EFFECTS OF INSECTICIDES ON *SITONA DISCOIDEUS* (GYLLENHAL) (COLEOPTERA: CURCULIONIDAE) AND ITS PARASITOID *MICROCTONUS AETHIOPOIDES* LOAN (HYMENOPTERA: BRACONIDAE)

A THESIS
SUBMITTED IN PARTIAL FULFILMENT
OF
MASTER OF AGRICULTURAL SCIENCE
AT
LINCOLN UNIVERSITY

BY
M. R. MCNEILL

LINCOLN UNIVERSITY
1991

Sitona discoideus and its parasitoid *Microctonus aethiopoides*.

Abstract of thesis submitted in partial fulfilment of the
requirements for the degree of M. Agr.Sc.

EFFECTS OF INSECTICIDES ON *SITONA DISCOIDEUS* (GYLLENHAL) (COLEOPTERA: CURCULIONIDAE) AND ITS PARASITOID *MICROCTONUS AETHIOPOIDES* LOAN (HYMENOPTERA: BRACONIDAE)

by Mark R. McNeill

Laboratory and field experiments were conducted to evaluate the effect of insecticides on the egg and adult stages of *Sitona discoideus* (Gyllenhal), and its natural enemy *Microctonus aethiopoides* (Loan). A range of insecticides were applied to *S. discoideus* eggs with the aim of assessing the ovicidal properties of the insecticides and to provide additional control to that already achieved against the adult weevil. The Potter tower direct spraying method was used to apply the insecticide at near field rates. Chlorpyrifos and fenitrothion exhibited the greatest activity followed by deltamethrin, diflubenzuron and γ-HCH. Summer oil and fenvalerate were ineffective as ovicides. Following treatment, chilling of eggs for 30 days below the threshold temperature for development, reduced the ovicidal activity of both chlorpyrifos and fenitrothion with the former showing an increase in LC_{50} and change in the log dose-probit slope, while the latter produced a shift in LC_{50} only.

The effect of chlorpyrifos and fenitrothion on the parasitoid *M. aethiopoides* (Loan) was investigated and compared to its host *S. discoideus*. Topical dosing with chlorpyrifos and fenitrothion showed that the parasitoid was more susceptible than the weevil. The selectivity ratio of LD_{50} parasitoid/ LD_{50} weevil confirmed that both insecticides were selective towards the parasitoid, with fenitrothion relatively more selective than chlorpyrifos. Fenitrothion was more toxic to aestivating than reproductive weevils, although the slopes of the log probit-dose line were similar. Possible reasons for the susceptibility of aestivating weevils relate to biochemical and physiological changes associated with aestivation, including greater sensitivity of cholinesterase or lack of food in the gut predisposing the weevil to the insecticide.

The tolerance of the pupal stage of *M. aethiopoides* to both chlorpyrifos and fenitrothion was demonstrated using the Potter tower and topical application. Direct application using the Potter tower, with cwncentrations in excess of field rate did not affect pupal survival. However, longevity and the ability to parasitise weevil hosts were impaired in females surviving chlorpyrifos

at 2.4 g/l. The effect of chlorpyrifos on parasitoid longevity was greater in males. The high tolerance displayed by the pupae was repeated when cocoons were topically dosed with methyl-ethyl-ketone solutions of chlorpyrifos and fenitrothion. An LD_{50} for pupae could not be determined for either insecticide at the range of concentrations tested.

A field experiment investigated the impact of chlorpyrifos applied at 0.3 kg a.i./ha on *M. aethiopoides*. Application in mid-May 1988 resulted in a 96.2% control of the weevil population leaving a post-spray density of 0.8 weevils m^{-2}. The parasitoids occurring as larvae inside the weevil were particularly susceptible to the insecticide at this time. Surviving weevils were 87.5% parasitised, and it is speculated that the resulting parasitoids would form the nucleus, albeit at low density, to attack any surviving weevils in the following spring.

In conjunction with the field study, laboratory experiments were conducted to assess the toxicity of field weathered chlorpyrifos residues on both the adult parasitoid and weevil. At 0.3 kg/ha, chlorpyrifos residues caused over 60% mortality of *M. aethiopoides* for three days after spraying, but activity declined rapidly thereafter. A more prolonged level of activity was recorded against *S. discoideus*, with c. 60% mortality one day following application declining slowly through to fifteen days post-spray. Chlorpyrifos at 1.0 kg/ha produced between 78-100% mortality in both weevil and parasitoid for ten days following application. Twenty-two days after spraying, mortality the 1.0 kg/ha rate still remained at $\geq$ 40% for both insects. There appeared to be no obvious interaction between weevil mortality and the presence of larval parasitoids. However 4th instar larva emerged and successfully pupated from dead weevils up to 24 hours after weevil mortality had been noted.

Based on the field experiment, timing of insecticide application is important for the survival of the parasitoid. Delaying application till June as opposed to May, would allow a portion of the parasitoid population to move from the vulnerable larval stage to the highly tolerant pupal stage. This would provide a nucleus of parasitoids to attack surviving weevils in spring.

However, despite virtual elimination of the parasitoid when lucerne is treated in mid-May, the management practice of applying a single spray in autumn combined with the limited areas being treated, ensures that reservoirs are maintained outside sprayed paddocks. The parasitoid is then reintroduced in the following autumn during the post-aestivatory reinfestation flights by the weevil.

CONTENTS

LIST OF FIGURES

FIGURE

LIST OF PLATES

PLATE

LIST OF TABLES

TABLE

CHAPTER 1

INTRODUCTION

Sitona weevil *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae) was accidentally introduced into New Zealand in the early 1970's (Esson 1975), the weevil having been in Australia since the 1950's (Chadwick 1960). Sitona weevil is a pest of *Medicago* species, and in New Zealand is a major pest of lucerne *Medicago sativa.*

The genus *Sitona* contains many cosmopolitan pest species which feed exclusively on *Medicago* spp. and are regarded as a major pest of leguminous plants throughout the world (Grossheim 1928). In Australia *S. discoideus* causes most damage in annual medics (Allen 1971), whereas in New Zealand lucerne is the crop at most risk (Kain and Trought 1982; Goldson et al. 1984)

As *S. discoideus* arrived in Australia and New Zealand without its associated natural enemies (Aeschlimann 1980), nor closely related competing species (Aeschlimann 1984), the weevil had the potential to have a major impact on *Medicago* crop production in Australia and New Zealand (Allen 1971, Goldson et al. 1984). As a consequence, a major research effort has been undertaken to evaluate the damage potential of *S. discoideus* in New Zealand, and its population dynamics (e.g. Goldson et al. 1985, 1988).

Lucerne is particularly important in Canterbury on light stony soils for sustaining livestock production when warm-dry conditions occur during January to March (Wall 1982). The presence of *S. discoideus* in lucerne was viewed with considerable concern by farmers who observed very high numbers of adults in December-January (Goldson et al. 1984). Feeding by these adults resulted in mid-season yield losses had the potential to be high (Allen 1971). Adult feeding activity is most marked during December to January and resulted in mid-season losses of up to 20-30% (Goldson et al. 1985). Because of the startling visual impact of adult feeding, their activity was initially given more importance than larval feeding (e.g. Wightman 1979; Kain and Trought 1982). However, it was subsequently shown that larvae were the most damaging stage causing yield losses of up to 43% with the effects lasting for up to 3 months (Goldson et al. 1985).

The biology and ecology of *S. discoideus* has been thoroughly researched by several authors (Frampton 1987; Goldson et al. 1984; Kwong Sue et al. 1980a,b; Wightman 1979, 1981), as has the economic loss caused by *S. discoideus* (Goldson et al. 1985; Goldson and Muscroft-Taylor 1988).

The agronomic basis for yield loss has been explored by Goldson and Proffitt (1985); Goldson et al. (1987) and Goldson et al (1988). Losses were attributed to a type of damage-induced dormancy and a loss in photosynthetic efficiency in lucerne (Goldson and Proffitt 1985), coincident with nitrogen stress induced by the larval destruction of the root nodules (Goldson et al. 1988). Additionally, there were some indications that repeated larval attack could lead to reduced plant vigour, and increased susceptibility to damage from even lower *S. discoideus* larval populations (Goldson et al. 1987; Proffitt and Goldson 1986).

Control of *S. discoideus* at the larval stage using soil drilled insecticides (Barratt 1985) and entomophagous nematodes (Bailey and Milner 1985) was unsuccessful. However, after the phenology of *S. discoideus* was understood (Goldson et al. 1984), a practical and effective recommendation was to apply one or two foliar applications against the adults in autumn (Goldson 1984). Chlorpyrifos and fenitrothion have been registered for use against *S. discoideus* on lucerne, and spray applications are timed to coincide with the end of the return flights back into lucerne during autumn, before the bulk of the egg laying took place. The timing of application could be left until late autumn as there is relatively little larval recruitment between May and mid August (Frampton 1984). However, as the intensity of egg laying is temperature dependent (Kwong Sue et al. 1980a), warm autumn temperatures combined with low saturation deficits, can mean that early autumn larval establishment can be particularly high (Frampton 1984).

Because there is the potential of yield loss in the susceptible 1-4 year old lucerne stands (Goldson and French 1984), from larvae eclosing before control measures are undertaken, it was therefore desirable that a contact insecticide effective against the adult weevil should also exhibit activity against the egg stage. To this end, experiments aimed at establishing what insecticides, employed primarily for the control of *S. discoideus* adults, were also ovicidal against the egg stage.

In 1982 *M. aethiopoides* Loan (Hymenoptera: Braconidae) a parasitoid of the adult *S. discoideus* was released and successfully established in New Zealand (Stufkens et al. 1987).

Initially, there were doubts about the ability of *M. aethiopoides* to effectively control *S. discoideus* population due to density dependent effects (Goldson and Proffitt 1986) and intergenerational gaps between the parasitoid and the weevil host (Goldson et al. 1984). Despite this, however, in mid-1987, it became evident that *M. aethiopoides* was becoming an effective control agent against *S. discoideus*.

Therefore a shift in the emphasis of this research project was necessary to accommodate a new need to preserve the biological control agent and reduce the insecticide inputs. Therefore, this research project was modified to include an assessment of the two insecticides currently registered for control against *S. discoideus*, on *M. aethiopoides*. The objectives of the research were therefore revised to:

Part I

To investigate the potential ovicidal activity of insecticides used for the control of *S. discoideus*.

Part II

1. To investigate the toxicity of chlorpyrifos and fenitrothion to *M. aethiopoides* adults and pupae, and compare the response to the host *S. discoideus*.

2. To determine the effect of applying insecticide to lucerne in autumn on the *M. aethiopoides* - *S. discoideus* interaction.

3. To establish the efficacy of chlorpyrifos applied to lucerne, on *S. discoideus* and parasitoid adults.

4. To suggest a possible means of reducing the impact of insecticide on the parasitoid.

CHAPTER 2

REVIEW OF THE LITERATURE

THE BIOLOGY OF *SITONA DISCOIDEUS* AND RELATED *SITONA* SPP.

INTRODUCTION

Sitona discoideus, commonly known in New Zealand as sitona weevil, belongs to the family Curculionidae, an important group in the Order Coleoptera, containing some 25 *Sitona* species (Aeschlimann 1984). As a group *Sitona* (Germar) attacks a wide range of leguminous plants including a number of important cultivated crops in Europe, the Near-East and North America (Melamed-Madjar 1966). Several species of the genus *Sitona* have been reported to specifically attack wild and cultivated *Medicago* L. spp. (Aeschlimann 1984).

In general, the larvae of the genus *Sitona* are root feeders, while adults attack the foliage (Morrison et al. 1974). The effect of larval feeding on the root system has been well documented (Morrison et al. 1974). Larvae feed on either root nodules, the root system or both (Aeschlimann 1986; Danthanarayana 1967; Grossheim 1928). For the broom weevil, *S. regensteinensis* (Hbst.), root nodules assume greater importance with larvae only consuming those nodules containing bacteria (Danthanarayana 1967).

The adults of *Sitona* attack the foliage, with feeding characterised by 'scalloping' of the leaf margins (Hopkins 1979; Wightman 1981). Adults have the potential to markedly reduce plant growth (Morrison et al. 1974), and heavy infestations can result in complete defoliation of the plant (Moulden 1973). For this reason the adults were sometimes considered the most destructive stage of the life cycle (Grossheim 1928).

Taxonomic position of *Sitona discoideus*

When *S. discoideus* was first found in Australia it was identified as *Sitona humeralis* Stephens (Chadwick 1960), but Roudier (1980) in examining the species and subspecies of the *S. humeralis* Stephens group, correctly identified the *Sitona* species occurring in Australia and New Zealand as *S. discoideus* Gyllenhal.

S. discoideus, *S. humeralis* Stephens, and *S. bicolor* Fahraeus constitute the three distinct species belonging to the *S. humeralis* group. All occur on *Medicago* species in the Western Palaearctic region with *S. discoideus* restricted to South Western Europe and North Africa (Aeschlimann 1984). *S. discoideus* occupies an ecological niche that is described by Aeschlimann (1984) as 'Mediterranean type'. The Mediterranean type life cycle is characterised by a spring emergence of adults, that undergoes a period of obligatory aestivation before sexual maturation occurs in autumn (Aeschlimann 1984). In the Mediterranean *S. discoideus* was found to be equally dominant on annual medics and cultivated lucerne. However, high indices of dominance on cultivated lucerne by *S. discoideus* was confined to the drier zones of the south western part of the Mediterranean region, where its principal competitor *S. humeralis* was absent or remained rare (Aeschlimann 1984).

The importance of nodules to larvae of the *S. humeralis* group of species has been the subject of some debate. El-Dessouki (1971), Kwong Sue et al. (1980a), and Goldson et al. (1985) reported that larvae require nodules to survive, whereas Aeschlimann (1986) and Stein (1967) stated that larvae were able to develop on rootlets only. It is possible that the artificial conditions used by Aeschlimann (1986) to conduct the feeding investigation may have lead to ambiguous behaviour by the larvae.

Arrival of *Sitona discoideus* into Australia and New Zealand

The arrival of *S. discoideus* in New Zealand was preceded by the accidental introduction of the weevil into Australia. *S. discoideus* was initially recorded in November 1958 damaging lucerne at a site near Camden, New South Wales (Chadwick 1960). The following year it was found some 160km southwest of the original discovery site. By 1966 the weevil was widespread throughout New South Wales (Greenup 1967) and had just reached South Australia (Allen 1971).

In New South Wales, *S. discoideus* caused considerable damage to lucerne but also was found to attack other legumes such as burr medic, white, red and subterranean clover and various vetches (Greenup 1967). In South Australia, annual medics (*Medicago* species) were the principal crop attacked. Annual medics supported the highest larval and adult densities of 200 larvae m^{-2} and 538 adults m^{-2} respectively (Allen 1971), although populations of up to 46.5 weevils m^{-2} were reported from lucerne stands (Allen 1969). Larval feeding caused almost 100% injury to root nodules of annual medics, the effect of this damage on nitrification considered to be potentially the most important aspect of *S. discoideus* feeding (Allen 1971). At that stage *S. discoideus* was considered to have the potential to become one of South Australia's most serious pasture pests, reducing seed yields and destroying newly sown annual medics or lucerne (Allen 1971).

S. discoideus was first reported in New Zealand in 1974 when larvae were found feeding on burr medic along 35km of narrow coastal strip at Awatoto near Napier (Esson 1975). The large population at the site indicated that the species may have been present for a number of years. It was believed that the weevil had been accidentally introduced from Australia via lucerne hay used for horse fodder (Esson 1975). In January 1976, *S. discoideus* was first recorded in Canterbury and Otago (Somerfield and Burnett 1976). However, the weevil had probably established in the South Island much earlier than that indicated by Somerfield and Burnett's 1976 paper (Wood 1980). In 1982, *S. discoideus* was reported from most parts of New Zealand where lucerne and annual medics were grown (Kain and Trought 1982).

Bionomics of *Sitona discoideus* in New Zealand

Early investigation of *S. discoideus* in Canterbury had lead to the conclusion that the species was bivoltine (Wood 1980; Wightman 1979). However, studies by Kwong Sue et al. (1980b) established that *S. discoideus* had only one generation per year in Canterbury. Longer-term research by Goldson et al. (1984) confirmed that *S. discoideus* exhibited univoltine aestivatory seasonality in Canterbury and Otago and was similar to that observed in South Australia (Allen 1971) and the primitive Mediterranean habitat (Aeschlimann 1979).

In Canterbury and Otago the new generation of teneral adults emerge from the soil in December/January, an event that is usually characterised by a distinct peak in eclosion. There follows a period of vigorous feeding by the adults, during which time the adults reach flight

competency and fly to aestivation sites (Frampton 1987). Aestivation sites are located along hedge rows and fence posts. Aestivation lasts for 7-8 weeks during which time the adults remain reproductively immature and do not feed.

(a) Post-aestivatory flights

Post-aestivatory return flights usually commence in the latter part of March and peak in mid-April (Goldson et al. 1984). Prior to the return flights, the filamentous indirect flight muscles redevelop until full-flight competence is obtained (Frampton 1987). The return to the lucerne is marked by reproductive maturation between the months of March and May (Goldson et al. 1984). Females oviposit through to October when the adult population density begins to decline due to ageing. By December the old generation has died out.

(b) Larval development

There are two main periods of *S. discoideus* larval establishment, these being early autumn and in spring (Frampton 1984). *S. discoideus* is active in winter and continue egg laying whenever the egg laying temperature threshold of 4^0C is exceeded (Wightman 1979). The majority of the larval establishment occurred during September, October and most of November when temperatures are warmer (Frampton 1984). Larvae are first found in soil during August and September (Wightman 1979), but appreciable numbers of larvae are only found from mid-September (Goldson et al. 1984). The eventual size of larval populations is related to the prevailing soil moisture conditions in September-October (Goldson et al. 1986). Larval populations peaked in mid-November after which time pupae can be found in the soil. Eclosion of teneral adults occurred in December and January with a distinct peak in population density (Goldson et al. 1984). This highly synchronised nature of emergence is attributed to the threshold temperature of 15^0C for final instar and pupal development (Kwong Sue 1978), which ensures that physiologically advanced larvae that established in autumn would remain pre-pupal until soil temperatures increased in spring (Goldson et al. 1984).

CONTROLLING *SITONA DISCOIDEUS* IN NEW ZEALAND

Introduction

In an insect's primitive habitat, among the natural checks and balances that regulate the population are a range of predators, parasites and pathogens. Consequently, in the primitive habitat of the Mediterranean, Aeschlimann (1980) recorded a rich and diversified complex of natural enemies (parasitoid, predators and diseases) acting sequentially and/or simultaneously on the populations of *Sitona* population. Once established in New Zealand, *S. discoideus* was able to exploit a habitat free of its natural enemies, although opportunist parasitism and predation was occasionally reported (Wightman 1979).

Although direct attempts to control of *S. discoideus* in New Zealand have generally been restricted to insecticides, several other non-chemical control methods for *S. discoideus* control have been investigated.

Cultural control

Neither haymaking nor rotational grazing proved successful in controlling *S. discoideus* populations (Kain and Trought 1982). Similarly, mob-stocking of sheep during winter as a control measure proved to be more detrimental to the lucerne than the weevil (Goldson and Wynn Williams 1984).

Biological control

When *S. discoideus* first arrived in New Zealand, none of the biological control agents that operated in the primitive habitat were present. Nevertheless, several predator and parasitoid species already present in New Zealand were found to attack *S. discoideus* albeit at low levels. In early studies of *S. discoideus* biology, Wood (1980) reported that starlings *Sturnus vulgaris*, were predators of the adult weevil and that a protozoan *Pleistophora* sp. was found to infect up to 20% of adult weevils. A pathogenic bacterium was also found in weevils collected at sites near Lincoln (Wightman 1979).

In the laboratory, *S. discoideus* larvae were shown to be susceptible to a number of nematode species with *Heterorhabditis* spp. having the greater infectivity (Bedding et al. 1983). However, field experiments in South Australia using *H. bacteriophora* (formerly *heliothidis*) (Poinar) and the fungus *B. bassiana* (Vuill.), were unsuccessful (Bailey and Milner 1985). In New Zealand no established parasitoids were found to significantly attack *S. discoideus*, although Wightman (1979) recorded finding three weevils parasitised by *Hippocampus (Perilitus) conninellae* Schrank.

Insecticides

Early attempts in the use of insecticides to control the adult *S. discoideus* were unsuccessful (Trought and Stringer 1976), if only because field trials had been carried out at the wrong time of the year. Similarly, the use of systemic insecticides to control larvae also proved unsuccessful (Barratt 1985) as neither oxamyl or fenamiphos reduced larval numbers during the root nodule colonisation phase. A possible reason was that the larvae inhabiting root nodules were protected from the insecticide (Barratt 1985).

Following the clarification of the bionomics of *S. discoideus* by Goldson et al. (1984), the recommendation was to apply insecticide after the post-aestivatory flights had taken place and before the onset of egg laying (Goldson 1984). Initially chlorpyrifos and fenitrothion were registered for use against *S. discoideus*, with deltamethrin recently registered as being active against the weevil (O'Connor 1990). Application could be delayed till late autumn - early winter as larval establishment arising from winter egg laying occurred mostly in spring (Frampton 1984). Similarly, in Australia spraying was timed during the period of incoming flights in Autumn (Hopkins 1985). However successful control was found to be very much dependent on the pattern of migratory flights. Where flights were short, treatment of the crop with insecticides with short residual activity gave adequate control. However, if incoming flights were prolonged, these chemicals did not effectively reduce weevil numbers (Hopkins 1985). This problem with regards to insecticide persistence was critical, as studies in South Australia had found that egg laying activity early in the season could result in approximately 1000 eggs/m^2 being deposited within two weeks of the post-aestivatory flights (Hopkins 1985).

MORPHOLOGY AND FUNCTION OF *SITONA DISCOIDEUS* EGG SHELL AND THE POTENTIAL USE OF OVICIDES

Introduction

The potential value of ovicides has been recognised as promising because the egg stage is a stationary target, and therefore allows control of a pest before it passes into the more damaging stages (larval, adult). The insect egg is in many cases the vulnerable link in the life cycle of an insect and therefore the most suitable stage against which chemicals can be effectively used (Smith and Salkeld 1966). Eggs may also be present in the environment for considerable period of time and in a vulnerable positions (Smith and Salkeld 1966). As a group, *Sitona* species are characterised by high fecundity, and protracted egg-laying. *S. discoideus* eggs therefore offered an ideal target as research had shown that oviposition occurred from autumn through into spring (Frampton 1984). Moreover, these eggs remained in the field for several months before eclosion (Frampton 1987). The implications are therefore that the eggs would be in a relatively exposed position making then susceptible to ovicides while the timing of application was not critical.

The morphology and function of the shell of *S. discoideus* eggs is little understood and generally there is a paucity of information on Coleopteran egg chorion structure and function. A brief outline of the function of the chorion and associated structures will therefore be presented as will key features that influence embryogenesis.

Sitona discoideus oviposition

(a) Fecundity of *Sitona* species

Sexual maturation and mating takes place at the completion of aestivation (Aeschlimann 1984; Goldson et al. 1984), with mating allowing females to fertilize eggs for up to six weeks (Aeschlimann 1984). Generally, *Sitona* spp. are characterised by a high fecundity (Aeschlimann 1975; Moulden 1973). According to Melamed-Madjar (1966), the egg production of *S. hispidulus* (Fabricius) and *S. lividipes* (Fahraeus) varied between 300 - 1200 eggs. Bigger (1930) found that field populations *S. hispidulus* in Illinois (USA) produced between 139 - 167 eggs/female. *S. discoideus* fecundity has been estimated at 1000 eggs/female (Allen 1971; Aeschlimann 1984), with up to 400 eggs/female/week being laid at 20^{0}C (Wightman 1979). However, Goldson et al.

(1988) found that through computer modelling, the field fecundity was considerably less with egg production per female ranging from between 234 to 559 eggs over the entire egg laying period. Similarly *S. regensteinensis* fecundity was found to fluctuate markedly over a two year study period (Danthanarayana 1969). Generally food source is believed to be important in determining the number of eggs produced by each species (Grossheim 1928; Melamad-Madjar 1966).

(b) *Sitona discoideus* oviposition behaviour

In New Zealand female *S. discoideus* become reproductive at the conclusion of the post-aestivatory flights back into lucerne (Goldson et al. 1984). *S. discoideus* oviposits while on or in the vicinity of the host plant (Aeschlimann 1979). Eggs are scattered indiscriminately on the soil surface (Allen 1971), with the majority of the eggs aggregated below the stem of the plant on which the weevil is feeding (Aeschlimann 1979). Eggs are spherical with a mean width of 0.310 and a mean length of 0.403mm (Goldson and Frampton 1983).

Weevils are winter active, with oviposition continuing through until the end of spring (Goldson et al. 1984). The long egg laying period found in *S. discoideus* appears common for many *Sitona* spp. (Aeschlimann 1980; Danthanarayana 1969; Melamed-Madjar 1966). Furthermore, environmental exposure of eggs prior to hatching does not appear to affect viability. Quinn and Hower (1985) reported > 91% viability of *S. hispidulus* eggs that had been in the field for over 5 months. Similarly, Melamed-Madjar (1966) found that *S. lividipes*, *S. hispidulus*, *S. crinitus* Herbst and *S. lineatus* Linnaeus egg viability approached 99.0%. Egg viability of *S. discoideus* is also found to be between 95 - 99% (Aeschlimann 1975; Frampton 1984).

Aeschlimann (1984) and Goldson et al. (1988) both found that while the *S. discoideus* egg laying temperature relationship was linear, the rate changed over the season giving distinct peaks in oviposition. In Canterbury the rate in the earlier part of the season (June - July) was about half that occurring in spring (September) (4.7 ± 1.7 vs 12.1 ± 3.1 eggs/day) (Goldson et al. 1988). This fluctuation in ovipositional activity through the season is also found in other *Sitona* spp. *S. hispidulus* and *S. crinitus* have a double peak in egg production, while *S. lividipes* and *S. lineatus* egg laying rose to a single peak before tapering off (Melamed-Madjar 1966). The ovipositional rhythm can be very complex. *S. lineatus* was found to have two major divisions in the ovipositional rhythm - a complete ovipositional rhythm and a diel ovipositional cycle (Schotzko and O'Keeffe 1986). The complete ovipositional rhythm consisted of three levels: 1) a second-

degree polynomial curve, 2) a 30-day cycle and 3) a daily oscillation and increase in amplitude of the daily oscillation as the ovipositional rhythm progressed. The diel ovipositional cycle was dominated by two general periods these marked by increased and decreased oviposition with intervening transitional periods.

(c) Colour changes in *Sitona discoideus* eggs

When first laid, eggs of *S. discoideus* are pale yellow but soon darken to grey then black (Wightman 1981). Eggs of *S. lineatus*, (Jackson 1920), *S. hispidulus*, *S. lividipes* and *S. crinitus* (Melamad-Madjar 1966) also undergo a similar colour change. This process of chorion melanisation during incubation is a feature of eggs that are exposed to variations in sunlight, moisture and air movement (Peterson 1969). In addition, melanisation may also ensure that eggs are camouflaged from egg parasitoids (Schotzko and O'Keeffe 1986). The rate of melanisation appears temperature dependent (Frampton 1987), taking 48 hours at 15^{0}C (Wightman 1981). Schotzko and O'Keeffe (1986) have also suggested that the nutritional quality of food ingested by the adult may affect egg melanisation rate. Often eggs are non-viable, the chorion does not melanise remaining translucent (Frampton pers. comm.; Melamad-Madjar 1966).

Chorion structure and function

Within the egg, the insect embryo is enclosed by two envelopes, the vitelline membrane and the chorion (Wigglesworth 1972). The vitelline membrane is the first layer that surrounds the oocyte plasma. The chorion or egg shell, is composed of several layers of proteinaceous material which is broadly divided into the endo- and exo- chorion (Hinton 1981). Together the endochorion and vitelline membrane form the key sections of the eggshell (Margaritis 1985).

Because of the requirements of embryogenesis the majority of terrestrial eggs have a network of cavities in the chorion that hold a layer of gas (Hinton 1981). In addition, all species that have such chorionic meshworks also have holes or aeropyles that extend through the shell and effect the continuity of the chorionic layer of gas with the ambient atmosphere (Hinton 1981). The form of this chorionic meshwork is very variable. In primitive insect types, the layer of gas is held between vertical columns in the inner shell. In other insect groups the meshwork is more complex, and specialisation to cope with unique environmental conditions has led to the

development of complex structures concerned with respiration and are restricted to selected positions on the egg (Hinton 1981).

The eggshell must not only protect the developing embryo from environmental hazards such as extremes in temperature and humidity and bacterial attack (Margaritis 1985), but must also allow respiration and water movement. Insect eggs can be categorised into three groups based on their environmental requirements during embryogenesis (Hinton 1981).

These are

1. Oxygen only - where the egg requires only oxygen from the ambient environment for embryonic and later pharate larva development;

2. Oxygen and water - in addition to oxygen the egg needs a plentiful supply of water in liquid phase. Eggs with these requirements increase in volume during development; and

3. Oxygen, water and nutrients - in addition to oxygen and water some eggs also take in nutrients during embryo development.

In the eggshell, organs specialised for the uptake of water are known as hydropyles (Hinton 1981). Three distinct kinds of hydropyles are known:

1. Serosal hydropyles;

2. Serosal cuticle hydropyles; and

3. Chorionic hydropyles.

Coleopteran eggshells tend to have a simple system of vertical columns, making up the intra-chorionic meshwork and it is these structures that form the respiratory system (Hinton 1981). With regard to the *Sitona* species, there is a paucity of information on chorion structure, although Hinton (1981) has classified the genus *Sitona* as possessing a primitive type of eggshell.

Factors controlling the rate of embryo development

The most important environmental factor affecting development is temperature (Hinton 1981). Humidity is also important but secondary to temperature as the environmental factor affecting development. Many coleopteran eggs are sensitive to desiccation (Margaritis 1985), and this is true with *Sitona* eggs which are stenohydric, and only survive within a narrow range of relative humidity (Hinton 1981). The exhaustive study by Anderson (1930), showed that *S. lineatus* eggs take longer to hatch as the saturation deficit increased. Corresponding with this retarded larval development, larval mortality is found to increase (Anderson 1930).

Ovicides against *Sitona discoideus* egg stage

(a) Barriers to ovicidal activity

A characteristic of most insect eggs is that the entire development process occurs within a closed system (cleidoic) where all the materials required for embryogenesis, with the exception of oxygen and in most cases water, are present (Smith and Salkeld 1966). The chorion is important in buffering the egg against environmental extremes (Margaritis 1985), and is also an important factor that influences the effectiveness of an ovicide (Salkeld and Potter 1953; Smith and Salkeld 1966). In addition the permeability of the enveloping layers surrounding the embryonic material changes with egg development (Beament 1949; Salkeld and Potter 1953). It is these changes in permeability that have correlated with differences in observed toxicity of insecticides (Beament 1949; Smith and Salkeld 1966).

(b) Mode of ovicidal penetration

Chemical penetration requires a degree of permeability in the egg structure. The aeropyles and micropyles are obvious sites of entry, as they serve to connect the inner chorion with the outside environment. The aeropyles are normally an appreciable fraction of a micron wide to several microns wide (Hinton 1981), and, while allowing for free movement of oxygen molecules, ave strong hydrofuge properties preventing penetration of water molecules.

There was considerable variation in uptake and penetration of insecticide by the chorion of different insect species (O'Brien and Smith 1961). The micropyles have been shown to be the

major penetration point for chemicals in the Hemipteran egg *Rhodnius prolixus* Stähl (Beament 1948), and while both aqueous and lipophilic liquids can eventually reach the vitelline membrane via the micropyles, the lipophilic liquids do so more quickly (Beament 1948). Little is known about the permeability of solid chorion, although oxygen diffusion through the continuous chorion may have an important role in embryo respiration (Hinton 1981). In Lepidopteran eggs, penetration by oil-soluble poisons can occur through the solid portion of the chorion (Beament and Lal 1957; Salkeld and Potter 1953). Tuft (1950) has shown that when the aeropyles of *R. prolixus* are blocked with shellac, the egg still continued to take up oxygen, although uptake is reduced to a tenth of normal. Blocking the aeropyles with agricultural oils is used effectively to control European red mite (Chapman and Pearce 1949) and Oriental fruit moth (Smith and Pearce 1948). Death is due to smothering of the embryo (Smith and Pearce 1948).

Selection of potential ovicides against *Sitona discoideus*

Many insecticide groups have shown activity against the egg stage of insects, although this activity can be restricted to certain insect groups or to a genus. Therefore description of insecticides will be restricted to those groups that have been indicated as active against coleopteran eggs, or are currently employed for pest control in lucerne. The majority of research on the effects of insecticides on insects has dealt with immature or adult stages, generally the stage that is destructive to the crop and the stage causing the economic loss. Research with insect eggs has mainly been concerned with the progress of embryo development (Counce and Waddington 1972) or the morphology and function of the egg shell (Hinton 1981). Approximately 90 percent of insecticides used today have been directed primarily at the disruption of the nervous system, with insect growth regulators, inorganic (lead acetate) and respiratory poisons (rotenone) accounting for the remainder (Lund 1985). Because in many cases embryo mortality occurs in the latter stages of development, or at the time of emergence (Smith and Salkeld 1966), it is necessary to discuss the main effects of insecticide poisoning as they relate to the post-egg stages.

(a) Organo-phosphate ovicides

Organophosphate insecticides interfere with the operation of the cholinergic synapse between neurons or nerve cells (Corbet et al. 1984). Activity is restricted to the central nervous system,

with little or no effect on the peripheral system (Lund 1985). Transmission of impulses from one neuron to another is via the synapse, and involves release of the transmitter acetylcholine (ACH) from the synapse, which then combines with a receptor on the post-synaptic membrane. Once the appropriate message has been passed, ACH is removed by acetylcholine esterase (ACHE) by hydrolysis of the ester bond. Organophosphates inhibit the esterase by replacing ACH as the substrate for the esterase. Consequently, there is a build up of ACH at the synaptic cleft. In response to a single synaptic stimulation there is repetitive firing of the ACH receptor proteins at the post-synaptic membrane, eventually leading to desensitization of the post-synaptic membrane (Eldefrawi 1985)

General effects on insects treated with organophosphates involve initial hyperactivity, followed by convulsions then paralysis (Lund 1985). Dehydration of vital tissue and subsequent degeneration is suggested as being the ultimate cause of death (Corbet et al. 1984).

Mortality of embryos poisoned with organophosphates was generally found to act at two stages of embryonic development (Salkeld and Potter 1953). In the first instance the embryo would develop to near completion, but death occurred before any attempt was made to emerge. The second stage occurred during emergence, where the larvae obtained a lethal dose while chewing through the chorion (Zschintzsch et al. 1965). Mortality was also observed to occur in the early stages of embryonic development (Salkeld and Potter 1953), but only when excessive concentrations were used.

The early stages of embryonic development involve germ band differentiation and blastoderm formation (Agrell and Lundquist 1973; Anderson 1972), and it is only during the latter stages of embryogenesis, that differentiation of the organ system including the nervous system takes place (Anderson 1972; Chapman 1975). The chemical development of the cholinergic system parallels the morphological development nervous system (Salkeld 1964). In eggs exposed to insecticides embryo death results from excessive levels of ACH, the consequence of cholinesterase inhibition (Mehrotra and Smallman 1957; Metcalf and March 1949; Smith and Wagenknecht 1956). For example, cholinesterase and ACH activity in *Musca domestica* L. eggs was only detected in the last quarter of the total development time, and when poisoned with parathion the concentration of ACH was found to be twice that occurring in the control eggs (Mehrotra and Smallman 1957).

Organophosphate compounds have been shown to kill insect embryos prior to the appearance of any detectable cholinesterase (Potter et al. 1957). Smith and Salkeld (1966) alluded to esterases of unknown function as being possible alternative targets of organophosphate. Lipases in insect eggs were reported as one possible site of action (Krysan and Guss 1971). Respiration has been observed to decline in insect eggs poisoned by organophosphorous insecticides (Smith 1955) attributed to the inhibition of embryonic lipases (Krysan and Guss 1971). Developing embryos of the milkweed bug *Oncopeltus fasciatus* (Dallas) use very little of their initial lipid store, in contrast to other insect species (Babcock and Rutschky 1961), and the fact that *O. fasciatus* eggs were totally insensitive to a number of organophosphate insecticides including parathion (O'Brien and Smith 1961; Zschintzsch et al. 1965) is further evidence that lipase inhibition may be important in poisoning of embryos by organophosphates.

(b) Pyrethroids

Pyrethroids are a large group of highly active insecticides. They were originally derived from a group of insecticidal esters, the pyrethrins, which can be extracted from the flower heads of *Chrysanthemum* species (Ruight 1985). Rapid degradation in sunlight initially limited the use of pyrethrins, but the discovery of the complex molecular structure of these compounds which was easily modified, allowed the manufacture of an immense range of synthetic pyrethroids (Ruight 1985).

Pyrethroids interfere with the insect's normal nervous activity, although the exact molecular basis for the mode of action is not yet known (Corbet et al. 1984). Pyrethroids have a disruptive effect on a variety of arthropod sensory preparations, motor neurons and the central nervous system (Ruight 1985). The neurosecretory system has shown to be vulnerable, with changes in hormonal state recorded for insects treated with pyrethroids (Miller and Adams 1982). The types of symptoms that occur and whether the site of action is the peripheral or central nervous system depends on the type of pyrethroid and dosage used (Miller and Adams 1982).

Pyrethroids have been divided into classes based on their effect on certain nerve groups and on the symptoms produced. Class I pyrethroids affect the cercal sensory nerve of *Periplaneta americana* (L.), with poisoning symptoms including restlessness, incoordination, hyperactivity, prostration and paralysis. Class II pyrethroids, including deltamethrin, did not stimulate the cercal

sensory nerve, and produced different symptoms including a pronounced convulsive phase (Gammon et al. 1981). Some compounds cause rapid knockdown, i.e. a state of incoordination and locomotor instability from which depending on dosage, complete recovery may occur (Sawicki 1962).

Stimulatory effects on isolated insect nerves in the presence of pyrethroid generate a massive discharge of nerve impulse, followed by spontaneous discharge of continuous activity, which may lead to blockage of impulse along the nerve fibre (Lowenstein 1942; Ruight 1985).
It is believed that pyrethroids, unlike organophosphates, prolong the depolarising after-potential of the nerve impulse by delaying the closing of sodium channels so that Na^+ ions still move into the axon (Corbett et al. 1984; Lund and Narahashi 1981a,b). There is also an effect on synaptic transmission (Adams and Miller 1979; Gammon et al. 1981). There are also indications that like DDT, and its analogues, pyrethrin and synthetic pyrethroids inhibit Na^+, K^+ ATPase and Mg^{2+}, Ca^{2+} ATPase (Ruight 1985).

Besides knockdown and kill, several pyrethroids have a repellent and ovicidal effect. Ovicidal activity, decreased oviposition, reduced fecundity and repellent effects were caused by deltamethrin against the boll weevil *Anthonomus grandis* Boheman (Moore 1980). Exposure to sublethal doses of pyrethroid has produced similar effects in the grain weevils *Calandra oryzae* L. (Chadwick 1962) and cigarette beetle *Lasioderma serricorne* F. (Tenhet 1947).

(c) Gamma-HCH

1,2,3,4,5,6-Hexachlorocyclohexane was first synthesized in 1852. In 1912 Van der Linden discovered 4 isomers of this compound, the most insecticidal being γ-HCH, which was named after him as lindane in honour of his work (Osborne 1985). γ-HCH has marked excitatory actions upon the nervous system of insects (Mullins 1955), although its neurotoxic activity is different from that of DDT. Whereas DDT disrupts the kinetics of the action potential γ-HCH modifies the ion fluxes (Osborne 1985).

γ-HCH has little or no effect on the peripheral nerves, and apparently does not affect the Na^+ and K^+ conductances or the action potential in the nerve fibre (Narahashi 1971). Lindane is not a

potent inhibitor of cholinesterase (Hartley and Brown 1955), but is believed to affect Ca^{2+} metabolism to produce its excitatory effects (Doherty 1985).

There is evidence that γ-HCH acts on the CNS by enhancing release of ACH from the pre-synaptic membranes of cholinergic synapses which are prevalent in this region (Uchida et al. 1975a,b). Biochemically, γ-HCH exerts its main toxic actions by inhibiting the Na^+, K^+, and Mg^{2+} ATPase from the nerve cord. It can affect transport of Na^+, reduce membrane permeability and produce a loss of K^+/Na^+ selectivity (Schefczik and Simonis 1980; Webb et al. 1979)

γ-HCH has not been used specifically as an ovicide, and its persistence in the environment have led to its discontinuation in many crops. It has now been withdrawn from sale in New Zealand (New Zealand Pesticides Board). However lindane's relatively high vapour pressure and persistence (Worthing and Walker 1983), were considered positive attributes that had application against *S. discoideus* eggs.

(d) Insect growth regulators

Several chemical agents are known to have adverse effects on cuticle, hence survival of the insect (Chen and Mayer 1985). Solvent oils or dusts exert their effects by causing water loss through disruption of the wax layer (Beament 1945; Wigglesworth 1942). However, it is the insect growth regulators which include juvenile hormone and the chitin synthesis inhibitors or benzoylphenylureas that have most promise as pest control agents (Chen and Mayer 1985). Against the mobile stages of insects, juvenile hormone analogues act on the epidermal cells producing abnormal cuticle and disruption of the cuticular architecture. The chitin synthesis inhibitors interfere with the deposition of normal cuticle during moulting (Mulder and Gijswijt 1973), with the adult stage seemingly unaffected (Chen and Mayer 1985).

(i) Juvenile hormone

Juvenile hormone(JH) and its analogues have been indicated as being active against coleopteran eggs (Retnakaran 1973; Walker and Bowers 1970) although there has been a wide variation in response compared to other insect groups (Staal 1975; Strong and Diekman 1973). Juvenile hormone blocks embryonic development, either when applied to the female adult or to freshly laid eggs (Riddiford 1972). Sláma and Williams (1966) were the first to describe the abnormal embryonic development in insect eggs after exposure to JH. This was subsequently

identified as juvabione (Bower et al. 1966), its effect being to disrupt the blastokinesis stage of embryo development (Riddiford 1972). Timing of JH was critical to the manifestation of ovicidal activity (Staal 1975). When applied after germ band formation, JH failed to prevent hatching, although it has a detrimental effect on post-embryonic development (Retnakaran 1973; Riddiford 1972).

Although juvenile hormone analogues have proven to be very effective against insects (Staal 1975), and insect eggs (El-Guindy et al. 1983; Walker and Bowers 1970), problems with insect resistance to JH compounds and the importance for critical timing of applicazion have limited their use as chemical control agents (Chen and Mayer 1985; Riddiford 1972).

(ii) Chitin synthesis inhibitors

Although adult insects are not affected, chitin synthesis inhibitors can be used as an ovicide through direct application to the egg or contamination of gravid females (Grosscurt 1978). Diflubenzuron which has been the most widely used chitin synthesis inhibitor, was the most effective compound against eggs of the cotton leafworm *Spodoptera littoralis* Boisd. compared to the juvenile hormones tripene and methoprene and three insecticides including chlorpyrifos (El-Guindy et al. 1983)

Ovicidal action is effected by interfering with the chitin deposition of the developing larva inside the egg (Grosscurt 1978). As an ovicide, diflubenzuron exhibited greater toxicity against immature eggs as opposed to more developed ones (El-Guindy et al. 1983; Grosscurt 1978). Ovicidal action through ingestion of treated plant material has produced a significant reduction in egg hatch of *Leptinotarsa decemlineata*(Say) (Grosscurt 1978), and white fringed weevil *Graphognathus leucoloma* (Boheman) (Henzell et al. 1979). Diflubenzuron (75g a.i./ha), applied to lucerne caused a significant reduction in egg viability of *S. discoideus* for 4 weeks after application (Frampton et al. 1987).

Techniques for evaluating potential ovicides

Several approaches have been used in evaluating potential ovicides. Salkeld and Potter (1953) and Potter and Tattersfield (1943) used the Potter tower (Potter 1941, 1952) to apply a measured dose of insecticide to eggs. Another approach is to dip eggs stuck to glass slides in chemical

solution for a measured time period. Immersion times range from 5 seconds (Radwan et al. 1983) to two minutes (Smith 1955). In a modification to this approach Potter and Tattersfield (1943) dipped eggs of *Aphis rhamni*, while still attached to twigs, for 10 seconds, to test the ovicidal potential of pyrethrum insecticides. A similar approach was used by Walker and Bowers (1970) for Mexican bean beetle *Epilachna varivestis*.

Topical application with a microapplicator syringe is also a technique that has been used successfully (e.g. El-Guindy et al. 1983; Retnakaran 1973; Walker and Bowers 1970). To measure fumigant effects' Walker and Bower (1970) treated one surface of a petri dish with various doses of JH. Eggs of *E. varivestis* were then attached to the adjacent surface, the petri dishes sealed and the eggs incubated until the control population had emerged.

THE BIONOMICS PHENOLOGY AND TAXONOMY OF THE GENUS *MICROCTONUS*

Introduction

After the accidental introduction and spread of *S. discoideus* in Australia (Allen 1971; Chadwick 1958), work begun on locating a possible biocontrol agent. A joint study between the CSIRO Division of Entomology and South Australian Department of Agriculture, was instigated to identify natural enemies of *Sitona* species in Europe and North Africa (Aeschlimann 1983a). Initial research and screening was carried out at the CSIRO station in Montpellier, France (Cullen and Hopkins 1982).

Anaphes diana Girault, a mymarid wasp was found to be a prominent egg parasitoid of a large number of *Sitona* species, as the biology of this wasp is well adapted to the life history of *Sitona* spp. (Aeschlimann 1977). However, the adult parasitoid *Microctonus aethiopoides* (Loan) was the first selection as a biological control agent. Earlier work had shown that this most effective parasitoid was distributed widely across the Mediterranean (Aeschlimann 1980). Additionally *M. aethiopoides* was found to be easily reared and multivoltine (Aeschlimann 1983a). The multivoltinism was considered to be important to the success of biological control as successive parasitoid populations impart increase control of the univoltine *Sitona* host (Aeschlimann 1983a). Importantly, Aeschlimann (1978) found that *M. aethiopoides* activity over a two year period caused a 50% reduction in weevil numbers, in Moroccan lucerne growing regions.

The genus *Microctonus* and their host

The *Microctonus* spp. belong to the subfamily Euphorinae within the family Braconidae. The Euphorinae is a cosmopolitan group of small koinobiont wasps of relatively basal lineage that use the adult stage of various insect species as their host (Shaw 1985, 1988). Some members of the genus have assumed some importance in agriculture as they have been shown to be effective biological control agents of several coleopteran pests (Loan 1983; Tobias 1965).

Microctonus aethiopoides

Formerly known as *M. aethiops*, Loan (1975), in a review of the Haliday species of *Microctonus* (Wesmael) found that a number of *Microctonus* species had been misidentified. While retaining the neotype *M. aethiops*, Loan (1975) determined that economic and taxonomic references to *M. aethiops* were in fact referring to *M. aethiopoides* (new species) whose hosts were restricted to the adult weevils of the genera *Hypera* and *Sitona*.

Distribution of *Microctonus aethiopoides*

M. aethiopoides is widely distributed throughout Europe (Loan 1975) and the Mediterranean (Aeschlimann 1980), being a prominent parasitoid of *Sitona* spp. in North Africa, Spain and southern France (Aeschlimann 1980). The wide distribution of the species is reflected in distinct behavioral and morphological differences between female *M. aethiopoides*. *M. aethiopoides* ecotypes have been shown to exhibit distinct host preferences (Aeschlimann 1983b; Loan and Holdaway 1961b, Sundaralingam 1986), differences in duration of the pupal stage (Aeschlimann 1983b) and adult longevity (Loan and Holdaway 1961b).

Morphology, adult size, colour and bionomics are aspects that have been used to distinguish *M. aethiopoides* biotypes (Adler and Kim 1985; Aeschlimann 1983b; Loan and Holdaway 1961b). Morphologically and morphometrically, biotypes can be distinguished by differences in the number of flagellar articles, number of segments in the labial palps and the extent of mesonotal areolation, size and colour (Adler and Kim 1985, Sundaralingam 1986).

The females of different *M. aethiopoides* biotypes can be distinguished by their colour variation particularly on the frons and thorax, although there is the confounding effect of distinct seasonal changes in colouration (Aeschlimann 1983b). Colour is therefore not a reliable aid to identification (Adler and Kim 1985).

Release of *Microctonus aethiopoides* in Australia and New Zealand

An initial population of *Microctonus aethiopoides* was sent from Morocco to Canberra (Aeschlimann 1978), and thereafter material was forwarded to South Australia and NSW (Cullen and Hopkins 1982). To ensure successful establishment, parasitoids were collected from areas climatically equivalent to the infested zones in Australia (Aeschlimann 1983a). Subsequent to the initial Moroccan shipment, parasitoids were sent from Greece as larvae inside field collected living host weevils (Aeschlimann 1983a). Releases in Australia involved field liberations of the parasitoid as adults, pupae and larvae within host weevils (Cullen and Hopkins 1982).

The introduction of *M. aethiopoides* into Canterbury, New Zealand took place in May 1982, with specimens being imported from South Australia (Stufkens et al. 1987). After a quarantine period they were released in October 1982, both as adults and larvae in parasitised weevils. A total of 30,450 parasitised weevils and 2390 adult parasitoids were released at 17 sites throughout Canterbury, Central Otago and Malborough (Stufkens et al. 1987). Subsequent monitoring confirmed that *M. aethiopoides* had become established in several lucerne growing areas in the South Island (Stufkens et al. 1987).

Early research on the phenology of the parasitoid in New Zealand, ascertained that there were possibly four parasitoid generations in Canterbury (Proffitt and Goldson 1987). Levels of parasitism in an aestivation site were recorded at 15% (Goldson and Proffitt 1986), with up to 40-55% parasitism in the field (Stufkens et al 1987). This compared favourably to levels of parasitism of between 0.04% and 6.5% - 49% at aestivation and field sites respectively in New South Wales, Australia (Cullen and Hopkins 1982). Initial indications were that where the parasitoid was active, weevil ground densities dropped significantly, compared with sites where *M. aethiopoides* was not present (Goldson and Proffitt 1986). However, Goldson and Proffitt (1986) considered that the ability of *M. aethiopoides* to reduce field populations of *S. discoideus* adults was probably not great. Goldson et al. (1984) also expressed concern that an absence of overlap

between weevil generations may represent an impediment to the success of the parasitoid. However, even at low densities parasitoids were able to successfully and efficiently bridge the weevil generation (Proffitt and Goldson 1987). Subsequent research revealed that an uncoupling of the strictly sympathetic aestivatory behaviour of *M. aethiopoides* enhanced levels of parasitism in the emerging weevil generation (Goldson et al. 1990).

LIFE-CYCLE AND PHENOLOGY *MICROCTONUS AETHIOPOIDES*

Introduction

M. aethiopoides lays its eggs into the haemocoel of the host through the membraneous area at the apex of the abdomen. Prior to oviposition, the female stalks its prey sometimes for a considerable period of time. When ready to oviposit, the female extends her ovipositor under and well in front of her head, and makes a quick thrust at the caudal end of the weevil's abdomen (Loan and Holdaway 1961b). An active host is preferred to a stationary one (Fusco and Hower 1973; Loan and Holdaway 1961b). This propensity to oviposit in active hosts has been observed in other *Microctonus* species (Loan 1967a; Smith 1952; Wylie and Loan 1984), and the related braconid *Perilitus rutilus* (Nees) (Jackson 1928). This behaviour may indicate a selection process for healthy individuals (Jackson 1928). Females do not discriminate between parasitised and unparasitised hosts (Loan and Holdaway 1961b) a behaviour similar to that observed in another *Sitona* spp. parasitoid *Pygostolus falcatus* (Nees) (Loan and Holdaway 1961a).

In the field, multiple parasitism is generally infrequent but during periods of high parasitoid activity the incidence of multiple parasitism is higher (Tobias 1965, M.R. McNeill unpublished data). Generally when multiple parasitism does occur only one individual will survive the competitors being eliminated by cannibalism or by enzymatic secretions of the rival (Smith and Peterson 1950). However, there are a small number of *Microctonus* species which are gregarious with a variable number of larvae emerging from a single host. *M. caudatus* (Thompson) (Luff 1976), *M. glyptosceli* Loan (Loan et al. 1969), *M. eleodis* (Viereck) (McColloch 1918), *M. vinelandicus* Loan (Loan and Holliday 1979) and *M. disonychae* Loan (Loan 1967b) are the five species known to exhibit this trait. Gregarious development is uncommon and appears to be restricted to large Coleoptera (Loan and Holliday 1979).

Parasitoid development in the host

(a) Egg stage

Eggs are restricted to the abdomen of the host but float free in the haemocoel. When first laid, eggs are long and slender, rounded at the capsule end and tapering gradually to a narrow pedicel (Loan and Holdaway 1961b). The evolution of a small egg is associated with the mode of endoparasitism in phanerozoic, mobile hosts and the need for rapid oviposition (Jackson 1928; Ogloblin 1913). After deposition in the host, egg development is marked by a rapid expansion in size, differentiation of the embryo and trophamnion (Loan and Holdaway 1961b). The change in *M. aethiopoides* egg size is dramatic with a 2790 fold increase in volume. In comparison, eggs of *M. vittatae* Muesebeck increase in volume some 1200 times (Smith and Peterson 1950), *M. sitonae* Mason x 68 times (Loan 1963) and 300 times in *M. melanopus* (Jourdheuil 1960: cited in Tobias 1965).

(b) Larval development

M. aethiopoides has five instars during development, the final instar emerging from the host. Loan and Holdaway (1961b) have meticulously described the morphological characteristics of the instars. The first instar is caudate in form and identified by the presence of a head capsule. It has powerful mandibles that are used to destroy other larvae if the host is reinfested (Tobias 1965). Both second and third instars are similar in appearance to the first but lack the head capsule. The fourth instar is unsclerotised and whitish yellow, with film-like cast skins of instars I-III adhering to the apex of abdomen. The fifth emerges from the host and is grub-like and pale yellow.

As larvae develop they expand to occupy the whole of the body cavity possibly extending into the thorax. Weevil body organs may become flattened and pushed to the side by the developing larva. The fifth instar emerges from the host by forcing the caudal end of the abdomen through the membrane describing the junction of the apical tergite and sternite.

Development thresholds of the combined egg plus larval stage and pupal stage were 9.8^{0}C and 8.2^{0}C respectively (Goldson et al. 1990). The day-degrees for development of the egg plus larval stage and pupal stage were 144.4 and 125.4 ^{0}D respectively (Goldson et al. 1990). At 23.3 ^{0}C development of the parasitoid from egg to emergence of the fifth instar was 12-13 days (Loan and Holdaway 1961b).

Effect of parasitism on the host

Parasitism does not appear to alter the external physical shape and behaviourial activity of the weevil (Loan and Holdaway 1961b), nor is there any difference in the appearance of the abdomen to that of the non-parasitised weevil. There are, however, physiological changes the most important occurring in the female host. Once parasitised, females cease egg laying after 1-2 days. Internal examination of the abdomen reveals that by this time the ovaries have begun to regress, as have the eggs within the oviduct (Loan and Holdaway 1961b). In contrast in male weevils not conditioned for diapause, parasitism does not have any effect on reproductive potency, the testes exhibiting no physical changes (Drea 1968; Loan and Holdaway 1961b). Similar observations have been reported from *M. caudatus* (Luff 1976), *M. hyperodae* (Loan and Lloyd 1974), *Pygostolus falcatus* (Loan and Holdaway 1961a) and *Perilitus rutilus* (Jackson 1928). Jackson (1928), reported finding testes containing living sperm in male weevils from which the parasitoid larva had emerged.

After emergence of the parasitoid fifth instar, the weevil may survive for up to one day, but while able to walk does not feed (Loan and Holdaway 1961b). The rear legs may also be partially paralysed. The abdominal cavity from which the parasite emerged is devoid of any fluid and fat bodies, while the trachea is distinct (Loan and Holdaway 1961b).

Diapause synchrony with host

The hosts of *M. aethiopoides* undergo an obligatory diapause during periods of unfavourable environmental conditions. During these periods the parasitoid undergoes sympathetic diapause as a 1st instar larva inside the host. This synchronisation (with the host) allows the parasitoid to survive the period when the host is absent from the habitat (Tauber et al. 1983). In weevils conditioned for diapause, parasitoid eggs develop through to the first instar at which stage the diapause state arrests further growth. These larvae are small and active with reduced brain ganglia (Loan and Holdaway 1961b). The ovaries of females are rendered functionless and the male weevil can be partially to completely castrated (Drea 1968). The effect on the male contrasts to the non-diapause situation, where the rapid development of the larva does not affect the functioning of the testes (Loan and Holdaway 1961b).

The cessation of weevil diapause is accompanied by a resumption in parasitoid development. Development appears to precede the cessation of the diapause state in non-parasitised weevils. For example, in Ontario *M. aethiopoides* larvae in *Hypera postica* (Gyllenhal) emerged from their hosts and pupated in the soil before the non-parasitised adult weevil became reproductively active (Abu and Ellis 1976). A similar behaviour has been observed with *M. aethiopoides* in aestivating *S. discoideus* (Goldson et al. 1990).

The period over which sexually immature hosts are present in the field largely determines the number of parasitoid generations, as such hosts inhibit the development of first instar *Microctonus* larvae (Aeschlimann 1983b). In the northern hemisphere, *S. discoideus* emerge during summer and hibernate before becoming sexually mature and laying eggs in the following spring. In Mediterranean climatic conditions, adult *S. discoideus* emerge towards the end of spring and aestivate before becoming sexually mature in early autumn (Aeschlimann 1979). In the Mediterranean, the bionomics of *S. discoideus* permits the completion of three parasitoid generations between March and June, with a further two generations from September to December (Aeschlimann 1983b). In New Zealand one generation is completed from April to October, with four generations occurring between October and March. (Goldson et al. 1990).

Microctonus aethiopoides adult stage

M. aethiopoides is arrhenotokous parthenogenetic; unfertilised females producing only male progeny, with mated females producing females as well as males. In the latter case the capacity of the spermathecal gland may be important in determining whether an egg is diploid or haploid (Fusco and Hower 1974).

The females are readily distinguished by patches of red on the head and abdominal region. The males are totally black with several structural differences distinguishing males from females (Loan 1975).

Mating is effected within minutes of adult emergence with the period of copulation varying from 31-45 seconds (Loan and Holdaway 1961b). Males mate only once or twice, and appear to be sexually active within the first 48 hours of emergence. Males will only mate or attempt to mate with virgin or newly mated females (Loan and Holdaway 1961b). Recently emerged *M.*

aethiopoides females appear to elicit a mating pheromone which strongly attract males. In the laboratory the male response is marked by the rapid vibration of the wings and antennal waving (Loan and Holdaway 1961b). In the field, males have been seen observed to aggregate downwind of a caged virgin female (M.R. McNeill unpublished data).

(a) Oviposition behaviour and fecundity

Female ovipositional activity occurs almost immediately after emergence from the cocoon. Ovaries are paired and each consists of 3-6 ovarioles (Loan and Holdaway 1961b). Under laboratory conditions *M. aethiopoides* has a mean productivity of 59.3 progeny (Fusco and Hower 1974). Loan and Holdaway (1961a) counted up to 96 mature eggs in *P. falcatus* females that were mated but had not oviposited. When ovipositing the number was reduced to between 48 - 77 both as deposited eggs and immature oocytes. Mature females of *M. stelleri* contain up to 600 eggs (Drea et al. 1972) *M. aethiopoides* females exhibit no host preference on the basis of sex and size, but ovipositional activity is influenced by the age of the weevil host (Fusco and Hower 1973).

Field persistence and dispersal

In Australia and the eastern USA, *M. aethiopoides* was reported to be able to persist at both very low population levels and host densities (Coles and Puttler 1963; Cullen and Hopkins 1982). The parasitoid was also found to have a strong dispersal ability (Day et al. 1971; Krueger and Radcliffe 1986; Stehr 1974). Aeschlimann (1983a) and Hopkins (1981) found that the parasitoid dispersed at least 10 - 15km in a year through active and passive movement, and five years after introduction it was widespread in South Australia (Hopkins 1988). This strong dispersal capability has been attributed to the evolution of parasitism of the mobile adult stage as opposed to the more immobile immature host stages (Shaw 1985).

BIOLOGICAL CONTROL AND THE CONCEPT OF INTEGRATED PEST MANAGEMENT

Introduction

All plants and animals have natural enemies (parasites, parasitoids, predators, or pathogens) that attack their various life stages (Caltigirone 1981). The impact of these natural enemies ranges from a temporary minor effect to the death of the host or prey (Stehr 1982). The term 'biological control' was first used by Smith (1919) to signify the use of natural enemies to control insect pests. When applied successfully to a pest problem, biological control agents can provide a relatively permanent harmonious and economical solution (Huffaker et al. 1984; Stern et al. 1959). In discussing biological control the concept of natural control is important. Natural control or the 'balance of nature' is defined as the maintenance of numbers (or biomass) over many generations within a restricted range by the combined effects of all factors and processes of the environment, including the population itself, and incorporating a density-dependent (negative feedback) feature (Huffaker et al. 1984). Biological control can act as an important factor in ensuring that the balance is maintained.

In many cases biological control by itself does not provide economically viable pest suppression in agricultural cropping systems (Bartlett 1956; Tauber et al. 1985; Wilson and Huffaker 1976). Conversely chemical controls generally involve only immediate and temporary decimation of localised populations and do not contribute to permanent density regulation (natural control) (Bartlett 1964; van den Bosch and Stern 1962). The intelligent application of chemicals without destruction of natural enemies requires the integration of biological and chemical control tactics.

Integrated control or integrated pest management (IPM) was first suggested by Bartlett (1956), but Stern et al. (1959), offered the first formalised concept of IPM. IPM as described by Stern et al. (1952), combines the advantageous features of both chemical and biological control methods (reducing the pest population while causing a minimal disruption of the natural enemy activity) thus achieving a more permanent pest suppression. The biological control component is an important element for reasons of economy, all round effectiveness and environmental harmlessness (Wilson and Huffaker 1976), with chemicals augmenting the biological control component (van den Bosch and Stern 1962).

IPM is a philosophy which has been developed and expanded over several years to include a variety of control tactics which are employed in a single co-ordinated pest management system to provide control of either a single or multiple pest target. As in-depth discussion of integrated pest management is beyond the scope of this thesis, further information can be obtained from the papers and books by van den Bosch and Stern (1962); McCarl (1981) and Metcalf and Luckman (1982).

Ecological and physiological selectivity of insecticides

An important element of integrated pest management is the characteristic of the chemical being applied to control the pest problem. Because chemicals come in a variety of formulations, with a range of activities, and modes of action, they can be selected to have minimal impact on beneficial insects.

Insecticide selectivity has been broken down into two categories: physiological selectivity and ecological selectivity (Croft and Brown 1975; Ripper 1956). Physiological selectivity concerns the inherent physiological difference in susceptibility of host and natural enemy to toxicant with the ideal being selectivity favouring the natural enemy, while ecological selectivity results from differential exposure of pests and natural enemies to the pesticide (Bartlett 1964; Hull and Beers 1985).

Of the three classes of pesticides, fungicides and herbicides have generally been found to be harmless to beneficial insects, while most insecticides have been harmful (Hassan et al. 1983; Hassan 1990). In fact, exceptional tolerance of natural enemies to insecticides is rare, with the converse situation of exceptional susceptibility frequently encountered (Bartlett 1964; Croft and Brown 1975). This lack of genuinely selective organic insecticides is seen as a major factor currently limiting greater application of integrated control (van den Bosch and Stern 1962; Hull and Beers 1985). As physiological selectivity is often difficult to achieve, other alternatives have been investigated that preserve the biological control component of IPM.

Ecological selectivity has been perceived as the best practical approach to ensure that natural enemies are preserved (Bartlett 1964). The successful application of ecological selectivity is achieved through exploiting the distinctive life cycles of the pest and the natural enemy (Bartlett

1964; Croft and Brown 1975; Newson et al. 1976), or by modifying the mechanics of insecticide application (Hull and Beers 1985). It does however require detailed study of the key pest - natural enemy relationships on the infested crop, and aims to uncover specialised or distinctive biological characteristics distinguishing the pests and their natural enemies (Bartlett 1964).

Where the distinctive life cycle stages of a pest and natural enemy(ies) cannot be exploited into IPM, the alternative is to use an insecticide that is least selective to the parasitoid (Plapp and Vinson 1977). This has been one of the aims of the International Organisation for Biological Control (IOBC), West Palaearctic Regional Section (WPRS). To determine the side effects of insecticides and identify selective chemicals for use in IPM programs, an effort has been made to develop standard tests for measuring the toxicity of pesticides. Standardised laboratory, semi-field and field test procedures (Hassan and Oomen 1985; Hassan 1990), have been developed to test a range of pesticides on important predators and parasitoids (Franz et al. 1980; Hassan et al. 1983).

Effects of insecticides on biological control agents

Insecticides affect beneficial insects in three ways: through direct toxicity, through toxicity or repellant action of chemical treatment considered inert and by elimination of beneficial populations by removal of host species (Debach and Bartlett 1951). Direct toxic effects result from exposure to the spray droplets, or to recently deposited residues (Elzen 1990). Differential toxicity of various classes of insecticides to parasitoids is evident, but is often predicted with difficulty (Elzen 1990). Toxicity has been found to be dependent on the type of insecticide (O'Brien et al. 1985), and differences in metabolic capabilities (Plapp and Bull 1988; Plapp and Vinson 1977). These differences in metabolic capabilities are related to diet and determine the route by which insecticides are metabolised (Croft and Morse 1979; Plapp and Bull 1988). The mode of insecticide application has also produced differential susceptibility to insecticides. For example, *Microplitis croceipes* (Cresson) has exhibited differential toxicity under topical application (Powell et al. 1986), spray-tower (Elzen et al. 1987) and field residues (Powell and Scott 1985).

Computer modelling has been used to predict likely outcomes of insecticides on the pest - parasitoid interaction (Hassell 1984; Waage et al. 1985). Further research, has examined the temporal and spatial side-effects of insecticides on beneficial organisms (Jepson 1988, 1990; Jepson and Thacker 1990). Modelling combined with laboratory and field experiments has been

used to examine the initial depletion and recovery of non-target organisms following insecticide application (Jepson 1990).

Sublethal effects of insecticides

In addition to acute mortality, insecticides cause various sublethal affects on parasitoids which might ultimately result in reduced population numbers and effectiveness in suppressing host populations (Croft and Brown 1975; Elzen 1990; Moriarty 1969; O'Brien et al. 1985). Sublethal effects can be manifested through behavioral and physiological means (Elzen 1990). Behavioral sublethal effects include alteration of foraging pattern, disruption of sexual communication and lack of host recognition (Elzen 1990). The physiological effects of pesticides at sublethal doses can be seen in altered reproductive potential, reduction in longevity and egg viability or fitness (Moriarty 1969). Sublethal effects may also include alteration to the genetic constitution of future generations (Moriarty 1969).

(a) General physiology

The toxicity of the insecticide to internal parasitoids is mediated by the physiology of the host, or determined by the ability to penetrate host associated structures to reach the feeding parasite (Croft and Brown 1975). Insecticides applied to hosts have produced varied effects on the internal parasitoid stage. Generally, survival of the parasitoid depends on the response of the host to insecticide (Croft and Brown 1975). If the insecticide kills the host, the parasitoid generally also dies. If the host survives, the effect of the insecticide is then mediated by the detoxification of insecticide in the host (Croft and Brown 1975). The insect growth regulators (juvenile hormone and analogues, and the chitin synthesis inhibitors) have been shown to be detrimental to larvae of endoparasitic wasps (Ascerno et al. 1980; Beckage and Riddiford 1982, Frampton et al. 1987; McNeill 1975; Vinson 1974). Methyl parathion-treated cotton foliage, when fed to parasitised *Heliothis zea* (Boddie), significantly reduced the emergence of *Microplitis demolitor* Wilkinson (Culin and DuBose 1987). Conversely, Tamashiro and Sherman (1955) reported that insecticides killed the fruitfly host but not the internal parasitoid. *M. aethiopoides* larvae were able to survive and develop inside *Hypera postica* surviving insecticide treatment (Abu and Ellis 1977; Dumbre and Hower 1976b), with advanced stage parasitoid larvae able to complete development in moribund *H. postica* (Dumbre and Hower 1976b)

(b) Reproduction and behaviour

Sublethal affects on parasitoid adults have included reduced longevity (Dumbre and Hower 1976b), egg laying and alteration to the sex ratio of offspring (Rosenheim and Hoy 1988; O'Brien et al. 1985). Sublethal doses of chlordimeform influenced the majority of *Bracon mellitor* Say females to paralyse the host larvae but not oviposit, any eggs that were laid being non-viable (O'Brien et al. (1985).

Behavioral changes can also result from exposure to sublethal doses of insecticide. Sublethal effects on the parasitoid *M. croceipes* (Cresson) resulted in reduced flight activity and impaired ability to orientate flight in an odour plume (Elzen et al. 1989). Sublethal doses of chlordimeform often caused both females and males of *B. mellitor* to imbibe honey water to an extent that the abdominal cuticle was burst (O'Brien et al. 1985), while Elzen et al. (1987) reported excessive grooming of *M. croceipes* surviving pyrethroid treatment.

INSECT BIOASSAY

Introduction

Bioassay is defined as the measurement of potency of any stimulant (e.g. insecticide) by means of the reactions which it produces in living matter (Hoskins and Craig 1962). The criteria for a bioassay includes working with insects in as nearly normal environment as possible and control of environmental conditions such as temperature, humidity and light.

Busvine (1971) has comprehensively reviewed the application and techniques of insect bioassay. Testing technique, site of application, choice of carrier, anesthesia and standardisation of insects are al aspects which need to be considered to ensure that variation and bias are minimised and that the result provides a true indication of likely response of a target to a chemical in field conditions.

Endpoint determination

The response of test insects to topical dosing have been graduated to describe responses ranging from unaffected, visibly affected and incapacitated and moribund or dead (Menusan 1948). Rosenheim and Hoy (1988) considered individuals dead if they were unable to maintain a normal posture or walk normally at a rate of at least 1mm/second. However, to reduce the source of error in the results and support the dosage-mortality curve, the criterion is to classify the response as either alive or dead, with moribund individuals categorised as dead (Metcalf 1958). Timing of recording of toxicity data is dependent on insect species, type of insecticide and method of exposure. The time at which effects are measured must be kept constant; usually the LD_{50} decreases and the slope increases as longer intervals are used (Hoskins and Gordon 1956). Generally, mortality is recorded at a predetermined time after treatment usually over a 24 hour or 48 hour period (Beard 1960). Weevil adult mortality has generally been recorded 48 - 72 hours after treatment (e.g. Abu and Ellis 1977; Dumbre and Hower 1976a). The toxicity response for parasitoid adults has been evaluated at 24 hours (Rosenheim and Hoy 1988), although for highly active insecticides measurements have been taken 6-12 hours post-treatment (Dumbre and Hower 1976a).

Analysis of results

If the percentage killed are plotted against dose, the resulting curve generally approximates to an asymmetrical S-shape (Busvine 1971). As it is difficult to use the sigmoid shape curves for either interpolation or extrapolation (Hoskins and Craig 1962), an improvement in the treatment of data was achieved by Gaddum (1933) who used standard deviations to express the spread of susceptibility within a population. By plotting log-dose verses standard deviations the crowding of individuals about the mean value is exactly balanced by the crowding of percentage about the standard deviation, and the line becomes straight. Since standard deviations can be plus or minus, Bliss (1935) suggested that the negative values can be eliminated by adding 5 to the standard deviation. This unit became known as 'Probits', the treatment of which was later refined by Finney (1971). The resulting line is termed the log dose-probit line (Ld-p), and is an expression of the reaction of the tested population to the chosen toxicant (Hoskins and Gordon 1956). Log-probit analysis is now a standard procedure for analysing the response of insects to chemicals over a range of doses. As the results of bioassays are almost always expressed using the log dosage -

probit system, LD_{50} and LC_{50} are determined by plotting the log dosage - probit line (Hoskins and Craig 1962).

Where mortality occurs during the course of an experiment from causes unrelated to the experiment a correction factor must be applied. If 'control mortality' is appreciable, it will affect the precision of the results (Busvine 1971; Finney 1971), especially where treatment mortalities are low (Busvine 1971). Abbott's formula (Abbott 1925) uses mortality in the control population to estimate the magnitude of mortality.

A linear dosage-probit relationship has both position and slope. Position signifies the value of one coordinate when the other is fixed and generally relates to the LC50 or LD50. The slope is more informative than the LC_{50} or LD_{50} value, as it gives an indication of what may be expected. Slope is defined as the change in probits per unit change in log dose (i.e. per tenfold change in dosage), and is equates to the coefficient b in the regression line equation (Hoskins and Gordon 1956).

In interpreting the log dose-probit line, a steep slope indicates that the population is highly homogeneous (i.e. a small variation in dosage produces a full range response) (Hoskins and Gordon 1956). Conversely, a flatter line results from the use of a population varying widely in susceptibility (i.e. greater heterogeneity within the population) (Busvine 1971; Finney 1971; Hoskins and Gordon 1956).

Finney (1971) used the Chi-square (χ^2) to test whether the data deviates significantly from the regression line. In a homogeneous population, χ^2 will on average be equal to the number of degrees of freedom (n-2), and assumes that the observed frequencies of response do not vary heterogeneously about their expectations, or that the points plotted on the log dose-probit figure do not deviate significantly from the regression line. A significantly large χ^2 means that this assumption breaks down. Where the calculated χ^2 is less than the 95% critical value, the line adequately fits the data, where the χ^2 exceeds the 95% critical value, the line fails to adequately fit the data.

CHAPTER 3

MATERIALS AND METHODS

POTENTIAL OVICIDES FOR THE CONTROL OF *SITONA DISCOIDEUS*

Collection of *Sitona discoideus* adults

For the experiments involving ovicides and topical dosing it was necessary to frequently collect *S. discoideus* adults from lucerne paddocks. The collection sites were near Darfield and Lincoln, Canterbury.

Weevils were collected using a modified high-powered vacuum cleaner which removed the litter around lucerne plants. The litter samples were then returned to the laboratory where the samples were sorted. Extraction of weevils involved coarse-sieving the litter to remove stones and leaf material. The resulting debris were then tipped onto a heated tray, the heat source provided by an overhead heat lamp. This ensured that the temperature at the surface of the tray was approximately 35°C. Weevils were quickly collected when they began moving in response to the heat.

Collected weevils were placed in containers in the laboratory. Two types were employed. The smaller 116 mm x 73 mm deep circular container were used for the majority of the studies, and parasitoid propagation. The larger 203 mm x 190 mm container was suitable for bulk storage of weevils. The floor of these two types of containers consisted of a fine nylon mesh which prevented weevils from escaping, but allowed eggs and parasitoid pupae to fall through to an aluminium collecting dish.

Collection of eggs

At regular intervals eggs were extracted from the collecting dishes (described above), by tipping the contents into a glass beaker. This was then tilted and rolled gently leaving behind any frass. For the ovicide studies, eggs that were black were selected, melanisation indicated that eggs were older than 24 hours and that these eggs were fertile. Egg production is greatest during the first 1-5 days after collection, after which time egg production rapidly declines. This can probably be attributed to artificial conditions of the laboratory and the influence of food quality and quantity.

Egg ultrastructure and moisture requirements

Prior to experiments with insecticides, tests were conducted to determine the moisture requirements of the egg, and observations made on egg melanisation and physical changes. Eggs of two age groups (1 and 10 days old) were placed on either dry filter papers or submerged beneath a layer of water, and their development time and mortality recorded. Eggs from which larva had emerged were examined under the scanning electron microscope (SEM). Eggs were first washed in distilled water, air dried and then fixed on an aluminium stub using double-sided adhesive tape. Eggs were coated with 500Å of gold using a POLARON 5000 and then examined under a Cambridge 250 MkII scanning electron microscope.

Application of insecticides using the Potter tower

The Potter tower (Potter 1941, 1952) was used to apply insecticide to *S. discoideus* eggs and *M. aethiopoides* pupae. The Potter tower has several attributes not the least of which are that a given quantity can be repeatedly deposited on a sprayed area, to produce an even deposit. In addition, there is little variation in spray deposits with change in temperature and humidity (Potter 1952), meaning that environmental conditions need not be rigorously controlled.

The Potter tower had been calibrated prior to these studies. A volume of 1.5 mls of insecticide solution applied at a pressure of 103.4 Kpa produced a wet deposit on plastic petri dishes of 0.86 x 10^{-3} g/cm^{-2}. Insecticide solution was added to the reservoir using a glass pipette. Once all the liquid had been atomised through the nozzle, there was a 8-10 second settling period after which the petri dish was removed. Dishes were then placed under a fume extractor to allow deposits to dry. Petri dishes were then covered and incubated at 15^{0}C in a controlled environment cabinet.

All ovicide experiments employed disposable 90 x 10 mm plastic petri dishes into which was placed a double layer of Whatman (No 1) filter paper. Eggs were first positioned on the filter paper that was slightly moistened with distilled water. In initial experiments eggs were manipulated with a fine Camel hair paint brush, but subsequently eggs were handled using the method described by Goldson and Frampton (1983). The device consisted of aluminium foil stretched over the mouth of a funnel and punctured in the central area with a minuten pin. A vacuum was created using a domestic vacuum cleaner and this trapped eggs in the holes. Once the holes were filled surplus eggs were then shaken off. The remaining eggs were then removed by interrupting the vacuum.

Insecticides tested

Preliminary evaluation consisted of several insecticides that were tested for ovicidal activity (Table 1). The chemicals to be tested were made up in distilled water just prior to experiments. Concentrations were based on recommended rates specified by the label or from the Agrichemical manual (O'Connor 1990). Laboratory temperatures under which the ovicide work was conducted ranged from 17^{0}C to 25^{0}C, but generally all experiments were performed at 20^{0}C.

Assessment of egg mortality

Larval emergence was recognised by the characteristic perforations through the chorion, where the larva had chewed an escape hole, or by the presence of larvae on the filter paper. Eggs were incubated until there was no further eclosion in the control population, however, as insecticides can delay eclosion several days were allowed to elapse before eggs were dissected to determine stage of death. In early experiments this interval was up to 26 days but with experience this was

refined to 5 days. All dissections were made under a variable power Zeiss binocular scope, and observations made on the stage of development of the embryo. In addition, notes on the behaviour of emerging larvae were made during and after eclosion.

Table 1: Chemicals used for the evaluation of ovicides against *S. discoideus* eggs.

Common name	a.i. and formulation	Trade name
chlorpyrifos	400g/litre emulsifiable concentrate	LORSBAN 40 EC
deltamethrin	25g/litre emulsifiable concentrate	DECIS
diflubenzuron	250g/kilogram wettable powder	DIMILIN 25 W
fenitrothion	600g/litre emulsifiable concentrate	CATERKILL 60
fenvalerate	200g/kilogram wettable powder	SUMICIDIN 20 WP
lindane	500g/kilogram wettable powder	LINDANE 50 W
spraying oil	970ml/litre mineral oil emulsifiable concentrate	SUNSPRAY

Effect of chilling on the ovicidal activity of chlorpyrifos and fenitrothion

As eggs oviposited winter can remain dormant for several months before eclosion (Frampton 1984), it was felt worthwhile to investigate the consequence that delayed egg development had on the ovicidal effectiveness of chlorpyrifos and fenitrothion. To determine the effect of chilling on the LC_{50} of *S. discoideus* eggs to chlorpyrifos and fenitrothion, eggs were collected over a three day period from a laboratory weevil population. To ensure eggs were of a similar physiological age, eggs collected on earlier dates were stored at 8°C. Prior to the experiments eggs were aged at 20°C. Five concentrations were tested for both chlorpyrifos and fenitrothion, ranging from 0.10 to 1.25 g a.i./l for chlorpyrifos and 0.10 to 1.88 g a.i./l for fenitrothion. The two treatments were designated as normal (uninterrupted development) and chilled (development suspended).

There were five replicates for each treatment with the number of eggs per dish ranging from 48-63 with a mean of 50 per dish.

To treat eggs under the Potter tower, the top layer of filter paper on which the eggs were positioned was removed from the petri dish and placed on a glass plate which in turn was located on the Potter tower stand. After the insecticide had been applied, eggs were returned to the petri dish which was then allowed to stand for 15 minutes in the laboratory. Eggs allowed to develop 'normally' were then directly transferred to a controlled environment cabinet and incubated at 18°C. Following treatment under the Potter tower, eggs that were to be chilled were incubated for 31 days at 6°C before being transferred to a 18°C controlled environment cabinet to complete development. To minimise any fumigant effects from the insecticide, the petri dish lids were only replaced after 16 hours in the controlled environment cabinets. During incubation both eggs and the inner surface of lids were periodically misted with distilled water. When eclosion was observed to occur in the control population, another five days were allowed to pass before final egg mortality was recorded.

TOXICITY OF CHLORPYRIFOS AND FENITROTHION TO *SITONA DISCOIDEUS* AND *MICROCTONUS AETHIOPOIDES*

Topical dosing of *Sitona discoideus* adults

For the topical dosing experiments on *S. discoideus*, only non-parasitised weevils were selected. Dumbre and Hower (1976) and Abu and Ellis (1977) had shown that the presence of immature parasitoids lowered the LD_{50} values of insecticides, and it was therefore important to eliminate this variable for these experiments. As weevils collected from the field were generally 40-60% parasitised, they were held at 20°C for 2 weeks to allow *M. aethiopoides* larvae to emerge. There was no attempt to separate the weevils by sex, although exceptionally small specimens were discarded. Prior to the dosing weevils were starved for 15-20 hours.

To expedite topical dosing and simplify handling, weevils were incapacitated with a 30 second exposure to CO_2, and then placed onto a plastic petri dish positioned on a bed of ice to delay

recovery. After weevils were dosed they were placed in petri dishes and supplied with a sprig of lucerne. A 15-20 minute recovery period was allowed before weevils were transferred to a controlled environment cabinet maintained at 15°C. Mortality was recorded 48 hours after treatment with weevils unable to make co-ordinated movement being classified as dead.

Collection of *Microctonus aethiopoides* pupae

To obtain sufficient parasitoid pupae and adults it was necessary to mass-rear *M. aethiopoides* in the laboratory. To achieve this 25-30 weevils were exposed for 24 hours to one mated female. Females were obtained from pupae that had emerged from field-collected weevils. The ratio of 25-30 weevils/female for 24 hours was based on Fusco and Hower (1974), who determined that this was the optimum density and ratio to mass-rear *M. aethiopoides* progeny.

Parasitoid larvae were allowed to pupate under strips of filter paper provided in the collecting dishes. *M. aethiopoides* pupae were recovered from the dishes every second day with only pupae that had completed cocoon formation taken at any one occasion. Jewellers forceps proved suitable for removing the cocoon from the filter paper. This handling did not affect the subsequent survival or longevity of the resulting parasitoid adults.

Cocoons were then placed on filter paper in a glass petri dish. A cotton bud was supplied moistened with distilled water to prevent desiccation. As pupae were sometimes collected over a period of 5-6 days, those cocoons collected originally were placed in a controlled environment cabinet set at 8°C to slow the rate of physiological development. Only pupae that had developed eyespots were selected for dosing studies.

Collection of *Microctonus aethiopoides* adults

Parasitoid adults were obtained by allowing laboratory collected pupae to eclose. Cocoons that were held at 8°C where transferred to 20°C and reared through to adults. Emergent parasitoids were placed in a plastic container supplied with a honey-water solution and a sprig of lucerne. The container was held at 15°C at L:D of 16:8. Both male and female adults were used in the experiment as preliminary observations had not shown any significant difference in

susceptibility. However, to ensure that there was no bias the two sexes were spread over the range of concentrations to be tested.

Although synchronous emergence was achieved to some degree by manipulating small groups of developing pupae at different temperatures, emergence of sufficient numbers of parasitoid adults for dosing tended to be prolonged over a 5 to 8 day period. For this reason topical dosing was carried out over several weeks using small groups of insects with the doses replicated. Topical dosing of parasitoid adults was carried out over a 20 day period in six groups for chlorpyrifos and four groups for fenitrothion.

Topical application

Topical dosing offers a precise and quantitative assessment of direct acute toxicity which in turn allows for the determination of the dosage mortality relationship to produce a LC_{50} or LD_{50} (Croft and Brown 1975). Topical dosing also has the advantage in that a known dose to individual insects is delivered with reasonable precision (Busvine 1971; Hoskins and Craig 1962), the only uncertainty being the measurement of the solution in the solvent (Hoskins and Craig 1962).

An Arnold micro-applicator was used in these experiments to drive either a 250ml or 100ml SGE syringe. Prior to dosing, adult parasitoid and weevils were exposed to CO_2 for 20 and 30 seconds, respectively. They were then tipped into a glass petri dish set over a bed of ice to delay recovery. A fine plastic tube attached to a water vacuum pump was used to manipulate the parasitoid adults. Each parasitoid was dosed by applying insecticide solution to the dorsal surface of the abdomen. Initially weevils were handled in the same way but for the majority of the experiments fine touch forceps proved to be the most suitable. Methyl-ethyl ketone proved to be the most suitable solvent for testing insecticides. MEK has the properties of superior wetting and solvent power while having a lower vapour pressure than acetone (Gast 1959; Hamilton et al. 1981; Heal and Menuson 1948). For all experiments, the solutions were made up just prior to use.

(a) Determination of dose size

Body weight of the test insect determined the volume applied. Although there is evidence that there may be a size effect there is no general rule that can relate dosage to body size (Busvine 1971). However the approach was to discard exceptionally small individuals (Dumbre and Hower 1976a), and apply a volume of solution in proportion to the size of the test insect (Abu and Ellis 1977; Dumbre and Hower 1976a).

A sub-sample of the insects to be treated were taken and weighed on a Sartorius electrobalance (R160D). Because of practical considerations, weevils were weighed in groups of 10, parasitoid pupae and adults in groups of 5 and 4 respectively.

Both the 250ml and 100ml syringes were calibrated using mercury. Calibrations were made before and after dosing to check for any changes in volume delivered. Weevils were dosed using the 250ml syringe that delivered a volume of 0.52 $\pm$ 0.02μl. A 100ml syringe was used for dosing *M.aethiopoides* adults and was calibrated to deliver 0.20 $\pm$ 0.02μl.

TOXICITY OF CHLORPYRIFOS AND FENITROTHION TO *MICROCTONUS AETHIOPOIDES* PUPAE

Introduction

To investigate the toxicity of chlorpyrifos and fenitrothion to pupae both the Potter tower and topical dosing were employed.

Potter tower application

The Potter tower was used to determine the response under field rates of chlorpyrifos and fenitrothion and to simulate field application conditions. Pupae were collected over a nine day period, with pupae collected at the earlier dates stored at 5°C. This ensured that physiological age was similar for all pupae, but to minimise any age effect the groups of pupae were randomized across all treatments. Only pupae $\geq$ 48 hours were used, their selection being made by visual assessment of their colour and presence of eyespots.

Prior to spraying, pupae were transferred to a 12°C controlled environment cabinet and left at this temperature for 18 hours before being transferred to 15°C for three hours. To apply the insecticide, cocoons were placed on a glass plate situated on the spray tower stand. Two mls of chlorpyrifos or fenitrothion was then sprayed through the atomizer. This produced a wet deposit of 1.15×10^{-3} gm/cm^{-2}. After treatment cocoons were air dried for 10 minutes which was followed by a further 20-25 minutes under the fume extractor. Cocoons were then moved to clean petri dishes for subsequent incubation at 15 °C. To minimise any fumigant effects from the insecticide, the lids were removed for the first hour only. To maintain humidity inside the petri dish, the underside of lids were periodically misted with distilled water.

There were three replicates for each chemical and concentration tested, each replicate consisting of nine pupae. Chlorpyrifos was applied at three concentrations of 2.4, 1.2, and 0.6 g a.i./l, and fenitrothion at 2.0, 1.0, 0.5 g a.i./l.

Pupae were checked every morning close to the predicted time of adult eclosion, and when emergence was imminent, pupae were checked twice daily. The treatment, sex of emerged adults and time of eclosion were recorded. Emergence was recorded as occurring one hour before collection time. This was based on the observation that soon after the light phase of the controlled environment cabinet was activated, parasitoid adults emerged from cocoons. Adults were transferred to a glass vial supplied with 1-2 trifoliate lucerne leaves. The end was plugged with cotton wool that was briefly dipped in a honey-water solution. To ensure that adult parasitoids did not become desiccated vials were placed on a bed of moist cotton wool. As with emergence, vials were checked 2-3 times a day and mortality recorded. Periodically the trifoliate leaves and cotton plug were replaced to maximise the optimum conditions for parasitoid survival. The parasitoid adults were held at 15°C, with a L:D ratio of 16:8.

After all adults had emerged, the remaining cocoons were dissected to determine the state of the cadaver, and a visual assessment made of the stage of morphological development. To test whether females surviving treatment were still able to successfully parasitise weevils, female parasitoids one to three days old were mated, then exposed to *S. discoideus* adults for 20-24 hours.

Topical application of *Microctonus aethiopoides* pupae

The collection and storage of pupae was described in the previous section was used for the collection and storage of pupae. Dosing was effected by placing the cocoon on a glass plate, and then bringing the cocoon into contact with the droplet formed at the end of the needle. Cocoons were orientated longitudinally to the syringe, with the insecticide applied to the rear dorsal surface of the cocoon. The rear dorsal region was determined by noting the position of the pupal eyespots. Based on an average weight of cocoons of 1.22mg (n= 113), the 250ml syringe was calibrated to deliver a volume of 0.33 $\pm$ 0.02 μl.

Following dosing the pupae were placed on filter paper moistened with distilled water. They were contained in 90 x 10 mm plastic petri dishes and incubated at 15^{0}C. Adults emerging from treated pupae were collected and placed in glass vials in an identical procedure to that employed for the Potter tower experiment. Time of emergence, sex and longevity were recorded and females were exposed to *S. discoideus* to determine whether they would parasitize hosts.

Analysis of results

All topical dosing experimentation and the ovicide studies investigating the LC_{50} of chlorpyrifos and fenitrothion under normal and chilled treatments used log-probit analysis for analysis of results. POLO, a computer program developed by Russell et al. (1977), was employed to determine LD_{50} values for *S. discoideus* and *M. aethiopoides* adults and LC_{50} values were determined for weevil eggs. Where POLO was not used, control mortality was corrected using Abbott's formula (Abbott 1925) and the results presented in table form.

In the experiments examining the toxicity of chlorpyrifos and fenitrothion to the pupae, the longevity of emerged adults were analysed using MINITAB (1989) to calculate the regression equation and coefficients of determination (r^2). For pupae treated under the Potter tower, the sex of emerging adults were recorded separately and the regression equations calculated for both sexes. No attempt was made to separate the sexes of adults emerging from pupae treated topically treated, the longevity data were pooled and the results analysed using MINITAB.

THE EFFECT OF CHLORPYRIFOS ON *MICROCTONUS AETHIOPOIDES* AND *SITONA DISCOIDEUS* IN THE FIELD

Introduction

Although laboratory tests can provide information on the likely response of natural enemies to insecticides, responses under field conditions are a more realistic indicator of the impact of insecticides on the natural enemy and its host. Insecticides when applied to a crop have been shown to totally eliminate the adult parasitoids, but the impact is generally less severe where the immature stages of the parasitoid are involved (Bartlett 1966; Croft and Brown 1975). To this end a field experiment was conducted at a site near Darfield-50km west of Christchurch, with the aim of measuring what impact a recommended field rate of chlorpyrifos had on the *M. aethiopoides* adult and immature stage and *S. discoideus*.

Field site and sampling program

The field site chosen was a three year old lucerne stand sown with cultivar WL318. Chlorpyrifos was applied during autumn to early regrowth lucerne after the return flights of Sitona weevil were completed as recommended by Goldson (1984). A vehicle mounted 6 metre boom was used to apply chlorpyrifos at the recommended rate of 0.3 kg a.i./ ha. Plots were 12 x 12 metres with a one metre buffer between each plot. The treatments were water only (control) and chlorpyrifos. There were seven replicates per treatment, and the treatments were randomised across the plots.

Sitona discoideus ground density and level of parasitism

The sampling program consisted of ground counts to estimate the density of adult weevils. To obtain an absolute population density, sampling utilised a modified high-powered vacuum cleaner to collect all the litter within a $0.2m^{-2}$ quadrat. Six samples were taken in each plot, returned to the laboratory for heat extraction of weevils. Weevils collected from each quadrat within a plot were bulked and a subsample of 12 weevils dissected to determine the level of parasitism by *M.*

aethiopoides, and the stage of parasitoid development within the host. A pre-spray ground count was made 5 days prior to spraying, with a post-spray sample taken one week after spraying.

Over the experimental period details of the daily weather conditions were obtained from the meteorological station (H32412) at Darfield.

Residual toxicity of chlorpyrifos

The length of time that insecticide residues remain toxic to natural enemies in the field can have important implications to reestablishment from uncontaminated areas, or eclosion from the pupal stage. To determine the residual toxicity of chlorpyrifos and the effect of weathering on the residual toxicity a further study was undertaken in conjunction with the above work. Both *M. aethiopoides* and *S. discoideus* adults were used in this experiment.

In addition to sampling from the two treatments used in the main experiment, lucerne was sampled from plots that received 1.0 kg a.i./ha of chlorpyrifos. The insecticide was applied on the same day as that applied for the main field experiment and formed part of a MAF Technology research project. These plots were sited 30 metres from the main experimental site.

At 1, 3, 6, 10, 15 and 22 days after application, lucerne sprigs were randomly selected from within the plots. The sprigs were bagged and taken to the laboratory and processed within two hours. Care was taken to select older leaves as they were assumed to have been present at time chemical application. This was particularly important later in the experiment when new growth had taken place in the field.

S. discoideus were selected from a laboratory population collected 2-3 weeks earlier from field sites near Darfield. They were of mixed sex, with an unknown level of parasitism. Thirteen to fifteen weevils were placed in 100mm x 100mm x 70mm deep polycarbonate containers. To ensure that weevils were confined in the vicinity of the foliage, the rim of each container was treated with FLUORN (Whitford Plastics Ltd). A sprig of lucerne was added to the container, the stalk wrapped in cotton wool liberally moistened with distilled water.

There were five replicates for each of the control, 0.3kg/ha and 1.0kg/ha treatments. Weevil mortality was assessed at both 24 and 48 hours. During the 48 hour exposure period emergence of pre-pupal 5th instar *M. aethiopoides* and successful pupation was recorded. Weevils that were classified as dead or alive were held separately for another 24 hours to observe whether parasitoid emergence took place. At the conclusion of this period weevils were then frozen, and later dissected to determine sex, whether or not there was a parasitoid present and the stage of parasitoid development.

To test the response of chlorpyrifos residues to *M. aethiopoides* two trifoliate leaves were removed from a lucerne sprig and then placed in a 50 x 10mm glass vial. One to two parasitoid adults were selected from a laboratory population and placed in the vials. Both male and females were used and where possible the sexes were paired. Vials were capped with a gauze lid, and then placed on moist cotton wool and held at 15^{0}C in a controlled environment cabinet. A fan built into the cabinet ensured continuous air movement and likely reduced any fumigant effects. Mortality was recorded after 24 hours.

The response of *S. discoideus* to chlorpyrifos residues was first corrected for control mortality and analysed using ANOVA, with a T-test applied to determine significant differences in mortality between treatments and sampling intervals. For *M. aethiopoides*, the 95% confidence intervals for the two insecticide treatments at each sampling date was determined using the calculation:

$$p^* = \frac{p(1-p)}{n}$$

where p^* is the variance of proportion in the population exposed,

p is the percentage mortality divided by 100

n is the number of parasitoids exposed in test.

The standard error (s.e.) determined by $\sqrt{p^*}$, with the confidence limits derived by multiplying the s.e. x 2.

CHAPTER 4

RESULTS

POTENTIAL OVICIDES FOR THE CONTROL OF *SITONA DISCOIDEUS*

Observations on *S. discoideus* egg development

S. discoideus eggs appear to take up water after deposition, marked by an increase in turgidity within the first 24 hours of deposition. This change is also associated with melanisation, with the black eggs possessing a rigid egg shell. Non-viable eggs do not develop the same rigidity in the chorion.

Water was shown to be important for egg development and viability. At high saturation deficits impairment of egg development and mortality of up to 68% occurred. By comparison, eggs that had been placed on moist filter paper experienced only 2 - 8% larval mortality and emerged 7-9 days before eggs that were subjected to moisture stress. Mortality arising from larval desiccation could be reduced if eggs that were allowed to develop under high saturation deficits were subsequently exposed to free water. Eggs treated in this manner responded rapidly, and the larvae eclosed within three hours. Superficially these eggs appeared dead, the chorion having collapsed and folded along the dorsal midline. Conversely, immersion of eggs in water was not found to impair either egg development or viability.

Structure of the egg chorion

Under the scanning electron microscope (SEM), the chorion and the outer waxy layer form the prominent structures of the egg (Plate 1). The waxy layer covering the chorion is 620ηm thick and is continuous across the entire egg. The chorion is between 1.5-1.7μm in width and appears to be constructed of distinct layers, perforated by cavities. These cavities are extensive across the inner surface of the chorion (Plate 2), although along the dorsal midline of the egg the density and diameter is reduced. The diameter of these cavities is between 450-1150ηm at the base, narrowing near the outer surface of the chorion. Cavities do not extend totally through the chorion, but

terminate 190-330ηm from the outer surface of the chorion (Plate 3). The narrowness of the chorion at these points can be gauged by the fact that when light was directed at the chorion, the silhouette formed by these cavities is readily discerned through the chorion (Plate 4). The outer surface of the chorion adjacent to the wax layer appears to be solid, although there is some indication that fine holes approximately 65ηm in diameter, arise from the distal surface of the cavity and extend fully through the chorion (Plate 3). The laminated nature of the chorion is also evident in Plate 4, with a network of fine cavities coincident in and running parallel with the general chorion itself.

Ovicides against *Sitona discoideus* eggs

The results of experiments with potential ovicides are shown below in Tables 2.0 to 6.0. Generally all insecticides exhibited some ovicidal activity against *S. discoideus* egg, with chlorpyrifos being the most active insecticide against a range of egg ages. Only summer oil applied at 3% did not cause any appreciable mortality, and was not significantly different to the control group. Fenvalerate exhibited poor ovicidal activity in tests with an average mortality of 12% (Range 0-22%) at field rate (25 g A.I./ha). The results obtained with each insecticide are presented separately in the following sections.

(a) Chlorpyrifos

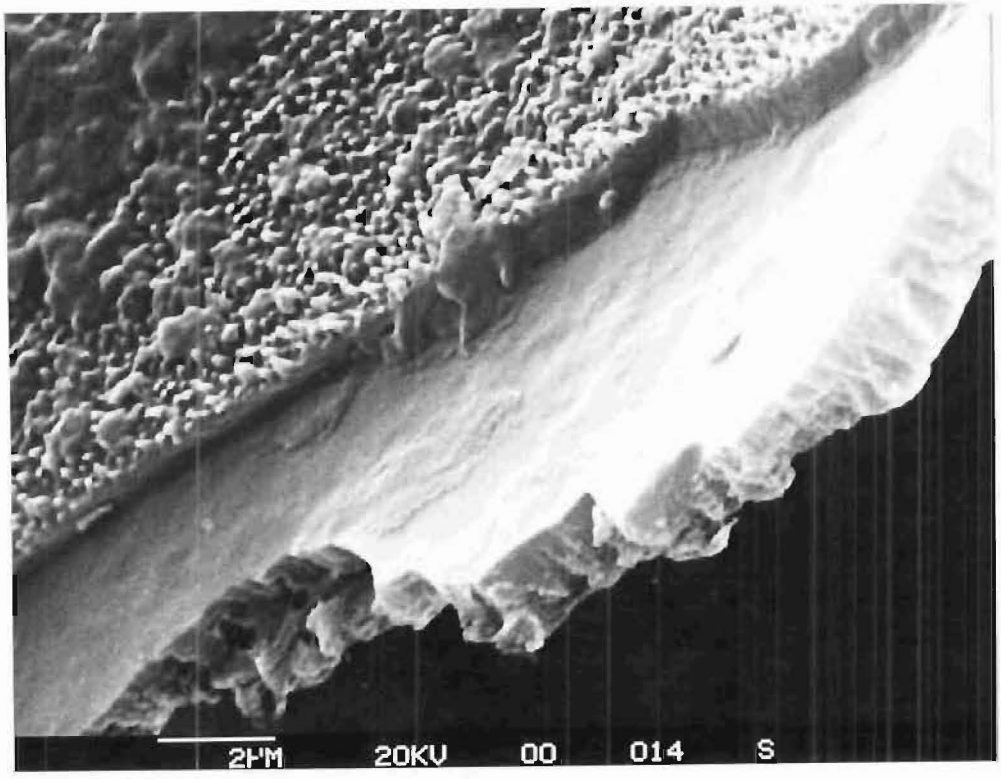
Chlorpyrifos exhibited high ovicidal activity both at 1.0 and 2.0 g/l (Table 2). This response was found over a wide range of egg ages from 1 day to 13 day old. Dissection of a subsample of 10 - 15 eggs per dish indicated that unhatched treated eggs contained either dead or moribund larvae.

(b) Fenitrothion

Fenitrothion was shown to have high ovicidal activity against eggs between 1-3 days old and at the two rates of 0.6 and 1.2 g/l (Table 3). Eggs dissected 18 days after last emergence of control population contained either dead or moribund larvae.

Plate 1: Oblique view of *Sitona discoideus* egg showing the outer wax layer and chorion.

Plate 2: Internal view of *Sitona discoideus* egg showing the extensive and abundant cavities across the inner surface of the chorion.


2PM 20KV 00 014 S

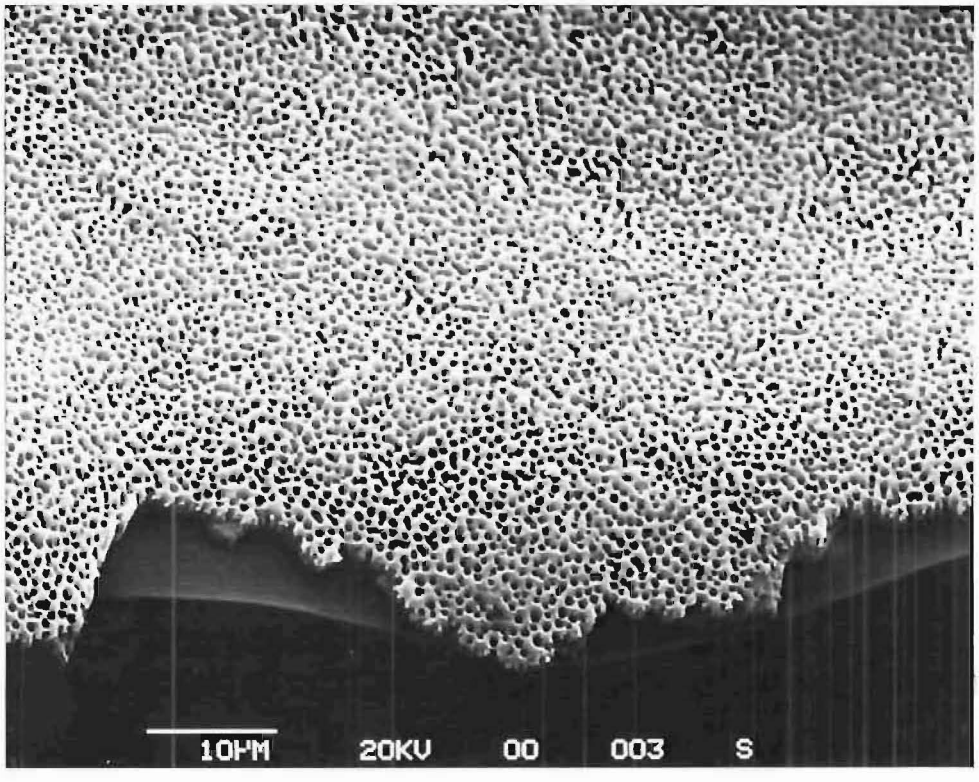
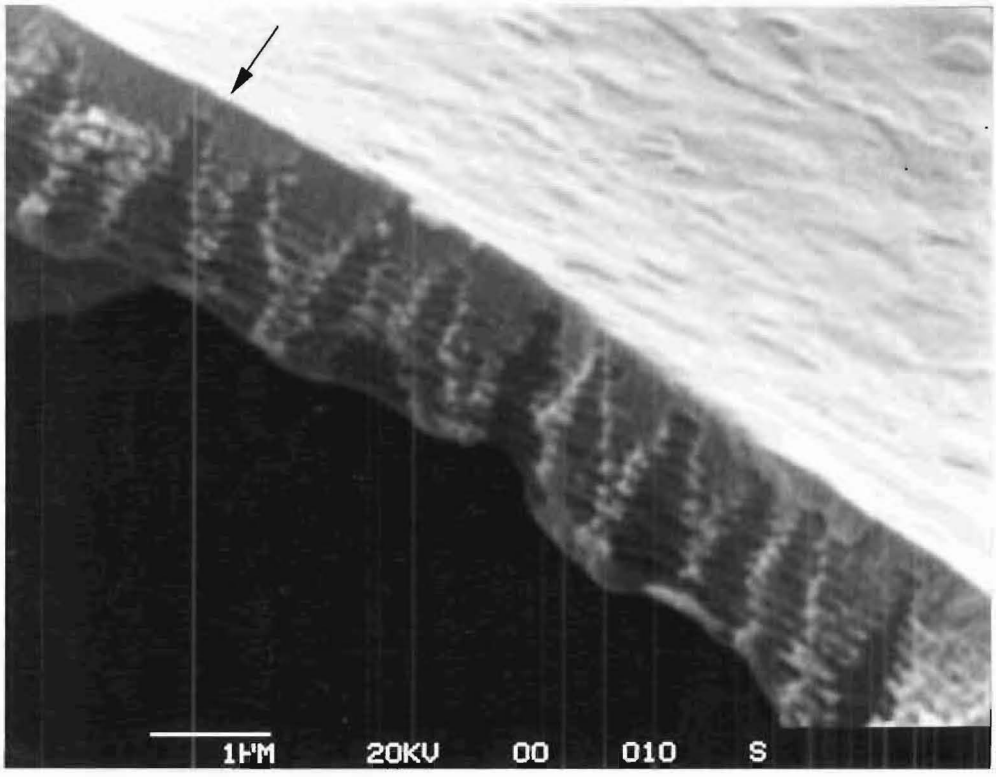

10PM 20KV 00 003 S

Plate 3: Transverse view of *Sitona discoideus* chorion showing the structure of the cavities, and the minute aeropyles extending from individual cavities to the outer surface of the chorion.

Plate 4: External view of the chorion indicating the position of the cavities ending near the outer chorion surface. The outer wax layer is evident in the bottom right hand corner of the picture.


1µM 20KV 00 010 S

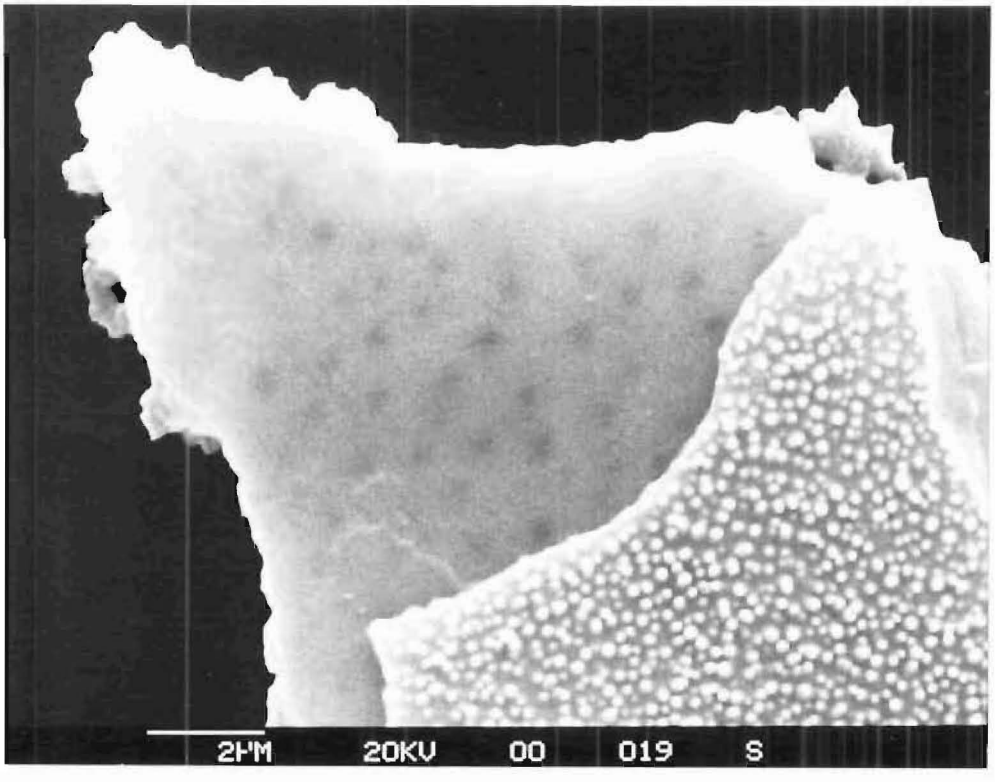

2µM 20KV 00 019 S

Table 2: The response of *S. discoideus* eggs to chlorpyrifos after treatment under the Potter tower.

Egg age (days)	Treatment	N[1]	NC[2]	Percentage mortality	95% C.I.
1 - 2 days	chlorpyrifos 1.0 g/l	50	40	100.0	-
6 days	chlorpyrifos 1.0 g/l	50	40	100.0	-
13 days	chlorpyrifos 1.0 g/l	50	40	100.0	-

1 - Number of eggs treated with insecticide.
2 - Number of eggs in the control.

Table 3: The response of *S. discoideus* eggs to fenitrothion after treatment under the Potter tower.

Egg age	Treatment	N	NC	Percentage mortality	95% C.I.
1 - 3 days	fenitrothion 0.6 g/l	74	70	98.3	7.2
	fenitrothion 1.2 g/l	78	70	100.0	-

Table 4: The response of *S. discoideus* eggs to deltamethrin after treatment under the Potter tower.

Egg age	Treatment	N	NC	Percentage mortality	95% C.I.
1 - 2 days	deltamethrin 5 mg/l	59	122	0.0	-
	deltamethrin 10 mg/l	85	122	12.7	12.1
	deltamethrin 20 mg/l	154	122	88.5	18.8
5 - 9 days	deltamethrin 20 mg/l	83	57	97.3	11.8

(c) Deltamethrin

Deltamethrin exhibited increasing ovicidal activity with increase in concentration (Table 4). At 5 mg/l there was no ovicidal activity, at 10 mg/l ovicidal activity produced a mortality of 12.7%. At 20 mg/l there was a 88.5% egg mortality. Although not significant ovicidal activity was more pronounced in older eggs with a 97.3% mortality of eggs 5-9 days old verses a 88.5% mortality in eggs 1-2 days old (Table 4).

(d) γ-HCH

γ-HCH exhibited poor ovicidal activity against *S. discoideus* at the two rates tested (Table 5). The 0.5 g/l rate of γ-HCH resulted in a mortality of 39.7% for eggs 1 - 2 days old. At 0.25 g/l egg mortality was reduced to 13.0%. Embryos not eclosing from eggs poisoned with lindane contained fully developed larvae. Many were moribund when excised from the egg 26 days after the last recorded emergence from the control group. Larvae were also observed to perforate the chorion but did not fully emerge. Moribund larvae that were removed from eggs exhibited uncoordinated activity indicating insecticidal poisoning.

(e) Diflubenzuron

Diflubenzuron was active against *S. discoideus* eggs aged between one to five days although effectiveness appeared to be slightly reduced with increasing egg age (Table 6). However, this was not significantly different. When a subsample of eggs was removed three days after treatment and subsequently incubated on fresh filter paper, much lower mortality occurred (Table 6.1). Dissection of a subsample of 10 - 17 eggs per dish, 10 days after the last emergence of larvae from the control population, indicated that unhatched treated eggs contained dead or moribund fully formed larvae. Only one to two eggs of the treatment group contained undifferentiated egg tissue, and was similar to that which occurred in the control population.

Table 5: Response of *S. discoideus* eggs to lindane after treatment under the Potter tower.

Egg age	Treatment	N	NC	Percentage mortality	95% C.I.
1 - 2 days	γ-HCH 0.25 g/l	87	92	13.0	14.3
	γ-HCH 0.5 g/l	130	92	39.6	9.4

Table 6: The response of *S. discoideus* eggs to diflubenzuron after treatment under the Potter tower.

Egg age	Treatment	N	NC	Percentage mortality	95% C.I.
1 - 2 days	diflubenzuron 1.2 g/l	117	67	100.0	-
3 - 5 days	diflubenzuron 1.2 g/l	112	105	92.8	11.9

Table 6.1: The response of *S. discoideus* eggs to diflubenzuron after eggs were placed on an uncontaminated surface three days after treatment.

Egg age	Treatment	N	NC	Percentage mortality	95% C.I.
1 - 2 days	diflubenzuron 1.2 g/l	30	30	75.9	27.8
3 - 5 days	diflubenzuron 1.2 g/l	30	30	23.9	38.1

Effect of chilling on the ovicidal activity of chlorpyrifos and fenitrothion

The toxicity of chlorpyrifos and fenitrothion to *S. discoideus* eggs and the effect of chilling on ovicidal effectiveness is shown in Tables 7 and 8, with the log dose - probit regression line for chlorpyrifos shown in Figure 1. The data for fenitrothion provided a provisional LC_{50} but was not robust enough to calculate the 95% confidence limits. For this reason a probit analysis was not performed on the fenitrothion data. A straight comparison of the LC_{50} values of the two chemicals showed that fenitrothion exhibited greater ovicidal activity compared to chlorpyrifos. This relationship was also found when eggs were chilled prior to incubation, although chilling increased the LC_{50} of both ovicides; 1.5 times for chlorpyrifos and 8.8 times for fenitrothion. The log - probit line and slope was also altered for both insecticides when eggs were chilled.

Table 7: LC_{50} of chlorpyrifos on *S. discoideus* eggs after treatment under the Potter tower and incubation at either 15°C or chilled at 6°C before incubation at 15°C.

Incubation regime	TN[1]	b[2]	Standard error	LC_{50} (mg/l)	95% C.I.	LC_{90} (mg/l)
15°C	1284	4.03	0.33	112.4	68.5 - 145.2	233.8
Chilling/15°C	1743	2.02	0.11	171.2	89.4 - 256.6	739.3

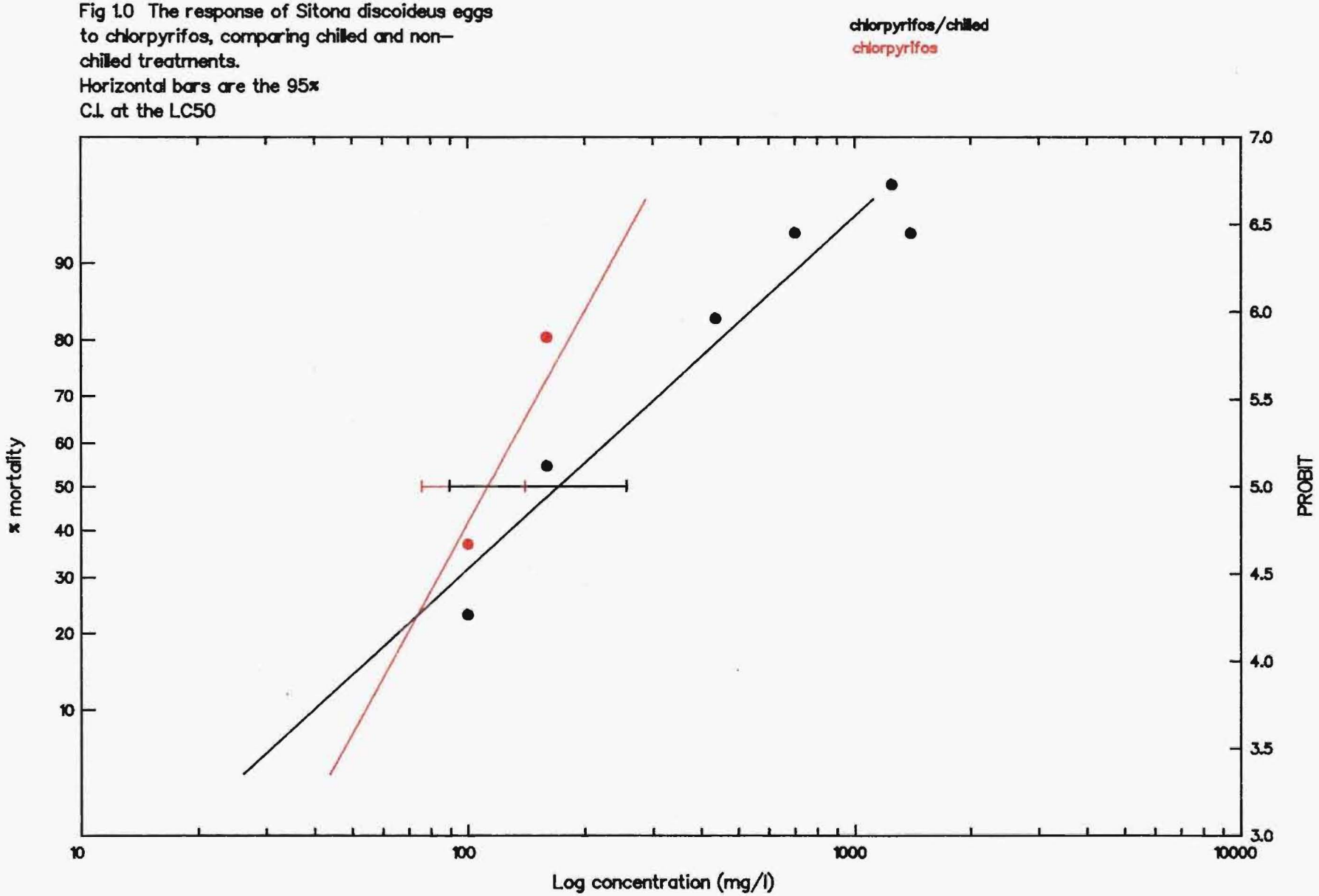
1 - Total number of insects treated
2 - Slope of the regression line

Table 8: LC_{50} of fenitrothion on *S. discoideus* eggs after treatment under the Potter tower and incubation at either 15°C or chilled at 6°C before incubation at 15°C.

Incubation regime	TN	b	Standard error	LC_{50} (mg/l)	95% C.I.	LC_{90} (mg/l)
15°C	1008	1.17	0.40	3.2	*	39.6
Chilling/15°C	1344	1.80	0.17	28.2	*	145.5

* - The data did not allow calculation of the confidence limits.

Fig 1.0 The response of Sitona discoideus eggs to chlorpyrifos, comparing chilled and non-chilled treatments.
Horizontal bars are the 95% C.L. at the LC50



TOXICITY OF CHLORPYRIFOS AND FENITROTHION TO *SITONA DISCOIDEUS* AND *MICROCTONUS AETHIOPOIDES*

Topical dosing

Figure 2 and Table 9 show the results of topical dosing of *S. discoideus* with chlorpyrifos and fenitrothion respectively. The toxicities of chlorpyrifos and fenitrothion to the parasitoid adult are shown in Figure 3 and Table 10. The LD_{50} values indicate that both insecticides were more toxic to *M. aethiopoides* adults, than *S. discoideus* adults. Both parasitoid and weevil exhibited similar susceptibility to both insecticides.

The LD_{50} response of reproductive *S. discoideus* to chlorpyrifos and fenitrothion were similar Slopes and intercepts were also not significantly different. Two LD_{50} values for fenitrothion were obtained from *S. discoideus*. Weevils collected in summer (December/January) produced a LD_{50} of 3.14 , while weevils collected in mid-spring (October) produced a LD_{50} of 5.80. There was a 1.85 times change in the LD_{50} between that of the January and October weevil populations. The response of the aestivating weevils is reflected in a significantly different intercept from that produced by reproductive weevils when dosed with chlorpyrifos and fenitrothion, although the log-probit lines for the three treatments were parallel.

Topically dosing *M. aethiopoides* adults produced almost identical LD_{50},s for chlorpyrifos and fenitrothion (Table 10). Comparisons of the log-probit - mortality line for chlorpyrifos and fenitrothion, indicated that slopes and intercepts were not significantly different.

An index of toxicity of insecticides to natural enemies is the selectivity ratio proposed by Metcalf (1958). The selectivity ratio is derived from the ratio of the LD_{50} non-target/LD_{50} of pest. The selectivity ratio for chlorpyrifos and fenitrothion to *M. aethiopoides* was 0.81 and 0.74 for respectively. Only the weevil population tested in mid-summer produced a selectivity ratio of 1.37, indicating that selectivity was favourable to the parasitoid.

Table 9: The toxicity of topically applied chlorpyrifos and fenitrothion to *S. discoideus* adults.

Insecticide	TN	b	Std. error (slope)	LD_{50} ($\mu g/g$)	95% C.I.	LD_{90} ($\mu g/g$)
Chlorpyrifos	399	6.34	0.69	5.36	5.02 - 5.72	8.54
Fenitrothion[1]	204	6.64	0.89	5.80	5.29 - 6.32	9.05
Fenitrothion[2]	1093	6.67	0.38	3.14	2.83 - 3.24	4.89

1 - Weevils collected in October
2 - Aestivating weevils collected in January

Table 10: The toxicity of topically applied chlorpyrifos and fenitrothion to *M. aethiopoides* adults.

Insecticide	TN	b	Std. error (slope)	LD_{50} ($\mu g/g$)	95% C.I.	LD_{90} ($\mu g/g$)
Chlorpyrifos	415	2.55	0.34	4.33	3.27 - 5.59	13.75
Fenitrothion (Oct)	311	2.86	0.75	4.29	3.57 - 5.55	12.03

Fig 2.0 The response of Sitona discoideus
to chlorpyrifos and fenitrothion
Topical application
Horizontal bars are the
95% C.I. at the LD50

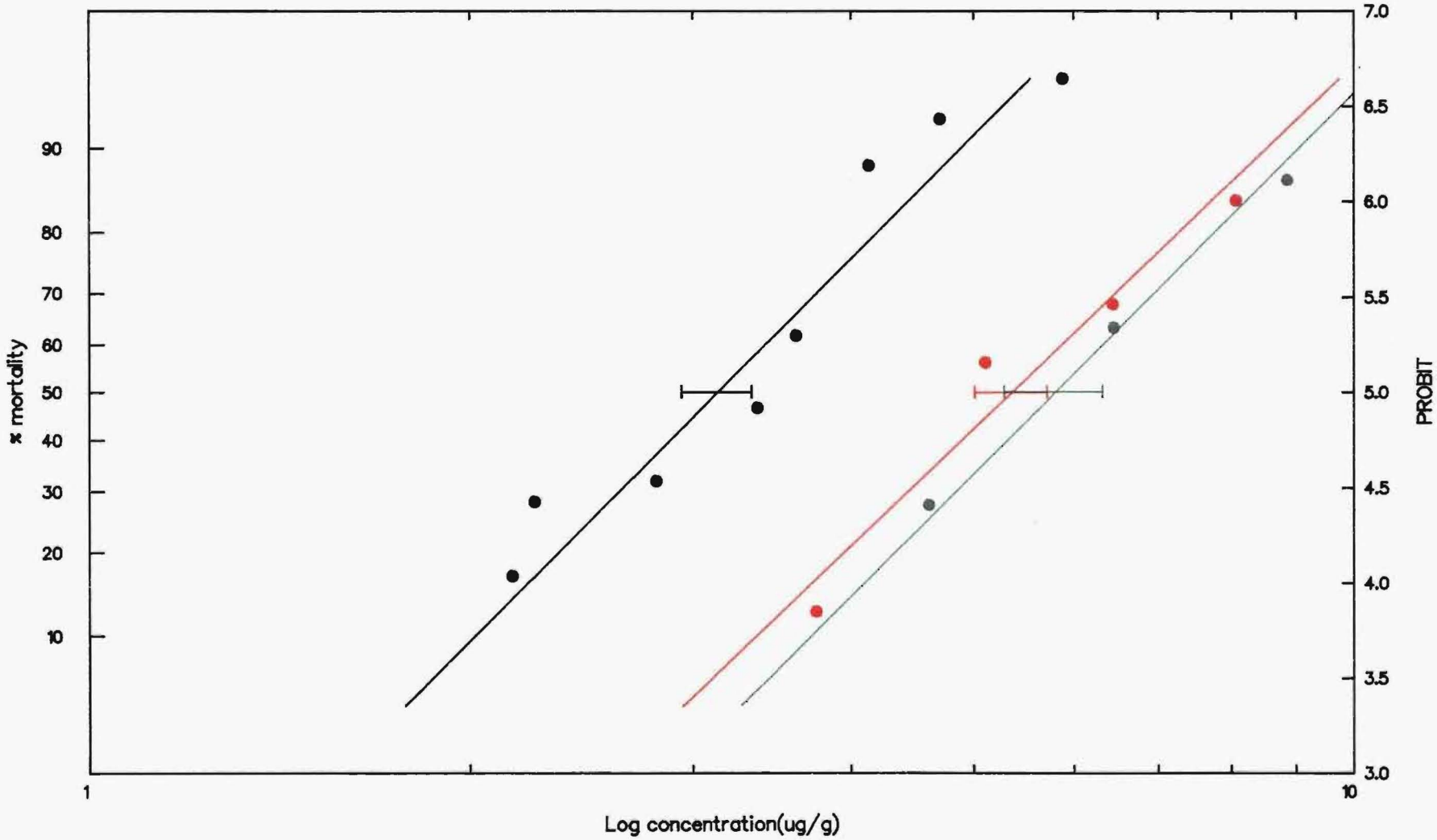
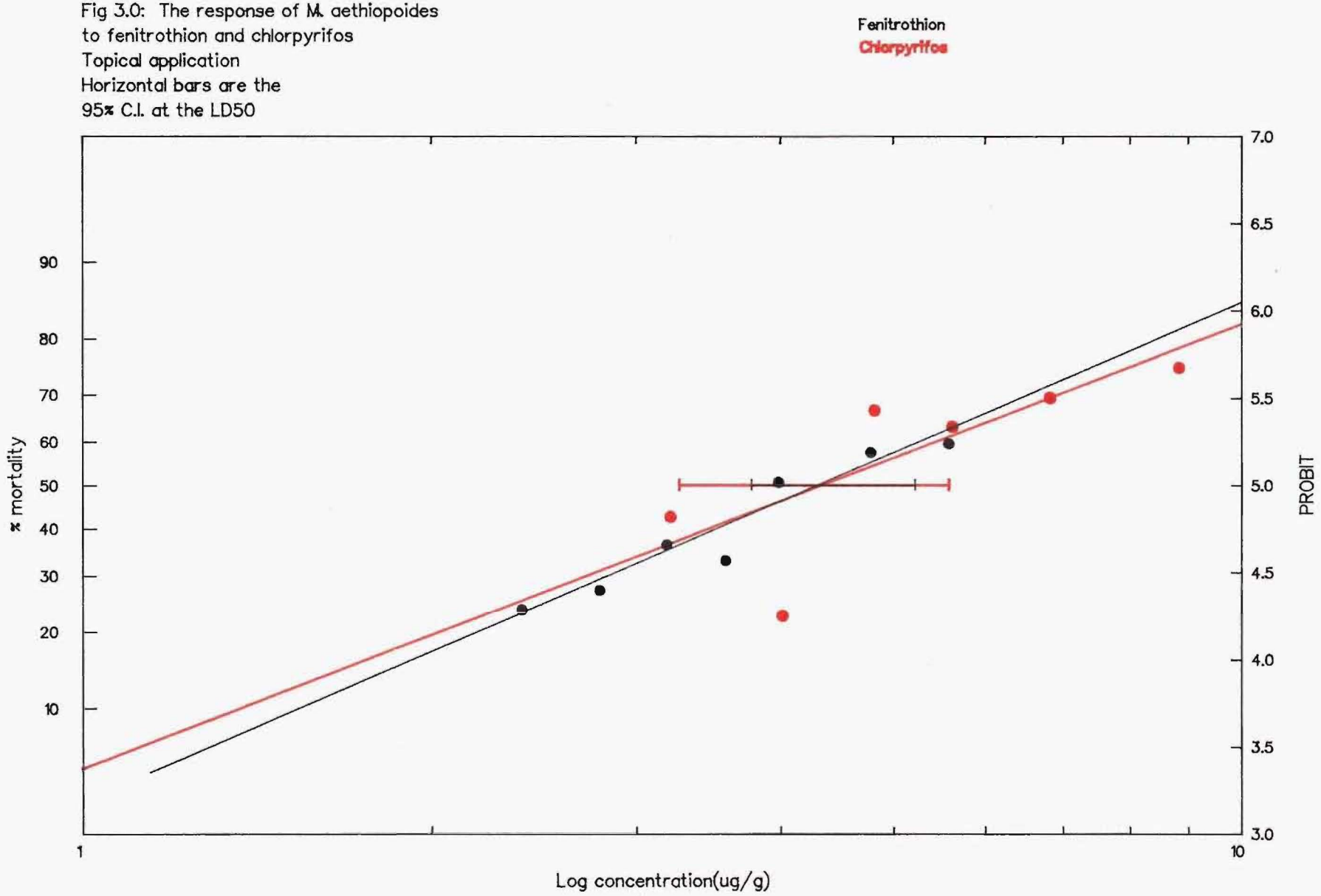


Fig 3.0: The response of M. aethiopoides
to fenitrothion and chlorpyrifos
Topical application
Horizontal bars are the
95% C.I. at the LD50



TOXICITY OF CHLORPYRIFOS AND FENITROTHION TO *MICROCTONUS AETHIOPOIDES* PUPAE

Potter tower application

There was low pupal mortality after direct spraying of cocoons with chlorpyrifos and fenitrothion (Table 11). Control mortality for chlorpyrifos and fenitrothion was only 14.8% and 12.5% respectively, with a mortality of 21.7% at the highest rate of chlorpyrifos. For cocoons treated with fenitrothion there was only 2.4% mortality of pupae at 1.0 g/l, with no mortality at 2.0 g/l. Dissection of cocoons revealed that in the majority of cases (88%, n=26), cadavers were either partially or fully developed. Three cocoons (n=10) that had been treated with fenitrothion were found to contain late 4th instar larva.

Longevity of *M. aethiopoides* adults emerging from treated cocoons

The longevity of parasitoids eclosing from treated pupae is shown in Figures 4 and 5. Chlorpyrifos produced the most marked response, with increasing concentration having a highly significant effect on the longevity of *M. aethiopoides* adults (Figure 4). The effect on longevity was most marked in the males with a highly significant difference between the sexes (Table 11). The separate regression equations for male and female parasitoids and the coefficients of determination are shown in Figure 4. The response of females was best described by a quadratic regression equation, although the effect was created by the unexpectedly high value obtained for females at the 1.2 g/l rate. The males response was best described by the linear regression. The general linear equation for *M. aethiopoides* males and females emerging from pupae treated with chlorpyrifos is:

Longevity = 11.8 - 0.004 Concentration + 3.3 MvsF

MvsF describes the correction factor for the male-female intercept, with M coded as 0 and F coded as 1. The term '+ female slope' listed in Table 12, refers to the difference in slopes for male and female adults.

The regression equation produced by fenitrothion is:

Longevity = 12.7 - 0.00093 Concentration + 0.4 MvsF

Increasing concentration of fenitrothion did not have a significant effect on longevity of emerging parasitoid adults although there was a slight trend towards reduced longevity with increase in concentration (Figure 5). Fenitrothion at all rates did not produce a significant differential effect on longevity between males and females. Also, the highest rate of fenitrothion did not have the same detrimental effect on *M. aethiopoides* adult longevity as had been observed with chlorpyrifos. The regression equations for both males and females indicated that there was no trend produced by increasing concentration (Figure 5).

Parasitism by adults emerging from pupae treated under the Potter tower

Because of low numbers of female parasitoids tests were not replicated. However, the results indicated that surviving females were able to parasitise weevils. Only at the high rate of chlorpyrifos was there any indication that the parasitoid's ability to successfully attack and parasitise weevils was impaired. The majority of female parasitoids surviving the 2.4 g/l rate of chlorpyrifos either died within 24 hours after eclosion or did not oviposit in *S. discoideus*. One female emerging at the highest rate did successfully oviposit in two weevils over a 24 hour period. Three females from the 1.2 g/l rate and two females from the 0.6 g/l rate also successfully parasitised *S. discoideus*, and produced a total of 11 and 2 offspring respectively. Two female *M. aethiopoides* from the control group produced a total of 11 pupae.

Six females emerging from pupae dosed with the highest rate of fenitrothion were tested to see how exposure affected their ability to parasitise. In all but one case, females were observed to pursue and subsequently oviposited in the weevils, with a total of 14 pupae recovered. Three females from the control treatment produced a total of 6 pupae.

Table 11: Percentage mortality of *M. aethiopoides* pupae to chlorpyrifos and fenitrothion after treatment under a Potter tower.

Insecticide	Concentration (g/l)	Number tested	Percentage mortality *
chlorpyrifos	0	27	
	0.6	27	0.0
	1.2	27	0.0
	2.4	27	21.7
fenitrothion	0	27	
	0.5	27	0.0
	1.0	27	2.4
	2.0	27	0.0

* - Mortality corrected using Abbott's formula.

Table 12: The interaction of longevity of *M. aethiopoides* adults emerging from treated pupae, to the parameters of insecticide concentration and sex.

Treatment	Chemical	Longevity vs	
Potter tower	chlorpyrifos	concentration	**
		male vs female	**
		+ female slope	NS
	fenitrothion	concentration	NS
		male vs female	NS
		+ female slope	NS
Topical	chlorpyrifos	concentration	NS
	fenitrothion	concentration	**

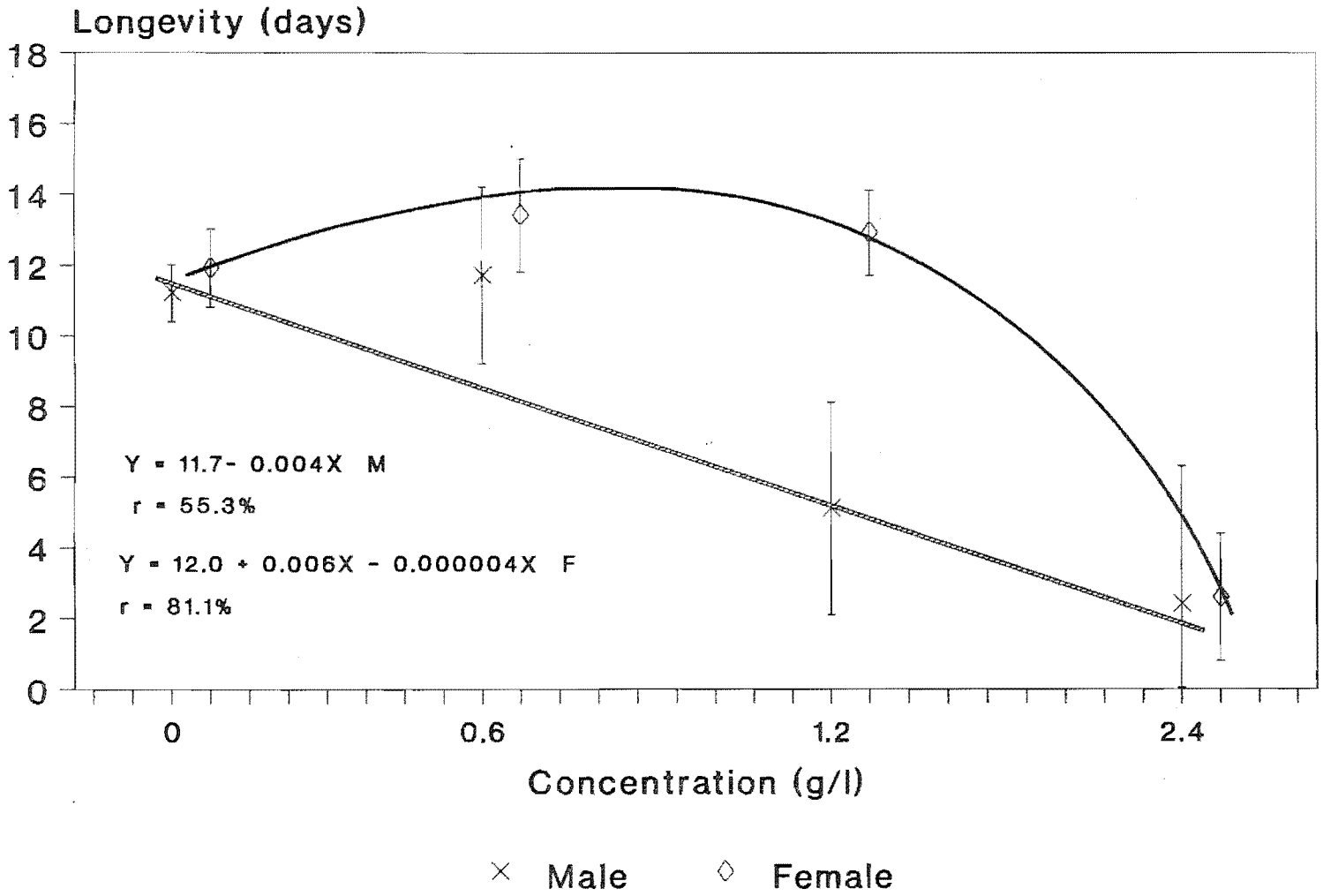


Figure 4: Regression relationship between longevity (days) with 95% C.I. and concentration, of *M. aethiopoides* adults emerging from cocoons treated with chlorpyrifos under the Potter tower.

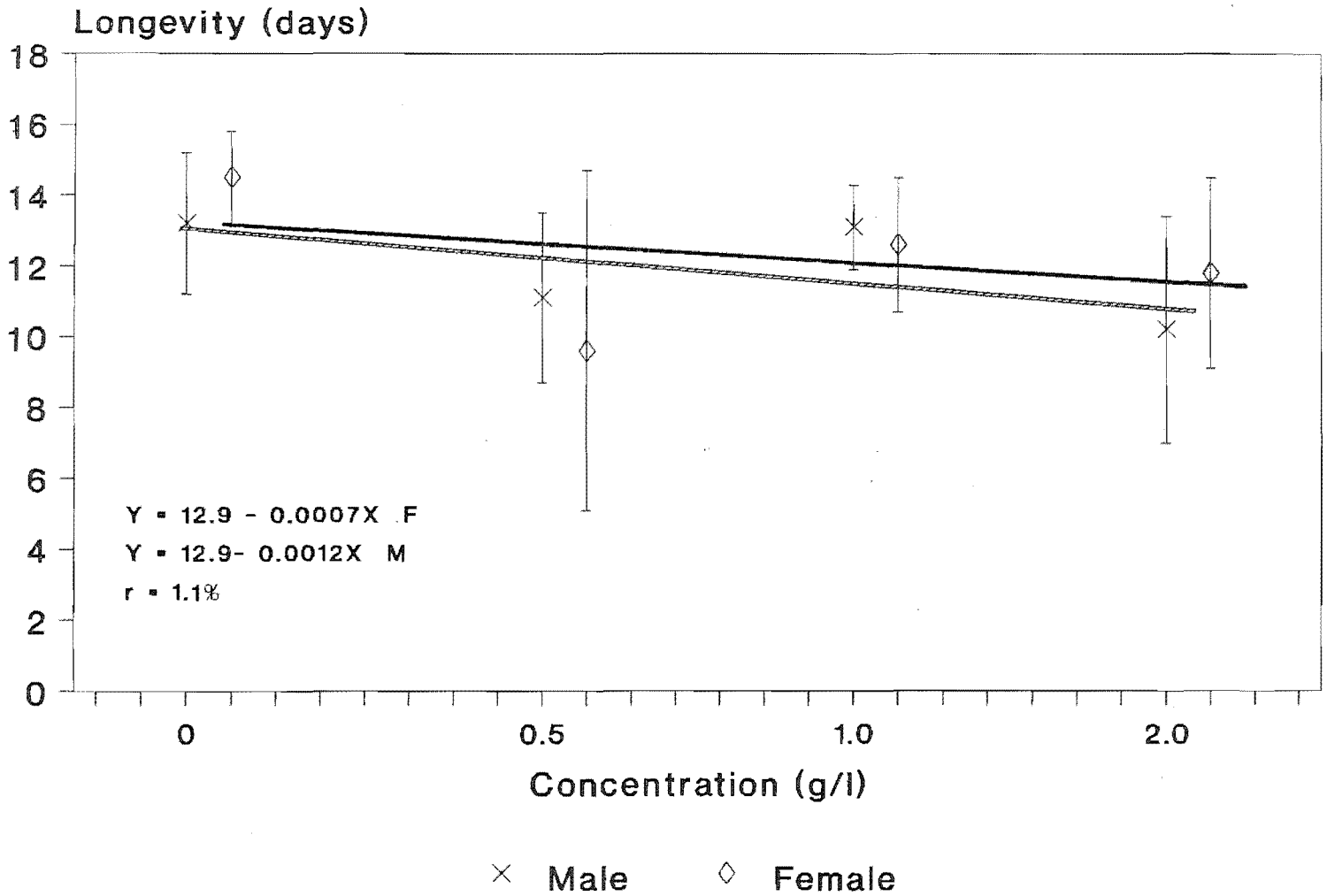


Figure 5: Regression relationship between longevity (days) with 95% C.I. and concentration, of *M. aethiopoides* adults emerging from cocoons treated with fenitrothion under the Potter tower.

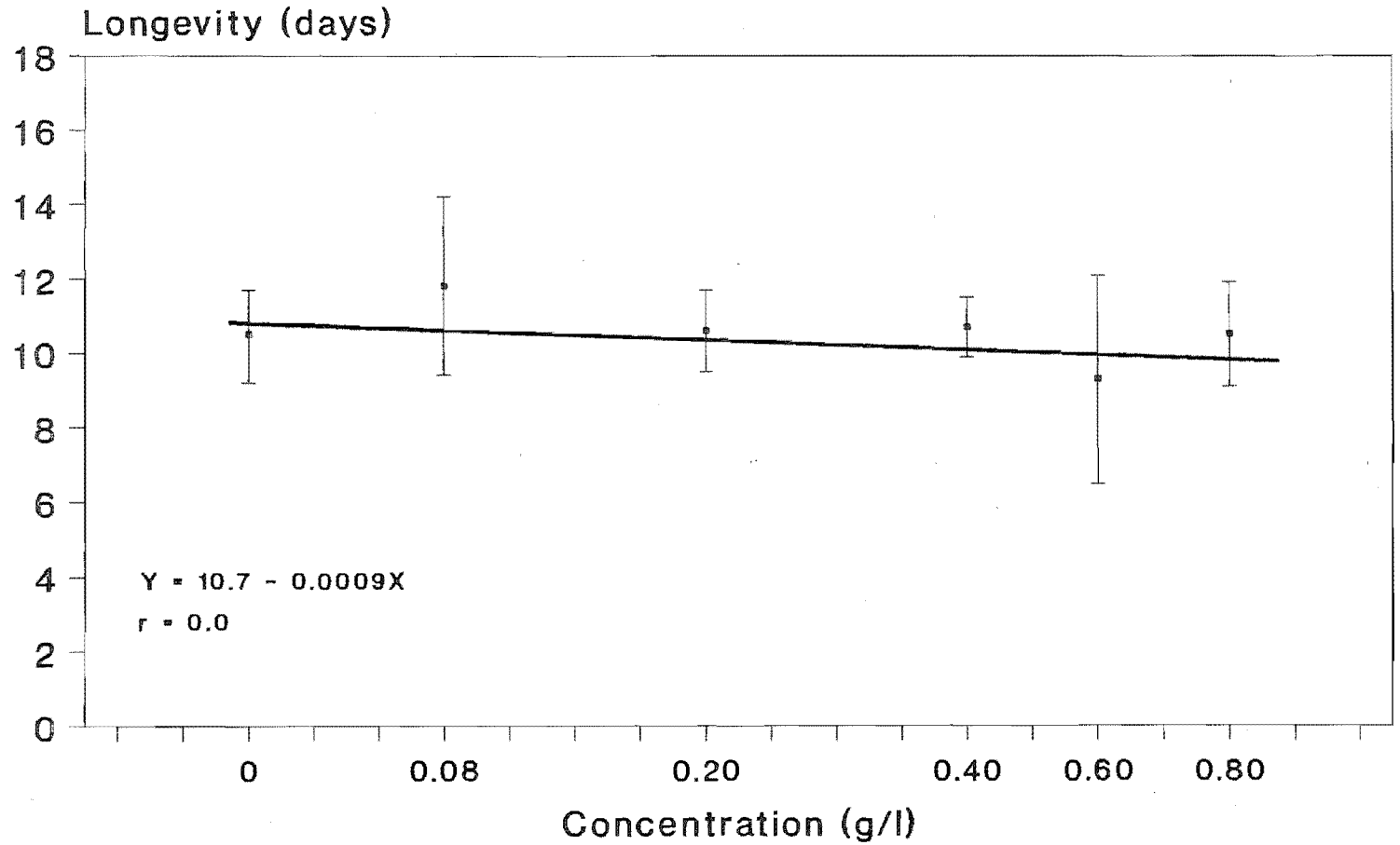


Figure 6: Regression relationship between longevity (days) with 95% C.I. and concentration, of *M. aethiopoides* adults emerging from cocoons treated topically with chlorpyrifos.

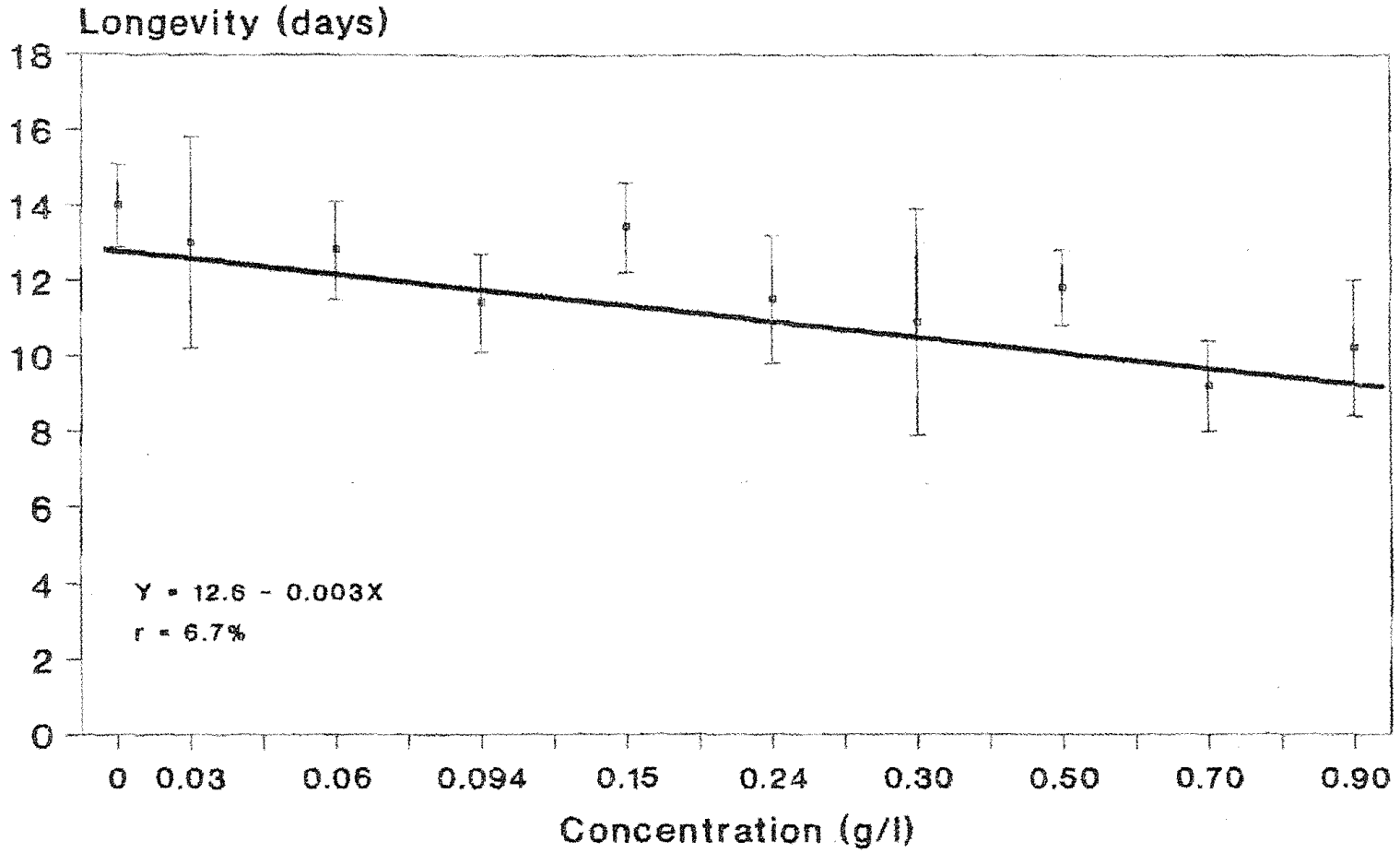


Figure 7: Regression relationship between longevity (days) with 95% C.I. and concentration, of *M. aethiopoides* adults emerging from cocoons treated topically with fenitrothion.

Topical dosing of *Microctonus aethiopoides* pupae

Determination of an LD_{50} value for pupae proved difficult. Even at the highest rates of each chemical, 0.8 g/l for chlorpyrifos and 0.9 g/l for fenitrothion, parasitoids successfully eclosed. Mortality at these concentrations was not significantly different from the control.

The regression equations and associated coefficients of determination for parasitoids emerging from pupae topically dosed with chlorpyrifos and fenitrothion are shown in Figures 6 and 7 respectively. At the six concentrations of chlorpyrifos tested there was no significant effect on longevity (Table 12). Increasing the concentration of fenitrothion produced a highly significant effect on longevity, although this significance has low predictive value given the large number of points and resulting variability at the lower concentrations tested.

Parasitism by adults emerging from treated pupae

As with the Potter tower experiments, parasitoid females emerging from treated pupae were mated, then supplied with weevils to observe whether they could successfully parasitize weevils. Parasitoids from pupae that had been treated with chlorpyrifos where able to parasitise *S. discoideus* at all rates, except those that had emerged from pupae treated at the highest rate. Females emerging from pupae dosed with fenitrothion produced offspring at all concentrations tested.

THE EFFECT OF CHLORPYRIFOS ON *MICROCTONUS AETHIOPOIDES* AND *SITONA DISCOIDEUS* IN THE FIELD

Field experiment

Spraying was conducted in ideal conditions with no wind and clear weather. Plate 5 provides a general view of the field site. The plastic structures sited in each plot (Plate 5) were eclosion traps for *M. aethiopoides*, but were not employed in this experiment. During the time that the

experiment was conducted, the mean temperature was 8.4°C, and -4.6°C was the lowest minimum recorded at the Darfield meterological station. An 8.5 cm snowfall was recorded on the 24 May, and the lowest grass minimum was -8.1°C was recorded 3 days later on the 27 May. During the period from the 13 - 31 May 45.4 mm of rain fell.

Ground density of *Sitona discoideus* adults

S. discoideus density in the pre and post-spray plots and the effective kill obtained by applying chlorpyrifos is shown in Table 13. In the pre-spray plots, sampling indicated that density, although fluctuating across all plots was not significantly different. After treatment, the density of the control plots declined by 29%, but the means were not significantly different as confidence intervals overlap. Conversely, treatment with chlorpyrifos had virtually eliminated *S. discoideus* from the sprayed plots (Table 13). The overall effectiveness of applying chlorpyrifos at 0.3 kg a.i./ha, was 96.2%.

Table 13: The mean pre-spray and post-spray ground density of *S. discoideus* adults and the effectiveness of applying chlorpyrifos at 0.3 kg a.i./ha to lucerne.

Treatment	Pre-spray density	95% C.I.	Post-spray density	95% C.I.	Percentage effectiveness[1]
Control	26.2	3.9	18.5	3.3	
chlorpyrifos	29.6	4.1	0.8	0.8	96.2

$$1 - \text{Percentage effectiveness} = 1 - \frac{T_a \times C_b}{T_b \times C_a} \times \frac{100}{1}$$

T_a = weevil density in chlorpyrifos plots post-spray.
T_b = weevil density in chlorpyrifos plots pre-spray.

C_a = weevil density in control plots post-spray.
C_b = weevil density in control plots pre-spray.

Level of parasitoid activity in the field

(a) Pre-spray parasitoid population

Dissection of weevils indicated that overall the level of parasitism was 45.2%; levels of parasitism in control and treatment plots were essentially the same (Table 14). In control plot 8 there was no parasitism recorded in the pre-spray sampling, although in the post-spray sample 14 days later, parasitism was 58.3%. A breakdown of parasitoid larval development stage indicated that in all pre-spray plots *S. discoideus* adults supported a mixed-aged immature parasitoid population, with a small number of weevils containing eggs (c. 8.0%).

(b) Post-spray parasitoid population

In the control plots, the relative proportion of eggs, 1st, 2nd-3rd and 4th-5th instar had not changed, from that recorded in the pre-spray population (Table 14). In the treated plots, 87.5% of the surviving weevils were parasitised. The parasitoid stages found in the surviving weevils ranged from egg to 4th-5th instars (Table 14). The proportion of surviving weevils having 1st instar parasitoids had increased, while there was a drop in the number of 2nd to 4th-5th instars. This was most evident with the 4th-5th instar larva, where the proportion had fallen from 40.5% to 14.3%.

Residual toxicity of chlorpyrifos

The effects of chlorpyrifos residues on *S. discoideus* adults is shown in Figure 8. At 0.3 kg/ha, 59.5% mortality occurred one day after spraying, with subsequent mortality declining gradually over the duration of the experiment. Twenty two days after treatment, mortality had fallen to 1.5%.

At 1.0 kg/ha, the toxicity of chlorpyrifos residue was more pronounced, with 91.8% mortality the first day after spraying, mortality remaining above 80% for six days after spraying. Ten days after treatment weevil mortality had dropped to 77.4%, with a gradual decline in activity similar to that shown for the 0.3 kg/ha treatment. At the completion of the experiment, mortality due to exposure to 1.0 kg/ha residue had only fallen to 40%.

Table 14: Percentage parasitism and stage of *M. aethiopoides* development in *S. discoideus* populations before and after spraying with chlorpyrifos.

	PRE - SPRAY		POST - SPRAY	
	Number	Percentage parasitism	Number	Percentage parasitism
control				
Weevils dissected	84		84	
Parasitised	39	46.4	43	51.2
Parasitoid stage				
eggs	3	7.7	4	9.3
1st instar	14	36.0	14	32.6
2nd - 3rd instar	11	28.2	13	30.2
4th - 5th instar	9	23.1	12	27.9
teratocytes	2	5.0	0	-
chlorpyrifos				
Weevils dissected	84		8	
Parasitised	37	44.1	7	87.5
Parasitoid stage				
eggs	2	5.4	1	14.3
1st instar	8	21.6	3	42.9
2nd - 3rd instar	12	32.4	2	28.6
4th - 5th instar	15	40.5	1	14.3
teratocytes	0	-	0	-

Although both rates of chlorpyrifos exhibited a decline in residue activity over the 22 day period, mortality due to residues within each treatment was not significant different between subsequent sample dates. Between the two insecticide treatments however, mortality was significantly different at all sampling dates, except on day one, and is attributed to a high variability in response between replicates in the 0.3 kg/ha treatment.

Response of *Microctonus aethiopoides* adults to chlorpyrifos residues

Figure 9 displays the percentage mortality of parasitoid adults at selected days after treatment. At the recommended field rate of 0.3 kg/ha, chlorpyrifos mortality was high for the first three days after spraying, with 78.0% mortality recorded on the day one, falling to 65.0% after three days. Mortality dropped significantly ($p > 0.05$) six days after treatment to 6.0%; after 15 days insecticide residues on the foliage were no longer toxic to *M. aethiopoides* adults. At the 1.0 kg/ha rate, the toxicity of residues remained high (> 80.0% mortality) for up to fifteen days after treatment although twenty-two days after treatment activity had declined to 50.0%. During the course of the experiment it was noted that generally higher mortality occurred in male *M. aethiopoides*, although this differential mortality was not significant except on day twenty-two and at the 1 kg/ha rate.

Effect of chlorpyrifos residues on emergence and survival of *Microctonus aethiopoides* pupae

(a) Parasitoid emergence during experiment

Fifth instar pre-pupae emerged on several occasions from laboratory cages in which the residual toxicity studies were being conducted. Failure to pupate was highest in the 1.0 kg/ha treatment and this effect persisted for up to day six. From day six emergence and pupation of parasitoid larvae were comparable to the control and the 0.3 kg/ha chlorpyrifos treatment. Pre-pupae emerging from weevils exposed to the 0.3 kg/ha treatment had overall a 82.4% success of pupation (n=17), comparable to the control with 86.7% (n=15). In contrast, only 47% (n=17) of pre-pupae emerging at the 1.0 kg/ha rate succeeded in pupating. Subsequent adult *M. aethiopoides* emergence from cocoons was similar for all treatments.

(b) Pre-pupal emergence from moribund/dead weevils

Parasitoid pre-pupae emerged and successfully pupated from dead or moribund weevils on six occasions, with emergence taking place 19 to 24 hours after the weevils had been collected. This occurred once on day 3 from a 0.3kg/ha treatment, the remaining pre-pupae emerging from the 1.0 kg/ha treatment on days 3(2), 6(2) and 10.

(c) Pre-pupal emergence from surviving weevils

Weevils that were alive at the completion of the experiment were held for a further 24 hours. Final instar larvae emerged and successfully pupated on four occasions, once from weevils surviving the 0.3 kg/ha treatment (22 day), and in three instances from weevils that had survived the 1.0 kg/ha treatment, two from weevils exposed to 15 day old residues and one from weevils exposed to 22 day old residues.

Effect of parasitism on mortality of *Sitona discoideus* adults exposed to chlorpyrifos residues.

The levels of parasitism and stage of parasitoid development in weevils that died during the 48 hour exposure period could not be directly related to the fall in residue activity over the duration of the experiment. Weevils dying during the 48 hour period were found to contain parasitoid stages ranging from egg to late 4th, and reflected a similar breakdown in parasitoid stages for surviving weevils. Overall, the level of parasitism in the control population rose slightly over the 22 day period, while levels of parasitism in weevils exposed to chlorpyrifos residues at either 0.3 or 1.0 kg/ha showed a decline. This trend was similar for weevils exposed to both rates and recorded as either dead or alive at the end of the 48 hour period.

Plate 5: General view of the lucerne stand on which the field experiment was conducted. View taken from the north-west corner of site.

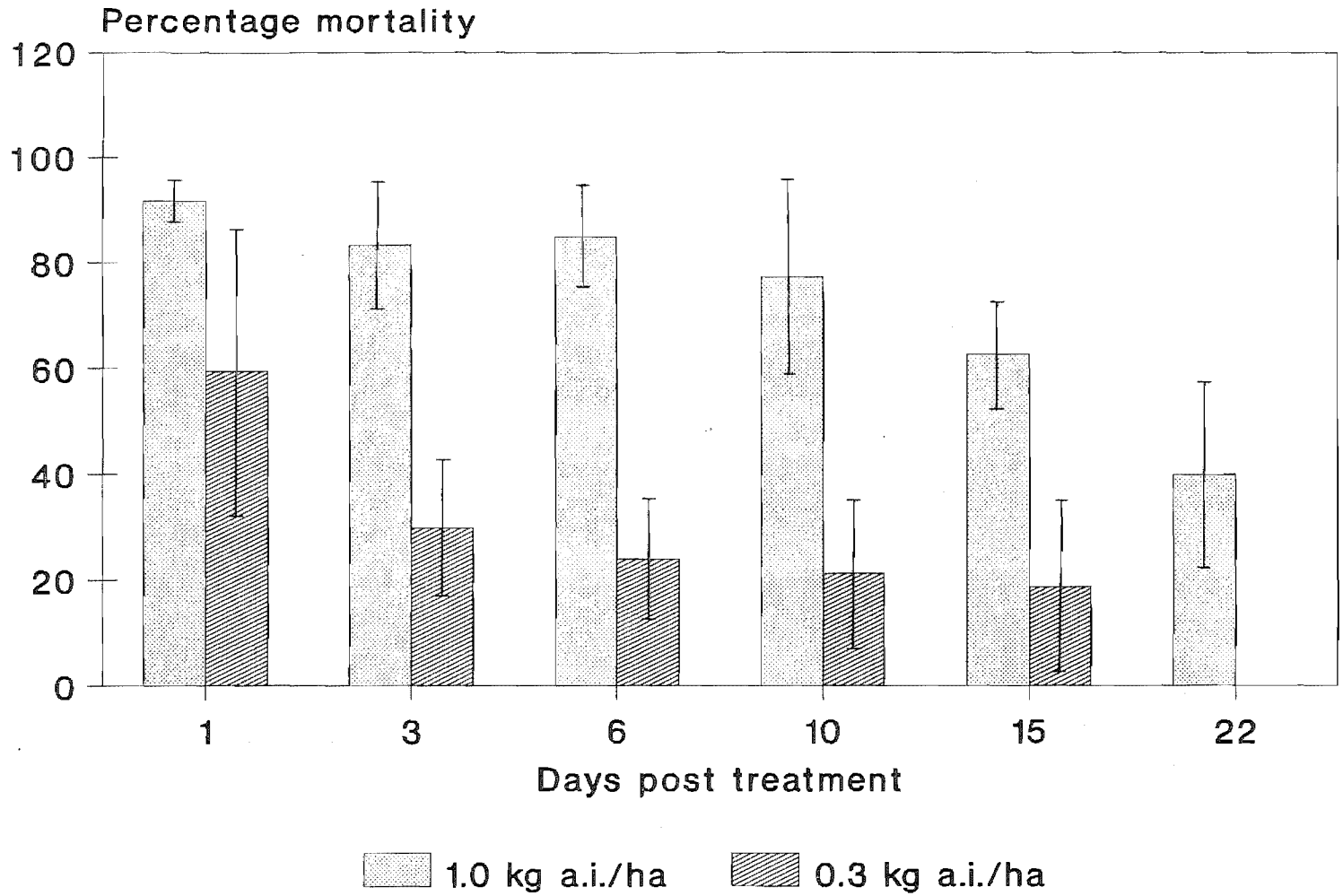


Figure 8: Residual activity of chlorpyrifos on lucerne relative to contact mortality of *S. discoideus* (95% C.I. shown).

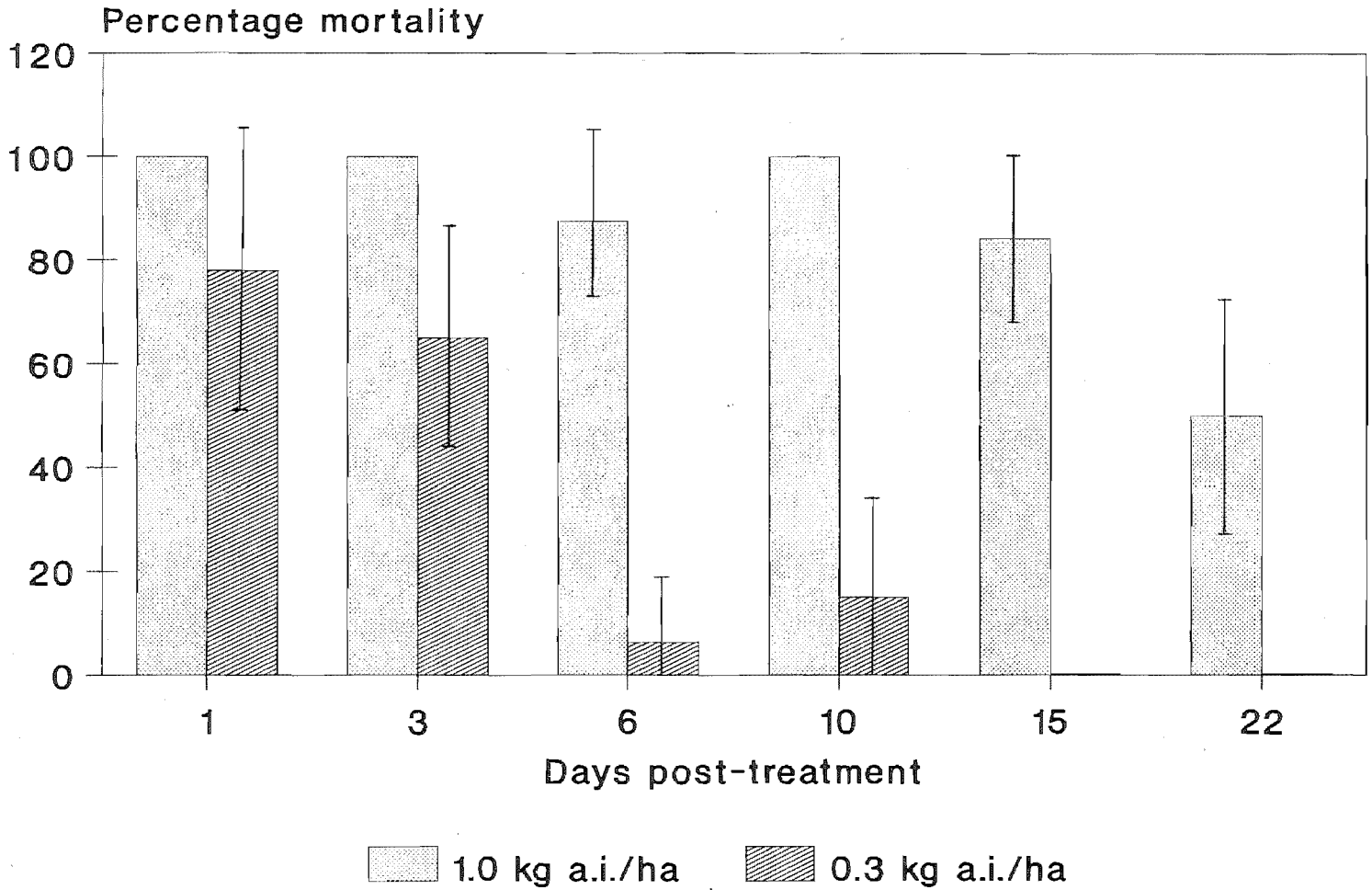


Figure 9: Residual activity of chlorpyrifos on lucerne relative to contact mortality of *M. aethiopoides* (95% C.I. shown).

CHAPTER 5

DISCUSSION

SITONA DISCOIDEUS EGG DEVELOPMENT AND STRUCTURE OF THE CHORION

Externally, the *S. discoideus* egg is uniform and continuous, with none of the sculpturing and specialised structures found on other coleopteran eggs (e.g. Hinton 1981). The waxy protective layer and chorion form two distinct features of the egg when fractured segments are viewed externally using the SEM (Plate 1).

The chorion of *S. discoideus* is relatively simple, composed of extensive cavities and microcavities but without the complex 'meshwork' found in other coleopteran eggs (e.g. Hinton 1981; Lincoln 1961). This type of respiratory system can be described as primitive, based on Hinton's (1981) interpretations.

(i). Oxygen requirements

Aeropyles do not arise from each cavity but rather appear to be sparsely scattered across the surface of the chorion. This may ensure that moisture loss is minimised as in their primitive Mediterranean habitat (Aeschlimann 1980) eggs would presumably be subjected to periods of high saturation deficits. What aeropyles are present ensure direct continuity between the external air and that held in the chorion, although oxygen diffusion across the solid chorion has been alluded to (Hinton 1981; Wigglesworth 1972).

How important oxygen movement across the egg chorion is for continued development of the embryo is difficult to determine. Not only is the total amount of oxygen passing through the chorion important, but the rate of movement also needs to be considered. Summer oil is known to exert an ovicidal effect in some species by smothering the developing embryo (e.g. Chapman and Pearce 1949). However, when oil was sprayed directly onto *S. discoideus* eggs there were no ovicidal effect. The implication is that either adequate oxygen for embryogenesis is retained within the chorion of the egg, or that the oil did not provide an effective smothering layer over the surface of the egg.

(ii). Moisture requirements

As with many coleopteran eggs, *Sitona* eggs, although lacking hydropyles are able to take up liquid water from the environment. Such absorption of water with a consequent increase in volume is considered to be a more primitive method of development than the converse strategy of doing without water and maintaining a constant volume (Hinton 1981). However, absorption of water is known to be a secondary specialisation among many recent groups of insects (Hinton 1981).

For *Sitona* spp., moisture would appear to be the main environmental factor affecting egg development and survival. Laboratory studies with *S. discoideus* indicated that larval eclosion was retarded and there was an associated increase in mortality when eggs were subjected to moisture stress. This is consistent with the findings of Anderson (1930) who, in a comprehensive study, demonstrated the importance of moisture to *S. lineatus* egg viability. Anderson (1930) showed that the duration of the incubation period of the egg (embryogenesis plus development of pharate first instar larva) was significantly affected by increased saturation deficits, and as the saturation deficit increased there was an associated rise in embryo mortality.

Two laboratory experiments investigating the role of water in egg development provided an indication of the role the chorion plays in regulating the immediate environment of the developing embryo and pharate larva of *S. discoideus*. In the first experiment, immersion of eggs in water did not drown the embryo or pharate larva. This may mean that the larva was able to survive while immersed in water, but what was more likely was that oxygen was retained within the chorionic structure and not displaced by water molecules. The second experiment revealed prompt eclosion of larvae occurred from eggs that had been subjected to moisture stress prior to exposure to free water. This suggests that water was transported to the pharate larva. Although little is known about such water absorption (active or passive), and the fine structure of the sites at which water is absorbed (Hinton 1981), it is probable that the aeropyles served to facilitate the observed rapid movement of water into the chorion to the vitelline membrane, aided by the strong moisture gradient that would occur between the chorion surface and the membrane surrounding the pharate larva. Saturation of the vitelline membrane may be the cue for the larva to commence eclosion, but it may also soften the chorion to allow the larva to cut it open.

In summary, it is apparent that the *S. discoideus* chorion, although relatively simple in construction, adequately serves to ensure that the requirements of protection, moisture and oxygen

retention are met. In addition, despite having narrow moisture requirements for survival (Hinton 1981) and being exposed for long periods to the climatic variables (Frampton 1984), *S. discoideus* eggs have a high level of viability (Aeschlimann 1975; Frampton 1984). This is consistent with the low egg mortality observed in other *Sitona* spp. (e.g. Danthanarayana 1969, Melamed-Madjar 1966).

Implications with respect to penetration of insecticides

Exposure of the embryo or pharate larva may be effected through two mechanisms: active invasion of toxicant into the egg, or passive, where the larvae encounters the toxicant during eclosion. Active movement of insecticide involves penetration of the chorion and vitelline membrane to reach the developing embryo. Having penetrated the chorion, ovicidal activity is influenced by the presence of sufficient quantity of toxicant to elicit the desired inhibition or disruption of the organelles (Brooks 1976; Winteringham 1969). There is also the requirement for a suitable site for the toxicant to act.

Given the paucity of information about coleopteran eggs with respect to structure and function it is difficult to draw definite conclusions about which route insecticides may take into the egg. The penetration may take place through either the aeropyles or solid chorion. The aeropyles of *S. discoideus* although not extensive could provide a direct pathway for movement of toxicant. For example, the micropyles of *R. prolixus* are the main route for ovicide penetration (Beament 1948). Movement across the solid chorion has been reported in *Pieris brassicae* (L.) (Beament and Lal 1957; Salkeld and Potter 1953), and given the thinness of the chorion adjacent to the cavities in the *S. discoideus* chorion, is a possible pathway for insecticide penetration.

Although not examined in this study, it is known that the composition of the layers surrounding the developing insect embryo change both in number, thickness and permeability (Beament 1949; Beament and Lal 1957). These changes in membrane permeability have in turn been correlated with differences observed in the sensitivity to ovicides during embryonic development (Beament 1949; Beament and Lal 1957; Salkeld and Potter 1953).

POTENTIAL INSECTICIDES FOR THE CONTROL OF *SITONA DISCOIDEUS* EGGS

In the Potter tower experiments described in this study, the ovicidal activity of a number of insecticides on *S. discoideus* was examined (Tables 2 - 6.1). Although the study was somewhat preliminary, it gave a general indication of which insecticides may be effective as ovicides. The laboratory-based ovicidal activity exhibited by all insecticides was expected. Of the ovicides tested, chlorpyrifos and fenitrothion were the most active, followed in descending order by diflubenzuron, deltamethrin and γ-HCH (lindane).

A characteristic effect of these insecticides was that the majority of tested eggs examined continued to develop until the pharate larval stage. This result is consistent with other findings inasmuch that in the early stages of development, inhibition of cholinesterase is not linked to any vital neurophysiological process (Mehrotra 1960; Zschintzsch et al. 1965). Of the neurotoxin insecticides, the organophosphates have been the most researched. From these studies it is known that cholinesterase is the main site of inhibition (e.g. Mehrotra and Smallman 1957; Smith and Wagenknecht 1956), although inhibition of egg lipase has also been implicated as a basis for ovicidal activity (Krysan and Guss 1971). For this reason, the embryo reaches a pharate larval stage before the neurotoxic effects take place. Other mechanisms may be involved, based on the observation that embryos under stress often reach maturity but die before eclosion (Smith and Salkeld 1966). The hypothesis is that muscular activity preceding hatching places additional stress upon an impaired organism causing an unidentified 'weakest link' to break under the load of insecticide poisoning. It has been further suggested by Smith and Salkeld (1966) that inhibition of enzymes or exhaustion of stored nutrients may be the cause of the response. In any event, the transition from an inactive developing embryo to a mobile form represents a critical stage in the growth and development of an organism.

Diflubenzuron was found to be an effective ovicide (Table 6). However, while exhibiting high activity against *S. discoideus* eggs, the age of eggs at the time of treatment was important. Toxicity was greatest in eggs 1 day old as opposed to eggs 3-5 days old. Eggs treated with diflubenzuron became mottled and wrinkled, and the cuticle of larvae was soft and pliable. This effect was also observed by Grosscurt (1978). Diflubenzuron acts by interfering with cuticle formation in the embryo resulting in an amorphous cuticular region instead of the normal lamellate cuticle deposition patterns. The decline in ovicidal activity with increasing age appears to be

another characteristic of diflubenzuron, and has been reported for the eggs of *Spodoptera littoralis* (El-Guindy et al. 1983) and other insect species (Grosscurt 1978).

Similarly, deltamethrin exhibited considerable ovicidal control of *S. discoideus* (Table 4). Deltamethrin belongs to a class of pyrethroid insecticides known to have broad spectrum activity against egg, larva and adult stages of other coleopteran pests (Elliot et al. 1978). Larvae that emerged from eggs sprayed with deltamethrin showed a unique modification in eclosion behaviour, where after piercing the chorion they would reverse their orientation and 'back out' of the egg. This response may be due to a repellent effect induced by many pyrethroids (Ruscoe 1977). The observation that eggs 5-9 days old exhibited higher mortality as opposed to eggs 1-2 days old may possibly be attributed to the more advanced state of development of the nervous system and the presence of a site of action.

Table 15 presents the values of vapour pressure and water solubility for the six insecticides tested. A comparison of vapour pressure and water solubilities for the insecticides tested, show that the most active ovicides (chlorpyrifos and fenitrothion), possessed high values for both properties. The low solubility and vapour pressure for diflubenzuron belies its high ovicidal activity against *S. discoideus* eggs and may lie with the fact that diflubenzuron was applied as a wettable powder, whereas the remaining insecticides tested were emulsifiable concentrates. Of the other insecticides tested, γ-HCH, an insecticide with relatively high vapour pressure (Table 15), exhibited poor ovicidal activity (Table 5). This was evidenced by the observation that many larvae emerged, but died after contacting residues on the filter paper. Those eggs that did not eclose contained moribund mature larvae. However γ-HCH has a low oil/water partition coefficient (Worthing and Walker 1983), a characteristic that may contribute to poor penetration across the waxy layer covering the chorion and into the chorion itself.

Insecticide penetration into the insect egg is a function of solvent type, insecticide polarity and chorion composition, as well as the concentration of insecticide at the surface of the egg (Hartley and Graham-Bryce 1980; Welling and Patterson 1985). As the chorion of insect eggs is largely proteinaceous (Hinton 1981, Margaritis 1985), penetration would depend on the lipophilic and hydrophilic nature of the ovicide (Beament 1948; Bracha and O'Brien 1966). Beament (1948) has described the various layers that make up the chorion of *Rhodnius prolixus*. Under a layer of lipid material there is a multilayered structure made up proteins or lipoproteins which do not allow large molecules to pass through. However, this structure is transversed by a large numbers of

micropyles and pseudomicropyles which have a comparatively lipophilic surface. As a result lipophilic compounds are able to penetrate the chorion via the aeropyle and/or micropyle in particular much more quickly than hydrophilic compounds (Beament 1948). Penetration into insect cuticle has been shown to be fastest for insecticides having the highest water solubility (Chio 1976, cited in Welling and Patterson 1985). Similarly when insect eggs were exposed to the vapour of different insecticides with roughly comparable oil/water partition coefficients, uptake was proportional to the logarithm of the vapour pressure (Bracha and O'Brien 1966; Zschintzsh et al. 1965), although for insecticides having very low vapour pressures a linear relationship was found between penetration and the partition coefficient of the insecticide (Bracha and O'Brien 1966).

Table 15: Vapour pressure and water solubility at 20°C (unless stated otherwise) for the insecticides tested against *S. discoideus* eggs. (adapted from Worthing and Walker 1983).

Insecticide	Vapour pressure	Solubility (water)
chlorpyrifos	2.5 Mpa at 25°C	2 mg/l
deltamethrin	2.0 μPa at 25°C	< 2 μg/l
diflubenzuron	< 13μPa	0.1 mg/l
fenitrothion	18 mpa	30 mg/l[1]
γ-HCH	5.6 Mpa	7 mg/l
fenvalerate	37 μPa at 25°C	< 1mg/l

1 - value obtained from Hartley, D., Kidd, H. 1983.

The effect of chilling on ovicidal activity of chlorpyrifos and fenitrothion

The results of the experiment where eggs were first treated under the Potter tower and then chilled for 30 days showed that chilling altered the LC_{50} of both insecticides when compared to eggs similarly treated but allowed to develop uninterrupted at 15°C.

Fenitrothion (Table 8) was more active than chlorpyrifos (Table 7) when comparing the two under both normal and chilled development. When eggs were allowed to develop without interruption fenitrothion exhibited greater ovicidal activity than chlorpyrifos. This relationship was maintained when development was suspended by chilling, but raised the LC_{50} of fenitrothion 8.8 times and chlorpyrifos 1.5 times. Despite this, ovicidal activity remained high for both chemicals.

An explanation of the shift in LC_{50} for both chemicals can be attributed to the temporal relationship between insecticide application and the morphological and neurophysiological development of the insect's nervous system. With a threshold of 8°C required for egg development (Kwong Sue 1978), the 30 days storage at 6°C induced no embryonic differentiation. The order of appearance of the materials necessary for nerve conduction is choline acetylase, cholinesterase (CHE) and acetylcholine (ACH) (Mehrotra 1960). CHE activity has been reported to appear at the midpoint of embryonic development and increases progressively until hatching occurs (Lord and Potter 1951; Salkeld and Potter 1953; Smith and Wagenknecht 1956), while the synthesis of ACH occurs near the end of embryonic development (Mehrotra 1960). It is possible therefore that the active ingredient of the ovicide may have penetrated the egg shell, while the CHE target site was not yet functional. Over this period both organophosphates would degrade so that by the time the ACH - CHE system became functional, the active residue would have broken down to non-active forms.

However it is suggested that the magnitude of the shift in LC_{50} recorded for both insecticides after chilling would be greater under field conditions, as the artificial conditions of the laboratory prolonged the efficacy of both insecticides. An indication that abiotic factors attenuated ovicidal activity had been demonstrated in the laboratory. Eggs placed on a soil base and then directly sprayed with either chlorpyrifos or fenitrothion recorded a drop in ovicidal activity of 17% and 3% respectively for the two insecticides, compared to eggs incubated on the double layer of filter paper (McNeill unpublished data). Further evidence that field conditions markedly reduce ovicidal activity was shown by Frampton (1987). Chlorpyrifos applied at 0.6 and 1.0 kg a.i./ha and sampled 3 days after field application produced mortalities that were comparable to the control population. Only bromophos at 1.5 kg/ha exhibited significant ovicidal activity (Frampton 1987),

The majority of *S. discoideus* eggs are found on the top 1 cm of soil (Aeschlimann 1979), and amongst or beneath the litter layer surrounding the lucerne plant. Chlorpyrifos is claimed to be

effective by contact, ingestion and vapour action and may persist in soil for between 60-120 days (Worthing and Walker 1983). However, the positions of eggs may mean that interception by plant material and adsorption and absorption onto organic matter would provide barriers to the active ingredient actually reaching the egg, and therefore render an ovicide ineffective.
Bromophos possesses a water solubility and vapour pressure (40 mg/l and 1.3 x 10^{-4} at 20^0C respectively) (Hartley and Graham-Bryce 1983), compared to that of chlorpyrifos (Table 15). A high vapour pressure and water solubility have been shown to be important for effective ovicidal activity (e.g. Bracha and O'Brien 1966; Chio 1976), and the field results of Frampton (1987) would confirm this. It would also indicate that fenitrothion, which has a comparable high water solubility and vapour pressure to that of bromophos, and was highly active as an ovicide in the laboratory, has the potential to be equally effective in the field.

Because of the shift in emphasis from insecticides as ovicides to insecticide effects on the parasitoid, many areas for further experimentation on ovicides was discontinued. The effect of formulation on ovicidal activity would be useful to understanding the mode of insecticide penetration. Wettable powders (WP) and emulsifiable concentrates (EC) have distinctive particle sizes and percentage active ingredients that may effect ovicidal activity. Other areas of investigation include refinement of the method for dosing eggs, duration of insecticide exposure in relation to ovicidal activity and the accurate determination of an LC_{50} and slope for each insecticide.

TOXICITY OF CHLORPYRIFOS AND FENITROTHION TO *SITONA DISCOIDEUS* AND *MICROCTONUS AETHIOPOIDES*

In the laboratory, both chlorpyrifos and fenitrothion proved highly active against both adult *S. discoideus* and *M. aethiopoides* adults, topical application producing LD_{50}'s of less than 6.00 ug/g (Tables 9 and 10 respectively).

The comparable responses shown by weevils collected in October (Table 9, Figure 2), indicated that both insecticides were equally effective under laboratory conditions. This contrasted to the response by aestivating weevils where the LD_{50} for fenitrothion was significantly lower

(Table 9). Despite this, the slopes of the log dose-probit lines were parallel for the three treatments (i.e. reproductive weevils dosed with either chlorpyrifos or fenitrothion and aestivating weevils dosed with fenitrothion only), indicating that the genetic heterogeneity expressed in the population was similar.

Aestivating weevils dosed with fenitrothion, produced a Chi-square (χ^2) of 19.00 compared to 0.85 for reproductive weevils (Table 15). This represented a 8.4 fold increase in heterogeneity between reproductive and aestivating weevils in the response to fenitrothion.

A significantly large χ^2 can be attributed to two causes: individual subjects in a batch receiving a particular dose may not react wholly independently of one another (i.e. members of a batch of insects may be genetically related to a closer extent than insects in a different batch and their responses may be correlated) or heterogeneity may arise from the subjects themselves (i.e. from uncontrolled factors such as saturation deficit which are constant for all subjects at a dose but may vary from dose to dose). Examination of the log dose-probit line for aestivating

Table 16: Chi-square values for both *S. discoideus* and *M. aethiopoides* adults in response to topical dosing with chlorpyrifos and fenitrothion.

Insect	Insecticide	Chi- square
S. discoideus	chlorpyrifos	3.65
	fenitrothion (reproductive)	0.85
	fenitrothion (aestivating)	19.00
M. aethiopoides	chlorpyrifos	9.51
	fenitrothion	1.63

weevils (Figure 2) showed that the dispersion of points about the line did not show any indication of a systematic curvilinearity. However, the comparatively large χ^2 expressed by aestivating weevils may be attributed to the fact that weevils were dosed in three batches over a two week period, and despite standardised conditions, variation may have occurred due to procedure and other uncontrolled factors. The effect of the three batches on the resulting heterogeneity was

determined using GENSTAT. Although significant at the 95% level, the batch effect only accounted for a small proportion of the total variation.

Finney (1971), emphasised that rarely can one distinguish clearly which form of heterogeneity, random or systematic, is causing a large χ^2. Even if the evidence points to a systematic trend, little can be done except suggest a larger experimental programme.

The greater susceptibility expressed in aestivating weevils to fenitrothion was unexpected, given that weevils in diapause are generally been reported to be more resistant to insecticides (Sun 1960). The characteristics that determine the summer resting state would presuppose that insecticide tolerance would be elevated in these weevils. Aestivation involves both behavioral and physiological changes (Masaki 1980) including suspension of gonad maturation, reduced metabolism and a sharp decline in feeding activity (Masaki 1980). Aestivating *H. postica* show reduction in the total gut dimensions, epithelial cytoplasm and musculature (Tombes and Marganian 1967), although feeding does not completely cease during aestivatory diapause (Bland 1971; Pienkowski 1976).

In teneral *S. discoideus* there are known to be relatively high lipid levels which increase further during the pre-aestivatory feeding phase (Frampton 1987), and decline gradually over the aestivation period (Frampton 1987). As summarised by Winteringham (1969), inert storage by indifferent tissue such as lipids is a means by which the toxicity of an externally applied insecticide may be attenuated; the relatively high accumulation of lipids in *S. discoideus* would act as a buffer against any toxicant that entered the haemolymph. Consistent with this mechanism, Bennett and Thomas (1963) established a negative correlation between lipid content and percent mortality of *H. postica* to malathion and heptachlor.

However, there is evidence that newly emerged weevils are more sensitive to insecticides. Discrepancies in the response of parasitised and non-parasitised weevils to insecticides reported by Dumbre and Hower (1976a) and Abu and Ellis (1977) were attributed in part to the use by Dumbre and Hower of newly emerged adults for toxicological studies (Abu and Ellis 1977). It is possible that biochemical differences between the two age groups may be related to the greater sensitivity to cholinesterase to inhibition in aestivating weevils. Such a mechanism has been reported between susceptible and resistant strains of *Tetranychus urticae* (Smissaert 1964; Voss and Matsumura 1965) and it may be that this differential sensitivity occurs between recently

emerged and reproductive 'older' *S. discoideus*. However there may be a more elementary explanation, relating simply to the presence of barriers to insecticide toxicity. *S. discoideus* do not feed during the eight week aestivatory period (Frampton 1987). As the amount of food in the gut is known to influence the toxicity of insecticides (Busvine 1971), the differential toxicity of fenitrothion between non-feeding aestivating and feeding reproductive weevils may be related to absorption of insecticide by food in the gut of the latter.

The potential exploitation of this susceptibility is precluded by a number of practical considerations based on the behaviour of the weevil. The first is that the majority of weevils disperse from the field to aestivation sites beyond the lucerne stand. These sites are often located under hedgerows and or along fence lines (Goldson et al. 1984). There is therefore the physical difficulty of locating and treating a widely dispersed and somewhat inaccessible target. The second factor, is the fact that post-aestivatory flights are widely dispersive (Allen 1971; Goldson et al. 1984) and weevils can fly several kilometres to reinfest the field. Insecticide treatment is unlikely therefore to preclude reinvasion from untreated areas.

Toxicity of chlorpyrifos and fenitrothion to *M. aethiopoides* adults

The results obtained in this study are consistent with research by Abu and Ellis (1977) and Dumbre and Hower (1976a,b) inasmuch as the parasitoid adult was extremely susceptible to insecticides. There was a marked difference in heterogeneity between to the two insecticide treatments applied to *M. aethiopoides* adults (Table 15). However, the large χ^2 resulting from the topical dosing with chlorpyrifos is explained by the large deviation between the observed and expected numbers responding to the second concentration tested (Figure 3). If this probit is ignored there is a close fit of the remaining probits about the line.

The log dose-probit line for *M. aethiopoides* was relatively horizontal (Figure 3) as evidenced by LD slopes of 2.55 and 2.86 (units of probit mortality per 10 units of log concentration) for chlorpyrifos and fenitrothion respectively. This is compared to slopes of 6.34 and 6.64 obtained for reproductive weevils (Figure 2). A high degree of care was needed to place a dose on the thorax of the minute parasitoid. This, combined with inherent variation in the volume produced by the Arnold applicator (Finney 1971) may have contributed to the different batches showing the wide response range. However, as care was taken to zero the applicator after each concentration

had been applied, and handling variations can be considered to be constant throughout the duration of a run, it is probable that the flatter slope truly indicates that there was a high degree of genetic heterogeneity within the population tested. Alternatively, the flatter slope may be attributed to the use of both sexes for topical dosing, as there can be considerable differences between sexes in their response to insecticides (Hoskins and Craig 1962; Sun 1960). Such findings are consistent with other dosing studies involving *M. aethiopoides*, where the use of both sexes produced a slope for methyl parathion of 1.66 $\pm$ 0.24 and an average slope of 1.70 $\pm$ 0.26 for four selected insecticides (Dumbre and Hower 1976a). When topical dosing has been restricted to females, the resulting slopes were much steeper with a slope of 3.80 $\pm$ 0.38 produced by malathion and an average slope of 4.30 $\pm$ 0.39 for five selected insecticides (Abu and Ellis 1977). Similarly, differential toxicity has been observed in *B. mellitor*, with azinphosmethyl and chlordimeform being more toxic to males than females (O'Brien et al. 1985).

The selectivity ratio (SR) is the ratio of toxicity of a compound to a natural enemy and the target pest species. Ratios $<$ 1 represents insecticides more toxic to the parasitoid than to the pest. Ratios $>$ 1 represent insecticides more toxic to the pest than to the parasitoid (Metcalf 1972). The SR's of 0.81 and 0.74 for chlorpyrifos and fenitrothion respectively were comparable, although fenitrothion was slightly more active against the parasitoid adult. Only when aestivating weevils were used to calculate the ratio did the SR tend to favour the parasitoid adult. Based on their SR's both chlorpyrifos and fenitrothion exhibited moderate selectivity towards *M. aethiopoides*, comparable studies elsewhere have shown that some widely used insecticides are even more active against the parasitoid. Topical dosing studies on *H. postica* and *M. aethiopoides* produced SR's of 0.19 and 0.35 for methyl parathion and carbofuran respectively (Dumbre and Hower 1976a) and an SR of 0.63 for malathion (Abu and Ellis 1977).

Organophosphate insecticides are known to be highly toxic to beneficial insects (e.g. Bartlett 1966; Croft and Brown 1975; Powell et al. 1986). Chlorpyrifos has a broad range of activity and is effective by contact, ingestion and vapour action, but is not systemic (Worthing and Walker 1983). Fenitrothion is also a potent insecticide with marked contact activity (Worthing and Walker 1983), registered for control of a range of pasture pests (O'Connor 1990). To this effect, chlorpyrifos has proven to be highly toxic against parasitoid adults (e.g. Morse and Bellows 1986; Plapp and Vinson 1977; Powell et al. 1986) and other natural enemies (e.g. Syrett and Penman 1980). This high susceptibility of adult parasitoids has been attributed to the mode of insecticide detoxification (Croft and Morse 1979; Plapp and Vinson 1977). Phosphorothionates (P=S) are

metabolised primarily through microsomal oxidative routes, whereas phosphate (P=O) compounds are degraded by hydrolytic processes (Plapp and
metabolise insecticides that rely on oxidative mechanisms for detoxification (Plapp and Bull 1988), consequently the P=S insecticides which include chlorpyrifos and fenitrothion exhibit high toxicity to parasitoid adults.

TOXICITY OF CHLORPYRIFOS AND FENITROTHION TO *MICROCTONUS AETHIOPOIDES* PUPAE

The cocoon stage of natural enemies has generally been shown to be tolerant in the laboratory to field insecticides (e.g. Bartlett 1966; Croft and Brown 1975). Reflecting this observation, *M. aethiopoides* pupae were found to exhibit strong tolerance to chlorpyrifos and fenitrothion both under the Potter tower and topical dosing.

The ability to survive direct application of insecticide was most obvious under the Potter tower treatment. Application of both chlorpyrifos and fenitrothion at concentrations above field rates did not affect pupal survival (Table 11). Sublethal effects on emerging adults were only evident at high insecticide concentrations. Effects on longevity were apparent when cocoons were treated with 1.2 and 2.4 g/l of chlorpyrifos (Figure 4). Male longevity declined significantly at 1.2 g/l, with both sexes showing similar responses to the 2.4 g/l application (Figure 4). At 2.4 g/l of chlorpyrifos several parasitoid adults were found dead near the cocoon or died soon after emergence. This result was attributed to the emerging adult receiving a toxic dose of insecticide, either when chewing through the pupal case or following contact with residues on the outer silk layer of the cocoon once it emerged (Dumbre and Hower 1976a,b). Because *M. aethiopoides* adults have been shown to be extremely susceptible to insecticides both in this study and elsewhere (Abu and Ellis 1977; Dumbre and Hower 1976a), only minimal doses are needed to kill adults in the process of emerging from their cocoons.

Although not presented in the text, topical dosing tests against the cocoons indicated that this stage of the life cycle would probably survive a field application of insecticide. Even at the highest rate of chlorpyrifos and fenitrothion tested (0.8 and 0.9 g/l respectively), adult parasitoids

successfully eclosed. Although numbers of test subjects at these rates were low (7 and 9), it provided an indication of likely response. Such pupal tolerance is attributable to the structure of the cocoon. The cocoon of *M. aethiopoides* has been described by Loan and Holdaway (1961b) as being composed of an exterior layer of silk and three inner layers of transparent, straw coloured material laid down by the larva. These multiple layers of the cocoon have been shown elsewhere (Dumbre and Hower 1976a) to confer a high level of tolerance to insecticides to the pupae. Dumbre and Hower (1976a) reported that topically dosed pupae were 18, 27 and 32 times more resistant than adult *H. postica* to methyl parathion, methidathion and carbofuran respectively. Results from the experiments described here would tend to indicate that *M. aethiopoides* pupae exhibited even greater tolerance to chlorpyrifos and fenitrothion. The topical dosing studies indicated that pupae displayed >80 times greater tolerance to both chemicals compared to *S. discoideus*: the results from the Potter tower study would seem to confirm this.

From experiments to test the ability of females successfully emerging from treated cocoons to parasitise weevils it appeared that those surviving high rates of fenitrothion (2.0 g/l) and chlorpyrifos (2.4 g/l) were still able to parasitise *S. discoideus*.

In the field, *M. aethiopoides* pre-pupae emerge from weevils and drop into the litter layer to pupate. If spun on soil, particles of soil and surface debris adhere to the cocoon making them difficult to locate (Loan and Holdaway 1961b). In the laboratory, pupating larvae make extensive use of lucerne litter and organic matter as attachment points on which to construct the cocoon. This behaviour may have adaptive value, in that the microenvironment created around the cocoon further protects the developing adult from extremes of temperature and saturation deficit. Using organic matter in the construction of the cocoon would also be important as camouflage. By coincidence, in an agricultural cropping system, this behaviour would provide additional protection from direct exposure to insecticide. These protected pupae could then form a nucleus of adults emerging in spring to attack surviving weevils.

THE EFFECT OF CHLORPYRIFOS ON *MICROCTONUS AETHIOPOIDES* AND *SITONA DISCOIDEUS* IN THE FIELD

Application of chlorpyrifos at 0.3 kg/ha caused a 96.2% reduction of *S. discoideus* populations, leaving a residual weevil density of 0.8 weevil m^{-2} (Table 13). The actual number of weevils that would survive through to spring would be even lower, as subsequent laboratory dissection showed that the majority were parasitised (Table 14). These adults would therefore carry the developing larva through to late winter-early spring (Goldson et al. 1990). Although it is possible that trivial movement of some parasitised weevils from the buffers into the chlorpyrifos-treated plots were included in the post-spray sample taken 1 week later, similar population decreases resulting from the application of chlorpyrifos have been reported by Frampton (1987).

The fact that a residual population of parasitised weevils did survive is a positive sign that a small parasitoid population would remain in the field. Of the surviving weevils, 87.6% were parasitised and assuming full parasitoid survival a population of 0.7 parasitoids m^{-2} would result. Given that just over half of the progeny would be female (Fusco and Hower 1974; Loan and Holdaway 1961b), a resulting adult female density of 0.40 m^{-2} could be expected. This low female density, coupled with the fact that weevil density was equally low, would suggest that the ability to locate and parasitise weevils widely dispersed within the lucerne crop was likely to be poor. Nevertheless, Cullen and Hopkins (1982) and Goldson et al. (1990) have reported that *M. aethiopoides* was able to survive at low host densities.

Residual toxicity of chlorpyrifos

The persistence of insecticides is an important factor in a parasitoid's ability to successfully re-establish in the field. Accordingly, the residue experiment was primarily designed to provide an indication of the likely interval before parasitoid adults could safely reoccupy the lucerne. The activity of chlorpyrifos against the weevil and the fate of immature parasitoids within weevils were also investigated.

Fifteen days after application, field-weathered chlorpyrifos residues (0.3 kg/ha) caused between 20-60 % mortality of *S. discoideus*, but by day 22 this insecticide activity had totally disappeared and caused no mortality (Figure 8). One day old residues (0.3 kg/ha) caused 79% mortality of parasitoid adults, although activity declined rapidly thereafter (Figure 9). There was a fall in activity observed on day 6, although this was not significantly different from parasitoid mortality recorded on day 10 (Figure 9). Although not statistically significant, chlorpyrifos at 0.3 kg/ha exhibited greater sustained activity against *S. discoideus* than against *M. aethiopoides*. This may be because unlike the parasitoid, the weevil ingested contaminated plant material. At 1.0 kg/ha, chlorpyrifos residues exhibited sustained activity against both weevil and parasitoid, with over 90% mortality recorded fifteen days after application against the latter species (Figure 9). The effect of insecticide concentration is to alter the foliar residue decay curve, with increased concentration reducing the rate of decay of foliar residues (Veierov et al. 1988). In relation to residue activity, this is expressed by a sustained and high level of mortality. Consequently, the mortality curve for both weevil and parasitoid at 1.0 kg/ha (Figures 8 and 9) exhibited a much more gradual decline over the experimental period.

Loss of active ingredient over time occurs through several mechanisms (Willis and McDowell 1987) and is expressed by a rapid decline in surface residues, followed by a slower asymptotic decrease (Veierov et al. 1988). An important feature of residue activity is that when the biological activity of insecticide residues is assessed over time, the percentage mortality of natural enemies often yield a decay curve which resembles closely that of the chemical deposit itself (Croft and Brown 1975). Persistence and activity can vary markedly between insecticides. For example methyl parathion while causing 100% mortality of *M. aethiopoides* adults immediately post-spray, rapidly degraded so that one day later only 32% mortality occurred (Dumbre and Hower 1977). While methoxychlor was the least toxic insecticide, it was the most persistent and was still active two weeks after application (Dumbre and Hower 1977). Persistence of residues in a crop is very much a function of several factors (Willis and McDowell 1987). While losses from foliage by volatilisation immediately after application can be both rapid and substantial (Willis et al. 1985), rain has the most dramatic effect on residues (Willis and McDowell 1987).

Although chlorpyrifos has shown high activity against parasitoids in the laboratory, Rosenheim and Hoy (1988) citing Luck et al. (1986) reported that chlorpyrifos had a relatively low degree of disruption in the field. This difference between laboratory and field results may be due to the relatively short field persistence of chlorpyrifos. Buck et al. (1980) and Leuck et al. (1968) found

that the half-life for chlorpyrifos varied from < 1 day on cotton to 2.9 days for bermuda grass. In this field experiment a comparable rapid decay in activity for chlorpyrifos residues was observed with a significant drop in activity occurring between days three and six.

Using the criteria developed by Hassan and Oomen (1985) and Hassan (1990), the effects of chlorpyrifos at field rates was shown in this experiment to be moderately persistent (16-30 days) to *S. discoideus*, but only slightly persistent (5-15 days) against *M. aethiopoides*. This is despite chlorpyrifos residues exhibiting greater activity against the parasitoid for the first 3 days after application. This rapid decay in activity against the parasitoid has implications for the successful reestablishment of the parasitoid. Because the period of residue activity is reduced, the interval before parasitoid adults can safely return to the crop is similarly reduced, with parasitoids able to move from non-sprayed reservoirs or from the protected cocoon stages within the crop. Consequently, the biological control component of IPM is maintained in the field.

Residues and the effect on the internal parasitoid

Theoretically, parasitised weevils would be more susceptible than non-parasitised, as physiological and biochemical changes (Vinson and Iwantsch 1980) and stress from rapid parasitoid development have been shown to cause increased susceptibility to insecticides (Dumbre and Hower 1976a). Dumbre and Hower (1976a), observed a correlation between parasitoid larval development and host susceptibility. Not only was the *H. postica* host more susceptible when parasitised but susceptibility increased between early and late instars of the parasitoid *M. aethiopoides*.

While no clear trend could be determined in this study between parasitism, development stage and susceptibility of weevils, a number of deductions can be made from the results. Where parasitoid development had reached late-4th instar, the prepupa (5th instar) was able to emerge from dead and moribund weevils. On the other hand, internal parasitoids in early stages of development died after their host was killed, due to a lack of requisites necessary to complete the parasitoid life cycle.

The lack of expected correlation between weevil mortality and parasitism in this study may be attributable to the behaviour of the weevil itself. Little is known about the physiological demands placed on the weevil or the requirements that the internal parasitoid makes on its host, particularly

near pre-pupal eclosion. It is hypothesised that physiological features such as feeding behaviour may change in a weevil supporting a parasitoid close to emergence. Another possible reason for the failure to correlate weevil mortality to parasitism could relate to the method used to expose the weevils to the residues. Because there was free choice for the weevil to either consume foliage and/or settle in the foliage, the dose received by an individual weevil could not be precisely controlled and thus intoxication was variable. Furthermore, the site of insecticide contact is important. Penetration along the dorsal surface is limited, as opposed to the abdomen or thorax where the interstitial points between tergites, joints and spiracles provide a much greater opportunity for insecticide ingress.

FIELD APPLICATION OF INTEGRATED PEST MANAGEMENT

From a practical viewpoint, timing of application is often the most effective and economical way of achieving differential insecticide selectivity (Newson et al. 1976). When applied at a stage where parasitoid activity and efficiency is at a minimum (Bartlett 1964) insecticide will cause the least disruption (Croft and Brown 1975).

Goldson (1984) recommended interim insecticidal control of *S. discoideus* at the end of the post-aestivatory flights in May - June which, based on the seasonal biology of the weevil (Goldson et al. 1984) was the best time to implement control. This timing coincided with the advent of winter and a consequent slowing down of insect development. Degree day accumulations over the critical period in autumn 1988 have indicated that spraying at this time occurs at a stage where *M. aethiopoides* had only reached the egg-larval stages (Goldson et al. 1990). This was confirmed by dissection (Table 14). Insecticide treatment at this time may therefore be seen as having the greatest detrimental effect on parasitoid survival.

Practical considerations or grower perceptions are often a problem to the successful implementation of IPM. *Microctonus colesi* (Drea) parasitises both larvae and adults of *H. postica*. The spray recommendation against *H. postica* was found to coincide with the occurrence of *M. colesi* as larvae in the adult weevils and resulted in a 98% reduction in weevil populations, with a consequent decline in adult parasitoid densities (Hower and Luke 1975). O'Brien et al.

(1985), commented on the fact that while chlordimeform had a less severe effect on the parasitoid *B. mellitor*, practical requirements meant that farmers used azinphosmethyl to control *A. grandis*, an insecticide to which the parasitoid was extremely susceptible. Similarly, Hower and Davis (1984) found that methoxychlor used to control the leaf hopper, *Empoasca fabae* (Harris), in lucerne, while highly selective, was applied by growers at a rate that was detrimental to the survival of *H. postica* and consequently the *M. aethiopoides* larvae within the weevil.

Conversely, IPM has been more successful when there are overlapping parasitoid generations present in the field, as the recovery of parasitoid numbers following insecticide application is much more rapid. Surgeoner and Ellis (1976) and Wilson and Armburst (1970) found that enhanced parasitism of *H. postica* by *B. curculionis* was due to adults emerging from protected pupae and reinvasion of parasitoids from surrounding areas. Similarly, Aeschlimann (1983a) reported that when all life stages of *M. aethiopoides* were present in the field, insecticides had minimal impact on the field population of the parasitoid.

Practical recommendations for preservation of parasitoids in the field

Insecticide application in May would effectively eliminate both adult *S. discoideus* and its parasitoid. Delaying insecticide spraying till late June would appear to be more favourable to parasitoid survival. According to day-degree accumulation (Goldson et al. 1990), a portion of the parasitoid population would have moved from the vulnerable larval stage into the highly tolerant pupal stage. Frampton (1984) concluded that an insecticide application could be delayed until mid-August without any reduction in the effectiveness against *S. discoideus*. This recommendation would suppress the damage potential of *S. discoideus* while preserving *M. aethiopoides*.

An important factor aiding the continued effectiveness of the parasitoid against *S. discoideus* in insecticide treated areas relates to the scale of control measures, an aspect that has been shown to have important long term side-effects on beneficial insects (Jepson 1988, 1990). Generally, insecticidal control of *S. discoideus* has been on a farm scale with treatment being restricted to 1-4 paddocks within a farm rather than regional (i.e., blanket insecticide application to several farms in a county). This limited spraying allows populations of *M. aethiopoides* to survive outside the treated paddocks and it is these sites which provide a source of parasitoids for recolonisation in the following season.

Current use of insecticides for the control of *S. discoideus*

Currently the three chemicals are registered for use against *S. discoideus* are chlorpyrifos, fenitrothion and deltamethrin at 0.3-0.4 kg a.i./ha, 0.6 kg a.i./ha and 6.25 g a.i./ha respectively (O'Connor 1990). While these are registered, the status of *S. discoideus* as a major pest of lucerne has changed markedly since 1982 when yield losses on dryland lucerne due to larval feeding and adult activity were first quantified (Goldson et al. 1985). This is principally the result of the effectiveness of *M. aethiopoides* in suppressing weevil populations to levels that are no longer economically damaging.

M. aethiopoides has had a substantial impact for several reasons. The parasitoid attacks adult weevils, with parasitism of females resulting in rapid sterilisation (Loan and Holdaway 1961b). This removes the female weevil at its highest reproductive value in terms of egg-laying potential and eliminates pest cohorts at the pre-egg stage, instead of the potentially damaging feeding stages (van Driesche and Gyrisco 1979). A further contribution to the success of the parasitoid can be attributed to the apparent importance that spring laid weevil eggs have on subsequent larval recruitment (Frampton 1984). A long time frame in which the parasitoid could act on the adult weevil stage is naturally provided. This is in sharp contrast to the situation in South Australia where, despite levels of parasitism reaching up to 90% by late July (Hopkins pers. comm.), substantial weevil oviposition by late May and June, did not allow enough time for useful suppression to occur (Hopkins 1988).

Perhaps the most significant factor that has enhanced the effectiveness of *M. aethiopoides* as a biological control agent has been the atypical behaviour observed in Canterbury populations of *M. aethiopoides* (Goldson et al. 1990). A breakdown in the obligatory aestivatory behaviour of *M. aethiopoides* has allowed increased levels of parasitoid activity over the summer period. Elsewhere the parasitoid undergoes a sympathetic obligatory diapause as first instar larvae inside the weevil host (Aeschlimann 1978; Hopkins 1981).

The impact of the parasitoid on the weevil can be gauged by comparisons of weevil density over time. Earlier work using low persistence insecticides (Goldson 1983; Goldson et al. 1985) had established that a weevil density of less than 20-25 m^{-2} resulted in larval densities below the damage threshold (e.g. 1500 larvae m^{-2}) (Goldson et al. 1985; Goldson and Proffitt 1985). In late May of 1989 initial ground densities at Darfield were reported at between 10-25 weevils m^{-2}, with

at least 50% parasitised, thus resulting in a reproductive population of only 5-12 weevils m^{-2}. (Goldson et al. 1990). This is in marked contrast to earlier work at the same site where densities were frequently of the order of 70 weevils m^{-2} (Goldson et al. 1985; Goldson et al. 1988).

CHAPTER 6

CONCLUSIONS

1. Several insecticides were shown to exhibit ovicidal activity against *S. discoideus*. Diflubenzuron, deltamethrin, chlorpyrifos and fenitrothion exhibited the most activity against eggs. γ-HCH, however, exhibited poor ovicidal activity. Diflubenzuron was most active against younger eggs; consistent with findings elsewhere. Chlorpyrifos and fenitrothion were highly effective ovicides against *S. discoideus*.

2. Post-treatment chilling of treated eggs affected the ovicidal activity of both chlorpyrifos and fenitrothion. Chilling increased the LC_{50} of fenitrothion, while for chlorpyrifos both the LC_{50} value and slope where increased. However, even after chilling, ovicidal activity remained high for both insecticides. While exhibiting ovicidal activity in the laboratory, previous research had shown that chlorpyrifos exhibited limited ovicidal activity in the field.

3. Topical dosing of *S. discoideus* and *M. aethiopoides* adults showed that chlorpyrifos and fenitrothion were highly toxic to both species. *M. aethiopoides* adults exhibited the greatest susceptibility to both insecticides this was reflected in the selectivity ratio of 0.8 and 0.6 for chlorpyrifos and fenitrothion respectively. Greater susceptibility of aestivating weevils is attributed to the biochemical and physiological conditions associated with aestivation.

4. In contrast to the adult stage, *M. aethiopoides* pupae exhibited strong tolerance to chlorpyrifos and fenitrothion. This tolerance was demonstrated when insecticides were applied both under the Potter tower and by topical dosing. While survival of pupae was not affected by direct dosing, longevity of surviving adults was affected in some cases. Chlorpyrifos when applied at 1.2 and 2.4 g/l under the Potter tower, significantly lowered the longevity of both male and females, with the former showing the greater reduction in longevity. Topical dosing with fenitrothion produced a significant reduction in longevity with increasing concentration. All the remaining treatments did not affect pupal survival or

longevity. Incomplete tests indicated that surviving *M. aethiopoides* females were capable of parasitising *S. discoideus*. Only adults surviving chlorpyrifos at 2.4 g/l under Potter tower application displayed any impairment of their ability to successfully parasitise hosts.

5. Field rates of chlorpyrifos applied to early regrowth lucerne in May virtually eliminated *S. discoideus* adults. Spraying was detrimental to *M. aethiopoides*, as at this stage of their life cycle the population had only reached the egg to late 4th instar stage. A residual population of parasitised weevils did survive, and it was considered that these weevils would act as a source for the parasitoid population to attack surviving weevils in spring.

6. Chlorpyrifos-treated lucerne residues exhibited activity over a 22 day period to both *S. discoideus* and *M. aethiopoides* adults. Activity against the weevil persisted for 15-20 days. After producing high mortality of parasitoids in the first 3 days, activity decreased rapidly thereafter and after 10 days residues were no longer toxic to *M. aethiopoides*. Differences in the mechanism of exposure are believed to be the reason for the differential mortality responses between the weevil and host. Parasitoid males, showed higher susceptibility to chlorpyrifos residues than females, but this was not significant at field rates. At 1.0 kg/ha, chlorpyrifos residues continued to be highly active for 15 days against both weevil and parasitoid. Twenty-two days after application and coinciding with the completion of the experiment, activity was still relatively high in the 1.0 kg/ha plots.

7. Investigation of the interaction between parasitoid larval development and weevil susceptibility to residues was inconclusive. Parasitoid larvae in advanced stages of development were able to emerge and successfully pupate from dead weevils, but dissection of weevils revealed that there was often a wide variation in larval development. This could not be related to weevil mortality or survival at the completion of each 48 hour exposure period.

8. Delaying insecticide application till June to control *S. discoideus* is more compatible with the IPM approach, as a portion of the parasitoid population would

have moved through to the highly resistant pupal stage. However, while applying insecticide in late May is less desirable, it is predicted that there would be no between-season suppression of parasitoid activity, as the limited scale of insecticidal control, the respective phenologies of the parasitoid and weevil and the widely dispersive post-aestivatory flights would allow reestablishment of the parasitoid in the field in the following season.

9. Because of the effectiveness of the parasitoid in suppressing *S. discoideus* to levels that are no longer economically damaging, the recommendation to apply insecticides to Canterbury lucerne is currently not made. This success of biological control is a positive sign, compatible with the low input nature of Canterbury pastoral farming and the concepts of sustainable agriculture.

ACKNOWLEDGEMENTS

I gratefully acknowledge the supervision and encouragement provided by Mr Bruce Chapman of the Department of Entomology, Lincoln University. Dr Stephen Goldson and Mr John Proffitt provided considerable assistance and support through all stages of this thesis. I also would like to recognise the help provided by Dr Ruth Frampton (MAF Technology, Lincoln), Mr Mike Bowie (Entomology Department, Lincoln University) on aspects of PROBIT and POLO, Messrs David Baird and David Saville (MAF Technology, Lincoln), for biometric advice, Mr A.H. Mander (Christchurch College of Education), for the skilfully constructed frontispiece and Ms S. Trotter. I am indebted to the late Bill Band, for allowing me the use of a paddock on which the field experiment was conducted.

A special thanks is offered to Ms Christine Galbraith for her time spent typing a large portion of this manuscript and her cheerful demeanour, and to both Ms Sue Trotter and Ms Rebecca Hurrell for their assistance in the typing.

I am grateful to MAF Technology, Lincoln for the ready access to a computer terminal, and to the many staff who have taken an interest in my progress.

On a personal note, thanks are due to the many friends and relatives who did not let me forget that I was completing a masters, to those who offered encouragement during the more chaotic periods of this research project, and to my flatmates Gordon and Martin for their understanding.

Finally, a special thanks to Sandra, who had the foresight to travel overseas during the completion of this research project, and to my parents for all their support.

REFERENCES

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18: 265-267.

Abu, J.F., Ellis, C.R. 1976. Biology of *Microctonus aethiopoides* a parasite of the alfalfa weevil, *Hypera postica*, in Ontario. Environmental Entomology 5: 1040-1042.

Abu, J.F., Ellis, C.R. 1977. Toxicity of five insecticides to the alfalfa weevil, *Hypera postica* and its parasites, *Bathyplectes curculionis* and *Microctonus aethiopoides*. Environmental Entomology 6: 385-389.

Adams, M.E., Miller, T.A. 1979. Site of action of pyrethroids repetitive "backfiring" in flight motor units of house fly. Pesticide Biochemistry and Physiology 11: 218-231.

Adler, P.H., Kim, K.C. 1985. Morphological and morphometric analyses of European and Moroccan biotypes of *Microctonus aethiopoides* (Hymenoptera: Braconidae). Annals of the Entomological Society of America 78(3): 279-283.

Aeschlimann, J.-P. 1975. A method for the extraction of *Sitona* (Col.: Curculionidae) eggs from soil and occurrence of a mymarid (Hym.: Chalcidoidea) in the Mediterranean region. Entomophaga 22: 111-114.

Aeschlimann, J.-P. 1977. Notes on *Patasson lameerei* (Hym.: Mymaridae), an egg parasitoid of *Sitona* spp. (Col. : Curculionidae) in the Mediterranean region. Entomophaga 22: 111-114.

Aeschlimann, J.-P. 1978. Heavy infestations of *Sitona humeralis* Stephens (Col., Curc.) on lucerne in southern Morocco. Annales de Zoologie-Écologie Animale 10: 221-225.

Aeschlimann, J.-P. 1979. Sampling methods and construction of life tables for *Sitona humeralis* Stephens (Col., Curculionidae) in Mediterranean climatic areas. Journal of Applied Ecology 16: 405-415.

Aeschlimann, J.-P. 1980. The *Sitona* [Col.: Curculionidae] species occurring on *Medicago* and their natural enemies in the Mediterranean region. Entomophaga 25: 139-153.

Aeschlimann, J.-P. 1983a. Sources of importation, establishment and spread in Australia, of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae), a parasitoid of *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae). Journal of the Australian Entomological Society 22: 325-331.

Aeschlimann, J.-P. 1983b. Notes on the variability of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae; Euphorinae). Contributions of the American Entomological Institute 20: 329-335.

Aeschliman, J.-P. 1984. Distribution, host plants, and reproductive biology of the *Sitona humeralis* Stephens group of species (Coleoptera, Curculionidae). Zeitschrift für Angewandte Entomologie 98: 298-309.

Aeschlimann, J.-P. 1986. Rearing and larval development of *Sitona* spp. (Coleoptera: Curculionidae) in the root system of *Medigago* spp. plants (Leguminosae). Zeitschrift für Angewandte Entomologie 101: 461-469.

Agrell, I.P.S., Lundquist, A.M. 1973. Physiological and biochemical changes during insect development. p 159-247. In: Rockstein, M. (ed.) The physiology of insects. Volume 1, second edition. Academic Press, New York.

Allen, P.G. 1969. Sitona weevil. Journal of Agriculture. South Australia 73: 80-81.

Allen, P.G. 1971. *Sitona humeralis* Steph. (Coleoptera: Curculionidae) in South Australia. South Australia Department of Agriculture Agronomy Branch Report No 35. 14 pp.

Anderson, K.T. 1930 Der einfluss der temeratur und der luftfeuchtigkeit auf die dauer der eizeit. I.Beitrag zu einer exakten biologie des linierten graurüsslers (*Sitona lineatus*). Zeitscrift für Morphologie und Ökologie der Tiere 17: 649-676.

Anderson, D.T. 1972. The development of holometabolous insects. pp 166-242. in Counce, S.J., Waddington, C.H. (eds). Developmental systems; Insects. Vol 1. Academic Press.

Ascerno, M.E., Smilowitz, Z., Hower, Jr. A.A. 1980. Effects of the insect growth regulator Hydroprene on diapausing *Microctonus aethiopoides* a parasite of the alfalfa weevil. Environmental Entomology 9: 262-264.

Babcock, K.L., Rutschky, C. 1961. Lipids in insect eggs: a review with new evidence from the milkweed bug, *Oncopeltus fasciatus* (Hemiptera, Lygaeidae). Annals of the Entomological Society of America 54: 156-164.

Bailey, P. and Milner, R. 1985. *Sitona discoideus*; a suitable case for control with pathogens? Proceedings of the 4th Australasian Conference on Grassland Invertebrate Ecology: 210-216.

Barker, R.J., Edmunds Jr., L.N. 1958. Toxicity of DDT in oil and in acetone to adult DDT-resistant house flies. Journal of Economic Entomology 51: 914-915.

Barratt, B.I.P. 1985. Research note; an attempt to control *Sitona discoideus* larvae with systemic insecticides. Proceedings of the 38th New Zealand Weed and Pest Control conference: 25-37.

Bartlett, B.R. 1956. Natural predators: can selective insecticides help to preserve biotic control? Agricultural Chemicals 11: 42-44, 1079

Bartlett, B.R. 1964. Integration of chemical and biological control. p 489-511. In: Debach, P. (ed.) Biological control of insect pests and weeds. Chapman and Hall, London.

Beament, J.W.L. 1945. The cuticular lipids of insects. Journal of Experimental Biology 21: 115-138.

Beament, J.W.L. 1948. The penetration of insect egg shells I. Penetration of the chorion of *Rhodnius prolixus* Stål. Bulletin of Entomological Research 39: 359-383.

Beament, J.W.L. 1949. The penetration of insect egg shells II. The properties and permeability of sub-choral membranes during development of *Rhodnius prolixus* Stål. Bulletin of Entomological Research 39: 467-488.

Beament, J.W.L., Lal, R. 1957. Penetration through the egg shell of *Pieris brassicae* (L.). Bulletin of Entomological Research 48: 109-125.

Beard, R.L. 1960. Post-exposure and determination of end-point. p 19-27. In: Shepard, H.H. (ed.) Methods of testing chemicals on insects. Burges Publishing, Minneapolis.

Beckage, N.E., Riddiford, L.M. 1982. Effects of methoprene and juvenile hormone on larval ecdysis, emergence, and metamorphosis of the endoparasitic wasp, *Apantales congregatus*. Journal of Insect Physiology 28: 329-334.

Bedding, R.A., Molyneax, A.S. and Akhurst, R.J. 1983. *Heterorhabditis* spp., *Neoaplectana* spp., and *Steinernema kraussei*; interspecific and intraspecific differences in infectivity for insects. Experimental Parasitology 55: 249-257.

Bennett, S.E., Thomas, Jr. C.A. 1963. The correlation between lipid content and percent mortality of the alfalfa weevil to heptachlor and malathion. Journal of Economic Entomology 56: 239-240.

Bigger, J.H. 1930. Notes on the life history of the clover root curculio, *Sitona hispidula* Fab., in central Illinois. Journal of Economic Entomology 23: 334-342.

Bland, R.G. 1971. Photoperiod-diapause relationships in the alfalfa weevil, *Hypera postica*. Annals of the Entomological Society of America 64: 1163-1166.

Bliss, C.I. 1935. Calculation of close/mortality curve. Annals of Applied Biology 22: 134-167.

Bosch, R. van den, Stern, V.M. 1962. The integration of chemical and biological control of arthropod pests. Annual Review of Entomology 7: 367-386.

Bowers, W.S., Fales, H.M., Thompson, M.J., Uebel, E.C. 1966. Juvenile hormone: identification of an active compound from balsam fir. Science 154: 1020-1021.

Bracha, P., O'Brien, R.D. 1966. The relation between physical properties and uptake of insecticides by eggs of the large milkwood bug. Journal of Economic Entomology 59: 1255-1264.

Brooks, G.T. 1976. Penetration and distribution of insecticides. p 3-58. In: Wilkinson, C.F. (ed.) Insect biochemistry and physiology. Plenum Press, New York.

Buck, N.A, Estesen, B.J., Ware, G.W. 1980. Dislodgable insecticide residues on cotton foliage: fenvalerate, permethrin, sulprofos, chlorpyrifos, methyl parathion, EPN, oxamyl, and profenos. Bulletin of Environmental Contamination and Toxicology 24: 289-295.

Busvine, J.R. 1951. Mechanisms of resistance to insecticide in houseflies. Nature 168: 193-195.

Busvine, J.R. 1971. A critical review of the techniques for testing insecticides. 2nd ed. 345pp. Commonwealth Institute of Entomology. Slough, England.

Caltigirone, L.E. 1981. Landmark examples in classical biological control. Annual Review of Entomology 26: 213-232.

Chadwick, C.E. 1960. *Sitona humeralis* Steph. (Coleoptera: Curculionidae) recorded from New South Wales. Australian Journal of Science 22: 453-455.

Chadwick, P.R. 1962. Studies on the sub-lethal effects of pyrethrins on the grain weevil, *Calandra oryzae* L. Pyrethrum Post 6: 20-26.

Chapman, P.J., Pearce, G.W. 1949. Susceptibility of winter eggs of the European red mite to petroleum oils and dinitro compounds. Journal of Economic Entomology 42: 44-47.

Chapman, R.F. 1975. The insects structure and function. English Universities Press, London.

Charnov, E.L.; Skinner, S.W. 1985. Complementary approaches to the understanding of parasitoid oviposition decisions. Environmental Entomology 14: 383-391.

Chen, A.C., Mayer, R.T. 1985. Insecticides: Effects on the cuticle. p 57-77. In: Kerkut, G.A., Gilbert, L.I. (eds.) Comprehensive insect physiology, biochemistry and pharmacology. Volume 12 Insect control. Pergamon Press, Oxford.

Chio, L.L. 1976. A comparative pharmacodynamic study of six insecticides in eight species. PhD Thesis, University of Illinois, USA.

Coles, L.W.; Puttler, B. 1963. Status of the alfalfa weevil biological control program in the eastern United States. Journal of Economic Entomology 56: 609-611.

Corbet, J.R., Wright, K., Baillie, A.C. 1984. The biochemical mode of action of pesticides, 2nd edition. Academic Press, London.

Counce, S.J., Waddington, C.H. 1972. Developmental systems; Insects. Volume 1. Academic Press, New York.

Croft, B.A., Morse, J.G. 1979. Research advances on pesticide resistance in natural enemies. Entomophaga 24: 3-11.

Croft, B.A., Brown, A.W.A. 1975. Responses of arthropod natural enemies to insecticides. Annual Review of Entomology 20: 285-335.

Culin, J.D., Dubose III, W.P. 1987. Insecticide interference with *Microplitis demolitor* (Hymenoptera: Braconidae) parasitization of *Heliothis zea* (Lepidoptera: Noctuidae). Journal of Economic Entomology 80: 1188-1191.

Cullen, J.M., Hopkins, D.C. 1982. Rearing release and recovery of *Microctonus aethiopoides* Loan (Hymenoptera Braconidae) imported for the control of *Sitona discoideus* Gyllenhal (Coleoptera Curculionidae) in South Eastern Australia. Journal of the Australian Entomological Society 21: 279-284.

Danthanarayana, W. 1967. Host specificity of *Sitona* beetles. Nature, London. 213: 1153-1154.

Danthanarayana, W. 1969. Population dynamics of the weevil *Sitona regensteinensis* (Hbst) on broom. Journal of Animal Ecology 38: 1-18.

Day, W.H., Coles, L.W., Stewart, J.A., Fuester, R.W. 1971. Distribution of *Microctonus aethiops* and *M. colesi* parasites of the alfalfa weevil in the eastern United States. Journal of Economic Entomology 64: 190-193.

Debach, P., Bartlett, B. 1951. Effects of insecticides on biological control of insect pests of citrus. Journal of Economic Entomology 41: 372-383.

Doherty, J.D. 1985. Insecticides and ion transport. p 79-113. In: Kerkut, G.A., Gilbert, L.I. (eds.) Comprehensive insect physiology, biochemistry and pharmacology. Volume 12, Insect control. Pergamon Press, Oxford.

Drea, J.J. 1968. Castration of male alfalfa weevils by *Microctonus* spp. Journal of Economic Entomology 61: 1291-1295.

Drea, J.J., Dysart, R.J., Coles, L.W., Loan, C.C. 1972. *Microctonus stelleri* (Hymenoptera: Braconidae, Euphorinae), a new parasite of the alfalfa weevil introduced into the United States. Canadian Entomologist 104: 1445-1456.

Driesche, R.G. van; Gyrisco, G.C. 1979. Field studies of *Microctonus aethiopoides*, a parasite of the adult alfalfa weevil, *Hypera postica*, in New York. Environmental Entomology 8: 238-244.

Dumbre, R.B. , Hower, Jr. A.A. 1976a. Relative toxicities of insecticides to the Alfalfa weevil parasite *Microctonus aethiops* and the influence of parasitism on host susceptibility. Environmental Entomology 5: 511-315.

Dumbre, R.B., Hower, Jr. A.A. 1976b. Sublethal effects of insecticides on the alfalfa weevil parasites *Microctonus aethiopoides*. Environmental Entomology 5: 683-687.

Dumbre, R.B. and Hower, A.A. 1977. Contact mortality of the Alfalfa weevil parasite *Microctonus aethiopoides* from insecticide residues in alfalfa. Environmental Entomology 6: 893-894.

Eldefrawi, A.T. 1985. Acetylcholinesterases and anticholinesterases. p 115-130. In: Kerkut, G.A., Gilbert, L.I. (eds.) Comprehensive insect physiology, biochemistry and pharmacology. Volume 12 Insect control. Pergamon Press, Oxford.

El-Dessouki, S.A. 1971. Der Einflub van der Gattung Sitona (Col., Curculionidae) auf einige Leguminosen. Zeitschrift für Angewandte Entomologie 67(4): 411-430. [abstract in Review of Applied Entomology 62: 496-497].

El-Guindy, M.A., Abdel-Sattar, M.M., El-Refai, A.R.M. 1983. The ovicidal action of insecticides and insect growth regulator/insecticide mixtures on the eggs of various ages of susceptible and diflubenzuron-resistant strains of *Spodoptera littoralis* Boisd. Pesticide Science 14: 253-260.

Elliot, M., Janes, N.F., Potter, C. 1978. The future of pyrethroids in insect control. Annual Review of Entomology 23: 443-469.

Elzen, G.W. 1990. Sublethal effects of pesticides on beneficial parasitoids. p 129-150. In: Jepson, P.C. (ed.) Pesticide and non-target invertebrates. Intercept, Wimborne.

Elzen, G.W., O'Brien, P.J., Powell, J.E. 1989. Toxic and behavioral effects of selected insecticides on the *Heliothis* parasitoid *Microplitis croceipes*. Entomophaga 34: 87-94.

Elzen G.W., O'Brien, P.J., Snodgrass, G.L., Powell, J.E. 1987. Susceptibility of the parasitoid *Microplitis croceipes* (Hymenoptera: Braconidae) to field rates of selected cotton insecticides. Entomophaga 32: 545-550.

Esson, M.J. 1975. Notes on the biology and distribution of three recently discovered exotic weevil pest in Hawke's Bay. Proceedings of the 28th New Zealand Weed and Pest Control conference: 208-212.

Finney, D.J. 1971. Probit analysis. 3rd edition. Cambridge University Press, London. 333 pp.

Frampton, E.R. 1984. The seasonal pattern of *Sitona discoideus* larval establishment in Canterbury lucerne and its implications for control. New Zealand Journal of Experimental Agriculture 12: 319-09322.

Frampton, E.R. 1987. The reproductive seasonality and flight capability of *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae) and its pattern of larval establishment in Canterbury lucerne. PhD Thesis, Lincoln College, New Zealand. 182 pp.

Frampton, E.R., Kerse, G.W., Goldson, S.L. 1987. The effect of diflubenzuron on sitona weevil and its parasitoid, *Microctonus aethiopoides*. Proceedings of the 40th New Zealand Weed and Pest Control conference: 216-218.

Franz, J.M., Bogenschütz, H., Hassan, S.A., Huang, P., Naton, E., Suter, E., Viggiani, G. 1980. Results of a joint pesticide test programme by the working group "Pesticides and beneficial arthropods". Entomophaga 25: 231-236.

Fusco, R.A., Hower, Jr. A.A. 1973. Host influence on the laboratory production of the parasitoid, *Microctonus aethiops*. Environmental Entomology 2: 971-975.

Fusco, R.A., Hower, Jr. A.A. 1974. Influence of parasitoid- host density and host availability on the laboratory propagation of Microctonus aethiops (Hym. Braconidae) parasitoid of Hypera postica (Coleop.: Curculionidae). Entomophaga 19: 75-83.

Gaddum, J.H. 1933. The use of transformed percentage response. p 268-270. In: Busvine, J.R. (ed.) A critical review for testing insecticides. 2nd edition. Commonwealth Argicultural Bureaux, London.

Gammon, D.W., Brown, M.A., Casida, J.E. 1981. Two classes of pyrethroid action in the cockroach. Pesticide Biochemistry and Physiology 15: 181-191.

Goldson, S.L. 1983. Field technique to establish a replicated range of sitona weevil densities. Proceedings of the 36th New Zealand Weed and Pest Control conference: 30-32.

Goldson, S.L. 1984. Sitona weevil in lucerne - biology and control. Aglinks FPP 548. Information Services, Ministry of Agriculture and Fisheries, Wellington, New Zealand.

Goldson, S.L., Dyson, C.B., Proffitt, J.R., Frampton, E.R., Logan, J.A., 1985. The effect of *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae) on lucerne yields in New Zealand. Bulletin of Entomological Research 75: 429-442.

Goldson, S.L., Frampton, E.R. 1983. A simple technique for rapid and accurate counting of *Sitona discoideus* eggs (Coleoptera: Curculionidae): note. New Zealand Entomologist 7: 468-469.

Goldson, S.L., Frampton, E.R., Barratt, B.I.P., Ferguson, C.M. 1984. The seasonal biology of *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae), an introduced pest of New Zealand lucerne. Bulletin of Entomological Research 74: 249-259.

Goldson, S.L., Frampton, E.R., Jamieson, P.D. 1986. Relationship of *Sitona discoideus* (Coleoptera: Curculionidae) larval density to September-October potential soil moisture deficits. New Zealand Journal of Agricultural Research 29: 275-279.

Goldson, S.L., Frampton, E.R., Proffitt, J.R. 1988. Population dynamics and larval establishment of *Sitona discoideus* (Coleoptera: Curculionidae) in New Zealand lucerne. Journal of Applied Ecology 25: 177-195.

Goldson, S.L., French, R.A. 1983. Age-related susceptibility of lucerne to sitona weevil *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae), larvae and the associated patterns of adult infestation. New Zealand Journal of Agricultural Research 26: 251-255.

Goldson, S. L., Muscroft-Taylor, K.E. 1988. Inter-seasonal variation in *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae) larval damage to lucerne in Canterbury and the economics of insecticidal control. New Zealand Journal of Agricultural Research 31: 339-346.

Goldson, S.L., Proffitt, J.R. 1985. Measurement of the imact of different larval densities of *Sitona discoideus* Gyllenhal on Canterbury lucerne. Proceedings of the 4th Australasian Conference on Grassland Invertebrate Ecology: 26-34.

Goldson, S.L., Proffitt, J.R. 1986. The seasonal behaviour of the parasite *Microctonus aethiopoides* and its effects on sitona weevil. Proceedings of the 39th New Zealand Weed and Pest Control conference: 122-125.

Goldson, S.L., Proffitt, J.R., McNeill, M.R. 1990. Seasonal biology and ecology in New Zealand of *Microctonus aethiopoides* (Hymenoptera, Braconidae), a parasitoid of *Sitona* spp. (Coleoptera: Curculionidae), with special emphasis on atypical behaviour. Journal of Applied Ecology 27: 703-722.

Goldson, S.L., Proffitt, J.R., Stephen, R.C. 1987. A long-term effect of sitona weevil damage in Canterbury lucerne. Proceedings of the 40th New Zealand Weed and Pest Control conference: 212-215.

Goldson, S.L., Wynn-Williams, R.B. 1984. The effect of winter mob-stocking on sitona larval populations in lucerne. Proceedings of the 37th New Zealand Weed and Pest Control conference: 110-112.

Greenup, L.R. 1967. The sitona weevil - a pest of lucerne *Sitona humeralis*. The Agricultural Gazette 78: 528-529.

Grossheim, H.A. 1928. Data for the study of the genus *Sitona*, Germ. Bulletin of the Mleev Horticultural Experimental Station no 17. 57pp. [abstract in Review of Applied Entomology 17: 434-436.].

Grosscurt, A.C. 1978. Diflubenzuron; some aspects of its ovicidal and larvicidal mode of action and an evaluation of its practical possibilities. Pesticide Science 9: 373-386.

Hartley, D., Kidd, H. 1983. The Agrochemicals Handbook. Royal Society of Chemistry, London.

Hartley, J.B., Brown, A.W.A. 1955. The effects of certain insecticides on the cholinesterase of the American cockroach. Journal of Economic Entomology 48: 265-269.

Hartley, G.S., Graham-Bryce, I.J. 1980. Physical principles of pesticide behaviour. Volume II. Academic Press, London.

Hassan, S.A., Oomen, P.A. 1985. Testing the side effects of pesticides on beneficial organisms by OILB working party. p 145-152. In: Hussey, N.W., Scopes, N. (eds.) Biological Pest Control. The glasshouse experience. Blandford Press, Poole.

Hassan, S.A. 1990. Testing methodology and the concept of the IOBC/WPRS working group. p 1-18. In: Jepson, P.C. (ed.) Pesticide and non-target invertebrates. Intercept, Wimborne.

Hassan, S.A., Bigler, F., Bogenschütz, H., Brown, J.U., Firth, S.I., Huang, P., Ledieu, M.S., Naton, E., Oomen, P.A., Overmeer, W.P.J., Rieckmann, W., Samsøe-Petersen, L., Viggiani, G., van Zon, A.Q. 1983. Results of the second joint pesticide testing programme bythe IOBC/WPRS - working group "Pesticides and beneficial arthropods". Zeitschrift für Angewandte Entomologie 95: 151-158.

Hassell, M.P. 1984. Insecticides in host-parasitoid interactions. Theoretical Population Biology 26: 378-386.

Henzell, R.F., Lauren, D.R., East, R. 1979. Effect on the egg hatch of white-fringed weevil of feeding lucerne treated with the insect growth regulator diflubenzuron. New Zealand Journal of Agricultural Research 22: 197-200.

Hinton, H.E. 1981. Biology of insect eggs. Three volumes. Pergamon Press, Oxford.

Hopkins, D.C. 1979. Sitona weevil. Department of Agriculture and Fisheries fact sheet No. 22/78. 2pp.

Hopkins, D. 1981. Establishment and spread of the sitona weevil parasite *Microctonus aethiopoides* in south Australia. Proceedings of the 3rd Australasian Conference on Grassland Invertebrate Ecology: 177-182.

Hopkins, D.C. 1985. Controlling sitona weevil, *Sitona discoideus* with insecticides. Proceedings of the 4th Australasian Conference on Grassland Invertebrate Ecology: 94-98.

Hopkins, D.C. 1988. Widespread establishment of the sitona weevil parasite, *Microctonus aethiopoides* and its effectiveness as a control agent in South Australia. Proceedings of the 5th Australasian Conference on Grassland Invertebrate Ecology: 49-54.

Hoskins, W.M., Craig, R. 1962. Use of bioassay in entomology. Annual Review of Entomology 7: 437-464.

Hoskins, W.M., Gordon, H. 1956. Arthropod resistance to chemicals. Annual Review to Entomology 1: 89-122.

Hower, A.A., Davis, G.A. 1984. Selectivity of insecticides that kill the potato leafhopper (Homoptera:Cicadellidae) and Alfalfa weevil (Coleoptera: Curculionidae) and protect the parasite *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae). Journal of Economic Entomology 77: 1601-1607.

Hower, Jr. A.A., Luke, J.E. 1975. Response of the alfalfa weevil parasitoid, *Microctonus colesi* (Drea) (Hymenoptera: Braconidae), to a recommended insecticide treatment in Pennsylvania. New York Entomological Society 83: 263-264.

Huffaker, C.B., Berryman, A.A., Laing, J.E. 1984. Natural control of insect populations. p 359-397. In: Huffaker, C.B., Rabb, R.L. (eds.) Ecological entomology. John Wiley and Sons, New York.

Hull, L.A. and Beers, E.H. 1985. Ecological selectivity: modifying chemical control practices to preserve natural enemies. p 103-122. In: Hoy, M.A., Herzog, D.C. (eds.) Biological control in agricultural IPM Systems. Academic Press Inc. Orlando.

Jackson, D.J. 1920. Bionomics of weevils of the genus Sitona injurious to leguminous crops in Britain (Part I). Annals of Applied Biology 7: 269-298.

Jackson, D.J. 1928. The biology of *Dinocampus* (*Perilitus*) *rutilus* Nees, a braconid parasite of *Sitona lineata* L. Part I. Proceedings of the Zoological Society of London part 2: 597-630.

Jepson, P.C. 1988. Ecological characteristics and the susceptibility of non-target invertebrates to long term pesticide side-effects. Field methods for the study of the environmental effects of pesticides. BCPC Monograph 40: 191-200.

Jepson, P.C. 1990. The temporal and spatial dynamics of pesticide side-effects on non-target invertebrates. p 95-127. In: Jepson, P.C. (ed.) Pesticide and non-target invertebrates. Intercept, Wimborne.

Jepson, P.C., Thacker, J.R.M. 1990. Analysis of the spatial component of pesticide side-effects on non-target invertebrate populations and its relevance to hazard analysis. Functional Ecology 4: 349-355.

Jourdheuil, P. 1960. Influence de quelques facteurs écologiques sur les flucuation de population d'une biocénose parasitaire. Annales de l'Institut National de la Recherche 2: 445-658.

Kain, W.M., Trough.t, T.E.T. 1982. Insect pests of lucerne in New Zealand. p 49-59. In: Wynn-Williams, R.B. (ed.) Lucerne for the 80's. Agronomy Society of New Zealand special publication 1.

Krysan, J.L., Guss, P.L. 1971. Paraoxon inhibition of an insect egg lipase. Biochimica et Biophysica Acta 239: 349-352.

Krueger, C.A., Radcliffe, E.B. 1986. Distribution and abundance of the alfalfa weevil parasite, *Microctonus aethiopoides* (Hymenoptera:Braconidae) in Minnesota. 30th North American Alfalfa Improvement Conference: 33.

Kwong Sue 1978. A rearing method and some biological studies of the weevil *Sitona humeralis* Stephens (Coleoptera: Curculionidae) in Canterbury. M. Hort. Sc. Thesis. Lincoln College. 93pp.

Kwong Sue, Ferro, D.N., Emberson, R.M. 1980a. Life history and seasonal ovarian development of *Sitona humeralis* (Coleoptera: Curculionidae) in New Zealand. New Zealand Entomologist 7: 165-169.

Kwong Sue, Ferro, D.N., Emberson, R.M. 1980b. A rearing method for *Sitona humeralis* Stephens (Coleoptera: Curculionidae) and its development under controlled conditions. Bulletin of Entomological Research 70: 97-102.

Leuck, D.B., Bowman, M.C., Beck, D.W. 1968. Dursban insecticide persistence in grass and corn forage. Journal of Economic Entomology 61: 689-690.

Lincoln, D.C.R. 1961. The oxygen and water requirements of the egg of *Ocypus olens* Müller (Staphylinidae, Coleoptera). Journal of Insect Physiology 7: 265-272.

Loan, C.C. 1963. The bionomics of *Sitona scissifrons* Say (Coleoptera: Curculionidae) and its parasite *Microctonus sitonae* (Hymenoptera: Braconidae). Annals of the Entomological Society of America 56: 600-612.

Loan, C.C 1967a. Studies on the taxonomy and biology of the Euphorinae (Hymenoptera: Braconidae). I. Four new Canadian species of *Microctonus*. Annals of the Entomological Society of America 60: 230-235.

Loan, C.C. 1967b. Studies on the taxonomy and biology of the Euphorinae (Hymenoptera: Braconidae). II. Host relations of six Microctonus species. Annals of the Entomological Society of America 60: 236-240.

Loan, C.C. 1975. A review of Haliday species of *Microctonus* [Hym.: Braconidae, Euphorinae]. Entomophaga 20: 31-41

Loan, C. 1983. Host and generic relations of the Euphorini (Hymenoptera:Braconidae). Contributions of the American Entomological Institute 20:388-397.

Loan, C., Holdaway, F.G. 1961a. *Pygostolus falcatus* (Nees) (Hymenoptera, Braconidae), a parasite of *Sitona* species (Coleoptera, Curculionidae). Bulletin of Entomological Research 52: 473-488.

Loan, C.C., Holdaway, F.G. 1961b. *Microctonus aethiops* (Nees) Auctt. and *Perilitus rutilus* (Nees) (Hymenoptera:Braconidae), European parasites of *Sitona* weevils (Coleoptera:Curculionidae). Canadian Entomologist 93: 1057-1079.

Loan, C.C., Holliday, N.J. 1979. Euphorine parasitic on ground beetles with descriptions of three new species of *Microctonus* Wesmael (Hymenoptera: Braconidae, and Coleoptera: Carabidae). Le Naturaliste Canadien 106: 393-397.

Loan, C.C., Klein, M.G., Coppel, H.C. 1969. *Microctonus glyptosceli* n.sp., a parasite of *Glyptoscelis pubescens* (F.) in Winsconsin. Proceedings of the Entomological Society of Washington 71: 230-233.

Loan, C.C., Lloyd, D.C. 1974. Description and field biology of *Microctonus hyperodae* Loan, n.sp. (Hymenoptera: Braconidae, Euphorinae) a parasite of *Hyperodes bonariensis* in South America [Coleoptera: Curculionidae]. Entomophaga 19: 7-12.

Lord, K.A., Potter, C. 1951. Studies on the mechanism of insecticidal action of organo-phosphorus compounds with particular reference to their anti-esterase activity. The Annals of Applied Biology 38: 495-507.

Lowenstein, O. 1942. A method of physiological assay of pyrethrum extracts. Nature 150: 760-762.

Luck, R.F., Morse, J.G., Moreno, D.S. 1986. Current status of integrated pest management in California citrus groves. p 533-543. In: Cavalloro, R., Di Martino E. (eds.) Integrated pest control in citrus-groves, proceedings of the experts meeting, Acireale. Balkema, Boston.

Luff, M.L. 1976. The biology of *Microctonus caudatus*(Thomson), a braconid parasite of the ground beetle *Harpalus rufipes* (Degeer). Ecological Entomology 1: 111-116.

Lund, A. 1985. Insecticides: effects on the nervous system. p 9-56. In: Kerkut, G.A., Gilbert, L.I. (eds.) Comprehensive insect physiology, biochemistry and pharmacology. Volume 12, Insect control. Pergamon Press, Oxford.

Lund, A.E., Narahashi, T. 1981a. Modification of sodium channel kinetics by the insecticide tetramethrin in crayfish giant axons. Neurotoxicology 2: 213-229.

Lund, A.E., Narahashi, T. 1981b. Kinetics of sodium channel modification by the insecticide tetramethrin in squid axon membranes. Journal of Pharmacology and Experimental Therapeutics 219: 464-473.

Margaritis, L.H. 1985. Structure and physiology of the eggshell. p 153-230. In: Kerkut, G.A., Gilbert, L.I. (eds.) Comprehensive insect physiology, biochemistry and pharmacology. Volume 1 Embryogenesis and reproduction. Pergamon Press, Oxford.

Masaki, S. 1980. Summer diapause. Annual Review of Entomology 25: 1-25.

McCarl, B.A. 1981. Economics of integrated pest management: an interpretative review of the literature. Special report 636. Agricultural Experimental Station, Oregon State University.

McColloch, J.W. 1918. Notes on false wireworms with especial reference to *Eleodes tricostata* Say. Journal of Economic Entomology 11: 212-224.

McNeill, J. 1975. Juvenile hormone analog: detrimental effects on the development of an endoparasitoid. Science 189: 640-642.

Mehrotra, K.N. 1960. Development of the cholinergic system in insect eggs. Journal of Insect Physiology 5: 129-142

Mehrotra, K.N., Smallman, B.N. 1957. Ovicidal action of organophosphorus insecticides. Nature 180: 97-98.

Melamed-Madjar, V. 1966. Observations on four species of *Sitona* (Coleoptera, Curculionidae) occurring in Israel. Bulletin of Entomological Research 56: 505-514.

Menusan, Jr. H. 1948. Comparative toxicity of insecticides administered in various ways to several species of insects. Journal of Economic Entomology 41: 302-313.

Metcalf, R.L. 1958. Methods of topical application and injection. p 92-113. In: Shepard, H.H. (ed.) Methods of testing chemicals on insects Vol. 1. Burgess, Minneopolis.

Metcalf, R.L. 1972. Development of selective and biodegradable pesticides. p 137-156. In: Pest control strategies for the future. National Academy of Sciences, Washington, DC.

Metcalf, R.L., Luckman, W.H. 1982. Introduction to insect pest management. John Wiley and Sons, New York.

Metcalf, R.L., March, R.B. 1949. Studies of the mode of action of parathion and its derivatives and their toxicity to insects. Journal of Economic Entomology 42: 721-

Millar, T.A., Adams, M.E. 1982. Mode of action of pyrethroids p 3-27. In: Coats, J.R. (ed.) Insecticide mode of action. Academic Press, New York.

MINITAB Reference Manual- 1989 . Release 7, Chapter 7.

Moore, R.F. 1980. Behavioral and biological effects of NRDC-161 as factors in control of the boll weevil. Journal of Economic Entomology 73: 265-267.

Moriarty, F. 1969. The sublethal effects of synthetic insecticides on insects. Biological Reviews 44: 321-357.

Morrison, W.P., Pass, B.C., Nichols, M.P., Armbrust, E.J. 1974. The literature of arthropods associated with alfalfa. II. A bibliography of the *Sitona* species (Coleoptera: Curculionidae). Illinois Natural History Survey Biological notes 88. 24pp.

Morse, J.G., Bellows, Jr. T.S. 1986. Toxicity of major citrus pesticides to *Aphytis melinus* (Hymenoptera: Aphelininidae) and *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae). Journal of Economic Entomology 79: 311-314.

Moulden, J. 1973. The biology of *Sitona* species with particular reference to *S. hunmeralis*. South Australia Department of Agriculture Agronomy branch report No 44. 50 pp.

Mulder, R., Gijswijt, M.J. 1973. The laboratory evaluation of two promising new insecticides which interfere with cuticle deposition. Pesticide Science 4: 737-745.

Mullins, L.J. 1955. Structure-toxicity in hexachlorocyclohexane isomers. Science 122: 118-119.

Munson, S.C., Padilla, G.M., Weissmann, M.L. 1954. Insect lipids and insecticidal action. Journal of Economic Entomology 47: 578-587.

Narahashi, T. 1971. Effects of insecticides on excitable tissues. Advances in Insect Physiology 8: 1-93

Newson, L.D., Smith, R.F., Whitcomb, W.H. 1976. Selective pesticides and selective use of pesticides. In: Huffaker, C.B., Messenger, P.S. (eds.) Theory and practice of biological control. Academic Press, New York.

O'Connor, B. 1990. New Zealand Agrichemical and Plant Protection manual 1990. SwiftPrint, Palmerston North.

O'Brien, R.D., Smith, E.H. 1961. The uptake and metabolism of parathion by insect eggs. Journal of Economic Entomology 54: 187-191.

O'Brien, P.J., Elzen, G.W., Vinson, S.B. 1985. Toxicity of azinphosmethyl and chlodimeform to parasitoid *Bracon mellitor* (Hymenoptera: Braconidae): lethal and reproductive effects. Environmental Entomology 14: 891-894.

Oglobin, A. 1925. Le rôle du blastoderme extraembryonaire du *Dinocampus terminatus* Nees pendant l'état larvaire. Českoslovenká společnost entomologická, Časposis 3. 27pp.

Osborne, M.P. 1985. DDT, γ-HCH and the cyclodienes. p 131-182. In: Kerkut, G.A., Gilbert, L.I. (eds.) Comprehensive insect physiology, biochemistry and pharmacology. Volume 12 Insect control. Pergamon Press, Oxford.

Peterson, A. 1969. Some eggs of insects that change color during incubation. The Florida Entomologist 45: 81-87.

Pienkowski, R.L. 1976. Behavior of the adult alfalfa weevil in diapause. Annals of the Entomological Society of America 69: 155-157.

Plapp, F.W., Bull,: D.L. 1988. Modifying chemical control practices to preserve natural enemies. p 537-546. In: King, E.G., Jackson, R.D. (eds.) International workshop biological control of *Heliothis* : increasing the effectiveness of natural enemies. Amerind, New Delhi.

Plapp, F.W., Vinson, S.B. 1977. Comparative toxicities of some insecticides to the tobacco budworm and its ichneumonid parasite. Environmental Entomology 6: 381-384.

Potter, C. 1941. A laboratory spraying apparatus and a technique for investigating the action of contact insecticides with some notes on suitable test insects. Annals of Applied Biology 28: 142-169.

Potter, C. 1952. An improved laboratory apparatus for applying direct sprays and surface films, with data on the electrostatic charge on atomized spray fluids. Annals of Applied Biology 39: 1-28.

Potter, C., Lord, K.A., Kenten, J., Salkeld, E.H., Holbrook, D.V. 1957. Embryonic development and esterase activity of eggs of *Pieris brassicae* in relation to TEPP poisoning. Annals of Applied Biology 45: 361-375.

Potter, C., Tattersfield, F. 1943. Ovicidal properties of certian insecticides of plant origon. (Nicotine, pyrethrins, derris products.). Bulletin of Entomological Research 34: 225-244.

Powell, J.E., King, E.G. Jr., Jany, C.S. 1986. Toxicity of fourteen insecticides in six classes to adult *Microplitis croceipes* (Hymenoptera: Braconidae). Journal of Economic Entomology 79: 1343-1346.

Powell, J.E., Scott, W.P. 1985. Effect of insecticide residues on survival of *Microplitis croceipes* adults (Hymenoptera: Braconidae) in cotton. Florida Entomologist 68: 692-693.

Proffitt, J.R., Goldson, S.L. 1986. The cummulative effect of sitona weevil larval damage on a Canterbury lucerne stand after three seasons. Proceedings of the 39th New Zealand Weed and Pest Control conference: 38-40.

Proffitt, J.R., Goldson, S.L. 1987. Overwintering biology and survival of the sitona weevil parasitoid *Microctonus aethiopoides*. Proceedings of the 40th New Zealand Weed and Pest Control conference: 23-26.

Quinn, M.A., Hower, A.A. 1985. Determination of overwintering survivorship and predicting time of eclosion for eggs of *Sitona hispidulus* (Coleoptera: Curculionidae). Environmental Entomology 14: 850-854.

Radwan, H.S.A., Assal, O.M., Abo-Elghar, M.R., Samy, M.A. 1983. Synergistic ovicidal action of organo-phosphorous and carbonate compounds when mixed with insect growth regulators in controlling cotton leaf worm. Indian Journal of Agricultural Science 53: 1055-1058.

Retnakaran, A. 1973. Ovicidal effects in the white pine weevil, *Pissodes strobi* of a synthetic analogue of juvenile hormone. Canadian Entomologist 105: 591-594.

Riddiford, L.M. 1972. Juvenile hormone and insect embryonic development: its potential role as an ovicide. p 95-111. In: Menn,J.J. Beroza, M. (eds.) Insect juvenile hormones: chemistry and action. Academic Press, New York.

Ripper, W.E. 1956. Effect of pesticides on balance of arthropod populations. Annual Review of Entomology 1: 403-438.

Rosenheim, J.A., Hoy, M.A. 1988. Sublethal effects of pesticides on the parasitoid *Aphytis melinus* (Hymenoptera: Aphelinidae). Journal Of Economic Entomology 81 476-483.

Roudier, A. 1980. Les *Sitona* Germar 1817 du groupe de *Sitona humeralis* Stephens 1831 [Col., Curculionidae]. Bulletin de la Societé Entomologique de France 85: 207-217.

Ruight, G.F. 1985. Pyrethroids pp. 183-262. In: Kerkut, G.A., Gilbert, L.I. (eds.) Comprehensive insect physiology, biochemistry and pharmacology. Volume 12 Insect control. Pergamon Press, Oxford.

Ruscoe, C.N.E. 1977. The new NRDC pyrethroids as agricultural insecticides. Pesticide Science 8: 236-242.

Russell, R.M., Robertson, J.L., Savin, N.E. 1977. POLO: A new computer program for probit analysis. Bulletin of the Entomological Society of America 23: 209-213.

Salkeld, E.H. 1964. Some physiological aspects of organogenesis in the insect embryo. Canadian Entomologist 96: 389-400.

Salkeld, E.H., Potter, C. 1953. The effect of the age and stage of development of insect eggs on their resistance to insecticides. Bulletin of Entomological Research 44: 527-580.

Sawicki, R.M. 1962. Insecticidal activity of pyrethrum extract and its four insecticidal constituents against house flies. III. Knockdown and recovery of flies treated with pyrethrum extract with and without piperonyl butoxide. Journal of the Science of Food and Agriculture 13: 283-292.

Schefczik, J., Simonis, W. 1980. Side effects of chlorinated hydrocarbon insecticides on membranes of plant cells. Pesticide Biochemistry and Physiology 13: 13-19.

Schotzko, D.J., O'Keeffe, L.E. 1986. Ovipositional rhythms and egg melanization rate of *Sitona lineatus* (L.) (Coleoptera: Curculionidae). Environmental Entomology 15: 601-606.

Shaw, S.R. 1985. A phylogenetic study of the subfamilies Meteorinae and Euphorinae (Hymenoptera: Braconidae). Entomography 3: 277-370.

Shaw, S.R. 1988. Euphorine phylogeny: the evolution of diversity in host-utilization by parasitoid wasps (Hymenoptera: Braconidae). Ecological Entomology 13: 323-335.

Slàma, K., Williams, C.M. 1966. 'Paper factor' as an inhibitor of the embryonic development of the European bug, *Pyrrhocoris apterus*. Nature 210: 329-330.

Smissaert, H.R. 1964. Cholinesterase inhibition in spider mites susceptible and resistant to organophosphates. Science 143: 129-131.

Smith, E.H. 1955. Further studies on the ovicidal action of parathion to eggs of the peach tree borer. Journal of Economic Entomology 48: 727-731.

Smith, E.H., Pearce, G.W. 1948. The mode of action of petroleum oils as ovicides. Journal of Economic Entomology 41: 173-180.

Smith, E.H., Salkeld, E.H. 1966. The use and action of ovicides. Annual Review of Entomology 11: 331-368.

Smith, E.H. and Wagenknecht, A.C. 1956. The occurrence of cholinesterase in eggs of the peach treeborer and milkweed bug and its relationship to the ovicidal action of parathion. Journal of Economic Entomology 49: 777-785.

Smith, H.S. 1919. On some phases of insect control by the biological method. Journal of Economic Entomology 12: 288-292.

Smith, O.J. 1952. Biology and behaviour of *Microctonus vittatae* Muesebeck (Braconidae) with description of its immature stages. University of California Publications in Entomology 9: 315-343.

Smith, O.J. 1953. Species distribution and host records of the braconid genera *Microctonus* and *Perilitus* Ohio Journal of Science 53: 173-178.

Smith, O.J., Peterson, A. 1950. *Microctonus vittatae*, a parasite of adult flea beetles, and observations on hosts. Journal of Economic Entomology 43(5): 581-585.

Somerfield, K.G., Burnett, P.A. 1976. Lucerne insect survey. Proceedings of the 29th New Zealand Weed and Pest Control conference: 14-18.

Staal, G.B. 1975. Insect growth regulators with juvenile hormone activity. Annual Review of Entomology 20: 417-460.

Stehr, F.W. 1974. Dispersal following establishment in Michigan of *Microctonus aethiops*, a parasitoid of adult alfalfa weevils. Environmental Entomology 3: 575-576.

Stehr, F.W. 1982. Parasitoids and predators in pest management. p 147-188. In: Metcalf, R.L., Luckman, W. (eds.) Introduction to insect pest management. John Wiley and Sons, New York.

Stein, W., 1967. Die Rüsselkäferfauna des Grünlandes und ihre phytopathologische Bedeutung. Zeitschrift für Angewandte Entomologie 60: 3-59; 141-181.

Stern, V.M., Smith, R.F., van den Bosch, R., Hagen, K.S. 1959. The integrated control concept. Hilgardia 29: 81-101.

Strong, R.G., Diekman, J. 1973. Comparative effectiveness of fifteen insect growth regulators against several pests of stored products. Journal of Economic Entomology 66: 1167-1173.

Stufkens, M.W., Farrell, J.A., Goldson, S.L. 1987. Establishment of *Microctonus aethiopoides* a parasitoid of the sitona weevil in New Zealand. Proceedings of the 40th New Zealand Weed and Pest Control conference: 31-32

Sundaralingam, S. 1986. Biological, morphological and morphometric analyses of populations of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae). M. Sc. Thesis. Pennsylvania State University, USA. 80pp.

Sun, Y-P 1960. Pre-test conditions which affect insect reaction to insecticides. pp 1-9. In: Shepard, H.H. (ed.) Methods of testing chemicals on insects. Volume 2. Burgess Publishing, Minneapolis.

Surgeoner, G.A., Ellis, C.R. 1976. Effect of field applications of carbofuran on *Hypera postica* (Coleoptera: Curculionidae) and its parasitoids. Canadian Entomologist 108: 649-654.

Syrett, P., Penman, D.R. 1980. Comparative toxicity of insecticides to lucerne aphids and their predator. Proceedings of the 33rd New Zealand Weed and Pest Control conference: 52-54.

Tamashiro, M., Sherman, M. 1955. Direct and latent toxicity to oriental fruitfly larvae and their internal parasites. Journal of Economic Entomology 48: 75-79.

Tauber, M.J., Tauber, C.A., Herzog, D.C. 1985. Biological control in agricultural IPM systems: a brief overview of the current status and future prospects. p 3-9. In: Hoy, M.A., Herzog, D.C. (eds.) Biological control in agricultural IPM systems. Academic Press, Orlando.

Tauber, M.J., Tauber, C.A., Nechols, J.R., Obrycki, J.J. 1983. Seasonal activity of parasitoids: control by external, internal and genetic factors. p 87-108. In: Brown, V.K., Hodek, I. (eds.) Diapause and life cycle stategies in insects. Junk, The Hague.

Tenhet, J.N. 1947. Effects of sublethal dosages of pyrethrum on oviposition of the cigarette beetle. Journal of Economic Entomology 40: 910-911.

Tobias, V.I. 1965. Generic groupings and evolution of parasitic Hymenoptera of the subfamily Euphorinae. Entomological Review 44: 494-507.

Tombes, A.S., Marganian, L. 1967. Aestivation (summer diapause) in *Hypera postica* (Coleoptera: Curculionidae). II. Morphological and histological studies of the alimentry canal. Annals of the Entomological Society of America 60: 1-8.

Trought, T.E.T., Stringer, G.C. 1976. Chemical control of sitona weevil on lucerne. Proceedings of the 29th New Zealand Weed and Pest Control conference: 28-30.

Tuft, R.H. 1950. The structure of the insect egg shell in relation to the respiration of the embryo. Journal of Experimental Biology 26: 327-334.

Uchida, M., Irie, Y., Kurihara, N., Fujita, T., Nakajima, M. 1975a. The neuroexcitatory convulsive and lethal effects of lindane analogues on *Periplanteta americana*. Pesticide Biochemistry and Physiology 5: 258-264.

Uchida, M., Irie, Y., Fujita, T., Nakajima, M. 1975b. Effects of neristoxin on the neuroexcitatory action on insecticide. Pesticide Biochemistry and Physiology 5: 253-257.

Veierov, D., Fenigstein, A., Melamed-Madjar, V., Klein, M. 1988. Effects of concentration and application method on decay and residual activity of foliar chlorpyrifos. Journal of Economic Entomology 81(2): 621-627.

Vinson, S.B. 1974. Effect of an insect growth regulator on two parasitoids developing from treated tobacco budworm larvae. Journal of Environmental Ecology 67: 335-337.

Vinson, S.B., Iwantsch, G.F. 1980. Host regulation by insect parasitoids. Quarterly Review of Biology 55: 143-165.

Voss, G., Matsumura, F. 1965. Biochemical studies on a modified and normal cholinesterase found in the Leverkusen strains of the two-spotted spider mite *Tetranychus urticae*. Canadian Journal of Biochemistry 43: 63-72.

Waage, J.K., Hassell, M.P., Godfray H.C.J. 1985. The dynamics of pest-parasitoid-insecticide interactions. Journal of Applied Ecology 22: 825-838.

Walker, W.F., Bowers, W.S. 1970. Synthetic juvenile hormones as potential coleopteran ovicides. Journal of Economic Entomology 63: 1231-1233.

Wall, T.J. 1982. Grazing management in practice - Canterbury. p 101-103. In: Wynn-Williams, R.B.(ed.) Lucerne for the 80's. Special publication No.1, Agronomy Society of New Zealand. Swiftcopy Centre Limited, Palmerston North.

Webb, G.D., Sharp, R.W., Feldman, J.D. 1979. Effect of insecticides on the short-circuit current and resistance of isolated frog skin. Pesticide Biochemistry and Physiology 10: 23-30.

Welling, W., Paterson, G.D. 1985. Toxicodynamics of insecticides. p 603-645. In: Kerkut,.A., Gilbert, L.I. (eds.) Comprehensive insect physiology, biochemistry and pharmacology. Volume 12 Insect control. Pergamon Press, Oxford.

Wigglesworth, V.B. 1942. Some notes on the integument of insects in relation to the entry of contact insecticides. Bulletin of Entomological Research 33: 205-218.

Wigglesworth, V.B. 1972. The principles of insect physiology. 7th ed. Chapter 1: Development in the egg. Chapman and Hall, London.

Wightman, J.A. 1979. *Sitona humeralis* in the South Island of New Zealand (Coleoptera: Curculionidae). Proceedings of the 2nd Australasian Conference on Grassland Invertebrate Ecology: 138-141.

Wightman, J.A. 1981. Sitona weevil, *Sitona discoidea* (Gyllenhal), life cycle. DSIR Information Series No. 105/40.

Willis, G.H., McDowell, L.L. 1987. Pesticide persistence on foliage. Reviews of Environmental Contamination and Toxicology 100: 23-73.

Willis, G.H., McDowell, L.L., Southwick, L.M., Smith, S 1985. Toxaphene, methyl parathion, and fenvalerate disappearances from cotton foliage in the Mid South. Journal of Environmental Quality 14: 446-450.

Wilson, F., Huffaker, C.B. 1976. The philosophy, scope and importance of biological control. p 3-15. In: Huffaker, C.B., Messenger, P.S. (eds.) Theory and practice of biological control. Academic Press, New York.

Winteringham, F.P.W. 1969. Mechanisms of selective insecticidal action. Annual Review Entomology 14: 409-422.

Wood, J. 1980. Notes on the sitona weevil, *Sitona humeralis* (Coleoptera: Curculionidae), a pest of lucerne in Canterbury. New Zealand Entomologist 7: 169-171.

Worthing, C.R., Walker, S.B. 1983. The pesticide manual. 7th edition. Britsh crop protection council, London.

Wylie, H.G., Loan, C. 1984. Five nearctic and one introduced euphorine species (Hymenoptera: Braconidae) that parasitize adults of crucifer-infesting flea beetles (Coleoptera: Chrysomelidae). Canadian Entomologist 116: 235-246.

Zschintzsch, J., O'Brien, R.D., Smith, E.H. 1965. The relation between uptake and toxicity of organophosphates for eggs of the large milkweed bug. Journal of Economic Entomology 58(4): 614-621.