# Chapter 1: Aspects of the ecology of *Helicoverpa punctigera* – what has changed?

## 1.1 Background

The genus *Helicoverpa* is part of the noctuid family of moths and has a worldwide range of species, which include *Helicoverpa armigera* (Hübner, 1805), *Helicoverpa assulta* (Guenée, 1852) *Helicoverpa atacamae* (Hardwick, 1965) *Helicoverpa fletcheri* (Hardwick, 1965), *Helicoverpa gelotopoeon* (Dyar, 1921), *Helicoverpa hardwicki* (Matthews, 1999), *Helicoverpa hawaiiensis* (Quaintance & Brues, 1905), *Helicoverpa helenae* (Hardwick, 1965), *Helicoverpa pallida* (Hardwick, 1965), *Helicoverpa prepodes* (Common, 1985), *Helicoverpa punctigera* (Wallengren, 1860), *Helicoverpa titicaca* (Hardwick, 1965), *Helicoverpa toddi* (Hardwick, 1965) and *Helicoverpa zea* (Boddie, 1850). As cotton bollworms and legume pod borers, larvae of *Helicoverpa* spp. are among the most significant crop pests worldwide (Fitt and Cotter, 2005).

There are two species of the genus *Helicoverpa* that are agriculturally significant in Australia, the cotton bollworm *Helicoverpa armigera* and the native budworm *Helicoverpa punctigera*. The cosmopolitan *H. armigera* and the Australian native *H. punctigera* are the most important pests of field crops in Australia (Zalucki *et al.*, 1986). Although the two species are closely related and share similar colouration and body sizes, each species has differing host-plant ranges, seasonal abundances and geographic ranges (Zalucki *et al.*, 1986, Fitt and Cotter, 2005). *Helicoverpa armigera* achieves 4-5 generations per season over most of Queensland and much of Northern New South Wales (Fitt and Daly, 1990). The diverse host range of *H. armigera* means that at any one time there may be a mosaic of subpopulations linked by adult migration, each with different mortality rates and fitness effects imposed by their environment (Dillon *et al.*, 1996).

*Helicoverpa zea* (closely related to *H. armigera)* is a pest in America, while *H. armigera* is a pest in Asia, Australia, Africa and Europe (Fitt and Cotter, 2005). *Helicoverpa armigera* and *H. punctigera* are referred to by a varied collection of common names, including bean pod borer, bollworm, climbing cutworm, common bollworm, corn earworm, cotton bollworm, flower caterpillar, *Heliothis* worm or grub, lucerne budworm, tobacco budworm, tomato grub or worm (Zalucki *et al.*, 1986). Some of these names can apply to both species without any meaningful distinction. To make matters worse, there has been taxonomic confusion between the two species in the past (Common, 1953), making publications before 1953 unreliable. The distinction between the two species is now more reliable, with a number of morphological differences cited in the literature that can be used for differentiation of species at various life stages (Zalucki *et al.*, 1986, Zalucki, 1991). As reflected in their joint common names both *H. armigera* and *H. punctigera* share a number of host plants, including cotton, chickpea, pigeon pea, oilseed, vegetable and fruit crops (Fitt and Cotter, 2005). The known host species for *H. armigera* and *H. punctigera* are reviewed by Zalucki *et al.* (1986), Zalucki *et al.* (1994) and Cunningham and Zalucki (2014). In terms of production losses from *H. punctigera* in Victoria and southern NSW, the most important hosts are chickpeas, field peas, faba beans, tomatoes and lucerne (McDonald, 1995, McDonald *et al.*, 2014).

Even though seasonal distributions of *Helicoverpa* species differ from year to year, some general distinctions on the arrival of different species on cultivated host plants can be made. As judged by trap catches of adults, in spring *H. punctigera* is more common, while *H. armigera* is more common in summer and autumn (Zalucki, 1991). While both *H. punctigera* and *H. armigera* share a variety of hosts, *H. armigera* is further distinguished by its capacity to feed on sorghum and corn, while *H. punctigera* is more frequently found on a variety of native legumes and daisies (Zalucki *et al.* 1986). Part of the reason for these differences may be that *H. punctigera* is better adapted to the environments where these native legumes or daisies grow rather than because *H. armigera* is not capable of using them as a host (P. Gregg Pers. Comm. 2014).

## 1.2 Life cycle and basic biology

Under summer conditions *Helicoverpa* spp. can take 4-6 weeks from egg to adult, while under winter conditions they may take as long as 8-12 weeks (Bailey, 2007). Adult moths have a wingspan of 30-45mm. Adults feed on nectar, living for approximately 10 days over which females can lay up to 1000 eggs, either singly or in clusters, on leaves, flower buds, stems and developing fruits (Zalucki *et al.*, 1986). Fertile eggs can hatch in three days at 25°C, or 6-10 days in cooler conditions. Eggs change from white to dark-brown black as the head of the larvae develops before hatching (Anon, 2005, Bailey, 2007). Harsh climatic conditions can affect the survival of young larvae and eggs by dehydration or frost.

Larvae eat through the eggshell and emerge when about 1-1.5mm long, as a white-yellow caterpillar with a dark head (Anon, 2005). Larvae (Fig. 1) initially feed on young foliage before moving on to flower buds, flowers, pods, fruits and seeds, as they mature.


*Fig. 1. The larval instars of* H. punctigera*. Photo: G. Baker, SARDI.*

## 1.3 *Helicoverpa* under changing climates and environments

Whether it is a changing climate or changing agricultural practices, different selection pressures will be put on *Helicoverpa* spp., which in turn lead to changes in population dynamics (Thrall *et al.*, 2010). Understanding these driving forces behind population dynamics is considered key to ecology (Betini *et al.*, 2013). Long term data are often lacking on the dynamics of agricultural pests, given that the primary drivers of agriculture and climate are changing at different rates (Ouyang *et al.*, 2014). Here I identify three time scales for perturbations, especially related to weather and climate, affecting insect populations: long term (many decades), medium term (many years) and short term (within one or a few years).

Human induced climate change is an example of a long term perturbation. It is the result of $CO_2$ and methane levels in the air rising to the highest levels in over 420 000 years, and they are set to increase further (Petit *et al.*, 1999). The exact effects of $CO_2$ on the atmosphere are rather more complex than the simple 'greenhouse effect' that equates the atmospheric $CO_2$ to a glasshouse, but the impact is broadly similar (Lindzen, 1997). Climate change has resulted in a 0.85°C global warming over the last 130 years and this may increase a further 1.5-4.8°C depending on $CO_2$ levels (IPCC, 2013). All living organisms, particularly ectotherms such as insects, have a small range of thermal tolerances within which they can survive, grow and reproduce (Huang and Li, 2015). When these tolerances are exceeded, organisms become physiologically stressed and may die (Lynch and Gabriel, 1987). A variety of factors can influence the abundance and survival of *Helicoverpa* spp. including host plants, irrigation conditions, soil moisture content, temperature, and soil layer construction, parasitism, predation and pathogens, and winter temperatures (Cullen and Browning, 1978, Duffield and Dillon, 2005, Ge *et al.*, 2005, Liu *et al.*, 2009, Liu *et al.*, 2007, Mironidis and Savopoulou-Soultani, 2008, Parajulee *et al.*, 2004). While any of these factors may be influenced by climate change, species with a large geographical range (such as *H. punctigera*) may be more resilient to

these changes (Bale *et al.*, 2002). Such species are able to shift their ranges north or south to find more favourable conditions as long term climatic conditions change (Bale *et al.*, 2002).

Climate change driven by increasing CO2 levels is only one kind of weather perturbation. Other perturbations can occur on different temporal scales. Short term weather variability such as storms or frosts can both create new opportunities for species or inflict catastrophic disturbance (Andrewatha and Birch, 1954), while medium term multi-year weather patterns can also have serious long-term consequences (Chapter 5.1.1). Weather patterns such as drought can reduce the abundance of parasitoids attacking *Helicoverpa* spp. (Noor-Ul-Ane *et al.*, 2015), as well as changing the competitive interactions between plant species, particularly between annual and perennial plants (Besaw *et al.*, 2011). An example of a medium term perturbation due to weather is the Millennium Drought over eastern Australia, and particularly inland Queensland, which started in 2001 and lasted until 2009 (Queensland Department of Science Information Technology and Innovation, 2015) and after this period host plants for *Helicoverpa punctigera* may have changed (Chapter 5.3). Observations of medium term climate perturbations over the course of several years are relatively rare compared to studies of short term impacts of weather, and modelling studies of the impacts of long term climate change. However, such perturbations can potentially result in scenarios (in the medium term) at least as extreme as some climate change scenarios. To fully understand potential long term effects of changeable climates on *Helicoverpa* spp., we also need to better understand how these medium-term changes affect them. Observations over many years, perhaps decades, are needed to identify medium and long-term regional population fluctuations, the size of density dependant interactions and drivers of population dynamics, and to quantify the contributions of those population drivers in *Helicoverpa* spp. (Ouyang *et al.*, 2014). There is currently a relative lack of such studies, but one such example is the HIRG (*Helicoverpa* Inland Research Group) study. The HIRG was an informal research collaboration between the University of New England (P.C. Gregg), CSIRO (G. P.

Fitt), University of Queensland (M. P. Zalucki) and Queensland Department of Primary Industries (P.H. Twine and D. A. H Murray) which operated between 1987 and 1993. It collected data on the abundance and distribution of *Helicoverpa* spp. larvae and adults in inland Australia, summaries of which were published by Zalucki *et al.* (1994) and Gregg *et al.* (1995), and incorporated in GIS models by Rochester (1998) to predict the sizes of inland *H. punctigera* populations and the potential for migration to cropping areas, on an annual basis.

Agricultural practices have changed radically over the last 60 years. The spraying of broad spectrum pesticides has been the most prevalent method used to manage arthropod pests on high value crops since the 1950s, increasing in use through the 60s and 70s before peaking in 1982 (National Research Council, 1996). The occurrence of pesticide-resistant pests has emerged as a consequence of overuse of pesticides, forcing growers to use more pesticide, often ineffectually, or take a more integrated approach to pest management (Kogan, 1998). Resistance to DDT in *Helicoverpa* spp. was first discovered in 1972/1973 and contributed to the cessation of cotton production in the Ord River region in 1974. High levels of resistance to commonly used pesticides have developed in *H. armigera* which has led to difficulties in controlling it, both in Australia and overseas (Forrester *et al.*, 1993, Holloway and Forrester, 1998, Kranthi *et al.*, 2002, Gunning *et al.*, 2007). This has often led to economic damage in crops. Pesticide resistance in *H. punctigera* was not as pronounced as in *H. armigera,* and *H. punctigera* was described as 'managing its own resistance risk', because it is assumed that amount of gene flow from populations outside of sprayed areas is so vast (Forrester, 1994). However, this assumption may be challenged by changes in the agricultural landscape. Other changes may be driven by changing markets and opportunities in agriculture. For example, a large change in the Australian agricultural makeup came as lupin production, *Lupinus angustifolius* L. (a host for *H. punctigera*), in Western Australia expanded in the 1980s-1990s in response to new export opportunities and new production techniques. Production spiked at 1.5M tonnes in 1999 and then decreased to 0.2 M in 2006 as export prices dropped (Paterson and Wilkinson,

2015). This example illustrates how changes in agricultural practices affect the landscape for insect pests, then reactions to market forces can rapidly change it again.

Taken together or separately, both these factors (climate variability and agricultural change) drive population dynamics. Density dependent mechanisms are important in a variety of different species (Brook and Bradshaw, 2006), and while there are limited data on *Helicoverpa* spp., it is clear that mortality factors which often operate in a density-dependent manner can be important in their population dynamics (Brook and Bradshaw, 2006). Furthermore, the importance of density dependence has been demonstrated in many insect populations such as butterflies (Nowicki *et al.*, 2009) and the greenhouse pest *Trialeurodes vaporariorum* (Westwood) (Jiang *et al.*, 1999). Disruption of long-established density-dependant mechanisms means that the incidence of pest outbreaks is likely to increase (Ouyang *et al.*, 2014).

## *1.4 Helicoverpa* management in Australia

In 1989, damage from *H. armigera* and *H. punctigera* was estimated as causing over $A25 million per year (Davidson, 1989). In 1997, *Helicoverpa* spp. damage was mitigated by control methods that cost on average over $A225.2 million per year, and without control, *Helicoverpa* spp. could have caused $A817.9 million per year (Adamson *et al.*, 1997).

In the late 1990s some cotton growers were faced with spiralling costs of up to $A1000/ha to control insecticide resistant *H. armigera* (Murray, 2005). Ingard® cotton, genetically modified to express a *Bacillus thuringiensis* (Bt) toxin that killed larvae of *Helicoverpa* spp., was introduced in 1996/1997, initially comprising 30 000 ha (10% of Australian cotton) and with a licence fee of $A245. Uptake increased to 30% of total cotton grown, with decreasing licencing fees of $A155 in 1998/1999. The acreage was capped at this level to help manage potential resistance to Bt

toxins (Murray, 2005). In 2003/2004, Bollgard II® expressing two Bt toxins, was introduced and the cap was removed, with quick adoption by the industry from 16% in 2003/2004 to 70% in 2004/2005, then 96% in 2013 (Wilson *et al.*, 2013). Licence fees jumped from $A190 to $A250 in that same period, and in November 2014 were priced at $A315/ha (Monsanto, 2014).

It is likely that the cost of genetically modified varieties has largely replaced the cost of insecticides and of lost production from insect damage. Although there are not current figures on the total economic cost of *Helicoverpa* spp., we can multiply the $A315/ha by the 392 kha of cotton grown in 2013/2014 (Dowling, 2015), and taking into account that some of that cotton is dryland cotton where licence fees are lower, then we can estimate the cost of *Helicoverpa* spp. is over $100 million per year in licence fees alone.

In the late 1970s, SIRATAC (C**SIR**O and Department of **A**griculture **TAC**tics) was developed to aid in cotton pest management (Fitt and Wilson, 2005). SIRATAC was a computerised decision support system that combined pest abundance with a *Helicoverpa* feeding model in order to predict the economic damage that might be caused, along with the effects of pest-control interventions (Brook and Hearn, 1990). Although SIRATAC was adopted on only 25% of the cotton area in Australia (Davidson, 1989), it paved the way for other integrated pest management practices. In 1992, the EntomoLOGIC decision support system was released by CSIRO. It incorporated the principles of SIRATAC, providing up-to-date decision-making support to growers based on the best available research at the time (Deutscher and Plummer, 1998). EntomoLOGIC was followed by the CottonLOGIC (Bange *et al.*, 2004, Mackrell *et al.*, 2009) suite of decision support products, including Palm OS, desktop and web apps designed to improve operations, ordering of sprays, automatic updating and sharing of data between client and consultants. Unfortunately, the closure of the Cotton Management Support Systems team caused development of CottonLOGIC to be halted in May 2007 (Bange *et al.*, 2007).

Another critical point in the adoption of integrated pest management practices was when *H. armigera* developed resistance to some insecticides, which caused a failure of control in 1983/84. Growers needed a new strategy to continue to control *Helicoverpa* (Forrester *et al.*, 1993). Resistance management schemes were developed, where chemical pesticides with different modes of actions were rotated, in order to reduce selection pressure on *H. armigera* towards resistance to all pesticides used (Forrester *et al.*, 1993). Hoque *et al.* (2000) demonstrated that using 'soft' pesticides, with reduced impacts on natural enemies of cotton pests, was 42-44% less expensive overall compared with the more damaging pesticides. Soft pesticides typically cost more than conventional chemicals per spray, but fewer sprays were needed for better yields. The average gross margin for Ingard® cotton was increased by 25% compared to 'hard' pesticides. Less harmful pesticides were shown to be effective at controlling cotton pests, while avoiding pesticide resistance issues by using different modes of action to conventional sprays (Hoque *et al.*, 2000, Hoque *et al.*, 2003). Overall use of all pesticides has been reduced, and in particular the use of broad spectrum insecticides has been reduced in favour of target-specific pesticides that have less impact on the natural enemies of pests (Fitt, 2000, Hoque *et al.*, 2000, Whitehouse *et al.*, 2005, Mansfield *et al.*, 2006, Baker *et al.*, 2008, Baker and Tann, 2014).

Integrated pest management (IPM) involves the utilisation of the most financially and ecologically effective means to combat a pest problem, leading to a stable and satisfactory solution (Kogan, 1998). Integrated control requires the application of chemical control used in the manner least disruptive of biological control (Stern *et al.*, 1959). At a grower level, IPM is relevant to all crops. IPM requires monitoring and better record-keeping, better hygiene, choosing better chemicals and using fewer chemicals (Goodwin, 2000). IPM practices are typically implemented at a site-management scale, but when considering multiple properties or an entire region, area-wide management can be a more coordinated strategy (Faust, 2008). For IPM to use an area-wide approach, each site needs a pest-management strategy including preventative, avoidance, detection and suppression measures (Stall,

1999). At an area-wide level, IPM can tackle pest problems on a scale larger than a single farm. Area-wide strategies can monitor the pest population in a region and take measures to prevent the outbreak of pests (Knipling and Stadelbacher, 1983), or even attempt to eradicate a pest from a region (Vreysen *et al.*, 2000, Smith, 1998). To obtain these outcomes, area-wide management practices have used traditional biological control, biorational pesticides, crop host resistance, cultural practices, physical and mechanical controls as well as both broad spectrum and reduced risk pesticides (Faust, 2008). These control measures are often combined with predictive models and expert systems (such as EntomoLOGIC and CottonLOGIC described above) in order to integrate and coordinate area-wide management strategies with growers' IPM schemes (Faust, 2008).

The uptake of transgenic plants expressing the toxin genes from the bacterium *Bacillus thuringiensis* (Bt) by the Australian cotton industry can be considered an area-wide control strategy targeting *Helicoverpa* species. By controlling how much Bt cotton can be grown on a property relative to refuge crops or conventional (non-GM) cotton, resistance management strategies can prolong the life of this highly effective control method (Gregg and Wilson, 2008).

Transgenic crop varieties have been successful in reducing pesticide use in crops such as cotton, corn, potato, rice, maize and oilseed rape (Romeis *et al.*, 2006). Cotton containing Bt toxin genes now represents more than 96% of Australian cotton (Wilson *et al.*, 2013). Bollgard II® expresses the Cry2Ab and Cry1Ac toxins to control key cotton pests worldwide. According to theoretical models, by combining two or more different toxin genes of the same variety in the same organism, a process known as "pyramiding", the development of toxin resistance in the target pests will be delayed (Roush, 1998). Despite this strategy, resistance to Bt toxins Cry1Ac and Cry2Ab has already been discovered in populations of *H. armigera* and *H. punctigera.* However, resistance frequencies have yet to rise to the extent that economic damage to crops occurs (Downes *et al.*, 2010b). While some surviving larvae of *Helicoverpa* spp. are occasionally seen in Bollgard II® crops, there is no

evidence that their survival is due to resistance. It is thought to be due to variable expression of the Bt toxins interacting with feeding behaviour of the larvae (Lu *et al.*, 2011). Downes *et al.*, (2010b) discovered that populations of *H. punctigera* from cropping areas had higher frequencies for resistance alleles to Cry2Ab than *H. punctigera* in non-cropping areas, and that the frequency of the allele was higher than alleles providing resistance to Cry1Ac. The presence of these resistance alleles in non-cropping inland populations could potentially be explained by either back migration or a natural exposure to Bt toxins in inland Queensland.

Resistance to Bt proteins probably occurred simply though random mutation. Mutation-selection theory predicts that a resistance gene could develop in the population and if the fitness cost of that mutation is low, then a non-zero equilibrium resistance allele frequency could develop in the population (Clark, 1998). In this scenario, Bt resistance alleles could have already been present in the population before the proteins were introduced into Australian cotton (Downes *et al.*, 2010b). An agent other than Bt cotton may have affected *H. punctigera* populations, contributing to selection for resistance alleles against Cry2Ab, a notion that is supported by the presence of Hp4-13-like resistance alleles in *H. punctigera* populations from non-cropping areas (Downes *et al.*, 2010a).

Management of resistance to Bt toxins is a major challenge to sustainability of Bt cotton, particularly when Bt resistance in one moth pest, *Plutella xylostella* L., has already evolved (Tabashnik, 1994, Raymond *et al.*, 2013), resistant *H. armigera* have been created in the lab (Kranthi *et al.*, 2000), and Bt toxin-resistance alleles for *H. punctigera* and *H. armigera* exist in Australian field populations (Downes *et al.*, 2010b). Refuges play an important part in resistance management by preserving a population of non-resistant *Helicoverpa* that can mate with potentially resistant individuals and dilute or delay the rise of resistant alleles in a population (Baker *et al.*, 2008, Downes *et al.*, 2010a, Baker and Tann, 2014). Pigeon pea appears to be the most important refuge crop, with a recent study evaluating it as the superior

refuge crop based on number of live pupae and pupal casings from emerged adult moths recorded from this crop (Baker and Tann, 2014).

## 1.5 Overwintering ecology

Although both *H. punctigera* and *H. armigera* are capable of long-distance migration (Farrow and Daly, 1987), the amount of migration into cropping area can vary considerably from year to year (Scott *et al.*, 2005). In irrigated areas of Northern New South Wales and Southern Queensland *Helicoverpa armigera* pupates in the autumn and undergoes a diapausing phase (Wilson *et al.*, 1979, Kay, 1982, Wilson, 1983), while reports of *H. punctigera* overwintering in the same region have historically been very rare (Wilson, 1983, Fitt and Daly, 1990). In early spring *H. punctigera* moths migrate to eastern cropping regions from inland Queensland (Gregg *et al.*, 1995). In Western Australia, *H. punctigera* build up in the central and southern arid zones on annual wildflowers that germinate after autumn and early winter rains (Walden, 1995). One generation is completed and the host plants senesce before moths emerge at the end of winter and migrate across Australia (Walden, 1995), potentially travelling over 2000 km over 2-3 nights (Gregg, 1993). Adult moths appear synchronously over the southwestern cropping regions of Western Australia at the beginning of spring in Western Australia (Walden, 1995).

Overwintering *H. punctigera* has historically been extremely rare in Namoi cotton crops from 1972-1983, with pupae only being found in one year of the nine studied (Wilson, 1983) and less than 1% of pupating *Helicoverpa* spp. being *H. punctigera* (Fitt and Daly, 1990). There are no comparable studies for overwintering of *H. punctigera* in recent years, but we know that from 1996 to 2003 throughout the season, 6.3-10.3% of total moth emergences from cotton or pigeon pea were *H. punctigera*, with population pockets in certain areas where up to 90% of pupae were *H. punctigera* (Baker *et al.*, 2008). In 2006-2012 this had increased to 21.4-

27.4% (Baker and Tann, 2014). Whether this represents an increase in overwintering populations remains to be determined.

When left unchecked, *Helicoverpa* pupae in the soil emerge as adults (early spring for *H. punctigera*, and mid to late spring/early summer for *H. armigera* (Zalucki, 1991)), to mate and find host plants to deposit eggs on, alongside *Helicoverpa* populations of immigrant origin (Fitt and Daly, 1990). In any population of *Helicoverpa* with both local overwintering and immigration contributing to the genetic makeup, there will be an increase in resistance alleles in the overwintering population and a reduction in resistant alleles from immigrants diluting the resistant population (Daly and Fitt, 1990). In the USA, changes in overwintering have been shown to influence resistance to transgenic Bt corn in *Diabrotica barberi* Smith & Lawrence (Chrysomelidae: Coleoptera) (Mitchell and Onstad, 2005). Overwintering larvae are subjected to selection pressure for resistance to Bt toxins which can carry over to the next season where their progeny can again be exposed to selection. To reduce the risk of toxin resistance becoming widespread, rigorous resistance management plans are used by Australian cotton growers. The area-wide management strategy known as “pupae busting” is one such strategy, which reduces the number of *Helicoverpa* pupae in the soil using mechanical means, eliminating potentially resistant adults before they reproduce (Duffield, 2004).

Studies on the effects of overwintering and diapause of *H. punctigera* have been largely absent from the literature over the last 20 years compared to *H. armigera*. The literature currently considers that winter breeding and winter-spring immigration occur in and from inland Australia, beyond cropping areas (Zalucki *et al.*, 1986, Gregg, 1995). Spring migrants from inland Australia (Gregg *et al.*, 1995) to these areas breed on spring weeds and crops of chickpea and canola before moving to other crops, including cotton, in late spring to early summer (Fitt and Daly, 1990). From mid-summer onwards the population gradually declines in abundance over the second half of summer. The magnitude of seasonal winter rainfall in inland western Queensland may determine the abundance of the migrant population of *H.*

*punctigera* in cropping areas in spring (Oertel *et al.*, 1999, Maelzer *et al.*, 1996). Winter rain has been described as having a positive effect on the size of second and third generation migrant populations while rain in spring and early summer has negative effects on migrant populations (Maelzer and Zalucki, 1999).

Data from pheromone traps and light traps near the Australian Cotton Research Institute (ACRI) in Narrabri NSW, collected from 1992-2002 by Baker *et al.* (2011) suggest *H. punctigera* is more common later in the summer growing season than originally thought. There is some disagreement between experts on the interpretation of the correlation found by Baker *et al.* (2011) between the numbers of *H. punctigera* at the start of a season and the numbers at the beginning of the following season. Baker *et al*. (2011) proposed that an increasing number of *H. punctigera* may be overwintering in cropping areas. However, an alternative explanation may be that this pattern is related to an overall decrease, across several seasons, in numbers of *H. punctigera* at all stages of the season (P. Gregg, Pers. Comm. 2014). Baker *et al.* (2011) also did not find evidence supporting the relationship between autumn and winter rainfall in central Australia and the abundance of *H. punctigera* moths in the following spring, that had been previously found by Oertel *et al.* (1999). There is debate about the relative importance of spring immigration from the inland versus local overwintering in contributing to the numbers of *H. punctigera* present in the following cotton season. Further data are required to determine the relative contributions of each source.

My thesis research project aims to contribute to understanding these issues by comparing data on the abundance of *H. punctigera* host plants in inland regions over the three years of candidature with the data collected during the 1980 to 1990s, when migration was thought to be extensive. It also aims to investigate the mechanisms and extent of overwintering in both cropping and inland regions.

## 1.6 Overwintering: diapause or quiescence?

Photoperiod and temperature are key factors in the regulation of seasonal cycles in insects (Orlova, 1998). Dormancy is a generic term that refers to a state of suppressed or arrested development (Danks, 1987) which can be induced by either cold or warm temperatures, which are often paired with short photoperiods or long photoperiods, respectively (Sarwan and Saini, 2007). Individuals in wild insect populations are exposed to decreasing photoperiods and lower temperatures in autumn and winter months, which may induce a state of dormancy (Tadmor and Applebaum, 1971). A state of dormancy is usually accompanied by metabolic suppression (Danks, 1987) and can be adaptive either ecologically or in the evolutionary sense.

Historically, use of the term diapause has been varied and often inaccurate (Kostal, 2006). Originally used to describe periods of arrest in ontogenic development, the term later developed two meanings, which are now defined as diapause and quiescence (Kostal, 2006). The cessation of development as a direct response to unfavourable conditions is now referred to as quiescence while the arrest of physiological or reproductive development is now referred to as diapause (Danks, 1987).

Quiescence is an immediate response to the decline of a limiting environmental factor below a physiological threshold with immediate resumption once conditions return to above the threshold (Saunders, 1982). Diapause, in contrast, is a programmed response to environmental factors, indirectly shifting the course of physiological development away from direct morphogenesis (Danks, 1987) and into an alternate sequence of physiological events (Kostal, 2006). Development does not always stop during this alternate sequence of events, but may continue at a slower rate, or growth may continue without development, or extra instar stages may be added to the larval development of the insect (Kostal, 2006). Typically, the onset of the diapause state precedes the start of adverse conditions and is most commonly

induced by reduced photoperiod and, at the population level, may result in the synchronisation of seasonal activities of that population (Saunders, 1982).

Dormancy can encompass quiescence and diapause and the term should be used when either diapause or quiescence may be occurring, or when neither of those alternative terms can be applied to the physiology of a given arthropod with certainty (Kostal, 2006).

By exposing larvae to different photoperiods Cullen and Browning (1978) determined the conditions under which diapause is induced in *H. punctigera*. When adults or eggs are exposed to long-day photoperiods, pupal diapause can be induced by exposing larvae to short day photoperiods (Cullen and Browning, 1978). Exposing pupae to temperatures in the range of 26-29°C can bypass this diapause response however.

## 1.7 Aims of the project

The aims of the work reported in this thesis were to:

1. Perform laboratory studies to confirm earlier work, or to determine if the physiological response to photoperiod and temperature, and the onset of diapause, in *H. punctigera* has changed. This project aim involved exploring some of the experiments of Cullen and Browning (1978) and confirming or refuting them with my own data to create a statistical model for the onset of diapause. These experiments involved exposing newly hatched *H. punctigera* larvae to constant photoperiod/temperature conditions and observing the development of the pupal stage where diapause can occur.
2. Investigate overwintering of *H. punctigera* in the local cotton growing region of the Namoi catchment in northern New South Wales. Although rare relative to *H. armigera*, *H. punctigera* does overwinter in cropping areas. This project aim sought to understand the conditions and timing of diapause under field conditions. Field studies used emergence cages to monitor the

timing of emergence of *H. punctigera* adults from the soil in cropping areas. This was combined with direct sampling of pupae through digging in fields.

3. Investigate how *H. punctigera* overwinters in inland Western Queensland before migrating south into cotton regions. This involved travelling to western Queensland and sampling vegetation in the region for *H. punctigera* larvae, in order to determine what hosts they survive on, as well as setting up emergence cages to determine the timing of emergence from pupae.
4. Aggregate historical survey data from the HIRG program (Gregg *et al*., 1995) with more recent *Helicoverpa* surveys of inland western Queensland and examine how the landscape may have changed using geographical information system (GIS) software. The HIRG project collected data on *Helicoverpa* from 1989-2000 while more recent surveys have been undertaken from 2009-2014. These data have never before been combined together in a cohesive database with appropriate weather data sets, in order to examine how the host plants of *H. punctigera* may have changed over the medium term, and what implications this may have had for its population dynamics.

# Chapter 2: Effects of varying temperatures and photoperiods on diapause induction under laboratory conditions

## 2.1 Introduction

### 2.1.1 Diapause in *Helicoverpa* spp. internationally

Diapause is the most important mechanism for winter survival in *Helicoverpa* spp. Knowledge of the conditions that can induce or terminate diapause assists in interpreting temporal patterns of diapause incidence and predicting post-diapause populations (Murray and Wilson, 1991).

Diapause in the American species, *Helicoverpa zea,* can be induced in pupae by exposing eggs, larvae or adults to a sequentially decreasing photoperiod (Pullen *et al.*, 1992). Diapause can be averted if pupae are exposed to 27°C, regardless of photoperiod (Pullen *et al.*, 1992). Lopez and Hartstack (1985) studied both American Heliothines together and found them to have similar diapause patterns and reported two phases of winter diapause. Phase 1 occurs at low temperatures during autumn and early winter that induces the diapause state in pupae and Phase 2 occurs at higher temperatures to terminate diapause (Lopez and Hartstack, 1985). While *H. zea* is limited to the Americas, the closely related species *H. armigera* is distributed over the continents of Australia, Asia, Africa and Europe (Fitt and Cotter, 2005), and more recently, it has also spread to the Americas as well (Kriticos *et al*., 2015). In comparison to *H. zea,* there are only slightly fewer 'papers' published on the topic of diapause/overwintering in *H. armigera* (66) compared to *H. zea* (73) (ISI Web of Knowledge search, 2014). African *H. armigera* can develop continuously in tropical climates, but undergo diapause at 22°C under a 12L:12D photoperiod, which can be averted by exposing pre-pupal or early-stage pupae to 26°C (Hackett and Gatehouse, 1982). A higher percentage of diapausing pupae is induced in a population at 18° under a 12L:12D than under 15L:9D or 9L:15D (Roome, 1979).

These onset conditions for diapause are an adaptation to autumn conditions to allow *H. armigera* to better survive cold, dry conditions over winter months (Roome, 1979).

### 2.1.2 Diapause in *Helicoverpa* spp. in Australia

In Australia, *H. armigera* have been recorded in autumn crops of cotton, sorghum, maize, soybean, mung bean and sunflower and subsequently overwintering (in a state of diapause) in the soil underneath those crops (Wilson, 1983, Lloyd *et al.*, 2008). Diapause starts being induced in populations from Toowoomba, Queensland (27.57S, 151.95E) in March, increasing in proportion to late April (Kay, 1982). Algorithms which relate daylength to latitude and season show that this translates to diapause starting to be induced at a ~12.5L:11.5D photoperiod (mid-March), and most of the population being in a state of diapause after a photoperiod of ~12L:12D (last week of March) (U.S. Military, 2014). Similarly, Wilson (1979) found that over 80% of *H.* armigera populations in the Namoi valley region, New South Wales, were diapausing from late April to May (~11L:13D photoperiod). Temperatures above 17°C were required to break diapause (Wilson *et al.*, 1979). These observations of Australian *H. armigera* appear to differ slightly from observations of African *H. armigera*, with Australian populations undergoing diapause at shorter photoperiods. This may be explained by the differing climates and photoperiod ranges between the two regions. Data on the specific onset conditions of diapause in *H. punctigera* have not been published since Cullen and Browning (1978), Browning (1981) and Murray (1991). *H. punctigera* undergoes diapause in response to either temperature or photoperiod, or a combination of the two (Cullen and Browning, 1978, Murray, 1991). The percentage of diapause in *H. punctigera* exposed to a specific photoperiod increases the closer the conditions get to 12L:12D (Cullen and Browning, 1978, Murray, 1991), and similarly, the percentage of diapause increases the closer temperature gets to 19°C (Cullen and Browning, 1978). A combination of a 12L:12D photoperiod and 19°C temperature induces at least 90% of a population to diapause (Cullen and Browning, 1978). Exposing pupae

to temperatures in the range of 26-29°C can bypass this diapause response however. Under field conditions at Toowoomba diapause in *H. punctigera* was found to be at its highest between mid-April and mid-June (Murray, 1991), where natural photoperiods in that region were between 12L:12D and 12.5L:11.5D at the time of the study (U.S. Military, 2014) . Murray (1991) examined diapause in *H. punctigera* and *H. armigera* in field cages at Toowomba, but only obtained limited data for diapausing *H. punctigera* because few individuals terminated diapause at the highest temperature tested (20.2°C), while none did at the lower temperatures tested (16-18, 19°C). Males and females of both *H. punctigera* and *H. armigera* were also found to have small but significant differences in the percentages of diapausing pupae under the same conditions (Murray, 1991). This observation is also present in *H. zea* (Pullen *et al.*, 1992) but is not explored by Cullen and Browning (1978) or Browning (1981).

### 2.1.3 Aims

1. This chapter aims to examine the effects of temperature and photoperiod and how they induce diapause in *H. punctigera* pupae under laboratory conditions.

2. At the same time, this chapter also considers the work of Cullen and Browning (1978), and to a lesser extent Murray (1991) as a starting point to first compare with, and then extend the knowledge of diapause in *H. punctigera.*

3. This chapter also aims to produce a predictive model for calculation of diapause in *H. punctigera*, as requested by the funding body, CRDC.

## 2.3 Materials and Methods

### 2.3.1 Determination of diapause

Cullen and Browning (1978) detailed two methods for the determination of diapausing *H. punctigera* pupae which were used in the experimental protocols in this chapter:

1. Eye spot method (Fig. 2)

When *H. punctigera* first pupates, a series of four 'eyespots' are initially visible (Stage A). As development progresses, the eyespots descend to the bottom of the eye (Stages B and C), disappear (Stage D), then the eye turns dark (Stage E). Diapausing pupae can clearly be determined if they have not passed Stage A after a certain period, which is 2-5 days at 28°C. Several authors have used the Cullen and Browning (1978) eyespot method to designate *H. armigera* pupae as being in diapause after 12-15 days with no movement of the eye spot (Chen *et al.*, 2014, Wu *et al.*, 2010, Mironidis and Savopoulou-Soultani, 2012).

2. Length-of-diapause method

The second method involves allowing a population of pupae to complete development. If pupal emergence took longer than 20 days at 28°C or 50 days at 19°C, they were considered in diapause. This method of diapause determination has the disadvantage of producing borderline diapause cases, where pupae on the threshold of the development time might provide false positive or false negatives (Murray and Wilson, 1991), and this may explain why recent publications (Chen *et al.*, 2014, Wu *et al.*, 2010, Mironidis and Savopoulou-Soultani, 2012) have not used this method in studies of diapause in *H. armigera*.

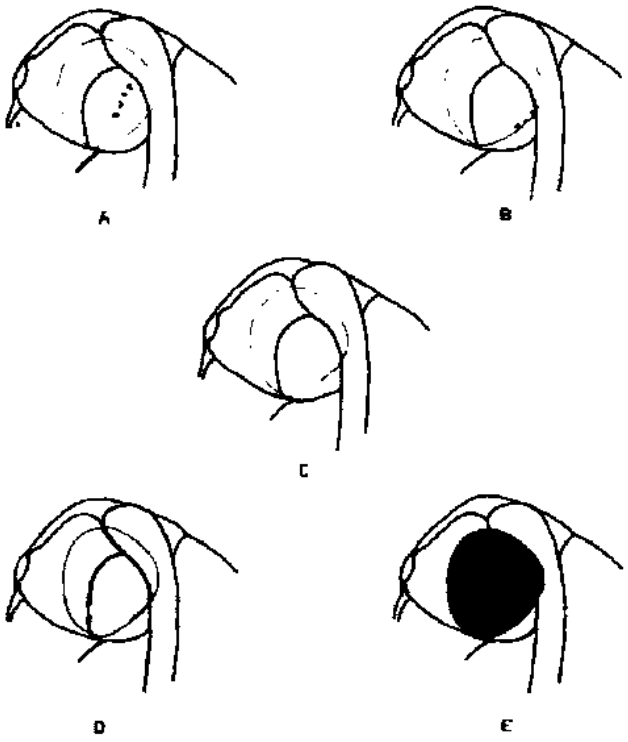



*Fig. 2. The stages of eyespot development in* H. punctigera *from A to E. From Cullen and Browning (1978).*

## 2.3.2 Insect cultures

Insects in culture were reared on an artificial diet similar to that described in Teakle (1991). Insect cultures received fresh genetic material in the form of *H. punctigera* larvae collected near Bedourie, Queensland (24.22S, 139.74E), approximately every nine months.

Up to 100 moths of mixed sexes were placed in a mating cage (Fig. 3) provided with dental wicks held in a plastic container with 10% sucrose solution as food. The cage consisted of a cylindrical frame with paper towel roll which served as oviposition substrate. The paper was scrolled daily to collect the eggs for rearing. The cages were left for 48h before eggs were used in the culture, to allow time for females to mate and lay fertile eggs. The paper towel with eggs was cut into pieces about 6 x

12 cm and  surface sterilized in 0.2% sodium hypochlorite (bleach) solution and carefully agitated with a soft brush for two minutes, then rinsed with distilled water onto filter paper using a Büchner  funnel. The eggs were placed in a 25°C controlled environment room in a 750ml round plastic takeaway food container (SK-30, SK Plastics Company Ltd, Shunde, Foshan, Guangdong Province, PR China) coated in artificial diet. Once the eggs hatched and the larvae were larger than 4mm they were transferred individually to a 35-ml plastic cup (Solo Cup Company, Urbana, Illinois, USA) containing 15ml of diet, using a soft haired brush. Once a larva was placed inside a cup, it was sealed with a plastic lid in which small holes were made to provide ventilation.

Three separate cultures of *H. punctigera* were maintained at different developmental stages to provide continuous supply of experimental larvae. Each batch of culture was periodically merged with the others in order to reduce the risk posed by genetic drift/inbreeding.

*Fig. 3. A mating cage used to obtain* H. punctigera *eggs by placing adult moths inside the chamber for a 3-5 day period. The paper sides of the cage were scrolled to obtain eggs while keeping the adults in the cage.*

### 2.3.3 Experimental cabinets

A number of controlled-environment cabinets were used for the experiments. Two TREIL 140-1-SD (Thermoline, Sydney, New South Wales, Australia) cabinets, one ICC18 (Labec, Sydney, New South Wales, Australia), and one retrofitted ICC36 (Labec, Sydney, New South Wales, Australia) were used to provide a range of photoperiods and temperatures (Table 1). These cabinets are hereafter referred to by the abbreviations given in Table 1.

In addition to these cabinets, which could only be set at one temperature at once, a water-jacketed multiple gradient temperature cabinet (Linder and May, Brisbane, Australia) with a horizontal trough and clear plexiglass removable box lids, was used to create three different temperature regimes under the same photoperiod (Table 1). Constant temperatures were produced in different compartments by adjusting the partitions made from PVC sheet. Two thermostatic controls at each end of the cabinet controlled the temperature gradients in the cabinet. Temperature and humidity in all cabinets were monitored and recorded using TinyTag View 2 TV-4500 data loggers (Gemini Data Loggers, Chichester, UK). The daily average fluctuations from the set temperature for each cabinet were calculated and listed in the table below (Table 1). Use of the TREIL cabinets was minimised once their lack of accuracy was known, and the ICC18 was purchased to replace them. The limitations of the TREIL cabinets are discussed further in Section 2.5.

### 2.3.4 Experimental protocol

Three trays containing 25 plastic cups were prepared, each cup containing 9ml of artificial diet. Excess diet was provided to experimental insects, relative to the insects reared for culturing, to ensure that neither desiccation nor lack of diet could affect the possible onset of diapause. Each cup received one larva, up to 24h old, which was sealed inside using the lid. Each tray of larvae was then placed in a controlled environment cabinet. Each treatment used 75 larvae, and between 12% and 52% of these reached the pupal stage (Table 3).

*Table 1. List of controlled environment cabinets and their average daily temperature fluctuations from the set value.*

| Cabinet | Alias | Accuracy |
|---|---|---|
| Retrofitted Labec ICC36 | 'Blue' | ± 1.0°C |
| 'Silver' Labec ICC18 | 'Silver' | ±0.6°C |
| TREIL Thermoline 140-1-SD | 'TREIL' | ±2.5°C |
| Linder and May Multiple Gradient Cabinet | 'MGC' | ±1.2°C |

Each larva was checked for pupation daily and, once pupation was observed, the pupa was placed in a new cup, covered in vermiculite and placed in a long-day controlled environment room (14L:10D, 19°C). The duration of the pupal stage was calculated by subtracting the date of emergence from the date of pupation. Pupae were checked once a day at 11:00h for pupal development and emergence. Eyespot movement was observed at the time of pupation and then every 48h afterwards using a microscope.

Only a limited number of controlled environment cabinets were available at any given time, so only a maximum of three different photoperiod/temperature combinations could be examined at any one time. The first series of experiments first examined a range of different photoperiods ('P1', 11:13, 12.5:11.5, 14:10), before setting the photoperiod at the optimum for inducing diapause while varying the temperature ('E1' to 'E8') (Table 2). The MGC was also only able to use one photoperiod at one time, but could use three temperatures at once. The MGC could not directly be set to a specific temperature, but rather needed to be calibrated by adjusting the heating/cooling elements using analogue controls and observing the temperature range produced in each compartment, prior to an experiment.

*Table 2. List of photoperiod and temperature regimes that larvae were exposed to after being reared at 14L:10D, 25°C.*

| **Experiment Code** | **Photoperiod Hours Light: Hours Dark** | **Temperature** | **Apparatus** |
|---|---|---|---|
| P1 | 11:13, 12.5:11.5, 14:10, 16:8 | 25°C | TREIL 140/Blue |
| E1 | 12:12 | 25°C, 20°C, 22°C | Blue/Silver/TREIL |
| E2 | 12:12 | 25°C, 22°C, 19°C | MGC |
| E4 | 12:12 | 22°C, 19°C, 16-18°C | MGC |
| E5 | 12:12 | 19-22°C | Blue |
| E6 | 14:10 | 25°C, 19°C, 17°C | MGC |
| E7 | 14:10 | 19°C, 20°C, | Blue/Silver/TREIL |
| E8 | 14:10 | Larvae at 19°C moved to 25°C. Larvae at 25°C moved to 19°C | Blue/Silver |

## 2.3.5 Statistical analysis

Confidence intervals at 95% were applied to the photoperiod/temperature regimes to show which treatments were significantly different from each other (Devore and Peck, 1993). A confidence interval for a population characteristic is an interval of plausible values for the characteristic, constructed so that the true value will be captured inside the interval, with a 95% probability in this case (Devore and Peck, 1993). The confidence interval was calculated using the following equation:

$$95\%\ \text{Confidence interval for mean}\ \mu = \bar{x} \pm 1.96\ \frac{\sigma}{\sqrt{n}}$$

Where $\bar{x}$ is the normally-distributed sample mean, $\sigma$ the standard deviation of the sample, $n$ is the number of values and + and – are the upper and lower limits of the interval.

### 2.3.6 Statistical modelling

In order to create a predictive model of *H. punctigera* diapause, several modelling techniques were used. Generalised linear models using R 3.1.2 (R Development Core Team, 2014) were used to model the data, as well as "Akima's polynomial method", a contour map representation of data, using Minitab Release 14 (Ryan *et al.*, 2006).

## 2.4 Results

Using the eyespot method, temperatures of 25°C induced the least amount of diapause in laboratory-reared *H. punctigera.* Within 25°C regimes, the highest percentage of diapause was induced at a 12L:12D photoperiod. Conversely, temperatures of 19°C or lower produced the highest percentages of diapause, even under a summer 14L:10D photoperiod. The highest percentages of diapause were induced when a 12L:12D photoperiod was combined with temperatures below 19°C (Table 3). When larvae and pupae were held under different temperature regimes, a decrease in temperature (larvae reared at 25°C moved to 19°C upon pupation) resulted in a higher percentage of diapause than at 25°C regime alone. An increase in temperature (larvae reared at 19°C then moved to 25°C upon pupation) did not appear to avert diapause (Table 4, 75% diapause).

When the two methods were compared, the eyespot method was found to detect a higher percentage of insects in diapause under autumn and winter photoperiods/temperatures. This was as much as a 14% difference depending on treatment, or up to ~4 pupae (Table 3).

## 2.5 Discussion

### 2.5.1 Diapause methodology

Of the two methods used to determine diapause, the eyespot method was more reliable at producing evidence of diapause than the pupal duration method. The pupal duration method suffered from the impracticality of observing diapausing pupae for months on end, until the diapause duration broke without external stimuli of rising temperatures or photoperiods. The longer that a pupa stays in diapause, the more likely it is to expire before it breaks diapause and emerges as an adult, reducing the overall number of insects available in the results.

*Table 3. Response of* H. punctigera *pupae to different photoperiod and temperature regimes, with diapause determined using the eyespot development and pupal duration methods outlined above. "-" indicates no data.*

| Photoperiod (Light:Dark hours) | Temperature (°C) | N | Diapause % (Pupal duration) | Diapause % (Eyespot) | 95% Confidence interval (Eyespot) |
|---|---|---|---|---|---|
| 11:13 | 25 | 29 | 3% | 3% | ±6.6% |
| 12.5:11.5 | 25 | 34 | 0% | 0% | - |
| 14:10 | 25 | 39 | 0% | 0% | - |
| 14:10 | 19 | 37 | - | 65% | ±15.3% |
| 14:10 | 20 | 39 | - | 12% | ±10.5% |
| 16:08 | 25 | 27 | 5% | 19% | ±14.7% |
| 16:08 | 20 | 36 | 36% | 47% | ±16.3% |
| 16:08 | 22 | 27 | 22% | 26% | ±16.5% |
| 12:12 | 25 | 16 | 6% | 18% | ±19.1% |
| 12:12 | 19 | 35 | - | 74% | ±14.5% |
| 12:12 | 22 | 19 | 42% | 47% | ±22.4% |
| 12:12 | 16-18 | 9 | - | 89% | ±20.5% |

*Table 4. Percentage diapause in* H. punctigera *when larvae were transferred to different conditions upon pupation under a 14L:10D photoperiod.*

| Larvae | Pupae | N | Diapause % (Eyespot) | Confidence interval (Eyespot) |
|---|---|---|---|---|
| 25°C | 19°C | 33 | 21% | ±13.9 |
| 19°C | 25°C | 51 | 75% | ±12.0 |

This accounts for the differences between the two methods, with some insects that would have been counted as being in diapause with the eyespot method, being counted as dead for the emergence method. Insects in diapause were more likely to die than those not in diapause, because the pupal duration was longer, sometimes resulting in results skewed away from diapause. Given this limitation and the fact that other authors did not use the diapause duration method in *H. armigera* but rather the eye spot method, (Chen *et al.*, 2014, Wu *et al.*, 2010, Mironidis and Savopoulou-Soultani, 2012), there is sufficient cause to reject this method for the study of diapause in Heliothine species. This method might be more successful if the limits of 20 days at 28°C and 50 days at 19°C (Cullen and Browning 1978) are strictly adhered to, and if pupae have not emerged after 50 days, pupae are marked as 'in a state of diapause', and are transferred to 28°C to emerge.

The question can be raised as to whether pupae that die before eclosing are viable, and should be counted in the eyespot method. As long as the pupae remained alive (as determined by them being responsive to a gentle probe from fine forceps) after three weeks of daily observations they were counted as being viable, otherwise they were discarded from the results. Most pupae that would later die showed anomalies in their bodies, before they died, such as splits in the pupal casing or excessively dark pupal casing, which were noted in the observations of each pupa.

### 2.5.2 Controlled-environment cabinets

The TREIL cabinets were found to be unduly affected by external factors, particularly ambient temperature in the summer when some experiments took place. For this reason, care had to be taken to calibrate the TREIL by setting the cabinets several degrees below the intended range, and monitoring them for several days before use. The MGC also had some issues, in that at temperatures below 19°C, even within a 30cm compartment, one end of the same compartment maintained 16°C while the end other maintained 18°C, even though the temperature was relatively constant over the duration of the experiment. This

concern was addressed by recording the temperature as “16-18” (Table 3) and randomly placing each cup back into the MGC compartment every time a cup was checked for pupation of the larva inside.

### 2.5.3 Comparison with earlier studies

The data from the various studies at constant temperatures reported above are broadly comparable to those of Cullen and Browning (1978), with larger proportions of insects entering pupal diapause the closer that conditions get to 12L:12D and 19°C. In order to compare these data with those of Cullen and Browning (1978), selected constant photoperiod/temperature regimes are reprinted here for comparison (Table 5) and directly compared (Fig. 4).

Cullen and Browning (1978) used maximum temperatures of 28°C, while my study used a maximum of 25°C before transferring to 19°C. My study found a much lower proportion of diapause in larvae reared at 25°C and moved to 19°C (21%) compared to that of Cullen and Browning (1978), who found that 100% of larvae were in diapause when reared at 28°C and then transferred to 19°C. As part of my study, transferring larvae reared at 19°C to 25°C was also investigated, with no direct counterpart from Cullen and Browning (1978). The results of these two regimes would have been expected to be reversed, with transfer from a lower temperature to a higher temperature being expected to avert diapause, while transfer from higher to lower being expected to induce it. Half of these results can be explained with the addition of one critical piece of information, that 26-29°C is required to avert diapause (Cullen and Browning, 1978). Larvae reared at 19°C but transferred to 25°C would never reach the diapause-breaking temperature threshold. The low percentage of diapause in larvae reared at 25°C and transferred to 19°C can be explained by the photoperiod, which can avert diapause.

Although the work of Cullen and Browning (1978) has provided extremely important information on diapause in *H. punctigera*, a major criticism of that work is the lack of *n* values for their studies, as well as the lack of information on the

statistical analysis used to show significance at P>0.05 or greater. The only mention of numbers of insects used are in the second figure of Cullen and Browning (1978), where 300 pupae were exposed to 28°C for the purpose of observing adult emergence. This oversight in statistical rigor makes it hard to make any meaningful comparison of Cullen and Browning with the data in this study, outside of estimation.

*Table 5. Constant photoperiod/temperature regimes used in Cullen and Browning (1978) that are comparable to the constant regimes used in this study.*

| Temperature (°C) | Photoperiod (Light:Dark hrs) | Diapause (%) |
|---|---|---|
| 16.5 | 13:11 | 6% |
| 22 | 14:10 | 3% |
| 28 | 14:10 | 0% |
| 19 | 14:10 | 14% |
| 19 | 11:13 | 38% |
| 19 | 11.5:12.5 | 37% |
| 19 | 12:12 | 92% |
| 19 | 12.5:11.5 | 85% |

## 2.5.4 Replication and pseudo-replication

In this study, effort was made to replicate each experiment temporally at least twice, with data loggers used to ensure that conditions were consistent and comparable between replicates. It was not practical to use higher numbers of insects in each single cabinet, and there were not enough cabinets available to undertake every photoperiod and temperature at once. In the experiments in this chapter, I argue that each self-contained cell has its own microclimate, and even within the controlled environment cabinets, the random placement of each individual cup is a source of variability. A potential criticism of this chapter is the lack of 'true' replication within the controlled temperature cabinets. This chapter has focused on using a small laboratory population to predict large-scale populations of *H. punctigera* in the changing seasons of Australia. There is often a

lack of replication in ecological studies, and when the cost of replication is large, experiments involving unreplicated treatment may be the best or the only option (Oksanen, 2001).

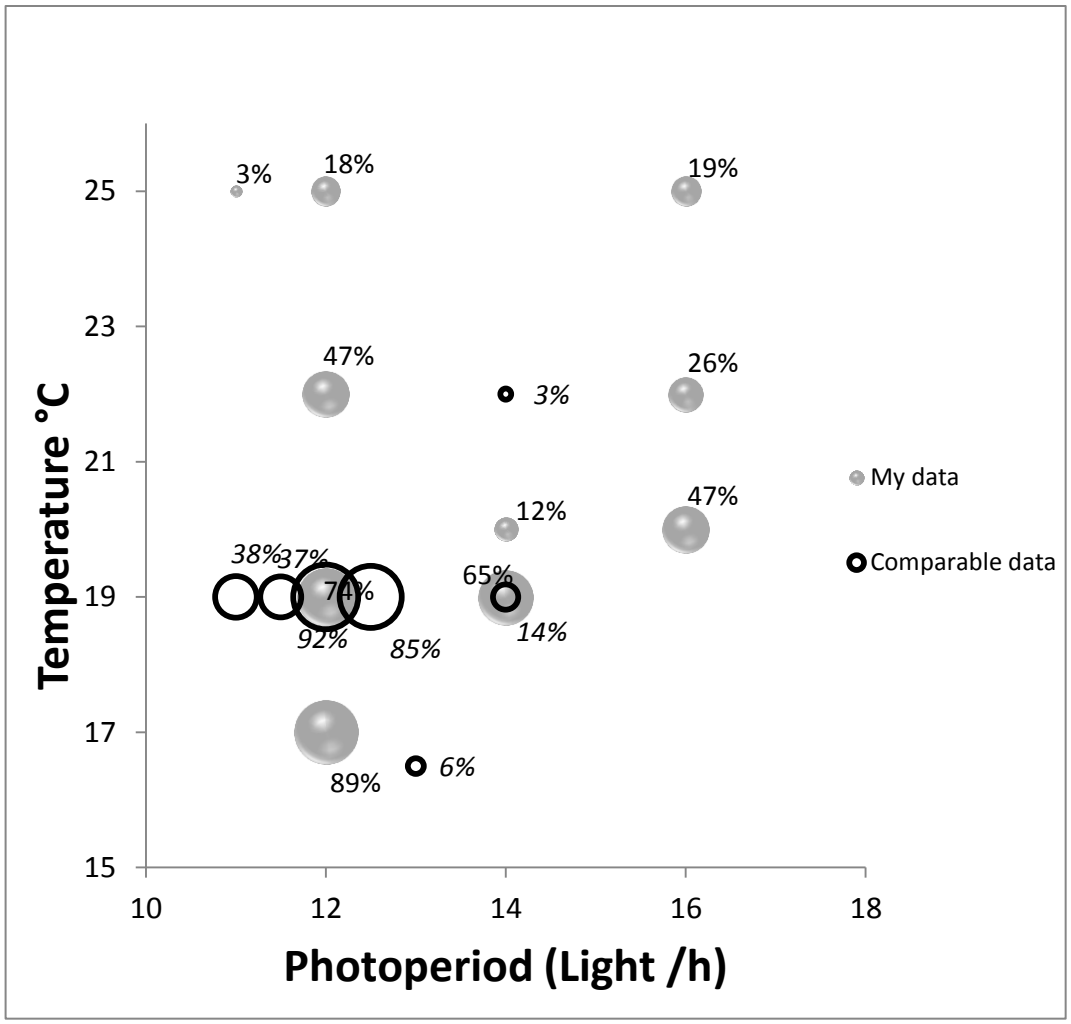



*Fig. 4. The results for photoperiod and temperature effects on diapause in* H. punctigera *from this study (grey bubbles) and comparable constant temperature data from Cullen and Browning (1978) (white circles). The size of the bubble denotes the % diapause.*

As long as tentative conclusions derived from unreplicated experiments are considered for what they are, rather than as a consequence of ignorance, we can make predictions based on these pseudoreplicated studies. There are no guarantees that microcosm studies like these, replicated or otherwise, can create

critical predictions of short-term ecological dynamics at massively reduced spatial scales (Oksanen, 2001), but they are useful to remove some conjectures on the ecology of *H. punctigera* and inform field research, such as those in subsequent chapters.

### 2.5.5 Statistical modelling of diapause in *H. punctigera*

Photoperiod and temperature were first explored as linear projections by displaying them as separate scatter graphs and applying a linear regression (Fig. 5). Comparable data from Cullen and Browning (1978) were added to the data from my study for comparison (Table 5). Linear plots of either factor are unlikely to truly represent the diapause response of *H. punctigera* if Cullen and Browning (1978) are correct and % diapause increases in a population the closer temperatures get to 19°C and 12L:12D. The data show that simple linear regressions fitted very poorly.

As expected there was very little correlation around photoperiod as a linear factor but a slightly greater, though still small, correlation with temperature. If diapause is expected to be distributed around the highest average of % diapause at 19°C and 12L:12D, we can apply a generalised linear model (GLM) within certain limits, to predict the % diapause of photoperiod/temperature regimes that were not present in the experiments.

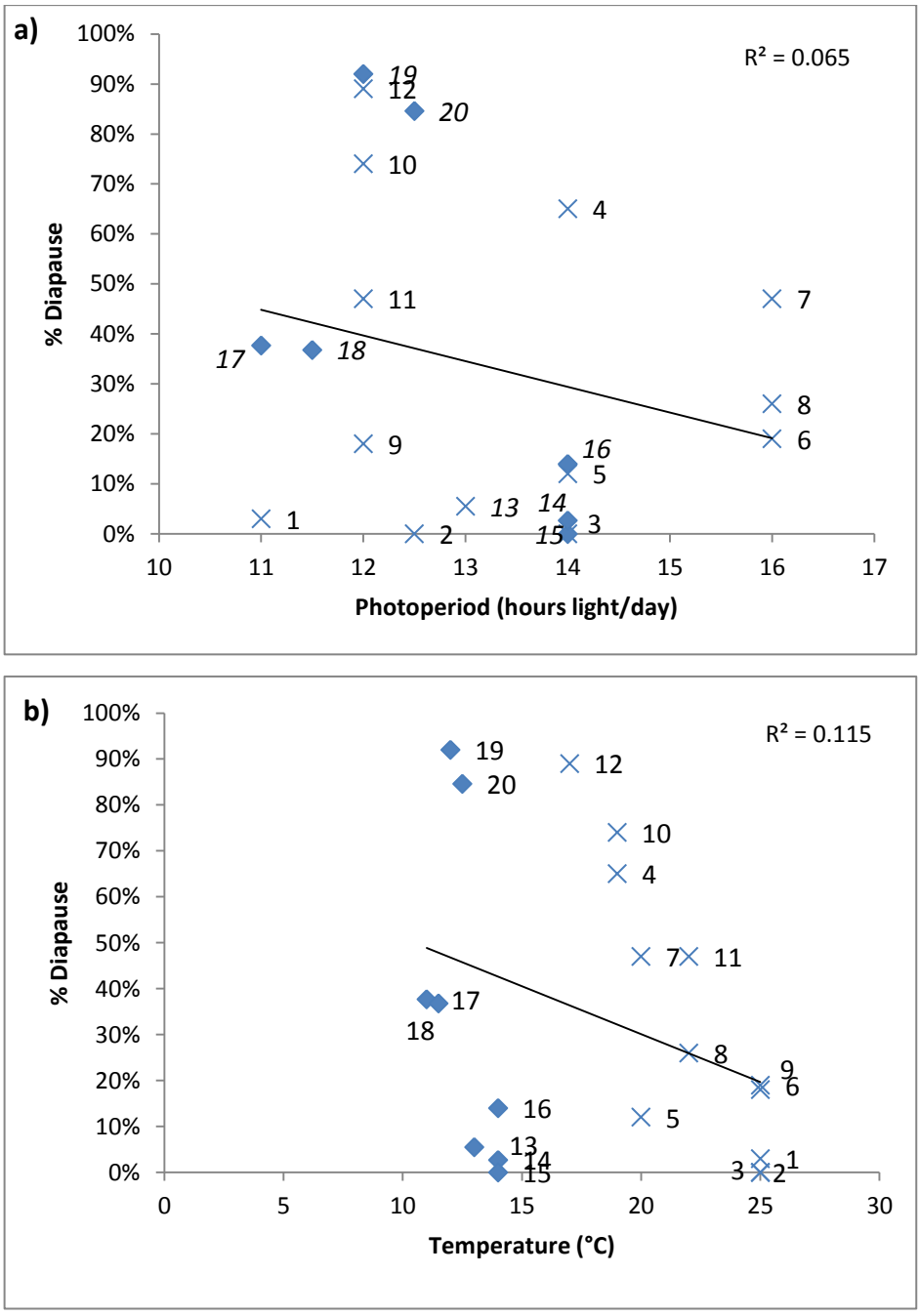



*Fig. 5. Photoperiod (a) and temperature (b) and the percentage of diapause induced in* H. punctigera*. Numbered points represent the same experiment in each graph. Points 1-13 are from Table 3 (X) while 14-20 are from Table 5 of Cullen and Browning (1978 (◆)*

### 2.5.6 Generalised linear model

The data were subjected to generalised linear modelling using a logit transformation and binomial errors (Table 6).

*Table 6. Output from a Generalised Linear Model of photoperiod and temperature, showing significant effects of temperature, photoperiod and temperature-photoperiod interaction. Null deviance: 143.775 on 11 degrees of freedom, residual deviance: 41.605 on 8 degrees of freedom. AIC: 81.58*

Coefficients:

| | Estimate | Std. Error | z value | Pr(>\|z\|) |
|---|---|---|---|---|
| (Intercept) | 36.05603 | 11.69439 | 3.083 | 0.00205 |
| Temp | -1.67588 | 0.54565 | -3.071 | 0.00213 |
| Phot | -1.93473 | 0.85452 | -2.264 | 0.02357 |
| Temp:Phot | 0.08697 | 0.03981 | 2.184 | 0.02893 |

The model was then used to predict the percentage diapause in over a range of scenarios based on the data, which was visualised in a graph (Fig. 5) and compared with Cullen and Browning (1978) (Fig. 4).

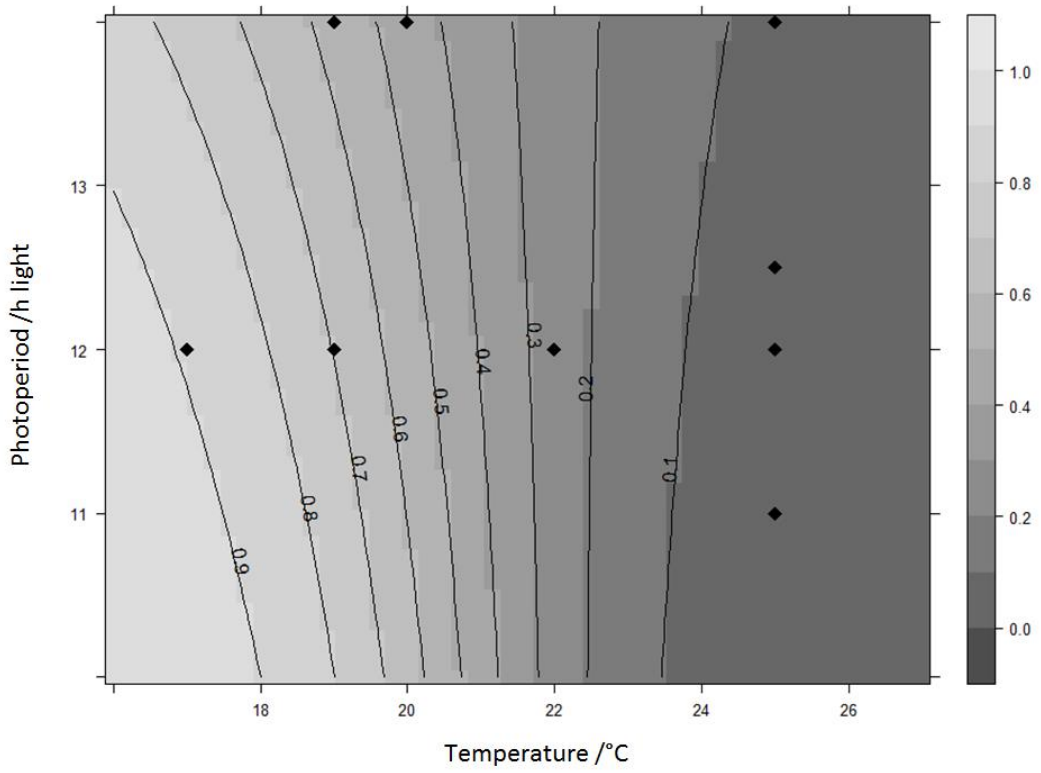



*Fig. 6. Generalised linear model predictions of diapause in* H. punctigera *based on the data in this study. Phot= Photoperiod, Temp = temperature (/°C) Study data are overlaid as diamonds.*

With this model describing the data, I attempted to improve the fit of the model by adding quadratic terms to the R equation (Table 7 and Fig. 7).

*Table 7. Quadratic Generalised Linear Model of photoperiod and temperature. In this model, temperature was no longer significant compared to the standard version. Both the standard and quadratic photoperiod model terms were significant, and the only significant role of temperature is in the photoperiod-temperature interaction term. Null deviance: 143.775 on 11 degrees of freedom, residual deviance: 29.106 on 6 degrees of freedom. AIC: 73.08*

Coefficients:

| | Estimate | Std. Error | z value | Pr(>\|z\|) |
|---|---|---|---|---|
| (Intercept) | 91.79186 | 25.90758 | 3.543 | 0.000396 |
| Temperature | -2.42602 | 1.73824 | -1.396 | 0.162813 |
| Photoperiod | -8.74341 | 2.23638 | -3.91 | 9.24E-05 |
| I(Temp^2) | 0.01292 | 0.04064 | 0.318 | 0.750525 |
| I(Phot^2) | 0.23975 | 0.06969 | 3.44 | 0.000582 |
| Temp:Phot | 0.09362 | 0.03937 | 2.378 | 0.017397 |

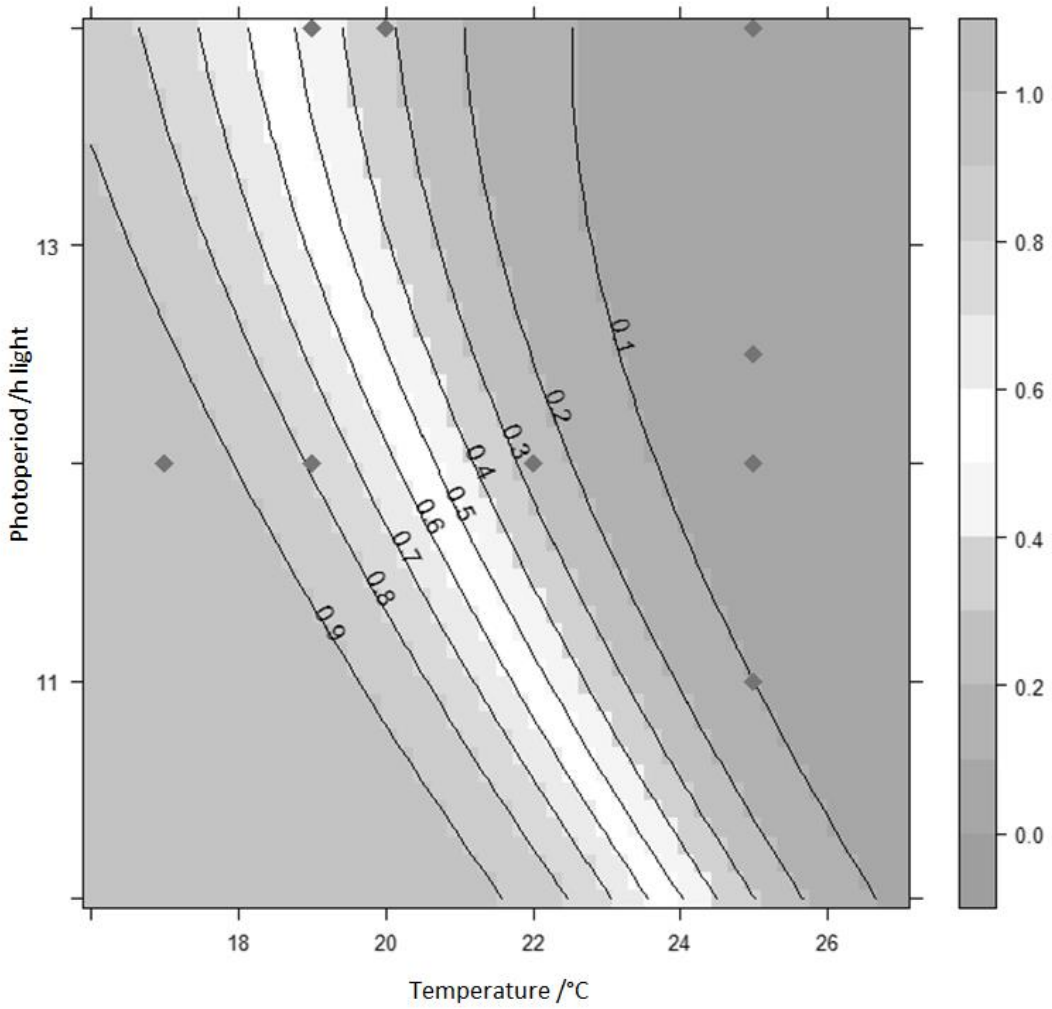



*Fig. 7. Generalised linear model predictions of diapause in* H. punctigera *based on the data in this study, using quadratic response terms*

This model above (Fig. 7) attempted to improve model fit by adding a quadratic term to the formula, but resulted in the temperature becoming non-significant within the model, so this model was rejected in favour of the previous one (Fig. 6).

Obviously the predictions from the model (Table 7), which are based on the study data, share the same differences with the Cullen and Browning (1978) data that the original data set does. In its current form, the model does not describe 12L:12D and 19°C as optimal diapause-inducing conditions, as Cullen and Browning (1978)

described (Table 8). The model finds optimal diapause conditions occur between 18°C and 10L:14D to 17°C and 12L:12D, which is biologically plausible but these conditions are outside the range of those tested in my work.

*Table 8. Comparison of model output (GLM diapause) with results from Cullen and Browning (1978) (C&B diapause).*

| Temperature (°C) | Photoperiod (Light:Dark hrs) | C&B Diapause (%) | GLM Diapause % |
|---|---|---|---|
| 16.5 | 13:11 | 6% | 87% |
| 22 | 14:10 | 3% | 25% |
| 28 | 14:10 | 0% | 21% |
| 19 | 14:10 | 14% | 56% |
| 19 | 11:13 | 38% | 75% |
| 19 | 11.5:12.5 | 37% | 72% |
| 19 | 12:12 | 92% | 70% |
| 19 | 12.5:11.5 | 85% | 67% |

The GLM model suffers from several outliers (14L:10D 20°C, 16L:8D 25°C and 16L:8D 20°C) which would change the predictive output of the model considerably if they were not present. More data are required to ensure that these outliers did not arise due to random chance. This model can also be described as overfitted, due to the lack of data points. This overall approach to diapause modelling is valid, and the while the data from my study are useful as a proof-of-concept, additional photoperiod/temperature ranges are required to enable this specific model to provide useful predictions. This approach would become more viable as more data are made available by future studies.

## 2.5.7 Contour-plot representation

Not a statistical model so much as a representation of the data, a contour plot, "Akima's polynomial method", was applied to the study data along with the data from Cullen and Browning (1978) and also the preliminary summer diapause data

from Chapter 4, using Minitab Release 14 (Ryan *et al.*, 2006). The biggest weakness of this representation is the lack of statistical rigor, as it does not take into account the number of individuals used in any of the studies. This does, however, allow me to incorporate the diapause data from Cullen and Browning (1978), where it could not be used in the GLM model (which requires the number of individuals per treatment) (Fig. 8).

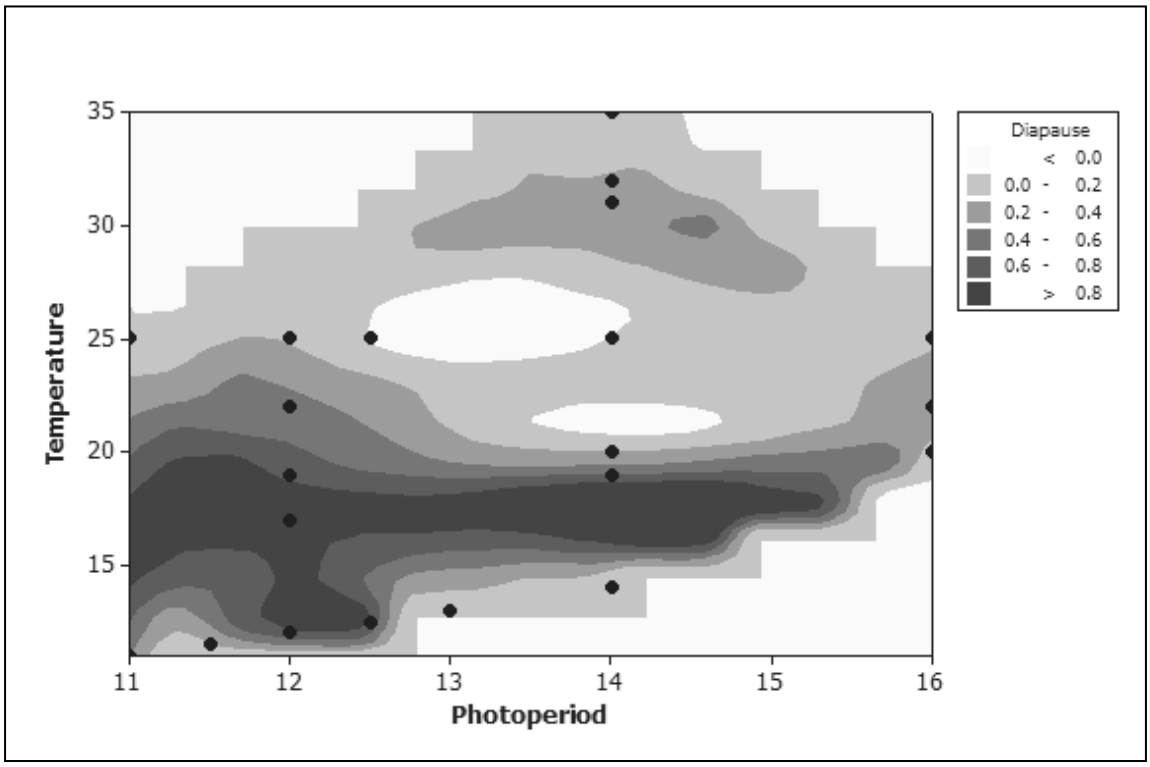



*Fig. 8. Contour plot of photoperiod (hours of light/24h) temperature data (°C) for the induction of diapause (proportion) in* H. punctigera*.*

The contour-plot shows the increasing likelihood of an insect in a population being in diapause at the photoperiod/temperature combinations used in the studies as well as those close to them. For example, under a 15h photoperiod, at 20°C, an individual *H. punctigera* pupa might have a probability of 0.2 to 0.4 of being in some sort of diapause even though that combination was not tested in the laboratory. As temperatures fall below 22°C, the incidence of diapause starts to climb under all

but the longest daylengths. At the highest temperatures (31-35°C) and the longest photoperiods (16h), we start to see some level of response that might represent summer diapause, which is discussed in Chapter 4. The contour-plot does give biologically useful data about *H. punctigera* and gives reasonable predictions on diapause induction at regimes close to those tested in the laboratory, but it can only interpolate on the available data, and cannot predict a diapause response below an 11h photoperiod, for example.

The contour plot was developed into a simple spreadsheet program for the prediction of diapause in *H. punctigera.* A sample of the input and output from this program is given in Fig. 9. This spreadsheet could be made into a simple tool, perhaps available online, to allow farmers and researchers to estimate diapause incidence in their regions in relation to the timing of farming operations. This will be explored more in Chapter 6.

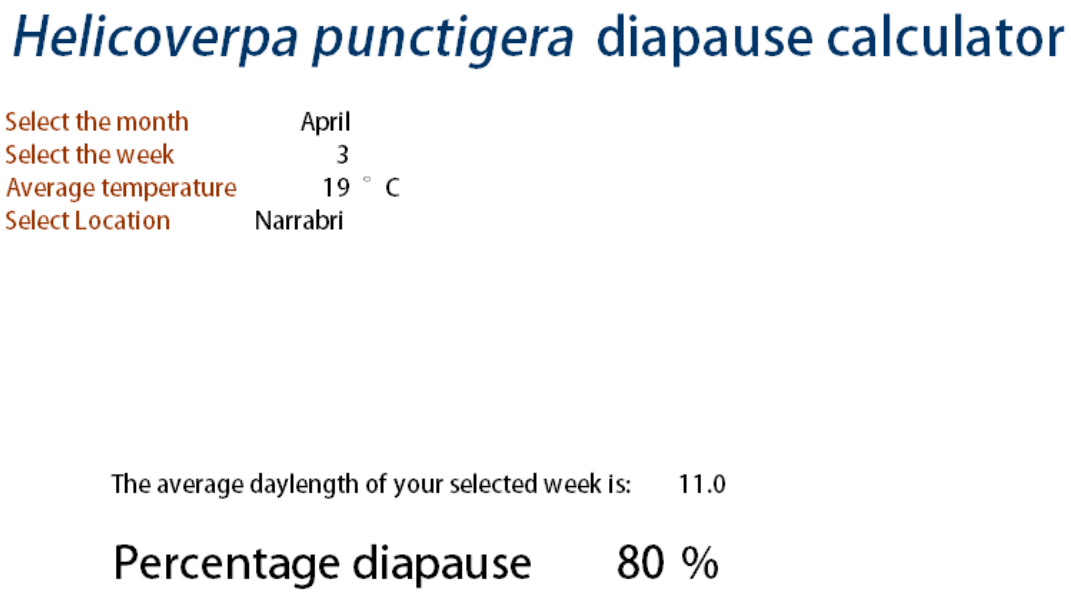



*Fig. 9. Screenshot of* H. punctigera *diapause prediction spreadsheet.*

A copy of this program can be obtained from https://www.dropbox.com/s/906h7qbj8unj15j/Punctigera%20contour%20model.xls?dl=0

The limited data, and resulting lack of statistical rigor involved in the creation of this tool limit its usefulness to growers, but in the absence of similar tools predicting diapause in *H. punctigera* it may still be useful. Further work needs to be done in order to determine the conditions under which diapause is broken once induced, particularly with regard to how long soil temperatures need to be above 25-27°C that is believed to break diapause (Cullen and Browning, 1978). Australian photoperiods can go as low as 10.5L:13.5D, which has not been considered in the experiments. If an insect is not in diapause by mid-winter, it is likely to be exposed to the coolest temperatures of the season without the protective physiological effects of diapause. As a result it was not considered a priority for the laboratory studies, but in light of the contour-plot prediction tool, exploring a 10.5L:13.5D would help fill in the gap. Fluctuating temperature studies would provide useful data on the thresholds where diapause might start to be induced under a closer approximation of field conditions rather than an 'average' simulated by a constant temperature. Studies with different day/night temperatures would provide additional insight into the diapause response if *H. punctigera* as well provide data for models that predict diapause under more realistic conditions.

# Chapter 3: Field studies on *Helicoverpa punctigera* overwintering

## 3.1 Introduction

### 3.1.1 Overwintering of *H. punctigera* in field crops

Chapter 2.1 set the scene for the state of the literature on diapause in *Helicoverpa punctigera*, in that there are few laboratory studies to reference. There are more field studies on overwintering and diapause in *Helicoverpa* spp*.,* though only a few specifically deal with diapause in *H. punctigera*. Murray (1991) established a *H. punctigera* culture from adults caught in a mercury-vapour light trap in Toowoomba, then reared them on artificial diet continuously in an open-air insectary from 1985-1988. A large proportion these individuals sampled during mid- to late- April were in diapause based on eye spot development (Murray, 1991). This suggested that diapause before late March is unlikely, but increasingly likely as photoperiods drop below 12.5L:11.5D (Murray, 1991), a conclusion also supported by the laboratory studies from Cullen and Browning (1978) and the results of Chapter 2.

In the past, the vast majority of *Helicoverpa* pupae found overwintering in northern New South Wales have been *H. armigera* rather than *H. punctigera* (Fitt and Daly, 1990). Wilson (1983) only found overwintering *H. punctigera* pupae in one year, at only one site, out of nine years of study in the Namoi Valley area. Fitt *et al.* (1989) found the distribution of adult *H. punctigera* (via pheromone trap catches) was clumped, with moths often found in small scale patches of 1-2km wide. The difficulties of simulating field conditions with regard to changing photoperiods and fluctuating temperatures under laboratory conditions have led Murray (1991) to use late-stage field-collected larvae in field cages or open-air insectaries. However,

the lack of late-stage larvae in the field during the photoperiods critical for diapause induction has limited the success of these strategies (Cullen and Browning, 1978).

The use of refuge crops such as pigeon pea to manage Bt resistance (Chapter 1.4) may have led to the apparent increase in the numbers of *H. punctigera* overwintering in cropping areas (Chapter 1.5), and as a result there is sufficient cause to attempt overwintering studies to monitor the incidence and fate of overwintering pupae.

Although studies have recorded *H. armigera* overwintering in the immature stages in western Queensland, it is much less common than *H. punctigera* (Gregg *et al.*, 1989). In contrast, *H. punctigera* populations often survive the winter months in large numbers in inland regions including western Queensland (Gregg *et al.*, 1989, Gregg, 1995, Oertel *et al.*, 1999). Late autumn/winter rainfall in some areas of inland western Queensland has been shown to be predictive of spring populations of *H. punctigera* (Oertel *et al.*, 1999, Maelzer and Zalucki, 2000), though more recently Baker *et al.* (2011) questioned this correlation. Rainfall in May, June and July germinates *H. punctigera* host plants, which in turn allow the build-up of populations of *H. punctigera* which can overwinter in both the larval and pupal stages, and migrate as adults in spring (Gregg, 1993, Oertel *et al.*, 1999).

As spring host plants dry off in inland western Queensland, *H. punctigera* moths are carried into cotton growing regions by westerly and north westerly winds (Gregg, 1993, Gregg *et al.*, 1993, Oertel *et al.*, 1999, Gregg *et al.*, 2001). These ecological patterns indicate a need to investigate overwintering of pupae in this region, which has not been directly attempted previously, though there are many observations of overwintering larvae (Zalucki *et al.,* 1994).

### 3.1.2 Methods in overwintering studies

One simple way of estimating the abundance of pupae in the field is the direct excavation of soil containing *Helicoverpa spp.* pupae, down to a depth of about

10cm (Titmarsh *et al.*, 1991). This method is less physically demanding than alternatives such as soil sieving, and has been used by several researchers in the past (Slosser *et al.*, 1975, Caron *et al.*, 1978, Wilson, 1983, Lopez and Hartstack, 1985).

Emergence cages are a non-destructive method for estimating pupal abundance. By confining a small area with a cage, all moths that emerge may be captured and counted, with the time of emergence recorded. This method estimates adult survivors rather than actual overwintering pupal numbers (Titmarsh *et al.*, 1991). Field cages have been used to study various aspects of the ecology of *Helicoverpa/Heliothis* moths in the USA (Caron *et al.*, 1978, Roach, 1981, Lopez *et al.*, 1984) and in Australia (Del Socorro and Gregg, 2001, Duffield and Chapple, 2001, Mahon and Young, 2010, Rogers and Brier, 2010, Sigsgaard *et al.*, 2002), although of these studies only Duffield and Chapple (2001) studied *H. punctigera*. These cages are often used in conjunction with pheromone traps when observing overwintering, as pheromone trap catches can be compared with emergence of moths within the cages (Lopez *et al.*, 1984). Previous techniques for emergence cages were not entirely appropriate for our needs. For example, Wilson (1983) used pyramid emergences cages, 1m x 1m x 1m, but these cages are not suitable for pigeon pea, the most numerous refuge where the highest *H. punctigera* populations are found (Baker and Tann, 2014). Pigeon pea refuges can be as tall as 2m, so a new emergence cage design was needed. Wilson (1983) also placed glass vials containing *H. punctigera* larvae and diet in the ground to monitor emergence, but this method does not simulate natural behaviour of H. punctigera well.

In order to demonstrate that *H. punctigera* does overwinter, direct observation of *H. punctigera* pupae emerging as adults is needed, rather than indirect monitoring of pheromone traps, where any *H. punctigera* adults detected in the spring could be immigrants rather than moths emerging from diapausing pupae.

### 3.1.3 Aims

The work described in this chapter aimed to:

1. Develop methods for sampling overwintering *H. punctigera* in the Namoi Valley and in far western Queensland.
2. Determine if *H. punctigera* are diapausing in Namoi Valley cotton refuges or in native host plants in far western Queensland.
3. Determine the timing for emergence of *H. punctigera* that are overwintering in the Namoi Valley and in far western Queensland.

There have been no studies on any aspect of *H. punctigera* overwintering as pupae in the western Queensland area, and no published studies directly investigating the abundance or timing of *H. punctigera* emergence. In this chapter I aimed to investigate how *H. punctigera* overwinters in inland western Queensland as well as in the cotton regions in the Namoi Valley in NSW. This was attempted using field cages to examine the timing of adult emergence, monitored manually as well as using nearby pheromone traps and temperature probes to record temperatures during the field experiments. When emergence cages failed to generate adult moths to sample, first self-contained emergence cells were attempted, and then a pupae-digging method was adopted to allow direct assessment of overwintering pupae.

## 3.2 Materials and Methods

### 3.2.1 Study areas

Field studies to investigate overwintering in *H. punctigera* were conducted in the Namoi Valley in NSW and in inland Queensland (Fig. 10). There were four sites in the Namoi Valley: “Kilmarnock” and “Milchengowrie” in Boggabri, NSW, “Drayton” and “Boondah” in Breeza, NSW. In inland Queensland, field studies were done at “Monkira” and “Cluny” near Bedourie, Queensland. Table 9 summarises the

location of each study site along with the methods used to explore overwintering in *H. punctigera.*

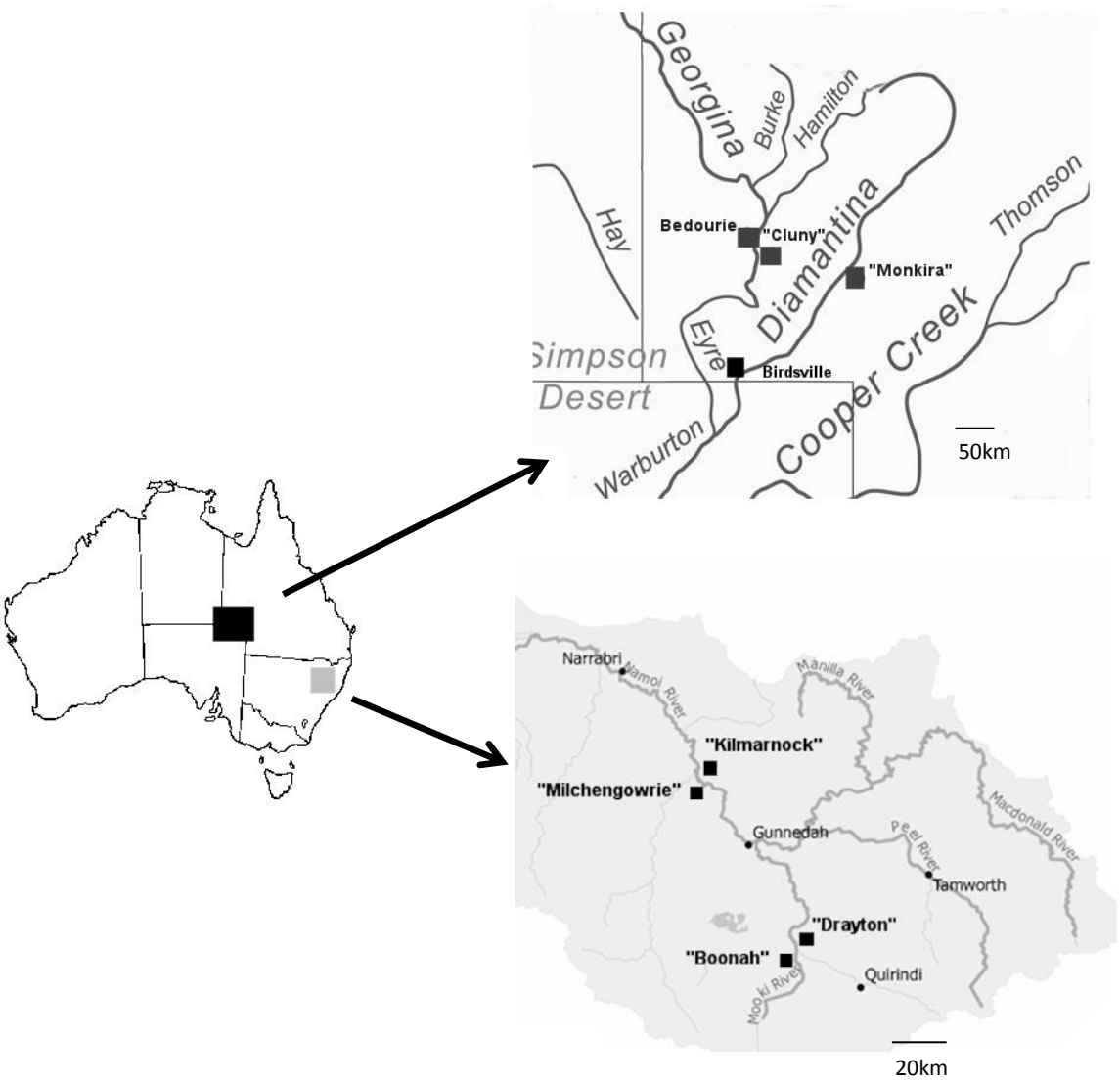



*Fig. 10. Map displaying field study sites in inland Queensland (top) and the Namoi Valley (bottom) (Murray-Darling Basin Authority, Diamantina Shire Council)*

*Table 9. Summary of field sites used to study* H. punctigera *overwintering from 2011-2014.*

| Year | Start Date | Location | Decimal Coordinates | Methods |
|---|---|---|---|---|
| 2011 | 10/03/11 | Namoi Valley, ACRI | 30.21S, 149.60E | Emergence cages |
| 2011 | 10/07/11 | Inland Queensland, ”Cluny” Station | 24.51S, 139.59E | Emergence cages |
| 2012 | 09/03/12 | Namoi Valley, “Milchengowrie” | 30.85S, 150.13E | Emergence cages |
| 2012 | 09/07/12 | Inland Queensland, “Monkira” Station | 24.84S, 140.56E | Emergence cages |
| 2013 | 26/03/13 | Namoi Valley, “Kilmarnock” | 30.85S, 150.13E | Emergence cages, Emergence cells |
| 2013 | 14/07/13 | Inland Queensland, “Cluny” Station | 24.51S, 139.59E | Emergence cages, Emergence cells |
| 2014 | 13/04/13 | Namoi Valley, “Drayton” | 31.27S, 150.44E | Emergence cages, pupae digging |
| 2014 | 13/04/13 | Namoi Valley, “Boondah” | 31.31S, 150.49E | Emergence cells |

### 3.2.3 Pheromone trapping

Universal canister pheromone traps (sometimes referred to as AgriSense traps, produced by Suterra LLC, Oregon, United States baited with lures produced by Suterra LLC, NSW, Australia and sourced from Entosol Pty Ltd, Roselands, NSW, Australia) were used to monitor the activity of *H. punctigera* moths in the study areas. For the inland studies two pheromone traps were placed at each field site for the duration of each emergence cage study. For comparison, data covering the same periods from similar traps that had been operating for some years in inland regions (Birdsville and Bedourie, P. Gregg and A. Del Socorro unpublished) and data

from the Namoi Valley obtained from available grower literature (Cotton Seed Distributors, 2012, Cotton Seed Distributors, 2013) and recently published pheromone trapping data (Baker and Tann, 2014) were used.

### 3.2.5 Emergence cages

Several designs of emergence cages were used for sequential years of field studies, each improving on the designs of the previous year.

The first design of the emergence cage used screen tents (Tasman 2, Oztrail, Eagle Farm, Queensland, Australia) (Figs. 11b and c), with the bottom cut out to expose the inside of the cage to the soil. These cages were placed around pigeon pea (*Cajanus cajan* L.) crops (Namoi Valley studies, Fig. 11b) or wild host plants (inland Queensland studies, Fig. 11c)). The host plants used in inland Queensland are described below in section 3.2.11. Once the cage was placed over the host plants the soil was piled around the edges of the cage to prevent access of *Helicoverpa* spp. larvae or natural enemies into or out of the cage. The capture apparatus was set up at the top of the tent. This apparatus consisted of an 80mm powder funnel attached to the lid of a 100 ml plastic culture vial using a rubber washer. The apparatus was held up by tensile cord tied to a metal bracket, supported by an iron pole hammered into the ground. Cloth tape was used to secure the funnel to the top of the cage. A solar powered garden LED light (Solar Stake Stainless Steel OX0633, Uncle Bills Imports, Silverwater, NSW, Australia) was disassembled and reassembled with an ultraviolet LED (5mm LED Waterclear 60mcd, Cree Inc, Durham, North Carolina, USA) for use as the lure at the top of the cages.

The second iteration of the apparatus used a larger screen tent (Tasman 4V, Oztrail, Eagle Farm, Queensland, Australia), without the fly. The Tasman 4V tent had twice the area of the Tasman 2, allowing twice the number of host plants inside. The Tasman 4V had a cross-section at the top, which the capture mechanism could be

attached to with plastic ties, replacing the supporting tensile cord, washer and iron pole combination (Fig. 12b).

The third iteration of the apparatus had a PVC plate custom made by the Science Engineering workshop at the University of New England, Armidale NSW. This plate had 5mm holes, two per corner, to secure the plate to the tent. The funnel was held in place, and held at the top of the tent, by a small screw thread. The culture vial lid was securely fastened to the plate with a small plastic insert that fitted into the lid exactly. The PVC plates could fit either the Tasman 2 or the Tasman 4 tents.


*Fig. 11. First iteration of field emergence cages used in the Namoi Valley and inland Queensland. Fig. a) shows the detail on the capture mechanism, two plastic culture tubes glued together, with an ultraviolet LED wired into a solar-array that activated after dark. A 45mm powder funnel was attached to the bottom culture tube via the lid of the culture tube and a rubber washer. One-way glass fibre mesh prevented moths from escaping the culture tube. Fig. b) shows the offset cages that the capture mechanism was used in, at Boggabri NSW. Fig. c) shows how the capture mechanism was attached to the cages, via an iron post, tensile cord and metal bracket.*

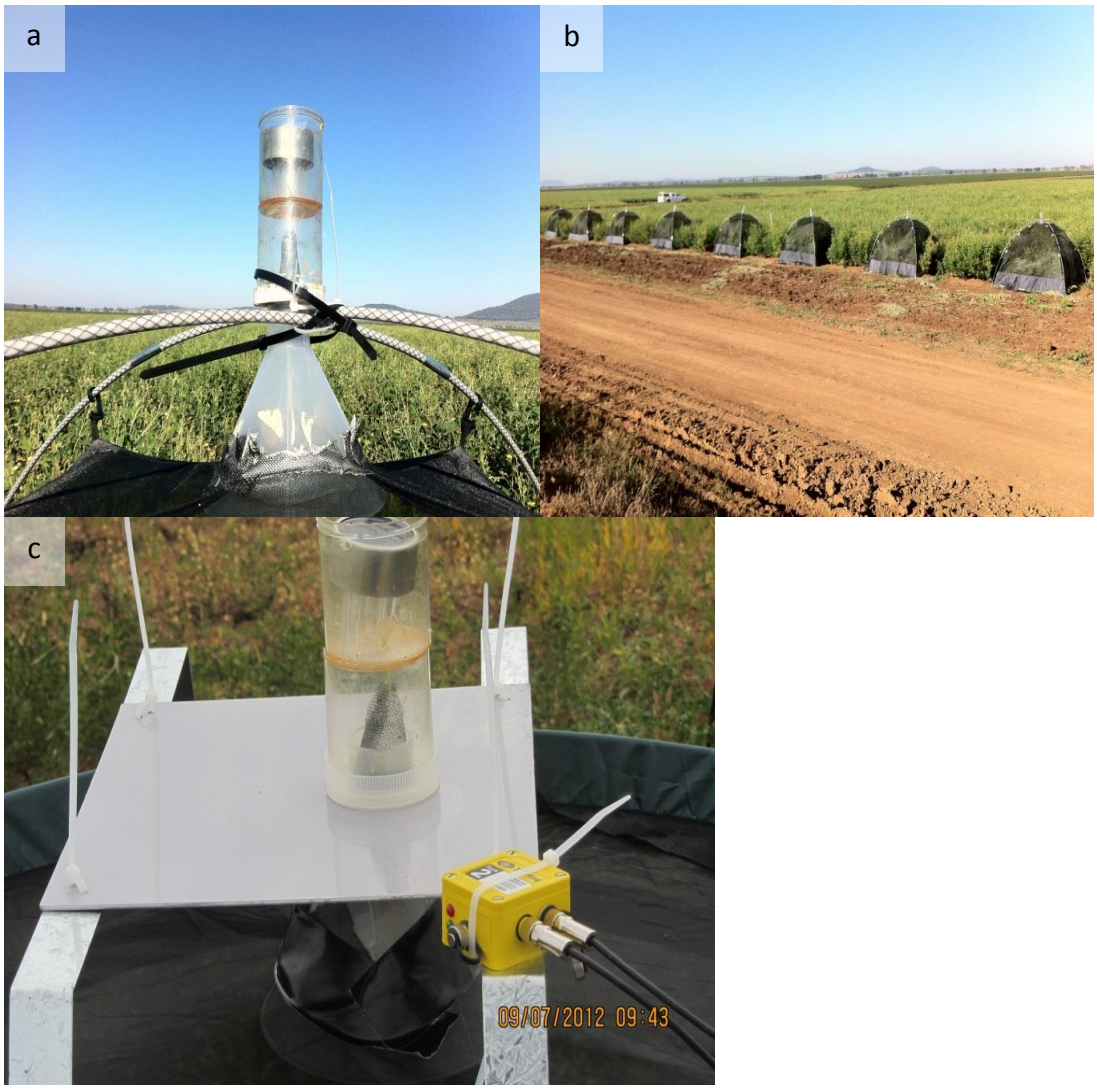



*Fig. 12. Second and third iterations of emergence cage design. Fig. a) shows that the post, tensile cord and bracket were discarded in favour of strapping the capture mechanism directly to the cages, Fig. b) shows the larger cages over multiple rows, and c) shows how a custom-designed PVC sheet was strapped to the edge of the cages using aluminium brackets, to secure the catchment cup and keep it upright, even in strong winds. A TinyTag dual probe data logger was also attached to at least two cages. (Photo: P. Gregg).*

### 3.2.6 Data loggers

Dual-probe TinyTag Plus 2 TGP-4520 data loggers (Gemini Data Loggers, Chichester, UK) were used to monitor temperatures in field trials. Each data logger had one probe in the soil at 10cm below the soil, at the level where pupae were likely to be. The second probe on a cage was secured at 1.5m above ground level to capture the air temperature. Secondary probes were used to explore differences between sites or to collect data at other locations (Table 9). In "Milchengowrie", 2012, probes were placed 1.5m above the ground and in the soil beneath the cage. "Monkira", 2012 also had probes in the same positions, but had two additional probes inside the soil of the cage and at the top of the cage, to allow comparison inside and outside the cage. "Kilmarnock" 2013 had probes in the same position as "Milchengowrie", 2012, but the probe suffered water damage and the data was unrecoverable. "Cluny" 2013 had probes outsides of cages, in the soil and 1.5m in the air, but also in the soil of the cages and inside the self-contained emergence cells. "Boondah", 2014 had a probe inside the self-contained cells, and a probe outside the cage in the soil (Table 9).

### 3.2.7 Self-contained emergence cells

In an attempt to improve the survival of larvae in emergence cage studies, a new protocol was developed, isolating a single *H. punctigera* larva inside a cell made of 40 mm PVC pipe, with a reservoir of artificial diet to sustain it over its development (Fig. 13a). The reservoir was filled with artificial diet and then sealed with either parafilm or beeswax, then placed inside the PVC cylinder encased with fine metal gauze. The cylinder was then half-filled with soil and the lid (a drain cowl) was placed over the top (Fig. 13b) with a single larva on the top. The cell was then placed with 80 others, inside a Tasman 2 tent with a data logger attached. At "Cluny" in 2013, temperature probes were placed inside a cell (a hole was cut in the bottom of the cell to allow access) and 10cm below the surface at an equivalent

depth to the cell. Each cell was considered a full replicate, as each cell was independent of the others.

*Table 10. Data logger locations and probe locations. Probes 1 and 2 were from the primary data logger and 3 and 4 from a secondary probe inside the cages.*

| Year | Region | Location | Probe 1 | Probe 2 | Probe 3 | Probe 4 |
|---|---|---|---|---|---|---|
| 2012 | Namoi Valley | "Milchengowrie" Farm 30.85S, 150.13E | 10cm below surface | 1.5m in the air | | |
| 2012 | Inland Queensland | "Monkira" Station S24.84, 140.56E | 10cm below surface | | 1.5m in the air | Soil surface |
| 2013 | Namoi Valley | "Kilmarnock" Farm S30.85, 150.13E | Logger failed | Logger failed | | |
| 2013 | Inland Queensland | "Cluny" Station 24.51S, 139.59E | 10cm below surface | 1.5m in the air | Inside cell | 10cm below surface |
| 2014 | Namoi Valley | "Boondah" Farm S31.31, 150.49E | Inside cell | 10cm below surface | | |
| 2014 | Namoi Valley | "Drayton" Farm S31.27, 150.44E | 10cm below surface | 1.5m in the air | | |

### 3.2.8 Bugdorm® cages with emergence cells

In the "Boondah" study in 2014, Bugdorm® 2 2120 Insect Rearing Tents, (MegaView Science Co., Ltd., Taichung, Taiwan) (Fig. 14) were used in combination with the emergence cells (Fig. 13a) described above. Emergence cells were buried in 10cm of soil, heat treated at >200°C for 8h to reduce the survival of potential pathogens, with a single first instar, laboratory-reared *H. punctigera* larva was placed in each cell.

### 3.2.9 Pupae digging

Pupae digging was done to collect *Helicoverpa* spp. pupae from the soil. To do this, the surface soil was cleared away, and the surrounding soil was carefully turned over and layered 2cm at a time, down to 10cm in depth (Titmarsh *et al.*, 1991). Often emergence tunnels dug by the pre-pupating larvae were visible, and a pupa could then be extracted from the end of the tunnel. In some cases where tunnels were not visible, pupae had to be retrieved from the loose soil.

### 3.2.10 Namoi Valley studies

The first Namoi emergence study was at the Australian Cotton Research Institute (ACRI) at Narrabri in 2011, the second at "Milchengowrie" at Boggabri in 2012, the third at "Kilmarnock" at Boggabri in 2013, and the final one at "Boondah" at Breeza in 2014 (Table 10).

In the Namoi Valley studies, crops of pigeon pea, grown as refuges in association with Bt cotton (Baker *et al.*, 2008), were first sampled using sweep nets. Emergence cages were placed over selected flowering crops with green foliage in order to provide sufficient nutrition to complete larval development. To facilitate plant growth and keep the foliage green, the cage enclosure was irrigated with 20mm of water spread evenly across the cage. Emergence cages were then seeded with *H. punctigera* larvae. Each cage was seeded with either 50 larvae caught from the surrounding pigeon pea crop, or with 50 larvae from the laboratory culture (see Chapter 2.3.2 for details), on the dates shown in Table 10. Two different cages (one with wild larvae and one with laboratory-reared larvae) were randomly chosen to receive a data logger, with one temperature probe outside the cage at 1.5m above ground and one probe inside the cage soil at 10cm below ground. Each type of cage was replicated four times, to allow comparison between wild larvae and field-caught larvae.

Prior to setting up emergence cages in 2014, it was necessary to first locate sites of *H. punctigera* larval populations. Each scouting location was subject to 5 x 20 sweeps using a 38 cm diameter net fitted with a bag made of polyganza. The numbers and size class (very small, small, medium and large) (Flower *et al.*, 2010) of *Helicoverpa* spp. larvae per 20 sweeps were recorded to compare the relative abundance at each site. *Helicoverpa* spp. larvae were collected and sorted to species by the criteria of hair colour on the first segment behind the head, using a hand lens. Black hairs in larvae above 20mm are indicative of the caterpillar being *H. punctigera* (Bailey, 2007). While this method was less reliable than examining the pupal cremaster spines to accurately determine species (Kirkpatrick, 1961), it was the only one available to separate the larval stages.

Another approach that was tried in the Namoi valley involved the direct collection of overwintering *H. punctigera* pupae. In 2011, 2012 and 2013, not enough late-season *H. punctigera* were found to attempt this, but in 2014 late season numbers were higher. In mid-April ten farms in the Namoi valley area were surveyed for larvae and overwintering pupae. The 2014 year of fieldwork was the most successful year for finding *H. punctigera* larvae. Almost every location that was sampled in 2014 was better than in previous years, where the ratio of *H. punctigera* larvae to *H. armigera* was generally less than 5%. “Drayton” Farm (31.19S, 150.42E, Fig. 10) was selected as the field site when the surveys discovered 2-3 times as many putative *H. punctigera* larvae in the pigeon-pea refuge crop compared to the other farms (Table 11). Pupae were collected from “Drayton” each week by digging 50-100 pupae from the soil, and separating the *H. punctigera* pupae from *H. armigera* based on the cremaster spines (Kirkpatrick, 1961).


*Fig. 13. a) Self-contained single-rearing unit constructed from PVC plastic and a drain cowl. b) The bottom of the cell was buried 8cm in the soil. The inner 'cup' was filled with artificial diet, preserved with a layer of wax or parafilm A single* Polycalymma stuartii *flower containing a* H. punctigera *larva was placed in each cell. c) Each cell was placed inside a screen tent. (Photos b & c: P. Gregg).*

*Fig. 14. Location and cage used in 2014 study at "Boondah".*

Pupae were held in a 50mm x 20mm cylindrical Perspex tube, with the lid fitted loosely, for transport back to the laboratory. Pupae were then incubated at either 19 or 25°C, and monitored for development and emergence each week. Individual pupae were kept in 30ml plastic soufflé cups (Chapter 2) with moist vermiculite for rearing. Half of the pupae collected were reared at 19°C and the other half at 25°C. The state of diapause was checked every 72h based on eye spot and emergence time (see Chapter 2 for details on diapause determination).

### 3.2.11 Inland Queensland studies

For the inland Queensland studies, native host plants were sampled for larvae using sweep nets as described by Zalucki *et al.* (1994). Emergence cages in inland Queensland were placed over host plants where *H. punctigera* were already present prior to the seeding of cages, though the numbers already present were much lower than the numbers that were added to the cages. Cages were seeded with larvae on the dates shown in Table 10. Since the area was remote, distant from the University of New England, and sparsely inhabited, it was necessary to choose sites where a local collaborator, from a nearby cattle station, could be recruited to monitor the cages. In 2011, the flowering daisy *Gnephosis arachnoidea* Turcz. was the most abundant source of *H. punctigera*, and was used as a field site when a collaborator at "Cluny" station was found. In 2012, the only source of *H. punctigera* found was on *Cullen cinereum* (Lindl.) J.W. Grimes at "Monkira" station, where a field site and a collaborator were established. In 2013, the poached egg daisy, *Polycalymma stuartii* (F.Muell. & Sonder) was abundant along the sand dunes next to "Cluny" Station, with most flowers found to be supporting 1-3 *H. punctigera* larvae, so cages were set up nearby and the same collaborator from 2011 was enlisted. Temperature probes were placed at the soil surface, 10cm below the surface and at 1.5m above the surface. The 2013 inland Queensland study used 90 emergence cells, placed at "Cluny" Station, which were to be checked for emergence by a local collaborator every week.

In the inland Queensland studies, pupae in the soil were only excavated when the cages were removed at the end of the study periods (06/10/11, 16/09/12 and 11/11/13 respectively). All plant materials in the cages were carefully extracted to search for moth wings or other evidence of predation on emerging adults. The soil underneath the cages was broken apart using a shovel and examined for evidence of pupal tunnels. Finally the soil was put through a sieve to find whole pupae and pupal cases. Any emergence from the cages and catches in the pheromone traps were monitored by a local collaborator during the study period.

## 3.3 Results

### 3.3.1 Namoi Valley studies

The Namoi Valley studies were hampered in all years by very high mortality in the late larval stages. Studies conducted in 2011 (ACRI) and 2012 ("Milchengowrie") yielded no moths in the emergence cages. When soil underneath the cages was dug no pupae were found, nor were any pupal tunnels or pupal casings found. Two different cages in 2012 had larvae hanging from the roof of the cage with symptoms indicative of death by infection with NPV (Poinar and Thomas, 1978). The 2013 study ("Kilmarnock") also did not yield any live adult moths, but 12 *H. punctigera* pupae were extracted from the soil underneath the cages. The fate of these pupae, after incubation at 25°C in the laboratory, is shown in Table 13.

The laboratory-reared larvae used in the single-cell emergence cages in 2013 all died as small-medium larvae. Another cage housing the 50 self-contained cells did not show any adult emergence, and only 2 dead pupae were recovered. Ants were discovered in two of the cells inside the cage, feeding upon the remains of a moist larva which had most likely died of NPV.

*Table 11. Pupae collected from emergence cages at "Kilmarnock", 2013.*

| **Laboratory-reared** | **Field-collected** |
|---|---|
| 1 killed by parasitoid | 1 killed by parasitoid |
| 3 killed by fungal pathogen | 3 killed by fungal pathogen |
| 2 emerged alive<br>1 killed in extraction | 1 emerged alive |

To conduct pupae digging in 2014, based on the number of *H. punctigera* candidates obtained from sweep net sampling, "Drayton" was chosen as the site for the study (Table 12).

*Table 12. Numbers of* H. punctigera *larvae found at different Namoi Valley farm fields over 80 sweeps in 2014.*

| **Farm and field** | **Coordinates (Lat,Long)** | **Number of *H. punctigera* in 80 sweeps** | **Percentage of *H. punctigera* on larval criteria** |
|---|---|---|---|
| "Gabo" 13 | 31.11S, 150.48E | 10 | 13% |
| "Kilmarnock" W9 | 30.85S, 150.21E | 15 | 19% |
| "Kilmarnock" C | 30.86S, 150.11E | 23 | 29% |
| "Milchengowrie" P1 | 30.85S, 150.13E | 2 | 3% |
| "Milchengowrie" B7 | 30.83S, 150.09E | 1 | 1% |
| "Milchengowrie" W6 | 30.81S, 150.08E | 22 | 28% |
| "Milchengowrie" W5 | 30.92S, 150.20E | 5 | 6% |
| "Drayton" A | 31.19S, 150.42E | 39 | 49% |

*Table 13. Pupal survey data from ~4h searches at "Drayton", 2014. Pupae recovered at different dates were split between 19°C and 25°C and the fates of* H. punctigera *pupae were recorded.*

| | Date | 12-05-14 | 22-05-14 | 29-05-14 | 25-06-14 | 09-07-14 | Total |
|---|---|---|---|---|---|---|---|
| 25°C | Alive | 3 | 0 | 0 | 4 | 1 | 8 |
| | Nonspecific death | 0 | 3 | 1 | 2 | 0 | 6 |
| | Diptera parasitism | 0 | 3 | 2 | 2 | 1 | 8 |
| | Hymenoptera parasitism | 0 | 0 | 0 | 0 | 0 | 0 |
| | Fungal pathogen | 0 | 0 | 1 | 0 | 0 | 1 |
| 19°C | Alive | 2 | 4 | 1 | 1 | 2 | 10 |
| | Nonspecific death | 1 | 1 | 1 | 3 | 0 | 6 |
| | Diptera parasitism | 0 | 1 | 1 | 2 | 0 | 4 |
| | Hymenoptera parasitism | 1 | 0 | 0 | 0 | 0 | 1 |
| | Fungal pathogen | 0 | 0 | 0 | 1 | 0 | 1 |
| | **Total** | 7 | 12 | 7 | 15 | 4 | 45 |
| | **Count of *H. armigera*** | - | 109 | 70 | 66 | 92 | |
| | **Total** | - | 121 | 77 | 81 | 96 | |
| | ***Percentage of H. punctigera*** | - | 10% | 9% | 19% | 4% | |

In the pupal digging studies at "Drayton" in 2014 the ratio of *H. punctigera* larvae surviving to pupae compared to *H. armigera* was much lower than the ratio of larvae collected by sweep netting (a maximum of 17% compared to 49%; Table 13 and Table 12). Pupae obtained from "Drayton" (Table 13) were all initially in stage A of development (Chapter 2.3.1), but the pupae reared in the laboratory at 25°C all developed to stage E within 5 days while those at 19°C remained in stage A. This suggests that all of the pupae were in diapause when collected, but exposure to 25°C at all times of collection broke the diapause and caused development to continue. Of the *H. punctigera* pupae extracted from "Drayton", 12 died of non-identifiable causes, while the 13 parasitised pupae found showed 92% Diptera (Tachinidae) and 8% Hymenoptera (Ichneumonidae) parasitism (Table 13).

## 3.3.2 Inland Queensland studies

### "Cluny" Station, 2011

The study at "Cluny" station in 2011 had only nine adults emerged from the cages (Table 14). The moths emerged in the week leading up to 21/08/11. When the soil beneath the cages was dug up on 6/10/11 pupal cases from which moths had emerged were found, and there were some emergence tunnels without cases. This suggested that adult moths emerged from their pupae, but were not captured in the collecting apparatus with the light. Several moth wings fragments were found in cages 3 and 4, but they were beginning to disintegrate and it was not possible to quantify them.

*Table 14. Pupal emergence obtained by assessing the soil beneath the field cages, "Cluny" Station, 2011. n = 50 per cage.*

| Cage | Moths emerged | Pupal cases | Emergence tunnels without pupal cases |
|---|---|---|---|
| 1 | 2 | 6 | 0 |
| 2 | 1 | 3 | 8 |
| 3 | 6 | 18 | 2 |
| 4 | 0 | 20 | 0 |

### "Monkira" Station, 2012

The 2012 "Monkira" study had a total of 64 moths emerged from the cages. There were two peaks of emergence 19/7/12 and 27/9/12, but many moths also emerged over the intervening period (Fig. 15). When the soil was dug up on 15/9/12, many pupal cases were recovered, but there were no live pupae.

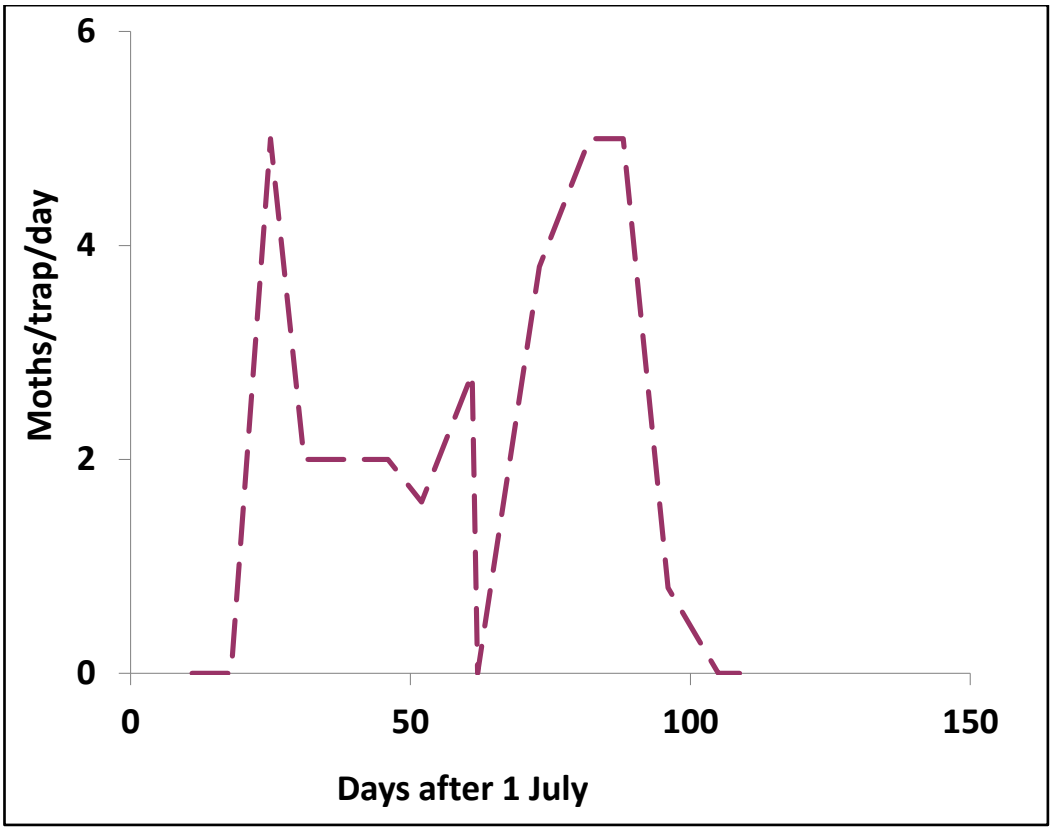



*Fig.15. Emergence of* H. punctigera *adults from cages at "Monkira" station, 2012.*

**"Cluny" Station, 2013**

The collaborator was unable to check the emergence of moths in the field cages during 2013, so no data on moth emergence timing were available. When the cages were examined on 11/11/13, of the 90 emergence cells used, 18 contained moths that had successfully emerged, while 72 failed to emerge. The screen-tent emergence cages did not have any moths in the catchment container at the top, although sieving the sand beneath the cages did recover some *H. punctigera* dead adults and pupal cases (Table 15).

*Table 15. Results of soil excavation beneath field emergence cages in" Cluny", 2013.*

| Cage | Adults (dead) | Pupal cases | Whole pupae (dead) |
|---|---|---|---|
| 1 | 0 | 0 | 0 |
| 2 | 0 | 6 | 0 |
| 3 | 1 | 0 | 0 |
| 4 | 0 | 0 | 8 |

### 3.3.3 Summary of results

The variety of methods used over the field seasons of 2011-2014 had varying successes and failures (summarised in Table 16). Each year, the methods/apparatus were improved in order to improve the chances at collecting data, but all studies in the Namoi Valley using emergence cages and emergence cells did not yield live moths, even when the same apparatus did in inland Queensland.

*Table 16. Summary of overwintering field studies, 2011-2014.*

| Year | Location | Methods | Results |
|---|---|---|---|
| 2011 | Namoi Valley | Emergence cages | No data. 100% mortality in emergence cages. |
| 2011 | Inland Queensland | Emergence cages | Emergence data/pheromone trap data collected. Local rat plague may have reduced emergence. 9 moths emerged in the cages. |
| 2012 | Namoi Valley | Emergence cages | No data. 100% mortality in emergence cages. |
| 2012 | Inland Queensland | Emergence cages | Emergence data/pheromone trap data collected. 64 moths emerged. |
| 2013 | Namoi Valley | Emergence cages, Emergence Cells | No larvae emerged in cages or cells, 3 emerged alive from soil under cages. |
| 2013 | Inland Queensland | Emergence cages, Emergence Cells | No timing data when emergence occurred due to collaborator failure. Emergence cells had 18 moths emerge out of 90 cells in total. |
| 2014 | Namoi Valley | Emergence cages, Emergence Cells, Pupae digging | Emergence cells unsuccessful, pupae digging yielded 18 live pupae all in diapause. |

### 3.3.4 Meteorological data during the overwintering trials

The temperature probe data showed how extreme the inland conditions were on the soil surface over the study period. The 2012 “Monkira” study had up to 50°C of temperature fluctuation in a single day (Fig. 16). Maximum temperatures commonly exceeded 40°C, even though the experiment was conducted during winter. However, it should be noted that these readings were from probes that were placed in the foliage and would have been exposed to sunlight for some time

during the day. They are therefore not directly comparable with temperatures that would have been recorded in a Stevenson screen, or with temperatures that might have been experienced by larvae which would have been able to seek shade. Minimum temperatures were much lower. Even at the surface of the soil, minimum temperatures still went below 0°C on some nights. At the soil surface, some of the more extreme upper temperatures were reduced, but lower winter temperatures were not reduced. Inside the cage and 10cm into the soil, temperatures were relatively cool but stable, and temperatures remained in a range that would maintain diapause rather than breaking it (Fig 18).

At “Cluny” Station in 2013, there was a similar pattern of extreme temperature fluctuation each day in the air, but once again, much of this was ameliorated by the soil. An important distinction is that in 2012 soil temperatures at the field site in “Cluny” rose above 25°C almost every day, above the threshold where diapause is broken (Fig. 17). In 2012 the effect of the cages on soil temperature was measured directly, with 1-2°C difference between soil in the cage and outside the cage (Fig. 22). Temperatures inside the cage emergence cells at “Cluny” were within 2-3°C of the surface temperatures inside the cages (Fig. 18), but the cage itself still provided an insulating effect (Fig. 22). The soil temperatures in “Monkira” were low enough to induce diapause, while the temperatures in “Cluny” were not.

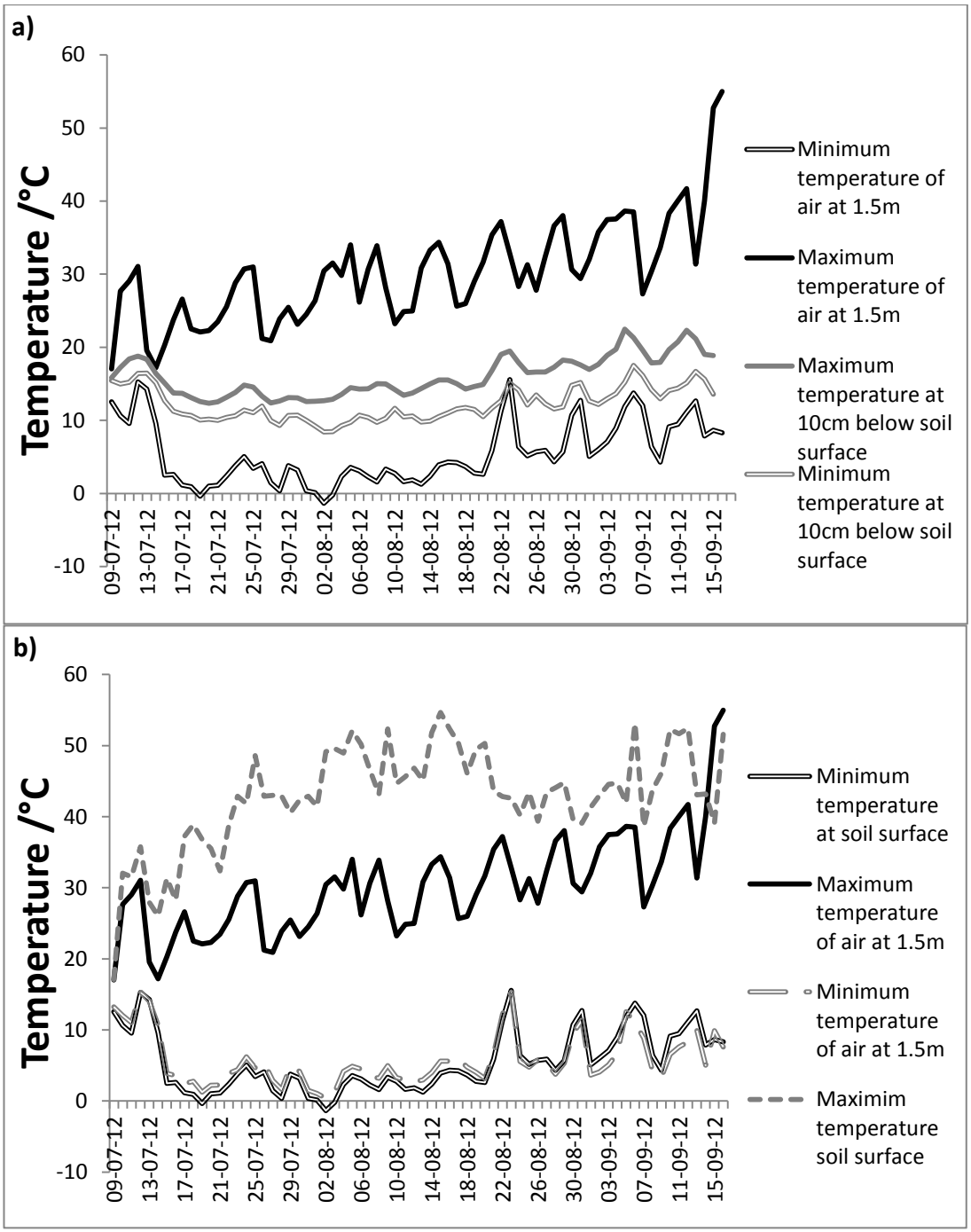



*Fig. 16. Daily maximum and minimum temperatures at "Monkira", 2012. Maximum daily temperatures 1.5m and 10cm below the surface (a), and soil surface, air 1.5m above cages (b).*

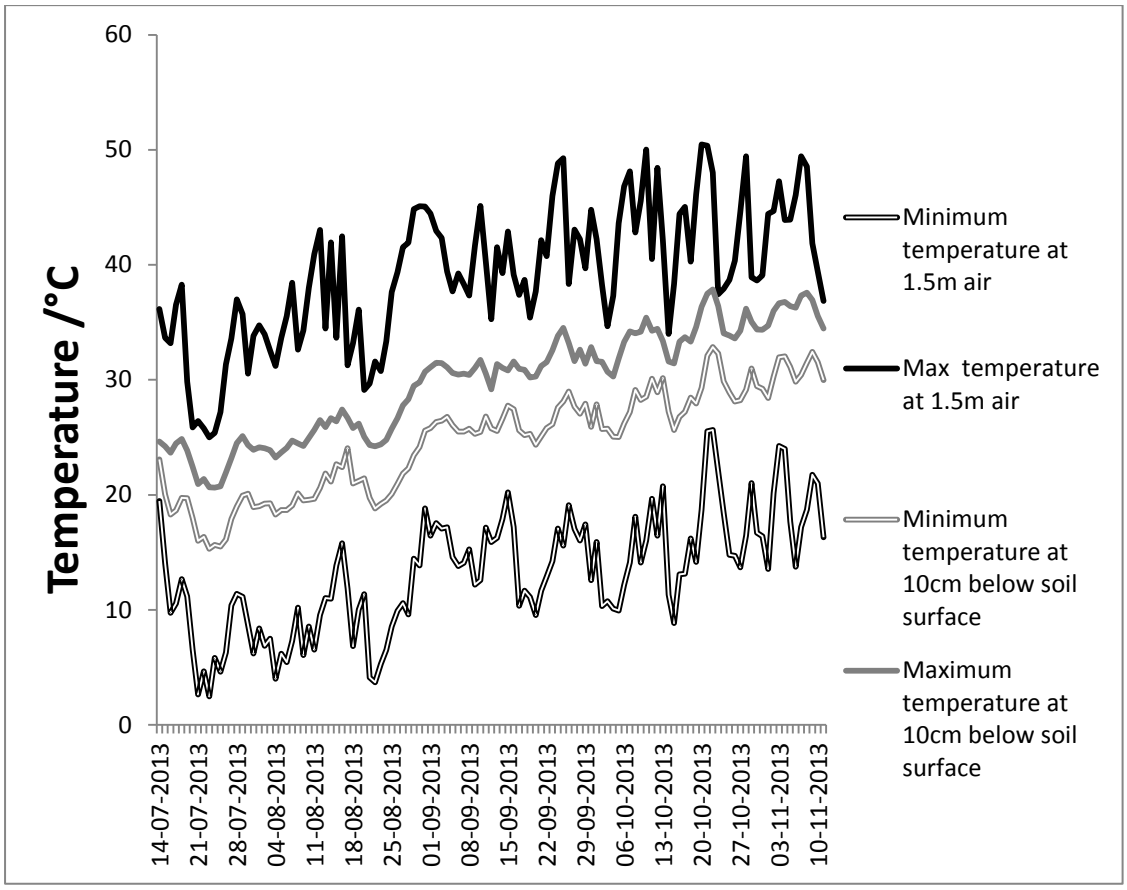



*Fig. 17. Daily maximum and minimum temperatures at "Cluny", 2013. The maximum and minimum temperatures collected at 1.5m outside the cages were much higher and lower respectively than the maximums and minimums inside the cages at 10cm below the soil surface.*

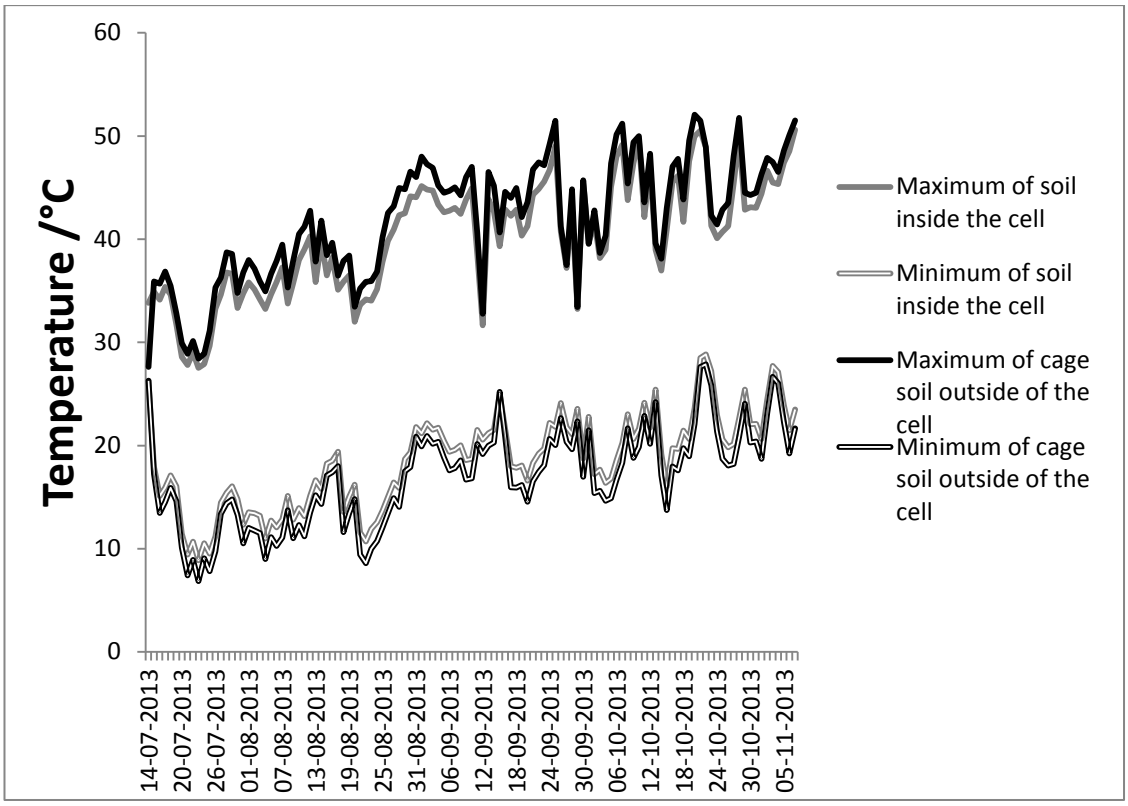



*Fig. 18. Daily minimum and maximum soil temperatures inside emergence cages and inside emergence cells at "Cluny", 2013. There was a slight insulation effect of the cells, but overall there was little difference between the soil in the emergence cages, and the cells inside the emergence cages.*

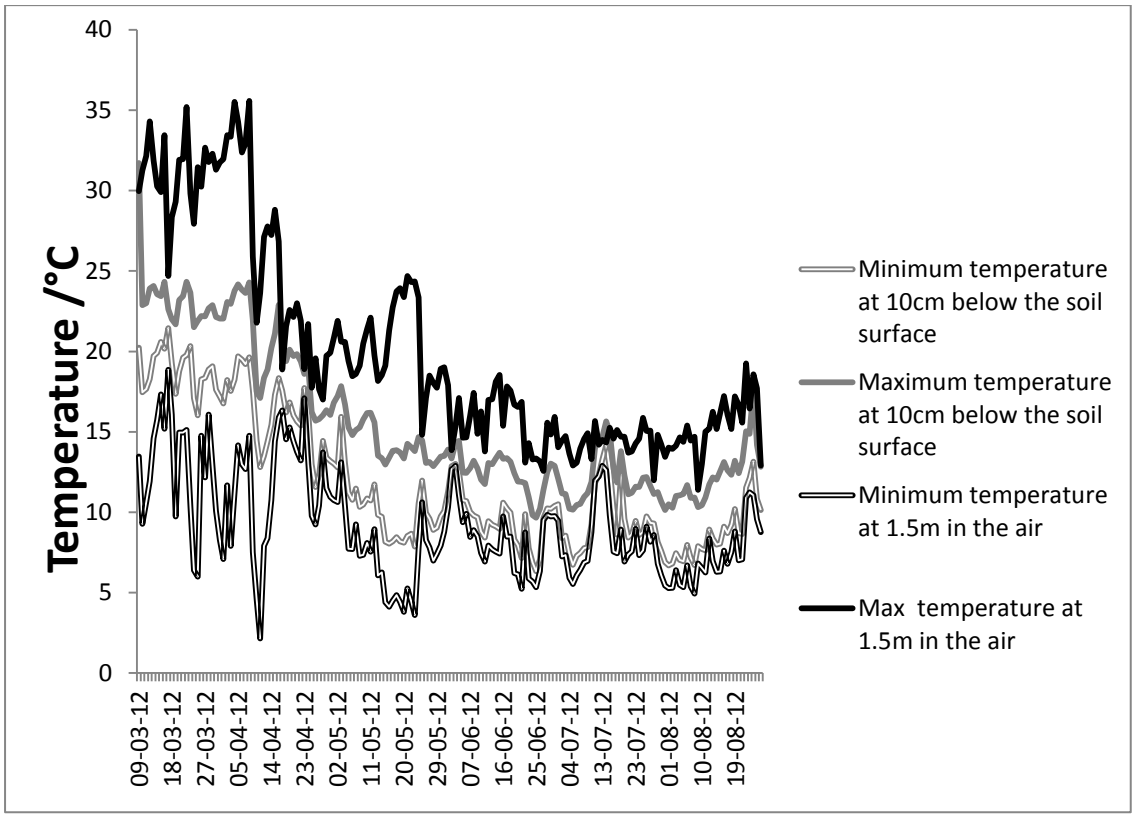



*Fig. 19. Daily maximum and minimum temperatures during the emergence study at "Milchengowrie" in, 2012.*

Air temperatures at "Milchengowrie" in 2012, where no larvae and very few pupae survived, had over 20°C of fluctuation on certain days, with temperatures going as low as 2°C from the start of recording on 9/3/12 through to 10/4/12 (Fig. 19). Maximum surface air temperatures in "Drayton", 2014, stayed above 20°C until 7/5/14, where temperatures dropped below a maximum of 15°C and remained there until 25/8/14 (Fig. 20).

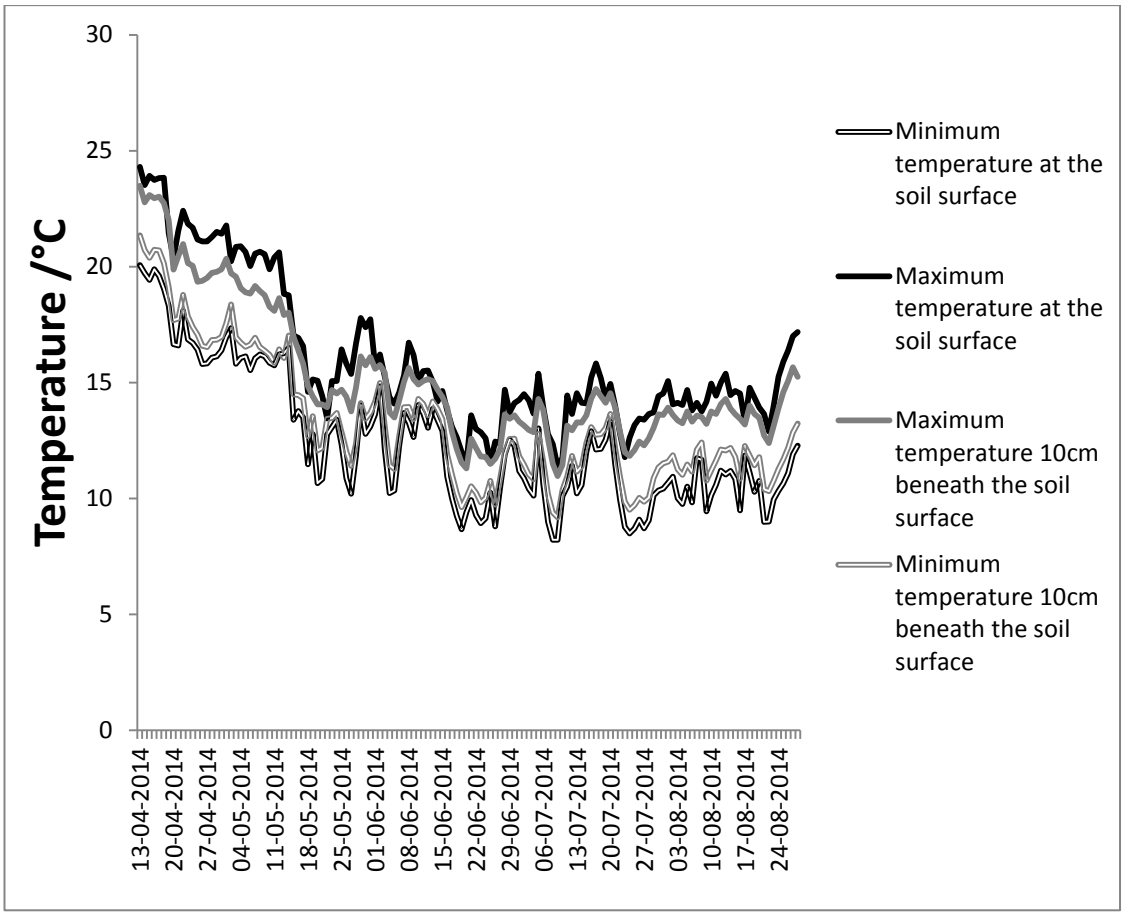



*Fig. 20. Soil temperatures at "Drayton" farm, the site of the 2014 pupae digging.*

Despite the geographical closeness to "Drayton", "Boondah" had lower temperatures overall, and greater daily temperature fluctuations but followed the same general temperature trends (Fig. 21). The new field cages and the soil did not seem to have a large effect on daily temperature fluctuations relative to the surface, particularly with the lower temperatures.

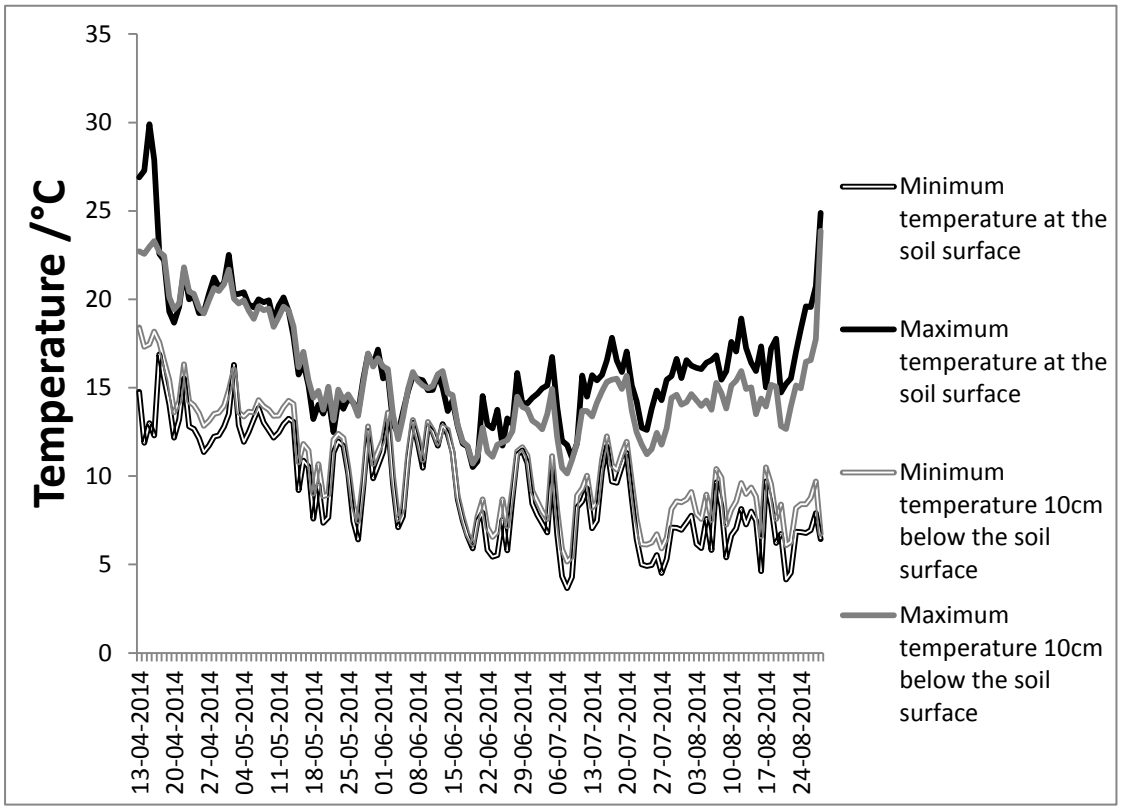



*Fig. 21. Soil temperatures at "Boondah", where the 2014 emergence cells were placed. The effects of the cage and the soil did not insulate the soil in the cage more than 1-5°C.*

The effects of cages on soil temperatures can be seen by comparison of probes inside and outside the cages at at "Monkira" 2012 and "Cluny" 2013 (Fig. 22). In general cage effects were relatively small. At "Monkira" the maxima were similar except on a few hot days in late winter and spring, where the peak was reduced probably due to shading. However there was a consistent increase in minimum temperatures of 1-2$^{o}$C, suggesting an insulating effect of the cages at night.

Conversely, at "Cluny" there appeared to be an insulating effect of 1-2$^{o}$C for maximum temperatures, but very little effect on minimum temperatures.

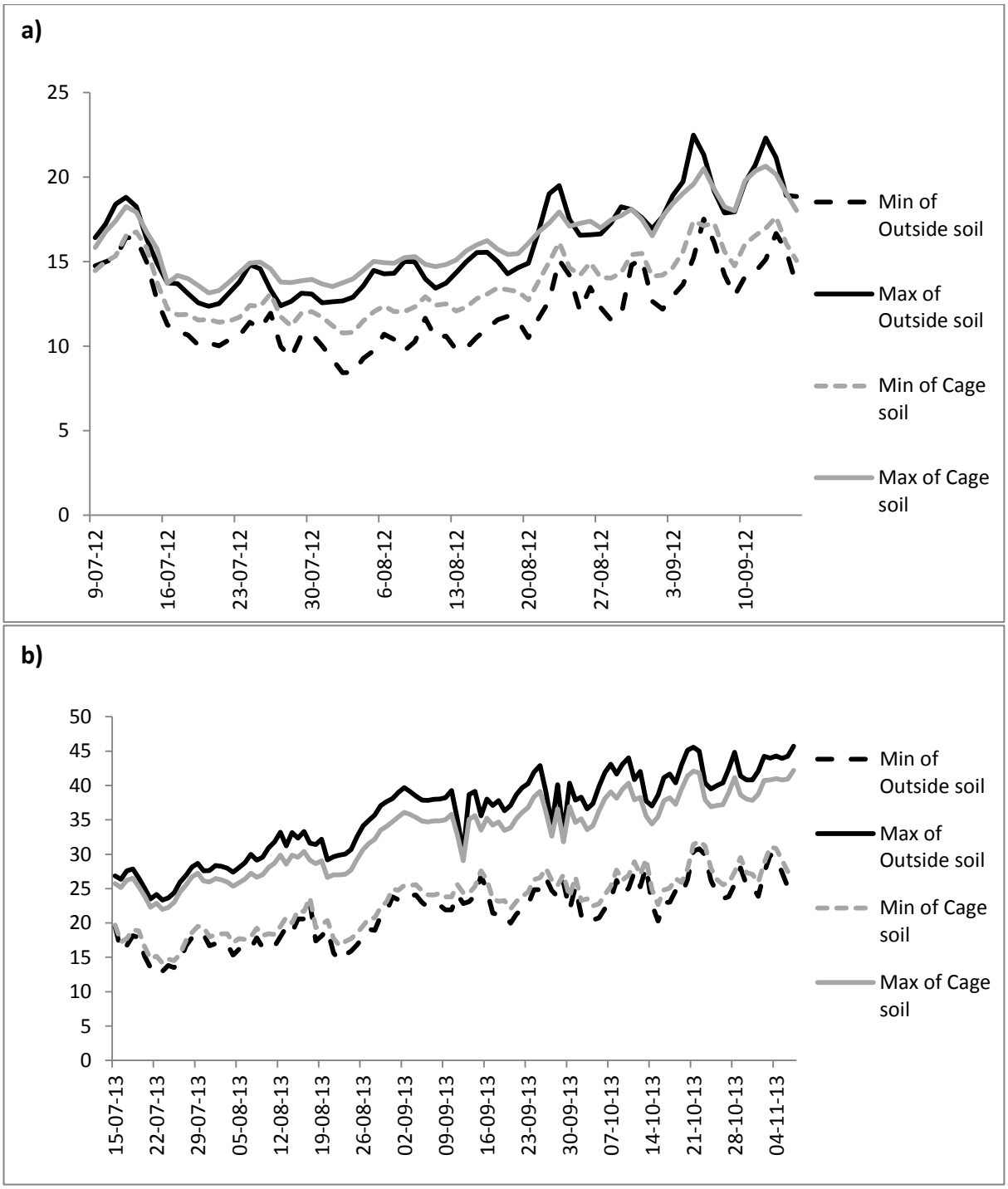



*Fig. 22. The effect of emergences cages on soil temperature at 'Monkira' 2012 (a) and 'Cluny' 2013 (b)*

## 3.4 Discussion

### 3.4.1 Evaluation of emergence cage methodologies

Emergence cage methods were developed for both local and inland field studies, and improvements were made each year aimed at increasing their effectiveness in field overwintering studies of *H. punctigera*. However, with the high mortality of larvae and pupae in the Namoi Valley, it appears that none of the emergence cages, nor the single emergence cell method, were able to sufficiently monitor large enough numbers of *H. punctigera* to obtain reliable data on the timing of emergence of local overwintering adults. In contrast, the same cage methods had much greater success in the field studies in inland Queensland. It is plausible that larval mortality factors such as potential predators, parasitoids and pathogens may be less important in the non-cropping inland Queensland areas compared to Namoi valley cropping regions. Compared to overwintering survival in inland Queensland, the best that can be said for these emergence cage methods in the Namoi Valley is that they served to demonstrate high natural mortality in the immature stages during the autumn, and they contained too few overwintering *H. punctigera* to provide any useful information on the extent and timing of diapause induction and termination, and subsequent moth emergence. Based on the meteorological data (Fig. 17, Fig. 18 and Fig. 19), it may have been better to bury the cells deeper than they were, as there may have not been enough insulation to truly capture soil temperature conditions, and this was a possible source of larval mortality in both the “Cluny” 2013 and “Boondah” 2014 studies.

### 3.4.2 Diapause in 2014 “Drayton” pupae

All of the 18 surviving pupae obtained (split between 19°C and 25°C) from pigeon peas at “Drayton”, were initially in diapause, but those held at 25°C broke diapause and continued development while those held at 19°C did not. This initially seems at odds with the results of Cullen and Browning (1978) and the conclusions of Chapter 2. This apparent contradiction may be resolved by considering the stages in which

diapause might be induced, maintained or terminated, using the process in *H. armigera* as a possible model.

I postulate that pupal development in *H. punctigera* proceeds along several paths with multiple stages, depending on environmental conditions (Fig. 23). In the first path, diapause is induced and maintained at low temperatures. In the second path, diapause is initially induced but broken at higher temperatures, and finally in the third path, conditions are in the optimal range and no diapause is induced. In *Helicoverpa zea*, once diapause has been induced, a period of cold exposure is needed to 'reactivate' the prothoracic glands, to complete development to adult stage (Tauber *et al.*, 1986).

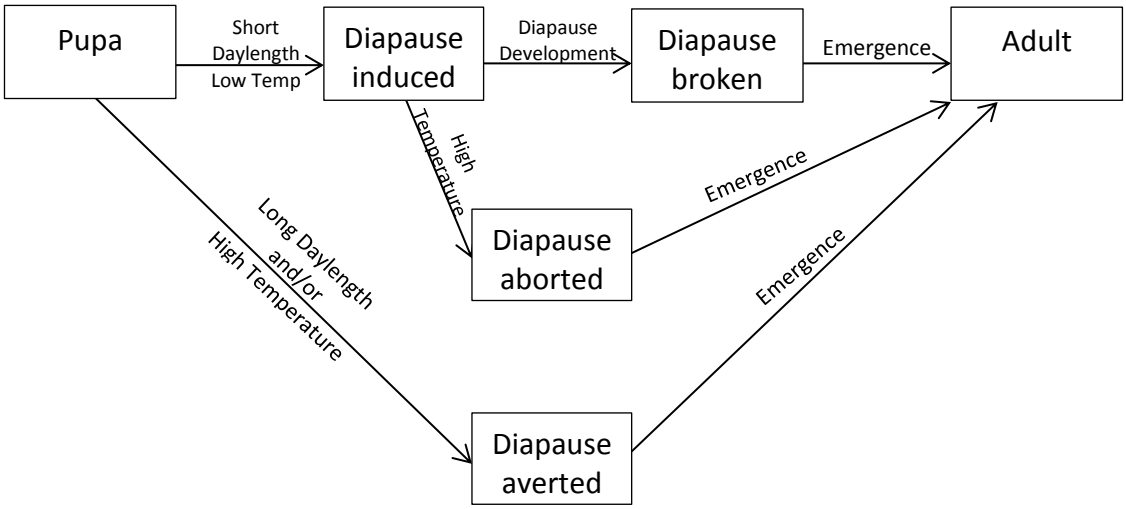



*Fig. 23. The possible routes of development to adult for a* H. punctigera *pupa, including standard development, diapause, and diapause aborted by high temperatures, based on comparisons with Cullen and Browning (1978) and the data in Chapter 2.*

The specific temperatures required for each of these 'high' or 'low' temperatures in Fig. 23 have not been accurately defined at this time, but based on the results of Chapter 2 and Cullen and Browning (1978), some values can be proposed. Chapter 2 explored the onset conditions for diapause, with a temperature of 19°C and a photoperiod of 12L:12D producing the greatest amount of diapausing pupae. However, most diapause-inducing conditions produce some proportion of pupae

not in diapause, so there appears to be no 'hard' set of conditions where every pupa is immediately switched into diapause development. Cullen and Browning (1978) found that diapausing pupae could have their diapause aborted early by exposure to 28°C, while Chapter 2 found that 25°C was not high enough to reproduce this response. A temperature regime of 25°C was enough to avert diapause in nearly all pupae, if diapause was not already induced (Chapter 2, Table 3). A month into the study (13/5/14), temperatures at "Drayton" dipped below a 19°C maximum, and the maximum did not rise above 17°C from 31/5/14 over the study period, ending 28/8/15 (Fig. 20). With the ~11h photoperiod and low temperatures in May, 80-100% of pupae would be in diapause if they were not already in that state before May. The 18 surviving pupae from "Drayton" were all in diapause when collected from the field, and based on the 4 surviving *H. punctigera* pupae collected on the first sampling trip (12/5/14), the conditions prior to that trip were sufficient to induce diapause. Daily maxima were already dropping (and staying) below 25°C.

### 3.4.3 Survival of *H. punctigera* in Namoi Valley field trials

The proportion of *H. punctigera* surviving the winter in the Namoi Valley area has historically been extremely low, with only 2/2182 *Helicoverpa* pupae recovered from cotton, pigeon pea and sunflower being *H. punctigera* compared to *H. armigera* from 1987-1988 (Fitt and Daly, 1990). The limited proportion of overwintering *H. punctigera* in local fields was also observed by Wilson (1978). More recent studies of *Helicoverpa* species show that the abundances of adult and pupal *H. armigera* and *H. punctigera* in Namoi Valley cotton refuges have dropped considerably from between the Ingard® era (1996-2004) to the Bollgard II® era (2005-onwards) (Baker and Tann, 2014). Whether this is due to changes in migration, seasonal differences or an increase in natural enemies since broad spectrum pesticides are no longer used in cotton is still not clear (see Chapter 1.4). However, despite the overall decrease, there are suggestions that the proportion of

*H. punctigera* relative to *H. armigera* has recently increased (Baker and Tann, 2014). My data from the Namoi Valley in 2014 support this suggestion. This fits with earlier data that suggested that *H. punctigera* numbers increased in some years (Wilson, 1983). The single good year of data collection (2014) may (tentatively) suggest that when and where conditions are favourable higher *H. punctigera* populations may be present than have previously been recorded by authors such as Fitt and Daly (1990) and Wilson (1983), but this incidence is either extremely patchy or subject to factors that we have not fully studied. Wilson (1983) noted that in one year where *H. armigera* populations were low, *H. punctigera* populations comprised 80% of larvae caught on late-grown cotton, although this has not been the case over the four years of field studies in this project.

The change in ratio of *H. punctigera* to *H. armigera* larvae caught compared to the same ratio for pupae collected suggests that far fewer *H. punctigera* larvae survive to the pupal stage than *H. armigera*, and this may help explain the lack of success in the Namoi valley field cage trials. However, an alternative possibility is that the methods for identifying *H. punctigera* larvae over *H. armigera* are unreliable, and some of the larvae collected from pigeon peas might have been incorrectly identified as *H. punctigera* when they were *H. armigera*.

In a dataset spanning from 1996 to 2003, Baker *et al*. (2008) observed that there was considerable variability in the abundance of *Helicoverpa (H. armigera* and *H. punctigera*) in both space and time. High abundance of *Helicoverpa* in the 1998-1999 growing season was likely due to heavy rains in winter-early spring generating abundant spring host-plants, which would have encouraged early plantings by farmers, and generated ideal sequence of host plants for *Helicoverpa* populations to develop on (Zalucki and Furlong, 2005, Baker *et al.*, 2008, Oertel *et al.*, 1999).

### 3.4.4 Biotic factors affecting overwintering survival

The relative lack of studies on natural enemies (pathogens, predators and parasitoids) of Australian *Helicoverpa* spp. in the pigeon pea refuges makes it extremely hard to quantify the direct impact of natural enemies on *Helicoverpa* populations in the field (Seymour and Jones, 1991). However, with the introduction of Ingard® and Bollgard II® Bt cotton, pupal parasitism in *Helicoverpa* has steadily increased as a result of refuge crops and reduced pesticide use (Baker and Tann, 2014).

Over 15 potential tachinid parasitoids and 8 ichneumonid parasitoids of *H. punctigera* have been described in Zalucki *et al.* (1986). My observations on parasitism at "Drayton" in 2014 are consistent with the results of Baker and Tann (2014), where the most common parasitoids of *Helicoverpa* spp. pupae were tachinid flies along with the ichneumonid wasp, *Heteropelma scaposum* (Morley). Of 67 *Helicoverpa* spp. pupae collected on pigeon pea at Getta Getta (North Star) in the Macintyre Valley in February 2008, 63% were parasitised while only 21% survived (Baker and Tann, 2014).

In addition to the parasitoids, there are a number of predatory insects from a number of diverse Orders including Dermaptera, Orthoptera, Heteroptera, Neuroptera and Coleoptera, as well as spiders from Orders Araneida, all of which prey upon *Helicoverpa* spp. (Zalucki *et al.*, 1986). It is very difficult to estimate levels of predation, or ascribe predation to a particular species, because predators often leave no evidence of their activities (Seymour and Jones 1991). However, in "Milchengowrie" in 2012 for the first two weeks of May, 10-20 brown lacewings (*Micromus tasmaniae* Walker) were caught inside the catchment containers of emergence cages. Both larvae and adults of *M. tasmaniae* are predators, and feed on small *Helicoverpa* larvae (Samson and Blood, 1980). Given the difference in size between *M. tasmaniae* adults and larvae, and late stage *H. punctigera* larvae already present in the field, it is unlikely that field-caught larvae were predated upon by *M. tasmaniae*. The laboratory-reared individuals however, were smaller

and might have been attacked. The presence of other predators was also possible. Predators, such as spiders hiding in the cracks in the soil, might have provided additional predation pressure. Little evidence of pupal predation was found overall, with very few 'headless' pupal casings found (as opposed to pupal cases from emerged moths), suggesting that much of the predation was confined to earlier larval stages. Studies on related *Helicoverpa* spp. have shown that mortality due to predation and parasitism is at its highest in eggs and early instar larvae, suggesting pupal mortality is less of an issue (Sansone and Smith, 2001, Zalucki *et al.*, 2002, Mohapatra and Sahu, 2005, Pustejovsky and Smith, 2006).

There are several pathogens which kill *Helicoverpa* spp., including naturally occurring *Bacillus thuringiensis*, nuclear polyhedrosis viruses (NPV) and fungal pathogens. NPV may be a large contributor to mortality of *H. punctigera*, with some NPV strains being effective enough to be formulated as biopesticides against *Helicoverpa* species (Teakle *et al.*, 1986, Dhaka *et al.*, 2010). Predators which feed on *Helicoverpa* spp. cadavers can spread NPV via excreta, further contributing to *Helicoverpa* spp. mortality (Cooper, 1981). Sampling *Helicoverpa* spp. larvae in refuges using sweep nets may have potentially been a source of NPV contamination. If cadavers hanging from a host plant are caught in a sweep net, it may inadvertently saturate the netting of the bag, potentially exposing all insects caught in the net to NPV particles. While there is no evidence for or against this hypothesis, it might help account for the high mortality of experimental insects in the Namoi studies in 2011, 2012 and 2013.

With a wide range of potential predators and pathogens present, it is understandable why there was poor survival in the Namoi Valley field cage studies. In comparison, higher survival in inland Queensland suggests lower levels of activity by natural enemies of *H. punctigera* there. In Australia there is general tendency for areas of patchy or intermittent rainfall to give rise to pest outbreaks (Drake, 1994). These patchy rainfalls cause a desynchronisation between pests and natural enemies so they may never reach an equilibrium, leading to the chance of pest

outbreaks becoming relatively high (Drake, 1994). This is a pattern that fits inland Queensland *H. punctigera*, which build up on vegetation germinated by patchy rainfall in the winter (Gregg *et al.*, 1995), seemingly largely unaffected by predators or parasitoids, before migrating into the cropping areas in the spring.

The 2011 inland Queensland study took place during a documented (and very rare) plague of the native long-haired rat, *Rattus villosissimus* (Waite) (Arthur and Harris, 2011). Rodent-sized access tunnels into the inside of two of the emergence cages were discovered, which may account for the large number of pupal cases inside the cages, along with the relatively low adult catches or lack of adult cadavers (Table 15). Such an occurrence is quite rare and rodent predation is not typically considered as an important factor in *H. punctigera* mortality in cropping areas.

### 3.4.5 Abiotic factors affecting survival

There are limited cold hardiness metrics on the life-stages of *H. punctigera.* There is an estimated developmental threshold of 10°C, compared to *H. armigera*, with a developmental threshold of 11-14°C (Allsopp *et al.*, 1991). However, without data on lower lethal temperature, time to death or other cold-hardiness metrics for *H. punctigera*, it is hard to determine whether or not *H. punctigera* can survive colder temperatures than *H. armigera*, or whether temperatures in the Namoi Valley ever approach lower lethal limits. Diapausing *Helicoverpa zea* pupae exposed to cold temperatures drop from surviving 115 days at 5°C to surviving 67 days at 0°C (Morey *et al.*, 2012), a change that could potentially be reflected in *H. punctigera* and prevent much of the overwintering population from surviving if temperatures are too cold for too long. In the case of the 2014 study at “Boondah” Farm, the larvae did not reach pupal stage, all dying as small and medium larvae sometime in mid-July. Although the laboratory-reared larvae had an optimal artificial food source, they did not develop to pupal stage in time, so were not in their cold-hardy pupal stage when the cold weather conditions hit (Fig. 21). The CLIMEX model for ecological suitability of an environment states that when an organism is below its

developmental minimum, it accumulates cold stress, with an excess of stress killing off an individual (Maywald and Sutherst, 1991). *H. punctigera* larvae in these field studies would likely be subjected to extended cold-stress when the temperature went below their developmental threshold, and eventually succumb to it. Larvae at "Boondah" were exposed to greater variability and lower daily average soil temperature and daily minimums than those at "Drayton" (Fig. 20).

The 2012 study at "Milchengowrie" had considerably more variable daily fluctuations (Fig. 19) than "Drayton", 2014 (Fig. 20), though the most severe fluctuations were ameliorated by the soil. Temperature alone was likely not severe enough to kill the experimental insects.

The insects in the cells at "Boondah" in 2014 experienced rather extreme temperature minimums and maximums throughout the study period. Although exposed to extreme temperature variability, typically as high as 20°C each day, this was unlikely to be the cause of the deaths of the experimental insects. Temperature fluctuations of this magnitude are unlikely to be lethal unless the upper or lower end of the range approaches lethal levels. One possible cause may have been that insects reared in the lab at 25°C were exposed to low temperatures soon after being placed in the cells (Fig. 21). The extended cold stress from this exposure may have caused them to die. It should be noted that a smaller type of screen cage was used for this study compared to previous years, which appears to provide less insulation from temperature extremes.

Given the temperatures at both 2014 sites, "Boondah" and "Drayton" (Fig. 20 and Fig. 21), it is almost certain that any pupae present in the soil would have not continued their development beyond the diapause stage throughout the winter, because temperatures never approached 25°C. The inland Queensland field studies showed differences between sites, and the soil types at "Cluny" and "Monkira" were also quite different from each other. The soil around "Cluny" is sandy, and the host plants in the area grow sparsely on nearby sand dunes. In "Monkira", the soil is firmer and floodplain vegetation can be found there. The vegetation cover on sand

dunes is typically much lower than on floodplains, and this is a potential reason for the difference between the temperatures at these two sites, with less shade at "Cluny" and higher temperatures. In both cases however, the soil protects any overwintering *H. punctigera* from the extremes of air temperature in inland Queensland. The differences in temperature from the different soil types may have had different effects on inducing diapause. The temperatures at "Cluny" were high enough to prevent diapause (Fig. 17) but at "Monkira", they were low enough to potentially induce diapause (Fig 16).

The emergence cells, buried in the soil as well as inside a screen-tent, also insulated the pupae from external extremes, though the effect was not as great as a pupa buried fully in the soil of an emergence cage (Fig. 18). Note that Fig. 18 is from a different data logger to Fig. 17, which experienced slightly different temperature conditions.

### 3.4.6 Overwintering, adult emergence and migration

Some tentative conclusions about the relationship between emergence and local pheromone trap catches in inland areas and in the Namoi Valley can be drawn from comparisons shown in Fig. 24 and Fig. 25.

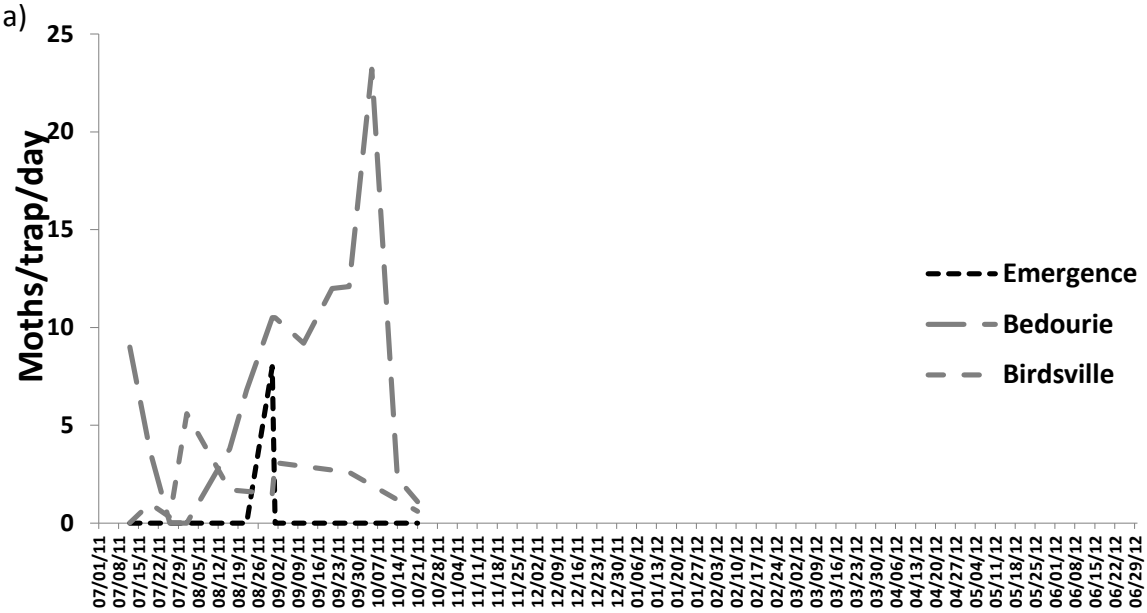
In 2011, the beginning of pheromone catches at "Cluny" corresponded with the limited emergence in the cages. However, the pheromone catches at Bedourie (about 25 km away) began before, and continued well after, emergence had stopped. This may suggest that moths originating from other surrounding areas were caught at Bedourie. Another possibility is that earlier and/or later emergence from the cages at "Cluny" was not detected because the moths were taken by predators (e.g. rats) before they were captured in the containers at the top of the cages.

In 2011, pheromone catches in the Namoi valley were well synchronised with those at Bedourie, and also followed the peak of emergence in the "Cluny" cages. Given

the large differences in soil temperatures between the Namoi and the inland (compare Fig. 19 and Fig. 20), this suggests that this early spring peak in pheromone catches in the Namoi was due to migration from warmer areas, such as inland Queensland, rather than local emergence (Fig. 24).

The 2012 study at "Monkira" showed slightly different results. Emergence was prolonged over a period of about 100 days, although the cages had been seeded on one day, with larvae of similar sizes. This suggests some type of "bet-hedging" mechanism, possibly related to diapause, that would allow a fraction of the population to emerge quickly and utilise any host plants remaining in late winter and early spring, while another fraction emerged later and probably emigrated. Lower temperatures suggested that diapause could potentially be induced in floodplain soils found at "Monkira", but not on the sand dunes at "Cluny" (Section 3.4.5 above). "Monkira" is roughly equidistant from both Birdsville and Bedourie, but Birdsville is in the same river catchment, and in 2012 that catchment flooded, whereas the one at Bedourie did not. Birdsville is therefore a more appropriate comparison site for this year. Despite the low numbers at Birdsville, there was synchronisation between early emergence at "Monkira" and Birdsville pheromone trap catches. However, later emergence at "Monkira" was not reflected in pheromone catches at Birdsville. As in 2011, there was a close synchronisation between a second peak in pheromone trap catches in western Queensland and in the Namoi valley around weeks 12-13 (early September), despite wide variation in soil temperatures, suggesting that in the Namoi early season catches may be associated with immigration rather than local emergence. However, the earliest peak of emergence in inland Queensland (mid to late July) was not mirrored in the Namoi. A possible explanation for these observations is the difference in temperatures between inland Queensland and the Namoi Valley. Temperatures during the first (July) peak may have been high enough to allow emergence of non-diapausing individuals in inland Queensland, which did not migrate from that region. Temperatures in the Namoi Valley did not allow pupae to complete diapause development.

a)




b)

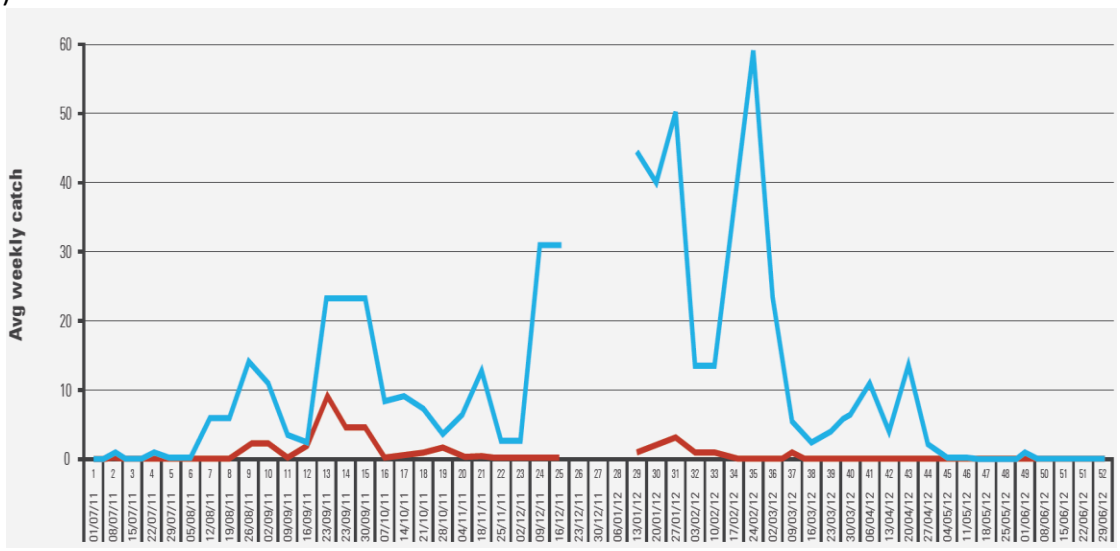



*Fig. 24. Emergence of* H. punctigera *adults from cages at "Cluny" along with pheromone trap catches at the nearby town of Bedourie and Birdsville, QL (top), and from the Namoi (bottom, red line) over the same period, aligned on the same temporal scale, 2011 (Cotton Seed Distributors, 2012).* Numbers for H. armigera *are also shown over this period (blue line).*

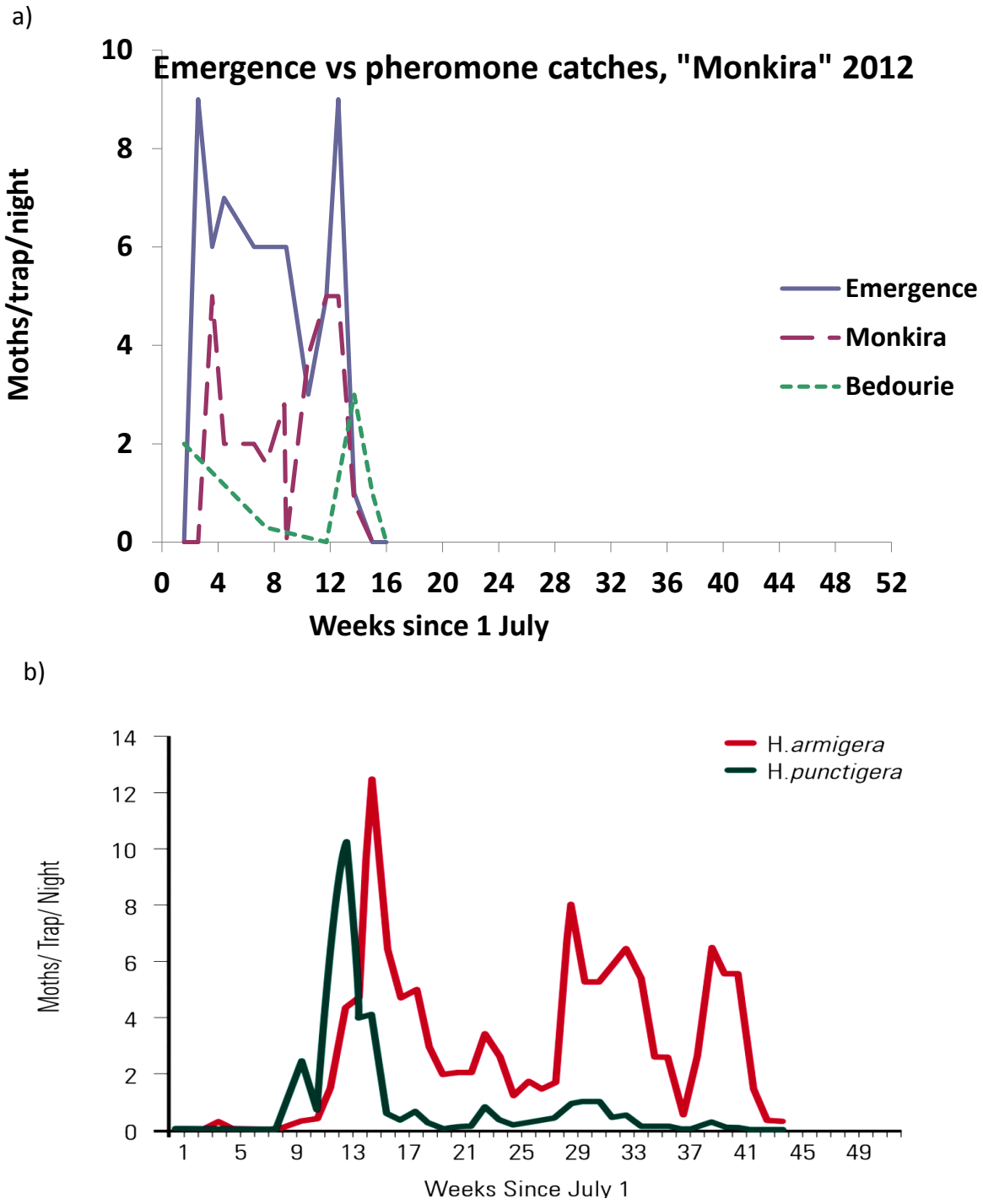



*Fig. 25. Emergence of* H. punctigera *adults from cages at "Monkira" along with pheromone trap catches at "Monkira" and the nearby town of Birdsville, QLD (a), compared with Namoi Valley pheromone trap catches over the same period, on the same scale, 2012 (b)(Cotton Seed Distributors, 2012).*

### 3.4.7 Summary

There are a variety of factors that may have contributed to larval mortality in the 2011-2013 Namoi Valley emergence cages, and thus greatly limited the number of pupae for study. These include rainfall affecting the availability of host plants (Baker *et al.*, 2008), temperature reducing the survival of pupae in the soil (Morey *et al.*, 2012), a changing cotton landscape (Ingard®/Bollgard®) which reduced insecticide use, thus increasing the abundance of predators and parasitoids while reducing the abundance of *Helicoverpa* (Baker and Tann, 2014). The unpredictable ratio of *H. punctigera* to *H. armigera* also made finding a suitable site for a study extremely time consuming. All of these factors potentially contributed to the failure of these field trials and emphasise the need for long-term studies.

Despite these setbacks, the same methods performed better in inland Queensland, where three years of field data were collected. Results in 2011 and 2013 were adversely affected due to a rat plague in 2011 and the collaborator not checking the cages in 2013. Nevertheless, emergence cages provided useful insights into the timing of overwintering moth emergence, and combined with local and remote pheromone trap data, the results suggest that immigration from inland Queensland made a substantial contribution to early season catches in the Namoi region.

Regular pupae digging, which provided immediate access to diapausing *H. punctigera* was the most effective method for obtaining *H. punctigera* pupae in the Namoi, at least in 2014. This method, combined with incubation at two temperatures, suggested that a high level of diapause was induced in late season larvae. Diapause was broken by exposure to low temperatures by early winter, but emergence was delayed because temperatures remained too low until spring.

Temperature probe data suggests a role of vegetation and soil types in the incidence of diapause in overwintering *H. punctigera* in inland Queensland. Pupae in the soil under sand dunes with sparse vegetation are less likely to be in diapause than those in the floodplains, where heavier vegetation cover may play a role in keeping soil temperatures below the threshold for diapause induction. This, and the

prolonged emergence at “Monkira” in 2012, suggests that a fraction of the overwintering population emerges early, in mid to late winter, and does not emigrate, while another fraction emerges in late winter to early spring, and does emigrate. This is in contrast to the Namoi Valley, where temperatures drop below the diapause-inducing threshold in May, and pupae found in the soil under refuge crops would be expected to be in diapause, and there is little emergence until early to mid-spring.

# Chapter 4: Preliminary studies on summer diapause

## 4.1 Introduction

The ecological function of summer diapause is to enable insects to endure harsh conditions due to high temperatures, dehydration and the lack of host plants. It is induced by lengthening photoperiods and higher temperatures, and breaks in response to cooler temperatures and shorter photoperiods (Masaki, 1980). Summer diapause is generally induced before the height of summer, terminated and followed by reproductive and feeding activities in the autumn and winter. Summer diapause has similar physiological responses to winter diapause, with diapausing life-stages exhibiting responses including hypertrophy of fat bodies, reduced rate of metabolism, increased resistance to desiccation and others (Masaki, 1980). Diapause, whether it be summer or winter diapause, can be described as an alternate sequence of physiological or developmental events in response to physiological stimuli (Kostal, 2006). Direct development (morphogenesis) is arrested during diapause, while physiological changes (physiogenesis) still continue along the alternate path (Kostal, 2006). Chapter 1 describes the general terminology of diapause in greater detail. Diapause in pupae is typically expressed as an extended pupal period caused by the prothoracic gland ceasing production of ecdysteroids needed to promote development, while in adults, diapause can cause cessation of egg maturation, degeneration of flight muscles, a halt in mating and atrophy of accessory glands (Denlinger, 2002).

There are less than 30 papers returned using the topic keywords '*Helicoverpa* summer diapause' using a Web of Science search (ISI Web of Knowledge search, June 2015) while winter studies of diapause in *Helicoverpa* spp. are more abundant (Chapter 2). There are no published studies reporting summer diapause in *H. punctigera*, although there are unpublished data suggesting *H. punctigera* pupae

do undergo summer diapause in the Namoi Valley (A.G.L. Wilson unpublished in Zalucki (1991)). Murray and Zalucki (1990) observed that temperatures >38°C induced a pupal quiescence that was terminated under laboratory conditions of 22°C, but this only occurred in 1 out of the 240 *H. punctigera* studied. Rainfall causes soil temperatures to decrease, and could be a potential diapause/quiescence-breaking signal (Murray and Zalucki, 1990). Murray (1991) describes what he calls a 'spring diapause' that occurs from December to March each year which exists as a mechanism to survive unfavourable seasons in a changeable environment. Unfortunately, the onset conditions for this type of diapause were not explored by Murray in laboratory studies (1991).

At least two other *Helicoverpa* species have been found to undergo some form of summer diapause. In China, *Helicoverpa assulta* (Guenée) larvae exposed to 35°C and a 16L:8D photoperiod produced diapausing pupae (Zhang *et al.*, 2014). Diapausing pupae had reduced respiration rates and fat-loss rates compared to non-diapause pupae (Zhang *et al.*, 2014). In *H. armigera*, summer diapause also occurs in the pupal stage, when larvae are exposed to temperatures in the range of 33-39°C (Liu *et al.*, 2004). In both *H. assulta* and *H. armigera*, males experience a higher percentage of diapause than females (Liu *et al.*, 2004, Zhang *et al.*, 2014). Nibouche (1998) found that in *H. armigera*, a 'hot thermal diapause' is distinct from either 'aestivation' or 'thermal photoperiodic diapause', noting that photoperiod did not affect 'hot thermal diapause' in this case.

Summer diapause in adult insects causes the maturation of the gonads to be suspended until autumn, with metabolic rate being maintained at a very low level of oxygen consumption (Masaki, 1980). Diapausing tropical butterflies (*Hypolimnas bolina* L. (Lepidoptera: Nymphalidae)) undergo adult diapause characterised by under-developed ovaries with no oogenesis and enlarged fat bodies, compared to non-diapausing adults (Pieloor and Seymour, 2001). Another species, the long lived moth *Caloptilia fraxinella* (Ely) (Lepidoptera: Gracillariidae) undergoes a nine-month reproductive diapause, with males no longer receptive to female pheromones until

diapause conditions are halted and become optimal for mating (Lemmen and Evenden, 2015). There are therefore at least two separate physiological responses within Lepidoptera that can stop adult mating while in a state of summer diapause.

Of particular interest on the topic of summer diapause, is trying to understand why *H. punctigera* moths are often caught in light traps in summer and autumn, but not in pheromone traps. An adult diapause response that stops males from responding to female pheromones, if present, might explain this response. Previous studies investigating this discrepancy between trap catches have suggested that competition between pheromone lures and presence of conspecific females may be the reason (Baker *et al.*, 2011). Whether the occurrence of summer diapause or quiescence in *H. punctigera* adults might explain this discrepancy warrants further study.

### 4.1.2 Aims

1. Determine any evidence of diapause or quiescence in *H. punctigera* in response to summer photoperiods and conditions.

The aim of this chapter was to conduct preliminary studies in the laboratory to investigate which, if any, life stages of *H. punctigera* undergo diapause/quiescence in response to summer conditions, and thus, identify areas for further study.

## 4.2 Materials and Methods

### 4.2.1 Pupal summer diapause study

The same apparatus and methodologies in Chapter 2 were used for these studies, with the following differences. Eighty freshly hatched larvae per treatment were exposed to temperatures of 31, 33, and 35°C under a single photoperiod of 14:10, for the entirety of their development. Diapause was determined using the eyespot and pupal timing methods used in Chapter 2. Only live pupae were counted with regard to pupal timing as an indicator of diapause.

### 4.2.2 Adult summer diapause study

Freshly hatched larvae were exposed to high temperatures in the same manner as the pupal diapause in chapter 2, using two temperatures, 25°C and 32°C. Emerging moths were transferred into a mating cage inside the controlled environment cabinets at 14L:10D (Fig. 2., Chapter 2). Moths were provided with fresh 10% sugar solution every 48h in the same manner as the insect cultures, until they died. Dead moths were stored in 100% alcohol prior to dissection to determine their mated status (Coombs *et al.*, 1993). Eighty moths per treatment were used, but these were pseudoreplicated due to the lack of cabinets available. See chapter 2.5.4 for a discussion on this topic.

### 4.2.3 Statistical analysis

Adult diapause data were subjected to Generalised Linear Modelling using an identity link and Gaussian errors, using R 3.1.2 (R Development Core Team, 2014). Pupal diapause data was subjected to Generalised Linear Modelling using a logit link function and binomial errors using R 3.1.2 (R Development Core Team, 2014).

## 4.3 Results

Data on pupal diapause and survival in *H. punctigera* larvae in response to the three summer temperatures tested are shown in Table 17.

*Table 17. Larval survival and pupal diapause at different temperature regimes under 14L:10D.*

| **Temperature** | **31°C** | **32°C** | **35°C** |
|---|---|---|---|
| Survival /80 | 49% | 50% | 29% |
| Diapause %/N | 21% | 30% | 17% |
| N | 39 | 40 | 23 |

Although the percentages of diapause at different temperatures were not significantly different from each other in the GLM model (p=0.55), mainly due to the relatively low survival rate and lack of replication, the fact that diapause, as indicated by eye spot movement, was detected in all three temperatures, suggests that temperatures in the range of 31-35°C can induce some form of summer diapause in *H. punctigera* pupae.

The adult diapause study showed a strong effect of the temperature treatments, with the majority of adult females remaining unmated at 32°C compared with 25°C (Table 18). All adult insects survived at least 6 days after emergence.

*Table 18. Number of times adult females were mated at two temperature regimes. N males refers to the number of males that were placed in the cage with the females.*

| **Number of times females were mated** | **25°C** | **32°C** |
|---|---|---|
| 0 | 21% | 73% |
| 1 | 36% | 13% |
| 2 | 0% | 13% |
| 3 | 21% | 0% |
| 4 | 14% | 0% |
| 5 | 7% | 0% |
| **N (females)** | 28 | 15 |
| **N (males)** | 44 | 18 |
| **% Survival to adult /80** | 90% | 41% |

*Table 19. Statistical analysis of diapause in H. punctigera adult females based on number of times mated. Null deviance: 104.279 on 42 degrees of freedom. Residual deviance: 81.457 on 41 degrees of freedom. AIC: 155.5.*

| Coefficients: | | | | |
|---|---|---|---|---|
| Estimate | Std. | Error | t | value |
| (Intercept) | 1.9286 | 0.2664 | 7.24 | 7.57E-09 |
| CatgorTemp32 | -1.5286 | 0.451 | -3.389 | 0.00156 |

The number of times females mated at the two temperature regimes were significantly different from each other at P=0.0016 (Table 19), suggesting some form of adult diapause or quiescence in response to high temperatures.

## 4.4 Discussion

The percentages of pupal diapause at different temperature treatments were not significantly different from each other given the numbers used in the study, however, based on both eye-spot movement and the pupal duration (Cullen and Browning, 1978), the observed response of the pupae suggests they were in a state of true diapause rather than quiescence, though this requires further testing with more replication. Certainly, it is worth further study to precisely determine the nature of this apparent summer diapause.

The 'spring diapause' described by Murray (1991) could certainly fit the results observed in this preliminary study and the timing of the spring diapause, from December to March, could protect it from the high temperatures in inland Queensland. Given this four-month window, it seems likely that this diapause

would be broken during this period once temperatures return to more optimal levels for *H. punctigera* to survive.

The results of the adult study clearly show that significantly less mating occurs at the higher temperature, with much less mating occurring at 32°C. Some long-distance migrating noctuid moths such as *Agrotis infusa* (Boisduval) aestivate during the summer months at high altitudes, ceasing to eat or reproduce until they return to breeding sites in the autumn (Common, 1954, Green, 2008). A reason for the lack of mating could be due to males not responding to female pheromones or females not producing pheromones or being receptive to mating, or both. The methodology of the study did not allow me to distinguish between these possibilities and is an area for further study.

Masaki (1980) notes that summer diapause suspends the maturation of the gonads until autumn, which fits what was anecdotally observed of the ovaries while performing the dissections of the spermatophores, but as the study did not examine male pheromone responsiveness we cannot rule out that insensitivity to female pheromones might occur in summer diapause (Lemmen and Evenden, 2015). This study has determined there is a physiological response to higher temperatures in adults, but whether this is diapause or aestivation requires further study.

While the studies in this chapter are preliminary, the results indicate the need for further work to define the parameters of possible summer diapause and/or quiescence in *H. punctigera* pupae and moths. Whether the observed dormancy in this species is true diapause or a form of quiescence is unclear (Kostal, 2006). The key difference between the two terms would be the conditions on how they are broken. The role of water in ending summer diapause needs further consideration, either by cooling the surrounding soil or by affecting the insect directly (Murray and Zalucki, 1990), particularly as this might be a mechanism by which *H. punctigera* emerges in response to winter rain. With regard to summer survival, the readings from inland Queensland temperature probes in Chapter 3 suggest that soil

temperatures may exceed 32°C on many days, even in winter, and that temperatures later in spring and during summer are likely to be much higher. Studies that focus on realistic temperature fluctuations, with periods of temperatures >35°C that would be lethal to the larvae under constant exposure, would provide better insight on how this diapause/quiescence is induced/broken. It is also not established whether Nibouche's (1998) observations on photoperiod playing no effect on summer diapause in *H. armigera* in France are true for *H. punctigera* in Australia. Temperature and photoperiod may have as complex interactions at high temperature as at low temperatures (Chapter 2).

Theoretically, if pupal summer diapause occurs based on temperature but not photoperiod as in *H. armigera* in France (Nibouche, 1998), it might be possible that a 'summer' diapause might be initiated at almost any time of year following periods of high soil temperature (Chapter 3). Given that 'spring' diapause could last from December through to March depending on conditions, terminologies like 'spring' or 'summer' diapause may not have a lot of meaning for *H. punctigera*, which seems likely to undergo some form of diapause whenever conditions are sufficiently harsh (Murray, 1991). The conditions for the onset and termination of this adaptive diapause could be a key difference between *H. punctigera* and *H. armigera* and may explain why *H. punctigera* is able to colonise areas further inland than *H. armigera*. It is likely that a smaller proportion of *H. punctigera* populations undergo spring/summer diapause in the inland compared to winter diapause in cropping areas, and this is another 'bet-hedging' adaptation to ensure that some of the population survive unpredictable periods of unfavourable conditions. Finally, the discovery of a spring or summer diapause in a *H. punctigera* life stage does not preclude the possibility of a quiescence also existing in the same life stage.

# Chapter 5: *H. punctigera* host plants in western Queensland – a GIS approach

## 5.1 Introduction

It is very difficult to obtain a robust understanding on the ecology of a species with studies conducted solely over a narrow time period typical of a PhD project. When the occurrence of the study species is patchy and/or subject to environmental factors we do not fully understand (Chapters 2 and 3), drawing conclusions from four seasons of field studies is likely to be difficult, because results may be unrepresentative of the whole system. In this chapter, the HIRG (*Helicoverpa* Inland Research Group) dataset is used in combination with more recent survey data. The HIRG was an informal research collaboration between the University of New England (P.C. Gregg), CSIRO (G. P. Fitt), University of Queensland (M. P. Zalucki) and Queensland Department of Primary Industries (P.H. Twine and D.A.H. Murray) which operated between 1987 and 1993. It collected data on the abundance and distribution of *Helicoverpa* spp. larvae and adults in inland Australia, summaries of which were published by Zalucki *et al.* (1994) and Gregg *et al.* (1995), and incorporated in GIS models by Rochester (1998) and used to predict the sizes of inland *H. punctigera* populations and the potential for migration to cropping areas, on an annual basis.

Similar data were obtained during the course of an ARC Discovery Research project between 1995 and 1998 (P.C. Gregg and V.A. Drake, unpublished), and in a Cotton Research and Development Corporation project between 2000 and 2001 (P.C. Gregg and G. P. Fitt, unpublished). No further surveys were conducted until 2009, when further work (P.C. Gregg and A.P. Del Socorro, unpublished) was initiated in projects funded by the Cotton Research and Development Corporation. I have been a participant in these projects since 2011. Thus, while the majority of these survey data were not captured by me, they have been largely unpublished and

unused (in some cases for decades), and this chapter attempts to use GIS software to visualise these data and perform novel analyses on them. These analyses were intended to detect if there was any changes in vegetation over the course of the entire suite of surveys, from 1989 to 2012.

### 5.1.1 The Millennium drought

Western Queensland, along with much of the eastern half of Australia, suffered a prolonged drought in the first decade of the 21st century which is sometimes described as the Millennium Drought. This drought, which was regarded as at least as unusual as a one in one hundred year phenomenon, lasted for most of a decade, starting in July 2001. The survey data for inland *Helicoverpa* spp. described above thus falls into two categories: pre-Millenium drought (1989-2001) and post-Millennium Drought (2009 on). No surveys were conducted during the Millennium Drought. Maps illustrating some of the rainfall deficiencies during this period are presented in Fig. 26. In inland Queensland the drought continued from 2001 before finally breaking in 2009 (Fig. 26). There appear to be no published studies describing the impact of this drought on the composition of the vegetation of inland Australia, though maps of vegetation conditions on the Long Paddock website (Queensland Department of Science Information Technology and Innovation, 2015) as well as many anecdotal reports in popular media indicate that the quantity of pasture vegetation was greatly reduced. Given the length and intensity of the drought, there may have been changes in the seed banks of inland hosts of *H. punctigera*. Parts of South Australia and Western Australia did not have such extreme drought conditions as inland Queensland (Fig. 26), and would have likely continued to be a source of *H. punctigera* into cropping areas of southern Australia over much of the Millennium Drought.

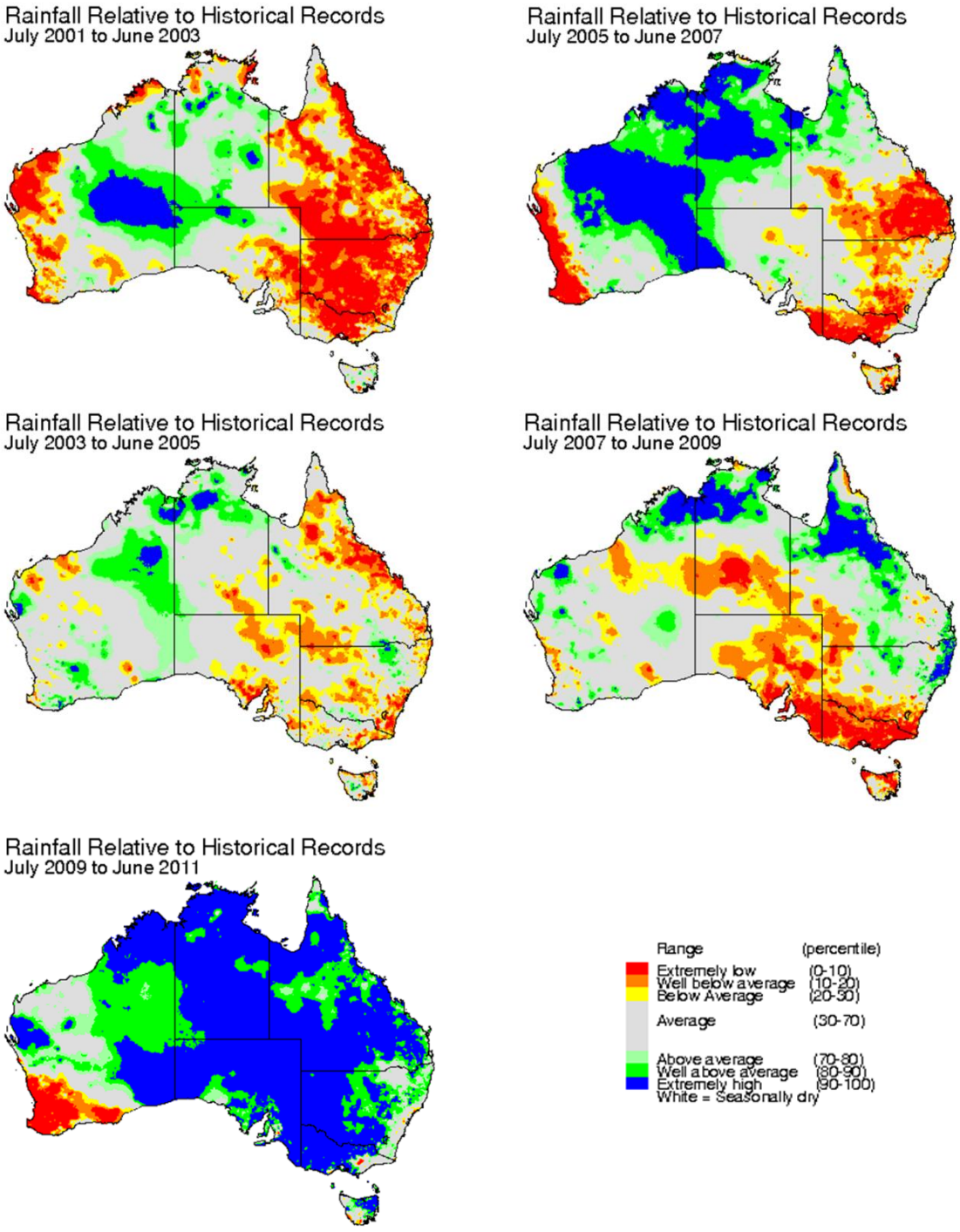



*Fig. 26. Long-term average rainfall across Australia 2001-2011. (Department of Science, Information Technology and Innovation, Queensland Government).*

### 5.1.2 GIS systems and *Helicoverpa* spp.

Using GIS software is not a method, so much as an approach to handling existing data, allowing comparisons to be made between database entries with comparative ease, as well as being able to compare maps (raster data represented as coloured pixels on a map) with data base coordinates (latitude/longitude coordinates with other attributes, such as host plants and *H. punctigera* abundance data).

Use of GIS approaches with regard to *Helicoverpa* spp. has been limited thus far. Holmstrom *et al.* (2001) took data from *H. zea* caught in blacklight traps in corn in the USA and used GIS to pair the population data with the corresponding map location. This was done as an extension tool to inform growers on weekly *H. zea* population numbers (Holmstrom *et al.*, 2001). Lu *et al.* (2006) used the law of thermal constant/day-degrees in combination with GIS to predict *H. armigera* emergence at different locations of the Xinjiang region of China. Moral Garcia (2004, 2006) used GIS with pheromone sampling data to locate sample points, characterise distribution patterns within a tomato field and map distributions of *H. armigera* in Extremadura, Spain. Kriticos et al. (2015) combined crop and irrigation databases in combination with the CLIMEX model to predict the range of *H. armigera* in the Americas. These limited examples using *Helicoverpa* spp demonstrate the largely untapped potential for GIS to act in a predictive or informative capacity.

Although modern GIS software was not available at the time, simulation models for predicting the migration of *H. armigera* and *H. punctigera* based on climatic data have been constructed (Rochester *et al.*, 1996, Dillon *et al.*, 1996) and validated by pheromone trap catches and radar data (Rochester, 1998). Rochester (1998)'s larval abundance model used the best available data at the time to predict migration in *H. armigera* and *H. punctigera*, but used only one set of assumptions based on a series of variables including temperature, rainfall, soil landscape class (a proxy for vegetation), NDVI for April and June and 12-year average NDVI. Rochester (1998) obtained NDVI satellite data from NOAA Advanced Very High Resolution

Radiometer (AVHRR), which had a resolution of 5km monthly (1982-90) and 1km fortnightly (1991-1993), and used those data to create 12-year average NDVI images for inclusion in his model. Rochester's (1998) model was created specifically to predict emerging moth populations, given a series of climatic data inputs. There have been no attempts to use GIS techniques for *H. punctigera* in Australia since the major drought of 2001-2009, which may have resulted in significant vegetation changes. In this chapter I endeavour to fill this gap. In contrast to the work of Rochester (1998), rather than using proxy-data for vegetation types, I use direct observations of host vegetation data based on long-term datasets, validated with comparison to vegetation land-use maps. I then use the findings to understand how the distribution of host plants may have changed after the drought, and what this may have meant for the population dynamics of *H. punctigera* in inland and cropping regions. The time frames of this question are longer than those of Rochester (1998) who used GIS to predict the probability of larvae being present at various locations in any one season.

Comparing survey data with remote sensing data for verification is not a novel method. Zhou et al., (1998) demonstrated that when given a consistent method, four field observers performing vegetation transects could produce results with a minimum correlation coefficient (R2 value) of 0.74 with two observers using image interpretation on photographs of vegetation in the same area. These experiments were done in locations and vegetation types similar to those in many of similar in scope to our surveys, and show good correlation between field observers and remote observers, but they were done on much smaller areas than those in this chapter.

### 5.1.3 Host plants

A host plant, in the context of the polyphagous *Helicoverpa* spp., is defined as a plant that supports the development of larvae and contributes to the abundance of reproducing adults. A proportion of the larvae need to complete development to

become reproductively viable adults, for this classification to be made (Cunningham and Zalucki, 2014). The quality of the host, described by terms such as 'good' or 'bad' is further specified by the relative abundance of eggs and larvae found on it (Walter and Benfield, 1994), as well as the host plant nutrition contributing to the percentage of larvae surviving to reproducing adults (Kyi *et al.*, 1991, Zalucki *et al.*, 2002).

Polyphagous *Helicoverpa* spp. have hierarchies of ovipositional host plant preferences which have been demonstrated to be stable over time and space (Jallow and Zalucki, 1996, Rajapakse and Walter, 2007, Zalucki *et al.*, 2012). However host plant preferences can be varied by learned responses, egg loads and relative abundance of hosts at certain times and places (Cunningham and Zalucki, 2014). The importance of different host plants for any polyphagous insect lies in their relations to (1) their distribution and relative abundance, (2) the seasonality of germination and growth in relation to the phenology of the insect (3) host-plant responses to weather variables, such as seasonal rainfall and flooding, (4) oviposition preferences of the insect, and (5) the abundance and survival of immature stages feeding on host-plants. In the case of *H. punctigera* in inland Australia there is a substantive body of work on the first three factors (Boyland, 1977, Purdie, 1990, Zalucki *et al.*, 1994, Alexander, 2005), but very little information on the last two.

The patchiness and unpredictability of the environment in inland Queensland, with frequent extremes of high temperature and drought, means that *H. punctigera* host plants are not present in that area for much of the year in large numbers. Many of the host plants, particularly daisies, are ephemeral species that only appear in response to seasonal rainfall (Hesse and Simpson, 2006). Most of the many daisy species in all three of the inland habitats defined in the next section of this chapter have seed dormancy mechanisms that prevent germination when conditions are hot, so the plants germinate in response to rain in cooler months (Mott and Groves, 1981, Hoyle *et al.*, 2008).

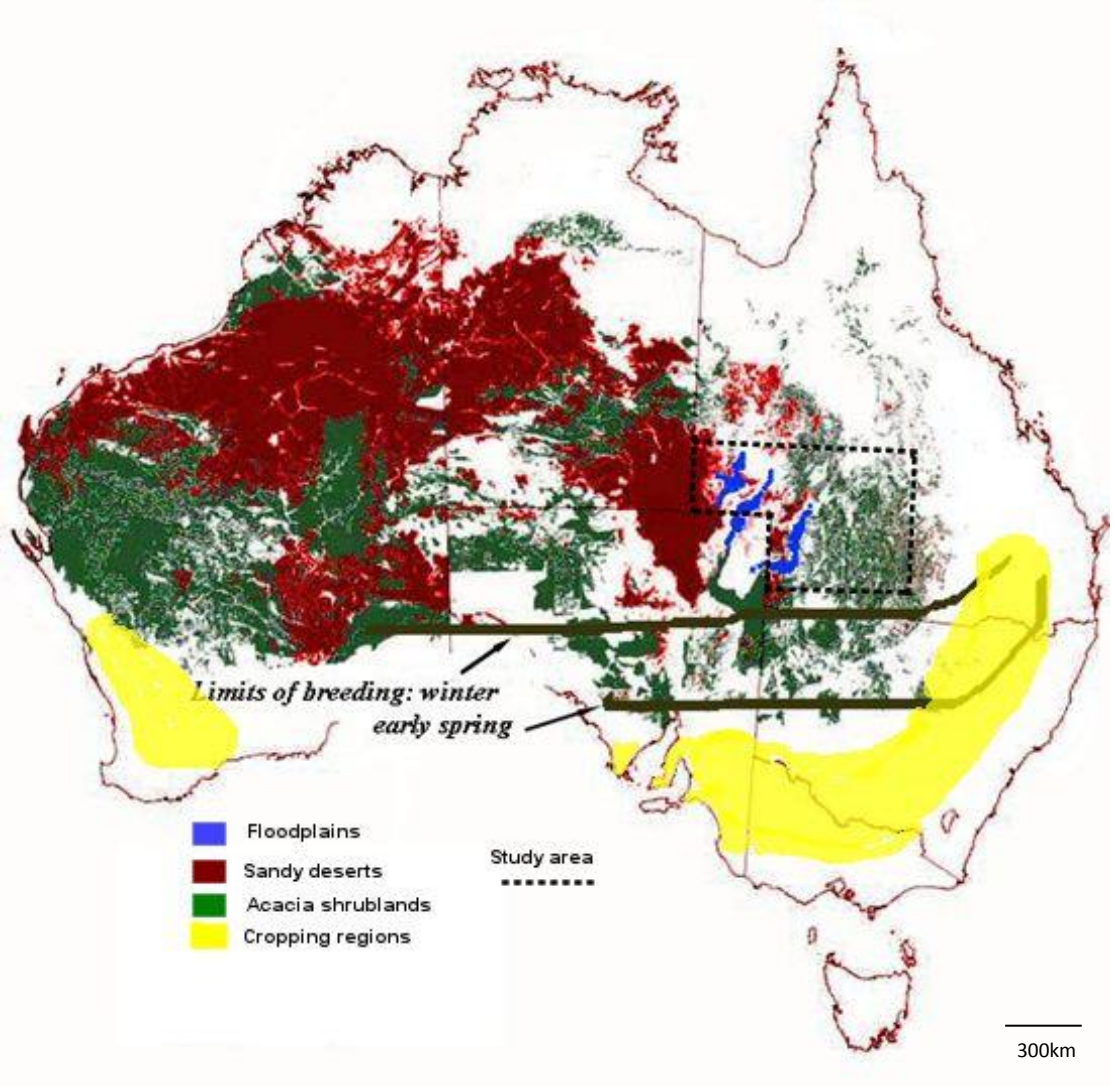



*Fig. 27.* Main H. punctigera *host plant vegetation types in Australia, showing the study area where surveys took place (P. Gregg).*

### 5.1.4 Major habitats

In inland Queensland, host plants for *H. punctigera* are found in three categories of major habitats, or 'vegetation types'. These habitats include the floodplains, the sandy deserts and the *Acacia* shrublands (also referred to as mulga) (Fig. 27) (Gregg *et al.*, 1995). Within these broad classifications there are many sub-categories (Boyland 1977).

#### Floodplains

Floodplains occupy about 1 x $10^6$ ha of the inland Queensland area, the main ones being Cooper Creek, the Diamantina and the Georgina/Eyre Creek systems. The dominant *H. punctigera* hosts in the floodplains belong to the family Fabaceae, although Asteraceae can also be common (Fig. 28) (Dawson and Boyland, 1974, Boyland, 1977, Mills and Purdie, 1982, Turner and Beeston, 1977, Mc Donald, 1982, Purdie, 1986, Zalucki *et al*. 1994). Fig. 28 shows an example of the border between sand dunes and floodplains.

*Fig. 28. Host plants found on Diamantina floodplains, 25°54'57"S, 139°23'45"E, near Birdsville, July 2011. The lower area (foreground) is dominated by* Cullen cinereum (Fabaceae), *(green) and further back are* Gnephosis arachnoidea (Asteraceae) *(yellow). These hosts have germinated in response to flooding resulting from rain hundreds of km to the north. (Photo: P. Gregg.)*

## Sandy Deserts

Sandy deserts occupy about 140 x $10^6$ ha in Australia. In western Queensland the largest area of sandy desert is the Simpson Desert which also extends into adjacent areas of South Australia and the Northern Territory. Here Asteraceae are the main *H. punctigera* hosts although Fabaceae can be present in localised areas (Fig. 29) (Dawson and Boyland, 1974, Boyland, 1977, Mills and Purdie, 1982, Turner and Beeston, 1977, Mc Donald, 1982, Purdie, 1986, Zalucki *et al.* 1994).


*Fig. 29. Host plants found on sand dunes-* Polycalymma stuartii *(Asteraceae)(white flowers) and* Senecio gregorii *(Asteraceae) (yellow flowers, after local rain has fallen, June 2009 (Photo: P. Gregg.)*

## *Acacia* shrublands (Mulga)

*Acacia* shrublands occupy about 160 x $10^6$ ha in Australia. In Queensland they are largely distributed in the "mulga lands" to the east of the floodplains and sandy deserts, and separate those habitats from the cropping areas which are located still further to the east. The main *H. punctigera* hosts in the *Acacia* shrublands are Asteraceae, while Fabaceae are very uncommon. There are also some host species in the Goodeniaceae, Malvaceae and other families (Dawson and Boyland, 1974, Boyland, 1977, Mills and Purdie, 1982, Turner and Beeston, 1977, Mc Donald, 1982, Purdie, 1986, Zalucki *et al.,* 1994). Examples of host plants found in the *Acacia* shrublands are shown in Fig. 30.

*Fig. 30. Host plants found on* Acacia *shrublands (mulgas)-* Velleia glabrata *(*Goodeniaceae), *yellow flowers and* Rhodanthe floribunda *(Asteraceae), white flowers, August 2009. (Photo: P. Gregg).*

### 5.1.5 Aims

1. Compile a database of historical survey data in a format that allowed comparisons to be made before and after the Millennium Drought,
2. Determine a way to verify subjective scores of vegetation made during the surveys against an objective remotely sensed index.
3. Interrogate the resulting GIS database to identify potential changes which might affect the ecology of *H. punctigera*, especially the roles of immigration and local overwintering in cropping regions.

## 5.2 Materials and Methods

### 5.2.1 Inland Queensland surveys

Since 1987, a network of pheromone traps across mid-western Queensland has provided information on the distribution of inland *H. punctigera* populations. These pheromone trap data in combination with information from local trap operators,


*Fig. 31. Sweep net sampling to collect* H. punctigera *larvae on sand dunes near "Cluny", 24°31'34"S, 139°34'54"E, 2013. (Photo: P. Gregg).*

meteorological data and NOAA satellite data, were used to determine if extensive growth of *H. punctigera* host plants had occurred, and whether an inland field trip was warranted to do vegetation and larval surveys (Zalucki *et al.*, 1994).

When surveys were undertaken, from one to five times per year, general vegetation conditions were recorded, the presence of specific host plants was noted, and sweep netting was used to determine the abundance of larvae (Fig. 31). Each trip involved a small number of collaborators from various institutions, and on those trips in which P. Gregg took part, vegetation along the route was documented with qualitative techniques described below.

Host plant survey data were captured using the methodology used by the HIRG surveys, and described by Zalucki *et al.* (1994). The surveys included in the GIS in this chapter derived from a total of 20 field trips covering 992 sites from 1989-2000 (pre-drought period) and 7 trips from 2009-2013, covering 535 sites. I was personally involved in the surveys from 2011 onwards.

Annual surveys done from 2009 typically followed the routes around Birdsville (139.35E, 25.90), Bedourie (139.47E, 24.35S) and Windorah (142.65E, 25.42S) in western Queensland. All vegetation surveys were in the form of audio recordings. From 1993 onwards, GPS data formed part of each recording, but in older reports a bearing (e.g. “20km North of Bedourie”) was used instead. Recordings were not made at exactly the same locations for every trip, but at fixed intervals (usually 10 or 20 km, as measured by the vehicle odometer) from landmarks such as road intersections, creek crossings and towns that were varied between trips. In this way a random sample of the survey sites was assured. Vegetation and tree assessments were recorded by P. Gregg in all cases, and consisted of a general description of the vegetation including trees as well as a numeration of the relative abundance of trees and herbaceous vegetation, separately. Vegetation including trees and herbaceous plants was scored separately through a scale of 0 to 10, with 0 for bare surface and 10 for complete surface cover. Only green vegetation was included in these estimates. While trees are not hosts for *H. punctigera*, their contribution to NDVI had to be removed, in order to determine the NDVI attributable to the herbaceous vegetation, which included the host plants.

These vegetation ratings were subjective. Given the large number of survey sites and the travel distances between them, quantitative vegetation cover measurement was not feasible. At each site, the presence of daisies or legumes was specifically noted, and the presence of known host plants was recorded at the species level.

The data were transcribed from audio reports by me into a spreadsheet and formatted into digital latitude/longitude coordinates that could be applied to map software. The survey database therefore contained, in addition to the time, date and coordinates of the survey, information on tree types and abundance, herbaceous vegetation type and abundance, species of host plants (where present), and details of any sweep-net larval surveys at each site.

Data were split into pre-drought (1989-2000) and post-drought (2009 onwards) subsets in order to examine the effects of the drought on the presence of *H. punctigera* host plants. The number of survey trips, and vegetation ratings, in each major vegetation type, pre- and post-drought are shown in Table 20. With the database complete, queries were made using pivot tables and lookup formulae.

### 5.2.2 Vegetation map layer

To provide an objective measure of the vegetation regions where survey data were collected, the survey coordinates were compared against the Western Arid Region Land Use Study, Parts 1-6 (Fig. 32) (Dawson and Boyland, 1974, Boyland, 1977, Mills and Purdie, 1982, Turner and Beeston, 1977, Mc Donald, 1982, Purdie, 1986).

*Table 20. Numbers of field trips and sites of vegetation ratings included in the database.*

| | Number of trips/year | Floodplain | *Acacia* shrublands | Sandy deserts | Total |
|---|---|---|---|---|---|
| **Pre-drought** | **20** | **137** | **695** | **140** | **992** |
| 1989 | 2 | 38 | 272 | 29 | 341 |
| 1990 | 2 | 24 | 137 | 11 | 174 |
| 1991 | 1 | 9 | 18 | 5 | 33 |
| 1992 | 2 | | 7 | 34 | 43 |
| 1993 | 2 | 14 | 41 | 29 | 86 |
| 1994 | 5 | 34 | 72 | 16 | 127 |
| 1995 | 1 | 2 | 23 | 0 | 26 |
| 1996 | 1 | | 12 | 5 | 18 |
| 1997 | 1 | 11 | 76 | 6 | 94 |
| 2000 | 3 | 5 | 37 | 5 | 50 |
| **Post-drought** | **7** | **114** | **364** | **51** | **536** |
| 2009 | 1 | 7 | 87 | 1 | 96 |
| 2010 | 2 | 25 | 103 | 12 | 142 |
| 2011 | 2 | 39 | 88 | 14 | 143 |
| 2012 | 2 | 43 | 86 | 24 | 155 |
| **Total** | **27** | **251** | **1059** | **191** | **1501** |

These maps cover the majority of inland Queensland, including the inland field sites from Chapter 3, and all the field survey sites used for the GIS in this chapter. The maps were created from aerial photography and ground surveys and used a 1:500 000 scale and the Transverse Mercator Projection. Each map has a number of colours, as well as each region having a letter/number code to further distinguish the colours representing each broad vegetation type (Fig. 33). These maps were used to determine the major vegetation type (e.g. sandy desert, *Acacia* shrubland, floodplains) where each of the surveys had been done.

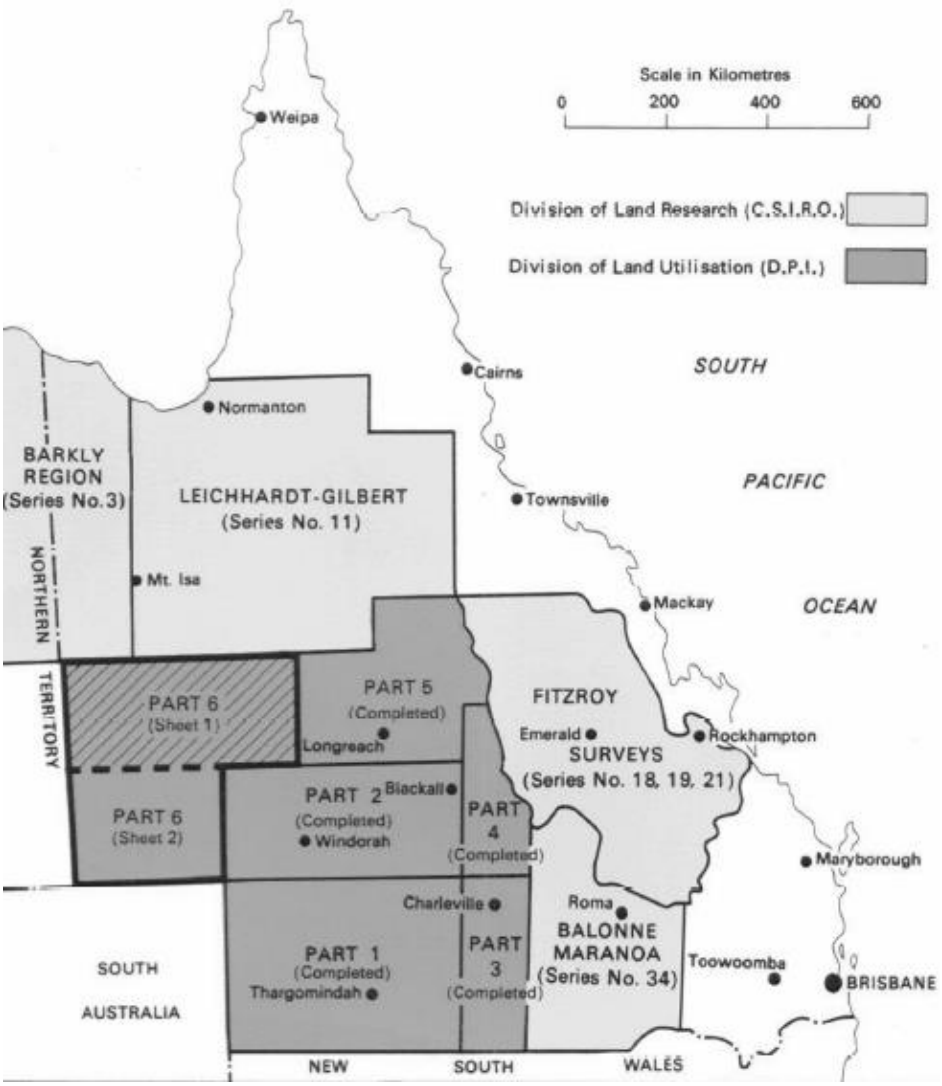



*Fig. 32. The areas covered by all six parts of the Western Arid Land Use maps. Creative Commons 3.0 AU (Boyland 1977).*

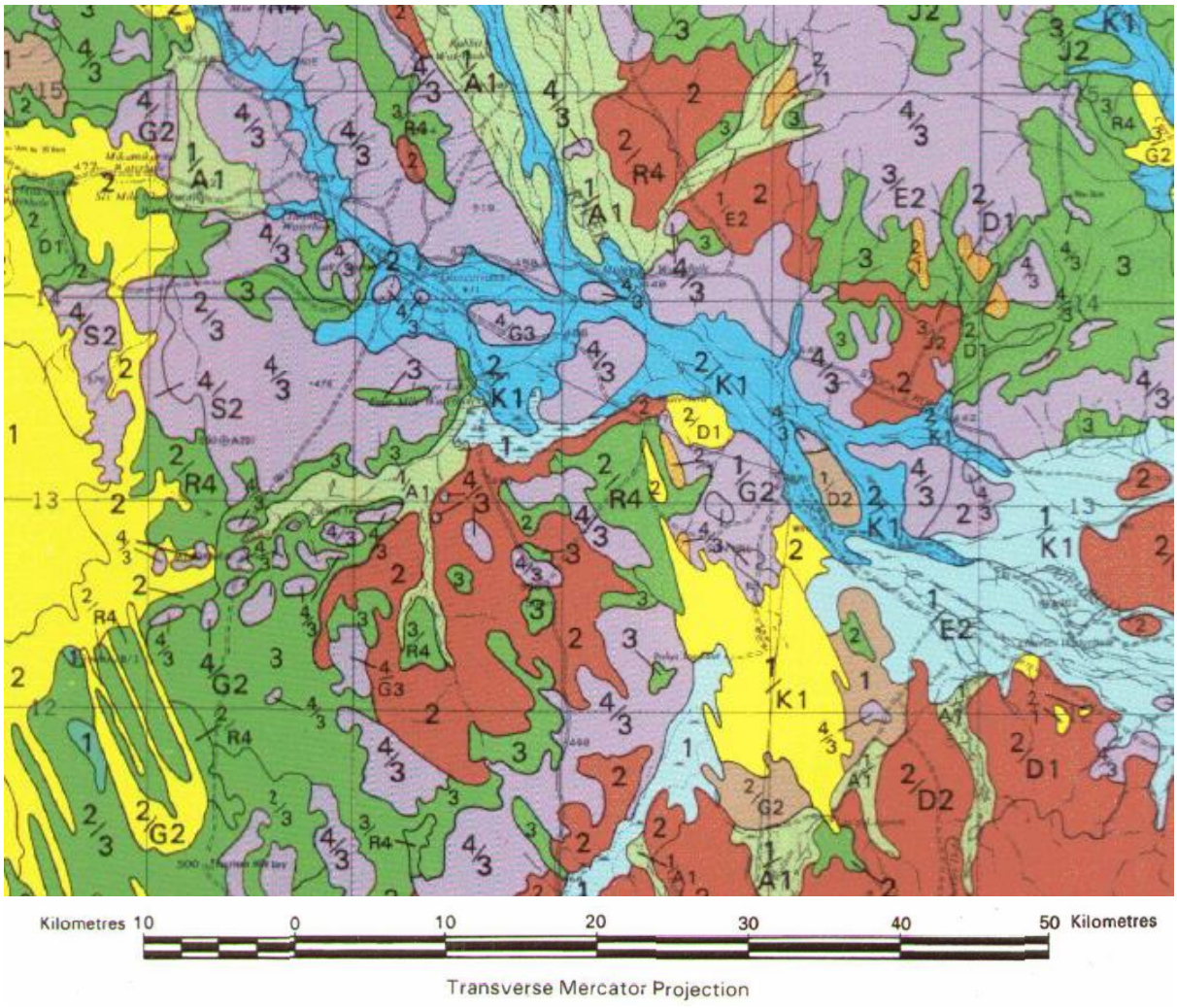



*Fig. 33. A small section (around Birdsville) of Part Six of the Western Arid Land Use map with the scale included. Creative Commons 3.0 AU (Boyland 1977).*

### 5.2.3 Landsat data

Landsat 7, launched in April 1999, is designed to refresh the global archive of satellite images. The Landsat 7 satellite uses Enhanced Thematic Mapper Plus to capture eight different wavelength bands (Table 21) (USGS, 2015). Each pixel in the NDVI images used represents 30m. Landsat 7 data are continuously collected across the region, with the same site being imaged every 16 days.

*Table 21. List of Landsat 7 bands (Source: USGS, public domain)*

| Landsat 7 | Wavelength (micrometers) | Resolution (meters) |
|---|---|---|
| Band 1 | 0.45-0.52 | 30 |
| Band 2 | 0.52-0.60 | 30 |
| Band 3 | 0.63-0.69 | 30 |
| Band 4 | 0.77-0.90 | 30 |
| Band 5 | 1.55-1.75 | 30 |
| Band 6 | 10.40-12.50 | 60 |
| Band 7 | 2.09-2.35 | 30 |
| Band 8 | 0.52-0.90 | 15 |

The data obtained from all Landsat satellites have been free for the public to access since January 2009, with a variety of both raw and processed images available to download (Irons, 2015).

### 5.2.4 NDVI data

Live vegetation absorbs visible light while reflecting energy from the near-infrared spectrum. Normalized Difference Vegetation Index (NDVI) is a calculation of the 'greenness' and density of growing vegetation. It is an index which compares the red and near infrared spectrum bands *rRed* and *rNIR*:

NDVI = (rNIR - rRed) / (rNIR + rRed)

The NDVI out value ranges from -1 to +1. The index has higher values to indicate greener, denser vegetation, while bare soil and snow have values close to zero. Water bodies are characterised by negative values.

By obtaining Landsat 7 images (Table 21, bands 3 and 4) from within 16 days of the time of the surveys and creating NDVI image composites calculated from the red and near infra-red Landsat image bands, the specific NDVI at the time of each survey was extracted (Fig. 34), to allow comparison with the subjective vegetation survey ratings recorded by P. Gregg, and to provide some quantitative estimate of the accuracy and repeatability of the scores he recorded. To ensure accuracy using these data, only the post-2000 year data using modern GPS coordinate collection were used for this comparison. Landsat 7 image data closest to the time of the survey was selected, but cloud cover could extend the time difference by up to a month.

### 5.2.5 MODIS EVI

An alternative to NDVI is the Global MODIS vegetation indices, which are designed to provide consistent spatial and temporal comparisons of vegetation conditions. MODIS EVI uses blue, red, and near-infrared reflectances of 469 nm, 645 nm, and 858 nm, to determine daily vegetation indices, using the equation:

$$EVI = G \times (NIR - RED)/(NIR + C1 \times RED - C2 \times Blue + L)$$

For MODIS EVI data, NIR/red/blue pixel values are atmospherically-corrected surface reflectance. L, C1, C2 and G are all constants, where L is canopy background adjustment, C1 and C2 are coefficients of the aerosol resistance term and G is the gain factor on the result. The MODIS EVI data, like other Landsat 7 data, are obtained every 16 days, but unlike direct colour bands, have a resolution of 250m rather than 30m (Fig. 35). EVI reduces the effects of tree canopy cover by calculating the non-linear, differential NIR and red radiant transfer through a

canopy, L in the equation above. This allowed me to discount the tree survey ratings (unlike with NDVI, where tree cover is a larger component), and focus on surface vegetation scoring.

While NDVI images were calculated in ArcMap 10 from the raw satellite bands, EVI images were obtained pre-calculated from the Landsat Archive.

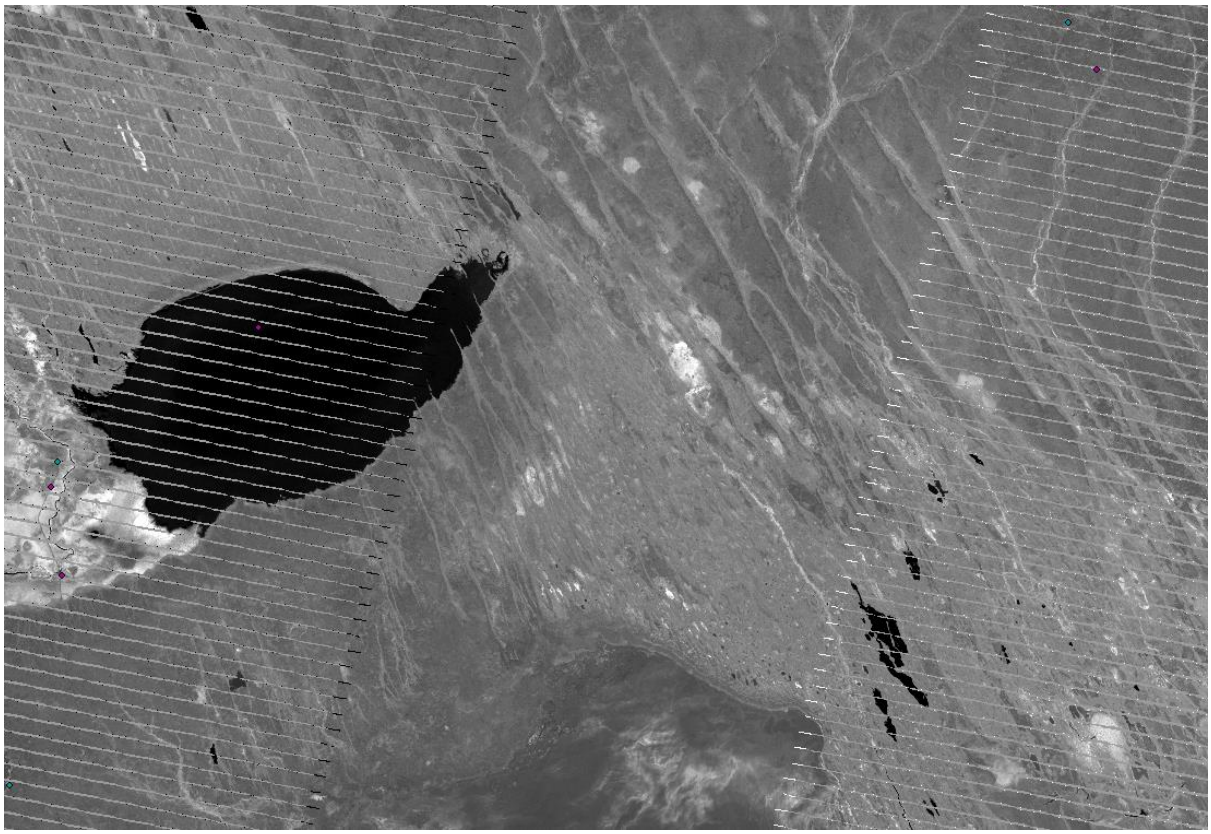


*Fig. 34. Partial image of one composite NDVI image (Lake Machattie, 50km SE of Bedourie, 24.82S, 139.78E), showing greyscale from 1 (white) to 0 (black) for vegetation, with a value of -1 for water (also visualised as black in this image). The scan-lines on the sides of the images are from Scan Line Correction mirrors failing in the Landsat 7 satellite after 2003.*

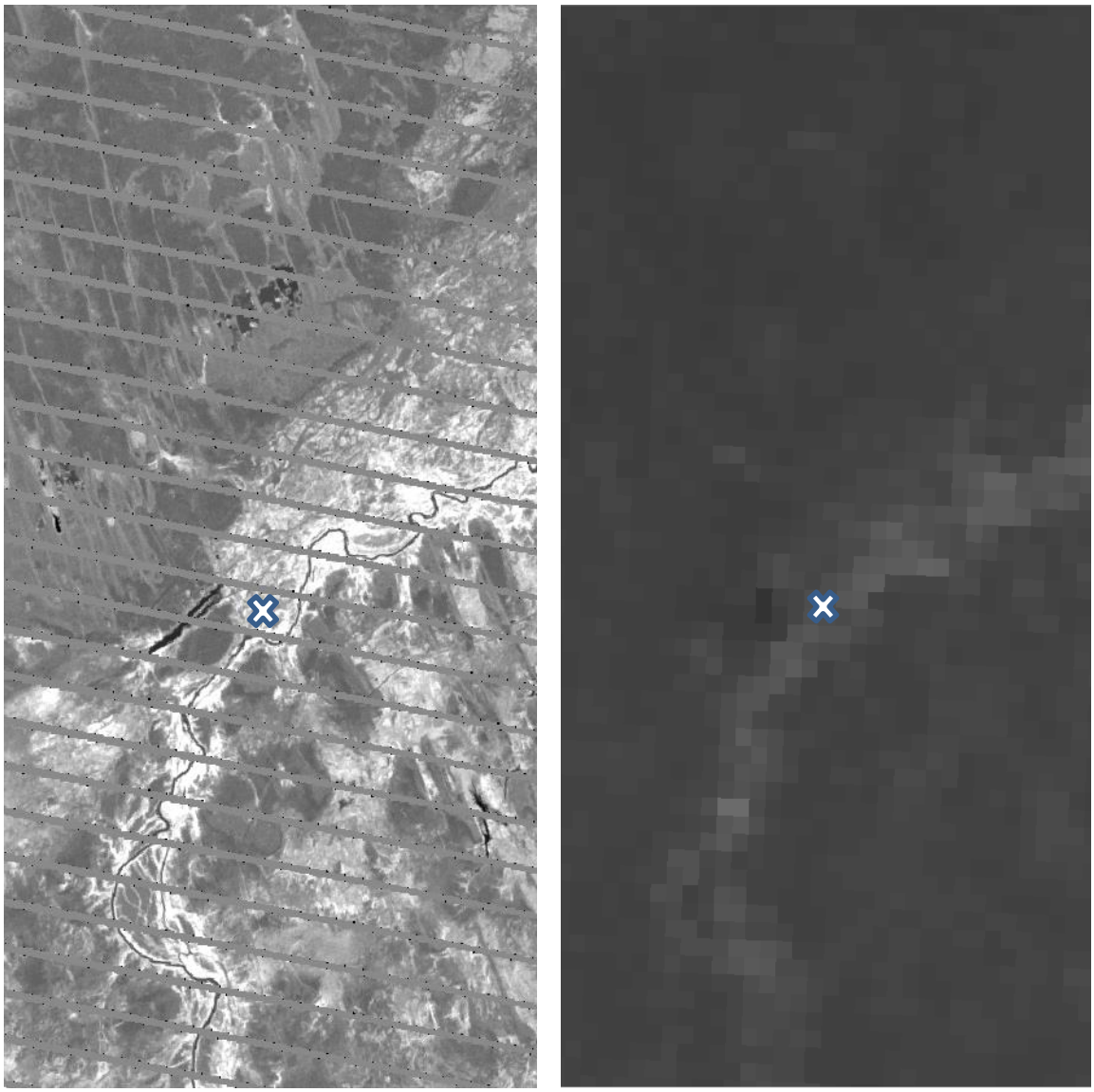


*Fig. 35. Comparison of a Landsat 7 NDVI image (left) with 30m resolution (and scan-line errors) and a MODIS EVI image (right) with 250m resolution. The X denotes the same site in Birdsville (25.9S, 139.4E).*

## 5.3 Results

### 5.3.1 NVDI comparison with subjective vegetation scores

The post-drought vegetation ratings and tree scores fitted poorly with the Landsat 7 NDVI data, and data transformations did not improve the correlation of linear

regressions. The expected relationship was a positive linear correlation, and this was not observed in these data sets, either for herbaceous vegetation (Fig. 36), or for trees (Fig. 37).

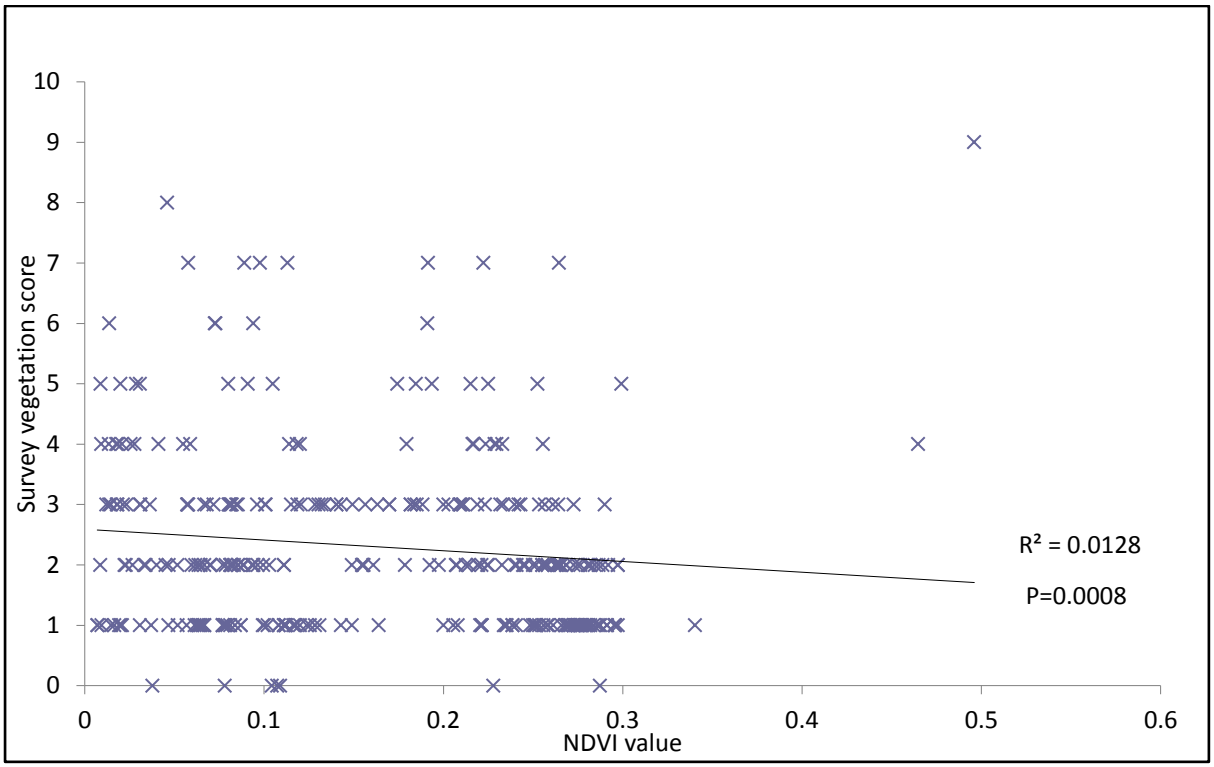



*Fig. 36. Comparison between Landsat 7 NDVI and survey vegetation ratings from 2009-2012.*

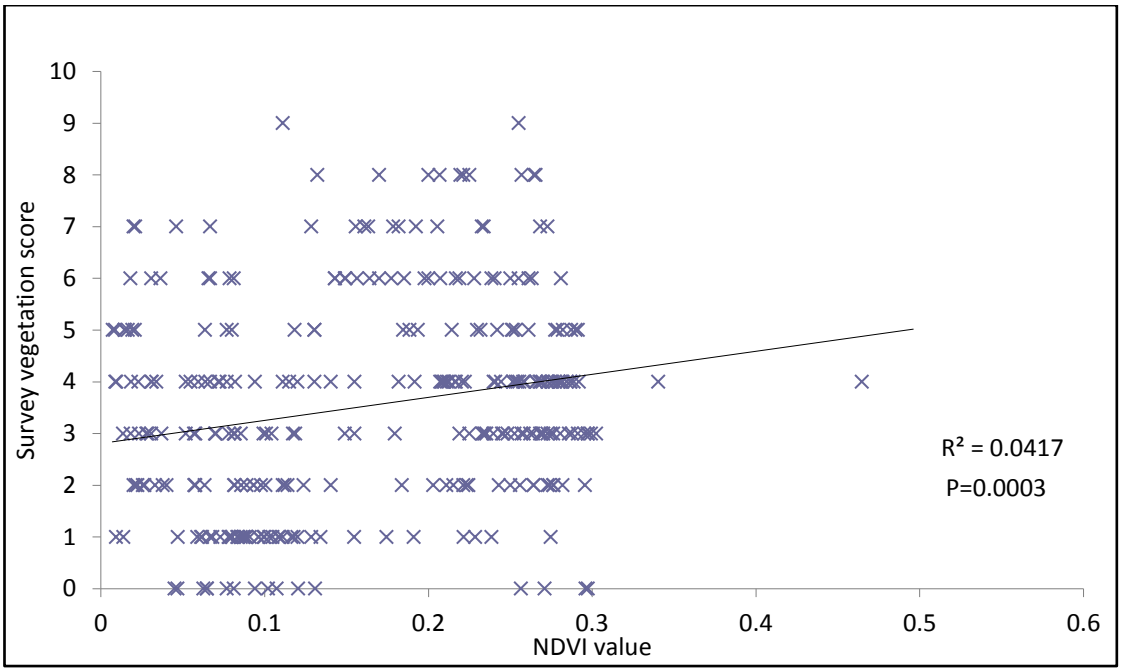



*Fig. 37. Comparison between Landsat 7 NDVI and survey tree ratings from 2009-2012.*

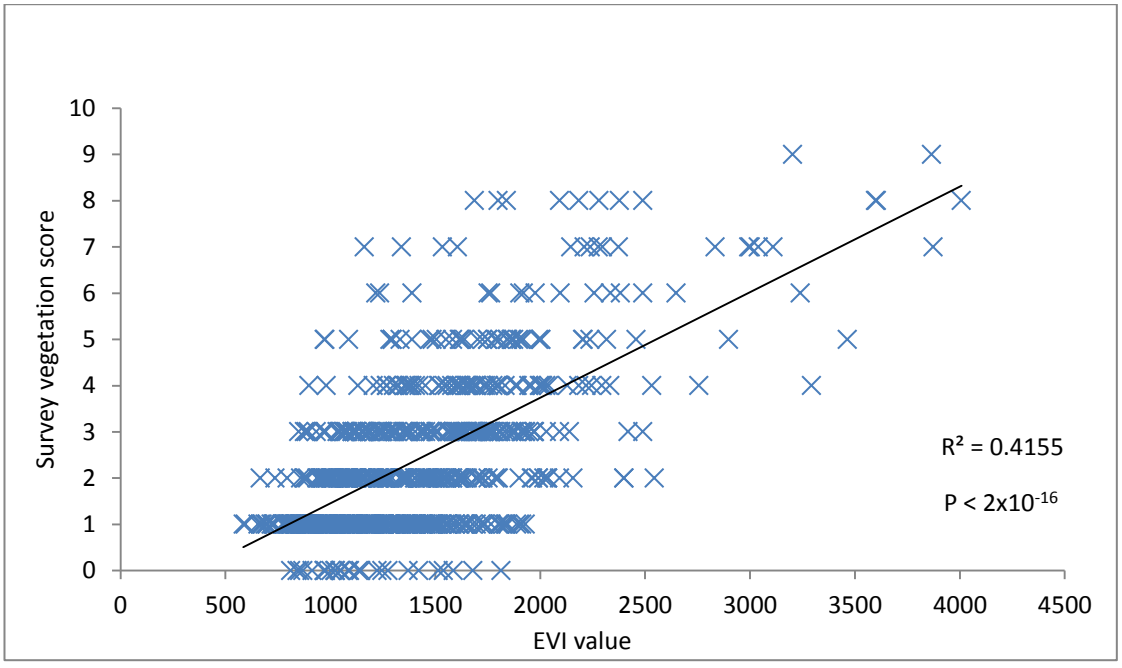



*Fig. 38. Comparison between Landsat 7 EVI and survey vegetation ratings from 2009-2012.*

### 5.3.2 Comparing vegetation surveys with EVI data

When NDVI did not provide adequate correlation with my survey data, I used EVI instead. When 250m MODIS EVI data was compared with survey data, the expected positive correlation emerged (Fig. 38). The NDVI dataset was therefore rejected in favour of the MODIS EVI dataset, which provided a correlation with the vegetation ratings. In an attempt to find a better fitting model by accounting for variation between different trips, a GLM was applied to the MODIS EVI/ vegetation call dataset.

*Table 22. Linear model comparing survey vegetation ratings and raster values from MODIS EVI maps. Residual standard error: 1.326 on 711 degrees of freedom. Multiple R-squared: 0.4155, adjusted R-squared: 0.4147 , F-statistic: 505.4 on 1 and 711 DF,*

| | Estimate | Std. Error | t value | Pr(>\|t\|) |
|---|---|---|---|---|
| (Intercept) | -0.82959 | 0.152575 | -5.437 | 7.44E-08 |
| EVI_value | 0.002284 | 0.000102 | 22.482 | < 2e-16 |

This simple linear model (Table 22) shows that the relationship between vegetation survey ratings and EVI was highly significant ($P<0.001$), with an $R^2$ value of 0.4155, which matches Fig. 38 exactly. The AIC function in R returned a value of 2429.8.

Akaike's information criterion (AIC) is a function defined as:

```
AIC = -2(log-likelihood) + 2K
```

Where K is the number of estimated parameters that are included in the model. The log-likelihood of the model given the data is readily available in statistical output and reflects the overall fit of the model (smaller values indicate worse fit) (Anderson et al., 1994).

Next the data were modelled using a GLM to account for possible differences between observations during different trips (Table 23). This model shows the same level of correlation with EVI, and showed that all but one trip in one year were not significantly different from each other. This suggests that there is a level of consistency between trips with regard to vegetation scoring and EVI. The AIC value (2296.2) was lower for the model which separated the trips, suggesting it is worse than the linear model at describing the data.

The survey trip in November 2010 had significantly higher average vegetation score than other trips, and this trip was both later in the year than other trips, and there had been considerably more rainfall in that year than the long-term average (Fig. 39). Because the assumptions on timing and rainfall were different to other years, for the purpose of matching EVI scores to vegetation survey data, these data were excluded and the model was run again and resulted with the same $P<0.000001$ value for Survey ~ EVI values. The AIC for this adjusted dataset was 1911.2, but was not comparable to the previous AIC values as the dataset had the November 2011 values redacted from it. Without the November 2011 data both the linear model and the generalised linear model had an AIC of 1943.1. Based on AIC values for each model and the fact that the scores from different trips were not significantly different from each other, we can simplify the models by removing the 'Trip month/year' term from the model, providing the best model to predict survey vegetation calls from EVI.

*Table 23. GLM model comparing survey vegetation score and raster values from MODIS EVI maps. Null deviance: 2138.9 on 712 degrees of freedom. Residual deviance: 1002.3 on 699 degrees of freedom. AIC: 2296.2.*

Coefficients:

| | Estimate | Std. Error | t value | Pr(>\|t\|) |
|---|---|---|---|---|
| (Intercept) | -0.31707 | 0.486398 | -0.652 | 0.5147 |
| RASTERVALU | 0.001729 | 0.000335 | 5.165 | 3.13E-07 |
| Field14aug10G | 0.047328 | 0.666935 | 0.071 | 0.94345 |
| Field14jul11G | -0.69999 | 0.601293 | -1.164 | 0.24476 |
| Field14jul12G | -0.59185 | 0.593755 | -0.997 | 3.19E-01 |
| Field14nov10G | 2.144493 | 0.656191 | 3.268 | 0.00114 |
| Field14oct11G | -0.61008 | 0.60739 | -1.004 | 0.31552 |
| Field14Sept12G | 0.593967 | 0.674486 | 0.881 | 0.37882 |
| RASTERVALU:Field14aug10G | 0.00049 | 0.000432 | 1.133 | 0.25762 |
| RASTERVALU:Field14jul11G | 0.000437 | 0.000391 | 1.117 | 0.26424 |
| RASTERVALU:Field14jul12G | 0.000324 | 0.000409 | 0.792 | 0.42843 |
| RASTERVALU:Field14nov10G | -0.00035 | 0.000418 | -0.826 | 0.40882 |
| RASTERVALU:Field14oct11G | 0.000575 | 0.00044 | 1.307 | 0.19161 |
| RASTERVALU:Field14Sept12G | -0.00077 | 0.000518 | -1.49 | 0.13661 |

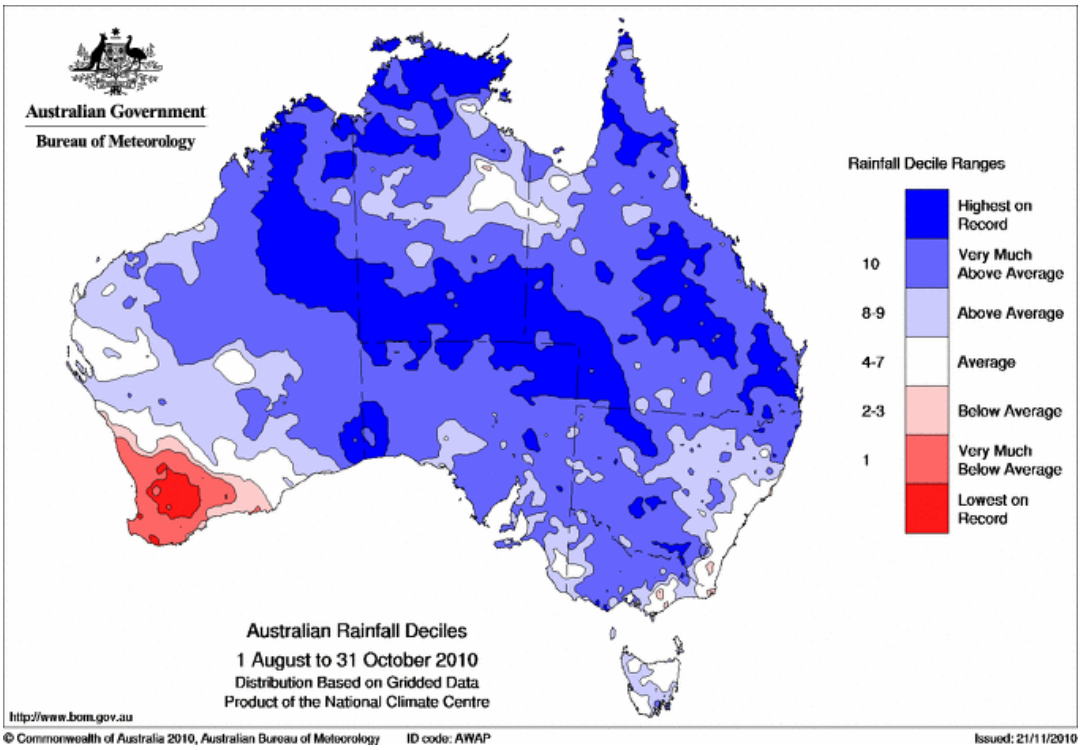



*Fig. 39. Long-term average rainfall across Australia prior to the November 2011 trip. (Department of Science, Information Technology and Innovation, Queensland Government).*

The statistical modelling process comparing survey data with objective EVI data allowed me to validate the survey results and provide the best model to relate EVI to survey data. The (joint) best model was the linear regression, with the characteristics above in Table 22.

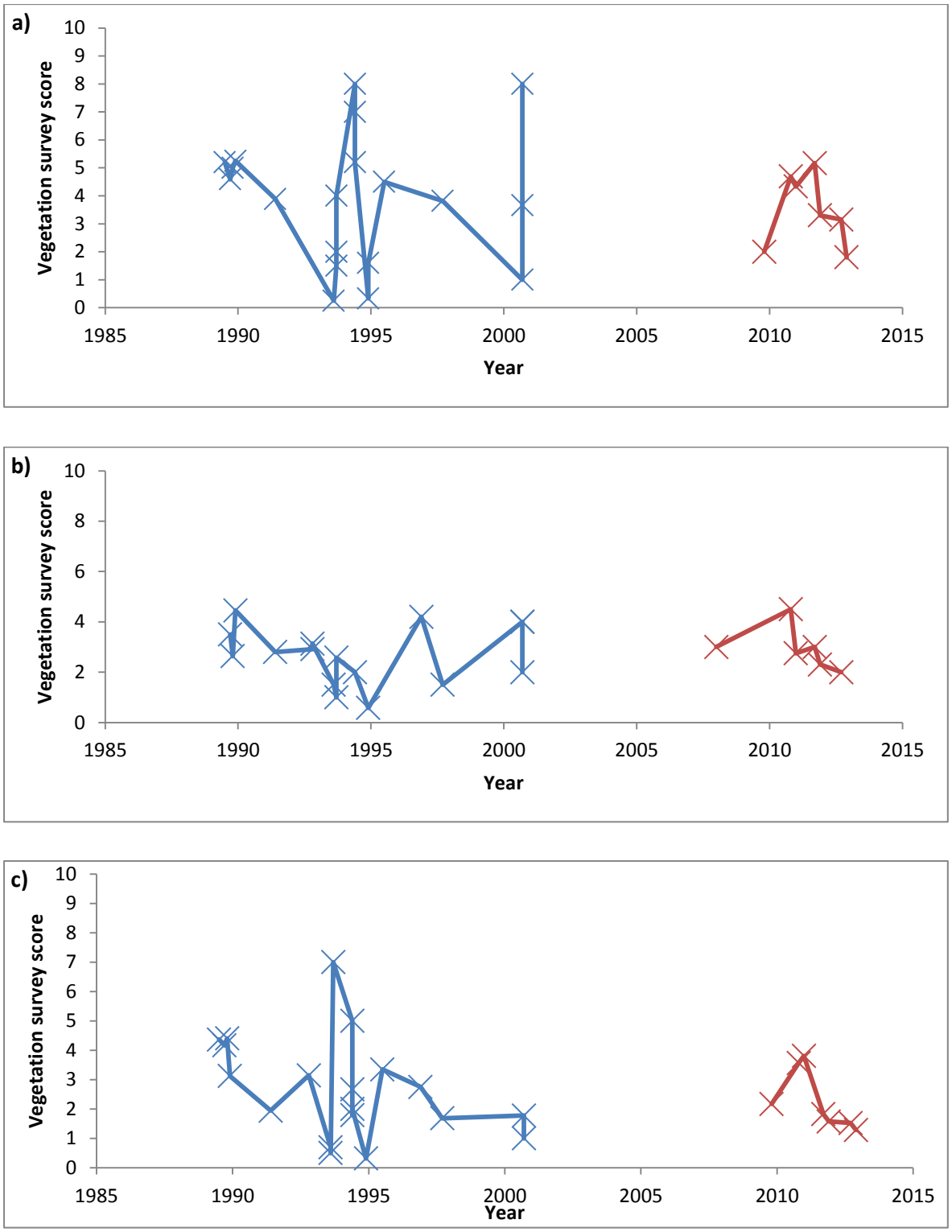



*Fig. 40. Average vegetation survey scores pre- (blue) and post- (red) drought. (a) floodplains, (b) sandy deserts, (c)* Acacia *shrublands*

In the floodplains during the pre-drought period, there were large variations in herbaceous vegetation ratings (Fig. 40, a). High scores were associated with major floods in 1994 and 2000, with lower scores due to minor flooding in other years and very low scores when there was no flooding in other years. During the post-drought period average herbaceous vegetation scores have not yet reached the same high levels as pre-drought, though significant flooding occurred in 2010 and 2011.

The sandy deserts showed little change in average herbaceous vegetation scores before and after the drought (Fig. 40, b). Ratings in this vegetation type were never as high as in the floodplains, reflecting lower vegetation cover even after good rainfall. The changes in vegetation generally reflected those of the floodplains, though there were some notable exceptions such as 1994 when floodplain scores were high but those in the deserts were low. In these years floods arose from rain which fell much further north, but not in the local area of the sandy deserts. However, very low scores were rare in the sandy deserts, and this probably reflects the ability of herbaceous vegetation in these areas to respond to small falls of rain.

Average vegetation scores in *Acacia* shrubland also fluctuated in response to local rainfall, but appear to have been trending downwards since the 1990s. Apart from a brief period in 2011, vegetation scores have been relatively low in the post-drought period (Fig. 40, c).

### 5.3.4 Changes in *H. punctigera* host plants

In addition to average vegetation survey score comparisons, differences in the presence/absence of specific host plants were calculated for survey sites within each major vegetation type. This allowed me to make comparisons between the presence/absence of different hosts before and after the drought. These differences, expressed as percentages of sites with a host compared to sites without that specific host, were calculated for each dataset (pre-drought and post-drought). Not all species of legumes (Fabaceae) or daisies (Asteraceae) were

included as distinct species within the tables. Some were excluded because either there were not enough of them in a vegetation type, or because they were known to be poor *H. punctigera* hosts.

The floodplains have had an increase in legumes with a specific increase in the good host plant *Cullen cinereum*, but daisies which were always less abundant than legumes, have decreased in abundance (Table 24). *Rhodanthe floribunda,* a good host plant, has not yet been seen in this vegetation type after the drought, nor have the poorer host daisies, *Craspedia* spp. There was a small increase in the presence of grasses in this vegetation type.

*Table 24. Changes in the presence of host plants recorded by inland vegetation surveys from pre-drought to post drought in floodplains, recorded as a percentage present in each survey.*

| | **Pre-drought** | **Post-drought** | **Change** |
|---|---|---|---|
| **Legumes (Fabaceae)** | 63.2% | 84.9% | +21.7% |
| *Cullen cinereum* (Lindl.) J.W. Grim | 47.1% | 86.3% | +39.2% |
| **Daisies (Asteraceae)** | 64.7% | 43.4% | -21.3% |
| *Rhodanthe floribunda* (DC.) Paul G. Wilson | 16.7% | 0.0% | -16.7% |
| *Craspedia* spp. | 17.7% | 0.0% | -17.7% |
| *Calotis multicaulis* (Turcz.) Druce | 8.8% | 3.9% | -4.9% |
| **Others** | | | |
| *Goodenia* spp. (Goodeniaceae) | 81.3% | 40.0% | -41.3% |
| *Malvastrum americanum* (Malvaceae)(L.) Torr. | 4.4% | 0.0% | -4.4% |
| **Grasses** | 40.9% | 55.7% | +14.8% |

As in the floodplains, the presence of *Rhodanthe floribunda* has decreased greatly in the sandy deserts (Table 25). *Calotis multicaulis* daisies have increased, but they

cannot support many *H. punctigera* larvae per plant (Zalucki *et al.*, 1994). Two daisies which are likely to be major host plants for *H. punctigera* in the sandy deserts, *Polycalymna stuartii* and *Senecio gregorii*, have also decreased. However, as in the floodplains, the incidence of legumes, especially *Cullen cinereum*, has increased. These species are generally associated with claypans between sand dunes. As in floodplains, the presence of grasses increased in sandy deserts.

In the *Acacia* shrublands (mulga) the presence of most host plants has substantially declined. This includes legumes, though they were never common in this vegetation type. Of most interest is the substantial decline in daisies, especially *R. floribunda* (Table 26). The only host plant to have substantially increased is *Sida platycalyx* (Malvaceae), which is a poor host that supports few larvae (Zalucki *et al.* 1994), and grows mainly in response to summer rain, so its presence during the winter months is mostly as senescent plants. Though not shown as individual species in the survey records, grasses have also increased between the pre- and post-drought periods. They are not hosts for *H. punctigera*. Compared to floodplains and sandy deserts, there was a larger increase in the presence of grasses in *Acacia* shrublands.

Overall, the presence of daisies has declined following the drought. Other hosts have increased in abundance, but host plants capable of supporting large numbers of *H. punctigera*, particularly the daisy *R. floribunda* appeared to be in decline. Grasses have increased, especially in the *Acacia* shrublands but these are not *H. punctigera* hosts.

*Table 25. Changes in the presence of host plants recorded by inland vegetation surveys from pre-drought to post drought in sandy deserts, recorded as a percentage present in each survey.*

| | **Pre-drought** | **Post-drought** | **Change** |
|---|---|---|---|
| **Legumes (Fabaceae)** | 15.4% | 52.9% | +37.5% |
| *Cullen cinereum* (Lindl.) J.W. Grimes | 12.8% | 47.1% | +34.3% |
| **Daisies (Asteraceae)** | 84.6% | 64.7% | -19.9% |
| *Rhodanthe floribunda* (DC.) Paul G. Wilson | 46.2% | 11.5% | -34.7% |
| *Calotis multicaulis* (Turcz.) Druce | 20.5% | 23.5% | +3.0% |
| *Polycalymma stuartii* F.Muell. & Sonder | 24.4% | 6.3% | -18.1% |
| *Senecio gregorii* F. Muell. | 11.1% | 3.1% | -8.0% |
| **Others** | | | |
| *Sida platycalyx* (Malvaceae) F. Muell. ex Benth. | 7.7% | 11.8% | +4.1% |
| **Grasses** | 22.7% | 43.6% | +20.9% |

*Table 26. Changes in the presence of host plants recorded by inland vegetation surveys from pre-drought to post drought in* Acacia *shrublands, recorded as a percentage present in each survey.*

| | **Pre-drought** | **Post-drought** | **Change** |
|---|---|---|---|
| **Legumes (Fabaceae)** | 4.9% | 2.7% | -2.2% |
| **Daisies (Asteraceae)** | 84.7% | 66.7% | -18.0% |
| *Rhodanthe floribunda* (DC.) Paul G. Wilson | 62.9% | 32.0% | -30.9% |
| *Calotis multicaulis* (Turcz.) Druce | 6.4% | 16.0% | +9.6% |
| **Others** | | | |
| *Sida platycalyx* (Malvaceae) F. Muell. ex Benth | 4.6% | 33.3% | +28.7% |
| *Goodenia* and *Velleia* spp. (Goodeniaceae) | 16.2% | 9.3% | -6.9% |
| *Malvastrum americanum* (Malvaceae)(L.) Torr. | 5.1% | 0.0% | -5.1% |
| **Grasses** | 44.3% | 86.6% | +42.3% |

The full dataset can be downloaded here:
https://www.dropbox.com/s/4n169p0f9lr2rk3/Full%20dataset%20veg_score%20average_2.0.xlsx?dl=0

## 5.4 Discussion

### 5.4.1 NDVI vs EVI

The exploration of models comparing vegetation scores with EVI has demonstrated that the linear model is the most appropriate way to describe the relationship between EVI and survey vegetation scores. It has also established the objectivity of these survey surveys relative to EVI indices, and has provided a formula for linking the older, pre-GPS vegetation surveys to EVI values for future database calls. The correlation is not perfect, as shown by the $R^2$ value of 0.42, but it is much better than obtained with NDVI. This may be due in part to the 250 m resolution of EVI compared to 30 m for NDVI. The GPS coordinates for each survey location were taken from the side of the road where the vehicle was parked, but the vegetation score evaluation took place from up to 50 m inside the vegetation itself, in order to minimise the impact of local disturbance. Rural roads and the graded area surrounding them are areas of extreme disturbance, favouring fast-growing plant species over the surrounding areas (Spooner, 2005).The survey GPS coordinates that were compared with NDVI images were well within this disturbed area, which might have thrown off the 30 m resolution readings, compared with the 250 m MODIS EVI images. I attempted to reduce the resolution of the NDVI data from 30 m down to 250 m using both the 'nearest neighbour' and 'bilinear' resampling functions of ArcMap 10, but the new NDVI image did not create a correlation with survey data. Given that the NDVI score and MODIS EVI were collected at the same time intervals, from the same Landsat 7 satellite, the differences in correlation between NDVI and MODIS EVI with vegetation survey score could also be due to how each index is calculated, possibly with regard to how EVI compensates for tree cover reflectance while NDVI does not.

### 5.4.2 Changes in host plants

Comparing host plant vegetation before and after the drought revealed some substantial changes to the vegetation of inland Queensland. The floodplains have had an increase in legumes with a specific increase in the good host plant *Cullen cinereum*, but daisies have decreased in abundance quite severely. This change is not surprising as legumes germinate in response to flood water. Floods still occurred at intervals throughout the drought, and because the floodplains drain areas much further north, they were less severely affected by the drought. Rain falls mostly in summer in these areas. In contrast, daisies germinate in response to autumn or winter rainfall further south of the study area, and this was severely restricted during the drought.

In the sandy desert vegetation areas there was a similar but more pronounced change in host plants, but not average vegetation scores. The paper daisy *Rhodanthe floribunda* used to be one of the most abundant host plants before the drought. It supported large numbers of larvae (Zalucki *et al.,* 1994). However, its presence has decreased greatly. Another daisy, *Calotis multicaulis,* has increased in abundance, perhaps because it appears to germinate under a wider range of temperature than most daisies (P. Gregg, Pers. Comm. 2012). However, they are poor hosts which support few caterpillars (Zalucki *et al.*, 1994, Gregg *et al.*, 1995). In the sandy deserts, legumes germinate on claypans or drainage lines between sand dunes, while most daisies germinate on the dunes themselves. Water from rain at any time of the year, including summer, can accumulate in the claypans areas and germinate hosts.

The *Acacia* shrublands show the same general trends, with the average vegetation levels lower in the post-drought period than the pre-drought period. The presence of legumes has decreased although they were never common in this vegetation type. Daisies in general have also become less common, especially good hosts for *H. punctigera* such as *R. floribunda*, while poor hosts such as the other daisy *C.*

*multicaulis* and *Sida platycalyx* (Malvaceae) have become more common. The presence of grasses in the *Acacia* shrublands doubled relative to either floodplains or sandy deserts. Overall, the *Acacia* shrublands may not be able to support the same abundance of hosts in the post-drought era.

Across all habitats, there has been an increase of plants that germinate in response to summer rain including legumes, grasses and *Sida platycalyx*. At the same time, there has been a decrease in plants that germinate in response to winter rain. An important limitation of these analyses of host plant presence data is that they do not consider the relative difference in abundance, which has been severely affected in some locations. Fig. 41 demonstrates how the vegetation has changed over 20 years at two sites in the *Acacia* shrublands, with a fraction of same host plants being present at the same location at the same time of year.


*Fig. 41. Pre-drought (top) and post-drought (bottom) conditions at two different locations at the same time of year, 20 years apart (1992 and 2012) Photos: P. Gregg.*

The changes in the *Acacia* shrublands may be of particular consequence because these areas, lying between the other habitats and the cropping areas (Fig. 42) may have been used by migrants from the inland as a "green bridge" between the sandy deserts/floodplains and cropping regions, that is, they may have supported one or more generations in late winter and early spring, prior to the invasion of cropping regions in late spring (P. Gregg, Pers. Comm., 2015). Thus, if this "bridge" is not functional, *H. punctigera* would only be able to migrate from inland Queensland floodplains or sandy deserts into Namoi valley cropping areas when the wind direction and strength were sufficient to bypass the *Acacia* shrubland regions.

Migratory *H. punctigera* flying at an altitude of 100-1000m with a median wind speed of 41kmh$^{-1}$ would travel a distance of around 980km over 12 hours (Drake *et al*., 2001). The long-term average of wind speed and direction from inland sites such as Birdsville (Fig. 43) suggests that the wind direction is often roughly in the right direction to send moths towards cropping areas in the Namoi Valley, but the surface wind speed is rarely fast enough to send migrants the full distance from inland Queensland to the Namoi Valley in one night. However migratory flights occur in the geostrophic layer above 100m where wind speeds above 41kmh$^{-1}$ occur frequently (Farrow and Daly, 1987). There is an approximately a 25° angle of wind directions towards the SE, with a minimum distance of about 1030km needed for *H. punctigera* to migrate directly from inland Queensland to the Namoi Valley (Fig. 42). In comparison, migrations from *Acacia* shrublands can occur over a 70° angle of SE wind speed and have a minimum distance of about 330km to travel. Given the larger distances, higher wind speed and lower angles of wind directions needed, it would be expected that, while direct migration from the sandy deserts and floodplains is feasible, suitable winds are likely to be less common than those for migration from the *Acacia* shrublands. Thus, if the latter are no longer functioning as breeding habitats for *H. punctigera,* spring migration to cropping areas such as the Namoi Valley is likely to be less frequent and may involve fewer *H. punctigera*.

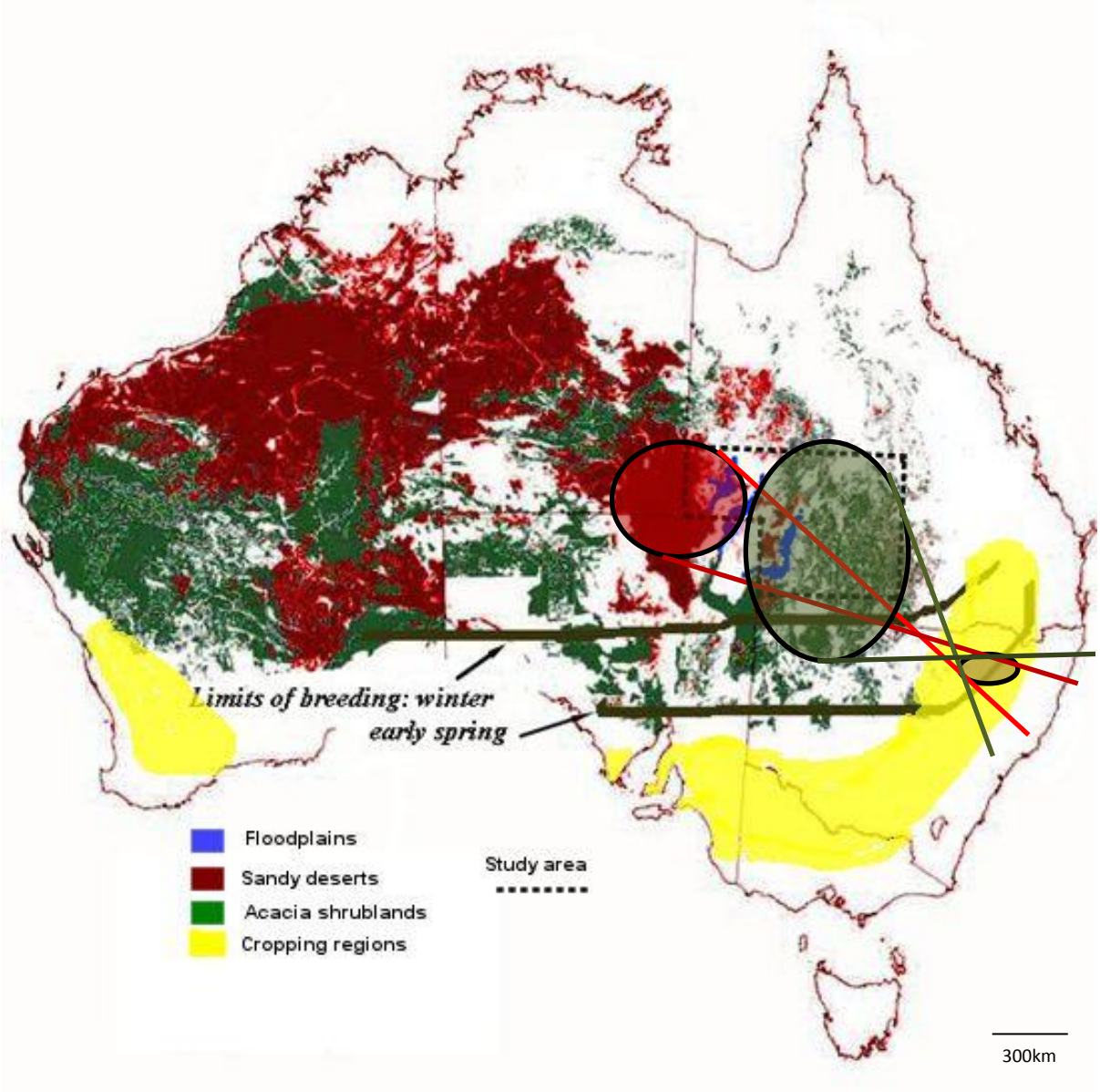



*Fig. 42. Potential wind directions needed to bring inland* H. punctigera *from inland Queensland (red ellipse) and* Acacia *shrublands (green ellipse) into the Namoi Valley cropping region (brown ellipse).*

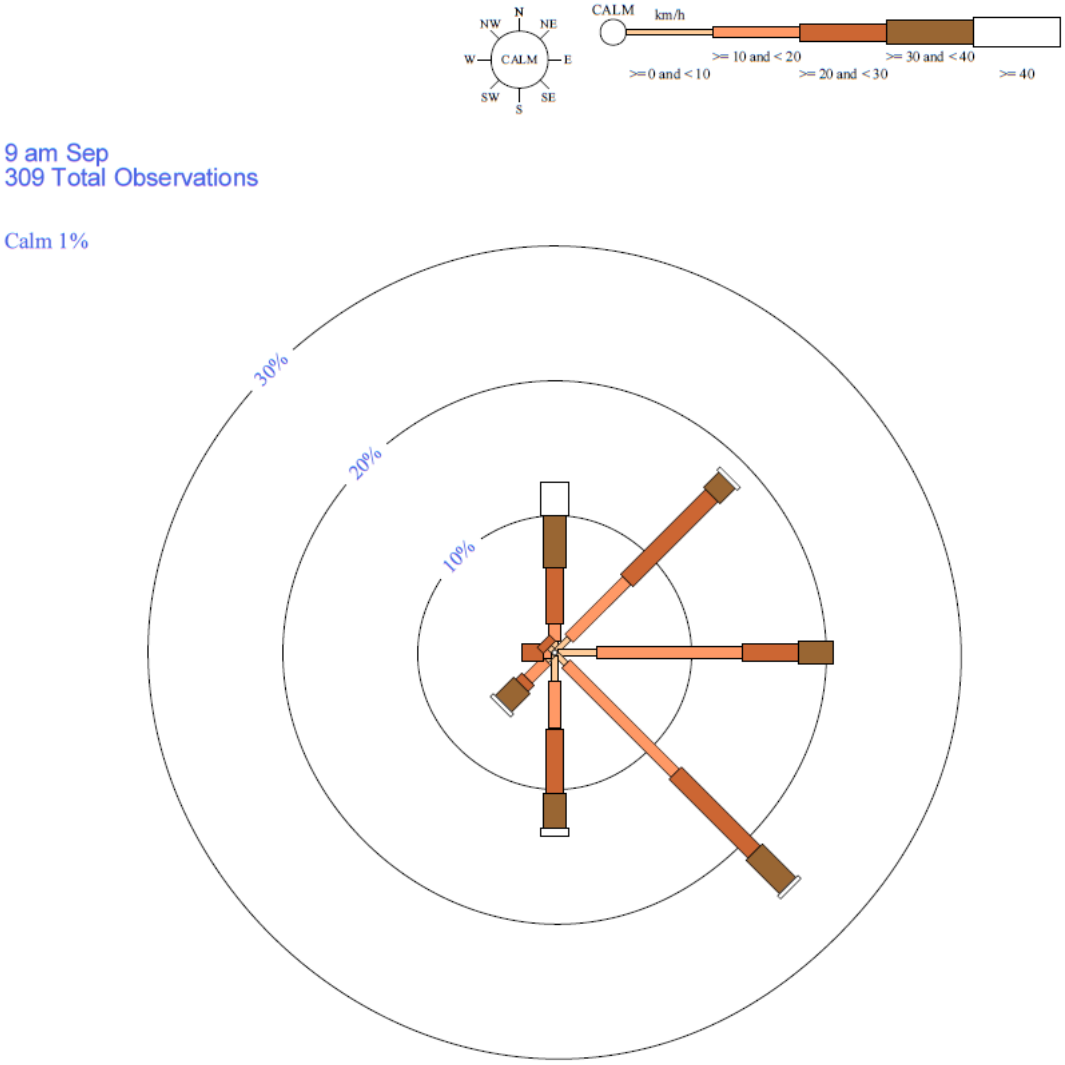



*Fig. 43. Rose of wind direction versus wind speed in km/h for the month of September (averaged from 2000 to 2010) at Birdsville airport (Bureau of Meteorology).*

Reduced migration is potentially a contributing factor in why spring populations of *H. punctigera* are being detected at lower levels in the Namoi valley in recent years (Baker *et al*., 2011) as one potential source of *H. punctigera* may have been eliminated or greatly reduced. Another potentially contributing factor is that rain has not been as regular in autumn and winter since the Millennium Drought, though in some years there has been substantial spring and summer rain (Queensland Department of Science Information Technology and Innovation, 2015).

Finally, another contributing factor may be that the seed bank of good hosts in the *Acacia* shrublands may have been reduced by the drought, and has not recovered.

## 5.5 Further work

The work in this chapter has only examined the surface of the survey data and important questions still remain, such as how the abundance of these host plants may have changed, rather than just presence/absence data from a habitat. Further survey work and follow up studies might determine if certain key host plant species are truly gone from major habitat types or simply dormant in the seed bank. The abundance of larvae relative to the host plant, habitat and location still needs to be addressed for pre- and post-drought periods. The database contains records of larval surveys of *H. punctigera*, and such records might prove useful in demonstrating how the abundance of *H. punctigera* larvae on specific host plants may have changed pre- and post- drought on the three major habitats.

Certain host plants, such as daisies and *Goodenia* spp., may only germinate with a combination of seasonal timing and rainfall, and this possibility should be explored through field trials in the inland area using artificial irrigation. Although survey data have been successfully modelled to apply an EVI value to survey data prior to GPS coordinates, there is more work to be done exploring the pre- and post- drought vegetation database in greater detail.

# Chapter 6: General discussion

## 6.1 Summary of major findings in the thesis

Laboratory studies were conducted under a range of temperatures and photoperiods, to determine under what conditions diapause occurs, and to compare the results with the only published study of diapause in *H. punctigera* Cullen and Browning (1978) (Chapter 2). Temperatures of 25°C induced the least amount of diapause in *H. punctigera* at 14L:10D, and the highest percentage of diapause was induced at 12L:12D at 25°C. Temperatures of 19°C or cooler produced the highest percentages of diapause, even under a summer 14L:10D photoperiod. At a 12L:12D photoperiod the highest percentages of diapause where induced at temperatures below 19°C. Larvae and pupae transferred from 25°C to 19°C showed an increase in diapause levels while larvae transferred from 19°C to 25°C did not. Results were similar to those of Cullen and Browning (1978) and confirmed that their findings were still highly relevant to *H. punctigera.* A statistical model was created from the study data, showing the significant effects of both temperature, photoperiod, and photoperiod-temperature interaction. However, the statistical model appeared not to have the same usefulness to growers and researchers for predicting the population of wild *H. punctigera* in diapause as the contour plot that was applied to the data. The contour plot of the data estimated the probability of *H. punctigera* pupae being in diapause based on the date and average temperature.

Attempts to study overwintering of *H. punctigera* in the Namoi Valley using emergence cages and cells were not successful mainly due to high mortality of larvae (Chapter 3). In only one of the three years was it possible to collect diapausing *H. punctigera* from the field by scouting pigeon pea crops at various locations in the Namoi Valley for populations of *H. punctigera* larvae. A field with a high ratio of *H. punctigera* to *H. armigera* was located at “Drayton” (49% putative *H. punctigera)*, which resulted in a ratio of approximately 10% of *H. punctigera* to 90% *H. armigera* as pupae in the soil. Pupae extracted from “Drayton” were all in

Stage A of development, indicating diapause. When half of these pupae were exposed to 25°C they all developed to stage E within 5 days, while the other half which were kept at 19°C, did not develop past stage A. These observations suggest that high levels of diapause were present in late-season pupae in the Namoi Valley, but diapause was broken by exposure to low temperatures by early winter, though emergence was delayed until temperatures rose in the spring.

Inland Queensland studies were more successful than the Namoi Valley studies. Emergence timing data combined with temperature probe data suggested that winter diapause does not occur in Inland Queensland on sand dunes with light vegetation, but can occur on floodplains with heavy soils and dense vegetation cover.

The 2012 “Monkira” study had a total of 64 moths emerged from the cages. There were two peaks of emergence, on 19/7/12 and 27/9/12, but many moths also emerged over the intervening period. There was no record of emergence timings at “Cluny” in 2013, but 18 of the 90 emergence cells contained moths that successfully emerged, while 72 failed to emerge. The timing of emergence within inland Queensland synchronised with pheromone trap catches in the Namoi Valley, potentially providing evidence that some of these inland moths migrate into cropping regions upon emergence in the spring.

Temperature probe data in winter and spring were useful in relating field conditions to the laboratory studies in Chapter 2. Potentially lethally cold temperatures were present in the Namoi Valley and lethally hot temperatures could occur in some habitats in inland Queensland. Maximum surface temperatures and 1.5 m air temperatures showed extreme daily fluctuations in the range of 40-50°C, compared to 10-20°C at 10 cm below the ground. Extremes of external temperature were ameliorated by being 10cm below the surface, but daily minimum and maximum temperatures were low enough to induce diapause in much of the population in the floodplain soils at “Monkira”.

Preliminary laboratory studies indicated the presence of a potential summer diapause in pupae (Chapter 4). In 17-31% of pupae arrested development was detected using both the eyespot method and the pupal duration methods at temperatures of 31-35°C. A potential reproductive diapause/quiescence was also detected in adults exposed to 32°C, defined by a 73% failure of females to mate, compared to 21% not mating at 25°C.

Possible long term changes in the vegetation of inland Queensland, potentially affecting the importance of immigration and local overwintering in the dynamics of *H. punctigera* populations in cropping areas, were investigated in Chapter 5. A largely unpublished collection of survey results dating back to the late 1980s was digitised into a database from audio recordings and paper records, and then compared with similar results obtained during the period 2009 to 2013, to investigate how *H. punctigera* ecology might have changed over the past three decades. Attempts to quantify historical subjective scorings of the amount of green host plant vegetation with remotely sensed data were made. NDVI data did not show any correlation with records of vegetation scoring from the surveys, but there was a correlation with MODIS EVI satellite data. A statistical model was constructed, and then used to compare mean vegetation scores over different major habitat types.

There were large variations in herbaceous vegetation ratings in the floodplains during the pre-drought period. Major floods in 1994 and 2000 have been associated with high vegetation scores in the floodplains and the *Acacia* shrublands, with lower values corresponding with minor floods or no floods. Post drought vegetation scores have not yet reached the same high levels as in pre-drought years even though significant flooding occurred in 2010 and 2011. The *Acacia* shrublands have had a downward trend in vegetation scores since the 1990s and have been relatively low post-drought, with the exception in 2011. The Sandy desert regions have shown little change in average vegetation scores before and after the drought, with lower vegetation cover being reflected by lower vegetation ratings even after

periods of increased rainfall. Very low vegetation scores are rare in the desert, which may reflect the ability of herbaceous vegetation in sandy soils to respond to small falls of rain.

Analysis of the presence or absence of major host plant species from the historical survey records compared with more recent observations was also undertaken. The results showed that the relative abundances of key host plants of *H. punctigera* have changed after the 2000-2009 drought, and that these changes have differed between the different major habitats. Overall, the presence of daisies has declined following the drought, especially in the *Acacia* shrublands, and while some other host plants have increased in abundance, hosts capable of supporting large numbers of *H. punctigera* have generally declined. In particular, *Rhodanthe floribunda*, a host plant capable of supporting many larvae, has decreased greatly in the *Acacia* shrublands, the floodplains and the sandy desert habitats.

## 6.2 Implications for management

Understanding both overwintering of *H. punctigera* in crops and the migration of *H. punctigera* into cropping systems is crucial to the management of this species. There is evidence that the amount of spring migration to the Namoi Valley has decreased over the last several decades, and the results presented in Chapter 5 may help explain why. For the cotton industry where rigorous resistance management plans are in place, immigration of *H. punctigera* in the spring from inland Queensland to the cropping regions is beneficial to dilute resistance alleles in the local population with non-resistant alleles from unselected immigrant moths. Unfortunately with the changes in vegetation associated with the Millennium Drought that were identified in Chapter 5, the *Acacia* shrubland habitat may not be functioning as the "bridge" between the sandy deserts and the floodplains where *H. punctigera* overwinters in large numbers (subject to autumn/winter rainfall), and the cotton growing areas. Fewer good host plants, especially in the *Acacia* shrublands, would most likely result in fewer migrants, and possibly a narrower

gene pool in migrants. This might in turn result in greater selection for adaptations to survive (notably resistance) in current (Bt dominated) crop environments in the Namoi Valley region by a resident population whose gene pool is less diluted by immigrant alleles.

From Chapters 2 and 3 and other studies, we know that overwintering survival occurs, but mortality is high. This overwintering population is subject to greater selection pressure to enable them to feed on Bt cotton, and more individuals may eventually become resistant to some Bt toxins (Chapter 1.3). Diapause in *H. punctigera*, be it summer or winter diapause, is critical for them to survive the changing conditions in Australia and the confirmed presence of a summer diapause/quiescence in *H. punctigera* pupae and adults would have implications for forecasting systems that predict survival of *H. punctigera* based on regional environmental conditions (Chapter 1.5). Such systems may be able to incorporate not only diapause thresholds that might delay spring migration into crops. The diapause calculation tool presented in Chapter 2 would be suitable for disseminating this information, particularly when further work determines the details of summer diapause/quiescence in greater depth.

The inland Queensland studies in Chapter 5 do not tell the complete story of *H. punctigera* migration and it is not just a pest of cotton crops and refuges in the Namoi Valley. Migrants can also move from South Australia and possibly Western Australia to cropping regions in Victoria on pre-frontal winds, where *H. punctigera* is the major pest of all grain legumes, also attacking most oilseed crops as well as tomato and sweet corn. There are important grain crops in Western Australia such as canola and lupins as well as other grains , and *H. punctigera* breeds in arid regions in winter and migrates to southwestern cropping regions in that state during spring (Chapter 1.5). The effects of the Millennium Drought on *H. punctigera* migrants into the Namoi Valley from western Queensland may have been partially offset by migration from South Australia and Western Australia (Chapter 5.4).

The research in this thesis is not just applicable to cotton management, but also to the cropping industries mentioned above, particularly in regions with similar dynamics of migration and overwintering. Large populations of *H. punctigera* can build up on native plants before migrating into crop plants, which can quickly cause a pest outbreak without regular monitoring.

## 6.3 Further work

There is an abundance of further work that could be done continuing the work on each chapter of this thesis.

The studies of Chapter 2 could be expanded upon by creating a better model of winter diapause that includes fluctuating temperatures. With the temperature probe data collected from both inland Queensland and the Namoi Valley, a more detailed model of diapause in *H. punctigera* could be constructed, and that might result in a better diapause prediction tool for researchers and growers. An exploration of potentially diapause-inducing daylengths of 10.5L:13.5D, the shortest natural photoperiod in the Namoi Valley region, would fill a gap in my results and improve the contour plot representation and the prediction tool based on it. This would also improve the statistical model at the same time.

If the incidence of overwintering *H. punctigera* in the Namoi Valley increases to the point where they become a concern for growers then determining the precise winter diapause termination conditions for *H. punctigera* might be justified. In light of the daily fluctuations in soil temperatures, a priority should be placed on examining diapause onset under fluctuating conditions. This information could be useful for the updating of forecasting models, allowing more accurate predictions of when *H. punctigera* might emerge from the soil. This would most likely be part of existing prediction systems for *Helicoverpa* spp. (e.g. Chapter 1.4). With the current levels of overwintering observed in this study, this is unlikely to be a high priority in the near future.

Given the high mortality present in Namoi Valley populations of *H. punctigera* indicated by field cage studies in Chapter 3, it is suggested that for future work either pupae digging is used from the start of an investigation, or else an open-air insectary is used to observe overwintering pupae. With regards to determining the cause of the high mortality, life-table studies would prove useful in determining exactly what caused the observed mortality and in what proportion. Understanding of overwintering in areas like the Namoi Valley could be improved with long-term ecological data collected in a manner similar to that which provided the database used in Chapter 5, but obviously that would be a significant undertaking that would require its own dedicated project. Studies by other authors are already providing useful information on *H. punctigera* ecology (Chapters 3 and 5). While pheromone and light trapping studies are useful in monitoring *H. punctigera* populations over the growing seasons, if the ratio of overwintering *H. punctigera* to *H. armigera* is increasing in the Namoi Valley, there may be merit in long-term field sampling of fields in order to inform future research directions and funding.

A priority should be expanding on the preliminary studies conducted on summer diapause (Chapter 4). There have been no attempts to ascertain the nature of summer diapause/quiescence responses in adults, which might help explain the difference in light and pheromone trap catches in summer months. The pupal summer diapause study should be expanded on by providing more replication so that possible differences between temperature treatments can be distinguished statistically, while the adult study needs to consider a wider range of temperatures and photoperiods to better understand the onset conditions, rather than simply the presence or absence of a summer diapause/quiescence. Termination conditions for summer diapause/quiescence should be investigated to make the distinction as to whether it is a true summer diapause or simply quiescence. The precise nature of how summer diapause/quiescence manifests in *H. punctigera* adults has not been determined and a standardised methodology for this, such as the eyespot method used in pupae, would prove useful for future researchers.

Once summer diapause/quiescence onset and termination conditions are determined, this information needs to be compared to both Namoi Valley and inland Queensland late-spring and summer soil temperatures, in order to explore how *H. punctigera* survives potentially lethal high temperatures and lack of host plants in spring and summer. The role of rainfall in cooling the soil should also be considered.

The database of survey results (Chapter 5) is being updated every year with new data from field trips, and this will expand the post-drought data set. The database of field studies dating back to 1989 has been given only the briefest of explorations in this thesis. Only vegetation survey score and presence/absence comparisons of host records have been considered so far. Locations where *H. punctigera* have been sampled need to be analysed to determine if they show the same or different patterns before and after the drought. With a statistical model linking EVI to vegetation scores, we can now use recent MODIS EVI images to predict locations where *H. punctigera* hosts might be present based on a combination of vegetation habitat type and EVI. When conducting a field trip, there is always a trade-off between the thoroughness of the investigation and the area that can be covered in the time allotted. Tools like publicly accessible EVI data in preference to or in association with NDVI might be used to improve the existing techniques used to better determine the itinerary of field trips to areas of interest, while avoiding areas where little-to-no hosts are present. In this regard, the CSIRO Australian Water Availability Project (AWAP) soil moisture anomaly maps might prove a valuable metric to pair with MODIS EVI. A way to somehow quantify the amount of host plants at a survey site would certainly prove useful for future studies, and while it is unlikely that a proxy for such a metric could be created from the existing survey data, a method for storing this information could potentially be devised for future surveys. Our group has already purchased an aerial photography drone which might be suitable for this purpose.

While there are limited survey data on the migration of *H. punctigera* from Western Australia and South Australia through to Victoria, and then potentially to New South Wales, further investigation into how habitats in these regions may have changed relative to inland Queensland habitats over the drought could prove crucial to understanding *H. punctigera* migration.

From the studies in this thesis, it seems likely that the ecology and overwintering behaviour of *H. punctigera* has not changed significantly over the last three decades, but rather weather perturbations such as the Millennium Drought may have affected the abundance and quality of host plants that *H. punctigera* uses to overwinter in inland Queensland. Reduced quality and abundance in host plants, particularly in the *Acacia* shrublands, may have reduced migration into cropping areas, which may in turn be a driver of Bt resistance in cotton crops. The incidence of summer diapause or quiescence has been detected in both the adult and pupal stages, and the onset/end conditions and ramifications of this discovery need to be further explored.

# References


ADAMSON, D., THOMAS, G. & DAVIS, E. 1997. *An economic estimate of Helicoverpa's effect on Australian agricultural production*: Report 1 in the *Helicoverpa* series. Brisbane, CRC for Tropical Pest Management.

ALEXANDER, R. 2005. *A field guide to plants of the Channel Country,* Queensland, Channel Landcare group Inc.

ALLSOPP, P. G., DAGLISH, G., TAYLOR, M. F. J. & GREGG, P. C. 1991. Measuring development of *Heliothis* Species. *In:* ZALUCKI, M. P. (ed.) *Heliothis research methods and prospects.* New York: Springer Verlag, 90-101.

ANDERSON, D. R., BURNHAM, K. P. & WHITE, G. C. 1994. *AIC model selection in overdispersed capture-recapture data*. Ecology, 75**,** 1780-1793.

ANDREWATHA, H. G. & BIRCH, L. C. 1954. *The distribution and abundance of animals,* London, The University of Chicago Press.

ANON 2005. *IPM - Understanding Helicoverpa ecology and biology in southern Queensland: Know the enemy to manage it better*. Brisbane, Queensland Department of Primary Industries.

ARTHUR, C. & HARRIS, J. 2011. *Native rats on the rampage* [Online]. Available: http://www.abc.net.au/local/stories/2011/08/25/3301755.htm [Accessed 16/02/15].

BAILEY, P. T. 2007. *Pests of field crops and pastures: identification and control,* Collingwood, Australia, CSIRO Publishing.

BAKER, G. H. & TANN, C. R. 2014. Refuge crop performance as part of the Bt resistance management strategy for *Helicoverpa* spp. (Lepidoptera: Noctuidae) in Australian cotton production systems. *Austral Entomology,* 53**,** 240-247.

BAKER, G. H., TANN, C. R. & FITT, G. P. 2008. Production of *Helicoverpa* spp. (Lepidoptera, Noctuidae) from different refuge crops to accompany transgenic cotton plantings in eastern Australia. *Australian Journal of Agricultural Research,* 59**,** 723-732.

BAKER, G. H., TANN, C. R. & FITT, G. P. 2011. A tale of two trapping methods: *Helicoverpa* spp. (Lepidoptera, Noctuidae) in pheromone and light traps in Australian cotton production systems. *Bulletin of Entomological Research,* 101**,** 9-23.

BALE, J. S., MASTERS, G. J., HODKINSON, I. D., AWMACK, C., BEZEMER, T. M., BROWN, V. K., BUTTERFIELD, J., BUSE, A., COULSON, J. C., FARRAR, J., GOOD, J. E. G., HARRINGTON, R., HARTLEY, S., JONES, T. H., LINDROTH, R. L., PRESS, M. C., SYMRNIOUDIS, I., WATT, A. D. & WHITTAKER, J. B. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology,* 8**,** 1-16.

BANGE, M., DEUTSCHER, S., LARSEN, D., LINSLEY, D. & WHITESIDE, S. 2004. A handheld decision support system to facilitate improved pest management in Australian cotton systems. *Computers and Electronics in Agriculture,* 43**,** 131-147.

BANGE, M., DEUTSCHER, S., LINSLEY, D., JOHNSTON, S., RICHARDS, D., THAKUR, L. & CLANCY, L. 2007. Delivering science to agribusiness: Cotton management support systems. Narrabri, NSW *Cotton Catchment Communities CRC Final Report,* 5.03.02.

BESAW, L. M., THELEN, G. C., SUTHERLAND, S., METLEN, K. & CALLAWAY, R. M. 2011. Disturbance, resource pulses and invasion: short-term shifts in competitive effects, not growth responses, favour exotic annuals. *Journal of Applied Ecology,* 48**,** 998-1006.

BETINI, G. S., GRISWOLD, C. K. & NORRIS, D. R. 2013. Carry-over effects, sequential density dependence and the dynamics of populations in a seasonal environment. *Proceedings of the Royal Society B-Biological Sciences,* 280, 20130110.

BOYLAND, D. E. 1977. *Western Arid Region Land Use Study Part 2*. Brisbane: The Division of Land Utilisation.

BROOK, B. W. & BRADSHAW, C. J. A. 2006. Strength of evidence for density dependence in abundance time series of 1198 species. *Ecology,* 87**,** 1445-1451.

BROWNING, T. O. 1981. Ecdysteroids and diapause in pupae of *Heliothis punctiger*. *Journal of Insect Physiology,* 27**,** 715-719.

CARON, R. E., BRADLEY, J. R., PLEASANTS, R. H., RABB, R. L. & STINNER, R. E. 1978. Overwinter survival of *Heliothis zea* produced on late-planted field corn in North Carolina. *Environmental Entomology,* 7**,** 193-196.

CHEN, C., XIA, Q. W., FU, S., WU, X. F. & XUE, F. S. 2014. Effect of photoperiod and temperature on the intensity of pupal diapause in the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Bulletin of Entomological Research,* 104**,** 12-18.

CLARK, A. 1998. Mutation-selection balance with multiple alleles. *Genetica,* 102-103**,** 41-47.

COMMON, I. 1954. A study of the ecology of the adult bogong moth, *Agrotis Infusa* (Boisd) (Lepidoptera: Noctuidae), with special reference to its behaviour during migration and aestivation. *Australian Journal of Zoology,* 2**,** 223-263.

COMMON, I. F. B. 1953. The Australian species of *Heliothis* (Lepidoptera:Noctuidae) and their pest status. *Australian Journal of Zoology,* 1**,** 319-344.

COOMBS, M., DEL SOCORRO, A. P., FITT, G. P. & GREGG, P. C. 1993. The reproductive maturity and mating status of *Helicoverpa armigera*, *Heliothis punctigera* and *Mythimna convecta* (Lepidoptera, Noctuidae) collected in tower-mounted light traps in northern New South Wales, Australia. *Bulletin of Entomological Research,* 83**,** 529-534.

COOPER, D. J. 1981. The role of predatory Hemiptera in disseminating a nuclear polyhedrosis virus of *Heliothis punctiger*. *Journal of the Australian Entomological Society,* 20**,** 145-150.

COTTON SEED DISTRIBUTORS 2012. *2012 Variety Trial Results*, Wee Waa, NSW Cotton Seed Distributors Ltd.

COTTON SEED DISTRIBUTORS 2013. *2013 Variety Trial Results*, Wee Waa, NSW Cotton Seed Distributors Ltd.

CULLEN, J. M. & BROWNING, T. O. 1978. The influence of photoperiod and temperature on the induction of diapause in pupae of *Heliothis punctigera*. *Journal of Insect Physiology,* 24**,** 595-602.

CUNNINGHAM, J. P. & ZALUCKI, M. P. 2014. Understanding Heliothine (Lepidoptera: Heliothinae) pests: What is a host plant? *Journal of Economic Entomology,* 107**,** 881-896.

DALY, J. C. & FITT, G. P. 1990. Resistance frequencies in overwintering pupae and the 1st spring generation of *Helicoverpa armigera* (Lepidoptera, Noctuidae) - Selective mortality and immigration. *Journal of Economic Entomology,* 83**,** 1682-1688.

DANKS, H. V. 1987. Insect dormancy: an ecological perspective. *Biological Survey of Canada (Terrestrial Arthropods), Monograph Series No. 1***,** 439pp.

DAVIDSON, S. 1989. Pyrethroid resistance in *Heliothis*: A genetic puzzle. *Rural Research C.S.I.R.O.,* 142**,** 11-17.

DAWSON, N. M. & BOYLAND, D. E. 1974. *Western Arid Region Land Use Study Part 1*. Brisbane: The Division of Land Utilisation.

DEL SOCORRO, A. P. & GREGG, P. C. 2001. Sunflower (*Helianthus annuus* L.) pollen as a marker for studies of local movement in *Helicoverpa armigera* (Hübner) (Lepidoptera : Noctuidae). *Australian Journal of Entomology,* 40**,** 257-263.

DENLINGER, D. L. 2002. Regulation of diapause. *Annual Review of Entomology,* 47**,** 93-122.

DEUTSCHER, S. & PLUMMER, C. 1998. *EntomoLOGIC - a computerised decision support system for cotton IPM,* In: Pest Management - Future Challenges, Vols 1 and 2, Proceedings. *6th Australisian Applied Entomological Research Conference*, Brisbane Australia, (A258-A262). Sep 29-Oct 02, 1998.

DEVORE, J. & PECK, R. 1993. *The exploration and analysis of data,* Belmont, California, Wadsworth Publishing Company.

DHAKA, S. S., SINGH, G., ALI, N., ARVIND, Y. & ADBHUT, Y. 2010. Field evaluation of insecticides and bio-pesticides against *Helicoverpa armigera* on tomato. *Annals of Plant Protection Sciences,* 18**,** 13-16.

DILLON, M. L., FITT, G. P., HAMILTON, J. G. & ROCHESTER, W. A. 1996. A simulation model of wind-driven dispersal of *Helicoverpa* moths. *Ecological Modelling,* 86**,** 145-150.

DOWLING, D. 2015. Cotton Yearbook 2015, Toowoomba, The Australian Cotton Grower.

DOWNES, S., MAHON, R. J., ROSSITER, L., KAUTER, G., LEVEN, T., FITT, G. & BAKER, G. 2010a. Adaptive management of pest resistance by *Helicoverpa* species (Noctuidae) in Australia to the Cry2Ab Bt toxin in Bollgard II® cotton. *Evolutionary Applications,* 3**,** 574-584.

DOWNES, S., PARKER, T. & MAHON, R. 2010b. Incipient resistance of *Helicoverpa punctigera* to the Cry2Ab Bt toxin in Bollgard II (R) Cotton. *PLoS ONE* 5(9): e12567.

DRAKE, V. A. 1994. The influence of weather and climate on agriculturally important insects - an Australian perspective. *Australian Journal of Agricultural Research,* 45**,** 487-509.

DRAKE, V. A., GREGG, P. C., HARMAN, I. T., WANG, H. K., DEVESON, E. D., HUNTER, D. M. & ROCHESTER, W. A. 2001. Characterising insect migration systems in inland Australia with novel and traditional methodologies. *In:* WOIWOD, I. P., REYNOLDS, D. R. & THOMAS, C. D. (eds.) *Insect Movement: Mechanisms and Consequences.* New York: Cabi Publishing. 207-233.

DUFFIELD, S. 2004. Evaluation of the risk of overwintering *Helicoverpa* spp. pupae under irrigated summer crops in south-eastern Australia and the potential for area-wide management. *Annals of Applied Biology,* 144**,** 17-26.

DUFFIELD, S. J. & CHAPPLE, D. G. 2001. Within-plant distribution of *Helicoverpa armigera* (Hübner) and *Helicoverpa punctigera* (Wallengren) (Lepidoptera : Noctuidae) eggs on irrigated soybean. *Australian Journal of Entomology,* 40**,** 151-157.

DUFFIELD, S. J. & DILLON, M. L. 2005. The emergence and control of overwintering *Helicoverpa armigera* pupae in southern New South Wales. *Australian Journal of Entomology,* 44**,** 316-320.

FARROW, R. A. & DALY, J. C. 1987. Long-range movements as an adaptive strategy in the genus *Heliothis* (Lepidoptera, Noctuidae) - a review of its occurrence and detection in 4 pest species. *Australian Journal of Zoology,* 35**,** 1-24.

FAUST, R. M. 2008. General introduction to areawide pest management. *Publications from USDA-ARS / UNL Faculty***,** Paper 645, Lincoln, Nebraska, USA.

FITT, G. P. 2000. An Australian approach to IPM in cotton: integrating new technologies to minimise insecticide dependence. *Crop Protection,* 19**,** 793-800.

FITT, G. P. & COTTER, S. C. 2005. The *Helicoverpa* problem in Australia: Biology and management. *In:* SHARMA, H. C. (ed.) *Heliothis/Helicoverpa Management:*

*Emerging Trends and Strategies for the Future.* 1 ed. Plymouth, UK: Science Publishers. 45-61.

FITT, G. P. & DALY, J. C. 1990. Abundance of overwintering pupae and the spring generation of *Helicoverpa* spp. (Lepidoptera: Noctuidae) in northern New South Wales, Australia: implications for pest management. *Journal of Economic Entomology,* 83**,** 1827-1836.

FITT, G. P. & WILSON, L. 2005. Integration of Bt cotton in IPM systems: an Australian perspective. *Second International Symposium on Biological Control of Arthropods, Davos, Switzerland, 12-16 September, 2005***,** 381-388.

FITT, G. P., ZALUCKI, M. P. & TWINE, P. 1989. Temporal and spatial patterns in pheromone-trap catches of *Helicoverpa* spp. (Lepidoptera: Noctuidae) in cotton-growing areas of Australia. *Bulletin of Entomological Research,* 79**,** 145-161.

FLOWER, K., MAAS, S., CEENY, S., CUNNINGHAM, Y., DEUTSCHER, S., DUGDALE, H., HARRIS, G., HICKMAN, M., HILL, J., KING, D., LARSEN, D., LEVEN, T., MCINTYRE, G., POWELL, J., VERWEY, P., WARK, J. & WEIR, D. 2010. *Cotton Pest Management Guide 2010-2011.* Toowoomba, Queensland, Greenmount Press.

FORRESTER, N. W. 1994. Resistance management options for conventional *Bacillus thuringiensis* and transgenic plants in Australian summer field crops. *Biocontrol Science and Technology,* 4**,** 549-553.

FORRESTER, N. W., CAHILL, M., BIRD, L. J. & LAYLAND, J. K. 1993. Management of pyrethroid and endosulfan resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Australia. *Bulletin of Entomological Research Supplement,* 1**,** 132pp.

GE, F., CHEN, F., PARAJULEE, M. N. & YARDIM, E. N. 2005. Quantification of diapausing fourth generation and suicidal fifth generation cotton bollworm, *Helicoverpa armigera*, in cotton and corn in northern China. *Entomologia Experimentalis et Applicata,* 116**,** 1-7.

GOODWIN, S. A. S., PARKER, R., TESORIERO, L., CONNELLAN, G., KESKULA, E., COWPER, B., MEDHURST, A., & RODRIGUEZ, C. 2000. Integrated pest management in ornamentals: Information guide. Horticulture Series:. Brisbane, Queensland.: Department of Primary Industries & Fisheries.

GREEN, K. 2008. Migratory Bogong moths (*Agrotis Infusa*) transport arsenic and concentrate it to lethal effect by estivating gregariously in alpine regions of the Snowy Mountains of Australia. *Arctic, Antarctic, and Alpine Research,* 40**,** 74-80.

GREGG, P. C. 1993. Pollen as a marker for migration of *Helicoverpa armigera* and *H. punctigera* (Lepidoptera: Noctuidae) from western Queensland. *Australian Journal of Ecology,* 18**,** 209-219.

GREGG, P. C. 1995. Migration of cotton pests: patterns and implications for management, In Constable,G.A. & Forrester, N.W. (eds) *Challenging the Future: Proceedings of the First World Cotton Research Conference, Brisbane, February 13-17. CSIRO, Canberra, pp.* 423-433.

GREGG, P. C., DEL SOCORRO, A. P. & ROCHESTER, W. A. 2001. Field test of a model of migration of moths (Lepidoptera: Noctuidae) in inland Australia. *Australian Journal of Entomology,* 40**,** 249-256.

GREGG, P. C., FITT, G. P., COOMBS, M. & HENDERSON, G. S. 1993. Migrating moths (Lepidoptera) collected in tower-mounted light traps in northern New South Wales, Australia - species composition and seasonal abundance. *Bulletin of Entomological Research,* 83**,** 563-578.

GREGG, P. C., FITT, G. P., ZALUCKI, M. P. & MURRAY, D. A. H. 1995. Insect migration in an arid continent. II. *Helicoverpa* spp. in eastern Australia. *In:* DRAKE, V. A. & GATEHOUSE, A. G. (eds.) *Insect migration: tracking resources through time and space.* Cambridge University Press, Melbourne.

GREGG, P. C., MCDONALD, G. & BRYCESON, K. P. 1989. The occurrence of *Heliothis punctigera* Wallengren and *H. armigera* (Hübner) in inland Australia. *Journal of the Australian Entomological Society,* 28**,** 135-140.

GREGG, P. C. & WILSON, L. J. 2008. The changing climate for entomology. *14th Australian Cotton Conference.* Broadbeach. August. Australian Cotton Grower's Research Association, Sydney.

GUNNING, R. V., MOORES, G. D., JEWESS, P., BOYES, A. L., DEVONSHIRE, A. L. & KHAMBAY, B. P. S. 2007. Use of pyrethroid analogues to identify key structural features for enhanced esterase resistance in *Helicoverpa armigera* (Huebner) (Lepidoptera : Noctuidae). *Pest Management Science,* 63**,** 569-575.

HACKETT, D. S. & GATEHOUSE, A. G. 1982. Studies on the biology of *Heliothis* spp in Sudan. *In*: REED, W., KUMBLE, V (eds.), *Proceedings of the international Workshop on* Heliothis *Management.* 15-20 November 1981, ICRISAT.Centre, Patancheru , India.

HENNEBERRY, T. J., BUTLER, G. D., LOEB, M. J. & WOODS, C. W. 1987. Diapause in the tobacco budworm, *Heliothis virescens* (Lepidoptera:Noctuidae): comparison of ecdysteroid titres in low and high temperature induced diapause. *Annals of the Entomological Society of America.* 12, 17-24.

HESSE, P. P. & SIMPSON, R. L. 2006. Variable vegetation cover and episodic sand movement on longitudinal desert sand dunes. *Geomorphology,* 81**,** 276-291.

HOLLOWAY, J. W. & FORRESTER, N. W. 1998. *Pyrethroid resistance in Heliothine pests of cotton: mechanisms and management* In: Pest Management - Future Challenges, Vols. 1 and 2, Proceedings. *6th Australaisian Applied Entomological Research Conference*, Brisbane Australia, (A171-A178). Sep 29-Oct 02, 1998.

HOLMSTROM, K. E., HUGHES, M. G., WALKER, S. D., KLINE, W. L. & INGERSON-MAHAR, J. 2001. Spatial mapping of adult corn earworm and European corn borer populations in New Jersey. *Horttechnology,* 11**,** 103-109.

HOQUE, Z., DILLON, M. & FARQUHARSON, B. 2003. *Three seasons of IPM in an area-wide management group - a comparative analysis of field level profitability,* Tamworth, Australia, NSW Agriculture.

HOQUE, Z., FARQUHARSON, B., DILLON, M. & KAUTER, G. 2000. Soft options can reduce costs and increase cotton profits. *Australian Cottongrower,* 21**,** 33-37.

HOYLE, G. L., STEADMAN, K. J., DAWS, M. I. & ADKINS, S. W. 2008. Physiological dormancy in forbs native to south-west Queensland: Diagnosis and classification. *South African Journal of Botany,* 74**,** 208-213.

HUANG, J. & LI, J. 2015. Effects of climate change on overwintering pupae of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *International Journal of Biometeorology,* 59**,** 863-876.

IPCC 2013. Climate change 2013. *In:* STOCKER T., DAHE Q. & GK., P. (eds.) *The physical science basis: contribution of Working Group I to the 5th Assessment Report of the IPCC.* Stockholm: IPCC.

IRONS, J. R. 2015. *Landsat 7* [Online]. USGS. Available: http://landsat.gsfc.nasa.gov/?p=3184 [Accessed 02/06/15].

JALLOW, M. F. A. & ZALUCKI, M. P. 1996. Within- and between-population variation in host-plant preference and specificity in Australian *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Australian Journal of Zoology,* 44**,** 503-519.

JIANG, N., XU, R., VAN LENTEREN, J. C. & VAN ROERMUND, H. J. W. 1999. Temporal and spatial analysis of greenhouse whitefly and its parasitoid *Encarsia formosa* in two types of greenhouse ecosystems. *Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie,* 123**,** 547-554.

KAY, I. R. 1982. The incidence and duration of pupal diapause in *Heliothis armiger* (Hübner) (Lepidoptera: Noctuidae) in southeast Queensland. *Australian Journal of Entomology,* 21**,** 263-266.

KIRKPATRICK, T. H. 1961. Queensland distributions and host records for *Heliothis* species (Lepidoptera:Noctuidae). *Queensland Journal of Agricultural Science,* 18**,** 195-202.

KNIPLING, E. F. & STADELBACHER, E. A. 1983. The rationale for area-wide management of *Heliothis* (Lepidoptera:Noctuidae) populations. *Bulletin of the Entomological Society of America,* 29**,** 29-37.

KOSTAL, V. 2006. Eco-physiological phases of insect diapause. *Journal of Insect Physiology,* 52**,** 113-127.

KRANTHI, K. R., JADHAV, D. R., KRANTHI, S., WANJARI, R. R., ALI, S. S. & RUSSELL, D. A. 2002. Insecticide resistance in five major insect pests of cotton in India. *Crop Protection,* 21**,** 449-460.

KRANTHI, K. R., KRANTHI, S., ALI, S. & BANERJEE, S. K. 2000. Resistance to 'CrylAc delta-endotoxin of *Bacillus thuringiensis*' in a laboratory selected strain of *Helicoverpa armigera* (Hübner). *Current Science,* 78**,** 1001-1004.

KRITICOS, D. J., OTA, N., HUTCHISON, W. D., BEDDOW, J., WALSH, T., TAY, W. T., BORCHERT, D. M., PAULA-MOREAS, S. V., CZEPAK, C. & ZALUCKI, M. P. 2015. The Potential Distribution of Invading Helicoverpa armigera in North America: Is It Just a Matter of Time? Plos One, 10.

KYI, A., ZALUCKI, M. P. & TITMARSH, I. J. 1991. An experimental study of early stage survival of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on cotton. *Bulletin of Entomological Research,* 81**,** 263-271.

LEMMEN, J. & EVENDEN, M. 2015. Environmental conditions terminate reproductive diapause and influence pheromone perception in the long-lived moth *Caloptilia fraxinella*. *Physiological Entomology,* 40**,** 30-42.

LINDZEN, R. S. 1997. Can increasing carbon dioxide cause climate change? *Proceedings of the National Academy of Sciences,* 94**,** 8335-8342.

LIU, Z. D., GONG, P. Y., HECKEL, D. G., WEI, W., SUN, J. H. & LI, D. M. 2009. Effects of larval host plants on over-wintering physiological dynamics and survival of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Journal of Insect Physiology,* 55**,** 1-9.

LIU, Z. D., GONG, P. Y., WU, K. J. & LI, D. M. 2004. Effects of parental exposure to high temperature on offspring performance in the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera : Noctuidae): adaptive significance of the summer diapause. *Applied Entomology and Zoology,* 39**,** 373-379.

LIU, Z. D., GONG, P. Y., WU, K. J., WEI, W., SUN, J. H. & LI, D. M. 2007. Effects of larval host plants on over-wintering preparedness and survival of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera : Noctuidae). *Journal of Insect Physiology,* 53**,** 1016-1026.

LLOYD, R. J., MURRAY, D. A. H. & HOPKINSON, J. E. 2008. Abundance and mortality of overwintering pupae of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) on the Darling Downs, Queensland, Australia. *Australian Journal of Entomology,* 47**,** 297-306.

LOPEZ, J. D., JR. & HARTSTACK, A. W., JR. 1985. Comparison of diapause development in *Heliothis zea* and *H. virescens* (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America,* 78**,** 415-422.

LOPEZ, J. D., JR., HARTSTACK, A. W., JR. & BEACH, R. 1984. Comparative pattern of emergence of *Heliothis zea* and *H. virescens* (Lepidoptera: Noctuidae) from overwintering pupae. *Journal of Economic Entomology,* 77**,** 1421-1426.

LU, B., DOWNES, S., WILSON, L., GREGG, P., KNIGHT, K., KAUTER, G. & MCCORKELL, B. 2011. Preferences of field bollworm larvae for cotton plant structures: impact of Bt and history of survival on Bt crops. *Entomologia Experimentalis et Applicata,* 140**,** 17-27.

LU, Z., TIAN, C., SHEN, Z. & CHEN, D. 2006. Prediction for cotton bollworm (Lepidoptera: Noctuidae) occurring date based on Web-GIS in Xinjiang Region of China. *Arid Land Geography,* 29**,** 582-587.

LYNCH, M. & GABRIEL, W. 1987. Environmental tolerance. *The American Naturalist,* 129**,** 283-303.

MACKRELL, D., KERR, D. & VON HELLENS, L. 2009. A qualitative case study of the adoption and use of an agricultural decision support system in the Australian cotton industry: The socio-technical view. *Decision Support Systems,* 47**,** 143-153.

MAELZER, D., ZALUCKI, M. P. & LAUGHLIN, R. 1996. Analysis and interpretation of long term light trap data for *Helicoverpa punctigera* (Lepidoptera: Noctuidae) in Australia: Population changes and forecasting pest pressure. *Bulletin of Entomological Research,* 86**,** 547-557.

MAELZER, D. A. & ZALUCKI, M. P. 1999. Analysis of long-term light-trap data for *Helicoverpa* spp. (Lepidoptera : Noctuidae) in Australia: the effect of climate and crop host plants. *Bulletin of Entomological Research,* 89**,** 455-463.

MAELZER, D. A. & ZALUCKI, M. P. 2000. Long range forecasts of the numbers of *Helicoverpa punctigera* and *H. armigera* (Lepidoptera: Noctuidae) in Australia using the Southern Oscillation Index and the sea surface temperature. *Bulletin of Entomological Research,* 90**,** 133-146.

MAHON, R. J. & YOUNG, S. 2010. Selection experiments to assess fitness costs associated with Cry2Ab resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Journal of Economic Entomology,* 103**,** 835-842.

MANSFIELD, S., DILLON, M. L. & WHITEHOUSE, M. E. A. 2006. Are arthropod communities in cotton really disrupted? An assessment of insecticide regimes and evaluation of the beneficial disruption index. *Agriculture Ecosystems & Environment,* 113**,** 326-335.

MASAKI, S. 1980. Summer diapause. *Annual Review of Entomology,* 25**,** 1-25.

MAYWALD, G. F. & SUTHERST, R. W. 1991. *User's guide to CLIMEX a computer program for comparing climates in ecology.* 2nd edition. CSIRO Australia, Division of Entomology Report No. 48, 51pp.

MC DONALD, W. J. F. 1982. *Western Arid Region Land Use Study Part 5*. Brisbane: The Division of Land Utilisation.

MCDONALD, G. 1995. *Native Budworm* [Online]. Available: http://www.depi.vic.gov.au/agriculture-and-food/pests-diseases-and-weeds/pest-insects-and-mites/native-budworm [Accessed 28/07/15].

MCDONALD, G., GOVENDER, A. & UMINA, P. 2014. PestFacts south-eastern. 12th October [Online]. Cesar Australia. Available http://cesaraustralia.com/sustainable-agriculture/pestfacts-south-eastern/past-issues/2012/pestfacts-issue-no-10-12th-october-2012/ [Accessed 18/09/15]

MICIC, S. 2015. *Diagnosing native budworm in canola* [Online]. Western Australia Department of Agriculture and Food,. Available: https://www.agric.wa.gov.au/mycrop/diagnosing-native-budworm-canola [Accessed 28/07/15].

MILLS, J. R. & PURDIE, R. W. 1982. *Western arid region land use study Part 3*. Brisbane: The Division of Land Utilisation.

MIRONIDIS, G. K. & SAVOPOULOU-SOULTANI, M. 2008. Development, survivorship, and reproduction of *Helicoverpa armigera* (Lepidoptera : Noctuidae) under constant and alternating temperatures. *Environmental Entomology,* 37**,** 16-28.

MIRONIDIS, G. K. & SAVOPOULOU-SOULTANI, M. 2012. Effects of constant and changing temperature conditions on diapause induction in *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Bulletin of Entomological Research,* 102**,** 139-147.

MITCHELL, P. D. & ONSTAD, D. W. 2005. Effect of extended diapause on evolution of resistance to transgenic *Bacillus thuringiensis* corn by northern corn rootworm (Coleoptera : Chrysomelidae). *Journal of Economic Entomology,* 98**,** 2220-2234.

MOHAPATRA, L. N. & SAHU, B. B. 2005. Age-specific life tables for American bollworm, *Helicoverpa armigera* (Hub.), on cotton. *Journal of Entomological Research,* 29**,** 9-14.

MONSANTO. 2014. *Cotton choices* [Online]. [Accessed [ONLINE] Available at: http://www.cottonchoices.com.au/price-discounts.html. [Accessed 06 November 2014].].

MORAL GARCIA, F. J. 2006. Analysis of the spatio-temporal distribution of *Helicoverpa armigera* Hb. in a tomato field using a stochastic approach. *Biosystems Engineering,* 93**,** 253-259.

MORAL GARCIA, F. J., RODRIGUEZ BERNABE, J. A., ARRANZ ROMERO, A., CRUZ BLANCO, J. I. D. L. & HONORIO GUISADO, F. 2004. Geostatistical methods were applied to obtain some maps of spatial dispersion in a tomato field of *Helicoverpa armigera* (Hübner) catches, monitored with 112 sex pheromone traps. *Boletin de Sanidad Vegetal, Plagas,* 30**,** 733-744.

MOREY, A. C., HUTCHISON, W. D., VENETTE, R. C. & BURKNESS, E. C. 2012. Cold hardiness of *Helicoverpa zea* (Lepidoptera: Noctuidae) pupae. *Environmental Entomology,* 41**,** 172-179.

MOTT, J.J. AND GROVES, R.H. (1981). Germination strategies, in *The Biology of Australian Plants*, eds J.S. Pate and A.J. McComb, 307–341, University of Western Australia Press, Nedlands, WA.

MURRAY, D. A. H.1991. *Investigation into the development and survival of Heliothis spp. pupae in South East Queensland.* Doctor of Philosophy thesis, University of Queensland.

MURRAY, D. A. H. & WILSON, A. G. L. 1991. Methods of studying diapause. *In:* ZALUCKI, M. P. (ed.) *Heliothis research methods and prospects.* New York: Springer Verlag, 102-108.

MURRAY, D. A. H. 2005. Cotton pest control in Australia before and after Bt cotton: Economic, ecologic and social aspects. *Proceedings of the 5th Brazilian Cotton Congress.* Salvador, Brazil.

MURRAY, D. A. H. & ZALUCKI, M. P. 1990. Effect of soil moisture and simulated rainfall on pupal survival and moth emergence of *Helicoverpa punctigera* (Wallengren) and *H. armigera* (Hübner) (Lepidoptera: Noctuidae). *Australian Journal of Entomology,* 29**,** 193-197.

NIBOUCHE, S. 1998. High temperature induced diapause in the cotton bollworm *Helicoverpa armigera*. *Entomologia Experimentalis et Applicata,* 87**,** 271-274.

NOOR-UL-ANE, M., ARIF, M. J., GOGI, M. D. & KHAN, M. A. 2015. Evaluation of different integrated pest management modules to control *Helicoverpa* for adaptation to climate change. *International Journal of Agriculture and Biology,* 17**,** 483-490.

NOWICKI, P., BONELLI, S., BARBERO, F. & BALLETTO, E. 2009. Relative importance of density-dependent regulation and environmental stochasticity for butterfly population dynamics. *Oecologia,* 161**,** 227-239.

OERTEL, A., ZALUCKI, M. P., MAELZER, D. A., FITT, G. P. & SUTHERST, R. 1999. Size of the first spring generation of *Helicoverpa punctigera* (Wallengren) (Lepidoptera : Noctuidae) and winter rain in central Australia. *Australian Journal of Entomology,* 38**,** 99-103.

OKSANEN, L. 2001. Logic of experiments in ecology: is pseudoreplication a pseudoissue? *Oikos,* 94**,** 27-38.

ORLOVA, N. A. 1998. Effects of photoperiod and temperature on diapause induction in *Chrysopa pallens* (Rambur) (Neuroptera, Chrysopidae). *Acta Zooogica Fennica* 209**,** 195-202.

OUYANG, F., HUI, C., GE, S. Y., MEN, X. Y., ZHAO, Z. H., SHI, P. J., ZHANG, Y. S. & LI, B. L. 2014. Weakening density dependence from climate change and agricultural intensification triggers pest outbreaks: a 37-year observation of cotton bollworms. *Ecology and Evolution,* 4**,** 3362-3374.

PARAJULEE, M. N., RUMMEL, D. R., ARNOLD, M. D. & CARROLL, S. C. 2004. Long-term seasonal abundance patterns of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera : Noctuidae) in the Texas high plains. *Journal of Economic Entomology,* 97**,** 668-677.

PATERSON, J. & WILKINSON, O. 2015. *Western Australian lupin industry* [Online]. Available: https://www.agric.wa.gov.au/grains-research-development/western-australian-lupin-industry [Accessed 26th of August 2015].

PETIT, J. R., JOUZEL, J., RAYNAUD, D., BARKOV, N. I., BARNOLA, J. M., BASILE, I., BENDER, M., CHAPPELLAZ, J., DAVIS, M., DELAYGUE, G., DELMOTTE, M., KOTLYAKOV, V. M., LEGRAND, M., LIPENKOV, V. Y., LORIUS, C., PEPIN, L., RITZ, C., SALTZMAN, E. &

STIEVENARD, M. 1999. Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. *Nature,* 399**,** 429-436.

PIELOOR, M. J. & SEYMOUR, J. E. 2001. Factors affecting adult diapause initiation in the tropical butterfly *Hypolimnas bolina* L. (Lepidoptera : Nymphalidae). *Australian Journal of Entomology,* 40**,** 376-379.

POINAR, G. O. & THOMAS, G. M. 1978. *Diagnostic manual for the identification of insect pathogens,* New York, Plenum Press.

PULLEN, S. R., MEOLA, R. W. & LOPEZ, J. D. 1992. Temperature as a sole factor for diapause induction after pupation in *Helicoverpa zea* (Lepidoptera: Noctuidae). *Environmental Entomology,* 21**,** 1404-1409.

PURDIE, R. W. 1986. *Western arid region land use study Part 5*. Brisbane: The Division of Land Utilisation.

PURDIE, R. W. (ed.) 1990. Vegetation. *pp. 49-92 in Wilson, P.R., Purdie, R.W. & Ahern, C.A. (Ed). Western arid region land use study – Part IV*: Division of Land Utilisation. Brisbane, Queensland Department of Primary Industries.

PUSTEJOVSKY, D. E. & SMITH, J. W., JR. 2006. Partial ecological life table of immature *Helicoverpa zea* (Lepidoptera : Noctuidae) in an irrigated cotton cropping system in the Trans-Pecos region of Texas, USA. *Biocontrol Science and Technology,* 16**,** 727-742.

QUEENSLAND DEPARTMENT OF SCIENCE INFORMATION TECHNOLOGY AND INNOVATION. 2015. *The Long Paddock,* [Online]. [Accessed 27/6/15 2015]. https://www.longpaddock.qld.gov.au/rainfallandpasturegrowth/

R DEVELOPMENT CORE TEAM 2014. R: A language and environment for statistical computing. *R Foundation for Statistical Computing.* Vienna, Austria.

RAJAPAKSE, C. N. K. & WALTER, G. H. 2007. Polyphagy and primary host plants: oviposition preference versus larval performance in the lepidopteran pest *Helicoverpa armigera*. *Arthropod-Plant Interactions* 1**,** 17-26.

RAYMOND, B., WRIGHT, D. J., CRICKMORE, N. & BONSALL, M. B. 2013. The impact of strain diversity and mixed infections on the evolution of resistance to *Bacillus thuringiensis*. *Proceedings of the Royal Society B-Biological Sciences,* 280, 20131497

ROACH, S. H. 1981. Emergence of overwintered *Heliothis* spp. moths from three different tillage systems. *Environmental Entomology,* 10**,** 817-818.

ROCHESTER, W. A. 1998. *The migration systems of Helicoverpa punctigera (Wallengren) and Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) in Australia.* Doctor of Philosophy thesis, Brisbane, the University of Queensland.

ROCHESTER, W. A., DILLON, M. L., FITT, G. P. & ZALUCKI, M. P. 1996. A simulation model of the long-distance migration of *Helicoverpa* spp moths. *Ecological Modelling,* 86**,** 151-156.

ROGERS, D. J. & BRIER, H. B. 2010. Pest-damage relationships for *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) on soybean (*Glycine max*) and dry bean (*Phaseolus vulgaris*) during pod-fill. *Crop Protection,* 29**,** 47-57.

ROMEIS, J., MEISSLE, M. & BIGLER, F. 2006. Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nature Biotechnology,* 24**,** 63-71.

ROOME, R. E. 1979. Pupal diapause in *Heliothis armigera* (Hübner) (Lepidoptera:Noctuidae) in Botswana: its regulation by environmental factors. *Bulletin of Entomological Research,* 69**,** 149-160.

ROUSH, R. T. 1998. Two-toxin strategies for management of insecticidal transgenic crops: can pyramiding succeed where pesticide mixtures have not? *Philosophical*

*Transactions of the Royal Society of London Series B-Biological Sciences,* 353**,** 1777-1786.

RYAN, B. F., JOINER, B. L. & CRYER, T. A. 2006. *Minitab Handbook. Updated for Release 14. ,* Belmont, California., Thomson Brooks/Cole.

SAMSON, P. R. & BLOOD, P. R. B. 1980. Voracity and searching ability of *Chrysopa signata* (Neuroptera: Chrysopidae), *Micromus tasmaniae* (Neuroptera: Hemerobiidae), and *Tropiconabis capsiformis* (Hemiptera: Nabidae). *Australian Journal of Zoology,* 28**,** 575-580.

SANSONE, C. G. & SMITH, J. W. 2001. Natural mortality of *Helicoverpa zea* (Lepidoptera : Noctuidae) in short-season cotton. *Environmental Entomology,* 30**,** 112-122.

SARWAN, K. & SAINI, R. K. 2007. Diapause in *Helicoverpa*/*Heliothis* spp. (Lepidoptera: Noctuidae) - a review. *Journal of Cotton Research and Development,* 21**,** 80-87.

SAUNDERS, D. S. 1982. *Insect Clocks,* Exeter, Pergamon Press.

SCOTT, K. D., LAWRENCE, N., LANGE, C. L., SCOTT, L. J., WILKINSON, K. S., MERRITT, M. A., MILES, M., MURRAY, D. & GRAHAM, G. C. 2005. Assessing moth migration and population structuring in *Helicoverpa armnigera* (Lepidoptera : Noctuidae) at the regional scale: Example from the Darling Downs, Australia. *Journal of Economic Entomology,* 98**,** 2210-2219.

SEYMOUR, J. E. & JONES, R. E. 1991. Evaluating natural enemy impact on *Heliothis*. *In:* ZALUCKI, M. P. (ed.) *Heliothis research methods and prospects.* New York: Springer Verlag, 80-89.

SIGSGAARD, L., GREENSTONE, M. H. & DUFFIELD, S. J. 2002. Egg cannibalism in *Helicoverpa armigera* on sorghum and pigeonpea. *Biocontrol,* 47**,** 151-165.

SLOSSER, J. E., PHILLIPS, J. R., HERZOG, G. A. & REYNOLDS, C. R. 1975. Overwinter survival and spring emergence of the bollworm in Arkansas. *Environmental Entomology,* 4**,** 1015-1024.

SMITH, J. W. 1998. Boll weevil eradication: Area-wide pest management. *Annals of the Entomological Society of America,* 91**,** 239-247.

SPOONER, P. G. 2005. Response of *Acacia* species to disturbance by roadworks in roadside environments in southern New South Wales, Australia. *Biological Conservation,* 122**,** 231-242.

STALL, W. M. 1999. IPM definition. *Journal of Vegetable Crop Production,* 4**,** 95-96.

STERN, V., SMITH, R., VAN DEN BOSCH, R. & HAGEN, K. 1959. The integrated control concept. *Hilgardia,* 29**,** 81-101.

TABASHNIK, B. E. 1994. Evolution of resistance to *Bacillus thuringiensis*. *Annual Review of Entomology,* 39**,** 47-79.

TADMOR, U. & APPLEBAUM, S. W. 1971. Adult diapause in the predaceous coccinellid, *Chilocorus bipustulatus*: Photoperiodic induction. *Journal of Insect Physiology,* 17**,** 1211-1215.

TAUBER, M. J., TAUBER, C. A. & MASAKI, S. 1986. *Seasonal adaptations of insects,* Oxford, Oxford University Press.

TEAKLE, R. E. 1991. Laboratory culture of *Heliothis* species and identification of disease. *In:* ZALUCKI, M. P. (ed.) *Heliothis research methods and prospects.* New York: Springer Verlag, 22-29.

TEAKLE, R. E., JENSEN, J. M. & GILES, J. E. 1986. Age-related susceptibility of *Heliothis punctiger* to a commercial formulation of nuclear polyhedrosis virus. *Journal of Invertebrate Pathology,* 47**,** 82-92.

THRALL, P. H., BEVER, J. D. & BURDON, J. J. 2010. Evolutionary change in agriculture: the past, present and future. *Evolutionary Applications,* 3**,** 405-408.

TITMARSH, I. J., ZALUCKI, M. P., ROOM, M. L., EVANS, P. C., GREGG, P. C. & MURRAY, D. 1991. Estimating the abdundance of adults and immatures. *In:* ZALUCKI, M. P. (ed.) *Heliothis research methods and prospects.* New York: Springer Verlag, 49-69.

TURNER, E. J. & BEESTON, G. R. 1977. *Western arid region land use study Part 4*. Brisbane: The Division of Land Utilisation.

UNITED STATES MILITARY. 2014. *The Astronomical Almanac. [ONLINE] Available at: http://asa.usno.navy.mil/. [Accessed 11 December 14].* [Online]. [Accessed].

USGS. 2015. *Landsat L7 ETM* [Online]. Department of the Interior. [Accessed 09/04/15 2015]. http://landsat.gsfc.nasa.gov/?p=3225

VREYSEN, M. J. B., SALEH, K. M., ALI, M. Y., ABDULLA, A. M., ZHU, Z. R., JUMA, K. G., DYCK, V. A., MSANGI, A. R., MKONYI, P. A. & FELDMANN, H. U. 2000. *Glossina austeni* (Diptera: Glossinidae) eradicated on the Island of Unguja, Zanzibar, using the sterile insect technique. *Journal of Economic Entomology,* 93**,** 123-135.

WALDEN, K. J. 1995. Insect migration in an arid continent. III. The Australian Plague Locust *Chortoicetes terminifera* and the Native Budworm *Helicoverpa punctigera* in Western Australia. *In:* DRAKE, V. A. & GATEHOUSE, A. G. (eds.) *Insect migration: tracking resources through time and space.* Cambridge University Press.

WALTER, G. H. & BENFIELD, M. D. 1994. Temporal host plant use in three polyphagous Heliothinae, with special reference to *Helicoverpa punctigera* (Wallengren) (Noctuidae: Lepidoptera). *Australian Journal of Ecology,* 19**,** 458-465.

WHITEHOUSE, M. E. A., WILSON, L. J. & FITT, G. P. 2005. A comparison of arthropod communities in transgenic Bt and conventional cotton in Australia. *Environmental Entomology,* 34**,** 1224-1241.

WILSON, A. G. L. 1983. Abundance and mortality of overwintering Heliothis spp. *Journal of the Australian Entomological Society,* 22**,** 191-199.

WILSON, A. G. L., LEWIS, T. & CUNNINGHAM, R. B. 1979. Overwintering and spring emergence of *Heliothis armigera* (Hübner) (Lepidoptera: Noctuidae) in the Namoi Valley, New South Wales. *Bulletin of Entomological Research,* 69**,** 97-109.

WILSON, L., DOWNES, S., KHAN, M., WHITEHOUSE, M., BAKER, G., GRUNDY, P. & MAAS, S. 2013. IPM in the transgenic era: a review of the challenges from emerging pests in Australian cotton systems. *Crop & Pasture Science,* 64**,** 737-749.

WU, S. B., WANG, M. Q. & ZHANG, G. A. 2010. Effects of putrescine on diapause induction and intensity, and post-diapause development of *Helicoverpa armigera*. *Entomologia Experimentalis et Applicata,* 136**,** 199-205.

ZALUCKI, M., MURRAY, D., GREGG, P., FITT, G., TWINE, P. & JONES, C. 1994. Ecology of *Helicoverpa armigera* (hubner) and *Heliothis punctigera* (Wallengren) in the inland of Australia - larval sampling and host-plant relationships during winter and spring. *Australian Journal of Zoology,* 42**,** 329-346.

ZALUCKI, M. P., 1991. *Heliothis research methods and prospects,* New York, Springer Verlag, 234pp.

ZALUCKI, M. P., CLARKE, A. R. & MALCOLM, S. B. 2002. Ecology and behavior of first instar larval Lepidoptera. *Annual Review of Entomology,* 47**,** 361-393.

ZALUCKI, M. P., CUNNINGHAM, J. P., DOWNES, S., WARD, P., LANGE, C., MEISSLE, M., SCHELLHORN, N. A. & ZALUCKI, J. M. 2012. No evidence for change in oviposition behaviour of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) after

widespread adoption of transgenic insecticidal cotton. *Bulletin of Entomological Research,* 102**,** 468-476.

ZALUCKI, M. P., DAGLISH, G., FIREMPONG, S. & TWINE, P. 1986. The biology and ecology of *Heliothis armigera* (Hübner) and *H. punctigera* Wallengren (Lepidoptera: Noctuidae) in Australia: what do we know? *Australian Journal of Zoology,* 34**,** 779-814.

ZALUCKI, M. P. & FURLONG, M. J. 2005. Forecasting *Helicoverpa* populations in Australia: a comparison of regression based models and a bioclimatic based modelling approach. *Insect Science,* 12**,** 45-56.

ZHANG, Y., FAN, J., YANG, L. & LIU, Z. 2014. The summer diapause rate, pupal weight, and metabolic rate of *Helicoverpa assulta* at 35°C. *Chinese Journal of Applied Entomology,* 51**,** 1274-1279.