS analysis of resistance of DBM to five insecticides

The map of insecticide resistance in Taiwan DBM populations was first completed by Cheng (1981). The sampled populations were scattered in a section of 300 km along the western side of Taiwan. TCh population was the only one collected in north-eastern Taiwan. Cheng (1981) found that Kao-Shu (KS) population in southern Taiwan is slightly resistant to insecticides, and TCh and TY populations in northern Taiwan are susceptible to insecticides. The remaining Taiwan populations are all resistant to insecticides including carbofuran, cartap, fenvalerate, permethrin and mevinphos. The data sets of insecticide resistance were transformed into log values and analysed by clustering dendrogram and MDS model.

The clustering dendrogram (Figure 4) shows that TCh is an outgroup, at a distance of 65% similarity, from the taxonomic unit of insecticide resistance. The operational taxonomic unit also has three clades separated at the distance of 0.75 in the similarity scale of the cluster. LC, in this case, was grouped with the southern clade. It is interesting to note that coincidences were found between the cluster of insecticide resistance and that of esterase isozyme: 1. susceptible DBM populations, TCh and JF in northern Taiwan stood respectively as outgroups from two different taxonomic units; 2. the ST population in northern Taiwan was grouped with the southern clade in both clusters; 3. the southern, central and northern clades in both clusters followed a decreasing gradient of temperature from south to north. However, geographical discordance among adjacent populations of DBM was also found in the clustering dendrogram of insecticide resistance. For example, KS DBM should retain a comparatively high level of resistance to insecticides compared with the northern

populations since this population is located in southern Taiwan. The KS DBM population is, however, clustered with two other susceptible populations as a member of the north clade. On the other hand, the ST DBM population in northern Taiwan was clustered as a member of the southern clade. All members of the southern clade had a higher frequency of EST 9b and a higher level of insecticide resistance, while all members of the northern clade had a low frequency of EST 9b and a lower level of insecticide resistance. The distribution of a DBM population in the cluster system seems to be dependent on three factors: 1. selection pressure of insecticide application; 2. latitude of the niche for the insect; 3. speed of dispersal of the resistant gene in the population.

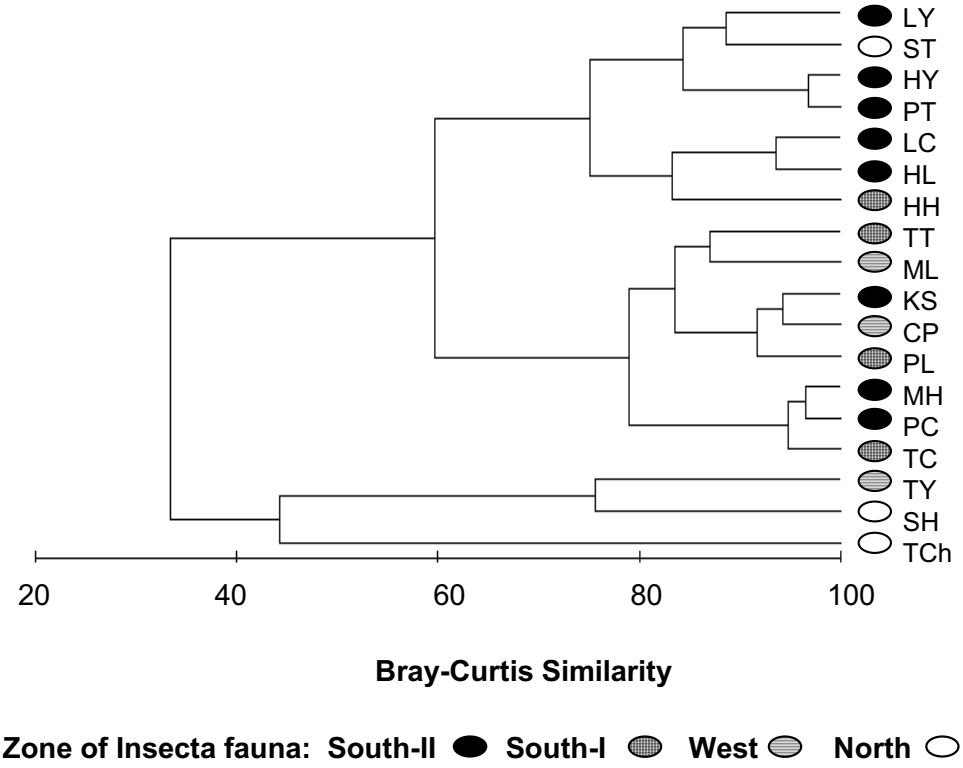

Figure 4. Dendrogram for hierarchical clustering (using group-average) showing relationships among 18 diamondback moth populations based on the insecticide-resistance of DBM to five insecticides (fenvalerate, carbofuran, mevinphos, permethrin and cartap). Similarity matrix was calculated using the Bray-Curtis similarity index. Data on insecticide resistance of DBM (Cheng 1981) were used.

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Shelton *et al.* (1993) suggested that insecticide resistance in DBM in North America originated from southern states. Similarly, we believe that the resistant gene in Taiwan population likely originated and subsequently spread from a southern population since the northern population, JF, has a lower frequency of this isozyme and retains its susceptibility to insecticide screening. In other words, the susceptible population found in northern Taiwan should be a better choice for determining the original development of the resistant gene. We created a culture of DBM that had EST 9a, instead of EST 9b, in the esterase zymogram, by sequentially interbreeding siblings of a single female-male pairing (Maa & Liao 2000). It is expected that a DBM population without EST 9b is possibly originally one that had EST 9b because of insecticide resistance. Unfortunately, the author was unable to find any wild DBM population with a zero frequency of EST 9b by as early as 1982. As Cheng (1981) indicated, all Taiwan populations are resistant to insecticides,

since development of resistance of this insect species to various pesticides were noticed early in the sixties (Tao 1973).

It is noted that the MDS configuration of insecticide resistance (Figure 5) shows a different trend from that of esterase isozyme. It shows that the sampling populations are aggregated in patches along a full tangent line (450) in the lower-left part of this two-dimensional model. In addition, the resistance MDS has a stress value of 0.03 indicating that the whole taxonomic unit was unable to be separated except for the TCh population.

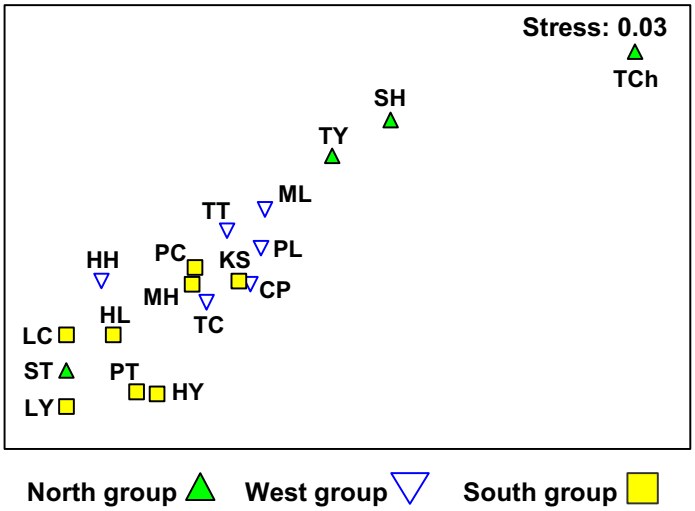

Figure 5. Multidimensional scale model of 18 diamondback moth populations based on the insecticide resistance of DBM to five insecticides (fenvalerate, carbofuran, mevinphos, permethrin and cartap). Similarity matrix was calculated using the Bray-Curtis similarity. Data on insecticide resistance of DBM (Cheng 1981) were used.

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Meanwhile, the esterase MDS (Figure 3) has a stress value of 0.18, suggesting that these three clades are separated and populations of the same clade (Figure 2) can be grouped together in this model. This difference between the two MDS reflect that esterase isozymes, or a detoxification mechanism associated with EST 9b of DBM is possibly playing a partial role in detoxification of insecticides. Meanwhile, the majority of the sampled DBM populations assayed by Cheng (1981) possibly had a common resistance mechanism.

It is accepted that resistance of DBM to different categories of insecticide varies depending on the detoxification mechanism of the insect. A broad spectrum of insecticide resistance observed in field populations is due to multiple resistance mechanisms, including detoxification of insecticides by microsomal oxidase, enhanced carboxylesterase, glutathion-S-transferase and target site insensitivity such as insensitive acetyl cholinesterase (Cheng 1986, Maa *et al.* 1997, Miyata *et al.* 1986, Sun *et al.* 1986, Yu & Nguyen 1992). Although Motoyama *et al.* (1992) suggested that permethrin shared no cross resistance with malathion or mevinphos in DBM, our synergistic test showed that fenvalerate was strongly synergised by Pb and malathion was depressed by TBPT (data not shown). Miyata *et al.* (1986) suggested that different populations have different resistance mechanisms. Cheng (1986) suggested that partial cross resistance was found between organophosphates and synthetic pyrethroids. Motoyama *et al.* (1992) found that the fenvalerate resistance of the revertant larvae could be restored by just one selection event with fenvalerate or even with malathion. It was concluded that there was an unknown factor(s) necessary to maintain the insecticide resistance in DBM, which cannot be explained by the conventional preadaptation theory.

The low stress value of the insecticide resistance MDS may hint that most Taiwan DBM had a common mechanism responsible for resisting different categories of insecticides. We found that correlation between EST 9b frequency of 1988/1989, or 1996/1997 populations and mevinphos/permethrin/fenvalerate resistance of 1979/1980 populations were all significant (refer to Tables 2 and 3) throughout this time interval. These results suggested that the titre or mechanism of insecticide resistance associated with DBM varied little during this period of time. The resistance level of any population may decrease when the pressure of selection was released. Nevertheless, a resistant gene in a heterogeneous form will be well-retained in the wild population.

Halpern and Morton (1987) found that malathion-selected *Drosophila melanogaster* produced fewer offspring and had defective larval development. Similarly, we found that an EST 9b homogeneous resistant pair, in a sibling interbreeding bioassay, produced fewer offspring when the parent male DBM had been either selected for highest frequency of EST 9b or had been selected under highest lethal dose of malathion (Maa & Liao 2000). It is easy to maintain an EST 9a homogeneous susceptible culture, but difficult to maintain an EST 9b homogeneous resistant culture in the laboratory when the culture is selected.

EST 9b for monitoring protein and 120 µg for diagnostic dose

Roush and Daly (1990) suggested that when two resistance genes are involved in resistance of an insect, a dose that kills 75% of the backcross is useful, since the most likely genotypes to be killed are the 75% that are most susceptible. In this case, a 99% kill of a susceptible population is necessary for a discrimination test. ST population selected with malathion for three to four generations produced defective offspring which either died before emergence or had only a few adults emerge. A routine rule for genetic analysis on the resistance gene was therefore not used in this study. Reports on the resistance mechanism of DBM revealed that it is a matter of multiple resistance. Tolerance of acetylcholinesterase found in malathion-resistant populations of Taiwan tend to be codominant to dominant in their inheritance (Maa *et al.* 1997). Our previous study revealed that malathion resistance of ST culture tended to be incompletely dominant in inheritance (Maa & Liao 2000). We also found cross resistance between malathion, an organophosphorous compound, and permethrin, a synthetic pyrethroid (Table 1). Yu and Nguyen (1992) indicated that DBM in Florida had high oxidative activity against various kinds of insecticides. Yu (1993) indicated that resistance to permethrin was inherited in an incompletely dominant, autosomal manner. We therefore chose 120 µg malathion per larva as the diagnostic dose for topical treatment. This dose is high enough to kill 95% of an intrabreeding susceptible culture of ST population (Maa & Liao 2000). In addition, nearly 75% of Taiwan populations had a LD_{50} lower than 120 µg/larva. This dose was also used for monitoring the population of 1990/1991 and 1996/1997.

Results of the study on the correlation between EST 9b frequency in the population and the malathion resistance of 1988, 1991 and 1997 populations (Figure 6) show that the slopes of the linear regression lines shift in tangent angle from 16.7°, 20.4° and 29.0°. All three slopes of 1988, 1991, 1997 intercepted approximately at zero frequency of EST 9b. We suggest that the association between malathion resistance and EST 9b frequency somehow increased gradually during 1987~1997. We expect that frequency of EST 9b would be a good indicating protein for monitoring malathion resistance in DBM in the field. The mechanism of malathion resistance associated with EST 9b will be studied with molecular techniques. However, the defect of a resistant homogeneous population is a disadvantage for survival in the long run.

Temperature effect

A rising temperature due to *El Niño* during 1998/1999 made the mean of EST 9b frequency increase from 69% in the 1997 population to 78% in the 2001 population. Correlation between EST 9b frequency and temperature is positively related. Correlation between survival rate of DBM to malathion is also positively related. This indicates that temperature is an important factor affecting the survival rate of the insect against malathion.

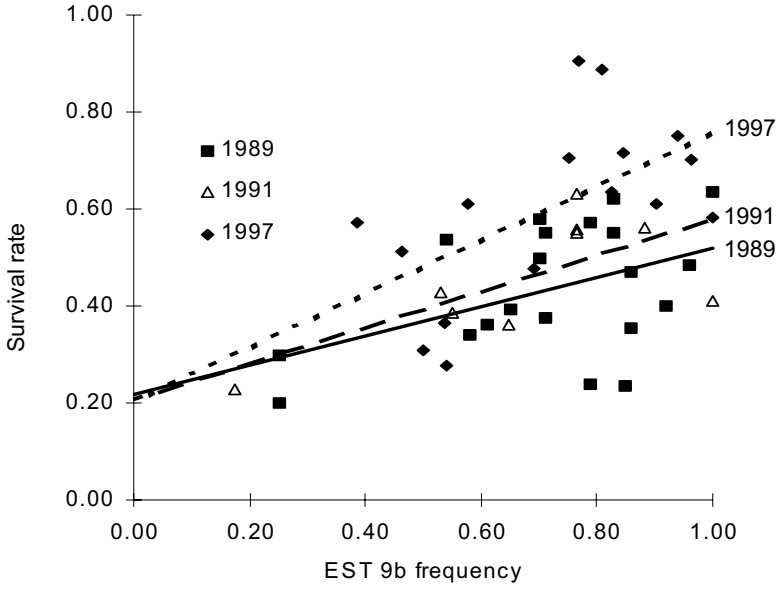

Figure 6. Linear correlation between frequency of EST 9b and survival rate of diamondback moth treated with 120 µg malathion per larva. 1989: y = 0.3001x + 0.2192; 1991: y = 0.3718x + 0.2062; 1997: y = 0.5535x + 0.2031.

Frequency, resistance, environmental gradients and time factor

Results of morphometric analysis on divergence of carboxyl esterase, frequency of the esterases and titre of insecticide resistance of DBM populations reveal that it is the sum of all selecting pressures including insecticide application, altitude, latitude, climate and time that makes the resistant population what it is. We assume that discontinuous geographical distribution of esterase isozyme patterns in adjacent populations of DBM was due to adaptation capability, survival rate and the ability of DBM to adapt to selection pressure from the environmental gradients. The dominant esterase isozymes, ESTs 3, 4, 8 and 9 are widely distributed in field DBM (Maa *et al*. 2000). These isozymes, unlike what was found in aphids (Devonshire & Moores 1982), are limited in quantity and are hardly able to function as major metabolic enzymes for degrading malathion or as major binding proteins for rendering malathion or its derivatives nontoxic. Doichuanngam and Thornhill (1989) suggested that there was a positive correlation between activity of carboxylesterase and malathion resistance. We argue that it is possible to simplify the often complex arguments of resistance mechanisms and detoxification systems using desirable general results and offer as proof, but without a powerful explanation, the actual patterns in nature. However, these isozymes are good monitoring proteins for malathion resistance for the following reasons: first, these isozymes are easily detected by 1-NA staining with a combination of paraoxon inhibition and second, ESTs 3b/9b are coded by incompletely dominant genes. It is a good quality for a monitoring protein since the associated resistance gene linked with EST 9b might be protected and thus be heritable even under extreme environmental stress. It was once expected that southern populations would obtain insecticide resistance faster than the northern ones. Although the high selective pressure of insecticide application against DBM would drive the adaptation of this insect to be highly resistant to insecticides, the south DBM, the heavily selected DBM, would adapt rapidly to pressure caused by insecticide application since there is higher temperature and less precipitation all year round in the niche of DBM. Nevertheless, this study revealed that the spread of EST 9b, or the resistance gene, in Taiwan populations is only a matter of time.

Acknowledgements

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Naturally derived chemistry (azadirachtin) for the control of crucifer pests in Australia

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Abstract

A field trial was established in cabbages to determine the efficacy of AzaMax®, a commercial formulation of 12 g/L azadirachtin (derived from neem kernels) against diamondback moth (*Plutella xylostella* (L.) Lepidoptera: Plutellidae), cabbage white butterfly (*Pieris rapae* (L.) Lepidoptera: Pieridae) and heliothis (*Helicoverpa armigera* (Hübner) Lepidoptera: Noctuidae). Results demonstrate the effectiveness of 0.5 L/ha, 1 L/ha, 2 L/ha and 3 L/ha of AzaMax® alone and in combination with a crop oil and a commercially available *Bacillus thuringiensis* (Bt) formulation. A comparison of the efficacy of AzaMax® with that of fipronil was also investigated.

Keywords

organic, *Plutella*, neem, AzaMax®

Introduction

Azadirachtin is a natural insecticide derived from the kernel of the neem tree (*Azadirachta indica* Juss). Azadirachtin has significant potential as a commercial insecticide, but stability problems have always restricted its performance and use in commercial formulations. Recently, Trifolio-M GmbH developed a method to extract azadirachtin directly from neem tree (*A. indica*) kernel. This process produces a very stable form of azadirachtin due to significant reductions in destabilising impurities. The powdered azadirachtin extract is commercially available as NeemAzal Technical and is used in various formulations for the control of a wide range of pests throughout the world. It has now found a niche within organic cropping situations as alternative chemistry to the use of Bt sprays.

NeemAzal Technical has the following specifications:

	%
azadirachtin A & B	40
azadirachtin H	2.3
salanin	2.8
3-deacetyl salanin	0.8
nimbin	0.7
deacetyl nimbin	0.4
Other limonoid compounds	16.7
Fatty acids	3.9
Partially characterised natural substances	31.3
Water	1.1
TOTAL	100

Through a joint venture between Organic Crop Protectants Pty Ltd (OCP) and EID Parry of India, OCP are developing the use of a 12 g/L formulation of NeemAzal Technical for the control of various crucifer pests in Australia, Tradename AzaMax®.

Materials and methods

A field trial was conducted in the Dandenong region of Victoria with various treatments (Table 1) from early February to mid March to compare the efficacy of AzaMax®, fipronil and a commercial *Bacillus thuringiensis* formulation. The target crop was Savoy Cabbage (Planted 31/1/00 – Harvested 1/4/00). All lepidopteran species that were present in any significant numbers were evaluated. The trial was set out as a randomised complete block design with four replicates. Plot size was 4 rows wide by 5 metres long. All assessments were made within the middle of each plot. Insect assessments were carried out immediately before each application of the treatments and 8 days after the final application.

Assessments were carried out by counting the number of diamondback moth (DBM), cabbage white butterfly and heliothis larvae on five randomly selected cabbage plants from the middle of each plot. The larvae were differentiated as: small <4 mm, medium 5-8 mm, large >8mm.

Treatments were applied using a gas operated hand-held sprayer with hand wand boom attached incorporating TX – 8 hollow cone nozzles (application 1) or five TX – 12 hollow cone nozzles (applications 2-6). One 1.25 m pass was made over the top of each row of cabbages at a pressure of 300 kPa, walking at 1 m/second. Total volumes of 290 L/ha (application 1) and 490 L/ha (application 2-6) were applied respectively. Details of application dates and spraying conditions are provided in Table 2.

Table 1. Insecticide treatments and rates for a field trial of of AzaMax®, fipronil and a commercial *Bacillus thuringiensis* formulation against pest Lepidoptera on Savoy Cabbage, Dandenong, Victoria, Australia, 2000

Treatment List	Rate Product/ha
1. AzaMax® + Synertrol® Horti Oil	0.5 L + 2.0 L
2. AzaMax® + Synertrol® Horti Oil	1.0 L + 2.0 L
3. AzaMax® + Synertrol® Horti Oil	2.0 L + 2.0 L
4. AzaMax® + Synertrol® Horti Oil	3.0 L + 2.0 L
5. AzaMax® + DiPel Forté + SHO	1.0 L + 500 g + 2.0 L
6. DiPel Forté DF^ + BS 1000	500 g + 0.1% v/v
7. Regent* 200 SC + BS 1000	250 ml + 0.1% v/v
8. Untreated control	

* Regent 200SC contains 200g/L fipronil and is a Registered Trademark of Aventis Corp, ^ -DiPel Forté DF contains *Bacillus thuringiensis* subspecies. *kurstaki*, strain HD-1 and is a Registered Trademark of Sumitomo Chemicals.

Table 2. Application dates and spraying conditions for field trial of of AzaMax®, fipronil and a commercial *Bacillus thuringiensis* formulation against pest Lepidoptera on Savoy Cabbage, Dandenong, Victoria, Australia, 2000

Date	Growth stage	Temperature °C	% relative humidity
08/2/00	8 leaf	34	53
15/2/00	9-10 leaf	32	68
25/2/00	10-14 leaf	34	52
03/3/00	16 leaf	21	91
09/3/00	3wk preharvest	26	71
16/3/00	2wks preharvest	25	63

Results

Diamondback moth

Results of DBM control are summarised in Figure 1 and represent cumulative mean numbers of larvae. A high to very high population of DBM was detected during the trial. Standard treatments of fipronil and Bt and AzaMax® @ 3L/ha provided good control of all sizes of larvae.

AzaMax® applications of 0.5 L/ha and 1.0 L/ha did not provide adequate control of DBM under the high pressure experienced during the trial. AzaMax® @ 2 L/ha was not significantly different from AzaMax® @ 3L/ha at the 95% level of probability.

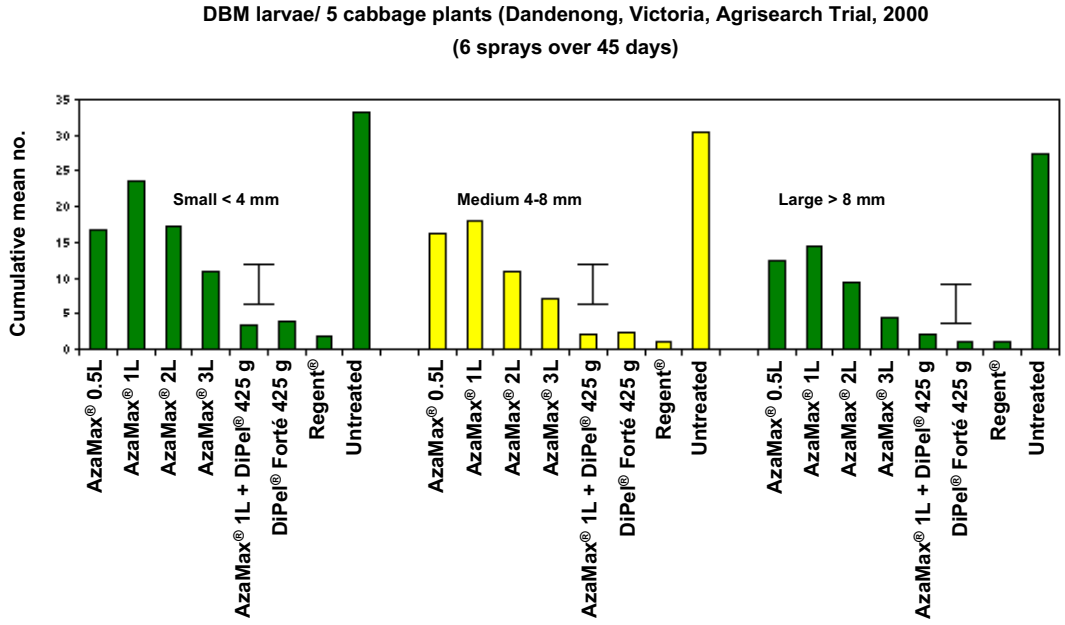

Figure 1. Cumulative mean numbers of DBM larvae/per 5 Savoy Cabbage plants in a field trial of AzaMax®, fipronil and a commercial *Bacillus thuringiensis* formulation, Dandenong, Victoria, Australia, 2000.

Heliothis

A small population of heliothis developed midway through the trial. Results of heliothis control are summarised in Figure 2. AzaMax® at application rates of 0.5, 1.0, 2.0 and 3.0 L/ha all controlled heliothis under a low infestation situation.

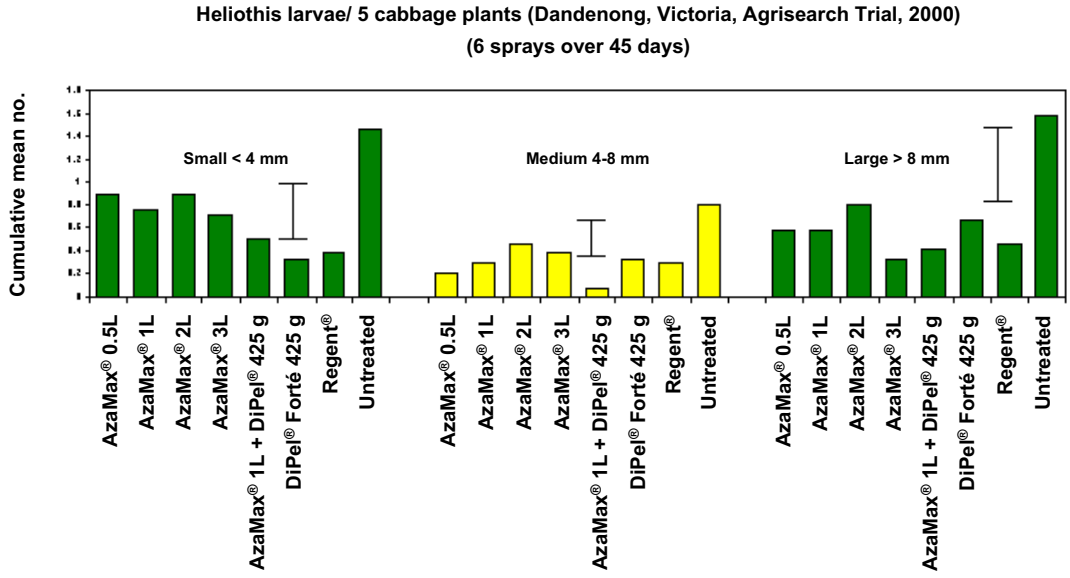

Figure 2. Cumulative mean numbers of Heliothis larvae/per 5 Savoy Cabbage plants in a field trial of AzaMax®, fipronil and a commercial *Bacillus thuringiensis* formulation, Dandenong, Victoria, Australia, 2000.

Cabbage white butterfly

A very small population developed mid way through the trial. Results of cabbage white butterfly control are summarised in Figure 3. All treatments provided similar levels of control of all larvae sizes excluding AzaMax® @ 0.5 L/ha which provided significantly poorer control when the total number of larvae were counted. No significant difference was detected for the level of control provided by AzaMax® at application rates of 1.0, 2.0 and 3.0 L/ha.

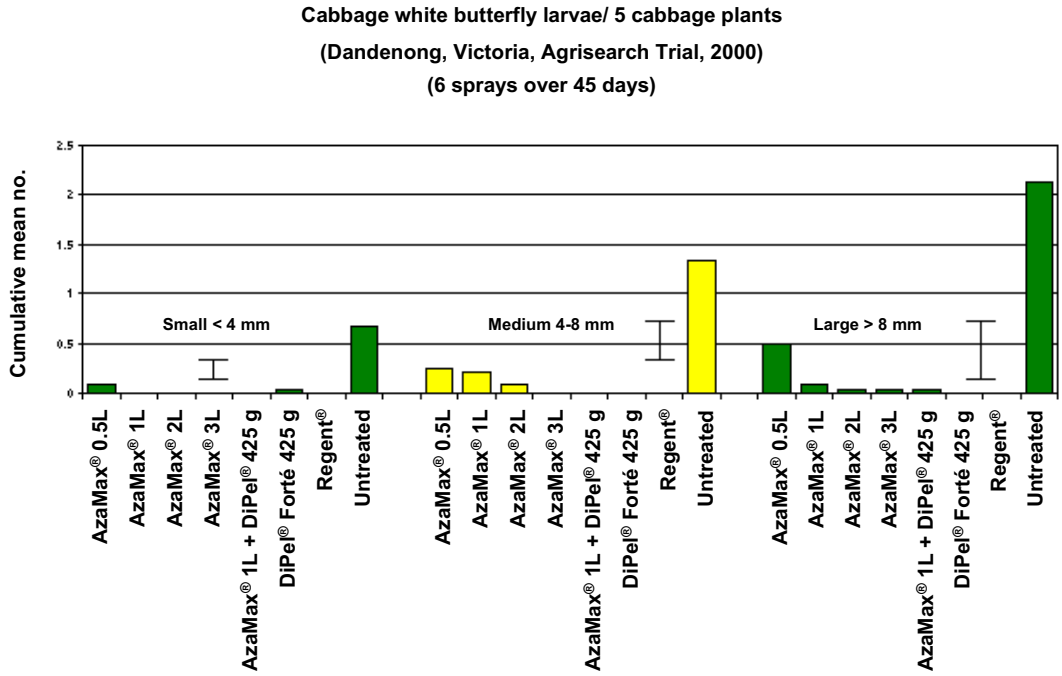

Figure 3. Cumulative mean numbers of cabbage white butterfly larvae/per 5 Savoy Cabbage plants in a field trial of AzaMax, fipronil and a commercial *Bacillus thuringiensis* formulation, Dandenong, Victoria, Australia, 2000.

Discussion

AzaMax® did not perform as well as Bt or fipronil under high populations of DBM. However the 3 L/ha rate of AzaMax® did offer similar levels of control to the standard treatments. There is evidence to suggest from this trial that AzaMax® will control heliothis and cabbage white butterfly, however its efficacy under medium to high pest pressure needs further investigation.

AzaMax® did not appear to enhance the efficacy of Bt in this trial probably because the antifeedant effect of azadirachtin was antagonistic to the ingestion of the Bt.

From this trial it appears that AzaMax® has significant potential as alternative chemistry for the control of DBM and other lepidopteran pests of crucifer crops. Furthermore its activity against other pests including aphids and thrips makes AzaMax® a very versatile product within Integrated Pest Management systems that utilise highly selective insecticides.

There is a definite need for a product like AzaMax® in organic production systems within Australia due to the lack of any alternative control options for DBM and other lepidopteran pests. Currently growers are forced to use Bt sprays on a regular basis, which will eventually cause resistance to develop and potentially jeopardize the life span of Bt in conventional agriculture.

Acknowledgements

Agrisearch Services Pty Ltd for conducting the Field Trial

Spinosad controls a range of lepidopteran pests in crucifers in Australia

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Abstract

Spinosad is one of the most widely used products for control of diamondback moth (*Plutella xylostella*) in Australia. Since the launch of Success* Naturalyte* in 1999, it has rapidly gained wide acceptance in all crucifer growing areas of the country. This has occurred, not only because spinosad is highly effective against diamondback moth, but because it also controls several other important lepidopteran pests such as heliothis (*Helicoverpa* spp.), cabbage white butterfly (*Pieris rapae*), cabbage centre grub (*Hellula hydralis*) and cabbage cluster caterpillar (*Crocidolomia pavonana*) at rates which provide growers with excellent value for money. Spinosad is highly active against loopers (*Chrysodeixis* spp.) and affords some control of cluster caterpillar (*Spodoptera litura*) and onion thrips (*Thrips tabaci*). There is no diamondback moth resistance to spinosad in Australia, the product has a favourable toxicological profile and it is selective to a range of beneficial predators and parasitoids.

The value of insect control is best gauged by assessing the quality of produce at harvest of the crop. In a small scale trial, a crop of broccoli infested with *P. rapae*, *P. xylostella, S. litura* and *C. pavonana* received a programme of six applications of spinosad at 7-10 day intervals. Spinosad at 48 g ai/ha resulted in 97.2% marketable heads, not significantly different ($P>0.05$) from the standard, prothiophos, at 750 g/ha which gave 100% marketable heads. Spinosad at 96 g ai/ha resulted in 98.7% marketable heads, reflecting superior control of *S. litura* at this higher use rate.

Keywords

Lepidoptera, yield, *Plutella xylostella*, *Helicoverpa*, *Pieris rapae*, *Hellula hydralis*, *Crocidolomia pavonana*, *Chrysodeixis*, *Spodoptera litura*, *Thrips tabaci*

Introduction

Spinosad is widely used for control of lepidopteran pests in crucifer crops in Australia. Success* Naturalyte* (containing 120 g/L spinosad, marketed by Dow AgroSciences) is labelled for use at 48 g ai/ha against diamondback moth (*Plutella xylostella* (L.) (Lepidoptera: Plutellidae)) and cabbage white butterfly (*Pieris rapae* (L.) (Lepidoptera: Pieridae), and at 48-96 g ai/ha for heliothis (*Helicoverpa* spp. (Hübner) (Lepidoptera: Noctuidae)).

There are several factors which have contributed to the market place acceptance of Success*, including cost-effectiveness against the key target pests *P. xylostella* and *P. rapae*, selectivity to a range of beneficial species and a favourable eco-toxicology profile.

In south-east Queensland, including the Lockyer Valley vegetable-basket region, brassicas are sporadically attacked by a number of lepidopteran pests in addition to those mentioned above. These include cluster caterpillar (*Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae)), cabbage cluster caterpillar (*Crocidolomia pavonana* (Fabricius) (Lepidoptera: Pyralidae)) and cabbage centre grub (*Hellula hydralis* (Guenée) (Lepidoptera: Pyralidae)). Ability to control this spectrum of lepidopteran *Brassica* pests is a desirable trait in any product in this market.

This paper reports a single multiple application trial conducted at the QDPI Redlands Research Station in December 1999 by John Hargreaves in which lepidopteran insect control and its effect on broccoli yield was assessed.

Materials and methods

Seeds of the broccoli cultivar Dome (Yates Seeds) were sown into speedling trays containing a soilless mixture of peat and vermiculite (1:1), plus 100 g dolomite, 50 g potassium nitrate, 50 g superphosphate and 50 g blood and bone per 25 L of mixture. Seeds were sown on 05 November 1999 and sprayed once with sodium molybdate 1 g/L, Solubor® 2 g/L and Nitofol® 1.9 mL/L as the seedlings developed.

The transplants were planted into a 700 m^2 border of the Redlands Research Station on 13 December 1999. The red krasnozem soil had been pre-drilled with a basal fertilizer (Granular 4®) at the rate of 0.1 kg/m. Rows were spaced 1.2 m apart and eight rows were drilled into the border with plants spaced 30 cm apart along the row. Four weeks after transplanting, plants were side-dressed with urea 9 g/m. Overhead irrigation was applied on demand and although conditions were wet through the period, overhead irrigation was generally applied once per week.

Four replicates of each treatment were laid out in a randomised block design. Plots were two rows wide by 30 plants long (24 m^2, 60 plants per plot). Five randomly selected plants from the middle of each plot were examined at each assessment.

Populations of pests built up slowly and no insecticides were applied until plots received their prescribed treatments. Plants were examined for the presence of larvae of all species as well as the eggs of *P. rapae, C. pavonana* and *S. litura*. The pupae of both *P. rapae* and *P. xylostella* were also counted.

Sprays were applied 7-10 days apart, depending on rain, from the 8th January. Initially 500 L of spray mix per hectare was applied by an Echo® power knapsack sprayer delivering 800 kPa pressure through a horizontal boom. Two hollow cone, sintered aluminum nozzles (Albuz® yellow 212) delivering 0.95 L/min, were spaced at 220 mm centres on the boom. An application speed of 1.9 km/h was maintained. On the 28th January, the boom was increased to 4 nozzles, spaced at 220 mm centres, but changed to Albuz® lilac 208 nozzles, delivering 0.45 L/min at 800 kPa. The wash volume was increased to 1000 L/ha. A non-ionic wetter was used with all treatments (Agral® 600) at the rate of 0.1 mL/L. At maturity, terminal flower heads were harvested progressively over the period 10th - 13th March. A flower head was considered "prime" when inspection of the surface and floret bases showed no larvae present nor obvious signs of gross feeding. Processing grade allowed only a degree of scarring to the floret bases, indicative of earlier feeding.

Data were statistically analysed by analysis of variance using the QDPI ranb programme with appropriate transformations.

Materials used:

- Agral® 600 a non-ionic surfactant containing 600 g/L nonyl phenol ethylene oxide condensate (CropCare Australia Ltd).
- Success* Naturalyte* containing 120 g/L spinosad as a suspension concentrate (* trademark of Dow AgroSciences (Australia) Ltd).
- Tokuthion containing 500 g/L prothiophos as an emulsifiable concentrate (trademark of Bayer Germany).

Results and discussion

No obvious phytotoxicity, with any concentration of spinosad, was noticed during the trial.

Crocidolomia pavonana *(Table 1)*

All treatments significantly reduced *C. pavonana* numbers at each assessment, but until the final assessment there was no difference between spinosad treatments and the standard, prothiophos. At the final assessment, spinosad at 6 g ai/ha gave a significant level of control, but itself was significantly poorer (P=0.05) than spinosad 12 g ai/ha and above, and prothiophos. Numerical equivalence with prothiophos was achieved with spinosad at 24 g ai/ha. Egg deposition by this species appeared to be unaffected by insecticide concentration.

Spodoptera litura *(Table 2)*

Spodoptera infestation was low at the start of the trial, but gradually increased over time. The peak of infestation occurred in early February with an average of 50 larvae per plant in the untreated controls. Prothiophos showed excellent activity maintaining numbers at a very low level, matched by spinosad at 96 g ai/ha. Lower rates of spinosad, although not significantly poorer, were less effective numerically. The egg deposition by cluster caterpillar, *Spodoptera litura*, appeared unaffected by chemical concentration.

Table 1. The numbers of cabbage cluster caterpillar, *Crocidolomia pavonana* (F.) larvae per broccoli plant after a range of insecticide treatments were applied to the foliage, Jan-Feb 2000

Treatment		Inspection date					
		7 Jan 00	17 Jan 00	27 Jan 00	7 Feb 00	17 Feb 00	28 Feb 00
Untreated		2.0 a	4.7 a	33.2 a	9.3 a	23.7 a	27.1 a
Success*	50 mL/ha	1.5 a	0.0 b	0.4 b	0.3 b	3.5 b	12.1 b
Success*	100 mL/ha	0.0 a	0.0 b	0.0 b	0.1 b	0.3 b	1.6 c
Success*	200 mL/ha	0.0 a	0.0 b	0.0 b	0.0 b	0.0 b	0.1 c
Success*	400 mL/ha	1.8 a	0.0 b	0.0 b	0.0 b	0.0 b	0.0 c
Success*	800 mL/ha	0.0 a	0.0 b	0.0 b	0.0 b	0.3 b	0.1 c
Tokuthion®	1.5 L/ha	0.0 a	0.0 b	0.0 b	0.0 b	0.0 b	0.1 c

All treatments applied with Agral® 600 at 10 mL/100 L. Values within columns, followed by a common letter, do not differ at the P=0.05 level of probability. Values quoted are re-transformed means from the $\sqrt{x}$ + 0.5 transformation.

Table 2. The numbers of cluster caterpillar, *Spodoptera litura* (L.) per broccoli plant, after a range of insecticide treatments had been applied sequentially to the foliage, Jan-Feb 2000

Treatment		Inspection date					
		7 Jan 00	17 Jan 00	27 Jan 00	7 Feb 00	17 Feb 00	28 Feb 00
Untreated	-	0.0 a	1.3 a	18.6 a	52.4 a	38.6 a	33.3 a
Success*	50 mL/ha	0.0 a	0.1 a	13.0 a	18.2 b	44.8 a	30.0 ab
Success*	100 mL/ha	0.0 a	0.0 a	1.2 a	5.8 bc	28.8 bc	18.4 ab
Success*	200 mL/ha	2.1 a	0.8 a	7.5 a	3.7 bc	12.4 cd	16.2 b
Success*	400 mL/ha	0.0 a	0.2 a	8.7 a	1.0 c	7.0 de	1.6 c
Success*	800 mL/ha	1.7 a	0.7 a	0.9 a	0.5 c	1.9 e	0.7 c
Tokuthion®	1.5 L/ha	1.6 a	0.1 a	3.8 a	0.2 c	3.2 de	2.2 c

All treatments applied with Agral® 600 at 10 mL/100 L. Values within columns, followed by a common letter, do not differ at the P=0.05 level of probability. Values quoted are re-transformed means from the $\sqrt{x}$ + 0.5 transformation.

Pieris rapae (Table 3)

Pieris rapae pressure was constant throughout the trial and numbers in the untreated plots ranged from 3.7 larvae/plant in early January to 6.5 larvae/plant in middle February. At 48 g ai/ha spinosad and above, control of *P. rapae* was similar to that given by prothiophos 750 g ai/ha. At lower rates of spinosad, control was less effective (although not significantly so). Numbers of pupae tended to be low and a less sensitive indicator of control.

Plutella xylostella (Table 4)

Plutella xylostella numbers were relatively low for the early part of the trial, although high numbers infested plants in the middle of February (14.1 larvae/plant in the untreated plots). Spinosad gave very good control of *P. xylostella* at 12 g ai/ha and above, and at 48 g ai/ha performed as well as prothiophos 750 g/ha. Although pupae were counted, these proved a less accurate measure of a treatment's performance than did larvae. The trial was harvested and produce graded according to commercial standards (Table 5). The highest proportion of "prime" broccoli came from the standard prothiophos plots (89.9%), although this was not significantly superior to rates of spinosad 48 and 96 g/ha which gave 76.4% and 87.5% of "prime" broccoli respectively.

The mean number of all species of larvae per plant found on broccoli after the fourth application of insecticide is shown in Figure 1. Combining "prime" and "processing" grade heads together to create a "marketable" category, the result was no different, with prothiophos giving 100% marketable heads, while spinosad at 48 and 96 g/ha gave 97.2 and 98.7% marketable heads respectively (Figure 2).

Table 3. The numbers of cabbage white butterfly, *Pieris rapae* (L.) per broccoli plant after a range of insecticides was applied to the foliage during Jan-Feb 2000

Treatment		Inspection date					
		7 Jan 00	17 Jan 00	27 Jan 00	7 Feb 00	17 Feb 00	28 Feb 00
Larvae							
Untreated		3.7 a	3.7 a	6.1 a	4.0 a	6.5 a	5.1 a
Success*	50 mL/ha	3.7 a	0.1 c	0.6 b	1.8 b	2.9 b	1.3 b
Success*	100 mL/ha	2.9 a	0.9 b	0.2 b	0.4 c	3.9 ab	0.7 bc
Success*	200 mL/ha	4.5 a	0.9 b	0.3 b	0.1 c	1.3 bc	0.1 cd
Success*	400 mL/ha	3.2 a	0.5 bc	0.4 b	0.2 c	0.5 c	0.0 d
Success*	800 mL/ha	2.3 a	0.1 c	0.6 b	0.3 c	0.4 c	0.0 d
Tokuthion®	1.5 L/ha	1.9 a	0.1 c	0.0 b	0.2 c	0.7 c	0.0 d
Pupae							
Untreated	-	0.0	0.5 a	0.8 a	0.8 a	5.8 a	1.0 a
Success	50 mL/ha	0.0	0.2 a	0.1 a	0.2 a	1.2 bc	0.5 abc
Success	100 mL/ha	0.0	0.2 a	0.1 a	0.1 a	1.3 bc	0.6 ab
Success	200 mL/ha	0.0	0.1 a	0.1 a	0.1 a	1.9 bc	0.1 bc
Success	400 mL/ha	0.0	0.1 a	0.5 a	0.3 a	2.9 b	0.2 bc
Success	800 mL/ha	0.0	0.1 a	0.1 a	0.2 a	0.5 c	0.1 c
Success	1.5 L/ha	0.0	0.0 a	0.4 a	0.2 a	0.1 c	0.1 c

All treatments applied with Agral® 600 at 10 mL/100 L. Values within columns, followed by a common letter, do not differ at the P=0.05 level of probability. Values quoted are re-transformed means from the $\sqrt{x}$ + 0.5 transformation.

Table 4. The numbers of immature diamondback moth, *Plutella xylostella* (L.) per broccoli plant after a range of insecticide treatments had been applied sequentially to the foliage

Treatment		Inspection date					
		7 Jan 00	17 Jan 00	27 Jan 00	7 Feb 00	17 Feb 00	28 Feb 00
Larvae							
Untreated		0.0	0.5 a	1.4 a	2.7 a	14.1 a	5.1 a
Success*	50 mL/ha	0.0	0.1 a	0.1 b	0.1 b	1.4 b	1.2 b
Success*	100 mL/ha	0.0	0.3 a	0.0 b	0.1 b	0.1 b	0.1 c
Success*	200 mL/ha	0.0	0.1 a	0.1 b	0.0 b	0.4 b	0.1 c
Success*	400 mL/ha	0.0	0.1 a	0.1 b	0.0 b	0.2 b	0.0 c
Success*	800 mL/ha	0.0	0.2 a	0.0 b	0.0 b	0.1 b	0.0 c
Tokuthion®	1.5L/ha	0.0	0.1 a	0.0 b	0.3 b	0.2 b	0.0 c
Pupae							
Untreated	-	0.0	1.0 a	1.3 a	1.6 a	5.5 a	3.5 a
Success*	50 mL/ha	0.0	0.1 b	0.1 b	0.2 b	0.7 b	0.8 b
Success*	100 mL/ha	0.0	0.0 b	0.2 b	0.2 b	0.6 b	0.1 b
Success*	200 mL/ha	0.0	0.1 b	0.2 b	0.1 b	1.4 b	0.1 b
Success*	400 mL/ha	0.0	0.1 b	0.1 b	0.0 b	0.1 b	0.1 b
Success*	800 mL/ha	0.0	0.1 b	0.1 b	0.0 b	0.2 b	0.1 b
Tokuthion®	1.5 L/ha	0.0	0.0 b	0.0 b	0.0 b	0.8 b	0.1 b

All treatments applied with Agral® 600 at 10 mL/100 L. Values within columns, followed by a common letter, do not differ at the P=0.05 level of probability. Values quoted are re-transformed means from the $\sqrt{x}$ + 0.5 transformation

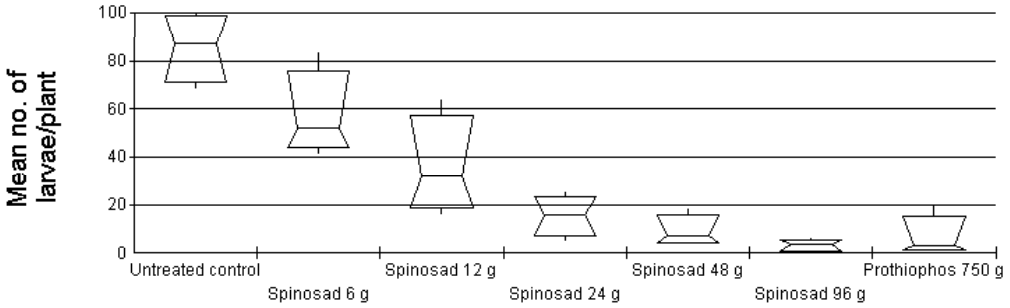

Figure 1. Number of lepidopteran larvae on broccoli assessed eight days after fourth application of insecticide.

Table 5. Quality assessments at harvest for 20 plants per plot of broccoli sprayed with a range of insecticides, in terms of caterpillar damage

Treatments		% broccoli heads by quality category		
		prime grade	prime + processing	Unmarketable
Untreated	-	0.3 c	6.2 f	93.8 a
Success	50 mL/ha	2.6 c	33.6 e	66.4 b
Success	100 mL/ha	41.0 b	87.5 cd	15.5 c
Success	200 mL/ha	52.7 b	90.7 bcd	9.3 cd
Success	400 mL/ha	76.4 a	97.2 abc	2.8 de
Success	800 mL/ha	87.5 a	98.7 ab	1.3 de
Tokuthion	1.5 L/ha	89.9 a	100.0 a	0.0 e

All treatments applied with Agral® 600 at 10 mL/100 L. Values within columns, followed by a common letter, do not differ at the P=0.05 level of probability. Values quoted are re-transformed means from the $\sqrt{x}$ + 0.5 transformation.

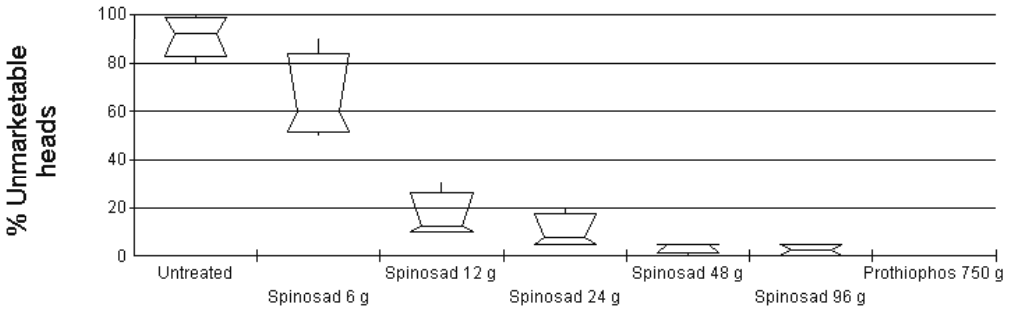

Figure 2. Percentage of broccoli heads classified as unmarketable due to insect feeding damage in field trial of spinosad.

Acknowledgements

This trial was conducted on behalf of Dow AgroSciences by John Hargreaves at the Queensland Department of Primary Industries research facility at Redlands.

References

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Studies of diamondback moth populations in Réunion Island (Indian Ocean)

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Introduction

Diamondback moth (DBM), *Plutella xylostella,* causes major damage to crucifer crops in tropical regions. In Réunion, these pests are currently managed by intensive chemical control methods, but this strategy generally does not sufficiently reduce crop damage levels and toxic pesticide residues can accumulate on consumed parts of the plants. We therefore conducted a field study to assess the impact of different chemical, biological and physical control methods on diamondback moth population levels.

Materials and methods

The following five field trials were carried out using Alta® cabbages:

Trial 1

We compared populations of DBM and associated parasitoids on two adjacent 300 m² plots: one plot was treated weekly with broad-spectrum pesticides (pyrethroids, organophosphates) and the other was an untreated control plot in which natural organisms could develop for potential biological control. These test plots were located at Bassin Martin, at 250 m elevation, in a region where monocropped sugarcane predominates. The test plots were monitored between March and May (at the end of the hot humid season).

Trial 2

As in the first trial, moth and parasitoid population patterns in a plot managed with supervised chemical pesticide treatments and in a control plot were compared. The treatments included alternating sprays with Batik® (*Bacillus thuringiensis*), Zolone® (phosalone) and Rocky® (endosulfan). The other experimental parameters were the same as in the first trial except that the monitoring period was between September and October (cool dry season).

Trial 3

DBM and associated parasitoid populations were monitored on a 400 m² plot located at Bras de Pontho, at 800 m elevation, in a region where vegetable and crucifer crops are mainly grown. No treatments were carried out on this plot, which was monitored from June to August (subtropical winter).

Trial 4

The test protocol was the same as the previous trial, but the plot was located at Piton Hyacinthe, at 1200 m elevation, with a monitoring period extending from April to June.

Trial 5

We assessed the efficacy of an insect net (Lutrasyl P17) to provide physical protection against adult DBM. Two plots planted with 10 cabbage plants from trial 1 were covered with the net immediately after planting.

Sampling

20 cabbage plants were sampled randomly once a week from each test plot, except in trial 5. II to IV instar larvae and cocoons were counted, isolated and reared in plastic boxes until parasitoid emergence after 15 days of incubation. Parasitism rates were calculated overall and separately for the main species. The dominant species of predators and hyperparasitoids were also recorded.

Results

Table 1 shows the auxiliary species detected in the different test plots. Three parasitoid species that are specific to *P. xylostella* and two Syrphidae (Diptera), including one undescribed species, were detected at

the four sites. However, the two nonspecific species of hyperparasitoid identified were only observed in plot 4, which was located at the highest elevation.

Table 1. Beneficial insects and hyperparasitoids observed on DBM in cabbage trials on Réunion Island

Category	Order	Species	Trials
Parasitoids	Hymenoptera	*Diadegma mollipla* (Holmgren)	ALL
		Cotesia plutellae (Kurdjumov)	ALL
		Oomyzus sokolowskii (Kurdjumov)	ALL
		Tetrastichus howardi (Oliff)	1, 2
Predators	Diptera	*Episyrphus* sp. nov.	ALL
		Melanostoma annulipes (Macquart)	ALL
Hyperparasitoids	Hymenoptera	*Notanisomorphella borborica* (Giard)	4
		Trichomalopsis oryzae (Risbec)	4

Figures 1 to 6 show the population dynamics of *P. xylostella* and its two main parasitoids (*Cotesia plutellae* and *Diadegma mollipla*) in trials 1, 2, 3 and 4.

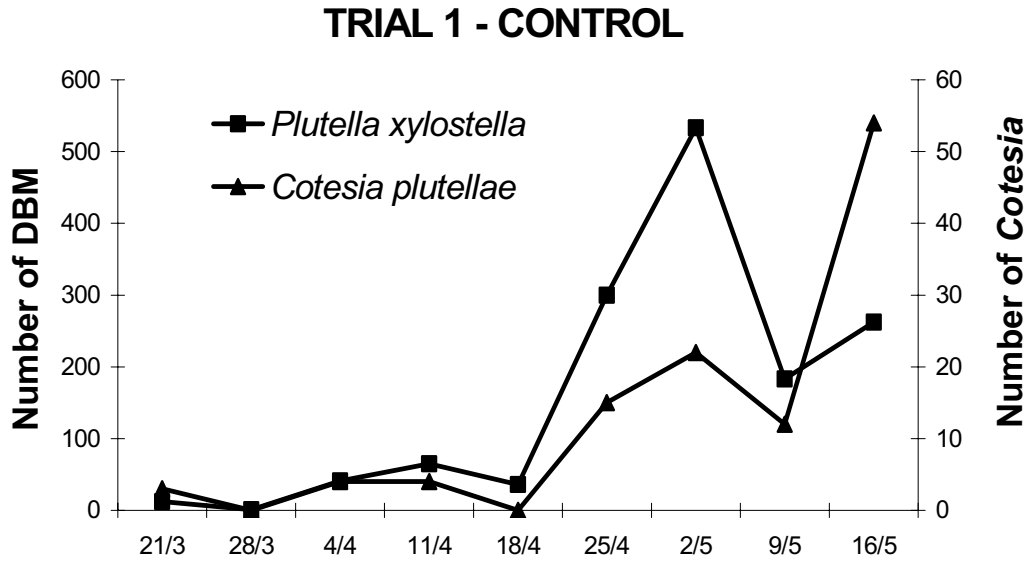

Figure 1. Dynamics of DBM and parasitoid populations in four trials on Réunion Island, a) Trial 1 - Control.

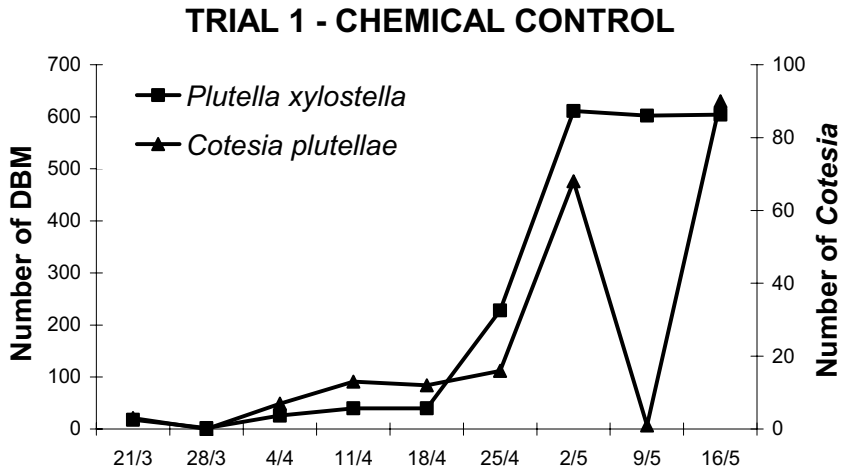

Figure 2. Dynamics of DBM and parasitoid populations in four trials on Réunion Island, b) Trial 1 - Chemical control.

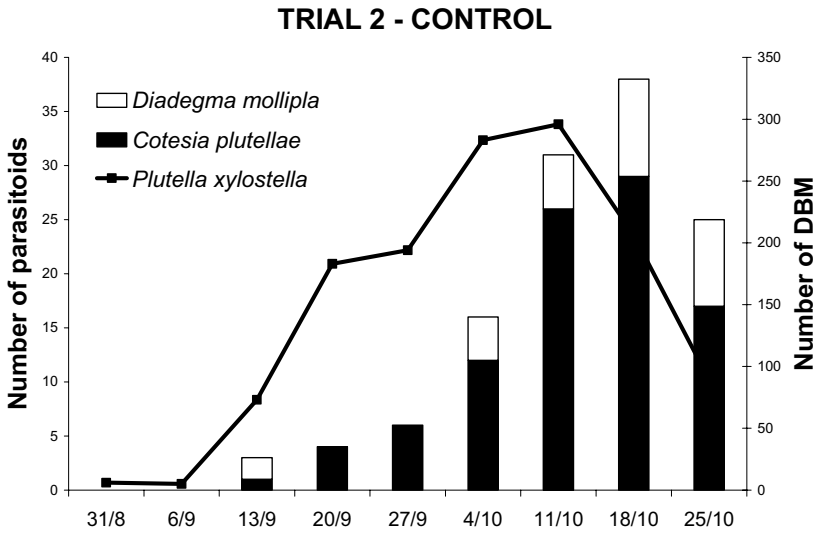

Figure 3. Dynamics of DBM and parasitoid populations in four trials on Réunion Island, c) Trial 2 - Control.

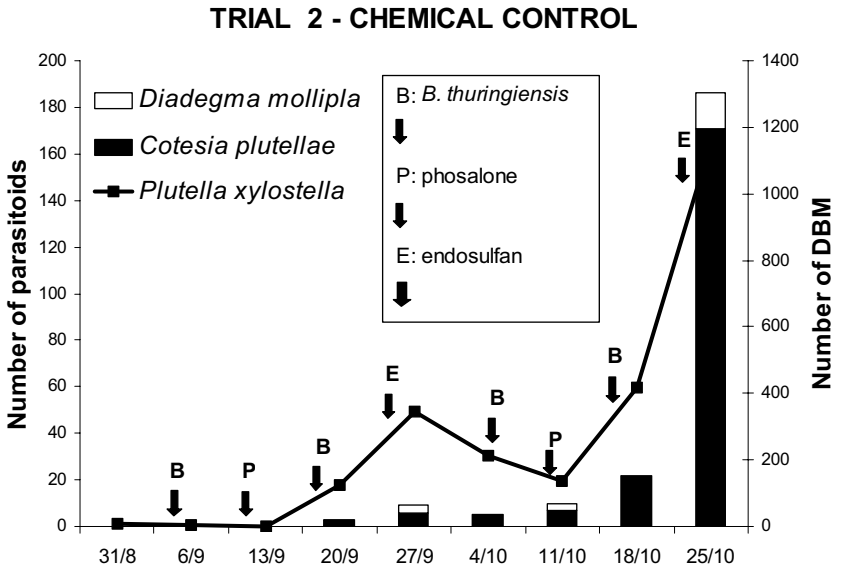

Figure 4. Dynamics of DBM and parasitoid populations in four trials on Réunion Island, d) Trial 2 - Chemical control.

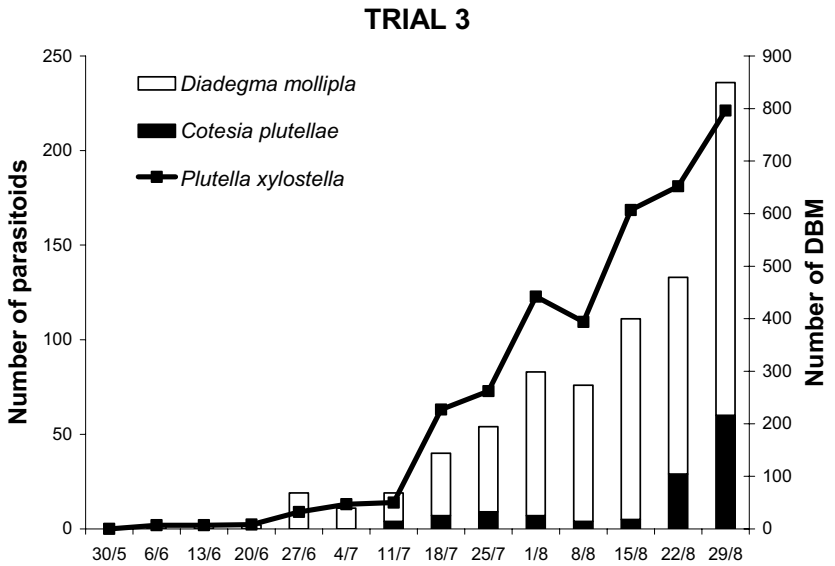

Figure 5. Dynamics of DBM and parasitoid populations in four trials on Réunion Island, e) Trial 3 - Control.

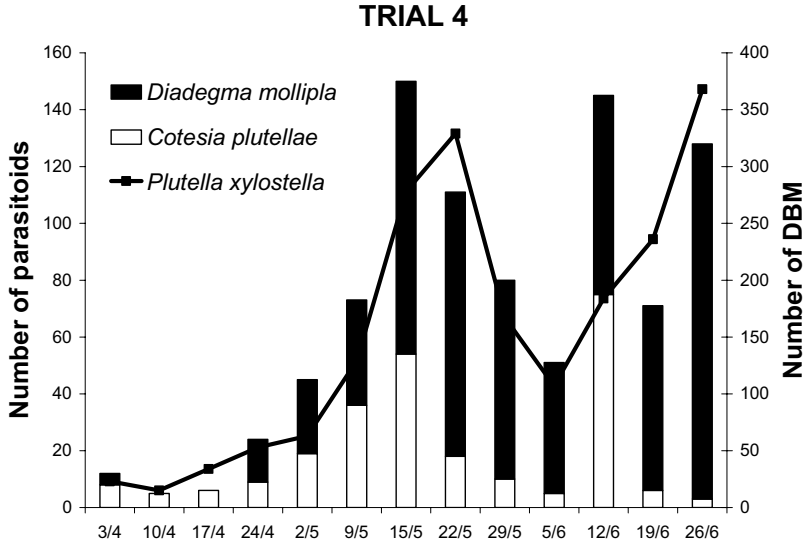

Figure 6. Dynamics of DBM and parasitoid populations in four trials on Réunion Island, f) Trial 4 - Control

Trial 1

D. mollipla populations were not taken into account in this trial. There was an increase in *P. xylostella* populations between the beginning of the cropping period until harvest, but with a more substantial increase in the chemically treated. This phenomenon could have been the result of the activity of auxiliary organisms unaffected by the pesticides. The *C. plutellae* population increased to a higher level in the treated plot, indicating that this parasitoid could be resistant to the broad-spectrum pesticides applied in this trial. Individuals derived from the control plot would nevertheless have been able to lay eggs between two pesticide sprays, therefore maintaining a high level of parasitism.

Trial 2

DBM populations gradually increased in the treated plot to reach a high level at the end of the cropping period (around 1200 larvae and cocoons on 20 plants). Only the first treatment with Rocky® seemed to temporarily reduce the populations, but this effect was not confirmed after the second treatment just prior to harvest. In the untreated plot, populations first increased and then sharply dropped at the end of the cropping period. Parasitism also increased in the two plots during the cropping period, but did not explain the

difference in patterns between these plots. We noted that *C. plutellae* accounted for most of the parasitism at this elevation (250 m).

Trial 3

On this plot, DBM and associated parasitoid populations steadily increased over the time course of the trial, but the impact of the latter was not sufficient to hamper crop damage. *D. mollipla* was the dominant parasitoid in this case.

Trial 4

Pest populations also generally increased in this trial, but there was a temporary decrease 1.5 months after planting. The parasitoid population patterns were similar, and *D. mollipla* was again the dominant species.

Trial 5

The results of this test were very disappointing, i.e. DBM was detected under the insect net (it was possibly not properly sealed, or eggs might have been laid on leaves in contact with the net) and the cabbages were substantially damaged (small size, yellow leaves, etoliated appearance that was probably due to poor lighting, temperature and humidity conditions).

Table 2 summarizes the number of parasitoids observed and parasitism rates calculated for the main species per plot, along with the overall rate. The parasitism rate ranged from 8 to 45% and increased with elevation.

Table 2. Parasitism rates (%) of DBM observed in four cabbage field trials on Réunion Island

Trial (altitude)	Chemical control	No of parasitoids	*Diadegma mollipla* (%)	*Cotesia plutellae* (%)	Global rate (%)
1. (250 m)	Yes (hard)	211	0.01	9.99	10
	No	116	0.01	7.99	8
2. (250 m)	Yes (soft)	235	1	9	10
	No	123	2	7	9
3. (800 m)	No	788	19	3	22
4. (1200 m)	No	901	33	13	44

Discussion

A relatively high number of auxiliary organisms were detected on *P. xylostella* in our study, whereas many other insect groups are scarce in Réunion due to the remoteness of this island. The hymenopteran *C. plutellae* was more common at low elevations whereas *D. mollipla* populations dominated at higher sites, as also confirmed elsewhere (*in litteris*).

In all trials, a high proportion of cabbages was not marketable due to damage caused by DBM, regardless of the pest management conditions (intensive chemical control, supervised control or natural biological control). These results demonstrate the inefficacy of the tested registered active ingredients against DBM populations in Réunion Island, indicating that these pests are likely resistant to several families of chemicals as a result of the longstanding unmanaged massive application of these compounds. Natural biological control is also not efficient enough, a situation that could worsen with the introduction of new beneficial insects. It was noteworthy that the chemical treatments seemed to have no impact on the parasitoids, indicating that these species could have also built up resistance.

The increase in the parasitism rate with elevation is perhaps associated with the fact that the cabbage cropping area is greater in the highlands (more suitable climate) and hence there would be a greater reservoir of parasitoids.

Insect nets are currently expensive, so their use on cabbage crops to hamper attacks by DBM would not be cost-effective. Moreover, the use of mini-tunnels to hinder moth egg laying and limit climatic constraints is also still too expensive to implement.

Implementing of an IPM programme for vegetable brassicas in New Zealand

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Abstract

In New Zealand, increasing levels of resistance in diamondback moth (DBM) to recommended synthetic pyrethroid and organophosphate insecticides were monitored for five years in vegetable *Brassica* crops until in 1997 they were associated with control failures in three regions. Subsequently, Crop & Food Research initiated a two-year IPM implementation programme with financial assistance from government and industry, including agrochemical companies. The IPM programme emphasizes the use of a reduced spray programme developed in the early 1990s based on a crop scouting system using presence/absence per plant of the major pests and a proven action threshold for cabbage. Further research has reconfirmed this cabbage action threshold, and broccoli and cauliflower action thresholds have been developed. The implementation phase involved training scouts and crop managers in insect identification and crop scouting techniques, and incorporated an accreditation system for trainees and a crop management recording system to document insecticide use in the IPM crops. Demonstration sites in three of the main *Brassica* growing regions were used for grower field days and to compare IPM practices with conventional pest management. Levels of resistance in DBM to the commonly used groups of insecticides were monitored and resistance levels were compared within a region as well as between regions. The IPM programme also recommends an insecticide resistance management rotation strategy with different chemical groups assigned to two different seasonal windows as well as the preferential use of selective insecticides to preserve natural enemies. An independent survey in November 2001 reported that 80% of growers in the main *Brassica*-growing region were using IPM and 96% were using crop scouting. Crop management records from IPM crops show an average saving of at least 50% in insecticide use compared with conventional crops.

Keywords

diamondback moth, integrated pest management, insecticide resistance

Introduction

Vegetable brassicas are the predominant fresh vegetable crop by area in New Zealand and have been one of the crops most frequently sprayed for pests. These frequent applications of insecticides led to the development of insecticide resistance to a level that has produced control failures for the key pest, diamondback moth (DBM), *Plutella xylostella* (Cameron & Walker 1998) and over-reliance on insecticides threatened the sustainability of *Brassica* crops. To counter this trend, an integrated approach to the control of pests in brassicas has been developed over the past ten years by Crop & Food Research (previously the Department of Scientific and Industrial Research (DSIR)) with funding from government and the New Zealand Vegetable and Potato Growers Federation (Vegfed). This integrated pest management (IPM) programme emphasizes the use of reduced spray programmes, alternation of insecticide groups, application of selective insecticides to preserve natural enemies, monitoring of insecticide resistance in DBM and improved communication with the industry.

The use of action thresholds and crop scouting in brassicas had previously been demonstrated to reduce pesticide applications by an average of 60% while improving crop quality (Beck *et al.* 1992). This research defined an infestation level at which spraying is economic (Beck & Cameron 1990) and provided an efficient crop scouting method to detect damaging populations (Beck & Cameron 1992). The scouting technique uses a "percent infested" threshold, which avoids the need to count pests and is easy for growers to use (Berry 2000). This technique was successfully demonstrated in commercial crops (Beck *et al.* 1992), but growers relied on increased levels of preventative sprays until insecticide applications began to fail to give control. An analysis of long-term trends in resistance from 1993 showed that by 1997, resistance in DBM to a standard synthetic pyrethroid, lambda-cyhalothrin, had reached levels in three regions well in excess of those associated with control failures in North America (Cameron & Walker 1998). The levels of

resistance recorded for the standard organophosphate, methamidophos, were also likely to cause control failures (Cameron *et al.* 1997).

The need for a specific implementation phase is crucial to the success of IPM programmes. Reviews of the world literature (e.g. Wearing 1988) indicate that successful adoption requires proof of both the IPM technology and an IPM implementation system, and emphasizes that the implementation phase requires repeated demonstration trials and training. For this purpose, an IPM implementation project proposal was developed by Crop & Food Research and funded by Vegfed and Technology New Zealand, a government agency. Funding was restricted to two years and the project began in November 1998. The project was based in Pukekohe, south of Auckland, the main vegetable *Brassica*-growing region in New Zealand, and in two regions on the east coast of the North Island, Gisborne and Hawke's Bay. The main objectives, activities and outcomes of the project are summarized.

Objectives and outcomes

Objective 1. Refining action thresholds

Field trials undertaken over the main growing seasons of 1998-1999 and 1999-2000 retested the original cabbage threshold and developed thresholds for broccoli and cauliflower. The trials at Crop & Food Research's Pukekohe Research Station tested thresholds at different crop growth stages and different times of the year and assessed the benefits of reduced spray applications and improved plant quality. Results of trials in year 1 confirmed the cabbage threshold developed by Beck and Cameron (1992) and a new broccoli threshold was proven (Berry, unpublished data). In year 2, the broccoli threshold was reconfirmed and cauliflower thresholds were tested.

The cabbage threshold is: Scout whole plant until head fill, then scout head plus four inner wrappers.

Action threshold for Lepidoptera: 15% infested plants; 12-14% infested, recheck in 3-4 days.

Action threshold for aphids: 10% infested with colonies.

The broccoli threshold is: Scout whole plant until past 6-8-leaf stage, then scout growing tip and inner leaves until floret formation. Protect the floret.

Action threshold for Lepidoptera: Seedling stage (30% infested), 6-8 true leaves to floret initiation (20% infested - growing tip only), then protect the floret (10% infested)

Action threshold for aphids: 10% infested with colonies. Results from trials in the 1999/2000 summer suggested that the threshold for broccoli could not be assumed to be the same for cauliflower, as previously proposed by Beck (1991). Observations of the scouting and harvest assessment data and the timing of insecticide applications suggest that the threshold for cauliflower should be: seedling (30%), 6-8 true leaf – curd initiation (20% - growing tip only); protect curd (5%). Further research is required to confirm this threshold before it can be endorsed. The results from the refinement of thresholds trials have been incorporated into an IPM manual for vegetable brassicas (Berry 2000).

Objective 2. Developing an insecticide rotation strategy

An accepted technique for limiting the development of insecticide resistance is restricting the use of particular insecticide groups to part of the year. Rotating the use of insecticides over an entire area in a "window strategy" based on calendar periods has proven to be an effective resistance management tactic (Roush 1989, Forrester *et al.* 1993). The implementation of this approach requires regional and national support for an agreed strategy while success requires the participation of a high proportion of growers.

A DBM insecticide resistance management-working group was formed in 1998. The working group comprised of researchers, growers, agrochemical company representatives and other industry personnel. An insecticide rotation strategy, based on options proposed for use in southern Australia, was recommended to ensure that DBM populations are not continually exposed to the same insecticides. This requires growers on a district basis to spray with chemicals in certain groups at certain times of the year (called windows). The strategy has two windows:
Early window - September to late January
Late window - February to August

This divides the year equally into the same number of DBM generations, based on average heat unit accumulations (P. J. Cameron, data not presented). Insecticides are assigned to windows depending on a number of factors, including their mode of action and cross-resistance patterns. Each of the windows includes a range of insecticides to provide a choice for controlling a range of pests (e.g. aphids and thrips) or for preserving natural enemies; Btk and Bta, for example, will kill caterpillars, but are harmless to other insects, including the predators and parasitoids of all the pest insects. This insecticide rotation strategy is presented in Table 1 and was published and updated in the New Zealand Commercial Grower (Walker 2001) and also in the IPM Manual (Berry 2000).

Table 1. Updated New Zealand diamondback moth insecticide resistance management rotation strategy for vegetable brassicas, November 2001

Early Window	**Late Window**
September - late January	February – August
Apply insecticides only in response to scouting thresholds	
Btk[1]	Bta[1] and mixture of Bta & Btk [1]
spinosad (Success Naturalyte®)	indoxacarb (Steward®)
	fipronil (Ascend®)
organophosphates	
	synthetic pyrethroids
endosulfan	
pirimicarb (aphids)	

[1]Apply Bt to small larvae on small plants

Objective 3. Training crop managers in insect identification and crop scouting

This objective was to train crop managers and potential commercial scouts in the necessary steps to identify pests, efficiently monitor their populations, assess natural enemies, select appropriate insecticides and apply them with good timing.

Fourteen scouts were trained in Pukekohe, six in Gisborne and four in Hawke's Bay. They undertook continuous, weekly scouting of crops, recording and reporting their findings to growers and giving spray or no spray recommendations using the Crop & Food Research action thresholds and following the insecticide rotation strategy. Trainees who successfully completed the scouting and reporting of two crop cycles and who passed laboratory and field assessments were accredited as crop scouts. If scouts were required by growers to recommend the application of insecticides it was a requirement of their accreditation that they were also "Growsafe" trained. The Growsafe course is a New Zealand quality assurance (NZQA) certified training course on the safe and effective use of agrochemicals.

Objective 4. IPM demonstration sites

The use of crop scouting was demonstrated annually in each region to quantify and illustrate the benefits of reduced spray programmes and insecticide rotation. The benefits were measured from scouting reports and audits by determining the frequency of spray applications, the extent of adoption of the insecticide rotation strategy, the quality of produce and also the degree of insecticide resistance. Demonstration sites were also used for scout training sessions; for practice in crop scouting, insect and disease identification; and for grower field days. Results from Pukekohe demonstration sites showed a 25 to 65% decrease in insecticide use in 1998-1999 and a 40 to 70% decrease in 1999-2000. Results from Gisborne showed a 50% reduction in insecticide use associated with crop scouting.

Objective 5. Monitoring insecticide resistance in diamondback moth

The susceptibility of 11-21 field populations of DBM to five insecticides was tested using a dose response leaf dip bioassay following techniques adapted by Cameron *et al.* (1997) from Tabashnik and Cushing (1987). Susceptibility levels were compared with those of the standard reference population (Pukekohe 1 strain) in New Zealand, which had not been exposed to any group of insecticides for at least 8 years. Trends were monitored over two years between and within four regions (Pukekohe, Gisborne, Hawke's Bay and Canterbury; South Island) using a synthetic pyrethroid, lambda-cyhalothrin (Karate®); an organophosphate,

methamidophos (Tamaron®); a Btk product (DiPel 2X®); a carbamate (Lannate®) and spinosad (Success Naturalyte®). Probit analysis was used to estimate the LC_{50} for each population and the standard population was included in each assay to minimize uncontrolled variables. To determine the degree of resistance, the LC_{50} of each population was compared with the standard New Zealand population and resistance ratios were calculated. Results for the standard synthetic pyrethroid and organophosphate are presented in Table 2. Some results from 1997 field surveys are also presented for comparison.

Table 2. Lambda-cyhalothrin (Karate®) and methamidophos (Tamaron®) resistance ratios for field populations of *Plutella xylostella* relative to the standard New Zealand population (Pukekohe 1) at 48 hours

		Lambda cyhalothrin			**Methamidophos**		
Collected	Population	LC_{50}	95% CI	RR	LC_{50}	95% CI	RR
1997	Pukekohe 2	0.006	0.004 – 0.009	4.9*	0.075	0.033-0.201	1.1
1997	Pukekohe 3	0.167	0.114 – 0.271	62.3*	0.246	0.188 – 0.323	2.7*
1998	Pukekohe 4	0.014	0.006 – 0.031	3.9*	0.204	0.113 – 0.385	1.9
1999	Pukekohe 5	0.008	0.003 – 0.019	3.7*	0.048	0.026 – 0.082	1.8
1999	Pukekohe 6	0.005	0.002 – 0.010	2.3	0.118	0.090 – 0.154	4.4*
2000	Pukekohe 7	0.010	0.007 – 0.013	13.2*	0.045	0.024 – 0.082	4.2*
2000	Pukekohe 8	0.022	0.012 – 0.039	29.2*	0.086	0.059 – 0.128	2.7*
2000	Pukekohe 9	0.091	0.055 – 0.152	9.7*	0.119	0.078 – 0.183	3.9*
1997	Gisborne 1	0.011	0.004 – 0.023	7.6*	0.204	0.083 – 0.501	1.7
1999	Gisborne 2	0.002	0.002 – 0.004	1.8	0.058	0.026 – 0.136	1.0
1999	Gisborne 3	0.001	0.0004 – 0.002	0.3#	0.092	0.064 – 0.132	1.7*
2000	Gisborne 4	0.012	0.007 – 0.021	6.4*	0.045	0.037 – 0.056	1.5*
1997	Hawke's Bay 2	0.006	0.003 – 0.009	4.2*	0.115	0.079 – 0.167	2.4*
1999	Hawke's Bay 3	0.001	0.0005 – 0.002	0.3#	0.065	0.030 – 0.125	1.2
1999	Hawke's Bay 4	0.002	0.0009 – 0.003	0.6	0.076	0.050 – 0.115	1.4
2000	Hawke's Bay 5	0.022	0.012 – 0.041	18.6*	0.034	0.021 – 0.054	2.1*
1999	Canterbury 1	0.008	0.002 – 0.025	8.5*	0.058	0.039 – 0.088	2.0*
1999	Canterbury 2	0.017	0.010 – 0.030	3.5*	0.158	0.052 – 0.481	3.6*

* significantly more resistant at the 95% significance level, # significantly more susceptible at the 95% significance level

Trends suggest that synthetic pyrethroid resistance is variable both between and within regions. Populations tested from Pukekohe (population 8) and Hawke's Bay (population 5) had levels of resistance that may be associated with control failures in the field. It is noteworthy that Pukekohe populations 5, 6 and 7 were collected within four kilometres of each other and had resistance ratios varying from a 2.3 to 29.2 fold difference from the standard population. These results demonstrate that resistance levels may be quite variable within a relatively small area. Two field populations (Gisborne 3 and Hawke's Bay 3) were more susceptible to lambda-cyhalothrin than the standard population. This is plausible because the susceptibility of the standard population has previously been compared with a standard susceptible north American DBM population (Geneva 88) collected from cabbage near Geneva NY. This New Zealand population (Pukekohe 1) was ten times more resistant to permethrin at the LC_{50} than the Geneva 88 population (Cameron *et al.* 1997). In addition, the susceptible population from Hawke's Bay (Hawke's Bay 3) was collected from an area that had been in organic production for ten years and where there had been little or no exposure to synthetic pyrethroid sprays.

Resistance levels to the standard organophosphate insecticide, methamidophos, appear to be relatively stable with a maximum of 4.4 fold resistance (Table 2). The range of resistance ratios for 13 field populations to the standard Btk (DiPel 2X®) was 0.4 to 2.4 fold. The resistance ratios for 11 populations ranged from 0.88 to 6.4 fold for the carbamate, methomyl, and 1.01 to 3.57 fold in 15 populations for spinosad (G.P. Walker and N.A. Berry, unpublished data).

Discussion

Action thresholds

Existing action thresholds have been improved to recognize different and more vulnerable plant growth stages, and now include thresholds for broccoli and proposals for cauliflower thresholds. The new IPM system reduces selection for insecticide resistance by defining pest thresholds that ensure spraying is initiated only when necessary. To detect these economic thresholds, efficient crop sampling systems developed by Beck (1991) have been instigated and methods are published in the IPM manual (Berry 2000).

Natural enemies

Reduced spraying and use of more selective chemicals have increased the control provided by natural enemies (G. P. Walker, unpublished data). Spray decisions can be modified to recognize the presence and build-up of these natural enemies. For example, if a pest population is close to the action threshold, but parasitoids and/or predators are in abundance, experience suggests that spraying may be delayed and the crop reassessed at a later stage.

Insecticide resistance in DBM

A key issue for the industry is managing or mitigating the effect of insecticide resistance in DBM. This can be achieved by growers and scouts adhering to the insecticide rotation strategy. Our surveys show that resistance levels vary within a region as well as between regions and between years. Therefore, growers need to be kept up to date with the insecticides that are working, and the locations and levels of resistance present. Continuous monitoring of levels of resistance in the field is necessary to provide growers and consultants with updated information.

Industry uptake of IPM for fresh market brassicas

In November 2001, an independent survey was undertaken to quantify the uptake of the IPM programme. Thirty *Brassica* growers in the Pukekohe region, those who contribute the large majority of the brassicas grown in the region, were asked a series of questions. Their responses are listed below.

- Are you scouting or having your crops scouted? – 29/30
- Are you using the Crop & Food Research action thresholds? – 24/30
- Are you using the recommended insecticide rotation strategy? – 27/30
- Are you rotating Success® and Steward®? – 24/30
- Are you rotating Btk with Bta or mixtures of Bta and Btk? – 17/30
- Are you using a scouting/consulting company? – 17/30

Using an IPM adoption definition that required the growers to answer yes to questions 1-4, 24 of 30 growers (80%) were considered to be using IPM and 96% were scouting their crops. In the other major *Brassica*-growing region, Gisborne, one company (Leaderbrand) now dominates the *Brassica* production in the district (growing 1000 ha of broccoli yearly). In Gisborne, more than 90% of the *Brassica* crops are being grown using the Crop & Food Research IPM programme. In Hawke's Bay, vegetable brassicas are not grown extensively, but key growers who were involved in the project are seeing the benefits of reduced spraying and are more confidently withholding sprays. They report a 50% reduction in insecticide use.

Benefits

We have demonstrated reductions in insecticide applications as well as increases in the use of more selective insecticides. The benefits of this approach include reduced selection pressure for insecticide resistance in pests as well as reduced exposure of applicators and reduced applications to adjacent cropping or non-cropping areas. In addition, appropriate rotation strategies extend the lifetime of new insecticide groups. Increased activity of biological control agents is associated with reductions in the need to spray. Finally, growers have benefited from improved marketing images for their crops as well as economic savings, mainly from reduced costs of insecticide applications.

Dollar savings are difficult to estimate. However, if the number of sprays required is reduced by 3-4 applications per ha, this reduction saves about $350 per ha. Costs of scouting at $30 per hour and savings from reduced spraying give a net saving of $125 per ha. About 4000 ha of cabbages, cauliflowers and broccoli are grown each year in New Zealand and approximately 65% are managed using IPM, giving total

savings from IPM scouting of crops of NZ$325,000 per year, not including qualitative benefits for the environment, worker health and in marketing.

The future for IPM in brassicas

The key to the success of the IPM programme is based on increasing uptake by growers who are prepared to continually monitor their crops, minimising the use of insecticides, and utilising the benefits of the insecticide rotation strategy in an area-wide, coordinated manner. The Crop & Food Research IPM programme for vegetable brassicas is now sufficiently developed for use by all cabbage, cauliflower and broccoli growers in New Zealand. For this technology to be continuously available to growers and other industry personnel (scouts, etc.), a key requirement will be to have adequately trained trainers available to train scouts to an acceptable standard.

IPM manual

The IPM manual for vegetable brassicas was published in November 2000 (Berry 2000). It documents the components of IPM in vegetable brassicas and includes photographs of all the insect pests and plant diseases and their natural enemies, methods and control strategies. It provides a standard reference against which the procedures used in a particular crop can be verified and audited as necessary. This manual ensures that the basis of decisions for the current programme and the improvement of any features for future modified IPM programmes are fully documented. It also provides a medium for accrediting scouts and involving growers, Vegfed and agrochemical companies in continually improving the technology in this programme.

In summary, IPM is now a major part of *Brassica* crop management, allowing year round sustainable production of high quality vegetable brassicas. The New Zealand vegetable industry is now in the unique position of being able to manage resistance and retain the use of insecticide groups that are now failing in some regions overseas.

Acknowledgements

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Integrating novel technologies for cabbage IPM in the USA: value of on-farm research

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Abstract

Integrated pest management (IPM), particularly for high-value crops, is often viewed by growers as a more risky approach compared to their current (conventional) pest control practices. In this article we present the results of a 3-year on-farm trial, which documents the expected benefits, and risks of IPM and a conventional pest management system for cabbage. In Minnesota, USA, *ca*. 500 ha of cabbage and cole crops are produced each year. Despite relatively few hectares, the high value of the crop (≈9,000 US$/ha), and high insecticide use (5-9 sprays/season), continues to create a demand for alternative pest management programs. Throughout the Midwestern states, cabbage is attacked by three important lepidopteran pests: *Pieris rapae*, *Plutella xylostella* and the cabbage looper, *Trichoplusia ni*. In the absence of insecticide resistance, as well as high parasitism rates of *P. xylostella* (80-90%), *T. ni* is often the most common, and most difficult insect pest to control. Much of our focus over the past 8 years, has been to: a) evaluate rapid presence/absence (binomial) thresholds that account primarily for *T. ni*, b) validate thresholds for use with "reduced-risk" insecticides such as SpinTor® (spinosad) and Proclaim®, with pyrethroid use as needed (e.g., lambda-cyhalothrin; Warrior®), and c) measure and communicate the value of IPM in on-farm trials.

Use of a 10% action threshold and the reduced-risk insecticides, consistently provided a lower proportion of plants infested with late-instar *T. ni* larvae and a higher percentage of marketable heads for the 3-year study (1998-2000), compared with a conventional system. The conventional system used by the grower included pest scouting only to determine the first spray. Thereafter, sprays were applied approximately every 10 days or when convenient. The IPM program resulted in the highest percentage of marketable heads, while reducing the number of insecticide applications in 1998 and 1999 (43-66%). Despite a slight increase in sprays in 2000 (3.0 vs. 2.6) for the IPM program, net profits were still highest for the IPM program in each of the three years, ranging from 16 to 107% over the conventional program. Expected utility analysis also revealed that the IPM program provided the highest expected net revenue ($973/ha) with the least risk, compared with the conventional strategy and untreated check. In summary, these results confirmed the economic benefit of an IPM approach by careful measurement of the benefits and risks of IPM and has been well-received by Minnesota growers.

Introduction

Integrated pest management (IPM) is a concept and practice that has the potential to improve productivity and/or reduce pesticide use for a variety of commodities in agriculture and forestry, as well as in schools, buildings and landscape settings within urban and residential areas. Essentially, IPM is a *management framework* that can be used by growers and consultants to employ all possible complementary pest control tactics to manage arthropod, pathogen, weed and vertebrate pests in a way that provides economic sustainability, is environmentally sound and is socially compatible. The IPM concept and components of IPM have received considerable research attention over the past 40 years (Kogan 1998). However, the research emphasis, often by necessity such as crises created by exotic pest outbreaks, has focused primarily on pest biology, economic injury levels, or the discovery and development of novel pest control tactics (e.g. Kogan 1998, Radcliffe & Hutchison 2002).

By contrast, there has been relatively little acknowledgement within the pest disciplines that the implementation of IPM *is a management, and specifically, a decision-making activity* (e.g. Carlson 1970, Moffitt *et al*. 1983, Plant & Stone 1991). Consequently, the benefits and risks of IPM are not always carefully measured or communicated to growers or end-users. As with other areas of agriculture, decision-making for pest control occurs with considerable uncertainty and risk, due to variable pest pressure, weather, crop price, etc. (Fleisher 1990). In addition, IPM is often more knowledge intensive than conventional systems and the burden to implement IPM is often left with the grower who must integrate new

IPM components with existing production systems (Plant & Stone 1991). Not too surprisingly, many growers, and those with high-value crops, often view IPM as a more risky option vs. conventional methods, which can include a substantial reliance on pesticide applications.

IPM risk-reward tradeoff

As one approach to illustrating the cost-benefit, or risk-reward value of an IPM approach, we adopted an economic risk analysis (Carlson 1970, Moffit *et al*. 1983) to analyse the results of a three-year on-farm implementation trial for cabbage IPM in Minnesota. Cabbage, broccoli and cauliflower continue to be important vegetable crops for Midwestern U.S. growers, for both processing and fresh-market (Eastman *et al*. 1995). Annually, approximately 500 ha of cabbage and cole crops are produced in Minnesota for fresh-market (grocers and roadside stands, WDH, unpublished data). Despite relatively few hectares in Minnesota, the high value of the crop (≈$9,000/ha) and traditionally high insecticide use of 5-9 applications/season, continue to create a demand for alternative insect management programs. In Minnesota, and throughout the Midwestern U.S., cabbage is attacked primarily by three lepidopteran pests: imported cabbageworm (ICW), *Pieris rapae*; diamondback moth (DBM), *Plutella xylostella* and the cabbage looper (CL), *Trichoplusia ni* (Eastman *et al*. 1995, Hines 1998). DBM is usually most abundant on early-season plantings, with ICW and CL becoming the dominant pests in late June to August. In the late 1960s to early 1970s, ICW was the dominant lepidopteran cabbage pest in Minnesota (Weires & Chiang 1973). However, throughout the 1990s, CL has become the most dominant insect pest (Hines & Hutchison 2001). In the absence of insecticide-resistant DBM, as well as high parasitism rates of DBM by *Diadegma insulare* (typically 80-100%; Hines 1998), our experience has been that CL is often the most difficult pest to manage in Minnesota. Thus, much of our focus over the past 8 years, has been to a) evaluate action thresholds that account primarily for CL and ICW, b) use simple-to-implement presence/absence thresholds (% of plants infested with one or more lepidopteran larvae), c) validate the thresholds for use with "soft" insecticides such as *Bacillus thuringiensis,* SpinTor®, and Proclaim®, and finally, d) assess the use, implementation and value of IPM in on-farm trials.

SpinTor® was initially selected as the primary alternative insecticide because of its known efficacy against CL (Liu *et al*. 1999) and the potential reduced impact on the diversity of natural enemies (predators and parasitoids) of the entire lepidopteran pest complex (Sparks *et al*. 1998). SpinTor® (spinosyns) is derived from the *Actinomycete* bacterium, *Saccharopolyspora spinosa* (Sparks *et al*. 1998).

In this article, we summarize an economic risk analysis for several novel, biologically-based insecticides, including SpinTor 2SC® (i.e. spinosad, Success®, Dow AgroSciences) and Proclaim® (Syngenta Corp.). Moreover, we also use these results to document the expected profits as well and economic risk (variability in profit) associated with the on-farm implementation of the Minnesota Cabbage IPM Program.

Materials and methods

Given the positive results from previous action threshold validation studies in 1996-1997 (Hines 1998, Hines & Hutchison 2001), we developed and implemented an IPM program with Pahl's Markets, one of the leading fresh-market growers in Minnesota. Our purpose was to compare an IPM program, based on previous experiment station (small plot) research, with conventional grower practices. The Pahls allowed us to use small sections of several commercial fields. During each of the three years (1998-2000) we established 6 commercial field sites (replications), with 3 treatments in each: IPM, Conventional and Untreated Check (= "Do Nothing strategy"). During each year of the study, plot size for each replication of each treatment was 390 m^2 (0.35 ha). All data were initially analysed using a randomised complete block design and one-way Analysis of Variance (SAS 1988). The 3-year data set was also analysed using expected utility analysis to determine average (expected) gross and net profit/ha, as well as the standard deviation of gross and net revenue/ha, as a measure of the risk associated with the IPM and Conventional pest management systems (Moffitt *et al*. 1983; Burkness *et al*. 2002).

The IPM program included the use of: presence/absence action threshold, the biologically based insecticides SpinTor 2SC® and Proclaim® (2000 only) early in the season to conserve natural enemies, and pyrethroids during late-season, in response to excessive insect pest pressure. Unlike previous threshold systems used in the U.S. and Canada, adjusted for pest species or vegetative vs. head growth stages (e.g. Eastman *et al*. 1995), we used a season-long action threshold of 10% of the plants infested with one or more CL early instar larvae/plant (Hines 1998). Despite this conservative threshold, validation studies indicated that because CL is the most difficult pest to control, the threshold worked well for CL as well as DBM and ICW

(Hines & Hutchison 2001). As noted in the current Minnesota guidelines, if CL is not present, and either DBM or ICW are present, we used the thresholds of 50% of the plants infested with larvae (early instars for ICW) until heading, then a 10% larval infestation from heading to harvest (Hines & Hutchison 2001).

All plots were monitored twice/week during July and August of each year, when pests were most active. For each replication of each treatment, a minimum of 30 heads was selected at random and examined for larvae of each pest species. The percentage of plants infested with one or more eggs and/or larvae for each pest was recorded. To better anticipate increasing CL infestations, the number of CL eggs was also noted. Pest monitoring and decision-making for the IPM plots was done independently of the grower, making every effort to not influence decision making by the grower. Every effort was made to keep the IPM decision-making confidential (blind test). The grower was responsible for all decision-making and spraying of the conventional plots. When treatment was necessary in the IPM plots, sprays were applied by one of the authors (ECB) using a back-pack sprayer fitted with a 3-m boom and 6 nozzles over 3 rows of cabbage (2 nozzles/row). This system was calibrated to apply 187 L water/ha. When treatments were necessary in the Conventional plots, one of the authors (GP) made the necessary applications using a tractor-mounted 19.3-m boom, with 38 nozzles. This system also included 2 nozzles/row and was calibrated to deliver 187 L water/ha.

At harvest, 10 heads/replication of each treatment were randomly selected, weighed and evaluated for marketability using a standard 1-6 scale (Greene *et al*. 1969), where: 1 = no damage; 2 = minor feeding damage on wrapper or outer leaves; 3 = moderate insect feeding on wrapper or outer leaves with no head damage; 4 = moderate insect feeding damage on wrapper leaves and minor head damage; 5 = moderate to heavy feeding on wrapper and head leaves with head having numerous scars, over 30% of leaf area eaten; and 6 = considerable insect feeding on wrapper and head leaves with head having numerous feeding scars, over 30% of leaf area eaten. These data were also analysed by one-way ANOVA.

Results and discussion

Use of the 10% action threshold and the biologically-based insecticides, SpinTor® (1998-2000) or Proclaim® (2000), in the IPM program, consistently provided a lower proportion of plants infested with late instar CL larvae (Figure 1, Table 1) and statistically higher marketability ratings (lower the number the better; usually <2.1) over the 3-year study (Table 1). Yields were significantly higher in the IPM plots vs. Conventional in 2000 only. The percentage of marketable heads, a function of both marketability rating and head weight, was more variable and not statistically different between the IPM and Conventional systems. However, the numerical differences in percent marketable heads, as illustrated in the frequency histogram (Figure 2) occur more consistently at the high end for the IPM program (3 years combined). Head contaminant levels were significantly lower in both the IPM and Conventional systems compared with the untreated check, for all 3 years of the study (Table 1).

Seasonal mean summaries of the impact of the IPM program, compared with the Conventional system are summarized in Table 2. Because of unique pest pressure each year, the number of sprays and control costs were variable, having a combined effect on final yields, marketability and profit. Despite higher control costs in 2000 for the IPM program, marketable yield was the highest ever (18.5% more than the Conventional). Subsequently, we observed the highest increase in net revenue in 2000, 107% higher than the Conventional system. Increases in net profit for the IPM program in 1998 and 1999, at 15.8 and 58.6%, respectively, are also notable. As noted in the 2nd row of Table 2, the IPM program incurs an annual, additional cost of $30/ha for scouting (pest monitoring fee). The added profits accrue despite this added cost, and the fact that the biologically based insecticides (SpinTor® and Proclaim®) are about three times more expensive than the pyrethroid (Warrior®) used by our grower.

We attribute the increased profit of the IPM program to more frequent, consistent scouting (twice per week), the use of a knowledgeable crop consultant (M.S. Entomology with 5 years vegetable IPM experience), and the ability to immediately apply an insecticide (within 24 h), when needed. To review, the Conventional system in this case relies on grower-based decisions (B.S. Agronomy; 20 years production experience), where scouting is typically employed only for the first spray; subsequent sprays are made every 10 days, or at times, when convenient for the grower. A good example of excellent timing, in a relatively low pest pressure year, was 1999, where only one IPM spray was applied (SpinTor®, Figure 1, Table 2).

Table 1. Mean (±SE) of seasonal infestation levels and yield and marketability data for the three-year on-farm trial; Apple Valley, Minnesota, U.S.A., 1998-2001

	1998			1999			2000		
	IPM	**Conv.**	**Check**	**IPM**	**Conv.**	**Check**	**IPM**	**Conv.**	**Check**
Cumulative season larval infestation[1]	4.29 ± 0.84 a	10.46 ± 1.51 b	21.04 ± 2.51 c	1.46 ± 0.28 a	11.41 ± 1.37 b	15.75 ± 1.97 b	3.44 ± 0.71 a	5.06 ± 0.83 a	10.33 ± 1.49 b
Marketability rating[2]	1.17 ± 0.04 a	1.53 ± 0.06 b	2.21 ± 0.08 c	1.47 ± 0.05 a	1.89 ± 0.08 b	4.02 ± 0.08 c	2.12 ± 0.08 a	2.67 ± 0.10 b	3.59 ± 0.09 c
Yield (kg/5 heads)	4.59 ± 0.22 a	4.28 ± 0.25 a	4.10 ± 0.21 a	4.91 ± 0.15 a	4.79 ± 0.21 a	4.93 ± 0.16 a	5.15 ± 0.17 a	4.49 ± 0.27 b	4.81 ± 0.20 ab
% Marketable heads[3]	97.83 ± 1.14 a	96.33 ± 1.96 a	87.33 ± 5.14 b	96.00 ± 1.84 a	91.00 ± 2.38 a	27.00 ± 6.06 b	81.83 ± 6.07 a	63.33 ± 10.78 a	38.33 ± 13.46 b
Contaminants[4]	0.00 ± 0.00 a	0.01 ± 0.01 a	0.11 ± 0.03 b	0.04 ± 0.03 a	0.03 ± 0.02 a	0.51 ± 0.16 b	0.09 ± 0.04 a	0.18 ± 0.06 a	0.77 ± 0.19 b

ANOVA based on 6 replications; means in a row, for each year, followed by same letter are not significantly different; REGWQ (*P*=0.05). Mean percentage of late instar cabbage looper and marketable heads were transformed using the arcsine transformation; contaminant counts were transformed using the square root transformation prior to ANOVA and mean separations using REGWQ (*P*=0.05); back transformed means are presented.

[1]Percentage of plants infested with late instar cabbage looper.

[2]U.S. 1-6 scale (Greene *et al*. 1969); 1 = no damage; 2 = minor feeding damage on wrapper or outer leaves; 3 = moderate insect feeding on wrapper or outer leaves with no head damage; 4 = moderate insect feeding damage on wrapper leaves and minor head damage; 5 = moderate to heavy feeding on wrapper and head leaves with head having numerous scars, over 30% of leaf area eaten; and 6 = considerable insect feeding on wrapper and head leaves with head having numerous feeding scars, over 30% of leaf area eaten.

[3]Number of marketable heads per 30 heads.

[4]Number of larvae and/or pupae per head (one or more pest species).

Table 2. Mean impact of the Cabbage IPM Program on number of sprays, control costs, marketable yield and increase in net revenue (profit), during three-year on-farm trial, Apple Valley, Minnesota, 1998-2001

	1998			1999			2000		
	Conv.	**IPM**	**Chk**	**Conv.**	**IPM**	**Chk**	**Conv.**	**IPM**	**Chk**
Number of sprays	7	4	--	3	1	--	2.67	3	--
Control costs $ /ha (spray/sampling costs)[1]	302/0	227/30	0/0	130/0	94/30	0/0	87/0	202/30	0/0
Marketable yield (%)[2]	--	+1.50	-9.00	--	+5.00	-64.00	--	+18.50	-25.00
Reduction in sprays (%)	--	43	--	--	66	--	--	+12	--
Reduction in control costs (%)	--	15	--	--	5	--	--	+167	--
Increase in net revenue (%)[3]	--	15.8	--	--	58.6	--	--	107.3	--

[1]Insect management costs for sprays is calculated using the following prices for Insecticides: SpinTor 2SC® (15 oz./ha) = $67.35/ha; Proclaim® (7.9 fl oz/ha) = $66.0/ha; Warrior® T (8 oz./ha) = $21.28/ha; Lannate® SP (2.5 lbs./ha) = $60.00/ha; and Surfactants: Bond (12 oz./ha) = $2.65/ha; Dyne-Amic (32 oz./ha) = $16.33/ha; Kinetic (8 oz./ha) = $5.45/ha; multiplied by the appropriate number of sprays. A $10.00/ha application fee was added for each application.

[2]Marketable yield refers to the increase or decrease in yield compared to the conventional plots.

[3]Increase in net revenue is calculated by comparing overall net revenue of a conventional system to an IPM system.

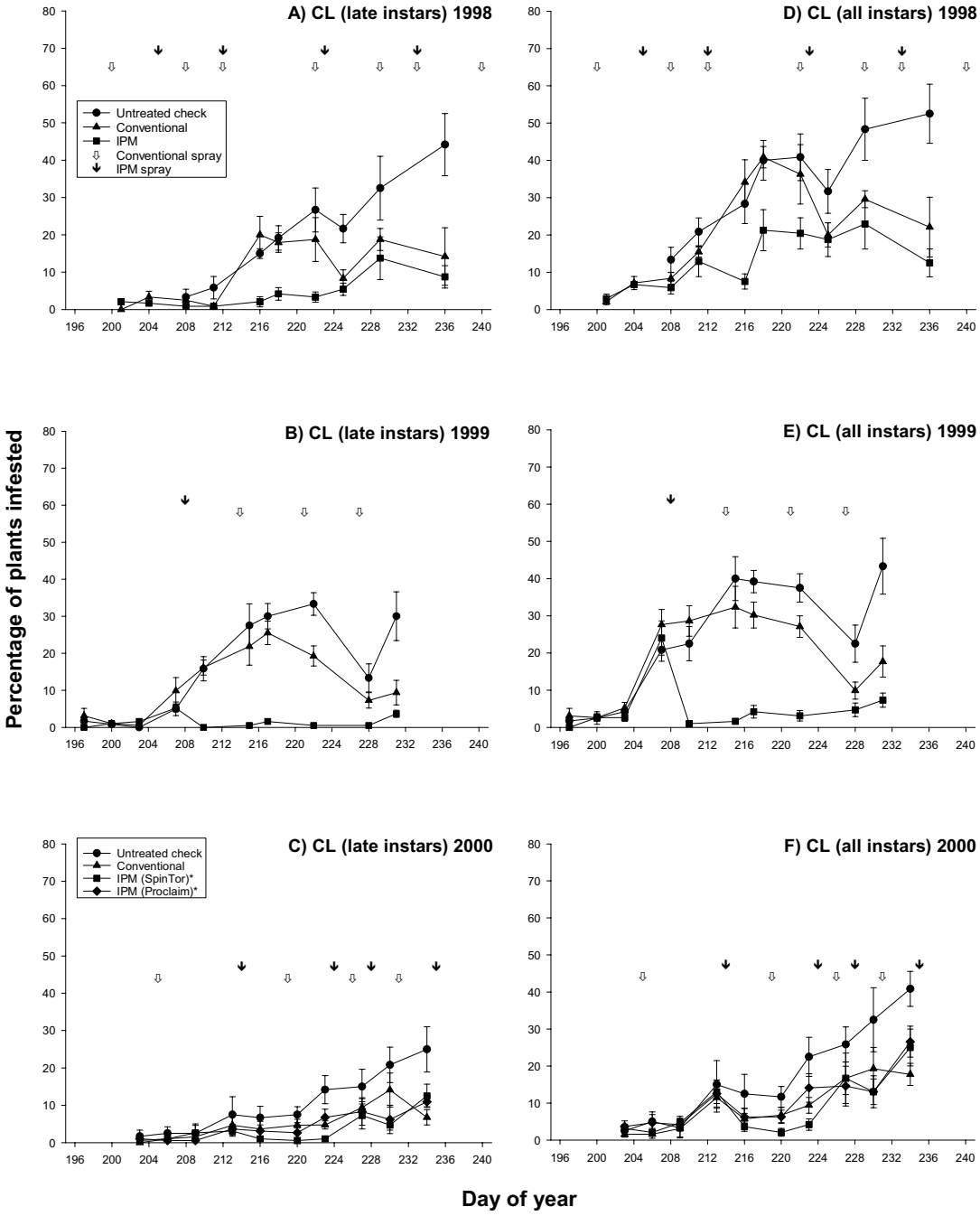

Figure 1. Phenology of cabbage looper (*Trichoplusia ni*) infestations for each management system, 1998-2000 (arrows refer to timing of sprays for each system, each year).

IPM risk-reward tradeoff

In addition to the traditional measures of economic impact, expected utility (Carlson 1970) and stochastic dominance (Moffitt *et al*. 1983, Burkness *et al*. 2002) methods were used to assess the overall risk of the IPM vs. Conventional programs. This type of analysis allows for a comparison of IPM and conventional control strategies, which incorporates economic benefits and a measure of risk (i.e. standard deviation and coefficient of variation of the expected value). This analysis (Table 3) revealed that the IPM program provides 6.4X higher profits ($973/ha) over the Conventional system ($151/ha). Moreover, the IPM program has about 46% less risk (lower standard deviation) over the Conventional system (Table 3). The untreated check, which reflects a "do nothing" strategy, shows that on average a grower would lose $2,949/ha by ignoring insect pests.

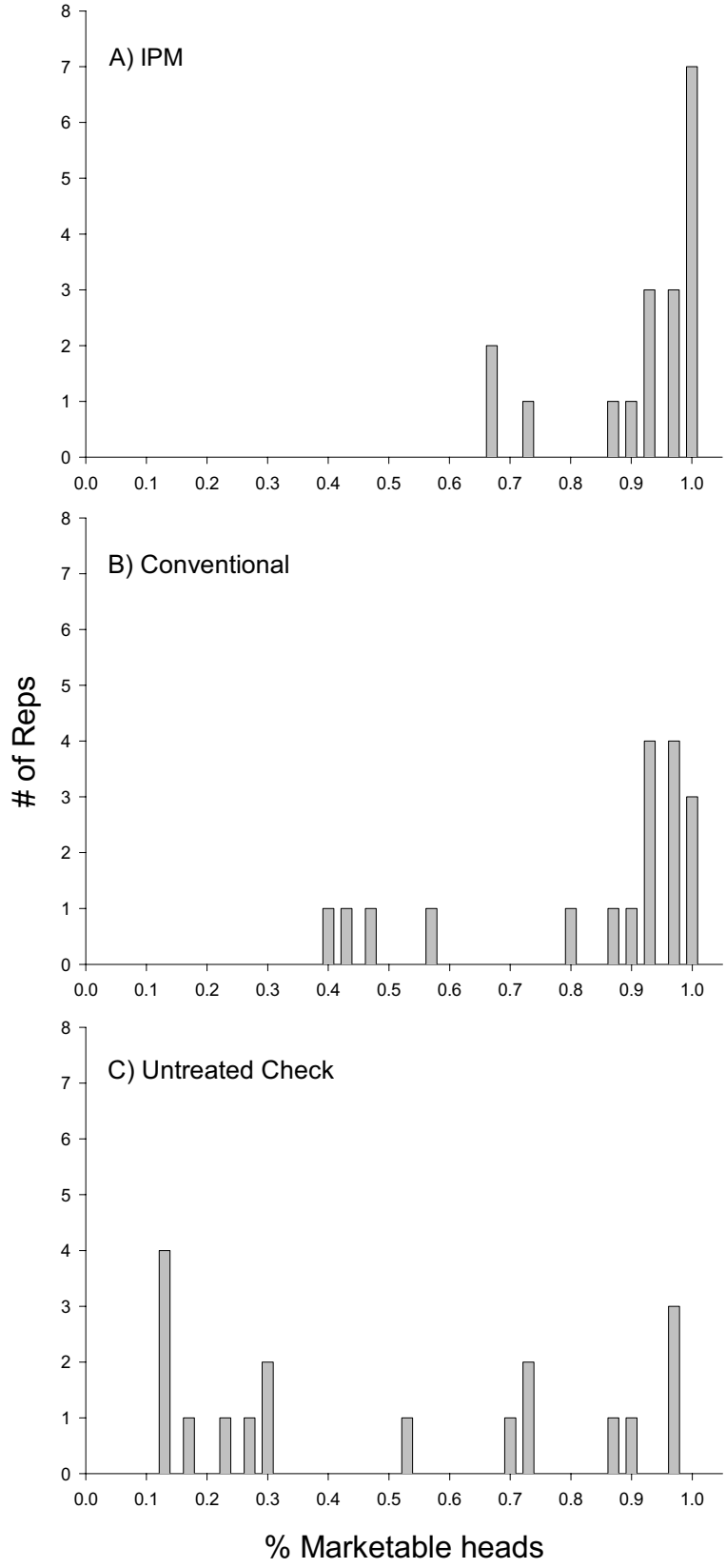

Figure 2. Frequency of marketable cabbage heads for three management systems, 1998-2000.

Table 3. Expected utility and standard deviation (risk) ($/ha) for various pest management strategies, cabbage on-farm trial, Apple Valley, Minnesota, USA, 1998-2000

	Expected value[1] (US$/ha)	Standard Deviation[1] (US$/ha; Risk)	Coefficient of Variation
Gross Revenue			
IPM	9,258	1109	0.12
Conventional	8,418	2062	0.25
Untreated Check	5,133	3317	0.65
Net Revenue			
IPM	973	1040	1.07
Conventional	151	1944	12.90
Untreated Check	-2,949	3285	1.11

[1]Expected value (utility) and standard deviation calculated according to the formula of Carlson (1970) and Burkness *et al*. 2002.

In addition to the expected utility results, we used the pooled 3-year data set to further assess the variability in percentage marketability (Figure 2) for each pest management system. These data were then used to calculate the distribution of gross and net revenue for each system. Here, we present the cumulative probability distribution functions (CDF) for gross and net revenue per ha (Figure 3). Formally, this analysis is known as stochastic dominance (Moffitt *et al*. 1983) and simply incorporates the variability (risk) of each system, for every possible revenue outcome. The results for this study indicate first degree stochastic dominance (FSD), with no overlap or intersection of the CDFs for each strategy. Thus, the CDF that is farthest to the right-hand side of the graph (IPM system) is the preferred, most profitable system.

Given these results, expected utility theory therefore suggests that both risk-averse and risk-taking growers should be very motivated to adopt an IPM approach for cabbage (e.g. Fleisher 1990). If the IPM approach had higher net revenue but also high risk (i.e., standard deviation greater than the Conventional), most risk-averse growers might be inclined to stay with the Conventional system. With this example, incorporating the field and pest infestation variability over three seasons, the risk and reward analysis is clearly in favour of the IPM program, with both the highest expected net profit and lowest profit risk.

In summary, the IPM program provided the highest expected profit with the least profit risk (net revenue variability) compared with the Conventional strategy and untreated check (Table 3). These results confirm that the economic benefits of an IPM approach for cabbage are sustainable over time, despite variable pest and weather dynamics. As a key component of this analysis, the results also highlight the value of having an experienced crop consultant involved with IPM decision-making. In this system, much of the consulting value was attributed to advanced IPM knowledge, but also availability (scouting twice per week when needed). The grower was often too busy with other production and marketing activities to devote the time necessary for pest monitoring. The initial, preliminary response by Minnesota vegetable growers to these results has been very positive. The results tend to fit their perspective on production risks, providing answers to questions like, …"IPM is too risky and crop consultants cost too much; how can I experience a profit?" or,… "how often will I see increased profits?" Via new outreach efforts, including field days and easy-to-use brochures, we plan to use this information to better communicate the value of IPM and further the adoption of cabbage IPM in the Midwestern U.S.

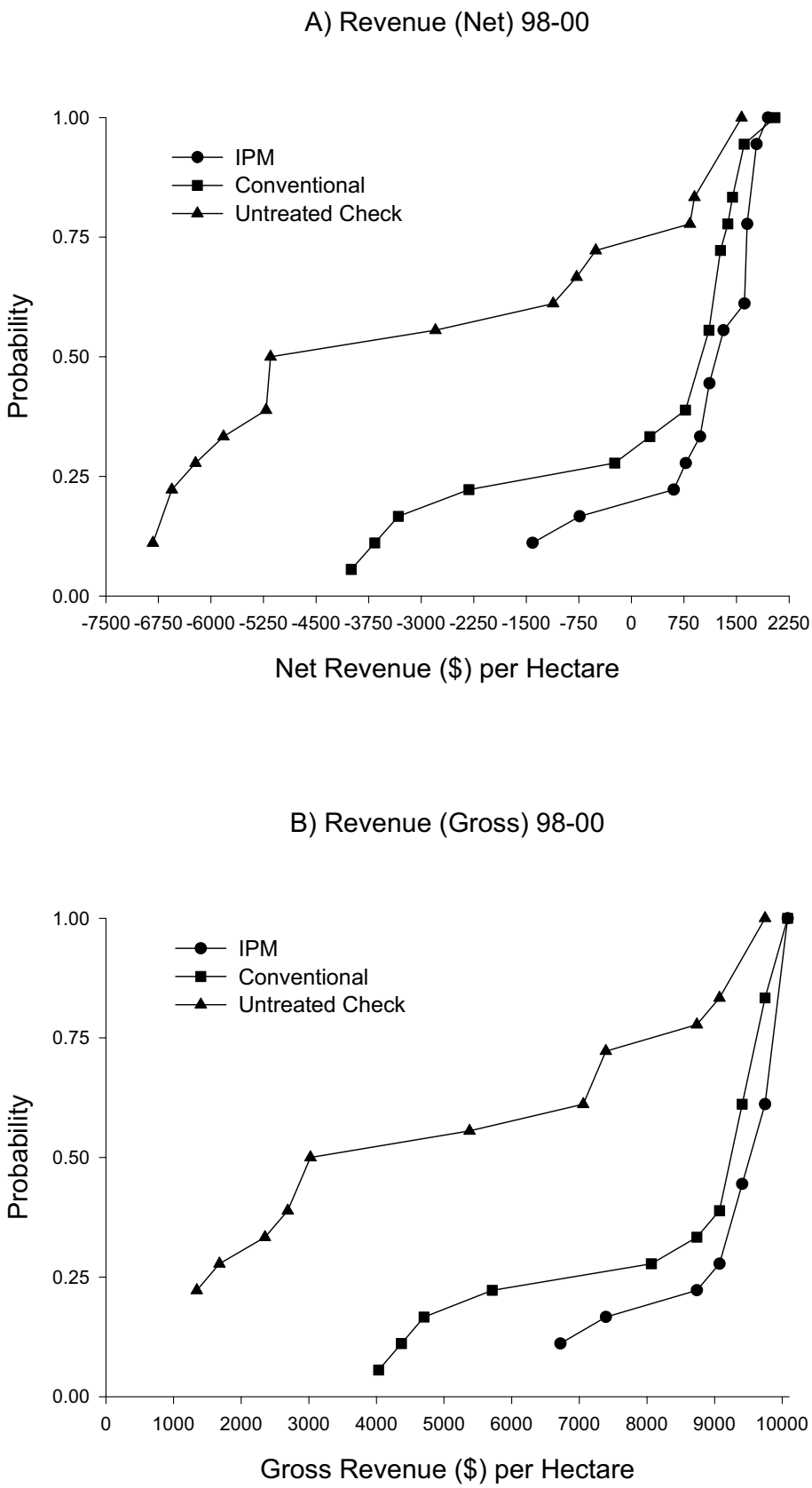

Figure 3. Cumulative probability distribution functions (CDF) for net and gross revenue based on observed frequency histograms for each cabbage management system, 1998-2000.

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Ecological impact of *Brassica* IPM implementation in Indonesia

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Abstract

In Indonesia, *Brassica* vegetables such as cabbage and Chinese cabbage are heavily infested with diamondback moth (DBM), *Plutella xylostella* (L.) and sometimes with cabbage head caterpillar (CHC), *Crocidolomia binotalis* (Zeller). *Diadegma semiclausum* Hellén is the most important larval parasitoid of DBM and becomes a key component of IPM programs on DBM. In 1999, studies were conducted at Lembang (RIV) and at Pangalengan in West Java to observe the ecological impact of IPM implementation versus a conventional pest management system on cabbage, using a paired treatment comparison. The faunal diversity in sweep net samples was higher in the IPM system compared with the conventional system (expressed by D values: 0.83 at Lembang and 0.59 at Pangalengan). The population of *D. semiclausum* increased in the IPM system by 34% at Lembang and 53% at Pangalengan. The abundance of soil dwelling predators (Coleoptera, Araneida, Hemiptera and Orthoptera) in the IPM system at Lembang and Pangalengan, increased by 84% and 68% respectively. Two species of soil microorganisms known as important biological control agents, namely *Trichoderma* sp. and *Bacillus* sp., were higher in the IPM system. IPM implementation on cabbage was superior to the conventional system in terms of (i) reduction of costs for insecticide usage by 79% at Lembang and 64% at Pangalengan and (ii) increase in marketable yield by 57% at Lembang and 19% at Pangalengan in the IPM system.

Keywords

cabbage, faunal diversity, *Plutella xylostella*, *Crocidolomia pavonana*, *Diadegma semiclausum*

Introduction

The Food and Agriculture Organization of the United Nations (FAO) (1989) defines sustainable agriculture and rural development as: “The management and conservation of the natural resource base, and the orientation of technological and institutional change in such a manner as to ensure the attainment and continued satisfaction of human needs for present and future generations. Such sustainable development conserves land, water, plant and animal genetic resources, in an environmentally, non-degrading, technically appropriate, economically viable and socially acceptable manner.” The definition stresses that management of resources can meet the human needs without destroying environment and should enhance the quality of the environment.

“Sustainable farming system” has become a popular term and is a most important issue in the field of agriculture to those people who have an interest in agricultural sustainability and who are concerned about conventional farming systems that rely heavily on synthetic chemicals and other off-farm inputs (Untung 1995). The problems associated with conventional farming practices are now widely recognised and include pest resistance to pesticides, the problem of pesticide residues in food, human and environmental hazards and harmful side effects for beneficial insects (Sastrosiswojo 1996b).

Arthropod biodiversity is an important component in the development of sustainable agriculture because of its role in maintaining soil fertility, crop pollination and control of arthropod pests (Sosromarsono & Untung 2001). Biodiversity forms an important part of an agroecosystem that includes both the living organisms and non-living components that interact within cultivated fields (Ooi 1997).

Overcoming the problems created by conventional farming systems has been by the implementation of integrated pest management (IPM), which is also considered as part of a sustainable farming system (Untung 1995). The general objective of IPM programs has been the development of improved ecologically-oriented pest management systems that optimise, on a long term basis, costs and benefits of crop protection (Huffaker & Smith 1980).

In Indonesia, the IPM concept was officially adopted in 1979 and was followed with the strengthening of the plant protection service. Following the Presidential Decree No. 3/1986, which banned 57 broad-spectrum pesticides for use on rice, the National IPM Programme was launched in 1989 for rice and secondary food crops (soy bean) and, in November 1991, for vegetables (Sastrosiswojo 1996a). An evaluation of the benefits of the IPM training program for cabbage growers in 1994 showed significant results such as: (1) reduction of insecticide applications (61–81%); (2) rate of DBM parasitism increased by up to 80%; (3) marketable yields were higher (8–16%) and (4) profit increased by $834/ha, in the IPM system versus the conventional system (Sastrosiswojo 1996b). Results of a recent study to assess the impact of IPM training on cabbage growers (Londhe *et al*. 1999) showed that IPM training had a positive and significant impact on cabbage yield. In addition, detailed costs and returns analysis by location (East Java, West Java and North Sumatra) showed higher yields and net revenues for farmers with IPM training.

Implementation of the IPM concept and technology on cabbage, through conducting IPM Farmer Field Schools (FFS) in ten provinces of Indonesia since 1992 to 1999, proved that the IPM system provided a higher net revenue when compared with conventional farmers' practice. The IPM system also required fewer numbers and amounts of pesticide sprays, but was able to secure healthy and higher quality of cabbage yields. More importantly, less pesticide residues were left on the harvested cabbages (Sastrosiswojo 1995, 1996b; Londhe *et al*. 1999). However, the ecological impact of cabbage IPM implementation has never been studied.

This paper evaluates the ecological impact of IPM implementation on cabbage in Bandung district, Indonesia. The biodiversity of insect fauna in the cabbage community treated with different pest control strategies (an IPM system versus a conventional system) was studied.

Materials and methods

In 1999, field experiments were conducted at two locations in Bandung District, West Java: at the Vegetables Experiment Station of Research Institute for Vegetables (RIV) at Lembang and in a farmer's field at Pangalengan. Cabbage (cv. Green Coronet) was planted on July 15, 1999 at Lembang and on August 17, 1999 at Pangalengan. Cabbages were harvested on September 24, 1999 (at Lembang) and on November 17, 1999 (at Pangalengan). In each location, the experiment used paired treatment comparisons (Chiarappa 1971) to compare the IPM system with the conventional system (designated Non-IPM) (Table 1). The treatments tested in each location were: (a) IPM technology developed at RIV Lembang (Sastrosiswojo 1995) and (b) Conventional system (Non-IPM), using application of agronomic factors and pest control commonly practised by the local farmers. Each plot size was 500 m^2 and contained approximately 1400 cabbage plants. Each plot was divided into five sub-plots, 100 m^2 each.

Assessments were made every week, starting from two weeks after planting. Organisms were counted or collected in small numbers to avoid disruption of the faunistic community. Three major groups were collected or counted: the fauna on cabbage plants, aerial forms and soil forms. Fauna was collected by hand or by sweep netting and then placed in killing containers. Aerial forms were collected using sweep nets, 20 sweeps per sub-plot. The soil inhabiting fauna was collected using pitfall traps, two traps per sub-plot. The value of species diversity (D) was determined by the following equation (Michael 1984):

$$D = \frac{\text{Number of species recorded}}{\sqrt{(\text{Total number of individuals})}}$$

Population study

Weekly counts of major insect species were conducted on 10 cabbage plants, which were systematically selected. Ten samples of IV instar DBM larvae were dissected to estimate levels of DBM larval parasitism by *D. semiclausum. S*oil samples were taken weekly from each sub-plot. Major soil microorganisms, thought to play an important role as biological control agents or decomposers of organic matter, were identified and their abundance estimated. The numbers of insecticide sprays and the amount of insecticide used during the growing season were recorded for each plot. The marketable yield of cabbage per plot was recorded at harvest time.

Table 1. Components of the IPM system and non-IPM systems applied to cabbage plots at Lembang and Pangalengan, 1999

IPM system	
A. Cultural control:	Use of Dolomite (4 t/ha).
B. Balanced fertilisation:	Stable manure: 30 t/ha; fertilisers used: 250 kg/ha TSP, 100 kg/ha Urea, 250 kg/ha ZA and 200 kg/ha KCl
C. Biological control:	*Diadegma semiclausum* is a core component of IPM.
D. Control threshold (CT):	DBM: 5 caterpillars/10 plants CHC: 3 egg clusters/10 plants
E. Monitoring of agroecosystem	Once per week Analyses of agroecosystem
F. Use of insecticide	Based on monitoring and control threshold level (CTL) of DBM and CHC Type of insecticide: selective (spinosad)
Conventional system (Farmers' practice)	
A. Cultural control	No liming
B. Fertilisation:	At Lembang 30 t/ha stable manure, 250 kg/ha TSP, 300 kg/ha Urea, 300 kg/ha ZA, 250 kg/ha KCl and 250 kg/ha NPK At Pangalengan 42 t/ha stable manure, 6 t/ha chicken manure, 20 t/ha mushrooms waste and 700 kg/ha NPK.
C. Biological control	Neglected
D. Control threshold	Neglected (calendar system)
E. Monitoring of agroecosystem	Neglected
F. Use of pesticides	At Lembang 2 times per week, mixtures of profenofos, *B. thuringiensis* var. *aizawai,* lambda cyhalothrin and antracol At Pangalengan 2 times per week, alternate use of fipronil, diafenthiuron, imidacloprid and abamectin

Results and discussion

Faunistic study

The diversity of the fauna sampled with a sweep net in the IPM plot was relatively higher than that of the Non-IPM plot (Table 2). This is indicated by the values of D in the IPM and Non-IPM plots (0.83 and 0.59 respectively). One possible reason was the use of the selective insecticide, spinosad in the IPM plots, while more toxic insecticides such as lambda cyhalothrin and fipronil were used in the Non-IPM plots. The toxicity of spinosad against humans and the environment is very low (Dow AgroSciences 2001).

The high diversity of fauna found in the IPM system in this study, and in cabbage communities in general, may increase the ecosystem stability. Consequently, IPM should also minimise pest outbreaks. It is very important to conduct studies of insect biodiversity before interventions are recommended (Ooi 1997).

Both macroarthropods and microarthropods were caught in the pitfall traps. The numbers of arthropods caught were always higher in the IPM plots than in the Non-IPM plots (Table 3). The data show that the use of the IPM system on cabbage crops increased the number of arthropods by 59% at Lembang and 96 % at Pangalengan compared with the conventional system.

Table 2. Diversity of insects sampled by sweep net from cabbage crops managed either with an IPM system or with a conventional (Non-IPM) system at Lembang and Pangalengan, 1999

Site (elevation)	Mean number of species		Total number of individuals		Species diversity (D)*	
	IPM	Non-IPM	IPM	Non-IPM	IPM	Non-IPM
Lembang (1250 m)	3.0	2.0	8.0	7.0	1.06	0.76
Pangalengan (1400 m)	6.0	4.0	101.0	89.0	0.60	0.42

* D values were counted based on formula given by Michael (1984)

Table 3. Mean number of arthropods caught in pitfall traps in a cabbage crop managed with either an IPM system or a Non-IPM system (conventional system) at Lembang and Pangalengan, 1999

Site (elevation)	Number of individuals caught in pitfall traps*		
	IPM system	Non-IPM system	Difference (%)**
Lembang (1250 m)			
Macroarthropods	87.5	43.1	103.0%
Microarthropods	4551.8	2876.7	58.2%
Total individuals	4639.3	2919.8	58.9%
Pangalengan (1400 m)			
Macroarthropods	62.5	57.7	8.3%
Microarthropods	4282.2	2160.8	98.2%
Total individuals	4344.7	2218.5	95.8%

*Total number of individuals from eight sampling occasions (means of five replications at each observation)
**$((N_{IPM}-N_{non\text{-}IPM})/N_{non\text{-}IPM)}$

Population study

At both Lembang and Pangalengan, the most important cabbage pests were DBM and CHC. At Lembang, the black cutworm (*Agrotis ipsilon* Hübner) was also common, while at Pangalengan, the potato leafminer *(Liriomyza huidobrensis* Blanchard) was common. In the IPM managed plots, the selective insecticide, spinosad, was applied only when the population of DBM larvae and/or egg clusters of CHC surpassed the control threshold. The thresholds used were 5 larvae/10 plants for DBM and 3 egg clusters/10 plants for CHC (Sastrosiswojo 1987, 1996a). Application of the IPM system on cabbage, both at Lembang and Pangalengan, effectively suppressed the population of DBM and CHC. In the IPM system, DBM numbers were reduced by 47% at Lembang and 34% at Pangalengan, while CHC numbers were reduced by 22% at Lembang and 62% at Pangalengan, compared with the conventional system (Table 4).

Table 4. Total numbers of DBM (*Plutella xylostella*) and CHC (*Crocidolomia pavonana*) in IPM and Non-IPM plots at Lembang and Pangalengan, 1999

	Lembang		Pangalengan	
	IPM	Non-IPM	IPM	Non-IPM
DBM	6.9	13.1	10.6	16.1
CHC	1.8	2.3	1.5	3.9

Diadegma semiclausum Hellén is the most important parasitoid of DBM larvae and was introduced into Indonesia in 1950 (Vos 1953). Now *D. semiclausum* is well established in almost all of the highland cabbage growing areas and has become the core component of IPM on cabbage (Sastrosiswojo 1996b).

In this study, *D. semiclausum* was the only parasitoid found in the cabbage plots at Lembang and Pangalengan. The fluctuations in numbers of *D. semiclausum* followed the populations of DBM at both

locations (Table 5). The use of a selective insecticide (spinosad) based on the control threshold of DBM in the IPM system, did not appear to disturb the role of *D. semiclausum* as a biological control agent of DBM. The parasitism of DBM by *D. semiclausum* increased by 34% at Lembang and 53% at Pangalengan in the IPM plots compared with the Non-IPM plot. *D. semiclausum* suppressed effectively the population of DBM during one growing season of cabbage.

Table 5. Mean number of *P. xylostella* larvae per cabbage (DBM) and % parasitism (% P) of DBM larvae by *D. semiclausum* in the IPM and Non-IPM plots at Lembang and Pangalengan, 1999

		Days after planting										
		14	**21**	**28**	**35**	**42**	**49**	**56**	**63**	**70**	**77**	**84**
Lembang												
IPM	DBM	0.4	0.3	0.6	1.6	0.4	1.8	0.5	0.2	0.8	0.2	0.3
	% P	0.0	0.0	45.0	10.0	37.5	11.1	25.0	25.0	11.1	25.0	50.0
Non-IPM	DBM	0.3	0.5	1.1	4.5	2.8	1.0	0.3	0.3	1.0	0.7	0.6
	% P	0.0	0.0	20.0	11.1	10.0	33.3	0.0	25.0	19.1	17.7	23.1
Pangalengan												
IPM	DBM	5.9	0.0	1.6	0.1	2.2	0.1	0.0	0.5	0.2	0.0	0.0
	% P	0.0	0.0	32.0	20.0	0.0	0.0	0.0	0.0	32.0	12.5	0.0
Non-IPM	DBM	2.7	5.2	4.9	0.4	0.7	0.8	0.3	0.3	0.8	0.0	0.0
	% P	0.0	0.0	17.3	20.0	0.0	25.0	0.0	0.0	0.0	0.0	0.0

Four orders of predatory arthropods were found in the pitfall trap studies: Coleoptera (43%), Araneida (24%), Hemiptera (18%) and Orthoptera (16%) (Table 6). Implementation of the IPM system increased the abundance of predatory arthropods by 84% at Lembang and 68% at Pangalengan (Table 6). The study suggests that the intensive use of insecticide usually practised by the cabbage farmers may have adversely affected the populations of soil predatory arthropods. Consequently, the populations of DBM, CHC and other insect pests may increase with intensive use of insecticides.

The soil fauna was observed on the soil surface and within the soil of cabbage communities treated with the IPM system and the conventional system (Non-IPM). The most common orders present were Collembola, Diplura and Acarina. There was a significant difference between the IPM system plots and the conventional system plots in terms of abundance and composition of soil fauna (Table 7).

At Lembang, the most abundant components of the fauna on the soil surface of the cabbage plots were Collembola (72%), followed by Diplura (26%) and Acarina (2%). In the soil samples, the most dominant component fauna was also Collembola (73%), followed by Diplura (22%) and Acarina (5%). In general, the application of the IPM system was able to increase the population of soil fauna by 59% on the soil surface and by 56% in the soil compared with the conventional system (Non-IPM).

At Pangalengan, results of the study indicated that the most abundant component fauna on the soil surface of cabbage community were Collembola (84%), followed by Diplura (14%) and Acarina (2%). Observation on the soil samples showed that dominant components of the fauna were Acarina (50%), Collembola (39%) and Diplura (11%). Similarly, implementation of the IPM system increased soil fauna by 71% on the soil surface and by 113% in the soil compared with the conventional system (Non-IPM).

Table 6. Population of predatory insects in IPM and Non-IPM (conventional system) plots at Lembang and Pangalengan, 1999

Populations	**Lembang**		**Pangalengan**	
	IPM	**Non-IPM**	**IPM**	**Non IPM**
Coleoptera	3.1	2.3	2.9	2.6
Araneida	2.2	0.6	2.4	1.4
Hemiptera	2.0	0.3	1.7	1.0
Orthoptera	1.5	1.2	1.4	0.3

Table 7. Mean abundance of soil fauna (a) on the soil surface and (b) in the soil of cabbage plots managed with IPM system or Non-IPM (conventional system) at Lembang and Pangalengan, 1999

Stratum sampled		Lembang		Pangalengan	
	Order	IPM	Non-IPM	IPM	Non-IPM
Soil Surface	Collembola	198.6	121.4	255.7	110.0
	Diplura	65.7	48.6	34.3	27.2
	Acarina	7.2	1.4	7.7	1.4
Soil	Collembola	138.8	80.0	102.5	57.5
	Diplura	36.3	28.8	25.0	18.8
	Acarina	8.8	7.5	132.5	77.5

Although there are many soil microorganisms that can play an important role as biocontrol agents of soilborne diseases, only two species were identified from the soil samples taken from the experimental cabbage plots. Counts of known species of biocontrol agents during the study revealed that *Trichoderma* sp. was by far the most common species present, followed by *Bacillus* sp. *Trichoderma* sp. is a soil fungus that actively decomposes organic matter in the soil by producing a cellulose destruction enzyme. The development of *Trichoderma* sp. and *Bacillus* sp. in the soil is much faster than other soil microorganisms, especially those responsible for soilborne diseases.

It is assumed that the mechanism in suppressing the population of soilborne diseases by both species is antibiosis. Results from the present study showed that implementation of the IPM system had only a minor effect, with the population of *Trichoderma* spp. in the soil increasing by only 6% at Lembang and 7% at Pangalengan compared with the conventional system (Table 8).

Table 8. Population of *Trichoderma* spp. in the soil sample taken from cabbage community treated with IPM system and Non-IPM (conventional system) at Lembang and Pangalengan, 1999

Populations	Lembang		Pangalengan	
	IPM	Non. IPM	IPM	Non IPM
Trichoderma spp.	5.87×10^4	4.71×10^4	4.88×10^4	4.26×10^4

The insecticide used in IPM plots, at both Lembang and Pangalengan, was spinosad (Success 25 SC). The insecticides used in Non-IPM plots (conventional system) at Lembang included profenofos (Curacron® 500 EC) and lambda cyhalothrin (Matador® 25EC), while at Pangalengan, fipronil (Regent® 50 SC), diafenthiuron (Pegasus® 500 SC), imidacloprid (Confidor® 200SL) and abamectin (Agrimec® 18 EC) were used in rotation (alternate use) every three days. The total number of insecticide sprays in the Non-IPM plots was 22 times during one growing season of cabbage. Insecticide application in IPM plots was only undertaken when the DBM population or population of egg clusters of CHC surpassed their control thresholds.

The study showed that implementation of the IPM system reduced the number of insecticide sprays by 82% at Lembang and Pangalengan, compared with the conventional system (Table 7). In addition, the amount of insecticides used was also reduced by 90% at Lembang and by 95% at Pangalengan. In terms of money, the cost for insecticides used was 79% at Lembang and 64% at Pangalengan. The marketable yield of cabbage in IPM plots was 13.7 t/ha at Lembang and 43.4 t/ha at Pangalengan (Table 9).

The marketable yield of cabbage in Non-IPM plots (conventional system) was 8.7 t/ha at Lembang and 36.6 t/ha at Pangalengan. The yield of cabbage at Lembang was very low, presumably because of low soil fertility and the high incidence of clubroot (*Plasmodiophora brassicae* Wor.). The figures show that marketable yield of cabbage in the IPM plots increased by 57% at Lembang and 19% at Pangalengan when compared with the conventional system.

Table 9. Use of insecticides and marketable yield of cabbage in the IPM system and the Non-IPM (conventional system) at Lembang and Pangalengan, 1999

Treatments	Lembang		Pangalengan	
	IPM	Non-IPM	IPM	Non-IPM
Number of insecticide sprays	4	22	4	22
Amount of insecticide used (L/ha)	2.25	66.00	2.25	41.25
Cost of insecticide (Rp x 10^6)	1.6	7.6	1.6	4.4
Marketable yield (t/ha)	13.7	8.7	43.4	36.6

Conclusions

Based on these studies, conclusions can be made regarding the ecological impact of the IPM implementation on cabbage at Lembang and Pangalengan as follows:

(a) Faunistic study: the IPM system increased faunal diversity: in consequence it will minimize pest outbreaks compared with the conventional system (Non-IPM).
(b) Population study: Major insect pests on cabbage during the study were the diamondback moth and cabbage head (cluster) caterpillar. The populations of DBM and CHC were effectively suppressed by IPM implementation. Rates of parasitism of DBM larvae by *D. semiclausum* increased by 34 to 53% in the IPM system. The abundance of soil inhabiting predators (macroarthropods) in the orders Coleoptera, Araneida, Hemiptera and Orthoptera was 68 to 84% higher in the IPM system. Implementation of the IPM system also increased the abundance of soil fauna (microarthropods) in the orders Collembola, Diplura and Acarina, both on soil surface and in the soil. The soil microorganisms (bio-control agents) identified in this study were *Trichoderma* sp. and *Bacillus* sp. The abundance of both species increased by 6% to 7% in the IPM system.
(c) Insecticide used: IPM implementation significantly reduced the number of insecticide sprays (82%), amount of insecticides used (90 to 95%) and the cost of insecticides used (64 to 79%).
(d) Cabbage yield: marketable yields were 57% at Lembang and 19% at Pangalengan higher in the IPM system compared with the conventional system.

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Development and implementation of *Brassica* IPM systems in the Lockyer Valley, Queensland, Australia

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Abstract

In the mid 1980s, *Brassica* vegetable production in southern Queensland was at crisis point with insecticide resistance in diamondback moth, *Plutella xylostella* (L.), leading to frequent spray failures. Through a succession of projects, an Integrated Pest Management (IPM) system targeted at the local *Brassica* pest complex was developed with farmers and industry using participative processes. Strategies included an extensive research and development program, on-farm trial work, demonstration sites, publicity, farmer and industry training, and information development and delivery. An evaluation process incorporated into project work in 1996 showed that changes in industry practice were occurring. Data were collected through a combination of surveys (1996, 1997, 1998, 2001) and focus group interviews (1997).

A key component of our implementation strategy was to ensure farmer and industry participation in the evolving IPM system by applying the concepts of adult education and action learning to project activities. A Brassica Improvement Group (B.I.G.) was formed in 1997 and this farmer-driven learning group has become an important vehicle for IPM extension efforts. This paper explores the contribution B.I.G. has made to positive IPM outcomes in the field. The concept of social capital is used to analyse the robustness of the group, its capacity to continue successful operation and ability to foster IPM with minimal agency support. This study indicates that surviving a change in leadership and focus appear to be critical factors for group sustainability.

Keywords

Brassica vegetable crops, integrated pest management, extension, social capital, evaluation

Introduction

The Queensland *Brassica* vegetable industry is estimated at Aus$30 million, which is about 20% of the Australian industry. On average, around 2800 hectares are planted to *Brassica* crops per year (ABS 1997, Harper *et al*. 1999). Crops are located primarily in the cooler southern regions of Queensland - the Lockyer Valley, Eastern Darling Downs and Granite Belt regions. Cabbage, cauliflower and broccoli are the major crops with smaller quantities of Chinese cabbage and other Asian vegetables grown.

The industry has seen some major changes in the past five years. These are being driven by the increasing market share of chain stores in the domestic markets, declining profitability of vegetable production and demands on farmers to implement more complex food safety, quality assurance and business management systems. This has resulted in some farmers leaving the industry and other farmers expanding their operations to achieve economies of scale. The total area planted to *Brassica* vegetable crops has increased over the past five years (ABS 1993, ABS 1997) and oversupply often has a negative impact on prices.

Within these changing production and marketing conditions, management of pests in *Brassica* crops has also seen some major changes, with Integrated Pest Management (IPM) now seen as best practice. Several factors facilitated this changed approach to managing pests. Traditionally, *Brassica* vegetable crops were grown year round in the Lockyer Valley and farmers relied heavily on scheduled sprays of broad-spectrum insecticides to manage a range of pests. This practice was called into question in the mid 1980s, when *Brassica* production in the Lockyer Valley was at crisis point due to insecticide resistance problems in diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). Wide spread spray failures, particularly in summer grown crops, mobilised industry and government to search for alternative techniques for managing diamondback moth (DBM).

Through a succession of projects, a practical IPM system was developed with local farmers and industry. DBM was the most difficult pest to manage, but as farmers reduced broad-spectrum insecticide use within an IPM framework, other pests became more problematic. A number of lepidopterous pests have the potential to cause significant crop damage during autumn and spring. These include centre grub, *Hellula hydralis* (Guenée) (Pyralidae: Lepidoptera); cabbage cluster caterpillar, *Crocidolomia pavonana* (Fabricius) (Pyralidae: Lepidoptera); cluster caterpillar, *Spodoptera litura* (F.) (Noctuidae: Lepidoptera) and heliothis, *Heliocoverpa* spp. (Noctuidae: Lepidoptera). Cabbage white butterfly, *Pieris rapae* (L.) (Pieridae: Lepidoptera) can be a sporadic problem in unsprayed crops. Thrips species have caused some concern over the past two to three years in crops where spraying with broad-spectrum insecticides has been significantly reduced. The IPM system of the Lockyer Valley is therefore aimed at managing the pest complex, not only DBM, at the cropping systems level.

The IPM system includes crop scouting, a break in production over summer, use of narrow-spectrum insecticides within a resistance management strategy, protection of natural enemies, release of predators and well-targeted spray application practices. Strategies for developing and implementing the system included an extensive research and development program, on-farm trial work, demonstration sites, publicity, farmer and industry training, and information development and delivery. The progress of IPM development can be divided into three different phases:

1988 to 1990 – implementation of an insecticide resistance management strategy.
1990 to 1995 – reducing reliance on broad-spectrum insecticides by introducing *Bacillus thuringiensis* into the cropping system coupled with a summer production break, crop scouting and improved spray application.
1995 to present – improving and building on the existing IPM system through research into natural enemies, pest monitoring protocols, insecticide spray coverage; and capacity building through developing decision-making tools and delivering training workshops to farmers and industry.

The approach and activities associated with project work from 1988 to 1996 are documented in greater detail by Heisswolf *et al*. (1997).

To increase responsiveness to industry needs and to assess projects against objectives, the IPM program has included a formal evaluation process since 1995. This process has provided information for decision-making to two consecutive projects funded by the Australian Centre for International Agricultural Research (ACIAR).

In early 1998, a local grower group was established to promote information sharing and facilitate IPM implementation. This grower driven-learning group, the Brassica Improvement Group (B.I.G.), has been meeting on a monthly basis during the production season since its inaugural meeting in February 1998.

Since implementation of the Extension Strategy Statement (Department of Primary Industries 1992), group approaches have become an important component of the public extension and farmer interface in Queensland. This has raised questions about group robustness and self-reliance. Groups can be seen as a part of the social capital of a community or industry. In 2000, we explored the dynamics of group formation and development within the context of social capital using B.I.G. as one of our case study groups.

The objective of this paper is to document changes in IPM practice that have occurred since 1990 and to explore the contribution that B.I.G. has made to this IPM outcome in the Lockyer Valley.

Materials and methods

Grower participation in project work

A critical component of research and extension work was to encourage grower and industry ownership in the evolving IPM system. Using participative processes based on adult education and action learning principles, we encouraged information flows between all sectors of the *Brassica* industry - researchers, consultants, farmers and agribusiness. The aim of this approach was to ensure research and development activities were well targeted and results could be tested, incorporated and adapted within existing farming systems.

The ACIAR project team actively sought industry involvement in setting research directions for both projects. In 1995, around 50 stakeholders took part in a problem specification workshop in Brisbane to

identify pest management problems and opportunities and to formulate strategic approaches for developing pest management solutions. Two industry representatives took part in a formal mid-term project review in 1996. At the conclusion of this first ACIAR project in 1998, a second industry workshop was held to review progress and identify new and emerging pest management needs using a modified SEARCH process (Dick 1990). Outcomes from this workshop were incorporated into the objectives of the second ACIAR project.

Extension and training activities were formulated using adult education and action learning principles. Adult education encompasses such concepts as learning from peers, building on past experience, taking a problem oriented approach and encouraging interaction and participation (Brookfield 1986, Knowles 1990). Strategies at field days and training activities therefore aimed to provide hands on experiences for participants, focused on small group processes and allowed time for discussion, interaction and questioning. Formal presentations of research results were incorporated within these adult education activities as appropriate.

The action learning cycle (McGill & Beaty 1992) provided a useful framework for structuring many activities. This cycle consists of four phases – plan, act, reflect and decide. For example, after a learning activity, the workshop design would encourage participants to move through the different phases of the action learning cycle by:

- Reflecting on what was learned,
- Deciding how this learning relates to their own situation,
- Planning how this new knowledge might be used on their own farm,
- And then applying this knowledge.

The Brassica Improvement Group

In 1997, a local farmer who had been working closely with the research and development team since 1993, decided to set up the Brassica Improvement Group (B.I.G.). Kevin Niemeyer brought back this idea after visiting cauliflower farmers in Western Australia with a project team member. After canvassing interest in establishing a similar group in the Lockyer Valley with other farmers, agribusiness and agency staff and discussing the potential aims of such a group at several meetings, the first general meeting of B.I.G. was held at the Gatton Research Station in February 1998. Kevin Niemeyer was elected chairman, a local crop scout was elected secretary/ treasurer and the executive was completed with the election of three farmer and two industry representatives. The role of agency staff was to support the group in its organisation and operation.

The objectives of the group were to provide a forum for sharing information, learning and discussion in a social atmosphere. Initially the group focused on IPM issues, but more recently the aim has been to address topical issues of the day. Since February 1998, B.I.G. has met monthly during the *Brassica* season, from February to October. Attendance numbers vary from 12 to 45, depending on the topic.

Social capital and the Brassica Improvement Group

The main objective in our study on social capital was to explore the factors that influence the establishment and development of effective farmer groups and identify strategies that we could use to support farmer groups become self-reliant and sustainable.

Like the terms “participation”, “extension” and “sustainability”, the concept “social capital” is difficult to define and therefore to measure. In the literature on social capital, different authors interpret the term social capital according to their own ideology and context (Dasgupta & Serageldin 1999, Wall *et al*. 1998) and there are many views and perspectives on “social capital” and little consensus on how it might be measured (Bourdieu 1986, Coleman 1990, Putnam 1995, Flora 1998, Grootaert 1998, Ostrom 1998, Pretty 1998, Uphoff *et al*. 1998). The term captures the idea that social bonds and social norms are an important basis for sustainable development. Its value was identified by Jacobs (1961) and Bourdieu (1986), later given a theoretical framework by Coleman (1988, 1990) and brought to wide attention by Putnam (Putnam *et al*. 1993, Putnam 1995). Solow (1999) describes social capital as “such things as trust, the willingness and capacity to cooperate and coordinate, the habit of contributing to a common effort”. These aspects of social structure and organisation are resources for individuals to use to realise their own personal interests.

For the purposes of our study on the factors impacting on the robustness of farmer groups, we used a typology of social capital developed by Pretty and Ward (2001) for assessing group maturity (Table 1).

These authors propose a series of criteria, which are organised into five discrete categories, for exploring the evolution of groups. Against each criterion, descriptions for assessing group maturity at different stages are given. Three stages of group evolution are proposed; Stage 1 Dependent, Stage 2 Independent and Stage 3 Interdependent.

In February to May 2000, we explored group robustness within the context of social capital using four farmer groups as case studies. One of these groups was the Brassica Improvement Group. A multi perspective approach (Van Beek & Nunn 1995) was used to select respondents for each group studied. The aim was to obtain information about each group from various perspectives, ranging from the group leader, to an outsider who was sceptical, but had good knowledge of the group.

A standardised questioning procedure was developed using the criteria from the Pretty and Ward typology (Table 1). Open-ended personal interviews were conducted over a three-month period in early 2000. Eight people interviewed per group. The qualitative data collected were analysed against the three stages of group evolution proposed by Pretty and Ward (2001) by assigning comments and phrases gathered during interviews into one of the five categories of the typology.

Table 1. Summary of a typology for assessing group maturity proposed by Pretty and Ward (2001). A dependent group is considered at Stage 1, an interdependent group at Stage 3 of group evolution

Categories of criteria	Descriptions for assessing group maturity
Worldviews & sense-making Ability and attitude to change, the values and beliefs of individuals within the group	A dependent group tends to be backwards looking, fearful of change with individuals fixed in their attitudes, beliefs and values. An interdependent group actively shapes its own future, accepts change as the norm and uses critical reflection and abstract thinking to develop new insights.
Internal norms & trust Value of the group to members, level of trust and commitment, sharing of ideas, development of rules and norms.	A dependent group tends to follow externally derived rules, distrusts the new, but shares some ideas and places some value on the group. An interdependent group develops its own rules and norms, expresses the social value of the group and shares ideas within the group as well as externally.
External links & networks Group links to external networks and sources of support and information	A dependent group has few links with other groups, tends to rely on an external facilitator and information flows are mainly top down. An interdependent group no longer requires an external facilitator, is able to encourage the formation of new groups and is well linked to many external information sources and agencies.
Technologies & improvements A group's capacity to experiment and generate solutions.	A dependent group continues to look for external and simplistic solutions to complex problems although there may be some experimentation. An interdependent group looks for solutions to problems within the group and externally, with experiments and redesign leading to adaptation and innovation.
Group lifespan The resilience of a group and the group's reason for being.	A dependent group tends to be initiated by an external agency or come together in response to a crisis. Groups under the same program look similar with groups likely to break down with the resolution of the crisis or withdrawal of the external agency. Interdependent groups have successfully achieved initial goals, are setting new goals and are engaged in different activities. They are unlikely to break down and have their own characteristics.

The evaluation process

A participatory planning process involving the ACIAR project team was used to define the evaluation approach and methods at workshops in March 1996 and January 2000. The evaluation hierarchy of Bennett (1975) was used as the model for structuring the evaluation plans. During these workshops, team members

listed their planned activities under the project objective to which the work contributed and then completed a table outlining:

- Resources required
- Anticipated people involvement
- Expected reactions from those involved
- Changes in knowledge, attitudes, skills and aspirations (KASA) which might come about as a result of their planned activities
- Changes in industry practice which would follow on from KASA changes
- Long-term outcomes which might eventuate

Key questions for the evaluation were formulated from workshop results and performance indicators were identified. The evaluation process was designed to integrate with normal project management activities with attainment of project milestones serving as one set of indicators. Evaluation activities designed specifically to monitor changes in pest management practices included:
Baseline and follow-up grower surveys (November 1996 and February 1998) – personal interviews with 20 *Brassica* farmers per survey.
Grower focus groups (October 1997) – two groups were interviewed, Group 1 consisting of 5 farmers with little personal contact with project team members, Group 2 consisting of 7 farmers with regular involvement in project activities.
Agribusiness survey (February 2001) – telephone interviews with 7 pesticide resellers and 5 pest management consultants.

Questionnaires used to review an insecticide resistance management strategy in 1990 (Heisswolf 1992), the 3V Strategy implemented in 1988 (Deuter 1989), served as a resource for survey development in 1995. In this earlier review, 37 Lockyer Valley broccoli farmers and 19 chemical resellers, field officers and consultants were interviewed using structured questionnaires. By linking the two processes, we planned to use results from the 3V Strategy review as baseline data for some of our evaluation outcomes.

Results and discussion

Changes in pest management practices

Evaluation results show that Lockyer Valley *Brassica* farmers have gradually implemented various components of IPM since 1990. Changes in pest management practice are summarised using several "IPM indicators" (Table 2). Over the past decade, 60 to 74% of farmers implemented a voluntary three month production break over summer (November to February). Our evaluation however indicates that this practice is coming under pressure due to low prices, with a trend for farmers to lengthen the season to capture higher prices and so shortening the production break.

Bacillus thuringiensis (Bt) use has increased dramatically since 1990, mixing of Bt with other insecticides has declined and in 2001, 90% of farmers were using narrow spectrum insecticides to manage pests. This indicates that farmers are aiming to target pest problems more effectively while minimising impact on natural enemies. This is supported by the increased use of crop scouts to make spray decisions and increased knowledge and use of natural enemies. According to survey results, overall there has been an increase in the use of IPM since 1995 although this of course depends on an individual's definition of IPM.

Is there a link between changes in practice and the establishment of B.I.G?

Results from the focus group interviews conducted in 1997 show that farmers involved with agency staff and project work were more advanced in implementing IPM practices than farmers with less direct contact. The former farmers had a higher tolerance to pest numbers in crops before spraying, were thinking about how to attract and release natural enemies, used crop scouts to make decisions and were looking for strategies to manage secondary pests. Half of these farmers were involved in the establishment of B.I.G.

Farmers with little direct agency contact were moving towards greater Bt use, but still relied on broad-spectrum insecticides. These farmers were aware of the potential of natural enemies and seemed prepared to change their spray practices to protect them. Half the farmers interviewed used crop scouts to cut down their spray costs. Farmers in this group were less likely to attend B.I.G. meetings. Both groups of farmers said that widespread implementation of a summer production break and increasing the acreage of crop being monitored for pests had helped with insecticide resistance management.

In the 2001 survey, 71% of chemical resellers and 20% of consultants listed B.I.G. meetings as one of the extension activities that they had attended during the season. This indicates that for some sectors of the agribusiness industry, B.I.G. meetings are an important avenue of interaction and source of information.

The link between IPM implementation and B.I.G. is far from clear although there are indications that the group has had an important influence on IPM development and adoption in the field. A follow up survey of *Brassica* farmers in 2002 will strengthen the evaluation process. Using personal or telephone interviews, data on the current level of IPM implementation, based on the IPM indicators from the previous surveys, will be collected and linked to involvement with B.I.G. since 1998.

Table 2. Changes in integrated pest management in Lockyer Valley *Brassica* crops based on percentage of farmers using various pest management practices

IPM indicator	1990[a]	1996[b]	1998[b]	2001[c]
Summer production break				
3 months	70%	74%	60%	44%
1 month	-	-	30%	51%
Rotating chemical groups	35%	26%	-	90%
Narrow spectrum insecticides	-	-	-	90%
Bt use overall	19%	95%	95%	-
Bt mixed with other pesticides	-	33%	17%	< in 1996
Improved spray application				
Avoid spraying in wind	-	70%	70%	-
Safety – filtered cabs	-	22%	44%	-
Crop scouting	30%	25%	50%	60%
Conscious protection of natural enemies	-	5%	-	30%
Release of predators	-	0%	-	10%
Use of IPM				
To some degree	-	60%	-	70%
"Advanced"	-	5%	-	10%

[a]Broccoli farmers, consultants and resellers interviewed, [b]Cabbage, cauliflower and broccoli farmers interviewed, [c]Consultants and resellers interviewed

The future of the Brassica Improvement Group

Our case study work on social capital and group sustainability in early 2000 showed that B.I.G. had the potential to evolve into a mature group, but that the group was facing some major threats to its sustainability. As part of the study analysis, we attempted to draw together qualitative data by categorising all words, comments or phrases given by the interviewees into the five sets of criteria proposed by Pretty and Ward (2001).

Within the limitations of this analysis process, B.I.G. appeared to fit into Stage 3 Interdependence for much of the criteria of the Pretty and Ward typology (Table 1), but there are some notable exceptions. The group was well linked to internal and external sources of information, was sharing experimental results and not reliant on an external facilitator for continued functioning, however the commitment of individuals to the group, the level of trust within the group and people's attitude to change varied.

The success of the group appeared to be strongly linked to the group leader, the initial goal of providing information on IPM had been achieved and there was some doubt as to the resilience of the group in the longer term. Our study indicated that the ability of a group to adapt to a change in leadership and to develop a sense of continuing purpose were critical factors for group evolution.

In 2000, B.I.G. appeared to be at a critical juncture in its development. The group had decided on a maximum of three terms for their executive early in group's formation and both the chairman and secretary/ treasurer were adamant that they would not break this rule and so stood down from the executive for the 2001 season. The agency's role was to support this decision particularly in light of the findings from our study on group robustness. Our aim was to assist the B.I.G. executive to review their achievements, refocus the aims of the group and resolve the issue of a change of leadership. After three crisis meetings of the

executive over the summer of 2000/ 01, a new executive was elected in February 2001 and, after a successful 2001 season, the 2002 executive was re-elected with only minor changes at the annual general meeting in October 2001.

B.I.G. is set for another season and appears to be much more robust after having survived its first change of leadership and change in focus. There are other indications that the group is likely to continue with little, if any, agency support:

- The core group of committed members has expanded
- External linkages have diversified
- The group produced a monthly newsletter during the 2001 season
- A member of the group was awarded a four month overseas scholarship
- The group achieved the Healthy Waterways award for South East Queensland in December 2001
- Some group members have formed an Environmental Management group to proactively address wider environmental concerns
- Topics covered at monthly group meetings address a range of issues
- The executive is planning to coordinate on-farm variety trials next season

Viewed against the descriptions in the Pretty and Ward typology (Table 1), these developments indicate that B.I.G. has moved further along the continuum of group evolution since our study in early 2000. For facilitators, this typology appears to be a useful tool for assessing group maturity and designing appropriate processes for supporting groups at different stages of their evolution. Our study of B.I.G. within the social capital context was valuable for highlighting the importance of providing adequate support at a critical period in the group's life - a change of leadership and focus.

There is some doubt as to whether B.I.G. will continue to play an important role in IPM implementation as the group is now focusing on other topics. It is the group's decision. In the absence of a pest management crisis, B.I.G. is more likely to focus on profitability issues such as prices, marketing, varieties and reducing input costs. From an agency perspective, B.I.G remains a key extension forum for input on research and development issues and for extending information on IPM and other topics. It will be up to agency staff to negotiate this two-way information flow with the Brassica Improvement Group.

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Towards biocontrol-based IPM for the diamondback moth in eastern and southern Africa

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Abstract

Effective chemical control of the diamondback moth (DBM), the key insect pest of crucifers in Eastern and Southern Africa, has become difficult. DBM has developed resistance to common insecticides and farmers increasingly use insecticide cocktails and spray more frequently. This is resulting in rising production costs, environmental contamination, health risks and high residues in produce. The recorded level of parasitism of indigenous parasitoids, including those of the genus *Diadegma,* is low compared with south-east Asia and South Africa. To enhance effective, economical and environmentally acceptable control of the pest, an IPM programme, based on the improved biological control of DBM, was initiated. This project tries to achieve improvements through a collaborative regional research effort in various steps: collection of basic information on distribution and efficiency of the indigenous natural enemy complex in Ethiopia, Kenya, Uganda and Tanzania; study of the taxonomy and bionomics of local parasitoids in comparison to parasitoids of proven value in south-east Asia and South Africa; importation, multiplication and release of superior parasitoids from Asia with support of the Asian Vegetable Research and Development Center (AVRDC) or South Africa. The organisational and operational set-up of the project is described.

Introduction

The main crucifer species grown in the Eastern and Southern African region (Ethiopia, Kenya, Tanzania, Uganda, Malawi, Zambia, Zimbabwe and Mozambique) are rape (*Brassica carinata* A. Br. and *Brassica napus* L.), kale *(B. oleracea* L. var. *acephala*), Chinese cabbage *(Brassica chinensis* L.) and cauliflower (*B. oleracea* L. var. *botrytis*). These vegetables are grown mainly for home consumption and domestic markets. They are valuable as relish in many homesteads, providing necessary dietary vitamins and minerals in a maize-based diet, as well as a source of cash for small scale farmers, particularly women and youths, in rural and peri-urban areas. However, production is often constrained by damage caused by a range of pests. Of the major pests, the diamondback moth (DBM), *Plutella xylostella* (L.) and aphids (*Brevicoryne brassicae* L.*, Lipaphis erysimi* Davis and *Myzus persicae* Sulzer) have been identified as the most damaging (Nyambo & Pekke 1995, Adane-Kassa & Abate 1995, Oduor *et al.* 1996, Seif & Löhr 1998) in the region.

To date, farmers in Africa depend solely on the use of insecticides, which are often applied on a calendar basis to control these pests, and it is becoming increasingly difficult and uneconomic to achieve effective control. DBM has developed resistance to a wide range of the insecticides commonly used (Mingochi *et al.* 1995; Kibata 1996; B. Nyambo, personal communication). In addition, incidence of turnip mosaic virus, which is transmitted by aphids, has been on the increase in the region in recent years (D. Mingochi, personal communication; A.A. Seif, personal communication). As a result, many farmers have resorted to the use and application of insecticide cocktails as well as increased spraying frequency in order to achieve control of DBM and aphids (Kibata 1996). This has led to an increase in the level of contamination of the farm environment, high pesticide residues in the produce as well as health risks to farm workers and increased cost of production.

To address the problem, a regional workshop in 1995 tried to identify knowledge gaps in the management of the major pests of crucifers and set regional research priorities (Nyambo *et al.* 1996). Three activities were given priority, as these would provide the much-needed basic information to formulate suitable IPM options to reduce over dependency on chemical insecticides. Among these activities was a study of the seasonality of DBM and aphids including an inventory of their indigenous natural enemies in Kenya, Malawi, Mozambique, Tanzania, Uganda, Zambia and Zimbabwe.

Few DBM parasitoids were recorded in the participating countries in these studies. These included *Diadegma* spp. and *Oomyzus sokolowskii (*Kurdjumov). However, their combined level of parasitism did not exceed 14.5% at any of the study sites except at Henderson-Zimbabwe where *O. sokolowskii* caused 40% parasitism in one single collection (Seif & Löhr 1998). A follow-up regional workshop in May 1998

discussed the research results and identified opportunities for future regional research activities (Seif & Löhr 1998). From the results, it was evident that DBM is the major insect pest of cruciferous vegetables in the region. Consequently, the workshop recommended that the issue of DBM and its natural enemy complex should be given more emphasis in future research activities in the region to generate more basic information as preparation for a classical biocontrol initiative against DBM. In particular, it was felt that more information is needed to explain why DBM parasitism by *Diadegma* spp. which has proven such a success in Asia, was so low at all study sites. To answer this question, more work on the taxonomy and biology of *Diadegma* was felt to be a prerequisite.

Judging from the experience in Asia with the implementation of biocontrol-based IPM approaches (Ooi 1990, Poelking 1990, Talekar *et al.* 1990, Biever 1996, Eusebio & Morallo-Rejesus 1996, Iga 1997), there is good potential in eastern and southern Africa to manage DBM with parasitoids as most of the production is also in the highlands. The aim of the project is to contribute to this process.

Expected results

ICIPE developed a project in cooperation with the national research institutions of the four partner countries. The proposal was submitted to the competitive grant facility for international agricultural research of the German Ministry of Cooperation and Development and awarded funding in early 2001. The following results are expected after the first project phase of three years:

- Baseline information on the indigenous natural enemies of DBM complemented through additional collections in collaborating countries
- Taxonomic status of the genus *Diadegma* in Africa is clarified and documented
- Comparative biological studies of the African and Asian DBM parasitoids conducted and promising strains for a classical biocontrol programme identified
- Classical DBM biocontrol pilot programme initiated in Kenya
- Exploration for additional pupal DBM parasitoids conducted in areas of DBM origin

Overall project coordination and management is based at ICIPE. The integration of AVRDC into the project ensures that maximum advantage is taken from its vast experience in a similar project in Asia. Major aspects of AVRDC involvement are the provision of parasitoids of proven quality from Asia and the coordination of exploration for additional pupal parasitoids in the areas of origin of DBM in partnership with the United States Department of Agriculture Biocontrol Station in Montpellier/France (Table 1).

The NARS second scientists as PhD students for the basic scientific studies suggested in the project, contribute with further collection of information about pests and local natural enemies and implement, with support from ICIPE and AVRDC, the importation, release and monitoring programme of the envisaged biocontrol programme. This will first be done in Kenya and later, in a second phase of the project, be extended to all countries in the region with an interest in cabbage production. An annual project coordination meeting is the main forum for planning and review of the regional project activities.

The project covers the crop and pests at the regional level and brings in both local and international expertise to alleviate a problem that has not been addressed by any country in the region. Cooperation should optimise use of scarce resources, avoid unnecessary duplication of efforts, enhance multi-disciplinary and team effort and enhance capacity and capability within NARS in eastern and southern Africa.

Table 1. Principal collaborators in the DBM biocontrol project in East Africa

Country	Institution	Responsibilities	Scientist involved
Kenya	ICIPE Horticultural Pests Programme KARI National Biocontrol Programme, Muguga KARI Regional Research Centre, Kisii	Overall coordination; molecular taxonomic methods; studies of indigenous parasitoids, introduction of exotic parasitoids; pilot site Taita Hills; impact analysis Survey Eastern & Central Kenya; pilot site Limuru; pilot release and monitoring Survey Western Kenya; pilot site Kisii; pilot release and monitoring	Dr. B. Löhr Dr. F. Nang'ayo Mr. Oscar Magenya
Ethiopia	EARO, Nazareth Research Station	National survey for indigenous parasitoids, pilot site studies, long-term population dynamics of DBM	Mr. Gashawbeza Ayalew
Tanzania	Plant Protection Division, Biocontrol Unit, Kibaha; German-Tanzanian IPM Project, Arusha	National survey for indigenous parasitoids, pilot site studies, pilot introduction	Mr. Oscar Mfugale Mr. William Mwaiko
Uganda	NARO Biocontrol Unit Namulonge Makerere University Kampala	National survey for indigenous parasitoids, pilot site studies	Dr. James Ogwang Ms. Florence Nagawa
South Africa	Plant Protection Research Institute, Pretoria	Studies of temperature adaptability of *Cotesia plutellae,* provide *C. plutellae* to Kenya	Dr. Rami Kfir, Mr. Robert Nofemela
Taiwan	AVRDC Taiwan	Provide *Diadegma semiclausum* and *C. plutellae* to Kenya; introduce and test heat-tolerant parasitoids	Dr. N.S. Talekar
France	USDA European Biological Control Laboratory (EBCL), Montpellier	Explore for more heat-tolerant parasitoids	Dr. Alan Kirk

AVRDC Asian Vegetable research and Development Center, Taiwan; EARO: Ethiopian Agricultural Research Organisation; KARI: Kenyan Agricultural Research Institute: NARO National Agricultural Research Organisation (Uganda)

Surveys for indigenous natural enemies

A training course for key national project collaborators was organised jointly by AVRDC and ICIPE in April 2001 in order to standardise methodology and research procedures at the beginning of the project to ensure proper data collection, preservation of specimens for identification and collation of information. Work plans and budgets for surveys and collections were also developed.

NARS collaborators in Ethiopia, Kenya, Tanzania and Uganda initiated national surveys and collections of DBM and its natural enemies in September 2001. Survey work is advanced in Ethiopia, Kenya and Tanzania and will be finished in all partner countries by May 2002. All relevant information is compiled in a database at ICIPE. Parasitoids are also curated and stored at ICIPE until identification is complete. So far, the surveys have not yielded any significant new parasitoids and confirmed the scarcity and ineffectiveness of local parasitoids. *D. mollipla, O. sokolowskii, Itoplectis* sp. and a few *C. plutellae* were the species collected so far. Overall parasitism rates were mostly below 10%. The Ichneumonidae were limited to highland conditions, *O. sokolowskii* and *C. plutellae* to warmer growing conditions (Löhr, unpublished survey data).

DBM moths collected from unparasitised larvae and/or pupae are also preserved and kept at ICIPE. The material may be very important for future studies on the diversity of the pest. Recent studies on other supposedly cosmopolitan species have shown that what appeared to be one species was actually a complex of closely related species (Munroe 1973) and this can have significant consequences for biological control efforts. Chang *et al.* (1997) have shown that in an area as limited as Hawaii, there is considerable variation in DBM and this can be expected to be much greater in the huge area covered by the proposed project.

Taxonomic status of the genus *Diadegma* in Africa

The genus *Diadegma,* the most efficient and widespread group of DBM parasitoids world-wide, has been recorded in Kenya, Tanzania, Uganda and South Africa (Seif & Löhr 1998, Kfir 1997). However, parasitism rates are generally low. Data from earlier field surveys in Kenya, Malawi and Tanzania showed low parasitism by a parasitoid initially identified as *D. semiclausum* (5–11.3%, 15% and 14.5% respectively) (Seif & Löhr 1998). There were some doubts about the correctness of this identification and Azidah *et al.* (2000) grouped all African DBM parasitoids of the genus under the species name of *Diadegma mollipla* (Holmgren), originally described as a potato tuber moth parasitoid. Doubts about the identity of the species persist as the genus *Diadegma* is not fully described (Fitton & Walker1990, Kfir 1997) and there seems to be variability in the material collected so far (R. Sithole, pers. communication; B. Wagener, pers. communication). Therefore, classical and molecular taxonomic methods are used in the project to clarify the taxonomic status of the local African *Diadegm*a spp.

Classical biocontrol of DBM for East Africa

There are three obvious candidates for introduction into East Africa: *D. semiclausum, C. plutellae* and *Diadromus collaris* Gravenhorst. The former was used widely in south-east Asia with good results and should perform equally well in the similar growing conditions of the east African highlands. A good complementary parasitoid for the same agro-ecological zone should be *D. collaris* which is completely absent from East Africa, even though it seems to be relatively common in South Africa (Kfir 1997). *Cotesia plutellae* is also relatively rare in East Africa. Parasitism rates observed in hundreds of collections in areas suitable for this species were very low in Kenya (Nang'ayo, Magenya, Gathu and Löhr, unpublished survey data) and the species was not found in Tanzania (Mwaiko, unpublished survey data). This contrasts with the situation of very high parasitism rates in the lowveld of South Africa (Kfir 1997) and makes the species a good candidate for introduction in semi-arid areas of East Africa. Studies on the temperature adaptability of the species are currently ongoing in South Africa. Another source for this species will be AVRDC Taiwan.

In preparation for an assessment of the impact of introduced parasitoid species, four pilot sites were established for detailed field studies of the population dynamics of DBM and its natural enemies. Pilot releases will be made into these sites after one year of continuous observations is completed. Establishment and impact of the released parasitoids will be assessed in these fields. In addition, a baseline study for an economic impact assessment is in preparation.

Exploration for additional parasitoids

AVRDC and European Biological Control Laboratory will work together to explore for parasitoids, especially pupal parasitoids, of DBM in the Mediterranean area, where the pest is believed to have originated (Talekar & Shelton 1993). DBM damage on crucifers in Europe is minimal because of the presence of a wide range of parasitoids in the continent. In Moldavia-Romania alone, almost 30 species of parasitoids have been listed and these reduce the DBM populations below economic damage levels (Mustata 1992). Discussions are under way with Prof. Mustata to revisit and reanalyse the historical data and initiate season-long collections in the Moldavia region to establish changes in parasitoid species composition with changing temperatures throughout the season.

Additional natural enemies are collected by EBCL staff on their regular collection missions. The collected parasitoids are shipped to AVRDC where they are purified, tested for their effectiveness under tropical lowland conditions and multiplied. These natural enemies will be maintained at AVRDC and made available for introduction in the project countries as well as any other interested countries in Asia.

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Developing a training and information package for IPM implementation in *Brassica* vegetable crops

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Abstract

The ability to correctly identify a pest or disease problem or to determine disorders within *Brassica* vegetable crops is critical to making a sound decision on corrective actions and the most appropriate strategy for a particular situation. Most research into problems of *Brassica* crops has been discipline based, focusing on identification and "best practice" recommendations for managing specific pathogens, insects, nutrient deficiencies or other disorders. A project to develop an electronic knowledge management system, based on Integrated Crop Management principles, aims to bring together insect, weed, disease and disorder information in a comprehensive and integrated multi-media product.

The information and training needs of farmers, advisers, students and scientists will determine the structure of this decision support tool. It will incorporate Lucid™ keys for diagnosing problems in *Brassica* vegetable crops, linked to "best practice" management strategies that can be delivered via CD and/or the Internet. The CD uses software (WebGIST), developed by the Centre for Pest Information Technology and Transfer at The University of Queensland, that enables the user to view, search and print the information in a format similar to current web browsers. The information is created in HTML so that it can easily be transferred and linked to the Internet. An English prototype CD has already been developed. A proposal has been submitted to the Australian Centre for International Agricultural Research to develop a final product, which will be bilingual (English and Mandarin), with the intention to develop a generic English version that can subsequently be customised for other languages and regions.

Keywords

decision support tool, Integrated Crop Management, problem diagnosis, Lucid™, best practice management

Background

A farmer's or adviser's ability to determine what is wrong with a particular *Brassica* vegetable crop, or to correctly identify a pest or disease problem, is critical to making a sound decision on the most appropriate strategy to take in a particular situation. By contrast, research to solve problems in *Brassica* vegetable crops has largely been discipline based, focusing on identification and 'best practice" recommendations for managing specific pathogens, insects, weeds, nutrient disorders or environmental effects. So while information or knowledge for solving a particular problem may exist, it may be in a form that is not easily accessible and immediately applicable for specific on-farm situations.

Pest management in tropical and sub-tropical *Brassica* vegetable crops has been particularly problematical for many years. The complex of insect pests, the quality issues regarding the level of control required, problems with insecticide resistance and the health risks to operators and consumers associated with excessive insecticide use all contribute to the intractability of the problem. Implementation of Integrated Pest Management (IPM) systems in vegetable crops is also difficult as it usually involves more complex decision-making processes when compared with calendar treatment with insecticides. For instance, in China, the greater use of biopesticides has increased the need for improved management skills as these products are often most effective when deployed as part of an overall IPM approach.

On the other hand, significant advances have been made in the development of IPM systems in Australia, China and some regions of south-east Asia. In particular, since 1995 two consecutive China/Australia projects funded by the Australian Centre for International Agricultural Research (ACIAR) have focused on

managing the insect pest complex affecting *Brassica* vegetables using an IPM systems approach. Activities were targeted at addressing priority research and development issues, but also incorporated a strong implementation component in work programs (Heisswolf & Bilston 2004, Liu *et al*. 2004, Heisswolf *et al*. 1997).

Combined with government initiatives, these efforts have had a considerable impact on the implementation of IPM in the project areas, and in Zhejiang Province and south-east Queensland as a whole. For example, an independent evaluation of project impact in Hangzhou, Zhejiang Province, in 2000 indicated that the proportion of growers keen on non-chemical measures for insect pest control reached 36% in a project area, compared to 20-23% in non-project areas in the same region (Liu & Qui 2001). In Queensland, Lockyer Valley, *Brassica* farmers have gradually implemented various IPM practices over the past ten years, with an estimated 60% of farmers using a crop scout to aid in decision-making and 70% of farmers using IPM to some degree (Heisswolf & Bilston 2004). The long-term impact and sustainability of these extension efforts could be considerably enhanced through the use of more efficient tools and systems for training and for integrating and delivering information to end users.

There is an opportunity to build on existing knowledge by integrating information on the identification and management of insect pests, diseases, disorders and weeds within one flexible information management system using an Integrated Crop Management approach. Our proposal is to develop an IT package that contains tools to correctly diagnose and evaluate problems in *Brassica* vegetable crops, backed up by "Best Practice" information, so creating a new information resource designed to get existing knowledge and technology into the field. This system will have a number of advantages over traditional paper-based materials:

- it will provide much greater flexibility and capacity than paper-based systems, allowing for a more stream-lined integration of knowledge from different disciplines within a problem-centred approach
- it can be used directly as an interactive training and decision support tool
- it will be easier to update and offers more potential for web site linkages
- it will be a resource for problem diagnosis and identification of "Best Management" options, with users having the option of printing off advisory leaflets and other products as needed

To ensure that the final IT product meets client needs, end users will be involved in the design and testing of prototypes.

In the past, extension material associated with crop protection problems has largely been in the form of booklets or pamphlets on specific problems or a class of problems. In China, a survey of literature of insects associated with *Brassica* vegetable crops in 1996 showed that although an extensive literature existed on individual species of pests, there had been little literature on *Brassica* IPM at the cropping systems' level (Liu & Yan 1998, Liu *et al*. 1996). There have been many manuals on insect pests and diseases in vegetable crops, however most of these manuals only give information on the morphology, biology and control of individual species, and only a few of them, such as the manual by Liu *et al*. (1995), offer limited coverage on the management of insects and diseases at the crop level.

In Australia, the availability of decision support tools that are aimed at the cropping systems level for *Brassica* vegetable crops is limited. Publications and Facts Sheets provided by state government departments are usually also discipline or species based, or provide agronomic information with limited detail for problem diagnosis and their solution. The exceptions are a field guide for identifying pests, natural enemies, diseases and disorders in *Brassica* vegetables (Donald *et al*. 2000) and a pest monitoring guide for these crops (Heisswolf & Brown 1997).

A non-computer based approach to dealing with knowledge management issues in horticulture is the approach adopted for the Agrilink Information kits produced by the Queensland Department of Primary Industries (DPI) (Anonymous 2001). These information kits were developed in response to an increasing need for "stand alone" information packages targeted at advisers, consultants, students and farmers. A key feature of these kits is the reorganisation of written and visual information in a form that reflects how decision-makers actually search for information.

The Agrilink kits encompass the principles of adult education, taking a problem-centred approach to presenting information, catering for different levels of user skills and preferred methods of searching for answers to problems. Each Agrilink kit is divided into sections which target various information needs, providing different entry points to a specific problem, which are then also cross referenced to other relevant

information within the package. The Problem Solver section of the kits represents a paper-based diagnostic key. It allows the user to work through a collection of images arranged according to symptoms seen in the field or nursery – the problem – rather than presenting images arranged along discipline lines – as a disease, pest or disorder.

The Queensland DPI is currently integrating information derived from the two China/Australia ACIAR projects within a *Brassica* Agrilink information package. Information on IPM and all other aspects of *Brassica* crop management is being brought together by tapping into expertise of departmental and industry staff in Queensland and linking with existing projects in other Australian states on diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae); Clubroot disease; and IPM implementation. The *Brassica* Agrilink publication will contain a large collection of images and drawings on which our proposed IT product can build.

The IT product will use software tools - WebGIST and Lucid™ - developed by the Centre for Pest Information Technology and Transfer. WebGIST is a software package that allows a CD product to have all the familiar functions of a web browser such as navigating the information screens and fact sheets, and printing and searching the content. Lucid™ software is a tool for building interactive multi-media identification keys (See www.lucidcentral.com). Lucid™ keys differ from traditional paper-based dichotomous or pathway keys by allowing the user the flexibility to begin an identification or diagnosis anywhere in the key, using whichever character is easiest to use. They are also not restricted by unanswerable couplets and can use various functions to progress through the key quickly to get an answer. For more information on Lucid™ identification keys and diagnostic keys, see www.lucidcentral.com and www.cpitt.uq.edu.au.

All the fact sheets on the CD product will be produced in Hyper Text Mark-up Language (HTML) that allows the user to navigate more easily through the CD content and also allows for future publication on the Internet. These fact sheets can incorporate text, images, sounds and video so that all information on a topic can be accessed from a single screen.

A prototype IT package for *Brassica* vegetable crops

A prototype CD utilising text and images from the draft *Brassica* Agrilink kit has already been developed for demonstration purposes as part of the current ACIAR project. The home page for this prototype is shown in Figure 1. The prototype contains rudimentary problem diagnosis and insect and weed identification keys. While the specific design of diagnostic and identification keys, and entry points and linkages to "Best Practice" information, will depend on the outcomes from participative planning processes with end users, three major keys are likely to be incorporated into the package:

- a problem specification key based on symptoms seen in the field e.g. diseases, disorders, pest damage etc. – this gallery type key is illustrated in Figure 2
- an insect identification key for both pests and natural enemies – this key may combine dichotomous and gallery keys (Figure 3) with a Lucid™ key
- a weed identification key based specifically on Lucid™. Figure 4 illustrates the layout of this matrix key

Figure 1. The home page of the prototype Brassica IPM CD illustrating the broad framework of the package

Figure 2. The problem diagnosis and identification key, illustrating the gallery key for spots and marks on leaves. Each image can be linked to a Fact Sheet or other relevant parts of the package.

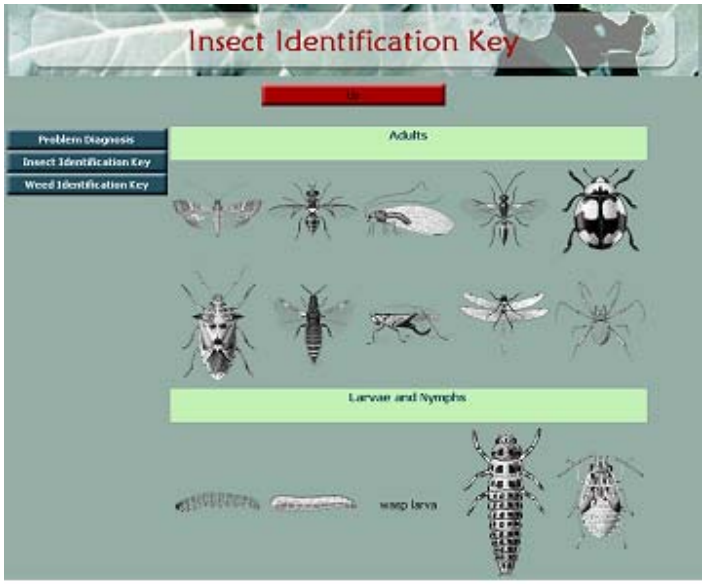

Figure 3. The entry point for the insect identification key, illustrating the gallery of drawings for major insect orders.

We estimate that between 100–120 "Best Practice" Fact Sheets on insect pests, natural enemies, diseases, weeds and disorders will be required to support the problem specification and identification keys. These information sheets will contain text and images, the likely format including an introduction, details on monitoring, the lifecycle of the pest or disease, damage and control – covering production breaks, crop hygiene, biological control options and pesticides. The prototype also contains a section outlining the principles and practices of IPM (Figure 5), a glossary and reference section and scope for integrating links to other sites.

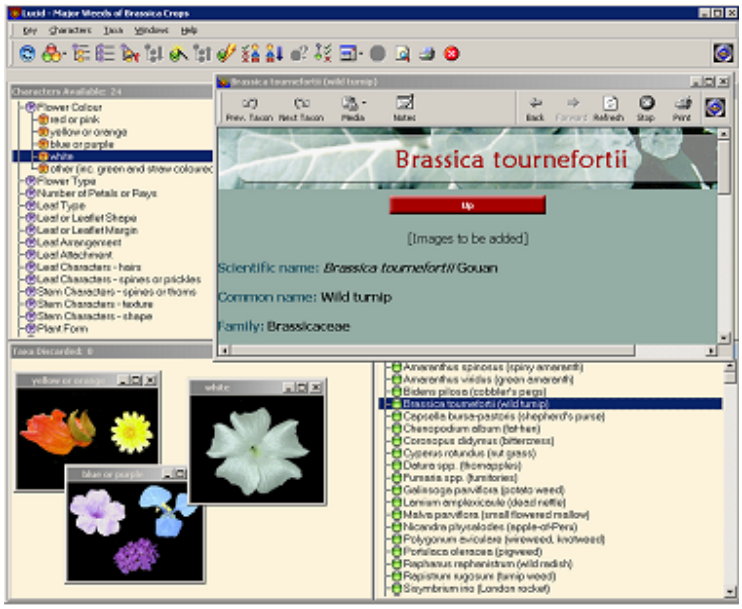

Figure 4. The Lucid™ matrix key for identifying weeds.

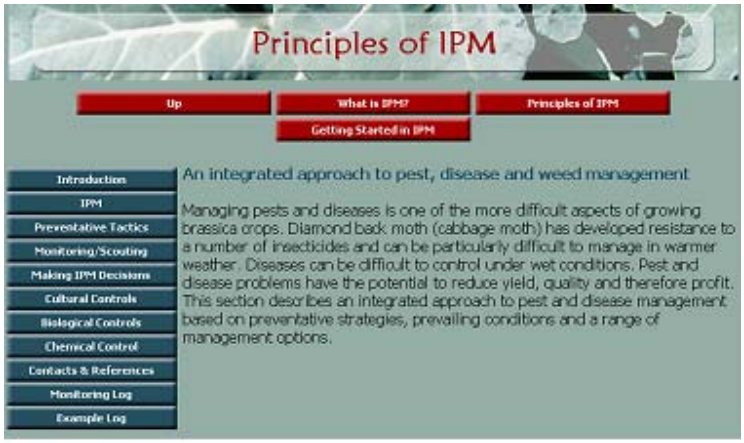

Figure 5. The section on the principles and practices of IPM in *Brassica* vegetable crops with major topics at the top and left hand side of the screen.

Plans for the future

A project proposal to develop the prototype CD into a field-tested, multi-media IT product ready for final production and distribution has been submitted to ACIAR. By providing an integrated resource base of information on the diagnosis and corrective treatment of crop problems in *Brassica* vegetable crops, the aim of the project is to contribute towards improved crop management by building the skills and capacity of decision-makers through several avenues:

- the use of the diagnostic and identification keys in training courses and by individual research and extension officers will lead to improved diagnostic skills and improved extension advice
- wider availability of "Best Practice" information, linked to crop diagnostic keys, will encourage improved decision-making and crop management skills on the part of research and extension staff, consultants and farmers
- images, keys and the interactive nature of the IT package will improve student and farmer training activities and, in the case of the latter, lead more directly to improved crop management

The main project activities will include design specification, integrating relevant text, images and other material to assist with identification, developing "best practice" fact sheets, building keys and testing the evolving training and decision support tool with researchers, extension officers, consultants, other advisers, students and farmers. An action research approach that involves end users in the design and testing of the IT

package will be incorporated as an integral part of the project. Based on adult education and action learning principles, this approach will ensure that the knowledge brought together from the different disciplines is placed within a cohesive framework that can be easily accessed and implemented by potential end users.

It is anticipated that the project will start in January 2003 and be completed by the end of June 2004. The Queensland DPI and Zhejiang University will coordinate the project and end-user participation in package design and testing, and provide technical expertise on Integrated Crop Management and "Best Practice" information on pests, diseases, disorders and weeds in this international collaboration. The Centre for Pest Information Technology and Transfer will facilitate the development of the multi-media diagnostic keys and provide software support and services. The Zhejiang Department of Agriculture will support development and testing of the package with end users in China through training workshops.

The chief output from the proposed project will be two field-tested, multi-media information management systems based on Integrated Crop Management principles. In the Chinese version, diagnostic keys and "best practice" management strategies will initially be developed for conditions in the east region of China, but there is potential for adapting the package to other regions in China. This IT product will be ready for final production and distribution in China with the aim of having it widely used as a training tool for students, researchers and extension officers; and to serve as a decision support tool for extension staff and farmers.

A second output from the project will be a field-tested generic IT package in English ready for customisation for other regions and languages, such as Indonesia and Vietnam. The Australian *Brassica* industry is interested in the concept of this multi-media package and our intention is to develop a process for customising the generic English version for Australian conditions, in collaboration with industry stakeholders.

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A change in attitude – seeing pests from a different perspective

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Abstract

This paper outlines a history of Integrated Pest Management (IPM) in *Brassica* vegetables from a grower's perspective. In the early 1990s, it had become very difficult to produce quality *Brassica* vegetables because of problems controlling diamondback moth (DBM) and exiting the *Brassica* industry all together was a definite possibility.

I was approached by an extension officer from the local DPI research station to try out IPM. This involved a lot of trial and error on farm over several years and this work continues today. It was lucky that we had good success in the first year, so that I could see that IPM could work, because the second year of trial work proved to be more difficult. We reviewed the season with other growers, ran a field day and planned our approach for the next season. Reviewing the past season and planning for the next season, what new strategies to try and how to improve our pest management program, has become a habit. We are continually trialing new methods that we can incorporate.

In February 1998, we had the inaugural meeting of the Brassica Improvement Group (B.I.G.) This group was formed to disseminate and share information that we had developed over the previous 5 years with other growers, industry and researchers. IPM level that we have reached today could not have happened without the team effort of people from all parts of the industry, from researchers to chemical companies, through to consultants and growers.

Why I changed to Integrated Pest Management

I need to take you back to where the problems started that made me look to IPM as an alternative pest management system. I started working on the family farm with my father on leaving school in the late sixties. At that time he grew potatoes, onions, pumpkins, lucerne, grain and ran a small dairy. Pests weren't a real problem then. My father didn't own a boom spray until the mid sixties when he built his own, which by today's standards is totally unworkable. It was a 200-litre fuel drum on the back of the tractor, a boom, and the calibration was to fill the drum and see how far it went. He then used chemicals like DDT which killed every thing. This we believed at the time was the answer to all pest problems. I remember the abundance of birds, (finches and Willy Wagtails) which slowly disappeared over the next few years. We were ignorant of the damage that we were causing to the environment.

I took the farm over in 1973 and continued a similar pest management program until DDT was finally banned. I would say I was one of the greatest critics of this action at the time, later to learn how wrong I was. In the early eighties I began to grow Brassicas. This went OK for a few years until DBM become uncontrollable due to chemical resistance. Our attitude at the time was, farm today for today. If we saw one grub we felt we'd missed a hundred, if it wriggled or flew we'd spray. We used new chemicals, as they became available with no consideration to the environment. If they worked we used them and when they didn't, we poured in another chemical. A new chemical might last 2 seasons. There was no thought to resistance management. I would mix two or three chemicals together and was spraying every second day, the moths were as thick as ever, harvest was about a 60% cut out and then the quality was poor, by today's standard unmarketable. I'd ask the chemical salesman why do we need to use mixtures? And the answer was, one won't work with out the other. Weren't we gullible?

In the late eighties, the DPI developed the 3-V strategy. The idea was to rotate the chemical families and to only use certain families at one time, thus having a period of time where each chemical family was not used. Unfortunately this strategy failed due to the ignorance and unwillingness of growers to cooperate. A summer production break was introduced and accepted by growers, only because it became uneconomical to grow brassicas due to the high costs of pest control and poor unmarketable produce.

Flowing on from this came the IPM trials. At this time I was seriously considering exiting the industry due to viability problems and the difficulty in producing a quality product. I became involved in IPM in the early nineties when an extension officer from the local DPI approached me to do commercial trials. As I had

nothing to lose, I said yes. Another grower from a different area in the district was also approached. He also agreed to run the trials. This proved to be a valuable decision as we were able to make comparisons between the two areas. So started a positive relationship between researcher and grower that still exists today.

It was lucky I had success in the first year. The second year proved to be more difficult while it was the opposite for the other grower. At the end of the second season we sat down with DPI to review the previous two years on the two farms involved. The interesting factor in this review was the approach that each farmer had taken. I started the successful first season with a soft approach, finding the second season more difficult when starting with heavy chemicals. The other grower did the opposite finding the first season difficult when starting with heavy chemicals, while having success in the second year when he took the soft approach to start that season.

It became obvious to me that a soft approach at the start of each season seemed to be a move in the right direction. I began to realise that the beneficials, although low in numbers after the summer break, were the breeding stock for that season. My season starts in February, planting every week for sixteen weeks with a double plant for two or three weeks in the middle of the season. To alleviate my lack of confidence I employed a professional consultant. I still use and recommend that all growers use a consultant. He is moving around the district and can be aware of pest pressures developing before the grower sees it on his own farm.

Having the advantage of advice and continual support from DPI and with an experienced consultant to monitor my crop, I decided to reduce the size of my first planting in the third year, take a soft approach, and if necessary sacrifice that planting. This I hoped would increase the numbers of predators for later in the season. This strategy, although we felt was very risky at the time, did work with no less in cut out than in previous years, and it did make pest control easier later in the season. This showed me that there was an alternative. We set out pheromone traps for DBM monitoring and numbers caught were consistently around the 240 moths per trap per week. Today DBM is no longer a major problem with moth counts reduced to an average of 7 per trap per week. All this was done in an intensive cropping area, with neighbours still using conventional style chemical technology and growing practices.

Farming for tomorrow today

I had gone through three years of criticism from my neighbours who told me I'm a nut, that this can't be done. The fourth year we were starting to put things together and now other growers were asking, how can we do it? How do we get started?

Changing from a purely chemical control program to a biological program does not happen overnight. The important step to getting weaned off chemicals was to learn to recognise pest damage symptoms and the correct identification of pests, predators and parasites. This we learnt by DPI holding pest ID Workshops for growers. We started to learn the principles of IPM. Step by step we learnt that we could tolerate higher pest numbers than previously thought possible. Because I sprayed only when needed, the number of sprays was reduced.

We saw the need to change to softer pesticides such as Bt which is more selective. We became aware that pest management with the use of predators and parasitoids is not only about controlling pests, but also about preventing them. Under a conventional program you create a sterile environment. With IPM, the grower is closely involved with all pest control actions, and because he knows better what's going on in his crop in terms of pest populations, the results with softer chemicals will be improved. Time is needed to build up the beneficial populations. An IPM program should be started at the beginning of the season. It may take one or two seasons before you see results, but it does become easier each year. A summer production break must be encouraged in our area.

We no longer spray with an ovicide. We carefully monitor the maturity of eggs to see if they have been parasitised and then spray at hatching to kill the larvae that come through, preferring to use Bt so as not to kill the parasitic wasp. Timing of this spray is critical and learning this was a major step forward. I now add molasses to the spray to encourage feeding. By doing this I have achieved good control of centre grub (*Hellula*). This draws the grub out onto the leaf to feed where the spray has been placed. I plant alyssum randomly throughout the crop. This encourages hover flies and parasitic wasps by giving them a nectar source needed to survive.

When spraying today, I consider what I don't want to kill, as well as what I need to kill. If I have to use a heavy chemical I spray the hot spots earlier so as to avoid spraying the whole crop. One spray with a heavy insecticide doesn't seem to have a large effect on beneficial numbers, but a second spray in succession can have a significant effect on these numbers.

Problems that we have encountered are that the secondary pests that were never a problem when using heavy chemicals are now coming through as primary pests. *Spodoptera* (cluster caterpillar) is one that we have found difficult to control. One area that I am trialing in the control of Spodoptera is root dipping of plants in a systemic insecticide before planting. Although showing promise this year, more work is needed before I could be certain that this is successful. Thrips have also become a problem towards the end of the season. The critical period for thrips in cabbage seems to be about fifty to sixty days after transplant, just as the leaf is starting to close over. We are controlling them by spraying dimethoate. This is hard on beneficials, but at the end of the season is not so critical. I would prefer to use a softer approach. I have trialed neem oil and soap sprays, but they showed little control. We still needed a dimethoate for control.

To enhance the efficiency of an IPM strategy you need to have a maximum of growers practising IPM. Those of us involved in this program saw a need to disseminate this information to other growers. In February 1998, the Brassica Improvement Group (BIG) was formed. This group meets once a month during the growing season to share and exchange information with researchers, industry and other growers. BIG has achieved industry and media recognition with widespread grower support.

Biological IPM has changed my whole approach to farming. I find I need to work with the environment, rather than think that I can control it. Be prepared for a change of attitude, this is most important. It is a change you will prefer in time. Those birds that I saw disappear in the sixties and seventies are starting to make a comeback. I watch with great delight as they swoop over my crops feeding on moths. I see small green frogs living at the bottom of cabbages feeding on small larvae. All this helps reduce pest pressures and significantly reduces the risk of chemical residue in the produce leaving my farm.

The healthier we can get our soils the healthier we can get our plants. By having healthier plants we have a better resistance to pest damage. Today I look at soil management rather than soil preparation. I'm using a yeoman plough rather than a mouldboard plough because a yeoman breaks the hard pan without soil inversion. A mouldboard inverts the soil and buries the microbes which live in the top two inches of soil.

Success of this IPM program can be measured on my farm by the fact that I was spraying cabbage every second day before starting IPM. Today, I average 5 sprays per planting for the season and most of these are with biological sprays. It is my dream before I finish farming, to grow a planting of cabbage without spraying an insecticide at all. I have achieved this in broccoli over the last 5 years. Now, I farm for tomorrow today.

The success of this program could not have been achieved without the team effort of researchers, the chemical companies, through to consultants and growers. To those who played a role in this program over the last ten years, you saved our industry. For this I am truly grateful, and on behalf of our industry in the Lockyer and further afield a big thank you. And to the organisers of this conference for allowing me the time to tell my story, also thank you.

Developments in IPM programmes for vegetable brassicas in Fiji, Cook Islands and Papua New Guinea

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Abstract

IPM extension workshops were held in Fiji in September 1999 and February 2000 for researchers and extension staff from Fiji, Cook Islands and Papua New Guinea. IPM teams were formed and new national programme activities are being supported by the Secretariat of the Pacific Community (SPC) Plant Protection Service. Technical recommendations include the introduction of a range of selective insecticides to maximise the impact of the key biological control agents of diamondback moth (DBM). Communication recommendations include the IPM teams lobbying government for support of IPM and also lobbying the agrochemical industry, importers and retailers for reduced costs and smaller packaging of selective insecticides. Activities underway in all three countries include training sessions for extension staff and farmers on recognition of key pests and beneficial organisms and proper use of insecticides. In Fiji, field trials are underway to develop action thresholds for the key pests, *Plutella xylostella* and *Crocidolomia binotalis* and to evaluate the use of selective insecticides, particularly those with short withholding periods. In the Cook Islands, IPM is included in official Ministry of Agriculture policy and has led to increased staff to support IPM activities, the importing and registration of "IPM-friendly" agrochemicals and the re-release of *Diadegma semiclausum* on Rarotonga Island. The Ministry of Agriculture has decided not to introduce *Cotesia plutellae* into the Cook Islands because of concerns over its specificity. In Papua New Guinea, establishment surveys undertaken after releases of *D. semiclausum* imported earlier from Fiji and the Philippines show that *D. semiclausum* has dispersed naturally up to 15–20 km from initial release sites in the Eastern and Western Highlands. The *Brassica* IPM programme in P.N.G. has been scaled down due to lack of funding and staff shortages. Work that is continuing includes mass rearing and releases of *D. semiclausum,* publishing IPM recommendations (extension leaflets) and field trials on new insecticides.

Cabbage caterpillars in New Caledonia: integrated pest management project

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Abstract

Several pest lepidopteran species have been identified in the archipelago. The most important is *Plutella xylostella* but, in some other biotopes (Loyalty Islands), *Crocidolomia binotalis* is frequently dominant. The other species are *Hellula undalis, Chrysodeixis* spp. and *Helicoverpa armigera.* The importance and the damage caused by these pests are outlined. With conventional chemical control, the farmers damage the environment, which is also risky for the consumers.

The project is the adjustment of the integrated pest management for our climatic conditions, including three coordinated operations: epidemiological studies of the pests, the determination of encountered parasitoids and predators, the evaluation of yield losses and efficacy of beneficial insects during a several-year long study. This part of the project has just begun and the first observations are given.

Introductions of parasitoids are projected during the next few years, with *Diadegma* spp., *Cotesia plutellae* and *Oomyzus* spp. For this operation, methods are described including field evaluations without pesticides during an all-year-round production system. The management of use of chemical control began several years ago (1994–1997) and some results have been published in a French journal "*Phytoma*". The chosen active ingredients are environmentally safe and not harmful to beneficial insects. For the moment, the active ingredients being tested are biological ones such as azadirachtin.