

**Optimising the establishment, persistence
and impact of *Trichogramma* on the
Liverpool Plains in northern New South
Wales**

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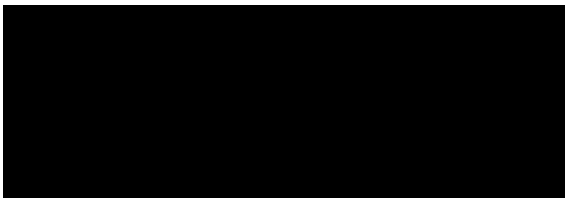
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Declaration

I certify that the substance of this thesis has not been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that, any assistance received in preparing this thesis, and all resources used, have been acknowledged in this thesis.



Christopher M Carr

29 January 2020

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I dedicate this thesis to my daughter, Samaya, who has an endless fascination for nature especially insects. May your gentle caring nature and passion for the environment forever drive you to discover and protect the wonders of this world.

Abstract

Egg parasitoids prevent larval hatch of insect eggs, making them ideal natural predators for economically destructive hosts. *Trichogramma* Westwood (Hymenoptera: Chalcidoidea) is utilised in crops around the world as a biological control agent for this purpose. In Australia, *Helicoverpa* Hardwick (Lepidoptera: Noctuidae) is a major lepidopteran pest of several spring and summer crops and has rapidly become resistant to broad-spectrum insecticides and *Bacillus thuringiensis* (Bt) proteins in genetically modified (GM) crops. However, little is known about native egg parasitoids of *Helicoverpa* on the Liverpool Plains, a highly productive and extensive cropping region on the north-western slopes of New South Wales. This thesis aimed to elucidate the potential of egg parasitoids such as *Trichogramma* to manage *Helicoverpa* species and how to optimise their effectiveness so they can be best utilised as a part of an integrated insect pest management program.

To establish the presence, impact and habitats of *Helicoverpa* egg parasitoids on the Liverpool Plains, we conducted a 3-year landscape-scale survey to determine the abundance of egg parasitoids and their seasonal phenology in crop and non-crop vegetation. We assessed egg parasitoid species abundance using water-pan traps (WPT) (white and yellow) and determined their impact by measuring mean parasitism rate of *H. armigera* egg cards in various habitats. To determine variations in annual egg parasitoid populations, we surveyed spring crops (canola, chickpea, faba bean, linseed, lucerne, sunflower and wheat) and native vegetation (forest, agroforestry and grassland/native pasture). In summer, we used a landscape approach to identify major crop (maize, cotton and sorghum) and forest habitats in two different but connected landscapes. Using the same methods, we surveyed potential overwintering habitats in forest.

We identified *Trichogrammatoidea bactrae*, *Trichogramma pretiosum*, *T. australicum*, *Telenomus* species and unidentified species of Trichogrammatidae.

Trichogrammatids and *Trichogrammatoidea bactrae* were the most abundant parasitoids in spring. Whilst *T. pretiosum* was the dominant egg parasitoid in late summer crops. Trichogrammatidae were more attracted to yellow traps than white whilst *Telenomus* spp. were only attracted to white traps. Egg parasitoid abundance and species differed significantly when compared between the Piallaway–Breeza and Carroona–Quirindi landscapes, indicating the composition of the landscape had an effect on parasitism impact of *Helicoverpa*. The abundance of different egg parasitoid taxa caught in water pan traps in the two landscapes was highly significant as was the interaction between individual taxon and landscape. In the forest habitats *T. australicum* and *Trichogrammatoidea bactrae* were equally abundant in the two landscapes, whereas *T. pretiosum*, *Telenomus* spp, and Trichogrammatids were more abundant in crops and non-crop vegetation in the Carroona–Quirindi landscape. The Carroona–Quirindi landscape had a greater proportion of native vegetation as well smaller and more diverse cropping systems, which conceivably leads less area affected by insecticides that greatly affect the potential of predators and parasitoids.

Egg parasitoids were abundant and diverse in crop and non-crop habitats on the Liverpool Plains. However, low abundance in spring crops could limit *Helicoverpa* control in these crops, and the late abundance of *T. pretiosum* in summer crops could limit its effectiveness as a reliable natural biological control agent. To address this, we pursued two different experimental approaches: one at a plant level and the other at a broader landscape level.

Highly variable rates of parasitised lepidoptera eggs have been reported between different cropping habitats, between different plants in the same crop and between different parts of the same plant. To investigate the effects of crop plant hosts, their combinations, and plant parts, we measured levels of parasitism by *T. pretiosum* of *H. armigera* eggs on a range of crop hosts that are regularly grown in northern New South Wales. Individual and mixed-species groups of three crop plants were

presented in small-cage trials to determine how plant mixes and egg position on the plant affected parasitism rates. We separated winter/spring crops into two groups (1: linseed, canola and wheat, and 2: lucerne, adzuki and faba bean) and investigated a third group of summer crops (cotton, maize and sorghum). The mean percentage parasitism across the range of crop plants varied from 7% (adzuki) to 42% (canola and faba bean). The group 1 crop plants had similar mean percentage parasitism levels: linseed (40%), canola (42%) and wheat (37%). Egg parasitism on group 2 crop plants differed between adzuki (7%), lucerne (26%) and faba bean (42%). Parasitism of summer crops also varied, with cotton (34%) preferred to sorghum (19%) or maize (9%). Crop configuration (i.e. different combinations of the three crops in each group) had no effect on parasitism rate, but plant part had a significant effect, with more egg parasitism occurring on leaves and stems than flowers in each group. Our results indicated that preferred crops are canola, faba bean, wheat and linseed.

To investigate *Trichogramma* utilising a landscape level approach, we identified the main cropping habitats on the Liverpool Plains in spring to act as a nursery crop to build early populations of these egg parasitoids. We then monitored *T. pretiosum* populations in summer crops adjacent to and 2 km from the release sites. In this approach, rather than employing inundative releases to control *Helicoverpa* (the main method used worldwide to combat pest outbreaks), we used inoculative releases to build up natural *T. pretiosum* populations to act as a preventative measure against seasonal variations of *Helicoverpa* attacks. Of the four winter crops preferred by *T. pretiosum* to parasitise *H. armigera* eggs in the cage experiment, wheat is the predominant crop grown on the Liverpool Plains followed by canola. We tested rates of early-season inoculative *T. pretiosum* releases at 30,000 wasps/ha and 60,000 wasps/ha in 5-ha plots every week for 3 weeks in wheat and canola across 12 farms in the Piallaway–Breeza and Caroon–Quirindi landscapes. We measured movement away from the release area in 50-m and 100-m intervals, and measured impact using *H. armigera* sentinel egg cards. We found high levels of egg card

parasitism in these crops within the release area. Between releases, average parasitism of *H. armigera* egg cards in high and low-release sites combined was higher in canola (57%) than wheat (35%), averaging 51% overall. Parasitism of egg cards peaked in the low-rate treatments at 88% in canola and 78% in wheat. However, we found that *T. pretiosum* did not move much further than 50 m from the release area. Distance from release area significantly affected egg-card parasitism, with average parasitism of 16% of egg cards at 50 m declining to 2% at 100 m.

We trapped egg parasitoids in white and yellow water traps and measured *Helicoverpa* sentinel egg card parasitism in cotton, sorghum, native forest and native grassland habitats in the subsequent summer in January and February in adjacent and more distant habitats. In January and February 2009, average egg card parasitism in four cotton crops within 50 m of the spring release crops was 27% and 33%, respectively, and in five sorghum crops it was 38.5% and 56.5%, respectively.

The main findings of this study were that the Liverpool Plains has a resident population of diverse *Helicoverpa* egg parasitoids in different habitats and landscapes. We found that the introduced but naturalised *T. pretiosum* parasitised *H. armigera* in wheat and canola and, in the absence of insecticides, were suitable nursery crops to facilitate localised population enhancement. Low inoculative release rates of *Trichogramma* may be the most cost-effective method of utilising *Trichogramma* as part of an IPM strategy. The limited application of this method in Australia highlights a significant future management option to control *H. armigera* in these crops. After the early season releases of *T. pretiosum*, increased abundance of this egg parasitoid facilitated natural pest control in nearby summer crops, cotton and sorghum, which are readily attacked by *H. armigera*. Whilst continued *Bt* protein (GM) advancements have limited the impact of *Helicoverpa* spp. in cotton, egg parasitoids should be an integral component of local insecticide and GM resistance management strategies, particularly for Australian grain crops. Furthermore, we have demonstrated that the impact of a very small parasitoid is comparable to other

predatory (beneficial) insects, which require larger scales than a single field. Consequently, individual farms and regions should be evaluated independently for effective targeted management of economic pests.



Pictures: Various *Trichogramma* spp. prepared for identification

Preface

This thesis has been written in the style of thesis by publication. Chapters 2-4 have been prepared as manuscripts and Chapter 2 and 4 has been prepared according to the style of journal; *Agriculture, Ecosystems and Environment*. I apologise in advance for some repetition between chapters as each is presented as a standalone manuscript. The formatting aligns with that suggested by the University of New England for consistency.

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Chapter 1. Introduction

1.1 Introduction

This thesis examines the ecology of the egg parasitoid *Trichogramma* on the Liverpool Plains in the Upper Namoi Valley (150° 28'E, 31° 13'S) of northern NSW. The importance of this parasitoid relates to its ability to parasitise the eggs of the two species of noctuid moth in the genus, *Helicoverpa*, major crop pests in the region, *H. armigera* and *H. punctigera*. Managing *Helicoverpa* resistance to insecticides has become critical in Australian field crops and prolonging the use of genetically modified crops expressing the *Bacillus thuringiensis* (Bt) proteins, such as cotton, via Resistance Management Strategies is a research challenge. *Trichogramma* species have an important role in Integrated Pest Management (IPM) programs. This thesis examines the role of *Trichogramma* by: firstly, establishing the habitats in which *Trichogramma* species occur and measuring their impact by evaluating egg card parasitism and, secondly, optimising the potential of *Trichogramma* populations that can be augmented by farmers as part of an IPM strategy in northern NSW.

I begin by providing the background for this research and reviewing the research literature around *Trichogramma* and the role of egg parasitoids in IPM related to this study. The chapter concludes with my research aims and objectives and a section describing the layout of the thesis.

1.2 Background

Trichogramma species are extensively used in biological control programs around the world (Smith, 1996; Zang *et al.*, 2021). There are over 600 or more species that parasitise the eggs of at least seven insect orders (Zang *et al.*, 2021). They prevent larval hatch of insect hosts, making them ideal biological control agents (Scholz,

2000). Most studies of *Trichogramma* relate to their use as biological control agents and augmentation in agroecosystems to combat pests (Li, 1994; Davies *et al.*, 2011b). In Australian cropping systems, particularly cotton, research has concentrated on the use of *Trichogramma* to control *Helicoverpa* (Davies *et al.*, 2011b). The development of insecticide resistance in *H. armigera* has necessitated further control options (Zalucki *et al.*, 2015). Whilst the advent of *Bt* cotton in 1996 has limited the use of insecticides to control *Helicoverpa* in cotton, resistance to the *Bt* proteins has required development of three new genetically modified varieties since (Downes *et al.*, 2017).

In northern Australia, in the Ord River Irrigation Area (ORIA, 15°47'S, 128°44'E), egg parasitoids were discovered in the 1960s (Richards, 1964) and have since reached very high levels of *Helicoverpa* egg parasitism. This occurred after Michael and Woods (1980) released imported *T. pretiosum* from the USA in the 1970s. However, *T. pretiosum* has not provided complete control of *Helicoverpa* in conventional cotton crops in this area (Davies *et al.*, 2011b). Nevertheless, the development of localised habitat manipulation techniques is ongoing and the viability of *Bt* cotton crops in the ORIA depends on *Trichogramma* for continued success in Resistance Management Strategies (Davies, 2006).

On the Darling Downs (27°33'S, 152°17'E) in southern Queensland, *T. pretiosum* was also released in the 1970s when there were no commercial mass rearing facilities in Australia (Twine, 1975). Inundative releases achieved successful parasitisation of *Helicoverpa* (49%) but not enough for economic control (Twine and Lloyd, 1982). In the 1980s, *Trichogrammatoidea bactrae* was the dominant egg parasitoid in the region (Scholz, 1990). Thus began the development of trials involving native egg parasitoids, but culturing and release proved difficult (Scholz, 1990). By the mid-1990s, research returned to focus on *Trichogramma pretiosum*, and through many subsequent releases, it has become the dominant parasitoid in the region (Scholz and Parker, 2004). More recently the use of *Trichogramma* has been adopted over

insecticidal treatments particularly in Bt cotton when *Helicoverpa* needs to be suppressed (Davies *et al.*, 2011b).

Trichogramma in northern NSW cropping systems received little attention prior to the last decade. Before this study, there was little knowledge of the presence of *Trichogramma* on the Liverpool Plains in the upper Namoi Valley on the North West Plains of NSW, with some studies suggesting that further north in the Lower Namoi (29°28'S, 149°51'E) was perhaps the southern geographical range limit of the parasitoid (Walker, 1999; Schellhorn *et al.*, 2000b). The factors affecting the distribution of *T. pretiosum* and critical to its persistence in northern NSW include climate, the use of insecticides and the availability of suitable refuges or habitats (Schellhorn *et al.*, 2000b). *Trichogramma* are opportunists and readily parasitise eggs of different species in the habitats where they occur (Roland, 2000). For this reason, *Trichogramma* species tend to be habitat rather than host specialists (Romeis *et al.*, 2005a; Michaud, 2018). Thus, establishing the habitats in which they occur and determining their impact by measuring egg card parasitism means farmers may be able to incorporate *Trichogramma* into their IPM and Insecticide Resistance Management strategies.

The first step in finding egg parasitoids on the Liverpool Plains requires a landscape approach identifying key habitats in each season. This includes documenting the overwintering of egg parasitoids, identifying the species complex and their parasitism rates in predominant crops and habitats in winter and summer. Once the natural populations are known, increasing the effectiveness of these parasitoids can occur, and recommendations for the conservation and utilisation of egg parasitoids can be established to guide growers and target future research. As Scholz (2003) and Bastos *et al.* (2010) recommended, future studies should address the potential for early-season, low-density releases of suitable egg parasitoids to increase their effectiveness if populations of a suitable egg parasitoid candidate are low.

1.3 Literature review

1.3.1 Introduction

In the first part of this review, I discuss the background to modern integrated pest management (IPM) and highlight the lack of the use of natural enemies (predators, parasites or pathogens) in IPM programs, and the disparity between research in developing IPM programs and the implementation of practical strategies that are useful to farmers. One aspect of this is the need for studies that not only assess natural enemy presence or abundance, but also measure impact. I review how previous research has focused on the effects of natural enemies at the scale of the field, whereas larger scales of natural enemy movement across landscapes and between habitats are important. I present the theoretical background to landscape effects on natural enemy diversity and review the recent expanding interest in this field. In the second part of the review, I discuss host location by parasitoids as well as *Trichogramma* identification and biology. I briefly review the extensive literature on biological control using *Trichogramma*, highlighting the areas where lack of knowledge or misevaluation has previously limited biological control by *Trichogramma*. I review the historical problems associated with inundative augmentative control of *Helicoverpa*. Lastly, I review the research around release introductions of *T. pretiosum* in Australia, the discovery of egg parasitoids on the Liverpool Plains in northern NSW and highlight the need for egg parasitoids to be incorporated into IPM strategies in this region.

1.3.2 Integrated pest management and conservation biological control

Damage by pest insects is estimated to cost US\$70 billion per year globally (Bradshaw *et al.*, 2016). Conventional management of pest insects in western agriculture is by means of synthetic insecticides, which provide short-term pest control in a measured and predictable way (Ekström and Ekbom, 2011). However,

the use of pesticides has many negative consequences (Carson *et al.*, 1962), and deprive agroecosystems of self-regulating functional components that provide their own pest control (Altieri, 1999). Broad-spectrum chemical pesticides are indiscriminate in killing not only the intended pest but affect all the insects in the ecosystem, as well as other wildlife, including endangered species and humans (Ruberson *et al.*, 1998). In addition, insect pests have repeatedly developed resistance to insecticides, requiring continued development of new chemistries (DeBach and Rosen, 1991; Zhao *et al.*, 2002; Umina *et al.*, 2019) and creating new pest problems.

Although pesticides can remove the threat of insect attack, it is not always an economically sound strategy (Ekström and Ekbohm, 2011). The routine use of pesticides can lead to ‘pest resurgence’ where, after pesticide application, insect pests can return to the former damage level (Waage *et al.*, 1985; Bale *et al.*, 2008). Additionally, secondary insect pests previously suppressed by natural enemies or competition may then increase (Metcalf, 1980), creating further insect pest recurrence and requiring repeated insecticidal control. Human and animal health, food quality and safety and environmental quality may all be compromised (Altieri, 1999). Barbosa (1998) has called these effects a global crisis, because whilst they contribute to high productivity, they may be too perilous for the environment, too risky for the consumer, and too problematic for the farmer.

In the late nineteenth and early twentieth centuries, before the advent of chemical controls, pest biology and cultural practices were used to control insect pests (Kogan, 1998). In a series of papers starting with Smith and Allen (1954), the term ‘integrated control’ was discussed in scientific papers. A seminal paper published by Stern *et al.* (1959) developed the ‘integrated control’ concept that underpinned the conceptual foundation of modern integrated pest management or IPM programs. This was an ecosystem approach for harmonising the use of chemical and biological pest control. In this approach, chemicals were only to be used where they were least disruptive to biological control. The four basic elements included (Stern *et al.*, 1959):

(1) the thresholds determining the need for control; (2) critical density sampling (to know whether the population density of an insect pest exceeds a critical level at which some treatment should be applied, (Iwao, 1975); (3) an understanding of the need to conserve the biological control capacity in the system, and (4) the use of selective insecticides or selective application methods when needed to augment biological control. This idea later evolved to incorporate all possible feasible tactics, including natural enemies, pesticides, cultural controls, host plant resistance and other biologically based methods (Ruberson *et al.*, 1998).

A United Nations Conference on Environment and Development in 1992 stated in agenda 21, article 15 that: '*Integrated pest management, which combines biological control, host plant resistance and appropriate farming practices and minimizes the use of pesticides, is the best option for the future, as it guarantees yields, reduces costs, is environmentally friendly and contributes to the sustainability of agriculture.*' However, pesticides have long overshadowed the importance of natural enemies in pest management programs (Ruberson *et al.*, 1998). The aim of IPM is to create an environment where natural enemies thrive and maximise their effects on pest control (Macfadyen *et al.*, 2015). However, the concept of integrated control has been an elusive goal for most modern-day IPM programs (Naranjo and Ellsworth, 2009). Ehler (2006) referred to IPM as integrated pesticide management and Zalucki (2009b) suggested that perhaps the dominant form of IPM in most crops is essentially 'sample, spray and pray'. Strickland *et al.* (1996) highlighted the need for a key focus of IPM in future to maximise the use of natural enemies. But natural enemies in IPM are rarely utilised (Thomas, 1999; Macfadyen *et al.*, 2015; Zalucki *et al.*, 2015), and most contemporary IPM programs are implemented with little consideration of ecosystem processes (Kogan, 1998). Such processes are difficult to model and incorporate into decision-making rules. However, they are important and extend beyond the scale of the field (Kareiva and Wennergren, 1995; Kogan, 1998; Tscharncke *et al.*, 2007b). However it is argued by Horne *et al.* (2008) that in south-eastern Australia broad acre cropping

(including wheat and canola) although there are not specific IPM strategies, there is enough information available for farmers and agronomists to start using an IPM approach. This is through changing practices and information transfer, which does not require detailed entomology.

To assess the impact of natural enemies on pests, studies are required at a scale relevant to farmers (Macfadyen *et al.*, 2015) and that measure pest mortality due to natural enemies at the appropriate ecological scale. Despite the knowledge and widespread acceptance of these processes, there are few studies demonstrating the ecological impact of predators or parasitoids on pest populations within the framework of pest-management programs. Furlong and Zalucki (2010) analysed 54 research publications in high-ranking agricultural journals between 2003 and 2008 to determine if predators had an impact on prey (pest) populations, and if the interaction was at an ecological scale relevant to pest management. They found that less than half (43%) of the field studies assessed the impact of predators on target pests. Most of the studies (76%) were conducted at the scale of experimental plots, which the authors argued is not the ecological scale that determines the population dynamics of pests and predators or that pest management decisions are made.

In many industries that are highly competitive, farmers favour systems that are high yielding with low risk (Tracy, 2015). Conservation biological control is more likely to be adopted at local and regional scales when the economics are clear (Gurr *et al.*, 2016). Similarly, conservation biological control can have multiple benefits that are not easily calculated and extend beyond pest management to other ecological services (Shields *et al.*, 2019). These include pollination, nutrient recycling, soil moisture retention, weed control and carbon capture as well as aesthetics (Wratten *et al.*, 2012). Ecological services research has been growing rapidly since ecosystem services were valued at US\$33 trillion per year globally (Costanza *et al.*, 1997). The ecosystem services concept encourages a proactive approach to biodiversity management (Reid *et al.*, 2006a) and requires a multidisciplinary approach.

Conservation biological control would benefit from a multidisciplinary approach, including agronomic, socio-cultural, economic and ecological considerations and political will (Michaud, 2018; Shields *et al.*, 2019). Farmer participation in habitat management is being aided by worldwide practical implementation, facilitated by farmer participation in research and the simultaneous delivery of ecosystem services other than just pest suppression (Gurr *et al.*, 2017). Conservation biological control has been integrated into existing farming practices, but there are inconsistencies across cropping systems, and pesticide resistance remains a problem worldwide (Begg *et al.*, 2017). Despite many techniques and interventions, the overall potential is unrealised as a result of economic, perceptual and communication barriers (Shields *et al.*, 2019) as well as government policies and the influence of agri-chemical companies (Rayl *et al.*, 2018). However, this presents an opportunity for conservation biological control by using locally present biological control agents where there are few regulatory hurdles and the end user can develop and implement ecologically based solutions to local pest problems (Shields *et al.*, 2019).

Whilst there has been some good success with insect-resistant crop plants (Stout, 2014), there is a lack of integration between insect-resistant crop plants and biological control (Smith, 2020). Current challenges and future opportunities in this area are well reviewed in Peterson *et al.* (2016) and several different aspects merit consideration. Host plant resistance can be defined as protection against herbivores or disease through selection, genetic modification or utilisation of plants with defensive traits (Peterson *et al.*, 2016). Conventional and transgenic insect resistance has the disadvantage of loss of function when virulent insect strains evolve high frequencies of resistant genes (Smith, 2020). For instance, virulence in Lepidoptera in both maize and cotton emerges after about 8 years (Ali *et al.*, 2006; Liu *et al.*, 2010; Campagne *et al.*, 2013; Fite *et al.*, 2020). However, polygenic resistance has been shown to be more durable in some crops. For instance, the brown planthopper *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) has been a major pest of rice, but

rice resistance to *N. lugans* has suppressed virulence for over 10 years (Mackill and Khush, 2018), as has been the case with wheat resistance to wheat curl mite *Aceria toschiella* (Khalaf *et al.*, 2019). In some crops like raspberry, this polygenic resistance has suppressed virulence in the raspberry aphid (*Amphorophora idaei*) for more than 30 years (Jones *et al.*, 2000). By feeding on insect-resistant plants, insect pests have decreased vigour, which in turn increases their susceptibility to insecticides and reduces the amount of chemical that needs to be applied for effective pest control (Smith, 2020). Insect-resistant plants fit well into IPM programs because the economic thresholds are raised and the emergence of virulent pest populations is reduced (Peterson *et al.*, 2018). Peterson *et al.* (2016) argued that it is important that plant breeders, entomologists, ecologists and extension professionals work collaboratively to optimise the levels of resistance that reduce pest populations but allow beneficial predators and parasitoids to function.

1.3.3 Landscape effects on natural enemy diversity

Landscape scale effects of non-crop vegetation and their spatial arrangement, connectivity and complexity is a large sub-discipline within conservation biological control (Begg *et al.*, 2017). A crop's ability to withstand pest attack is influenced by the ecological processes in the surrounding landscape as well as the phenology and health of the crop (Tschardt and Brandl, 2004; Schmidt and Tschardt, 2005; Schweiger *et al.*, 2005). Whilst the term landscape implies a large area, a landscape approach to insect studies can also involve small spatial scales (Wiens *et al.*, 1997). Ecological landscapes are mosaics of patches, and landscape size differs among organisms, being the spatial scale intermediate between an organism's normal home range and its regional distribution (Dunning *et al.*, 1992). Landscape ecology is partly the study of habitat composition within the context in which patches occur, and the resulting connectivity of the landscape (Forman, 1995). The spatial and temporal patterns of crop-associated resources such as adjacent unmanaged habitats can be critical to the survival of natural enemies (Barbosa, 1998). However, the landscape

diversity of agricultural areas has declined with increasing mechanisation, which favours large and uniform production units (Altieri, 1999). Pimentel et al. (1992) argued that the majority of the world's biodiversity occurs in ubiquitous human-made ecosystems. In modern agricultural landscapes, the loss and fragmentation of semi-natural habitats have led to a reduction in species richness and abundance (Haenke *et al.*, 2009; Mangel, 2017), which in turn affects biological control.

Although little is known about the relationship between diversity and biological control (Marino and Landis, 1996), biodiversity enhances and maintains ecosystem functions in ephemeral agricultural ecosystems (Hawkins, 1993). Prey diversity, for example, can have a powerful effect on the nutrition, reproduction and survival of natural enemies (Symondson *et al.*, 2006). The management of agricultural landscapes (e.g. habitat conservation) has been shown to mitigate the effects of agricultural intensification (Altieri, 1995; Wratten and Van Emden, 1995; Menalled *et al.*, 1999a; Landis *et al.*, 2005; Colazza *et al.*, 2017).

In the mid-western USA, simplification of landscapes has resulted in less effective natural enemy control and higher pesticide use at a regional scale (Meehan *et al.*, 2011). It is generally thought that structurally simplified landscapes have reduced natural enemy activity within crops (Schmidt *et al.*, 2005; Tschamntke *et al.*, 2007a; Schellhorn *et al.*, 2008b; Flick *et al.*, 2012). For example, Thies and Tschamntke (1999a) found increased parasitism of the rape pollen beetle (*Meligethes aeneus*) and lower crop damage in structurally complex landscapes than in simple landscapes with a high percentage of agricultural use.

Numerous reviews have highlighted the effects of habitat fragmentation on insects in agricultural landscapes (Thies and Tschamntke, 1999b; Debinski and Holt, 2000; Tschamntke and Brandl, 2004; Bianchi and van der Werf, 2005; Haynes *et al.*, 2007; Tschamntke *et al.*, 2012; Schellhorn *et al.*, 2014). Bianchi et al. (2017) discussed the conceptual and empirical approaches to investigate landscape features that affect the spatial distribution of arthropods. The first approach is the use of mechanistic

models not based on quantitative sample data of natural enemies and pests but incorporating population dynamics and dispersal processes to generate predictions of arthropod distribution in relation to habitat features. For example, Bianchi *et al.* (2010) generated 1000 computer-simulated landscapes and examined four predator groups with different dispersal abilities as biological control agents. The authors found that the most mobile and aggregating predators were the most effective at pest suppression (control) and that the source habitat for natural enemies of agricultural pest species has the most effect on their potential to colonise crops. The second empirical and most prevalent approach is to use correlative studies to determine local population measurements using metrics of the surrounding landscape (Thies and Tschardtke, 1999b; Tschardtke *et al.*, 2005; Haenke *et al.*, 2009; Perović *et al.*, 2010). These are often in the form of land-use classes, such as the proportion of crop and non-crop habitat. However, the complexities involved in determining population processes and population densities of natural enemies may not always be captured by the underlying drivers of biocontrol (Bianchi *et al.*, 2017). Disruptive factors include insecticide application, the presence of different plant species in the same land-use class confounding outcomes, and meteorological events.

Dunning *et al.* (1992) outlined a general framework of four broad classes of ecological process that affect population abundance and dispersion at landscape scale. The first of these processes, landscape complementation, is where a single species requires at least two different resources at the same point in the species' life cycle. Thus, individuals must travel between non-substitutable patches of different types and so the distance and functional connectivity between these patch types influence the species' population size in the landscape. An example is (1) nesting sites *versus* (2) nectar and pollen for bees (Holzschuh *et al.*, 2007). The second process, landscape supplementation, is the response of an individual to the distribution of patches with substitutable resources nearby, such as alternate less

productive habitats. Third, source/sink relationships occur when relatively productive patches serve as a source of emigrants, which disperse to less productive patches (sinks) where populations cannot persist. Fourth are neighbourhood effects: this is where a species' abundance in a 'focal patch' is more strongly affected by the physiognomic characteristics of a nearby patch than patches further away (Dunning *et al.*, 1992). For example, Summerville and Crist (2004) found that small diverse forests can support comparable numbers of moth species to those in less diverse, large forest stands because the habitat quality and local patch size were more important in determining community structure of forest moths than the landscape context. These few categories are not mutually exclusive and multiple processes can apply. For example, Ouin *et al.* (2004) demonstrated that woody habitats close to grassland patches had a significant positive effect on butterfly communities, involving landscape supplementation and landscape complementation as well as source/sink relationships. Similarly, by providing nectar resources of flowering plants next to crops (Géneau *et al.*, 2012), parasitoids are affected via landscape complementation, source/sink relationships and neighbourhood effects. Dunning *et al.* (1992) concluded that an understanding of the inter-relatedness of the parts is required to predict the changes in population dynamics or community structure when there is a change in landscape structure. However, many functional mechanisms that might explain and predict how landscape features influence spatial distribution of insects via landscape-scale processes remain unexplored (Tscharrntke *et al.*, 2012), and progress is limited (Kremen, 2005).

The four landscape processes above assume temporally stable conditions and focus on the spatial interactions between habitat elements in insect landscapes. However, temporal changes in resource availability and insect population sizes are a feature of agricultural landscapes. Non-crop habitats are generally stable and crop habitats are generally unstable (Schellhorn *et al.*, 2014). This is due to crop establishment growth and harvest activities, as well as periodic agricultural inputs including pesticides,

herbicides, nutritional sprays and the alike. (Kennedy and Storer, 2000). The result of these processes is that natural enemies and their hosts are subjected to frequent potential mortality events. However, persistent populations have traits that enable them to exist in ephemeral and disturbance-prone habitats such as short life cycles and the ability to move to refuges outside the crop (Schellhorn *et al.*, 2014). Frequent disturbance may affect the abundance of natural enemies in crops, even when with suitable prey or host densities are present. In the case of the egg parasitoids of four phytophagous pentatomids bugs in soybean, Hirose *et al.* (1996) found that the host-specific egg parasitoids exhibited low levels of parasitism later in the season as measured by egg card parasitism. The highest parasitism was recorded by the generalist egg parasitoid, *Ooencyrtus nezarae*, which parasitised all four pentatomids, with 60 - 70% of egg masses parasitised. However, in the second half of the soybean season, ruling out competition effects, *O. nezarae* emigrated from the crop and dispersed to other habitats. Dispersal was measured by sticky traps, which showed that *O. nezarae* had a female-biased sex ratio in the first half of the season, but there was a male-biased sex ratio in the latter half of the season as the females had already dispersed. Hirose *et al.* (1996) concluded that *O. nezarae* exploited mobile hosts, but in terms of effective naturally occurring biological control, protecting the latter stages of crop development from pentatomids was problematic. *O. nezarae* hosts have a similar development time. If *O. nezarae* was to stay in the crop, it could not successfully colonise other habitats so this is possibly a strategy to exist in ephemeral habitats (Hirose *et al.*, 1996).

The composition, context and connectivity of a given landscape can differ for two parasitoid species, which then affects their respective efficacy at finding hosts (Hochberg and Ives, 2000). Insect communities and populations in ephemeral habitats depend on large-scale regional exchange between populations. There are several reviews that examine the role of biological control at the field and landscape scale (Gurr *et al.*, 2003; Roschewitz *et al.*, 2005; Tschamntke *et al.*, 2007b). Given the

highly dynamic nature of agricultural landscapes, Schellhorn et al. (2014) proposed that the functional categorisation of agricultural landscapes for supporting natural enemies should integrate the spatial and temporal heterogeneity of the landscape, and explicitly account for disturbance regimes and their variations. Furthermore, Schellhorn et al. (2008c) highlighted the limited knowledge of natural enemies in Australia and stressed the importance of future research to include (1) identifying habitats that act as sources of natural enemies and their pests; (2) understanding dispersal from habitat sources into crops, and (3) acquiring quantitative knowledge of the tipping points and spatial scales at which the key processes operate.

1.3.4 Estimating parasitoid conservation and biological control potential using egg card percentage parasitism

Differences in life-history strategy in natural enemies, in particular predators and parasitoids, influence their control potential. Parasitoids (Hymenoptera and Diptera) are important biological control agents of agricultural pests and differ from predators by utilising just a single prey individual during their development. An assessment of parasitoids usually includes the time taken to host emergence and parasitism rate (i.e. the percentage of the prey population parasitised by the parasitoid). The three main methods of estimating parasitoid impact in the field includes collecting and rearing (Scholz, 1990; Davies, 2006; Furlong *et al.*, 2008), molecular identification (Furlong, 2015) and exposure of sentinel egg hosts to egg parasitoids (Scholz and Parker, 2004; Carr *et al.*, 2009). The latter method is particularly useful where there are limited host numbers in the field, enabling parasitoid activity to be monitored all year round regardless of host density. Another advantage is that sentinel egg host parasitism can be compared across different crop types across time and multiple habitats in agricultural landscapes in a standardised way (Thomson and Hoffmann, 2013). However, percentage parasitism alone does not necessarily reflect impact on pest population growth. Additional

factors incorporating immigration, emigration, reproduction rates and other mortality factors need to be considered (Macfadyen *et al.*, 2015).

1.3.5 Host selection by parasitoids in agricultural landscapes

There are four spatial scales at which parasitoids navigate: the scale of the landscape, plant community level, individual plant and individual plant parts. Salt (1935) defined this in parasitoids as the separation between ecological (searching) and physiological (acceptance) mechanisms. Following eclosion (emergence), a female parasitoid wasp faces a filtering hierarchy (Keller 1999) of locating habitats, locating hosts and accepting hosts for successful parasitism (Doutt 1964; Flanders 1953; Vinson & Iwantsch 1980). This is in addition to the complexities of biotic and physical factors that affect foraging cues.

The innate host-finding attributes of parasitoids are well developed and many parasitoids may be considered habitat specialists rather than host specialists (Barbosa & Benrey 1998). Some parasitoids are attracted to volatiles exuded by the food plants of their host (Altieri *et al.*, 1981a; Nordlund *et al.*, 1985b; Vet and Dicke, 1992) or detect the presence of plant chemicals in the frass of their hosts (Nordlund *et al.*, 1981; Quickie, 1997). Loon *et al.* (2000) found the first empirical evidence that attracting parasitoids benefits the reproductive success of plants. Parasitoids use plant-derived stimuli as cues to locate both host habitats and hosts (Vinson, 1980). Vet and Dicke (1992) argued that plant-derived stimuli are more influential as they are available in larger quantities and emitted over larger distances than host egg cues, but are not infallible indicators of host presence. Several studies have described differential responses to plant-derived stimuli where parasitism may occur on some plants but not on others of the same species, or at different heights on the same plant (Martin *et al.*, 1981; Vet *et al.*, 1990; Kester and Barbosa, 1991; Loon *et al.*, 2000). For egg parasitoids, host feeding does not necessarily indicate the presence of host eggs (Vet and Dicke, 1992) but more recent studies have shown that plant volatiles induced by egg deposition attract egg parasitoids (Colazza *et al.*, 2004;

Hilker and Meiners, 2006). In addition, odours from the mating of adult hosts, larval frass and host egg kairomones are indicators for foraging female *Trichogramma*. Boo and Yang (2000) found that scale and egg odours from *Helicoverpa assulta* and *Ostrinia furnacalis* attract *T. chilonis* and that male extracts of the moths increase parasitism rates when sprayed on host eggs. Similarly, Reddy et al. (2002) found that *T. chilonis* has a positive association with the sex pheromone and larval frass volatiles from *Plutella xylostella* in addition to cabbage leaf volatiles. Ahmad et al. (1996) demonstrated when releasing *T. chilonis* in the field that the addition of host pheromones increased parasitism rates of the pink bollworm (*Pectinophora gossypiella*) but not of the spotted bollworm (*P. scutigera*).

Host foraging by adult female *Trichogramma* is influenced by the quality of the host that the parasitoid develops in (Keasar et al., 2001) as well as by the position of the host egg on the plant (Wang et al., 1997). Host egg size, age and availability are also important factors in parasitism efficiency by *Trichogramma*. A larger egg (depending on host species) allows *Trichogramma* to lay more of its own eggs into it. Grenier et al. (2001) found that *T. evanescens*, *T. pretiosum* and *T. exiguum* laid three to four eggs in large host species' eggs, but only one in smaller host eggs. The emerging parasitoids were of similar size independent of host egg size. *Trichogramma* adult life span may also be affected by the eggs they develop in. Nogueira and Parra (1994) found that *T. pretiosum* reared in *Helicoverpa zea* (its natural host) and *Ephestia kuehniella* (factitious host, i.e. a host other than the target host) had similar development times and early parasitism efficiency after emergence but the longevity of *T. pretiosum* emerging from *E. kuehniella* was greater than from *H. zea*. The raising of *Trichogramma* in factitious hosts, however, does not necessarily change their preference for host eggs. Takada et al. (2001) found that *T. dendrolimi* raised on 12 generations of *E. kuehniella* still preferred its original and larger host, *Mamestra brassicae*. Host egg age is important for egg selection, and female *Trichogramma* generally prefer freshly laid eggs that are less than 24-hours old (Scholz, 2003). Monje and Ohnesorge (1997) and

Calvin et al. (1997) found that *T. pretiosum*, given a choice, preferred young eggs to older ones.

The role of infochemical use by egg parasitoids has been reviewed by Fatouros et al. (2008). Host location behaviour is important information for the targeted application of parasitoids in field crops, where the mere presence of host eggs is not enough to guarantee successful biological control. Fatouros et al. (2008) recommended in their review that infochemical-exploiting strategies used by foraging egg parasitoids are best studied by examining plant-host-parasitoid interactions at different scales in the field since parasitoids can act differently in the laboratory.

1.3.6 *Trichogramma* biology and identification

Trichogramma is a genus of egg endoparasites in the superfamily Chalcidoidea (Hymenoptera: Trichogrammatidae). They prevent the larval hatch of herbivorous insect hosts, so the herbivore no longer causes host plant damage. Typically, they attack a host life stage that is immobile, and are thus termed idiobiont parasitoids. For this reason, they have become the most widely utilised natural enemies in biological control programs (Smith, 1996), despite their small size ranging from 0.2 to 1.5 mm (Pinto and Stouthamer, 1994). A recent review of *Trichogramma* biology can be found in Consoli et al. (2010). There are approximately 210 *Trichogramma* species worldwide and they are one of 80 genera in the Trichogrammatidae.

Trichogramma species occur naturally in almost every terrestrial habitat (Almeida, 2000), and attack a wide range of pests of field crops, forests and horticultural crops. They are among the most studied parasitoids worldwide (Stinner, 1977).

Trichogramma species have been recorded parasitising eggs of over 400 species belonging to at least seven insect orders and 44 families (Smith, 1996). Silva and Stouthamer (1999) highlighted that all *Trichogramma* species are capable of parasitising more than one host species. Many *Trichogramma* are polyphagous (Pinto and Stouthamer, 1994), attacking hosts indiscriminately (Fye and Larsen, 1969).

Pinto (2006) undertook a review of the New World genera of Trichogrammatidae. The minute size, fragility and logistical problems of collection and curation of specimens has meant that taxonomy is often difficult (Pinto, 2006), in addition to their uniformity, causing difficulty in identifying separate species (Nagarkatti and Nagaraja, 1977). The main collection techniques of chalcid wasps has been detailed by Noyes (1982), and includes sweeping, suction sampling, beating, pyrethrum spray, rearing, Malaise traps, yellow pan traps, suction traps, pitfall traps and extraction from leaf litter or grass tussocks. Ideally parasitised eggs are collected from host plants where they are naturally laid or from sentient egg cards if host eggs can be laboratory cultured. Specimens then need to be slide-mounted and viewed with a light microscope up to at least 600x magnification (Pinto, 2006). Traditionally, morphological features of the male genitalia and antennal structures are used for identification (Nagarkatti and Nagaraja, 1977). However, phenotypic plasticity in morphology (Pinto *et al.*, 1989b) and reproductive compatibility (Pinto *et al.*, 1991) as well as cryptic species (Pinto *et al.*, 1986) and interspecific hybridisation (Pintureau, 1990) cause difficulties in accurate species identification.

The development of molecular techniques for the identification of *Trichogramma* has provided a viable alternative to time-consuming and expensive morphological approaches. However, early approaches to molecular-processing techniques were equally as expensive and time-consuming (Pinto *et al.*, 2003). Some DNA-based studies have involved simple sequence repeats (SSR) to differentiate between species (Pizzol *et al.*, 2005; Fu *et al.*, 2006), but polymerase chain reaction (PCR) is more widely used. The main advantage of PCR over morphology-based identification is that individual specimens can be identified quickly and cheaply by non-specialists (Stouthamer *et al.*, 1999). Once a *Trichogramma* specimen is identified, further investigation is needed to determine host preference, which may be influenced by species strain or environmental factors that are difficult to assess in the field (Ashley *et al.*, 1974). Similarly, the uncertainty of the host range in the field can also make it

hard to determine if *Trichogramma* species are generalists or specialists (Fatouros *et al.*, 2008).

In Australia, native egg parasitoids of *Helicoverpa* spp. belong to two hymenopteran families, Trichogrammatidae and Platygasteridae (Scholz, 1990; Masner, 1993). The Platygasteridae are represented by *Telenomus* spp., which are rarely identified to species level and about which there is little documented. There are ten identified native *Trichogramma* species recorded in Australian cotton-growing areas, and another two native species of uncertain status (*Trichogramma* spp.) and an introduced American species, *T. pretiosum* (Fig. 1.1).

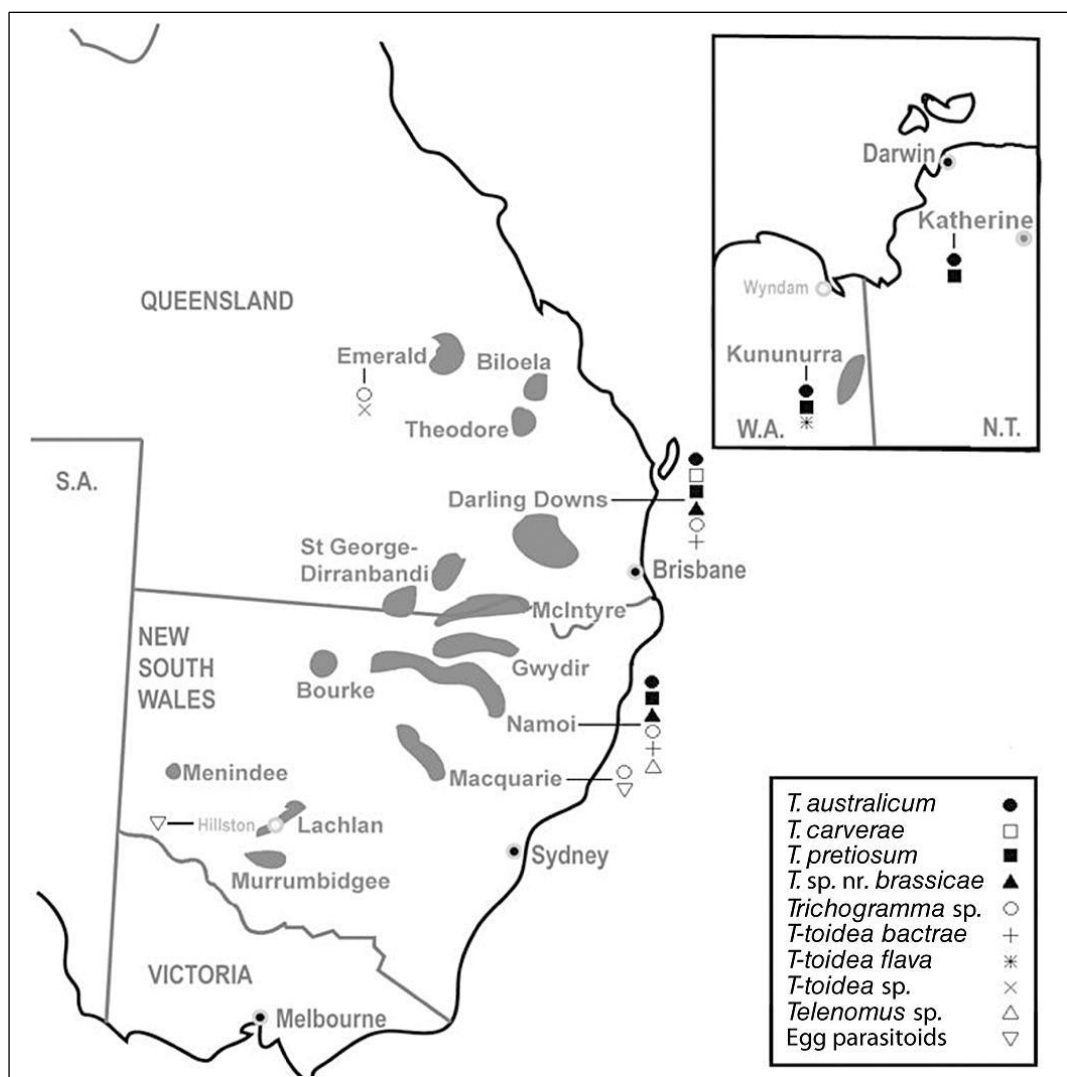


Figure 1.1: Distribution map of egg parasitoids recorded in cotton-cropping regions in Australia (adapted from Davies *et al.* 2011)

1.3.7 Factors affecting biological control using *Trichogramma*

Most of the ecological research on *Trichogramma* relates to their use as biological control agents with a focus on their augmentation for the control of insect pests in agroecosystems (Li, 1994; Smith, 1996). In 1926, Flanders (1927) successfully reared *Trichogramma* on a factitious host (*Sitotroga cerealella*), which enabled greater efficiency and ease of multiplication for biological control. Now *Trichogramma* species are used in over 30 countries to control pests in horticulture, field crops and forestry (Li, 1994). The development of biofactories for mass production has been taken up particularly in Russia and China, where 27.6 million ha and 2.1 million ha, respectively, of agricultural crops were treated in the 1990s (Li, 1994). Although there is great difficulty in estimating world production of *Trichogramma* and various authors differ in their estimates, Smith (1996) estimated approximately 32 million ha of crops have *Trichogramma* wasps applied as biocontrol agents.

Early attempts at using *Trichogramma* in applied biological control programs were not successful as multidisciplinary aspects of biocontrol were not observed (Parra, 2009). Parra and Zucchi (2004) highlighted seven areas where lack of knowledge or misevaluation limited the effectiveness of *Trichogramma* since the 1930s, including: (1) unsuitable species for host; (2) not evaluating egg density of the target pest or availability of host eggs; (3) lack of quality control in rearing *Trichogramma* species; (4) the habitat and plant specificity of *Trichogramma*; (5) competition with other biocontrol agents; (6) effects of insecticide use, and (7) insufficient numbers of parasitoids released or the method of release. Failure in one or many of these seven areas has resulted in the efficiency of *Trichogramma* being discredited (Parra *et al.*, 1988). However, improvements have led to a resurgence of research activity in the last 30 years targeting these problem areas.

The suitability of *Trichogramma* species for control of a host pest varies with local conditions. Li (1994) found, for example, that the effectiveness of *T. evanescens* in

controlling *Pieris* spp. was good in western provinces in China, but no *Trichogramma* species were able to parasitise *Pieris* spp. in the eastern province of Guangzhou.

Knowledge of the target pest egg density per square metre can help evaluate if there are enough eggs present to parasitise and the rate at which the parasitoid should be released. Parasitoids that respond to hosts in a density-dependent manner have the capacity to quickly suppress pest populations below economic thresholds (Paull *et al.*, 2014). A suitable egg parasitoid must also be able to find their host at low densities. Even with high host abundance, poor parasitism may be observed because of possible host discrimination influences. For instance, if a preferred host is abundant and available elsewhere, the egg parasitoids may ignore abundant non-preferred pest species or, conversely, parasitise low egg densities at a high rate where the host egg is favoured (Davies *et al.*, 2011b). Host foraging by adult female *Trichogramma* is influenced by the quality of the host that the parasitoid developed in (Keasar *et al.*, 2001), as well as by the position of the host egg on the crop plant (Wang *et al.*, 1997). Release quality control involves rearing *Trichogramma* on either the natural host, factitious hosts or *in vitro* (on artificial diets). The International Organization for Biological Control (IOBC) has developed standard methods to assess sex ratio, number of females released per hectare, longevity, fecundity and capacity to parasitise the natural host (Hassan and Zhang, 2001). A general review of quality-control issues can be found in Parra (2009) and Hassan (1993).

The habitat and plant specificity of different *Trichogramma* species was reviewed by Romeis *et al.* (2005a), addressing the increasing evidence of the strong impact of the latter on parasitism efficiency. Habitat affects parasitism rates, which may vary among the different food plants that parasitoids are collected from (Pinto and Oatman, 1998). Host species can vary widely depending on the host plants where eggs are found (Kuhar *et al.*, 2004; Gingras *et al.*, 2008b; Feng *et al.*, 2015). Similarly, habitat complexity influences parasitism levels (Gols *et al.*, 2005) as do the physical and chemical characteristics of the plant (Andow and Prokrym, 1990; Süverkrupp,

1997; Romeis *et al.*, 1998a). The prevalence of *Trichogramma* in certain habitats, and the effects of plant surface area, plant density and the structural complexity of the habitat are all important factors in augmentative releases (Romeis *et al.*, 2005a).

Competition effects include predation by other biological control agents. Parasitised host eggs take longer to develop than unparasitised eggs and so they are exposed to a wide range of predators (Scholz, 2003; Davies *et al.*, 2011b). Additionally, superparasitism where individuals of the one primary parasitoid species attack the same primary host individual influences female fitness and subsequent progeny (Duval *et al.*, 2018). Intraguild predation (IGP) has the capacity to negatively affect parasitoid abundance and affect augmentative releases (Traugott and Symondson, 2008). Chailleux *et al.* (2013) found that *Trichogramma* was affected by the mirid predator, *Macrolophus pygmaeus*, in small laboratory cages due to the probing of freshly parasitised eggs, but this decreased when predators and parasitoids were not confined with parasitised eggs to small arenas. Chailleux *et al.* (2013) concluded that *Trichogramma* parasitoids may significantly increase the level of control of the pest in the field over that attained by *Macrolophus pygmaeus* alone. Naranjo (2007) examined field life-table data of generalist parasitoids and found that IGP has only small negative effects on total generational mortalities. Overall, host impact via IGP is less relevant for egg parasitoids as the host is killed after parasitism, and the impact on the host will not change if the egg is subsequently attacked by a hyperparasitoid or predator (Sarah Mansfield, pers. comm.).

The impact of chemicals on *Trichogramma* varies widely depending on the individual species and the development stage of the parasitoid. Bueno *et al.* (2008) found that some fungicides and herbicides increased *T. pretiosum* mortality and in some cases had similar toxicity as broad-spectrum insecticides. Potrich *et al.* (2017) found that entomopathogenic fungus *metarhizium anisopliae* in a free-choice test was repellent to *Trichogramma pretiosum*. However, in a no-choice test fungal application before and after parasitism had no effect on the number of eggs parasitised by *T. pretiosum* or

the sex ratio of emerging adults. Carmo et al. (2010) found that in another egg parasitoid, *Telenomus remus*, herbicides were harmless on the first day of parasitism, but caused various levels of reduction of *T. remus* parasitism on the second day. Bull and Coleman (1985) found differences in chemical effects on individual *Trichogramma* species based on the host eggs that they emerged from. Most chemical insecticides are harmful to both developing and immature and adult *Trichogramma* (Llewellyn, 2000; Scholz and Zalucki, 2000; Davies, 2006; Consoli et al., 2010). Addressing the impact of chemicals on *Trichogramma* is critical to developing an appropriate IPM strategy employing multiple tactics for control of pest insects. Some of the discrepancies in the effects of chemicals on *Trichogramma* appear to be due to methodological inconsistencies with insecticide bioassays (Davies, 2006), and differences between laboratory and field studies (Bastos et al., 2010). For example, sunlight breaks down chemicals in the field, but this may not be able to be replicated in the laboratory in the same way. Laboratory experiments should be the pre-cursor to field trials that more accurately reflect the direct and indirect impact on *Trichogramma*. Several studies have shown that broad-spectrum insecticides are most toxic to *Trichogramma* (Hassan et al., 1987; Fitt, 1994; Hewa-Kapuge et al., 2003; Bastos et al., 2006a; Wang et al., 2014; Khan and Ruberson, 2017) and that selective insecticides are less disruptive (Wright and Verkerk, 1995; Scholz and Zalucki, 2000). The selective use of targeted applications of chemicals can be incorporated into biological control programs.

1.3.8 Conservation biological control and augmentation of Trichogramma

Biological control involves the management of biological control agents to reduce pest populations and their effects. There are two main strategies that are interrelated: conservation of indigenous populations (conservation biological control) and augmentation, which is the release of biological control agents in selected areas where populations are sparse. Nordlund (1996) suggested that 'conservation' includes the action taken to protect or maintain existing populations of biological

control agents. Conservation of naturally occurring *Trichogramma* has many advantages in that parasitoids do not have to be purchased or applied (Scholz, 2000). This can include the use of alternate crops or hosts as breeding sources of *Trichogramma* (Díaz *et al.*, 2012), cultural practices (Schellhorn *et al.*, 2000a), feeding supplements (McDougall and Mills, 1997b; Wäckers, 2003), behavioural semiochemicals (Ashley and Gonzalez, 1974; Mensah *et al.*, 2014) and the release of supplemental hosts at times of low natural host activity (Parker and Pinnell, 1972; Linkous, 2013). The release of natural enemies assumes that their population is insufficient to provide optimal biological control, and thus can be increased to control pests via augmentation.

There are two alternative strategies for population augmentation, inundation and inoculation. O'Neil *et al.* (1998) termed these as corrective or preventative augmentation, respectively. Inundation involves releasing mass natural enemies to have an immediate effect on the pest population, which is already at near-damaging levels, and is achieved exclusively by the released parasitoid themselves (Sherif, 1993). Inoculative release is the expectation that the released organism will control the target after multiplication (Eilenberg *et al.*, 2001). *Trichogramma* is commonly referred to as an inundative biological control agent, an approach which historically has been evaluated as a stand-alone tactic to achieve high egg mortality immediately following release (Scholz, 2000). For some insects such as heliothine pests, this method of control has proved mostly ineffective (King *et al.*, 1985; Suh *et al.*, 2000b; Scholz and Parker, 2004). Augmentative release of several *Trichogramma* species has occurred in Australian crops to control *Helicoverpa* spp. with limited success (Davies, 2006). *Trichogramma* is best suited to inundative releases when the host has few ovipositional flights per season and lays its eggs in masses (Scholz, 2000). However *Helicoverpa* species are multivoltine, lay their eggs singly (Fitt, 1989) and detection in pheromone catches is unreliable (Gregg and Wilson, 1991). These factors make inundation tactics difficult because they affect *Trichogramma* release times. However

Scholz (2000) argued that *Trichogramma* is best thought of as part of an IPM program that increases the level of egg parasitism above naturally occurring levels, which is much more achievable and may have sound economic benefits.

In broadacre crops such as cotton, the advent of *Bt* cottons expressing the Cry1ac endotoxin from *Bacillus thuringiensis* in 1996 led to significant reductions in insecticide use (Downes *et al.*, 2017). This is an important factor for conservation and augmentative control with *Trichogramma*, because heavy insecticide use prior to 1996 severely limited natural enemy effectiveness in cotton-growing areas (Davies *et al.*, 2011b). Like insecticidal chemicals, genetic modification faces recurring insect resistance requiring ever-increasing gene additions. This has brought IPM into more focus for such industries, and *Trichogramma* has become valuable in resistance management (Fitt and Wilson, 2005). Inoculative release has been shown in some horticultural crops to be successful in preventative pest control over a whole season (Li, 1994) although the utilisation of this technique in broadacre crops is limited. However, with inoculative releases, relatively few parasitoids are required early in the season, possibly independent of the ovipositional period of the pest (Smith, 1996), which is an advantage in the timing of *Trichogramma* releases for *Helicoverpa* control. Sherif (1993) argued that the effectiveness of low-level releases of *Trichogramma* is improved when the pest population is low and when the pest is just establishing in the field. Michaud (2018) considers inoculative control to be wholly more sustainable than other forms of augmentation and much better than inundation.

1.3.8 *Trichogramma* in Australian broadacre crops

In Australian cotton-growing areas, *Trichogramma* has had a significant impact on *Helicoverpa* parasitism (Davies *et al.*, 2011b). Despite the diversity of native egg parasitoid fauna, all inundative releases have been of *T. pretiosum* (Scholz, 1990). High levels of egg parasitism by indigenous species were recorded in Western Australia including parasitism levels of 60% in cotton and 90% in sorghum (Michael

and Woods, 1978). In the Ord River region of northern Australia, *T. pretiosum* which was introduced in the 1970s has now almost superseded the endemic *T. australicum* and *Trichogrammatoidea flava*. *Trichogramma pretiosum* has dispersed to areas beyond the Ord River Irrigation Area where it accounts for 90% egg mortality in conjunction with the insecticidal qualities of Bt cotton (Davies, 2006). Similarly on the Darling Downs in southern Queensland, the widespread introduction of *T. pretiosum* in the 1980s led to parasitism levels of *Helicoverpa* peaking at 90-100% in cotton and sorghum (Scholz, 2003). Furthermore, *T. pretiosum* parasitised more *Helicoverpa* eggs in this region than any other egg parasitoid. In northern NSW, fewer releases have occurred and natural egg parasitism levels have been recorded as 'low' in the Namoi Valley (Schellhorn *et al.*, 2000b). Schellhorn *et al.* (2000b) gave three possible explanations for the rarity of egg parasitoids in the Namoi Valley. Firstly, this region may be the southern limit of the geographical distribution of *Trichogramma* species based on their baiting results at Warren, NSW. However, the dominant introduced egg parasitoid, *T. pretiosum*, is Nearctic and specimens from the USA from where the parasitoid was imported were mixed with cultures from Quebec where flight initiation or parasitism at temperatures below 15°C may be adaptive, especially in early and late summer (Fournier and Boivin, 2000). Furthermore, *T. pretiosum* was released successfully in the Sydney basin and the central west of NSW (Llewellyn, 2000). The other two options suggested by Schellhorn *et al.* (2000b) were the poor availability of habitats during autumn and winter combined with broad-scale insecticide use. The latter two explanations warrant further consideration in the Namoi Valley.

The upper Namoi Valley includes the northern Liverpool Plains, which was thought to not have many egg parasitoids and about which little is documented in the literature. Like the mid and lower Namoi Valley, the Liverpool Plains has broadacre cropping including cotton. Cotton growing reaches its south-eastern climatic limit south of Caroonna due to low soil temperatures. However, smaller crop areas and

more diverse crop varieties occur on the Liverpool Plains, particularly in the Carroona to Quirindi districts, where maize, canary seed, soybeans, mungbeans and lucerne are more frequently grown and less insecticide is used compared to the mid and lower Namoi Valley (Mick Duncan, agronomist pers. comm.). Landscape habitat complexity in the form of diverse crops in autumn and winter gives more available habitat in the agricultural landscape for egg parasitoids to overwinter.

1.4 Research aims and objectives

This thesis aims to assess the presence of *Trichogramma* in northern NSW broad-acre field-crops, determine their preferred habitats and consider how the potential of *Trichogramma*, in integrated pest management (IPM) of *Helicoverpa* can be optimised in Liverpool Plains cropping systems.

The specific objectives of the thesis are to:

- 1) Review the substantial literature highlighting the success and failure of *Trichogramma* as the most widely used natural enemy worldwide in biological control programs and identify the past factors limiting *Trichogramma* effectiveness in IPM (Chapter 1)
- 2) Determine the abundance, diversity and seasonal phenology of *Trichogramma* and other egg parasitoids of *Helicoverpa* in crop and non-crop vegetation, including summer landscapes with different proportions of native vegetation. (Chapter 2)
- 3) Evaluate *Trichogramma* crop choice preferences, combinations of crops and plant parts to determine if there are differences in parasitism, (Chapter 3)
- 4) Assess the role of certain crops as nurseries for the population build-up of *Trichogramma*, and the potential for supplementing these populations to boost population abundance measuring two different release rates and movement from the release site (Chapter 4).

- 5) Measure the persistence and impact of *T. pretiosum* into the summer cropping season after inoculative releases in spring nursery crops in crop and non-crop habitats (Chapter 4).
- 6) Synthesise the results and present conclusions and recommendations to improve IPM in the region (Chapter 5).

1.5 Layout of thesis

Whilst *Trichogramma* wasps are the most widely used natural enemies worldwide, several questions need further investigation to determine the environmental constraints on *Trichogramma* survival in northern NSW. I present three research papers (Chapters 2 - 4) that firstly identify local *Trichogramma* populations and their habitats, then I explore techniques to maximise their potential in IPM in ways that are practical for growers to consider. A concluding chapter (Chapter 5) summarises findings from this thesis and provides management implications and recommendations for future research.

In Chapter 2, I firstly look at the Liverpool Plains landscape and determine the predominant crop and non-crop habitats. Whilst native vegetation habitats are relatively stable, the mosaic of crops changes across the landscape within and between years. Thus, I surveyed egg parasitoids in the predominant spring and summer crops and native vegetation over 2 years. To determine egg parasitoid population abundance I used water pan traps and determined impact by measuring *Helicoverpa* egg card parasitism. I then used the same methods to assess native vegetation as an overwintering habitat for local egg parasitoid populations. In summer, I identified two landscapes that have different proportions of native vegetation and determined if *Trichogramma* abundance and diversity differed between the two.

In Chapter 3, I changed spatial scales and looked more closely at the individual crop level. I evaluated if local *Trichogramma pretiosum* would parasitise eggs in each crop grown on the Liverpool Plains given the same number of *Helicoverpa* eggs under caged conditions. I assessed if crop mix affected parasitism rate and measured these differences on individual plant parts (stem, leaf and flowers).

In Chapter 4, I drew on the preceding experiments to increase the effectiveness of *Trichogramma* control of *Helicoverpa* on the Liverpool Plains. *Trichogramma* reached peak activity when most of the summer crops had already reached maturity and are at levels below those considered effective for economic control of *Helicoverpa*. One approach was to utilise crops as nurseries to boost spring populations *T. pretiosum*. Wheat is the primary crop in the landscape at this time of year and canola is consistently attacked by *Helicoverpa*. Thus, I determined if this technique can significantly reduce *Helicoverpa* populations at the egg stage in these crops, decreasing economic damage. Furthermore, I also investigated if the effects of supplementing *T. pretiosum* populations in spring could boost population numbers in summer crops earlier, giving sustained protection from *Helicoverpa* egg hatch and reduce the need for unnecessary chemical applications.

Chapter 5 presents a synthesis of Chapters 2–4 and draws conclusions regarding the potential to utilise *Trichogramma* and other egg parasitoids of *Helicoverpa* on the Liverpool Plains as part of an IPM program. Contributions of this research to current theoretical and practical knowledge are explained and limitations of the research are discussed. Recommendations for future research are provided.

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Chapter 2. Landscape ecology of *Trichogramma* and other egg parasitoids on the Liverpool Plains, northern New South Wales

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2.0 Abstract

The noctuid moth *Helicoverpa* is a major pest of several spring and summer crops in Australia, but little is known about native egg parasitoids of *Helicoverpa* in northern New South Wales cropping systems. We conducted a 3-year landscape-scale survey on the Liverpool Plains in northern NSW to determine the abundance of egg parasitoids and their seasonal phenology in crop and non-crop vegetation. In the first 2 years of the study, we compared two landscapes, Piallaway–Breeza and Caroon–Quirindi, which vary in the amount of native vegetation present. We assessed egg parasitoid species abundance using white and yellow water pan traps and determined their impact by measuring mean parasitism rate of *H. armigera* egg cards in various habitats. To determine variations in annual egg parasitoid populations, we surveyed spring crops (canola, chickpea, faba bean, linseed, lucerne, sunflower and wheat) and native vegetation (forest, agroforestry, grassland/native pasture). In summer, we surveyed crops (maize, cotton and sorghum) and forest. Using the same methods, we surveyed potential overwintering habitats in forest. We

identified *Trichogrammatoidea bactrae*, *Trichogramma pretiosum*, *T. australicum*, *Telenomus* species and unidentified species of Trichogrammatidae. In the third year, after inoculative release of *T. pretiosum* in wheat and canola in spring, we assessed changes in egg parasitoid assemblages in adjacent and more distinct summer habitats.

In spring 2006–07, linseed had the highest levels of mean (± 1 s.e.) egg card parasitism ($3.3 \pm 1.2\%$), followed by sunflowers and faba beans ($1.7 \pm 0.8\%$ each). In 2007–08, agroforestry and lucerne had the highest rate of egg card parasitism (0.034 ± 0.014 each). Native vegetation (forest and plains grass *Austrostipa aristiglumis*), linseed and faba bean were also preferred habitats. No egg parasitoids were found in chickpea in any year. Trichogrammatids and *Trichogrammatoidea bactrae* were the most abundant parasitoids in spring. Trichogrammatidae were more attracted to yellow traps than white, whilst *Telenomus* spp. were only attracted to white traps.

Egg parasitoids were similarly abundant in cotton, maize, sorghum and forest in each of the first two summers. *Trichogramma pretiosum* was the most abundant egg parasitoid in summer and preferred crops to forest. *Trichogramma australicum* and *Telenomus* spp. preferred forest and were less abundant than other egg parasitoids. In 2006–07, mean egg card parasitism peaked in late February in sorghum ($14 \pm 4\%$), followed by cotton ($13 \pm 4\%$), maize ($5 \pm 1\%$) and forest ($2 \pm 1\%$). In 2007–08, mean egg card parasitism peaked in late January and February in sorghum ($29 \pm 6\%$), cotton ($26 \pm 11\%$), maize ($23 \pm 6\%$) and forest ($7 \pm 2\%$). In 2009, two summer surveys in cotton, sorghum, forest and grassland habitats were conducted after inoculative release of *T. pretiosum* in spring. Average egg card parasitism was highest in sorghum ($47 \pm 14\%$) followed by cotton ($25 \pm 13\%$), forest ($13 \pm 7\%$) and grassland ($5 \pm 2\%$). *T. pretiosum* was the most abundant parasitoid after the spring releases and there was a slight reduction in *Trichogrammatoidea bactrae* in crop habitats compared to the previous summer. In winter, all egg parasitoids found in spring and summer habitats occurred in forest in low abundance.

Egg parasitoid abundance and species differed significantly between the Piallaway–Breeza and Caroon–Quirindi landscapes. There was a greater number of egg parasitoids in the Caroon–Quirindi landscape, which had a greater amount of native vegetation close to crops. There were differences in the species composition between the landscapes. In summer 2006–07 and 2007–08, the capture rate of egg parasitoids in the Caroon–Quirindi landscape was greater than in the Piallaway–Breeza landscape. Although there was no difference in egg card parasitism between landscapes in summer 2006–07, the effect of landscape was significant in 2007–08.

Overall, this study found that Trichogrammatids and *Trichogramma* are present on the Liverpool plains and they were also found to overwinter in native vegetation, which was previously not documented. The region with more non-crop vegetation had a richer and more stable populations of egg parasitoids. The results are an important baseline for increasing the abundance and effectiveness of egg parasitoids in IPM on the Liverpool Plains to enhance their effectiveness.

2.1 Introduction

Parasitic Hymenoptera are ubiquitous in most terrestrial environments with 31 000 species estimated to occur in Australia (Yeates *et al.*, 2003). These parasitoids play a significant role in regulating populations of many herbivorous insects including agricultural pests (La Salle and Gauld, 1993). It is estimated that each insect herbivore has approximately five species of insect parasitoid (Hawkins, 1993), and these may attack any stage of the host's lifecycle. These host–parasitoid relationships play a key role in conservation biological control in agricultural landscapes. In modern agricultural landscapes, the loss and fragmentation of semi-natural habitats have led to a reduction in species richness and abundance of parasitoids (Haenke *et al.*, 2009; Mangels, 2017), which in turn affects biological control. Structural simplification of the agricultural landscape may be unfavourable to certain parasitoid species that require non-crop resources (Menalled *et al.*, 1999b). In addition to prey, parasitoid survival depends on over-wintering habitats, suitable

microclimates, food for adults (such as nectar and pollen) and alternate hosts (Thies and Tscharntke, 1999b; Landis *et al.*, 2005). The spatial and temporal patterns in crop-associated resources such as adjacent unmanaged habitats can be critical to the survival of parasitoids (Barbosa, 1998). In like manner, the composition and connectivity of agricultural landscapes are relevant to a parasitoids efficacy and resulting levels of parasitism (Roland, 2000).

The noctuid moth, *Helicoverpa armigera*, is a major pest of modern agriculture in the Old and New World. *Helicoverpa* control and crop damage cause losses in excess of US\$5 billion each year across Asia, Europe, Africa, America and Australia (Pearce *et al.*, 2017). In Australia, native egg parasitoids of *Helicoverpa* spp. belong to two hymenopteran families, Trichogrammatidae and Platygasteridae (Masner, 1993; Scholz, 2000). *Trichogramma* (Hymenoptera: Trichogrammatidae) is a genus of idiobiont endoparasites of the eggs of other insects, principally Lepidoptera, Hemiptera, Coleoptera, Thysanoptera, Hymenoptera, Diptera and Neuroptera. They prevent the larval hatch of herbivorous insect hosts, reducing the amount of host plant damage. Furthermore, in species such as *T. pretiosum*, for each single *Helicoverpa* egg parasitised, one to three adult wasps emerge. For this reason, *Trichogramma* species have become the most widely utilised natural enemies in biological control programs (Smith, 1996). To maximise the biological control potential of this egg parasitoid, it is important to study native and naturalised *Trichogramma* species, their abundance and their ecology (Barnay *et al.*, 2001) and why natural *Trichogramma* populations only partially control *Helicoverpa*.

Little is known about *Trichogramma* in northern NSW cropping systems, despite inundative egg parasitoid releases using exotic *T. pretiosum* on the Darling Downs in southern Queensland in the late 1970s and 1980s. Vacuum sampling surveys by Stanley (1997) that compared overall predator and parasitoid abundance and diversity between 'soft' and conventionally managed cotton crops around Narrabri (149°46'E, 30°19'S) in northern NSW recovered 12 times as many trichogrammatids

on average in the former. Further surveys by Schellhorn et al. (2000b) suggested that egg parasitoids were relatively rare in Namoi Valley crops, as only 1–2% of 22 152 *Helicoverpa* eggs, either field-collected or placed out on sentinel egg cards, were parasitised in a survey of lucerne, lupin, pigeon pea, sorghum, cotton and *Echium* spp. The recovered egg parasitoid complex consisted of two unidentified *Trichogramma* spp. and one *Telenomus* sp. and, though rare, was considered ubiquitous across the lower Namoi Valley region (Schellhorn *et al.*, 2000b). More recently, Whitehouse et al. (2007) surveyed conventional and *Bt*-transgenic cotton crops across the mid–lower Namoi Valley and found 3% and 6%, respectively, of *Helicoverpa* eggs on sentinel cards parasitised by *Trichogramma* spp. Interestingly, higher egg parasitism rates (10%) were found further south at Pilliga (30°45'S, 149°00'E) (M. E. A. Whitehouse, unpubl. data 2007).

To understand the role of egg parasitoids in the upper Namoi Valley in northern NSW, this study aimed to discover what species occurred on the Liverpool Plains. To determine which habitats they occurred in, we used a landscape approach to identify major crop and non-crop habitats in two different but connected landscapes. We assessed species abundance (trap rate) using yellow and white water-pan traps, and measured impact by assessing the percentage parasitism of *H. armigera* egg cards. To assess the variations in annual populations we surveyed spring crops, summer crops and non-crop overwintering habitats over 2 years. Then after inoculative release of the introduced *T. pretiosum* in spring 2008 (see Chapter 4), we assessed changes in egg parasitoid assemblages in summer habitats in the third year of the study.

2.2 Methods

Egg parasitoid surveys were conducted between September 2006 and March 2009 over an area of 60 km between Gunnedah (150°15'E, 30°59'S) and Quirindi (150°41'E, 31°31'S) on the Liverpool Plains, northern NSW. An additional site at Boggabri (150°02'E, 30°42'S) was surveyed in July 2007 for the overwintering survey. The

main winter/spring and summer crops surveyed were wheat (*Triticum aestivum*), canola (*Brassica napus*), faba beans (*Vicia faba*), chickpeas (*Cicer arietinum*), lucerne (*Medicago sativa*), sorghum (*Sorghum bicolor*), cotton (*Gossypium hirsutum*), sunflowers (*Helianthus annuus*), maize (*Zea mays*) and linseed (*Linum usitatissimum*). The native grassland was predominantly plains grass (*Austrostipa aristiglumis*) along stock routes and was similar at all sites. The open forest was generally dominated by white box (*Eucalyptus albens*), with silver-leaved ironbark (*E. melanophloia*), white wood (*Atalaya hemiglauca*), bull oak (*Allocasuarina luehmannii*), white cypress pine (*Callitris glaucophylla*) and other *Eucalyptus* spp. with a grassy understorey.

The study area consisted of black fine-textured alluvial plains of two types. The landscapes were adjacent but separated by several kilometres of mostly native pasture and some native forest on less fertile cracking clay soils. The field survey locations for 2006–07 and 2007–08 were different each year due to crop rotations but were in a similar area or farm (Appendix 1). The Piallaway–Breeza landscape had few wooded areas, and where they did occur, trees were sparse (Appendices 2 and 3). The vegetation in this landscape was mainly grassland and herbaceous crops. The Caroon–Quirindi landscape had grassland and herbaceous crops but a greater extent of forest (Appendices 4 and 5). Crop and habitat types were determined by visiting each farm in the region and having landowners use coloured dots to represent planted crops and crops that were going to be planted in the upcoming season. This information was then transferred to a digital map. The location of sites was chosen based on crops and habitats at least 2 km apart, as well as ease of access to sites.

2.2.1 Collection methods

The water-pan traps (WPT) consisted of 21-cm diameter plastic dinner plates filled with water and a drop of detergent and left for 24 hours before collection in a container of 70% ethanol. Where there was excess water left on the plate, it was drained through a small piece of loosely woven polyester voile so that the small

micro-hymenoptera would not be lost and the voile was then placed in the container with ethanol. In the spring 2006 surveys, the water traps were placed on a tripod made of garden stakes just above crop height, secured by wire and left for 24 hours. This method was refined in subsequent surveys, with the traps placed on the ground in each habitat and secured by 3 bamboo skewers as this was a more efficient system. Insects have been shown to be more attracted to certain colours than others (Reza and Parween, 2006). Romeis et al. (1998b) conducted experiments with different-coloured sticky traps and found that yellow was best at attracting *Trichogramma*, although white attracted more males. White water funnels were used by Barney et al. (2001) to successfully capture native *Trichogramma* in an orchard. Because male *Trichogramma* are best for determining morphological identification via antennae (Pinto and Stouthamer, 1994), we used three white and three yellow plates. *Helicoverpa* sentinel egg cards were sourced from an *H. armigera* colony that was maintained in the agronomy laboratory at the University of New England. Pupae were sourced from the entomology laboratory at the Tamworth Agricultural Institute (NSW Department of Primary Industries). Pupae were placed in modified containers that were fitted with regular paper towelling around a cylinder. The method has been used in previous studies (Gregg and Wilson, 1991) to obtain *Helicoverpa* eggs on paper towelling. Clusters of fresh *Helicoverpa* eggs, 1–2-days-old, were cut out in approximately 1-cm shapes with at least 10 moth eggs and stapled to a yellow card, 5 x 8 cm. Thirty egg cards were used in each habitat in 2006–08, and 40 in the 2008–09 summer surveys. These were kept cool in an esky with ice bricks until placed in the field by pegging them to thin bamboo stakes to a height of up to 50 cm (determined by plant height) or stapled to the leaves when the plants were bigger. In grasslands and forest sites the egg cards were always at 50 cm on bamboo stakes. The cards were laid out in two rows approximately 20 m apart in the middle of the crop. Each card was placed approximately 5 m apart in the two rows with a buffer of 10 m from the edge of the habitat. Upon collection, 24 hours after deployment in the

crop, egg cards were stored carefully for transport to the laboratory where they were maintained at 25°C until eclosion. After 5 days, blackened eggs were considered parasitised. Each card was recorded as either parasitised or not parasitised and the numbers of eggs that were parasitised or not parasitised on each card was recorded. If moth eggs were missing (from predation or other factors), they were considered not parasitised.

Collection of lepidopteran eggs was conducted on 10 April 2007 in the forest sites with four scientists searching for eggs in native vegetation for 30 minutes. A further survey on 5 July was also conducted in the same habitats with two scientists searching for lepidopteran eggs for 30 minutes.

Bucket light traps described by Baker et al.(2011) were located at the NSW Department of Primary Industries research station at Breeza during the field surveys and later replaced by a smaller 12-V DC 8-W bucket with a black-light fluorescent tube purchased from Australian Entomological Supplies™. Inside the funnel, egg cartons soaked in 1,1,2,2-tetrachloroethane for quick subjugation of specimens were collected at dawn, placed in a container and kept in a freezer. *Helicoverpa* spp. were identified and other Lepidoptera assigned to morphospecies to distinguish species abundance.

2.2.2 Egg parasitoid identification

Egg parasitoids were identified using the morphological features of antennae, wings and male genitalia, with the latter requiring the slide mounting of specimens in Canada Balsam and viewed under 600× magnification (Nagarkatti and Nagaraja, 1977; Pinto *et al.*, 1989b; Pinto and Stouthamer, 1994). Most specimens were not slide-mounted for identification due to the time required for preparation, but a subsample was mounted to confirm species. The wings, antennae and identifying features of Trichogrammatidae (size 0.3–1.2 mm, funicle with not more than two segments, tarsi three-segmented, colour not metallic and often yellow-brown with red eyes) were confirmed, and where possible further identification to species was

made. If further species identification was not certain, as only male characteristics are diagnostic for *Trichogramma* species (Pinto *et al.*, 1989a), they were simply referred to as Trichogrammatidae. Identified species were *Trichogrammatoidea bactrae*, *Trichogramma pretiosum* and *T. australicum*. *Telenomus* species (Hymenoptera: Platygasteridae) were identified using the morphological features of the antennae, thorax and wings (Masner, 1993): they characteristically had black eyes and body.

2.2.3 Spring crop field surveys for egg parasitoids

In 2006, two field surveys were conducted on 29–30 September and 18–19 October using 30 sentinel egg cards, three yellow water-pan traps (WPT) and three white WPT per site across 19 farms. The dry conditions limited the number of crops that were grown in the area at the time. However, five forest sites, two sites each of lucerne, chickpea, plains grass (grassland), faba bean and wheat, and one site each of canola, agroforestry, linseed and sunflower were surveyed.

In 2007, two surveys were conducted on the 8–9 October and 30–31 October using 30 sentinel egg cards, three yellow WPT and three white WPT per site across 19 farms. These comprised five forested sites, two sites each of: linseed, lucerne, plains grass, wheat, faba beans, chickpeas and one site each of sunflowers and agroforestry. There was no canola in the study area in the 2007 season.

In spring 2006 and 2007, egg parasitoid abundance was calculated per WPT for each habitat and pooled for each year. To compare the effect of trap colour (white or yellow) on the number of individuals of each species caught, a chi-square (χ^2) test was used, with expected values assuming equal likelihood of capture in white and yellow traps. Where species numbers were small, Fisher's exact test was used to determine significance. In spring 2006 and 2007, the percentage parasitism of *Helicoverpa armigera* sentinel egg cards was used to compare egg parasitoid activity between habitats. Percentage parasitism was calculated by dividing the number of parasitised cards by the number of cards deployed and multiplying by 100. Similarly

trap capture rate of water pan traps was measured by dividing the number of traps containing egg parasitoids by the number of traps set.

2.2.4 Agricultural landscape structure effects in summer egg parasitoid habitats

In summer 2006–07 and 2007–08, habitats in the Piallaway–Breeza (Appendices 2 and 3) and Caroona–Quirindi (Appendices 4 and 5) landscapes on the Liverpool Plains were compared. Three crops each of summer crops (cotton, maize and sorghum) and three forested sites in the Piallaway–Breeza landscape were compared with two crops each (cotton, maize and sorghum) and two forest sites in the Caroona–Quirindi landscape. The uneven distribution of habitats among landscapes was the result of there only being two well-separated cotton habitats in the Caroona–Quirindi landscape. These sites were surveyed every 3 weeks on six survey dates from 25 November 2006 to 14 March 2007 and from 23 November 2007 to 1 March 2008. The total number of egg parasitoids per trap per site was measured on each survey date each year in each landscape. A two-way analysis of variance (ANOVA) with replication (i.e. the six survey dates) in Excel® was used to compare the effect of landscape and habitat on trap rate of egg parasitoids. Two-way ANOVA with replication (i.e. date) was also used to compare the effect of landscape and taxon on trap rate.

2.2.5 Summer survey after a spring *T. pretiosum* inoculative release

Three months after spring releases of *T. pretiosum* in canola and wheat in 2008 (Chapter 4), neighbouring crops of either sorghum (five sites) or cotton (five sites) were monitored for egg parasitoid abundance using *Helicoverpa* sentinel egg cards and WPT in the same way as egg parasitoid activity had been monitored in previous summers. An additional two sites for each crop were monitored approximately 3–5 km from release sites. Forty egg cards were deployed in each crop on two sampling dates, 23–24 January and 25–26 February 2009. Mean trap rate of egg parasitoids and egg card parasitism per habitat was calculated to compare with past years.

2.2.6 Overwintering survey of egg parasitoids

On 27–28 July 2007 and 2–4 July and 22–23 July 2008, six forest sites were surveyed for egg parasitoids. In addition, a grassland site with some trees was surveyed at Boggabri in 2007, and an agroforestry site at Breeza in 2008. In 2007 and 2008, 30 and 40 *Helicoverpa* sentinel egg cards, respectively, were deployed at each forest site. In addition, three yellow WPT and three white WPT were deployed per site to determine the presence of egg parasitoids.

2.3 Results

2.3.1 Spring surveys of egg parasitoids using WPT

The first survey for egg parasitoids on 28–30 September 2006 yielded 18 specimens from 114 WPT. Most were identified as Trichogrammatidae (11 specimens) whilst *Trichogrammatoidea bactrae* (four specimens) was collected in linseed, lucerne and forest. *Telenomus* spp. (three specimens) were restricted to forest and grassland. In the second spring survey on 18–19 October 2006, there was a slight increase in egg parasitoid captures (23) in WPT. Ten specimens were trichogrammatids, whilst nine specimens of *Trichogrammatoidea bactrae* were identified in faba bean (2), linseed (1), lucerne (1), grassland (1) and forest (4). A single *Trichogramma pretiosum* specimen was found in agroforestry and five *Telenomus* spp. were found in the native vegetation (forest and grassland) sites. No egg parasitoids were found in chickpea in either survey. Trichogrammatidae were the most frequently caught egg parasitoid (Fig. 2.1) over both survey dates. Trichogrammatidae were more attracted to yellow WPT than white (chi-square statistic = 3.857, $P < 0.05$) whilst the few *Telenomus* spp. were only caught in white traps (Fisher's exact test, $P = 0.077$).

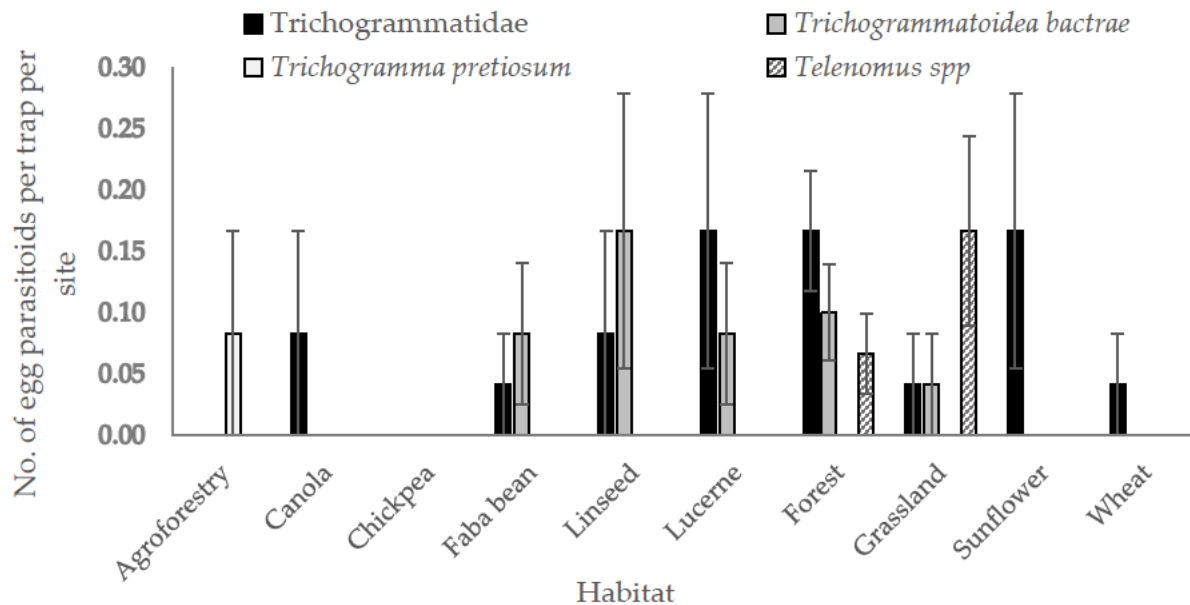


Figure 2.1 Mean trap rate (± 1 s.e.) of egg parasitoids in various habitats on the Liverpool Plains in spring 2006, pooled over yellow and white water-pan traps and two dates (28–30 Sep and 18–19 Oct).

The first survey for egg parasitoids on 8–9 October 2007 yielded 24 specimens from 114 WPT. *Trichogrammatoidea bactrae* was most frequently identified (11 specimens) occurring in every habitat except for chickpea and agroforestry. Trichogrammatids (eight specimens) were found in most habitats except for chickpea, sunflower and wheat. *Telenomus* spp. were restricted to native vegetation (3 specimens), one specimen of *T. australicum* was found in forest, and one specimen of *T. pretiosum* in agroforestry.

In the second spring survey on the 30–31 October 2007, 31 specimens were caught in WPT. Most were identified as trichogrammatids (15), occurring in all habitats except for chickpea, linseed and grassland. *Trichogrammatoidea bactrae* (8 specimens) was identified in faba bean, linseed, lucerne and forest (2 specimens per habitat). *Telenomus* spp. (5 specimens) were found in native vegetation. *Trichogramma pretiosum* (2 specimens) were found in sunflowers and agroforestry (1 each). A single *T. australicum* specimen was found in grassland. No egg parasitoids were found in chickpea on either survey date. Trichogrammatidae were the most frequently caught egg parasitoid over both survey dates (Fig. 2.2). Unlike spring 2006, WPT colour was

not significant in determining Trichogrammatidae catch ($\chi^2 = 0.391$, NS).

Trichogrammatoidea bactrae was attracted significantly more to yellow traps than white (Fisher's exact test, $P < 0.05$), while *Trichogramma australicum* and *T. pretiosum* were too rare for a colour preference to be detected. However, *Telenomus* spp. were only attracted to white plates (Fisher's exact test, $P = 0.038$), as in spring 2006.

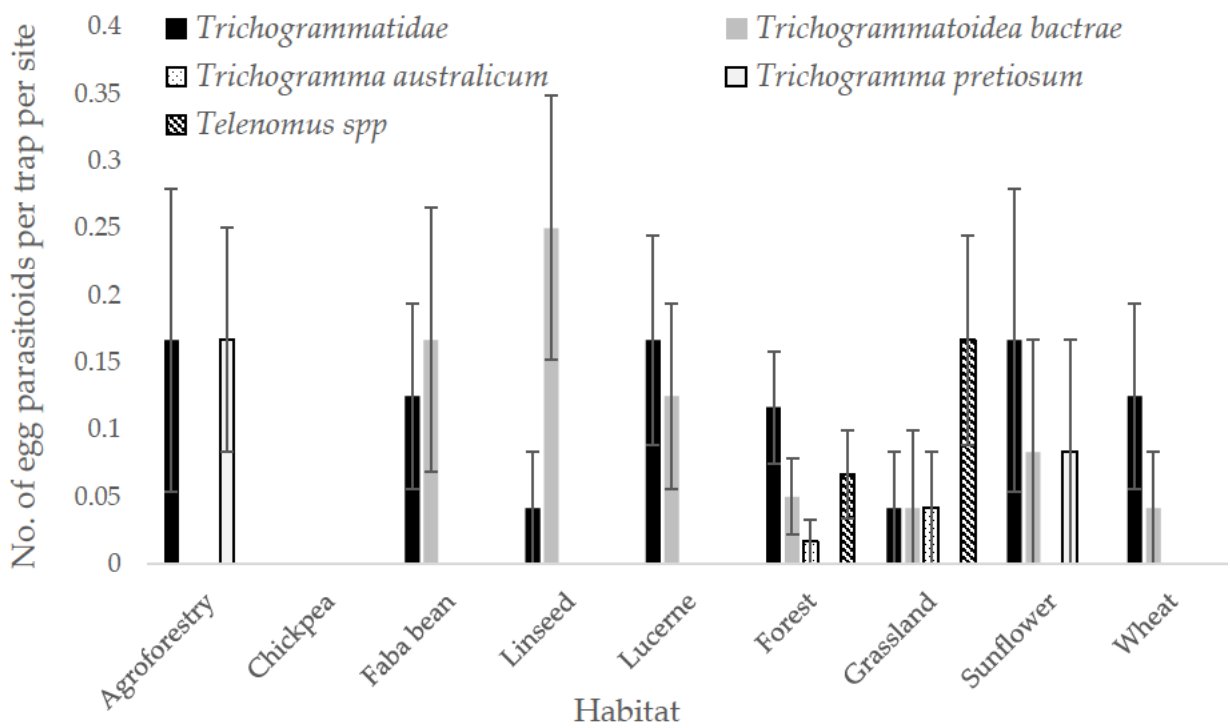


Figure 2.2: Mean trap rate (± 1 s.e.) of egg parasitoids in various habitats on the Liverpool Plains in spring 2007, pooled over yellow and white WPT and two dates (8–9 Oct and 30–31 Oct).

2.3.2 Spring surveys of egg parasitoids using sentinel egg cards

In spring 2006, only six of the 1140 egg cards deployed were parasitised. The field survey on 28–30 September had one parasitised egg card each in linseed, faba bean and forest. In the second spring survey on 18–19 October, one parasitised egg card each was found in linseed, sunflower and forest. *Trichogrammatoidea bactrae* emerged from the parasitised eggs in the forest habitat (one specimen) and trichogrammatids (one each) emerged in the crop habitats.

In spring 2007, 15 of the 1140 egg cards deployed were parasitised. All habitats had at least one parasitised egg card except for chickpea. Two egg cards were parasitised but did not hatch. *Trichogrammatoidea bactrae* emerged from faba (two), lucerne

(three) and forest (two). Trichogrammatids were found in lucerne (one), forest (one), grassland (one), sunflower (one) and wheat (one). *Trichogramma pretiosum* emerged from an egg card in agroforestry (one) and lucerne (one) in the second field survey.

2.3.3 Light traps

On 28–30 September 2006, three *H. armigera* moths were captured in the light trap and 11 other moths were caught comprising six different morphospecies. On 18–19 October 2006, one *H. punctigera* and one *H. armigera* were caught, along with 23 other moths representing 10 morphospecies. On 8–9 October 2007, two *H. armigera* were captured in the light trap and another 16 moths were caught comprising eight morphospecies. On 30–31 October 2007, no *Helicoverpa* spp. were caught but 24 other moths of 13 morphospecies were caught.

2.3.4 Summer field surveys for egg parasitoids using WPT

In summer 2006–07, 241 egg parasitoids were caught in 720 (white and yellow) WPT. They were identified as trichogrammatids (106 specimens; Fig. 2.3), *Trichogrammatoidea bactrae* (33; Fig. 2.4), *Trichogramma australicum* (two specimens, one on 25 November 2006 and one on 23 February 2007), *T. pretiosum* (32; Fig. 2.5) and *Telenomus* spp. (8 specimens; Fig. 2.6). In the crop habitats, trap rate increased as the crops matured, but an increase toward the end of the summer was less clear in the forest (Fig. 2.7). Egg parasitoid taxon abundance was dependent on habitat type as the interaction between taxon and habitat was significant (ANOVA, $P = 0.004$; Fig. 2.8). Average trap rate combining all egg parasitoids in 2006–07 was >0.335 per trap per site, and trichogrammatids and *Trichogramma pretiosum* were more abundant than *Trichogrammatoidea bactrae* or *Trichogramma australicum*.

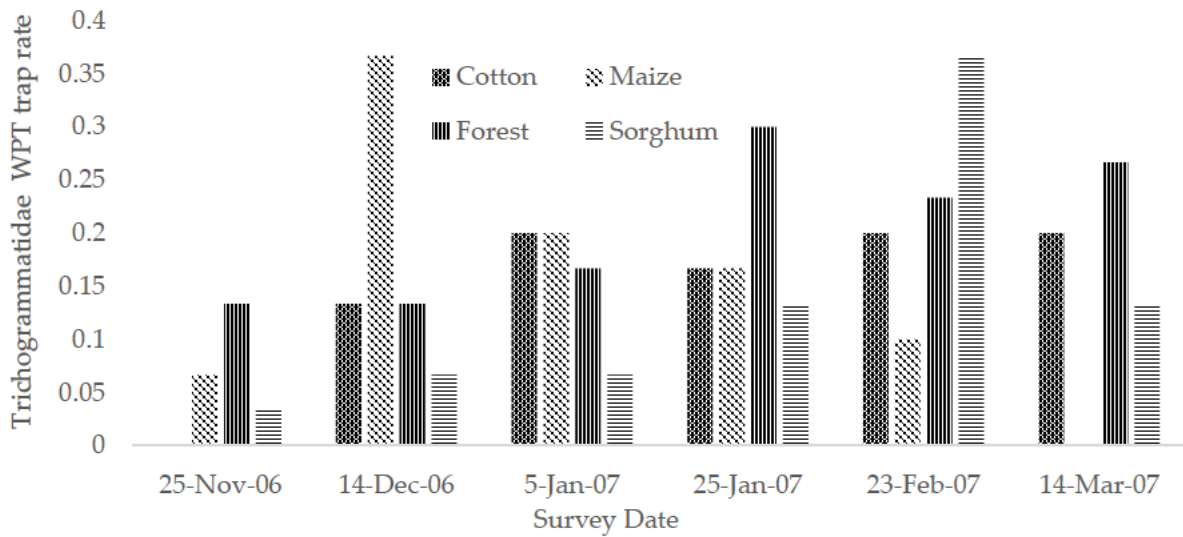


Figure 2.3: *Trichogrammatidae* trap rate in various habitats on the Liverpool Plains in summer 2006–07 over six survey dates (from 25 Nov 2006 to 14 Mar 2007).

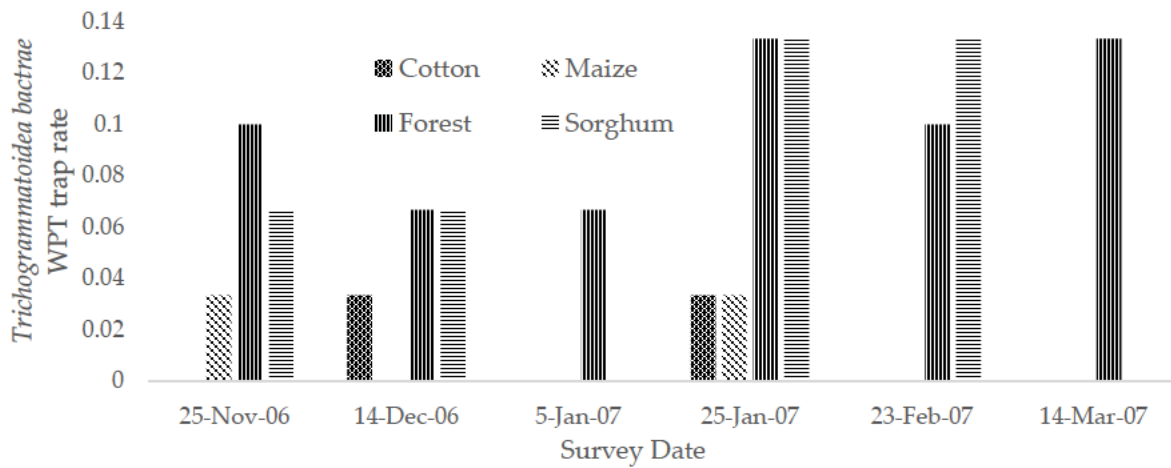


Figure 2.4: *Trichogrammatoidea bactrae* trap rate in various habitats on the Liverpool Plains in summer 2006–07 over six survey dates (from 25 Nov 2006 to 14 Mar 2007).

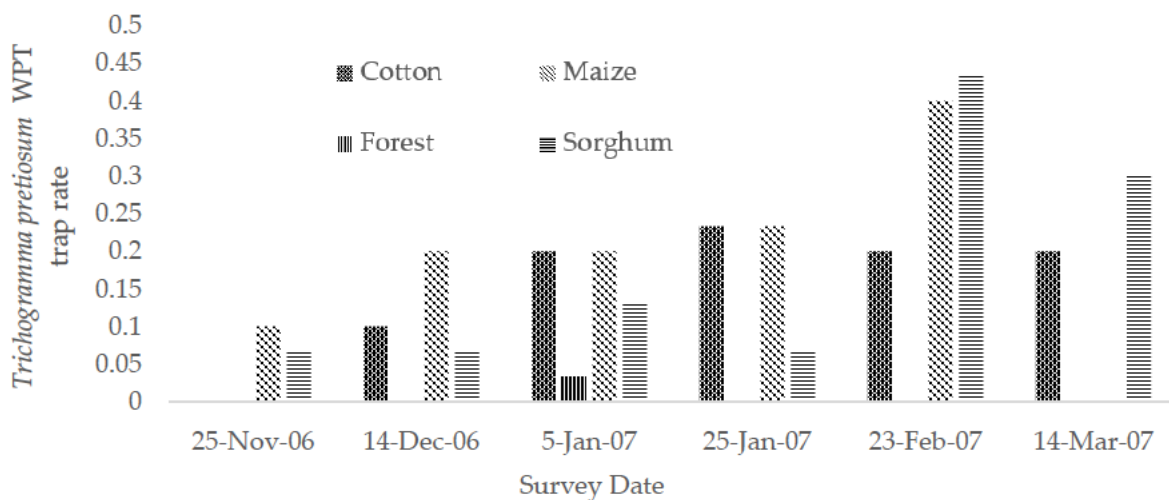


Figure 2.5: *Trichogramma pretiosum* trap rate in various habitats on the Liverpool Plains in summer 2006–07 over six survey dates (from 25 Nov 2006 to 14 Mar 2007).

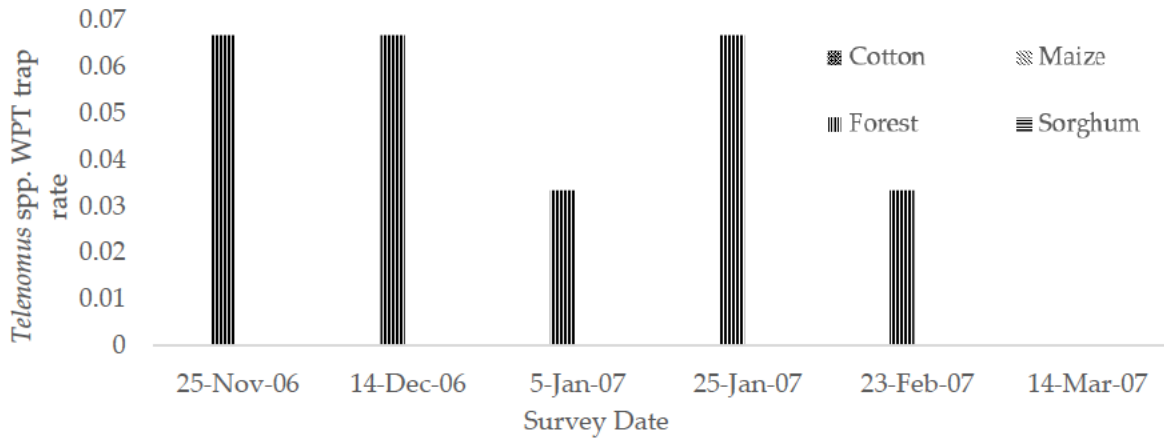


Figure 2.6: *Telenomus* spp. trap rate in various habitats on the Liverpool Plains in summer 2006–07 over six survey dates (from 25 Nov 2006 to 14 Mar 2007).

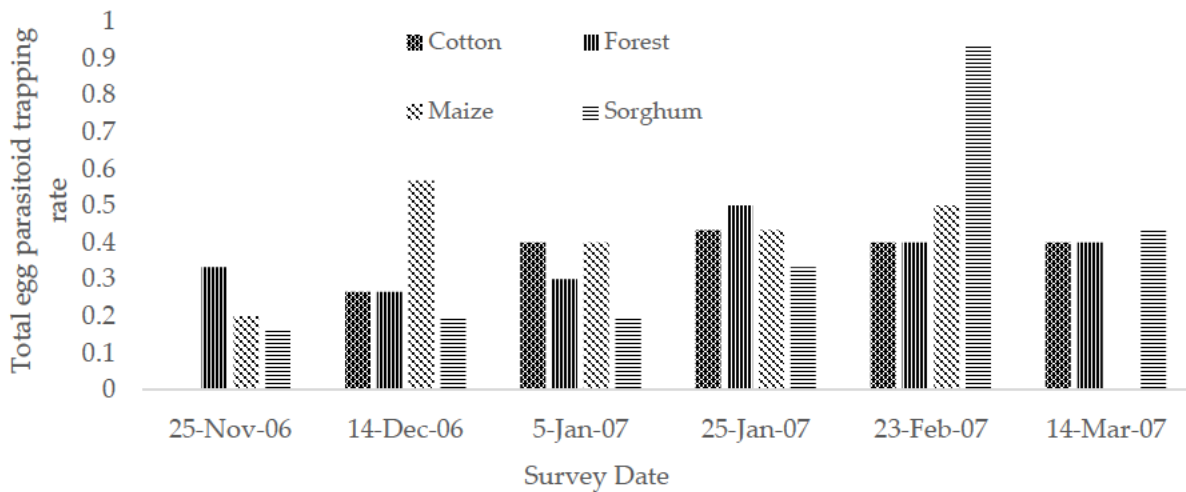


Figure 2.7: Pooled WPT trap rate in various habitats on the Liverpool Plains in summer 2006–07 over six survey dates (from 25 Nov 2006 to 14 Mar 2007).

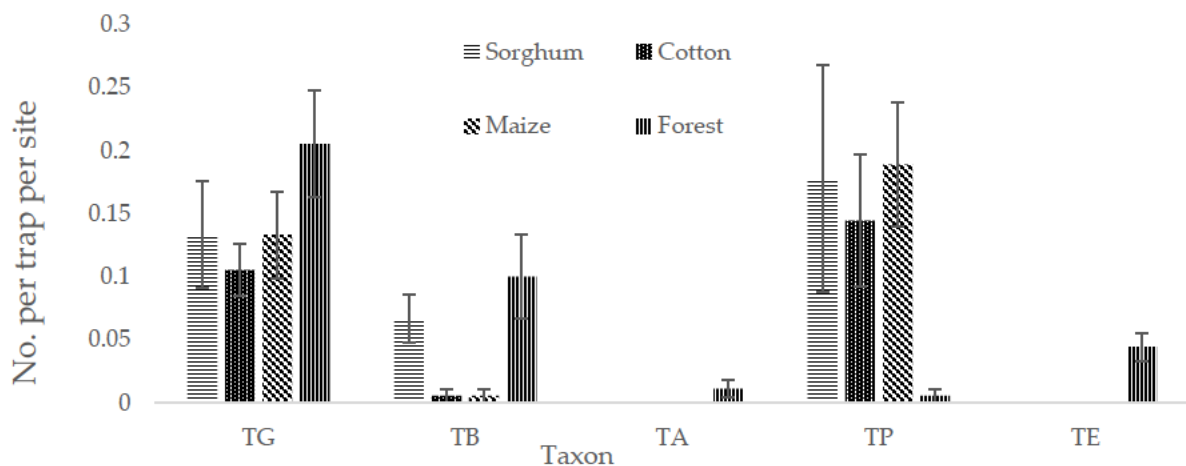


Figure 2.8: WPT trap rate (± 1 s.e.) pooled for each habitat of egg parasitoids (TG = Trichogrammatid, TB = *Trichogrammatoidea bactrae*, TA = *Trichogramma australicum*, TP = *Trichogramma pretiosum*, TE = *Telenomus* spp.) on the Liverpool Plains in summer 2006–07.

Summer WPT surveys in 2007–08 returned 406 egg parasitoids from 720 (white and yellow) WPT. Trichogrammatids (106 specimens; average trap rate 0.16) varied among summer habitats and reached peak abundance in late January (Fig. 2.9).

Trichogrammatoidea bactrae (73 specimens; average trap rate 0.08) was found most in forest and maize habitats, less in sorghum and least in cotton (Fig. 2.10).

Trichogramma australicum (seven specimens; average trap rate 0.009) was only found in forest (Fig. 2.11). *Trichogramma pretiosum* (204 specimens; average trap rate 0.28) was the most abundant egg parasitoid in crops, increasing with crop maturation and was found least in forest (Fig. 2.12). *Telenomus* spp. (14 specimens; average trap rate 0.02) were only found in forest habitats (Fig. 2.13). Abundance of egg parasitoids as determined by WPT trap rate gradually increased and peak trap rate occurred at end of January then declined gradually (Fig. 2.14).

In summer 2007–08, egg parasitoid taxon abundance (measured by trap rate, Fig. 2.14) was significant (ANOVA, $P < 0.001$, $df = 4$) and the interaction between species and habitat was also marginally significant ($P = 0.068$, $df = 12$). *Trichogramma pretiosum* was the most abundant parasitoid in crops but was virtually absent from forest. In contrast, *T. australicum* and *Telenomus* spp. were scarce and confined to forest (Fig. 2.15).

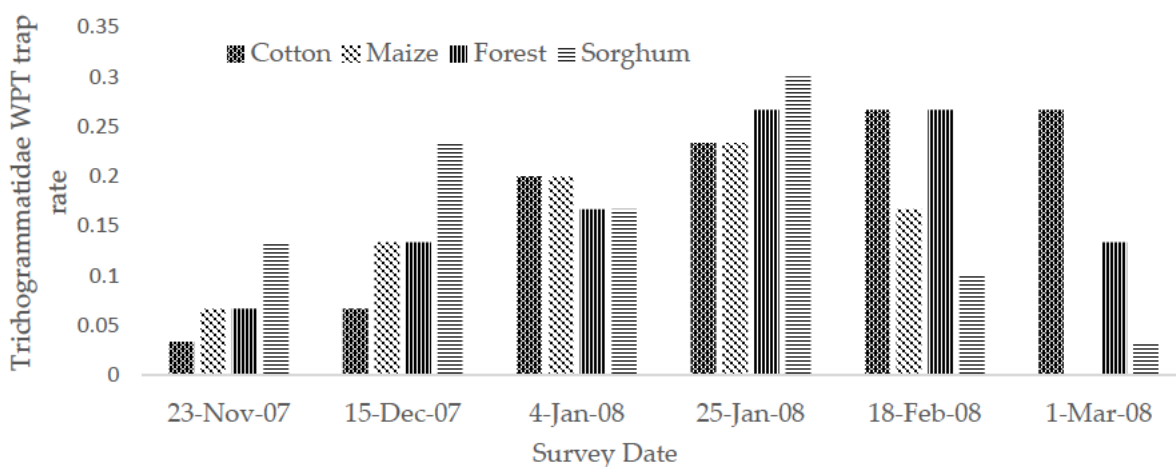


Figure 2.9: Trichogrammatidae trap rate in various habitats on the Liverpool Plains in summer 2007–08 over six survey dates (from 23 Nov 2007 to 1 Mar 2008).

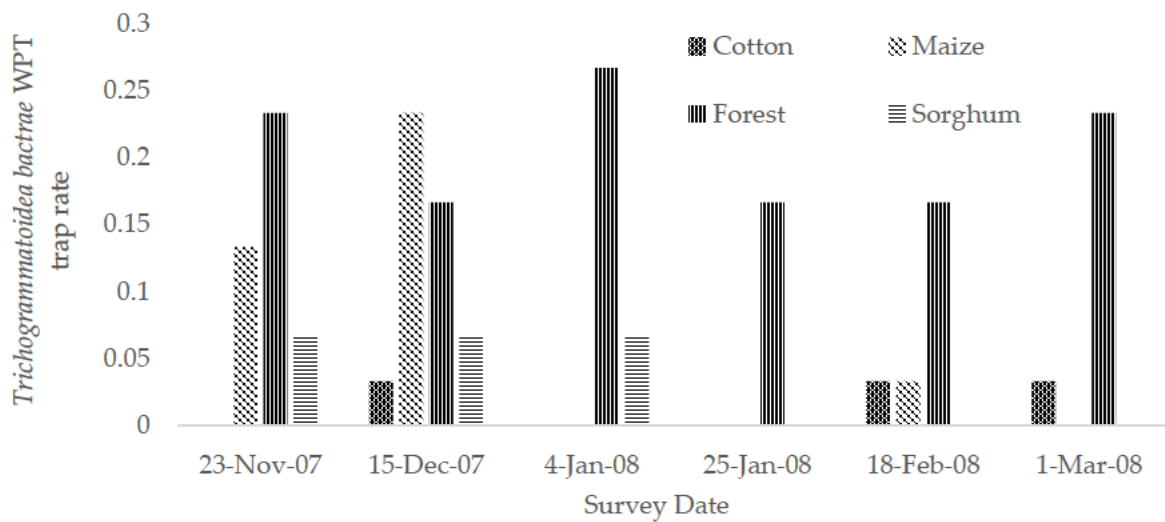


Figure 2.10: *Trichogrammatoidea bactrae* trap rate in various habitats on the Liverpool Plains in summer 2007–08 over six survey dates (from 23 Nov 2007 to 1 Mar 2008).

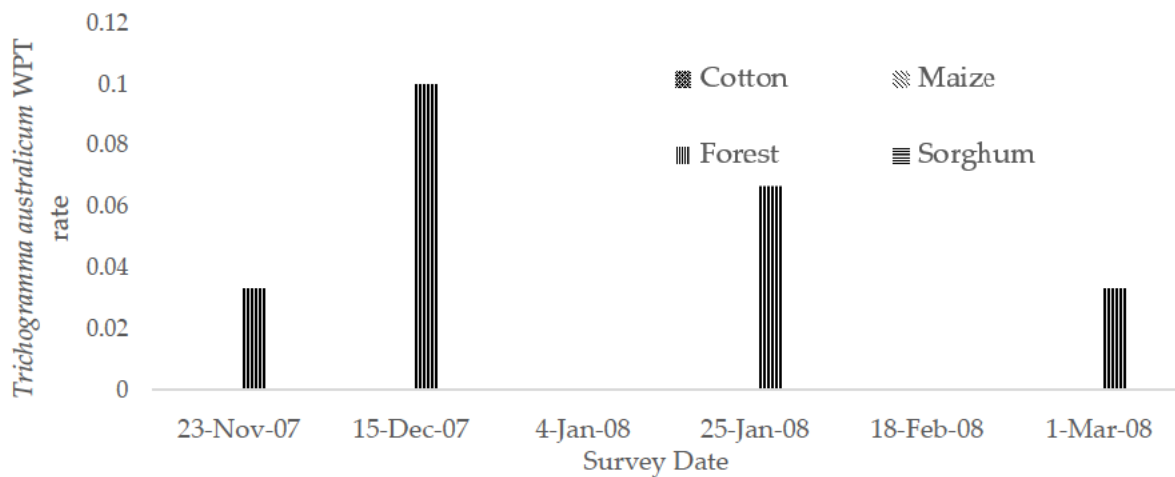


Figure 2.11: *Trichogramma australicum* trap rate in various habitats on the Liverpool Plains in summer 2007–08 over six survey dates (ranging from 23 Nov 2007 and 1 Mar 2008).

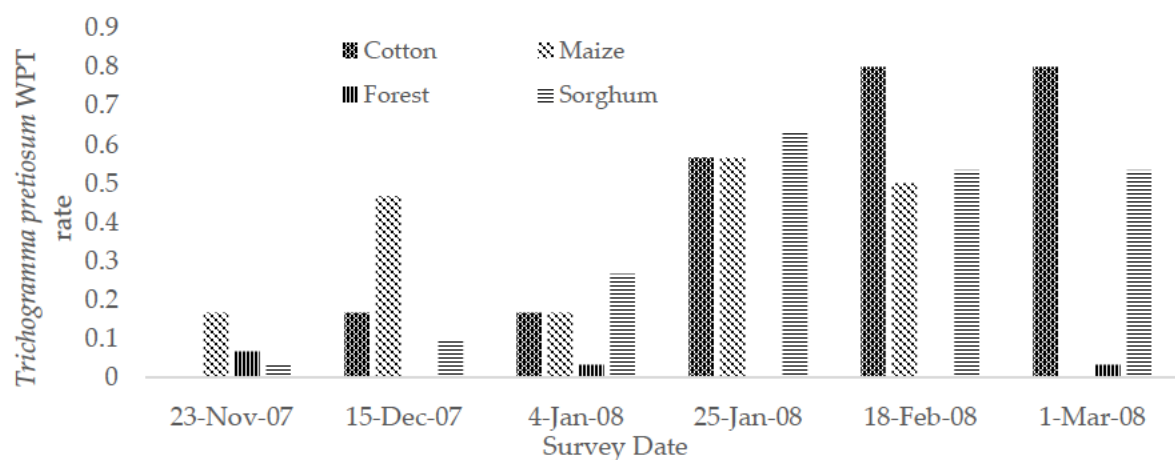


Figure 2.12: *Trichogramma pretiosum* trap rate in various habitats on the Liverpool Plains in summer 2007–08 over six survey dates (from 23 Nov 2007 to 1 Mar 2008).

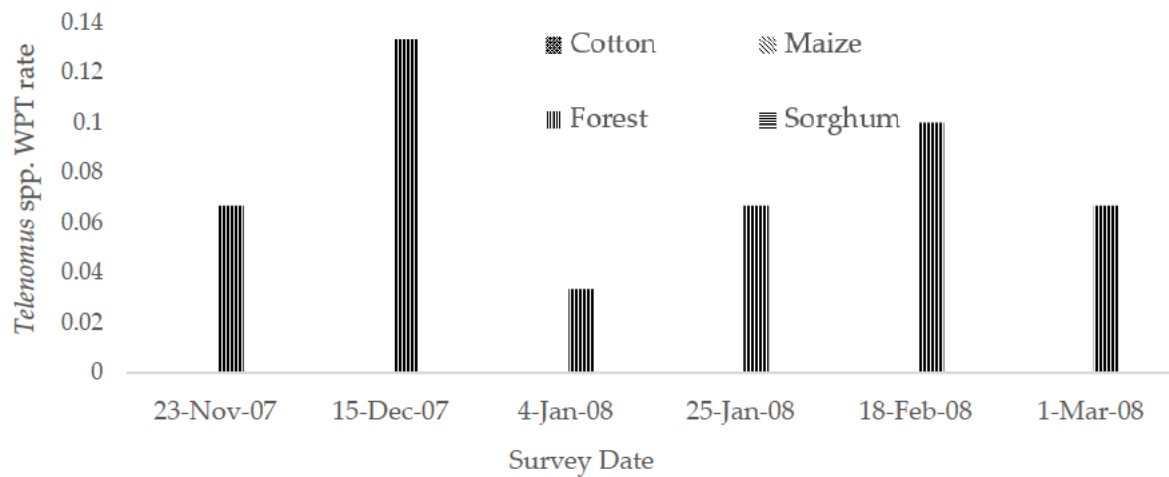


Figure 2.13: *Telenomus* spp. trap rate in various habitats on the Liverpool Plains in summer 2007–08 over six survey dates (from 23 Nov 2007 to 1 Mar 2008).

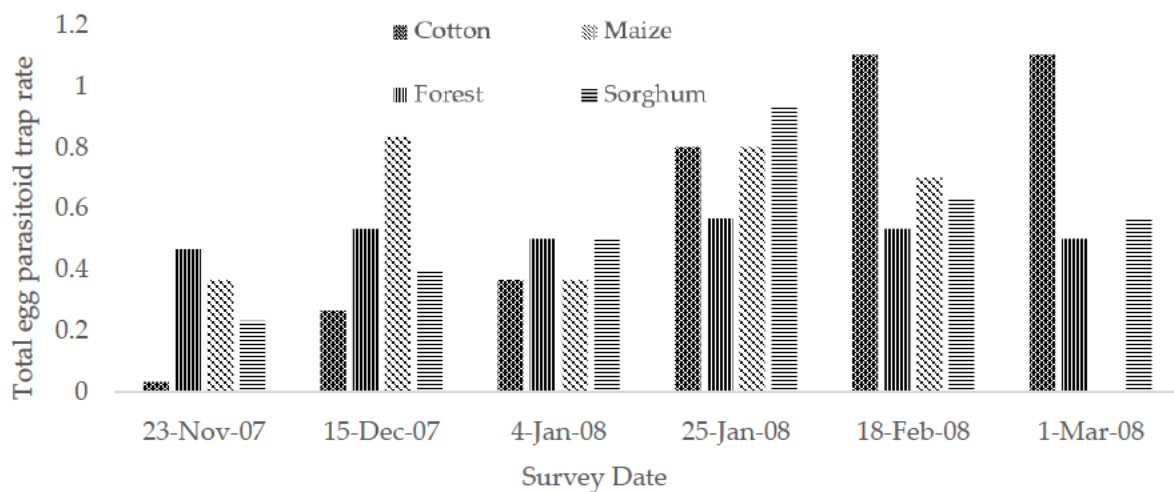


Figure 2.14: WPT trap rate of all egg parasitoids habitats on the Liverpool Plains in summer 2007–08 over six survey dates (from 23 Nov 2007 to 1 Mar 2008).

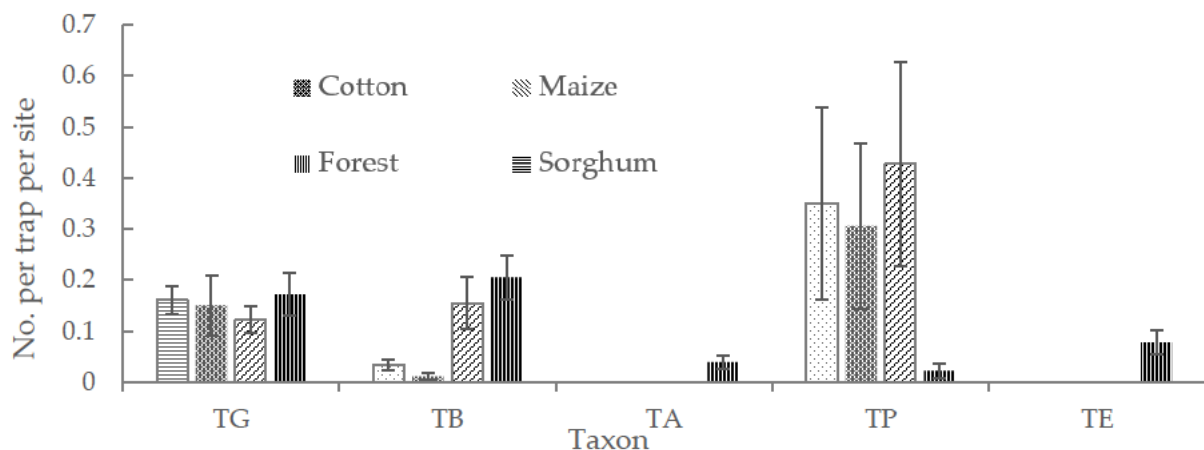


Figure 2.15: 2007-8 WPT trap rate (± 1 s.e.) pooled for each habitat of egg parasitoids (TG = trichogrammatid, TB = *Trichogrammatoidea bactrae*, TA = *Trichogramma australicum*, TP = *Trichogramma pretiosum*, TE = *Telenomus* spp.) on the Liverpool Plains in summer 2007–08.

In summer 2009, trap rate increased from past surveys in crop habitats mainly due to *Trichogramma pretiosum*, which was released in spring in wheat and canola (Fig. 2.16).

2.3.5 Summer field surveys for egg parasitoids using egg card parasitism

In summer 2006–07, egg card parasitism gradually increased with crop maturation and was highest in February in sorghum (mean \pm s.e., $14 \pm 4.0\%$), followed by cotton ($13 \pm 7.0\%$), maize ($5 \pm 1.7\%$, in late January) and forest ($2 \pm 0.8\%$ in both late January and February) (Fig. 2.17). In 2007–08 average egg card parasitism peaked in late January and February in crops (sorghum, $29 \pm 6.2\%$; cotton, $26\% \pm 10.6\%$; maize, $23 \pm 5.6\%$) and in early March in forest ($7 \pm 2.4\%$). Egg card parasitism doubled between 4 Jan and 25 Jan 2008 (Fig. 2.18). In January–February 2009, egg card parasitism peaked in all habitats in late February (sorghum, $47 \pm 7.5\%$; cotton, $25 \pm 6.3\%$; forest, $13 \pm 6.2\%$; grassland, $5 \pm 1.4\%$; Fig. 2.19).

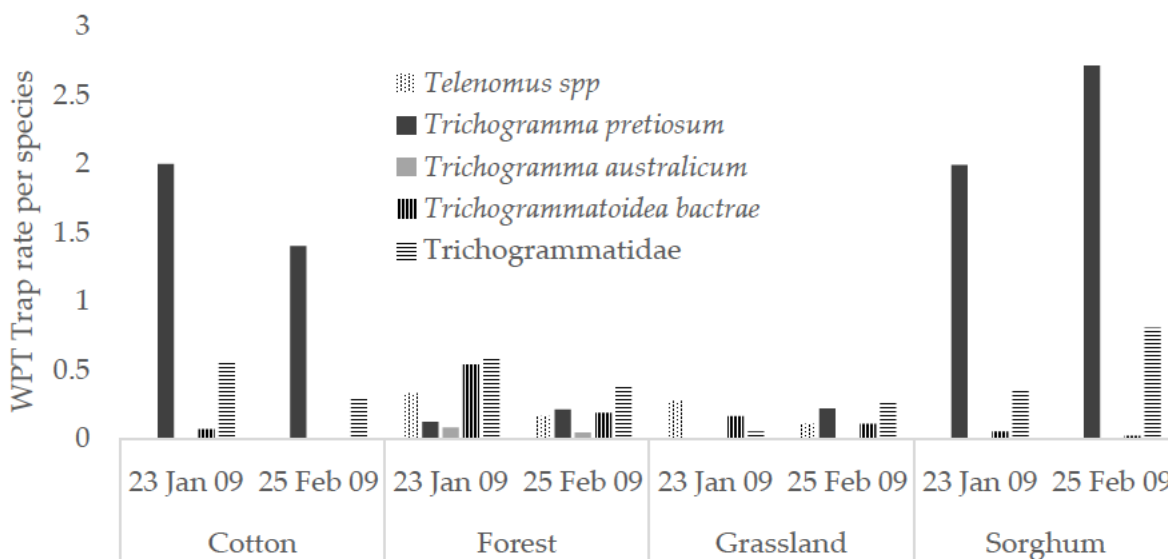


Figure 2.16: Trap rate of egg parasitoids per habitat across a range of habitats on the Liverpool Plains in summer 2009.

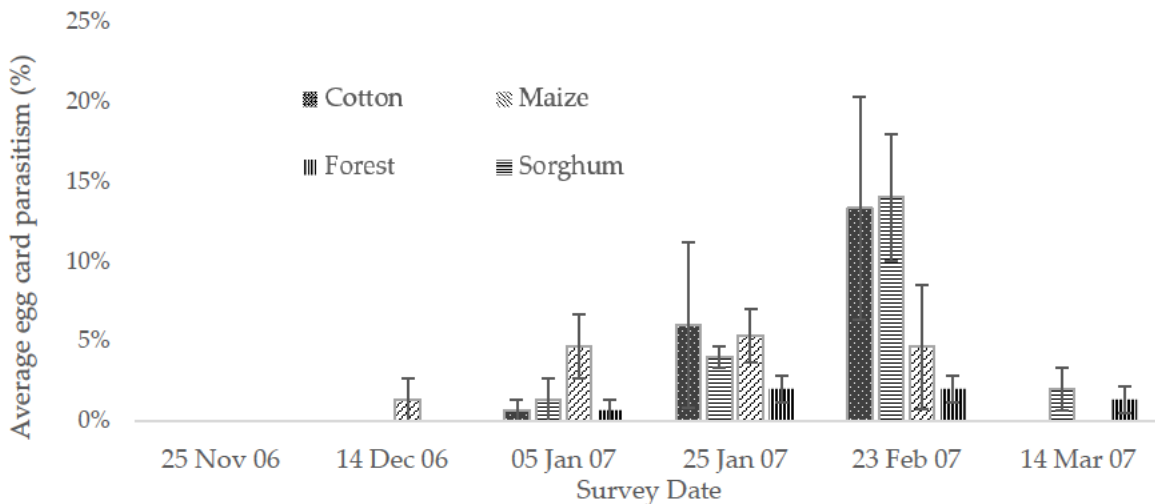


Figure 2.17. Average (± 1 s.e.) egg card parasitism rate in habitats on the Liverpool Plains in summer 2006–07 over six survey dates (ranging from 25 Nov 2006 to 14 Mar 2007)

2.3.6 Agricultural landscape structure and summer egg parasitoid abundance

In 2006–07, the effect of landscape on the abundance of egg parasitoid taxa was significant ($P = 0.005$) with a greater abundance of egg parasitoids in WPTs in the Caroon–Quirindi (CQ) landscape (avg. trap rate = 0.47) than in the Piallaway–Breeza (PB) landscape (avg. trap rate = 0.24) (Fig. 2.20). This was most apparent in sorghum (avg. trap rate: CQ, 0.71 and PB, 0.16) followed by cotton (avg. trap rate: CQ, 0.38 and PB 0.18), whereas trap rates were similar for maize between the two landscapes (avg. trap rate: CQ, 0.32 and PB, 0.34). Forest had a higher WPT trap rate in the CQ landscape (0.49) than the PB landscape (0.29). The interaction between landscape and habitat was not significant ($P = 0.092$). However, there was a marginal interaction between taxon (species) and landscape ($P = 0.067$) as *Trichogramma australicum* was only found in the PB landscape while *Trichogramma pretiosum*, *Trichogrammatoidea bactrae* and the remaining taxa were more abundant in the CQ landscape. Averaging over all taxa, trap rates varied with habitat among dates (Fig. 2.20) but the differences were not consistent nor significant ($P = 0.513$), with average (\pm s.e.) trap rates per habitat varying between 0.28 ± 0.07 (cotton) and 0.43 ± 0.14 (sorghum).

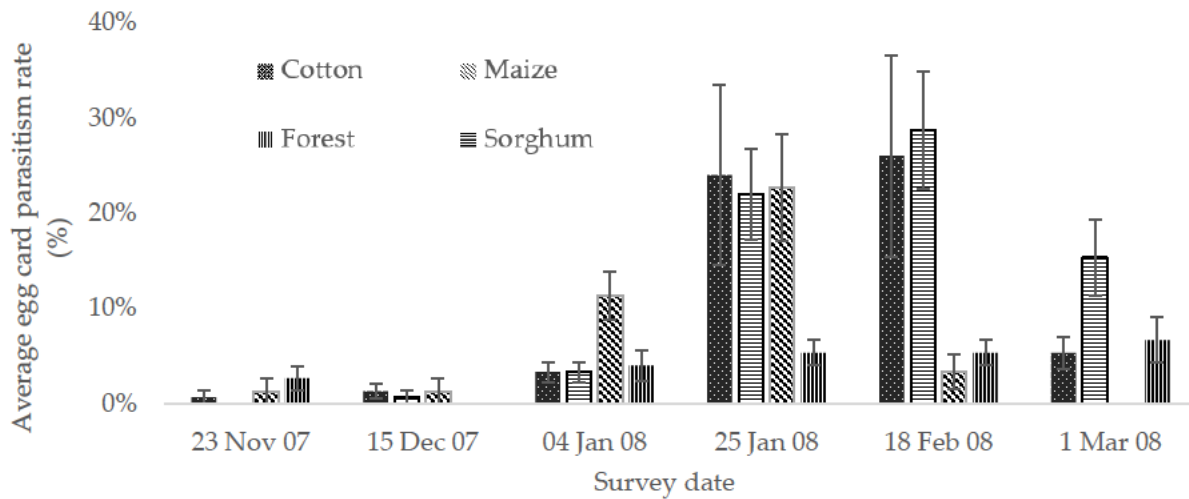


Figure 2.18: Average (± 1 s.e.) egg card parasitism rate in habitats on the Liverpool Plains in summer 2007–08 over six survey dates (ranging from 23 Nov 2007 to 1 Mar 2008).

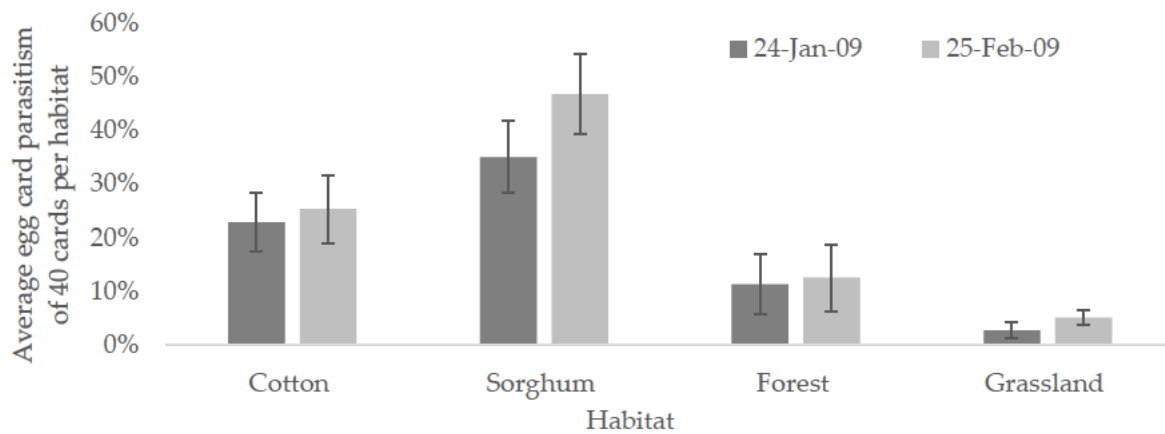


Figure 2.19: Average (± 1 s.e.) egg card parasitism rate in habitats on the Liverpool Plains in summer 2009 over two survey dates (24 Jan and 25 Feb).

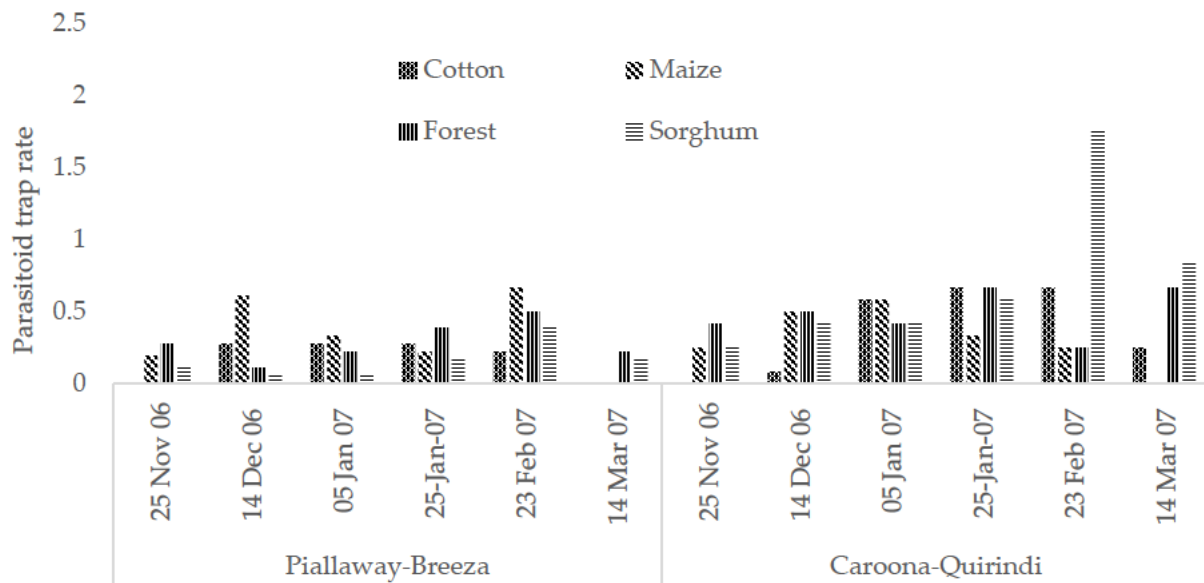


Figure 2.20: Total egg parasitoids per trap (trap rate) in various habitats across two landscapes, Piallaway–Breeza and Carroona–Quirindi, on the Liverpool plains in 2006–07.

In summer 2007–08, egg parasitoids were more abundant than the previous year, as judged by WPT rates, and the effect of landscape was again significant ($P < 0.001$), with greater trap rates again in the Carroona–Quirindi (CQ) landscape (avg. trap rate = 0.93) than in the Piallaway–Breeza (PB) landscape (avg. trap rate = 0.31; Fig. 2.21). Average egg parasitoid capture rates per vabitat were two (forest) to five (sorghum) times greater in the Carroona–Quirindi than the Piallaway–Breeza landscape. WPT rates did not differ significantly between habitats ($P = 0.50$), with average WPT rates (\pm s.e.) varying between 0.53 ± 0.17 (cotton) and 0.78 ± 0.18 (maize). Nor was the landscape–habitat interaction significant ($P = 0.542$). The effect of taxon, landscape and their interaction were all significant at $P \leq 0.001$, with *Trichogramma pretiosum* seven times more abundant in the Carroona–Quirindi (0.57 ± 0.14) than the Piallaway–Breeza (0.08 ± 0.02) landscape but *T. australicum* and *Trichogrammatoidea bactrae* similarly abundant in both landscapes (0.01 ± 0.01 and 0.09 ± 0.02 in CQ, and 0.01 ± 0.003 and 0.11 ± 0.02 in PB, respectively) albeit less abundant than *Trichogramma pretiosum*.

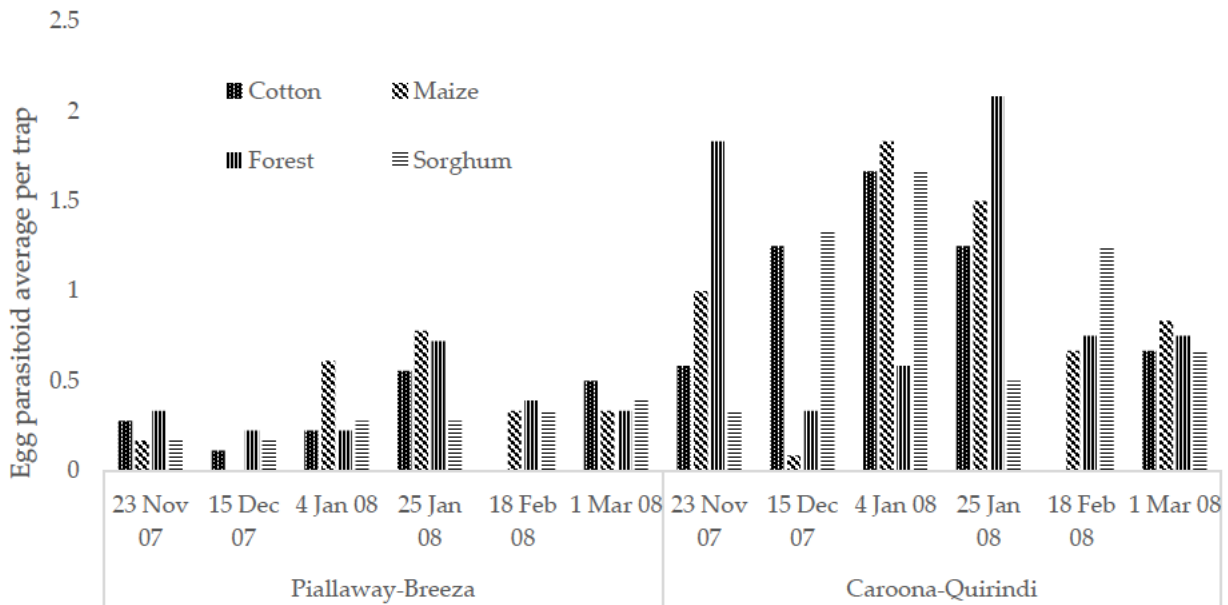


Figure 2.21: Average trap rate in a variety of habitats across two landscapes; Piallaway–Breeza and Carroona–Quirindi, on the Liverpool plains in 2007–08.

Unlike the WPT data, there was no significant difference ($P = 0.11$) in egg card parasitism between the two landscapes in 2006–07 although average parasitism was greater in the Carroona-Quirindi landscape (average egg card parasitism \pm s.e. = $4.2 \pm 0.01\%$) than in the Piallaway–Breeza landscape ($1.6 \pm 0.005\%$) owing to high levels of parasitism in cotton and sorghum in late January and late February in the former landscape (Fig. 2.22). The effect of habitat was not significant ($P = 0.53$) nor was the interaction between landscape and habitat ($P = 0.38$).

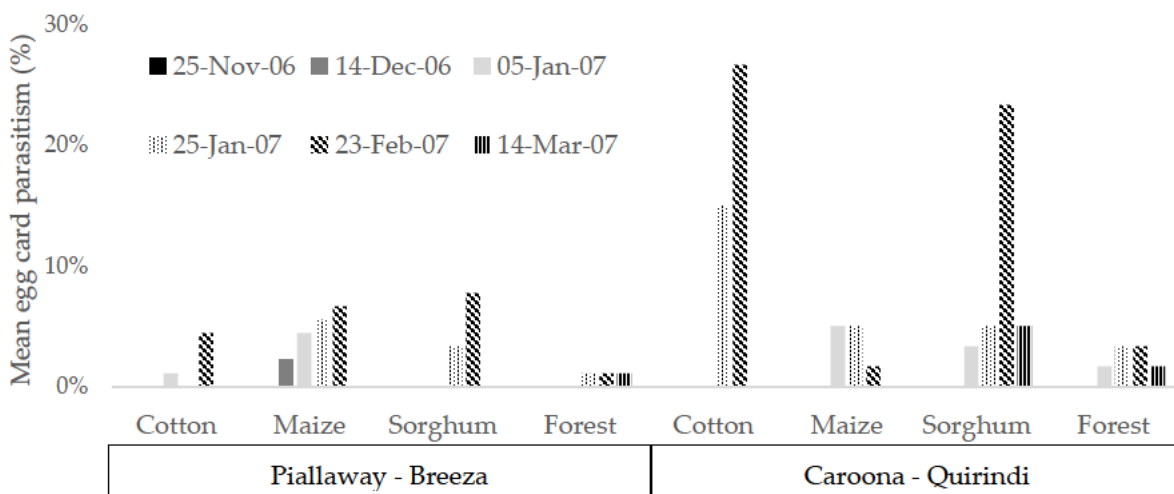


Figure 2.22: Comparison of mean egg card parasitism per habitat over summer habitats across two landscapes Piallaway–Breeza and Carroona - Quirindi, on the Liverpool plains in 2006–07.

In the summer of 2007–08, egg card parasitism across habitats was significantly ($P = 0.022$) greater in the Carroona–Quirindi landscape ($13 \pm 3.1\%$) than the Piallaway–Breeza landscape ($5 \pm 1.2\%$) (Fig. 2.23). The effect of habitat was not significant ($P = 0.31$), nor was the interaction between habitat and landscape ($P = 0.79$).

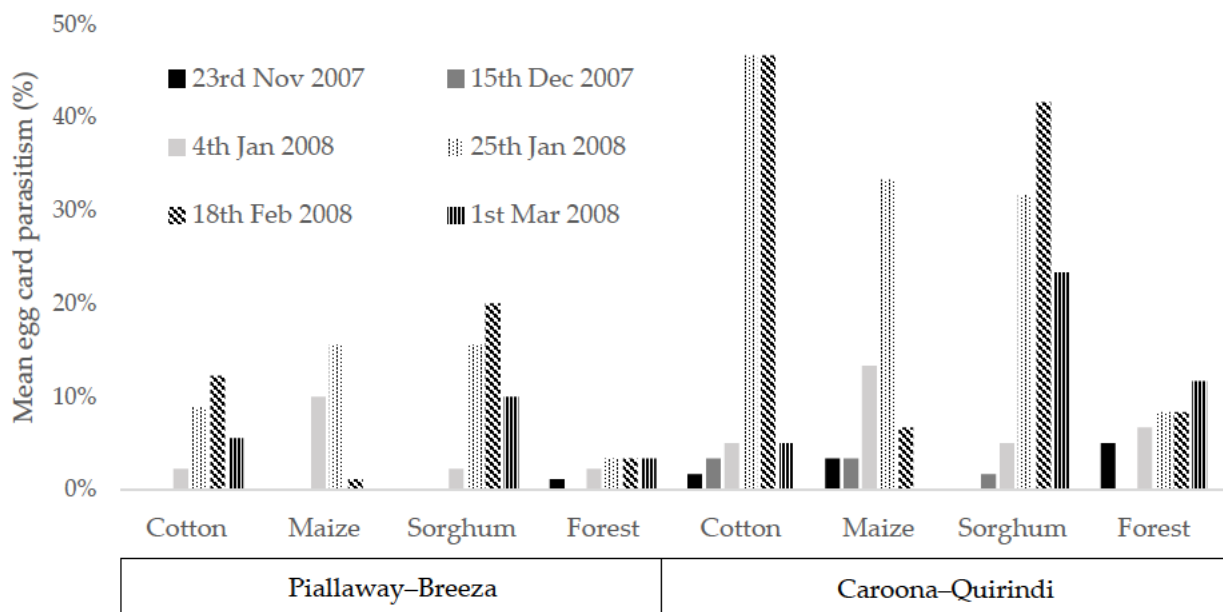


Figure 2.23: Comparison of mean egg card parasitism per habitat over summer habitats across two landscapes, Piallaway–Breeza and Carroona–Quirindi, on the Liverpool plains in summer 2007–08.

In the two surveys in January and February 2009 after the release of *T. pretiosum* in the preceding spring, there were more egg parasitoids in crops than forest or grassland habitats ($P \leq 0.001$), but there was little difference between landscapes (Figure 2.24).

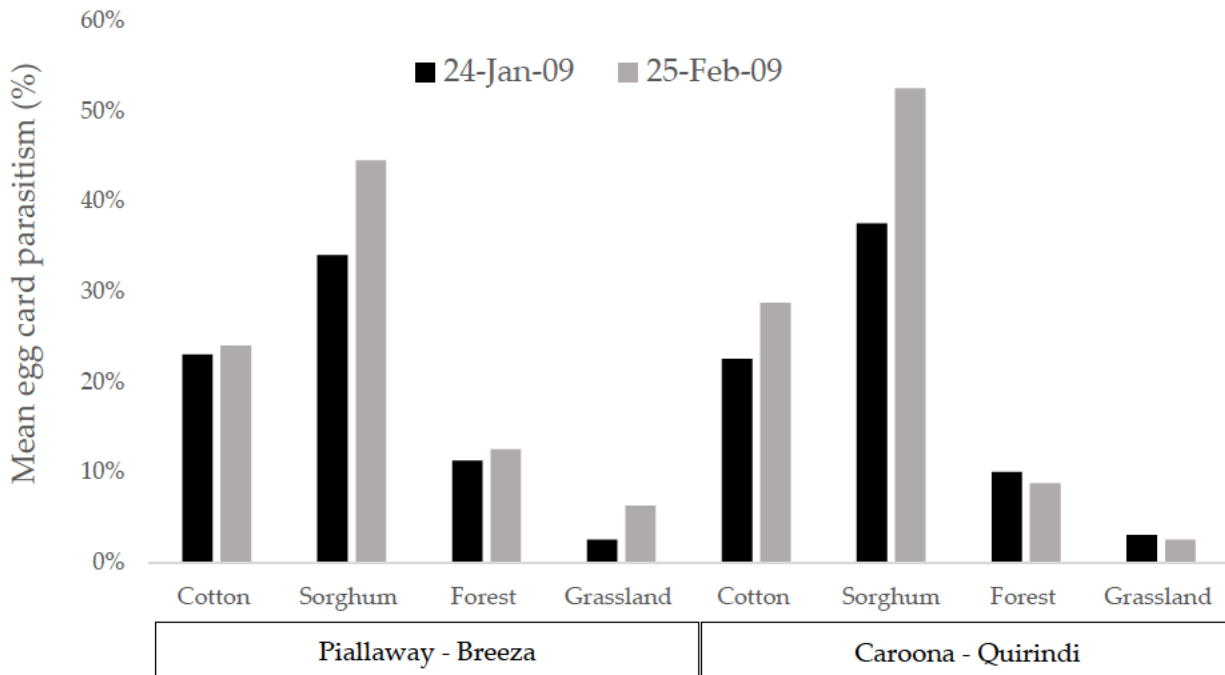


Figure 2.24: Mean parasitism (%) of egg cards across two landscapes, Piallaway–Breeza and Caroona–Quirindi, over two sampling dates (23–24 Jan and 24–25 Feb 2009) on the Liverpool Plains.

2.3.7 Egg parasitoid overwintering survey

In the winter 2007 survey of egg parasitoids in forest habitats, only two of the 180 egg cards deployed were parasitised. One did not hatch and the emerging parasitoid from the other egg was *Trichogrammatoidea bactrae*. In the WPT, 11 specimens were identified (Fig. 2.25): Trichogrammatidae (4 specimens), *Trichogrammatoidea bactrae* (3), *Trichogramma australicum* (1) and *Telenomus* spp. (3). In the first winter survey in 2008, two egg cards of the 240 egg cards deployed were parasitised, one by *T. pretiosum* and the other by *Trichogrammatoidea bactrae*. In the second survey in 2008, three of the 240 cards were parasitised and identified as Trichogrammatidae and a fourth as *Trichogrammatoidea bactrae*.



Figure 2.25: WPT trap rate, winter 2007 survey (27–28 Jul 2007). Taxa: TG = Trichogrammatid, TB = *Trichogrammatoidea bactrae*, TA = *Trichogramma australicum*, TP = *Trichogramma pretiosum*, TE = *Telenomus* spp.

In the first survey in 2008 (2–3 July), WPT yielded 12 specimens: trichogrammatids (4), *Trichogrammatoidea bactrae* (3), *Telenomus* spp. (2) and one each of *T. australicum* and *T. pretiosum*. In the second winter survey in 2008, 14 egg parasitoid specimens were caught in WPT: trichogrammatids (5) *Trichogrammatoidea bactrae* (3) and *Telenomus* spp. (6).

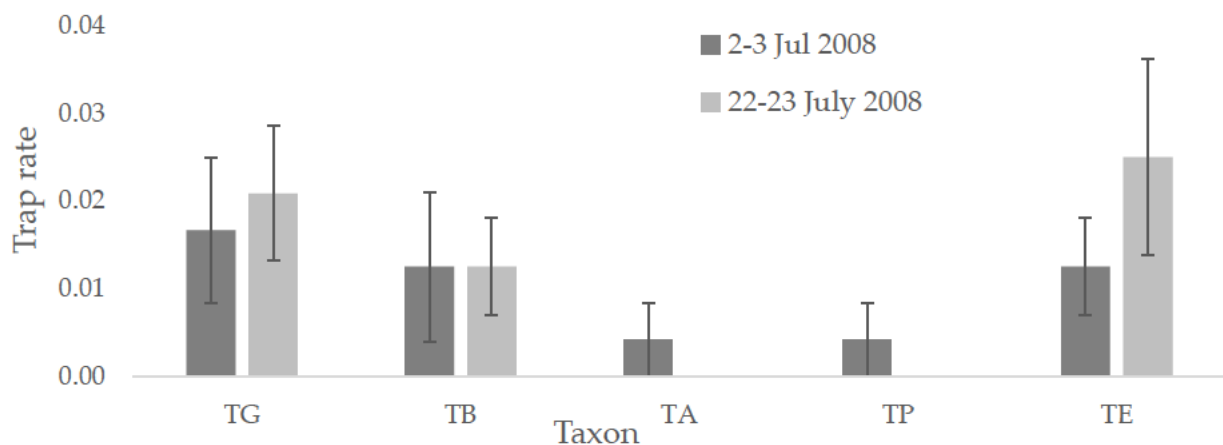


Figure 2.26: Winter 2008 egg parasitoid WPT rate in forest on two survey dates (2–3 Jul and 22–23 Jul 2008). Taxa: TG = Trichogrammatid, TB = *Trichogrammatoidea bactrae*, TA = *Trichogramma australicum*, TP = *Trichogramma pretiosum*, TE = *Telenomus* spp.

2.3.8 Autumn–winter 2007 natural egg collection

In April 2007, 11 lepidopteran eggs were collected from the Breeza and Quirindi forest sites. Although at least two were parasitised, no egg parasitoids hatched out

from them. The July 2007 survey yielded eight lepidopteran eggs, but none were parasitised.

2.4 Discussion

This study has demonstrated that egg parasitoids are common and diverse in crop and non-crop habitats on the Liverpool Plains. Impact measured by parasitisation of *Helicoverpa* egg cards can reach relatively high levels in some crops and in non-crop habitats in summer. At the beginning of the survey in 2006, drought affected the region, and this reduced the abundance of egg parasitoids. In subsequent years, parasitism of *Helicoverpa* egg cards and abundance of egg parasitoids increased. Sampling in northern Australia by Davies and Zalucki (2008) similarly collected specimens of *Trichogramma* in native habitats in a comparatively poor (i.e. dry) wet season.

In the present study on the Liverpool plains, the relatively stable habitats of native grassland and forest harboured a diversity of egg parasitoids that did not fluctuate as much during the season as in the cropping habitats, particularly in summer. Previous work by Perovic et al. (2010) in the Macquarie Valley in central NSW showed that *T. pretiosum* parasitism was higher near shelter belts and determined that spill-over into cotton crops was occurring. In our survey, we found lower mean WPT trap rates of *T. pretiosum* in forest sites and higher trap rates for other Trichogrammatids. We did, however, detect *T. pretiosum* parasitising *Helicoverpa* egg cards in winter forest habitats. This suggests that *T. pretiosum* may utilise native vegetation as an overwintering refuge but is clearly more abundant and prefers crop habitats during late spring and summer. Its populations may have a greater ability to increase in crop habitats than forest on the Liverpool Plains. The lack of early populations of *T. pretiosum* in spring crops may be an effect of limited availability of host eggs in native vegetation where competition with native egg parasitoids is likely to be high. In spring 2006 and 2007, *Trichogrammatoidea bactrae* was the dominant egg parasitoid in forest habitats and crops (Figs 2.1 and 2.2). However, in

summer crops, *T. pretiosum* was more abundant than any other egg parasitoid, but less so in forest (Figs 2.8, 2.15 and 2.19).

In the analysis of the summer WPT trap rates in 2006–07, the interaction between taxon and habitat was significant ($P < 0.01$), indicating that the abundance of taxa depended on the habitat. In 2007–08, the interaction between taxon and habitat was again marginally significant ($P = 0.068$). Comparatively, lower rates of egg card parasitism and lower trap rates in all habitats in 2006–07 was attributed to the drier conditions, whereas 2007–08 represented a more normal season for habitat quality and parasitoid–host relationships. In 2007–08, the analysis of WPT data confirmed that some taxa such as *T. pretiosum* were more abundant than *T. australicum* or *Telenomus* species, the difference being highly significant ($P < 0.001$). As noted above, the interaction between taxon and habitat was marginally significant, with *T. pretiosum* being rare in native vegetation and *Telenomus* spp. and *Trichogramma australicum* rare or absent from crops.

After inoculation of *T. pretiosum* in wheat and canola in spring 2008 (Chapter 4), trap rate of *T. pretiosum* increased in the subsequent summer (average WPT rate \pm s.e. = 1.08 ± 0.37). However, in the same season, there was reduced *Trichogrammatoidea bactrae* abundance (0.145 ± 0.06) and activity in crops (Fig. 2.19) compared to the previous summer (1.10 ± 0.04). Scholz and Parker (2004) showed a reduction in *Trichogrammatoidea bactrae* egg card parasitism after releases of *Trichogramma pretiosum* on the Darling Downs. *T. pretiosum* has also been recorded to displace native egg parasitoids in cotton crops in northern Australia (Michael and Woods, 1978).

Combining WPT data from half yellow and half white traps in summer 2009 compared with separate WPT colours in the previous year may have influenced *Trichogrammatoidea bactrae* catch rate. The extent to which some egg parasitoids are attracted more to water traps than other species is not well known. In 2009, water pan trap abundance correlated with egg card parasitism (Chapter 4). However, this

was not observed in previous years of the study. *Telenomus* spp. were only ever detected in white water pan traps and did not parasitise any of the *Helicoverpa* egg cards throughout the 3 years of surveys. Previous studies have indicated that parasitoid catches are higher in yellow traps (Dowell and Cherry, 1981; Storck-Weyhermüller, 1988; Romeis *et al.*, 1998b) although experiments by Pollet and Grootaert (1987) found species abundance in woodland was better detected by white water traps. Physiological conditions may also influence water pan trap effectiveness, through differences between the sexes, as found in *Trichogramma* (Romeis *et al.*, 1998b) and other parasitoids (Wäckers, 1994). For example, Wäckers (2004) found females of the parasitoid, *Cotesia rubecula*, searched for yellow targets more readily when starved than when fed on a sugar solution. Hickman *et al.* (2001) found that parasitoids were caught more readily when they were newly emerged or hungry. Visual cues, such as certain colours, may be used for host habitat location in egg parasitoids (Jervis, 2007). Somchoudhury and Dutt (1988) found that longevity and fecundity were not increased when *T. perkinsi* and *T. australicum* were provided with flowering maize or sorghum. Their study suggested that *Trichogramma* species are unable to utilise these pollen sources. Hence, some *Trichogramma* may not necessarily target flowers for nectar or pollen. However, our survey results have shown that in spring, flowering habitats such as linseed and faba beans that have white flowers attracted a greater abundance of *Trichogrammatoidea bactrae* in yellow traps while *Trichogramma pretiosum* was readily caught in white-coloured traps in sunflowers with yellow flowers (Figs 2.3 and 2.4).

In our study there was a gradual build-up of egg parasitoid populations in crop habitats in summer. *T. pretiosum* was the most abundant egg parasitoid caught in water pan traps in all summer crops (Figs 2.8, 2.15 and 2.19). The decrease in egg card parasitism in maize crops in February was attributed to crop senescence. Interestingly, in 2007–08, mean trap rate of egg parasitoids in maize was higher than in either cotton or sorghum (Fig. 2.17), but average parasitism of egg cards per

habitat in sorghum and cotton were higher (Fig. 2.21). Differences in height, with WPT on the ground and egg cards attached to the upper part of the maize plant, may have contributed to this. Several studies have demonstrated that plant height can affect *Trichogramma* parasitism percentage (Andow and Prokrym, 1990; Gingras and Boivin, 2002; Gingras *et al.*, 2008a), and *T. pretiosum* is found in the lower canopy more than other *Trichogramma* species (Ables *et al.*, 1980; Fournier and Boivin, 2000).

In the first year of this study, *Trichogrammatoidea bactrae* was similarly abundant to *Trichogramma pretiosum* in sorghum habitats from the end of November to the end of January in WPT and was absent from maize (Figs 2.6 and 2.8). The following year, *Trichogrammatoidea bactrae* trap rate (abundance) for the same period was approximately half that of *Trichogramma pretiosum* (Figs 2.13 and 2.15). At the start of the season, *Trichogrammatoidea bactrae* and *Trichogramma pretiosum* abundance was similar, but by the end of January, *Trichogrammatoidea bactrae* trap rates started to decline (except in sorghum, Fig. 2.4) when *Trichogramma pretiosum* trap rate was at its maximum. Notably in 2009, *Trichogrammatoidea bactrae* was almost absent from crops. It is possible that the inoculative release of *Trichogramma pretiosum* in spring impacted on *Trichogrammatoidea bactrae* abundance in summer crop habitats. However, *Trichogrammatoidea bactrae* abundance in forest in the same period remained relatively stable. Further research is warranted on the impact of releases on the population dynamics of native egg parasitoids, and if there are cascading effects relevant to future conservation biological control.

There were significant differences in egg parasitoid activity between the Caroon–Quirindi landscape compared to the Pialloway–Breeza landscape in successive summer seasons. We recorded a significantly higher WPT catch rate in the Caroon–Quirindi landscape than the Pialloway–Breeza landscape, this effect not being due to habitat as this effect was not significant. When comparing egg card parasitism, landscape was not significant in the first summer but was in the second year ($P = 0.022$). The differences in species of egg parasitoids (taxa) were marginally

significant in 2006–07 but highly significant between the two landscapes in 2007–08. In 2006–07, for example, there were more trichogrammatids and *Trichogramma pretiosum* than *T. australicum* or *Telenomus* spp. Overall there were a greater number of egg parasitoids in the Carroona–Quirindi landscape. The marginal interaction between landscape and species suggests that certain species were more abundant in one landscape than the other. For example, *Trichogramma australicum* was only found in the Piallaway–Breeza landscape and *T. pretiosum* and to a lesser extent trichogrammatids were more abundant in the Carroona–Quirindi landscape. In 2007–08, landscape ($P = 0.001$) significantly affected WPT catches. *Trichogramma australicum* and *Trichogrammatoidea bactrae* were similarly abundant in the two landscapes, but *Trichogramma pretiosum*, *Telenomus* spp. and trichogrammatids were more abundant in the Carroona–Quirindi landscape. In this study we did not directly measure distance or proportion of native vegetation in the two landscapes surveyed. However, visually, the Carroona–Quirindi landscape (Appendices 4 and 5) had a higher proportion of forested areas than the Piallaway–Breeza landscape (Appendices 2 and 3). The Carroona–Quirindi landscape also had a greater proportion of crops such as maize, lucerne and canary seed that were generally not sprayed with insecticide (Mick Duncan, agronomist, pers. comm.). In addition, individual crop areas were smaller than in the Piallaway–Breeza landscape. In summer 2006–07, there was an unexpected decrease in *Telenomus* spp., *Trichogrammatoidea bactrae* (measured by trap rate) in forest habitats (Fig. 2.22). This is while there was a significant increase in abundance and egg card parasitism in sorghum and cotton in the Carroona–Quirindi landscape. Both *Trichogrammatoidea bactrae* and trichogrammatids decreased in the forest and increased in cotton and sorghum in the Carroona–Quirindi landscape at this time. We can only speculate about the movement of egg parasitoids into surrounding crops or other reasons for a sudden decrease in abundance. Egg parasitoid abundance and diversity is likely affected by processes operating at different spatial scales (Tschardt et al., 2007b; Schellhorn et al., 2014). However, there are substantial logistical problems associated

with measuring the effects of landscape structure on parasitoid–host relationships (Menalled *et al.*, 1999a). Although non-crop habitats have been shown to be a source of parasitoids in agroecosystems (Landis and Marino, 1999; Letourneau *et al.*, 2012; Macfadyen and Muller, 2013; Bianchi *et al.*, 2015), the findings with respect to non-crop habitat improving pest management have been inconsistent (Karp *et al.*, 2018). Processes such as the frequency of insecticide application can have greater impacts than resource availability (Jonsson *et al.*, 2012). Nevertheless, future research into the mechanisms and patterns of *Trichogramma* colonisation from source habitats is warranted, as is the attraction and retention of egg parasitoids using inter-cropping and dedicated insectary patches within crops.

Before this study, it was unknown if *Trichogramma* and other egg parasitoids overwintered on the Liverpool Plains. There was a diversity of taxa with low species abundance in the forest habitats over winter. Trichogrammatids made up the bulk of specimens, but both *Trichogramma pretiosum* and *Trichogrammatoidea bactrae* parasitised *Helicoverpa* egg cards. *Telenomus* spp. were widespread, and *Trichogramma australicum* was in low abundance (Figs 2.27 and 2.28). More than likely, our attractant-based methodology did not detect all species of egg parasitoids, and our attempts with natural egg collection were not fruitful in finding any parasitised eggs. The discovery of overwintering egg parasitoids has practical implications for the preservation of non-crop habitats in the landscape and was a key question of growers in the area. The threat of mining in the areas of hilly native vegetation in the study area (Franks *et al.*, 2010; Thampapillai, 2011) would threaten the effectiveness of future biological control opportunities in crops on the Liverpool Plains.

Trichogramma and other egg parasitoids of *Helicoverpa* not only provide added benefits to conventional pest control, but are important in the management of the endemic, insecticide-resistant *H. armigera*. Furthermore, the continued viability of Bt-transgenic cotton varieties depends on appropriate management strategies that

minimise resistance. *Helicoverpa* resistance management in grain and oilseed crops remains an ongoing issue on the Liverpool Plains and other surrounding regions as well as the reduction in chemistries (Umina *et al.*, 2019). In addition to resistance management this is due to health and environmental concerns as well as export markets such as Europe which have already banned some of the chemistries still available in Australia. Such chemicals may in the near future become unavailable for these reasons. Consequently, *Trichogramma* and other egg parasitoids should be integral to the insecticide resistance management strategy for the Liverpool Plains.

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We, the Research Master/PhD candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated in the *Statement of Originality*.

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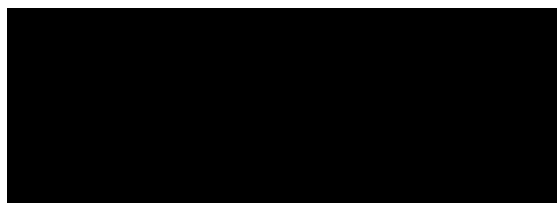
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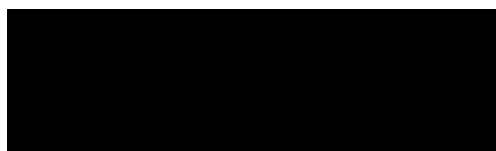
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Chapter 3. Crop and plant-part preferences of *Trichogramma pretiosum* parasitising *Helicoverpa armigera* under glasshouse conditions

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3.0 Abstract

Highly variable rates of parasitised Lepidoptera eggs have been reported between different cropping habitats, between different plants in the same crop and between different parts of the same plant. In this study we measured levels of parasitism by *Trichogramma pretiosum* of a polyphagous pest *Helicoverpa armigera* on a range of its crop hosts which are regularly grown in northern New South Wales. Individual and mixed-species groups of three crop plants were presented in small-cage trials to determine how plant mixes and egg position on the plant affect parasitism rates. We separated winter/spring crops into group 1 (linseed, canola and wheat) and group 2 (lucerne, adzuki and faba bean) and investigated a third group of summer crops (cotton, maize and sorghum). The mean percentage parasitism across the range of crop plants varied from 7% (adzuki) to 42% (canola and faba bean). The group 1 crop plants had similar mean percentage parasitism levels: linseed (40%), canola (42%) and wheat (37%). Egg parasitism on group 2 crop plants differed between adzuki (7%), lucerne (26%) and faba bean (42%). Parasitism of summer crops also varied with cotton (34%) preferred to sorghum (19%) or maize (9%). Crop configuration (i.e. different combinations of the three crops in each group) had no effect on parasitism

rate, but plant part had a significant effect, with more egg parasitism occurring on leaves and stems than flowers in each group. The results have implications for *Trichogramma* preferences for crop habitats and plant part specificity, and the role of *Trichogramma* in biological control is discussed. Specifically, the density of releases may need to be adjusted for different crops based on the movement of *T. pretiosum* around the crop plant. The combination of crop plants had little effect on parasitism rate on individual plants, which suggests inter-cropping or cover cropping does not change individual plant searching behaviour under glasshouse conditions. Field observations are warranted to confirm this.

3.1 Introduction

Chalcid wasps in the genus *Trichogramma* (Hymenoptera) are egg parasitoids and important economical biological control agents of lepidopteran pests (Smith, 1996). *Trichogramma* host location involves a hierarchy of behavioural activities and decisions to seek resources (Keller, 1999). Most *Trichogramma* species are generalists (Pinto *et al.*, 1989b), and their hosts such as *Helicoverpa* (Hardwick) attack a wide variety of plant species (Fitt, 1989). Several studies have suggested that *Trichogramma* species are more habitat-specific than host-specific (Salt, 1935; Andow and Prokrym, 1990; Kester and Barbosa, 1991; Barbosa and Benrey, 1998; Romeis *et al.*, 2005b; Fatouros *et al.*, 2007). This may be a co-evolutionary outcome of the host actively avoiding being detected, so that the parasitoid needs to use more than host stimuli to locate a host (van Lenteren *et al.*, 2006). The role of plant cues is important in host location by *Trichogramma* (Altieri *et al.*, 1981a; Nordlund *et al.*, 1985a; Bjorksten and Hoffmann, 1998), including plant morphology traits such as plant volatiles, trichomes, and floral and extrafloral nectaries (War *et al.*, 2016).

Trichogramma species are useful biocontrols in a range of pest and host systems and an understanding of their behaviour in relation to crop choice is essential for optimising their use in biological control programmes (Bjorksten and Hoffmann,

1998; Gurr *et al.*, 2012). For example, biological control agents may not be able to manage pest insect populations successfully due to limited host-searching capacity (Murali-Baskaran *et al.*, 2017). Factors such as plant diversity play a defining role in host availability and their natural enemies. Root (1973) called this the 'enemies hypothesis' where increased plant diversity (polyculture) enhances the actions of natural enemies by providing more resources for natural enemies than monocultures. The hypothesis assumes a greater range of alternative hosts or prey, plant-derived foods and a more favourable microclimate in polycultures than monocultures (Landis *et al.*, 2000; Jervis, 2007; Iverson *et al.*, 2014). In addition, certain plants may contain chemicals that directly attract or retain parasitoids in diversified cropping systems (Andow and Risch, 1987). Some plants like tomatoes have been shown to stimulate parasitism by *Trichogramma* (Nordlund *et al.*, 1985b) and in combinations of tomato, bean and maize it is not clear if these additional plants help retain or attract *Trichogramma* (Nordlund *et al.*, 1984). Previous work by Altieri *et al.* (1981b) recorded that parasitisation of *Helicoverpa zea* eggs by *Trichogramma* species was significantly higher in soybean–maize intercropped plots (48%) than in weed-free soybean monocultures (22%). Letourneau (1987) showed that *T. pretiosum* parasitism rates of *Diaphania hyalinata* eggs were higher on squash interplanted with maize and cowpea (18%) than in squash monoculture (9%). More recently Manandhar & Wright (2015) found variable rates of parasitism on corn silks comparing three treatments: intercropping corn with sunn hemp (48%), corn monoculture with release of *T. pretiosum* (63%) and a maize monoculture control (15%). They concluded the intercropped plots with higher parasitism was due to enhanced activity of naturally occurring parasitoids, encouraged by floral resources. On the other hand, Andow & Risch (1987) demonstrated increased *T. minutum* parasitisation by a factor of two in monoculture maize – bare ground fields (90%) than in polyculture fields of maize, bean and squash (45%). Seasonal phenology of parasitism, however, was similar in both.

Trichogramma parasitisation also varies with the plant structure or part where the host eggs have been laid. This may be due to their preference for landing on certain parts of the plant (Süverkrupp, 1997). Once on a plant, *Trichogramma* wasps have been observed to walk and undertake short jumps around the different structures of the plant (Schmidt *et al.*, 2003), which affects their ability to negotiate different plant parts and therefore find and parasitise eggs. This has implications as to how *Trichogramma* regulates host populations because the relative amount of different plant parts could affect their ability to find hosts at a high enough rate for successful biological control.

In this study we measured the parasitism of *H. armigera* by *T. pretiosum* on different groups of crop plants and their parts to answer the following questions: (1) does the mix of crops influence the level of parasitism in three crop-choice combinations – (a) wheat, linseed and/or canola, (b) adzuki bean, faba bean and/or lucerne, and (c) sorghum, maize and/or cotton? (2) Is the rate of parasitism affected by the position of *H. armigera* eggs on the plant (that is, on flowers, leaves or stems)?

3.2 Materials and methods

3.2.1 Parasitoid

Trichogramma pretiosum wasps were sourced from Bugs for Bugs™, Mundubbera, Queensland. Bugs for Bugs obtains wild type *Trichogramma* each year from south-eastern Queensland to mix into its colony and was the only commercial supplier of *T. pretiosum* in Australia at the time of the experiment. *T. pretiosum* arrived as parasitised *Sitotroga cerealella* (grain moth) eggs on Post-it® notes. The newly emerged *Trichogramma* were conditioned to search for *H. armigera* eggs rather than *Sitotroga cerealella* eggs by rearing them through two generations on *H. armigera* eggs to reduce the association with *Sitotroga cerealella*. Mansfield and Mills (2004) showed that *Trichogramma* have innate behaviour to search for the host they emerged from. Once the adult *T. pretiosum* wasps emerged, both males and females were caged with

a honey and water-soaked cotton dental wick for an initial period of 6 hours. Strips of *Helicoverpa armigera* eggs on paper towelling were then hung on the inside of each container and were parasitised. When the next generation of *T. pretiosum* emerged 9–12 days later, having been maintained at $21^{\circ} \pm 2^{\circ}\text{C}$, they were again given *Helicoverpa armigera* eggs to parasitise. The ensuing *T. pretiosum* generation was used in this experiment. The newly emerged female wasps were put into 250-mL containers that had plastic strips with smeared honey solution and could mate overnight with six males at $21^{\circ} \pm 2^{\circ}\text{C}$ with a photoperiod of L16:D8 in the rearing laboratory. The mated and fed female *T. pretiosum* (7–10 hours old) were captured in empty gelatin capsules (size 00) and the capsules marked with batch number and sex. The capsules were placed in a plastic container and the wasps released from the capsules inside each cage. The cage was sealed and *H. armigera* eggs collected 10 hours later and placed in numbered capsules with small pin holes. The number of parasitised eggs was recorded at 5 days and emergence after ~10 days. Temperature in the glasshouse was on average 18.2°C in replicate one and 17.2°C in replicate two.

3.2.2 Egg host

Helicoverpa armigera pupae were obtained from the NSW Department of Primary Industries' (NSWDPI) Tamworth Agricultural Institute from a laboratory colony fed on a soybean composite diet. *Trichogramma* can successfully parasitise infertile eggs within the first 48 hours before egg collapse (Calvin *et al.*, 1997). To avoid this, pupae were separated into male and female containers and once they hatched, approximately 25 male and female pairs were raised in each laying chamber in a laboratory at the University of New England. *H. armigera* moths laid on nappy-liner fabric, which was then cut into segments (2×2 mm) each containing a single *H. armigera* egg and stuck on the plant using a flour and water mix to avoid commercial glues with unknown volatile effects. Use of eggs on the nappy liner meant retention of a small amount of *H. armigera* scales to mimic field conditions. Hence both oviposition and kairomone cues used by *Trichogramma* to locate host eggs were

present. Segments were glued to the plant: five eggs on the stem, 10 on the leaves and five on or near flowering parts, and the position of the host eggs was recorded. Eggs were placed at least 2 cm apart as *Trichogramma* cannot immediately detect eggs further than 4 mm apart (Wajnberg *et al.*, 1994) and so re-searching was key to finding eggs.

H. armigera eggs were collected on the second day of laying to increase the probability of fertile eggs and were still white when used in the experiment.

Helicoverpa development time is only 3–5 days depending on temperature (Scholz, 1990), thus 1–2-day-old eggs gave the best chance of host egg parasitism. Pizzol *et al* (2012) demonstrated that 1–2-day-old host eggs were four times more likely to be parasitised than 3–4-day-old eggs by *Trichogramma* in *Lobesia botrana*. Ruberson and King (1993a) showed that suitability of *H. zea* eggs for parasitism by *T. pretiosum* declined with age, and that adult emergence did not differ when parasitised at 14 hours (97%) or 38 hours (93%) but had declined significantly (42%) in 62-hour-old host eggs.

3.2.3 Plant hosts

Helicoverpa armigera host plants were grown in a heated greenhouse at the University of New England. The seeds were sourced from NSW DPI research trials at Breeza Research Station and represent those commonly grown on the Liverpool Plains, NSW: cotton, sorghum and maize (typical summer crop plants), and faba beans, adzuki beans, lucerne, wheat, canola and linseed (typical spring crop plants). Crop plants were sown in early February 2008 and were given AquaGro L® (25.5 L ha⁻¹, Aquatrols®, Paulsboro, New Jersey, USA) every 3–4 weeks. Plants 8–12 weeks in age at the early flowering stage of each species were used, except that the adzuki plants did not flower. Whole plants were used in experiments and each pot was uniquely numbered and contained two of the same crop plant in each pot. No insecticides were used throughout the duration of the experiment. Organic pest control involved the physical removal of aphids and other insects.

3.2.4 Experimental design

The experiment was run over two consecutive 10-day periods: replicate 1 from 12–16 May and replicate 2 from 21–25 May 2008. Cages (2 m tall, 1 × 1.5 m) were made from white loosely woven polyester voile. Each cage was placed on a steel bench top with the top corners of the cage suspended with rope from the ceiling. One benchtop had the winter crop plants (four cages) and another had the summer crop plants. Crop plants were separated into three groups: group 1, linseed, canola and wheat; group 2, lucerne, adzuki and faba bean, and group 3, cotton, maize and sorghum (Table 3.1).

Table 3.1: The crop and cage combinations used in each group of experiments. Group 1: linseed (1), canola (2) and wheat (3); group 2: lucerne (4), adzuki (5) and faba bean (6); group 3: cotton (7), maize (8) and sorghum (9).

Group 1				Group 2				Group 3			
#	Cage	Combination	Day	#	Cage	Combination	Day	#	Cage	Combination	Day
1	1	111	1	1	3	444	1	1	5	777	1
2	1	112	2	2	3	445	2	2	5	778	2
3	1	113	3	3	3	446	3	3	5	779	3
4	1	222	4	4	3	555	4	4	5	888	4
5	1	223	5	5	3	556	5	5	5	889	5
6	2	221	1	6	4	554	1	6	6	887	1
7	2	333	2	7	4	666	2	7	6	999	2
8	2	332	3	8	4	665	3	8	6	998	3
9	2	331	4	9	4	664	4	9	6	997	4
10	2	123	5	10	4	456	5	10	6	789	5

3.2.5 Statistical analysis

Generalised linear mixed models (GLMM) were used to analyse the proportion of eggs parasitised in each group of experiments (Table 3.1) using a logit link function and a binomial error distribution in ASReml 4.1 (Gilmour *et al.*, 2015). Restricted maximum likelihood (REML) estimates variance components in mixed models assuming Gaussian random terms and has been widely adopted (Gilmour *et al.*, 1995). In series of models analysing each group of three crops in turn, crop and crop combination were treated as fixed effects. In the other series of analyses, crop

species, plant part and their interaction were treated as fixed effects. In all analyses, replicate, day nested within replicate and cage nested within day nested within replicate were treated as random effects. To investigate the relative amount of egg parasitism among just the flowers of each crop species in each group, *t*-tests were undertaken in Excel, comparing the percentage egg parasitism among the flowers of each plant (pooling across replicates, $n = 20$) between each pair of species. In the combinations where there was only one plant type (e.g. canola x 3, or CCC) these were averaged. Nectar has been well established in the literature as important for parasitoid and specifically *Trichogramma* fitness and thus we considered it relevant to focus on this treatment effect.

3.3 Results

The parasitism rates by *T. pretiosum* varied among the different crop species across the three groups. Mean percentage parasitism varied from 7% (adzuki) to 42% canola and faba bean (Fig. 3.1). There was consistently higher parasitism in replicate 2 than in replicate 1, but the relative differences between species were preserved (Fig. 3.1). The group 1 crop plants had similar mean parasitism levels, the effect of crop being non-significant: linseed (40%), canola (42%) and wheat (37%). Group 2 crop plant parasitism levels were more variable, with crop being significant ($P < 0.001$): lucerne (26%), faba (42%) and adzuki (7%). Egg parasitism of the summer crops also varied (effect of crop, $P = 0.002$), with cotton (34%) attracting more egg parasitism than sorghum (19%) or maize (9%) (Fig. 3.1). Crop combination within crop was not significant in analyses of any of the three groups of crops (group 1, $P = 0.896$; group 2, $P = 0.742$; group 3, $P = 0.124$), meaning that parasitism did not vary with different combinations of crop plant in each group (Fig. 3.2).

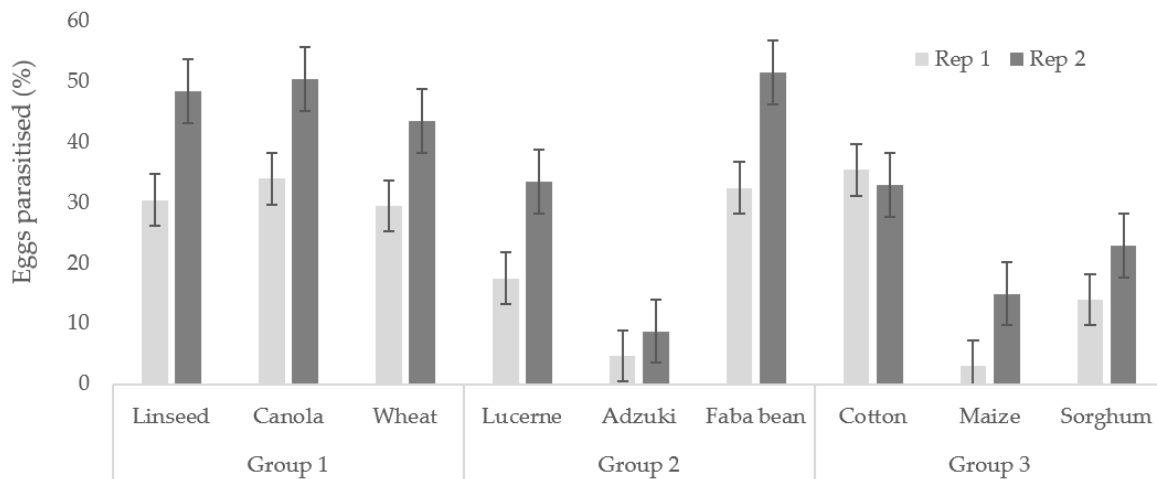


Figure 3.1: Percentage parasitism of *H. armigera* eggs by *T. pretiosum* on nine crops in two different replicates of each combination of crop plants per each group. Egg parasitism data for stems, leaves and flowers were pooled. Data are mean parasitism percentage \pm 1 s.e.m.

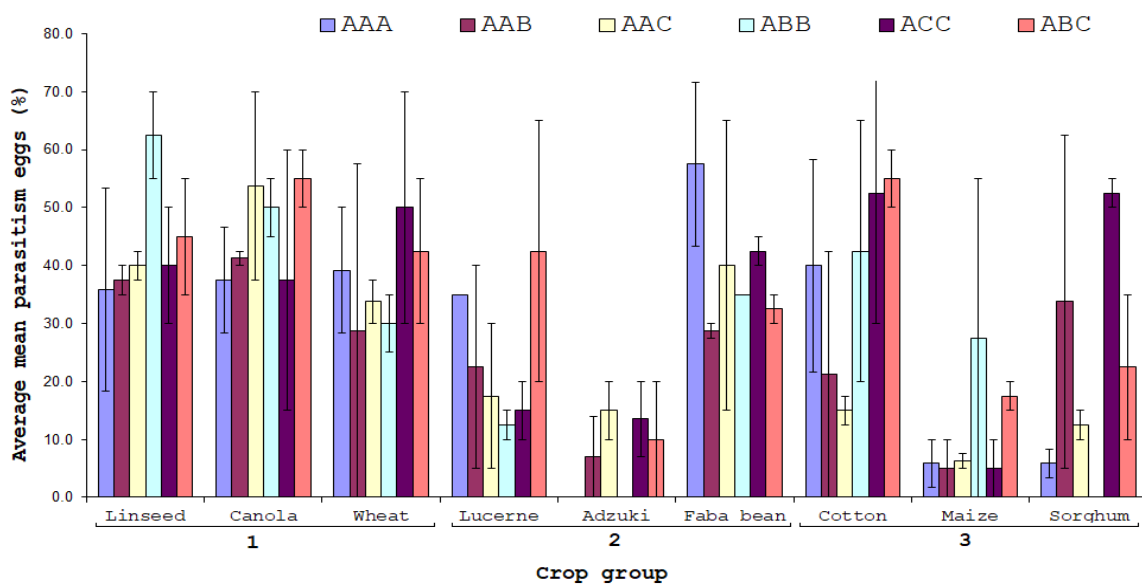


Figure 3.2. Mean percentage parasitism (\pm 1 s.e.m.) of *H. armigera* eggs in three groups (1–3) of three crop plant species (A–C) each in six combinations (AAA, AAB, etc.). Each crop species is species A in the six combinations shown per species. Data are pooled over plant part.

Plant part had a significant effect on parasitism levels in all three groups of experiments. Overall, *T. pretiosum* preferred to parasitise eggs on stem and leaves rather than flowers (Fig. 3.3), an effect that was consistent across crop species as the interaction between crop and plant part (leaves, stems, flowers) was not significant

in analyses of any of the three crop groups. In group 1 (linseed, canola and wheat, the effect of plant part was highly significant ($P < 0.001$), with egg parasitism on flowers (17%) much less than levels recorded on leaves (44%) and stems (49%). In group 2 (lucerne, adzuki and faba bean), flowers (11%) were again less favoured for egg parasitism than leaves (20%) or stems (25%; $P = 0.011$) as was the case with group 3 crops (cotton, maize and sorghum): flowers (5%), leaves (19%) and stems (19%; $P < 0.001$). Although not significant at $P = 0.05$, the interaction between crop species and plant part was close to being significant in the group 1 experiment ($P = 0.076$), and inspection of Fig. 3.3 suggested that the relative levels of parasitism among flowers varied most between the three species. Mean parasitism of *Helicoverpa* eggs (± 1 s.e.) among linseed and canola flowers was $29 \pm 7\%$ and $20 \pm 5\%$, respectively, and significantly greater than egg parasitism among wheat flowers ($10 \pm 3\%$) in the case of linseed ($t = 2.57$, $P = 0.014$, $n = 38$). In the other groups, similar tests suggested that egg parasitism among flowers of faba bean ($33 \pm 6\%$) was greater than for lucerne ($12 \pm 6\%$; $t = 2.57$, $P = 0.014$, $n = 38$), and floral parasitism in cotton ($20 \pm 3\%$) was greater than in maize ($3 \pm 2\%$; $t = 2.48$, $P = 0.021$, $n = 38$).

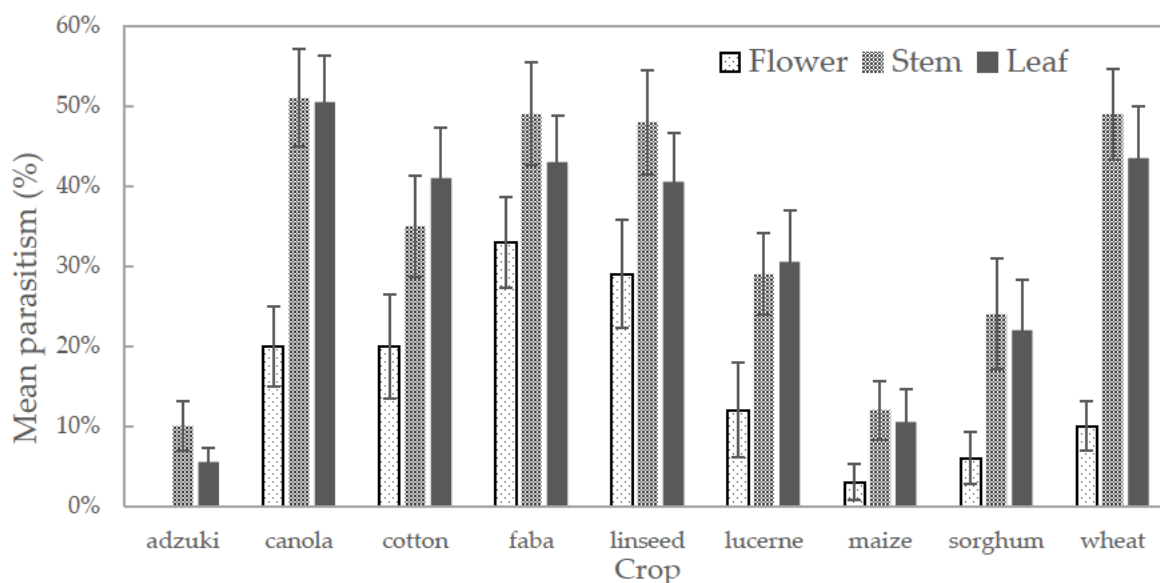


Figure 3.3. Mean percentage parasitism (± 1 s.e.m.) on leaves, stems and flowers of nine crop species. Replicates 1 and 2 were pooled across nine crop plants in the experiment

3.4 Discussion

3.4.1 Combinations of crop plants

In our study, different combinations of the three crop species in each group did not affect the level of egg parasitism on individual plant species. In the various combinations of sorghum, maize and cotton, egg parasitism by *T. pretiosum* on each crop species was consistent (crop combination nested within crop term, $P = 0.124$), meaning that egg parasitism on a given crop species was not affected by the identity of its companion crop plants, although mean parasitism varied between cotton (34%), maize (9%) and sorghum (19%). We did not observe higher rates of parasitism in cotton when mixed with sorghum unlike Scholz and Parker (2004), who found higher parasitism rates when sorghum (42%) was intercropped with cotton (56%). In the other two groups, there was similarly no effect of crop combination on *T. pretiosum* parasitism levels. This suggests that *T. pretiosum* will readily parasitise *Helicoverpa* eggs in canola (42%), linseed (39%) and wheat (36%), or faba bean (42%) and (to a lesser extent) lucerne (25%) and adzuki (7%), regardless of the proximity of the other crops in each group. This is relevant to cover cropping or inter-cropping with species other than the main crop type, which is becoming more widely used in the grains and cotton industry in northern NSW. The experiment aimed to see if *T. pretiosum* would be distracted by other crop plants that may be more or less favourable. However, we didn't see significant differences in having a mix or the same species in close proximity. Although combinations of crop plants did not significantly affect parasitism of the stem, leaf and flower of our crop plants, field conditions are very different to the glasshouse. In theory, chemicals contained in certain plants can attract and help retain parasitoids in diversified cropping systems (Altieri *et al.*, 1981a; Nordlund *et al.*, 1984). Prior to the introduction of industrial chemical insecticides 65 years ago, crop combinations in conjunction with other cultural practices were widely utilised in a holistic approach to farming (van Lenteren *et al.*, 2018). Combinations of different plant cultivars may improve biological control, but there is insufficient research in relation to this (Stenberg *et al.*,

2015). More recently Bustos-Segura et al. (2017) demonstrated in small 4 × 4-m plots with three different plant populations of *Brassica* that the parasitoid, *Diaeretiella rapae* (Hymenoptera: Braconidae), was more abundant in higher diversity plots than those containing a single variety of *Brassica*. By measuring parasitism in different crop combinations, we determined if certain plants reduce the biological control capability of *T. pretiosum* due to limited host-searching capacity.

At a larger spatial scale, the movement of natural predators into crops as well as the design of agricultural landscapes that reduces pests and encourages natural predators is a goal of agroecology (Gagic et al., 2018). Certain crops in the landscape can encourage *Trichogramma* as has been demonstrated by Perović (2009) in cotton, which benefits from an increased area of planted cotton. Several studies have shown cotton harbours high levels of *Trichogramma* (Lopez et al., 1982; Scholz, 1990; Kring and Smith, 1995; Davies et al., 2011a). Sorghum has been highlighted as a crop that supports a wide range of natural predators (Schellhorn et al., 2008a) and *Trichogramma* has reached high parasitism levels in sorghum (Scholz, 1990; Romeis et al., 1999a; Scholz and Parker, 2004), keeping pests below economic thresholds. The volatile chemicals from the plants themselves have been attributed to attracting *Trichogramma*. For example, Romeis et al. (1997) demonstrated that volatiles emitted by sorghum panicles during flowering and the soft dough stage attracted *T. chilonis*, but concluded that host kairomones and host eggs must also be present to sustain a high parasitoid population. A similar effect has been reported for *T. pretiosum* in maize, where volatiles emitted by the plants have been shown to increase parasitisation rate (Scholz, 2000). Future studies could establish if cultivar type affects *T. pretiosum*, which may have additional benefits for the retention of parasitoids within crops after a biological control release.

3.4.2 Parasitism on stem, leaf and flower

In our study, plant part had a significant effect on *T. pretiosum* parasitism levels, with significantly less parasitism of *Helicoverpa* eggs among flowers than leaves or stems

in all three groups of crops. This was a consistent finding in each experiment (Fig. 3.3). Although the interaction between crops species and plant part was not significant in any experiment, the interaction was marginally significant for group 1 (canola, linseed, wheat), and inspection of Fig. 3.3 suggested that the principal difference was the relative amount of parasitism among flowers of the three different crops species (egg parasitism among the flowers of: canola, 20%; linseed, 29%, and wheat 10%). Wheat flowers are minute compared to linseed or canola which have white and yellow flowers, respectively. Similarly, among the group 2 plants, faba bean (33%) with white flowers had higher mean parasitism of eggs located among inflorescences than lucerne (12%) with purple flowers. In the group 3 plants, cotton (20%) with white flowers had higher mean parasitism of eggs among inflorescences than either of the cereals (sorghum, 6%, or maize, 3%). *Trichogramma* wasps are known to be influenced by flower colour in their choice of plants, although studies that determine flower recognition are limited. In a study by Begum et al. (2004) using flight cage experiments, *Trichogramma carverae* preferred alyssum (*Lobularia maritima*) with white flowers to cultivars with light pink, dark pink or purple flowers. Whilst we did not test specifically for flower colour, the apparent preference of *T. pretiosum* for parasitising eggs among the white flowers of linseed and faba bean compared to canola, lucerne and cereals may be due to colour effects.

Trichogramma are known to utilise pollen and nectar, which increase longevity and fecundity (Andow and Risch, 1987; Gurr and Nicol, 2000; Heimpel and Jervis, 2005; Tian et al., 2016). The *T. pretiosum* used in this experiment had been fed a water/honey mix prior to the choice trials and were only left in cages containing crop plants for 10 hours, so there could have been little necessity for wasps to visit flowers for extrafloral nectar (sugar searching), providing time for host searching. Wäckers (1994) demonstrated in the larval parasitoid, *Cotesia rubecula* (Hymenoptera: Braconidae), that food-deprived wasps chose flower odours while sugar-fed individuals preferred host-associated odours. There is a trade-off between host

searching and carbohydrate searching in *Trichogramma* (Tenhumberg *et al.*, 2006). Egg parasitoids can decide whether to use a particular host for food or reproduction (Heimpel and Collier, 1996) and most studies have examined parasitoid species that do not engage in host-feeding (Balzan and Wäckers, 2013). Begum *et al.* (2006) found increased survival in *T. carverae* on shoots and flowers than on plants with flowers removed or with no shoots. In studies by Somchoudhury and Dutt (1988), *T. perkinsi* and *T. australicum* longevity and fecundity did not increase when provided with flowering maize or sorghum, suggesting that the two *Trichogramma* species are perhaps unable to utilise these pollen sources. Wäckers (2004) suggested that some flowers may even be repellent to *Trichogramma*, and this warrants further study. Our study demonstrates that flowers with host eggs are not parasitised as readily as other plant parts by *T. pretiosum*. This would suggest that *T. pretiosum* capsules be attached in the field to leaves and stems rather than flowers for biological control. This result also has implications for choosing flowers as an attractant in habitat manipulation. For instance, Witting *et al.* (2007) measured a significant difference in *Trichogramma* abundance attributed to flower species and height when comparing six habitat plants (black-eyed Susan, buckwheat, fennel, cock's comb, Shasta daisy and yarrow), but when the flowers were removed there was no effect on abundance. Witting *et al.* (2007) concluded that *Trichogramma* was attracted to these plants but flowers did not appear to be responsible for this attraction.

Overall the average mean parasitism by *T. pretiosum* was similar on leaves (32%) and stems (34%) compared to flowers (15%). Micro-climate is an important factor for *Trichogramma* survival due to their small size and vulnerability to weather events (Romeis *et al.*, 2005a). Bouchier and Smith (1996) argued that this can hinder their ability to parasitise hosts irrespective of host and biological parameters, such as fecundity and host acceptance. *T. pretiosum* may for instance avoid wet substrates when looking for host eggs during rain (Keller *et al.*, 1985) or can be affected by high temperatures (Ramesh and Baskaran, 1996). We found in cotton that mean

parasitism on leaf (41%) and stem (35%) was similar. Keller and Lewis (1985) found that flight activity of *T. pretiosum* when released to control *Helicoverpa* was higher within the plant canopy of cotton which they attributed to sheltering from high temperatures and ambient winds. In the group 1 plants (canola, wheat, linseed), egg parasitism ranged from 40% to 51% and was similar on stems (49%) and leaves (44%), but there was no statistical difference between them. The effects of spatial differentiation in the preference of *T. pretiosum* parasitising host eggs has implications for their control potential in the field (in terms of enemy-free space).

We recorded low rates of parasitism on adzuki bean most likely because of the hairy nature of the plant surfaces. The hairy leaves and stem may reduce *Trichogramma* walking speed and possibly entrap them, reducing their ability to locate a host (Rabb and Bradley, 1968; Keller, 1987). Chickpea also has hairy plant surfaces and is not a favoured *Trichogramma* habitat (Scholz, 1990; Llewellyn, 2006). However, chickpea is distinct in that the species has acidic trichomes and the exudates are secreted on all green parts of the plant, making it a lethal surface for surface-roaming insects (Romeis *et al.*, 1999c; Wakil *et al.*, 2009). Low egg parasitism by *Trichogramma* has been recorded on other plants with trichomes, including tobacco (Rabb and Bradley, 1968), wild tomato (Kauffman and Kennedy, 1989) and pigeon pea (Romeis *et al.*, 1999b). However, adzuki bean does not have acidic trichomes like chickpea, which is why we recorded some parasitism on stems ($10 \pm 3\%$) and leaves ($5 \pm 2\%$). If *Helicoverpa* moths prefer to oviposit where *T. pretiosum* are unlikely to parasitise their eggs (e.g. on hairy trichomes or flowers), then the biological control role of *Trichogramma* could be hindered.

We recorded lower rates of parasitism in lucerne on leaves (29%) and stems (30%), than for canola, faba bean, linseed, wheat, or cotton (35–51%), although lucerne has previously been recorded as attractive to *Trichogramma pretiosum* (Scholz, 1990). Altieri *et al.* (1982) used an extract of lucerne to increase *T. pretiosum* parasitism of faba bean from 2.3% to 4.7%. Differences between our experiment and the field

might be due to our use of cultured parasitoids, cultured eggs and artificial egg placement as well as not assessing all stages of crop phenology.

The temperatures in the glasshouse where the experiments were conducted could ideally have been warmer as we are unable to rule out the possibility that low temperatures reduced the movement of *T. pretiosum*, as has been demonstrated by Pratioli et al. (2004). They found the optimum temperature to be 22–25°C. However, the second replicate had higher parasitism rates across most crop species despite being conducted under slightly lower temperatures than replicate 1, suggesting that there may have been a problem with the quality of the *Trichogramma* used in the first replicate. Another potential cause that was not measured was *Helicoverpa* egg quality, which may have been impacted by a change in host generations. Overall, the study suggests that inter-cropping will neither enhance nor detract from effective egg parasitism by *T. pretiosum* in a crop situation. Potentially, *T. pretiosum* could be released in canola, linseed, faba bean and wheat and have similar levels of parasitism and these crops may be useful for releasing egg parasitoids in spring to build up their populations as part of an IPM strategy to reduce chemical control of *Helicoverpa*. This is part of a wider strategy to improve conservation biological control on the premise that mixing different crop species increases prey diversity and explicitly brings together complementary natural enemies (Snyder, 2019). We found that crop combination did not affect parasitism rate at a micro-scale, but it may produce different effects at field scale as *Helicoverpa* choice of crops for oviposition is affected by factors such as predator avoidance and host plant nutrition.

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STATEMENT OF AUTHORS' CONTRIBUTION

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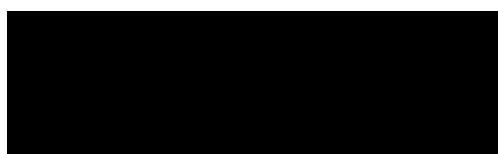
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Chapter 4. Inoculative release of a beneficial wasp egg parasitoid, *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae), to supplement natural wasp populations for control of *Helicoverpa* in northern New South Wales field crops

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4.0 Abstract

Increasing the impact of natural enemies to combat pest insect populations requires not only conserving natural enemies but utilising methods to increase their populations to be effective against pest outbreaks. On the Liverpool Plains in northern New South Wales, *Helicoverpa* (Hardwick) is a seasonal pest of several winter and summer crops. Chickpeas, linseed, canola, sorghum and maize are particularly vulnerable to attack above economic thresholds, requiring sometimes repeated insecticide applications. Naturally occurring populations of the egg parasitoid, *Trichogramma pretiosum* (Westwood), have been identified on the Liverpool Plains, but reach peak activity when most of the summer crops have

already reached maturity and are at levels below those considered effective for economic control. In the weeks when *Helicoverpa* historically immigrate in large numbers on the Liverpool Plains, we tested rates of early-season inoculative *T. pretiosum* releases at 30,000 wasps/ha and 60,000 wasps/ha in 5-ha plots every week for 3 weeks in wheat and canola across 12 farms in the Breeza–Quirindi landscapes. We measured movement away from the release area in 50 and 100-m intervals. We found high levels of *Helicoverpa* sentinel egg card parasitism in these crops within the release area. However, we found that *T. pretiosum* did not move far from the release area beyond 50 m. Furthermore, we trapped egg parasitoids in white and yellow water traps and measured *Helicoverpa* sentinel egg card parasitism in cotton, sorghum, native forest and native grassland habitats in the subsequent summer in January and February in adjacent and more distant habitats. We also compared natural egg parasitoid populations from seasonal surveys from the previous 2 years. We found a significant increase in *T. pretiosum* following the inoculative spring release in summer crops, compared with the previous 2-year survey. We found *T. pretiosum* had higher populations in cotton crops near the spring release sites and that populations decreased over 2 km away from the release sites, but we found less of an effect in other habitats away from the spring inoculative release sites.

Our results suggest that supplementing *T. pretiosum* populations in spring by inoculative release in wheat and canola may lead to a significant reduction in *Helicoverpa* populations at the egg stage in these crops and decreased economic damage. Furthermore, the effects of supplementing *T. pretiosum* populations in spring boost populations, which persist in summer crops and peak earlier, may give sustained protection from *Helicoverpa* egg hatch and a reduced need for chemical applications.

4.1 Introduction

In spring on the Liverpool Plains in northern New South Wales (150° 28'E, 31° 13'S), the foremost crop insect pest is the native, migratory, multivoltine, polyphagous moth, *Helicoverpa* (Hardwick). It emerges locally as well as immigrating up to 2000 km to infest canola and leguminous crops predominantly, and attacks cotton, sorghum and maize through the summer cropping season. Two endemic species of economic importance on the Liverpool Plains are *Helicoverpa armigera* (Hubner), an Old World species, and the facultative migrant *Helicoverpa punctigera* (Wallengren)(Downes and Mahon, 2012). They are seasonally dynamic, with four to five generations per season and a winter pupal diapause in northern NSW (Fitt, 1989). Control and crop damage causes losses in excess of US\$5 billion each year across Asia, Europe, Africa, America and Australia (Pearce *et al.*, 2017). The primary means of controlling *Helicoverpa* in cotton is genetic modification (GM) using *Bt* toxins. Resistance to insecticides is a recurrent problem in the management of *Helicoverpa* (Zalucki *et al.*, 2009a) and, in particular, *H. armigera*, which has become increasingly difficult to control due to widespread resistance to synthetic chemical insecticides (Downes and Mahon, 2012). Continued sustainability of GM cotton production in northern Australian cotton-growing areas is dependent on effective parasitoid biological control (Davies *et al.* 2011). The same could apply on the Liverpool Plains for the same reasons, but the situation is less studied.

Trichogramma is a genus of idiobiont egg parasitoids in the Trichogrammatidae (Hymenoptera) and is specialised to develop in the eggs of several other insect orders, including Lepidoptera. *Trichogramma* species occur naturally in almost every terrestrial habitat, and attack a wide range of pests in field crops, forests and horticultural crops (Almeida, 2000). They are one of the most used biological control agents as they are easily reared on alternative hosts (Parra, 2009). In Australia, native egg parasitoids of *Helicoverpa* belong to two Hymenopteran families,

Trichogrammatidae and Platygasteridae (Scholz, 1990; Masner, 1993). The Platygasteridae are represented by *Telenomus* spp. about which there is little documentation and that are rarely ever identified to species level. *Trichogramma* is an egg parasitoid of *Helicoverpa*. It develops in a *Helicoverpa* egg, taking 7–14 days to emerge, and has a short 5–14-day free-living adult phase searching for eggs to parasitise. Two or three *Trichogramma pretiosum* can emerge from one *Helicoverpa* egg (Scholz, 2000). This makes this parasitoid an effective beneficial insect as it can build up numbers rapidly. There are ten identified native *Trichogramma* species recorded in Australian cotton-growing areas, and another two native species of uncertain taxonomy as well as the introduced American species, *T. pretiosum* (Davies *et al.*, 2011b).

Whilst *Trichogramma* has generally failed to suppress *Helicoverpa* when released in cotton-growing regions (Davies *et al.*, 2011c), the traditional means of inundative release may not be best suited to *Helicoverpa* control (King and Powell, 1992; Scholz and Parker, 2004). The landscape of the Liverpool Plains cropping area is a broad mosaic of winter and summer crops, with few non-crop refuges to promote and sustain natural enemies amidst the ever-changing pattern of short-season cropping. Along with frequent disturbance due to cultivation, insecticide application and harvest in the system, the lack of suitable refuges for natural enemies increases the frequency of pest outbreaks (Landis *et al.*, 2000).

4.1.1 Insect pest management (IPM) and *Trichogramma*

Development of integrated pest management (IPM) principles in agroecosystems has encouraged the increased use of natural enemies for pest control since its inception (Smith and Allen, 1954). IPM promotes the use of insecticides with minimal non-target impact along with spray thresholds and (soft) insecticide options that favour natural enemy populations (Macfadyen *et al.*, 2015). However, IPM has low adoption despite widespread awareness, and the use of natural enemies in

contemporary IPM is rarely utilised (Zalucki *et al.*, 2015). In other words, its adoption at a scale relevant to farmers is lacking (Macfadyen *et al.*, 2015).

Many studies that aim to conserve natural enemies do not assess pest mortality (Furlong and Zalucki, 2010). Information such as parasitism rates of target pest insects is valuable information for farmers to judge the effectiveness of utilising and supplementing natural enemies. The commercial availability of natural enemies means that adoption is limited by confidence in the efficacy of natural enemy releases and their availability from suppliers. For example, there is a limited subset of natural enemies in Australia compared to Europe or North America (Begum *et al.*, 2017). In a similar way to insecticides, which offer short-term pest control in a measured and predictable way (Ekström and Ekbohm, 2011), natural enemy releases can be thought of as a purchasable product to be applied (O'Neil *et al.*, 1998).

Augmentation of natural enemies consists of two alternatives, corrective or preventative (King *et al.*, 1986). The corrective method is to release large numbers of beneficial insects to combat a particular pest. This inundative release method with *Trichogramma* has not been very effective in *Helicoverpa* management (Davies *et al.*, 2011b). The preventative method is to release small numbers of beneficial insects when the pest population is low or developing. Inoculative releases are particularly useful where the parasitoid doesn't commonly exist, or at a time of the year when it is not abundant (Stehr, 1982) or parasitism levels are below 30% (Llewellyn, 2006). Coexistence of pests and natural enemies at low density is an essential feature of inoculative control methods (Pak, 1992). Inoculative release has the potential to be effective against *Helicoverpa*. Less research has been done on inoculative than inundative releases possibly because of the ecological complexity involved and lack of funding (Smith, 1996). Most contemporary IPM programs are implemented with little consideration of ecosystem processes (Kogan, 1998). Ecosystem processes are difficult to model and incorporate in decision-making rules, but they are important and extend beyond the ecological scale of the field (Kareiva and Wennergren, 1995;

Kogan, 1998; Tschardtke *et al.*, 2007b). For this reason, we have gone beyond the field to the larger landscape level in this study to measure the effects of distance and adjacent habitats.

4.1.2 *Trichogramma* in northern NSW

On the Liverpool Plains, peak egg parasitoid activity occurs at the end of January when the summer crops are reaching maturation (Carr, 2009). Before this period *Helicoverpa* parasitoid activity is too low for effective economic control. Whilst other studies have established benefits of native vegetation in harbouring egg parasitoids in Australian field crops (Schellhorn *et al.*, 2008b; Perović *et al.*, 2010), the intensive cropping areas on the Liverpool Plains have little native vegetation adjoining crops. Consequently, one approach is to use the nearby available crops as *Trichogramma* nursery crops. The potential importance of habitat reserves or nursery crops for *Trichogramma* has been highlighted by Scholz (2003) and Díaz *et al.* (2012). Known habitats for low numbers of egg parasitoids in spring on the Liverpool Plains are native vegetation, linseed, canola, sunflowers, faba beans and wheat (Carr, 2009). At this time of the year, the most ubiquitous landscape habitat on the Liverpool Plains is wheat. Although *Helicoverpa* subsists in wheat, it rarely causes economic damage, so insecticidal control of *Helicoverpa* in this crop is limited. The wheat crop habitat is akin to a banker plant system (Frank, 2010) where pests can exist below damaging levels and their natural enemies can build up. Before crop senescence and harvest in December, the egg parasitoids can proliferate into surrounding crops that are by now increasingly prone to increased *Helicoverpa* pressure such as maize, cotton and sorghum, which are the main summer crops on the Liverpool Plains. The key to this concept is to time such releases with *Helicoverpa* emergence, thus giving the best opportunity for *Trichogramma* to establish. Hassan (1989) has suggested that to increase the effectiveness of natural enemies, they should be released when the pest population is low and at the beginning of the occurrence of the pest in the field. Canola is another suitable crop in the Liverpool plains, but is usually less

widespread. This crop is highly attractive to *Helicoverpa*, which often causes economic damage. Canola's advantage as a parasitoid habitat is its floral nectar resources as well as abundance of hosts including the diamond-backed moth, *Plutella xylostella* (Liu *et al.*, 2004). Its disadvantage as a nursery crop is that it is often sprayed with broad-spectrum insecticides because of damaging levels of *Helicoverpa*. This, then, is detrimental to sustaining parasitoids, which are sensitive to chemical applications (Hegazi *et al.*, 2004).

Recent studies have highlighted the need for research identifying the potential of early-season, low-density releases of *T. pretiosum* in cotton landscapes (Scholz and Parker, 2004; Bastos *et al.*, 2010). The idea is not new, having been suggested as a result of theoretical simulation modelling by Knipling and McGuire (1968). To counter the imbalance in pest populations versus the slower build up in populations of natural enemies, the latter could be encouraged to build up in surrounding habitats. On the Liverpool Plains, options for semi-permanent habitat are limited. We propose that it is possible to utilise a whole crop as a natural enemy nursery, which can be effective for natural enemy development at the scale of an individual farm level but also at the regional cropping landscape scale. Early-season low-density releases (inoculation) may be practical, both ecologically and economically, to boost local populations of *Trichogramma*. In this research, we examined the following questions: (1) can early season low-density releases (inoculation) of *T. pretiosum* parasitise *Helicoverpa*? (2) What density is effective? (3) How far does *T. pretiosum* move when released in a crop, and (4) does *T. pretiosum* persist into the summer cropping season if released in a preceding winter nursery crop?

4.2 Materials and Methods

Experiments were conducted between September 2008 and March 2009. Study sites in spring were nine winter wheat crops and nine canola crops ranging from 10 to 100 ha, and approximately 5 km apart over an area 60 km south of Gunnedah (150° 15'E, 30° 59'S) and north of Quirindi (150°41'E, 31°31'S) on the Liverpool Plains, northern New South Wales. The study area was divided up into two landscapes, the Breeza landscape (appendices 6 - 7) and the Carroona–Quirindi landscape (Appendix 8).

Both landscapes are defined by agricultural black alluvial flats of many kilometres in extent along the Mooki River and surrounding hills that are predominantly grassy woodland-forest. The Carroona–Quirindi landscape is characterised by smaller crop paddocks and greater proportion of non-crop vegetation than the Breeza landscape.

The main winter and summer crops grown were wheat (*Triticum aestivum* L.), canola (*Brassica napus* L.), faba beans (*Vicia faba* L.), chickpeas (*Cicer arietinum* L.), lucerne (*Medicago sativa* L.), oats (*Secale cereal* L.), barley (*Hordeum vulgare* L.), sorghum (*Sorghum bicolor* L.), cotton (*Gossypium hirsutum* L.), sunflowers (*Helianthus annuus* L.), mung beans (*Vigna radiata* L.), maize (*Zea mays* L.), pigeon pea (*Cajanus cajan* L.), soybeans (*Glycine max* L.), sweet pea (*Lathyrus odoratus* L.), canary (*Phalaris canariensis* L.) and linseed (*Linum usitatissimum* L.). The native grassland was predominantly plains grass (*Austrostipa aristiglumis* L.) and was similar at all sites.

The open forest was dominated by, white box (*Eucalyptus albens*), with silver-leaved ironbark (*Eucalyptus melanophloia*), white wood (*Atalaya hemiglauca*), bull oak (*Allocasuarina luehmannii*), and ironbark (*Eucalyptus* spp.), with a grassy understorey.

The experiment was a factorial design of two crops x three rates of *Trichogramma* release (high, low and none) x three sampling dates x three replicates. Temperature and wind directions were recorded (La Crosse WS2350 weather station) in two locations, Piallaway (150°30'E, 31°05 S) and Quirindi West (150°28'E, 31°28'S).

4.2.1 Releases

Releases were conducted over a period of 4 weeks between 23 September and 25 October 2008. Releases were timed to coincide with the historical emergence of *Helicoverpa* on the Liverpool plains and were monitored using a 12-V D.C. 8-W light trap (Australian Entomological Supplies, Bangalow, NSW) and pheromone traps (Agronomy Department, University of New England). Studies that compare trap catch, oviposition and parasitism efficacy have shown consistently that the best results are achieved when *Trichogramma* is released a few days before, rather than at the start of, oviposition (Kanour and Burbutis, 1984; Smith, 1996).

4.2.2 Release rates

Populations of *T. pretiosum* were supplied by Bugs for Bugs™ (Mundubbera, Queensland) and reared on an alternate host, *Sitotroga cerealella*. New *T. pretiosum* specimens from the Darling Downs in Queensland are annually introduced into the mass-reared colonies. The suggested medium rate of inundative release in sweet corn (Scholz *et al.*, 1998; Scholz, 2000) in Australia is 60 caps/ha, with each cap containing approximately 1000 unhatched wasp-parasitised moth eggs (60,000 wasps/ha). In our inoculative approach, we used this rate as our high rate. We then used 30 caps/ha (30,000 wasps/ha) as our low rate.

The release area was located in the approximate middle of each crop in a 250 × 250-m marked area. Releases were conducted each week in each crop for 3 weeks. Egg parasitoid sampling was conducted 1 week before the first release (before release), 2 weeks after the first release (mid release) and 1 week following the last release at each site (post release). This achieved relatively consistent populations of *Trichogramma* emerging at contrasting times across the release period. The average release time for *Trichogramma* emergence is typically 7–14 days (Scholz *et al.* 1998, Smith, 1996). Egg parasitoid activity was determined using *Helicoverpa* sentinel egg cards evenly spread along four crop rows with eight egg cards each in the 250 × 250-m release area (Fig. 4.1). One row of four egg cards was used to monitor

parasitism at 50-m and 100-m intervals away from the release area in all cardinal directions (N, S, E and W). The *Helicoverpa* sentinel egg cards were sourced from a *H. armigera* colony that laid eggs on paper towelling. Clusters of fresh *Helicoverpa* eggs, 1–2-days-old were cut out in approximately 1-cm shapes with at least 10 moth eggs and stapled to a yellow card, 5 × 8 cm. These were kept cool in an esky with ice bricks until placed in the field by stapling them to crop plants. Upon collection, 24 hours after deployment in the crop, egg cards were stored carefully for transport to the laboratory where they were maintained at 25°C until eclosion. After 5 days blackened eggs were considered parasitised. Each card was recorded as either parasitised or not parasitised and the numbers of eggs that were parasitised or not parasitised on each card was recorded. If moth eggs were missing (from predation or other factors) they were considered unparasitised. A sample of two parasitised egg cards per crop were placed into a 250-ml container so emerging *Trichogramma* could be identified to species level.

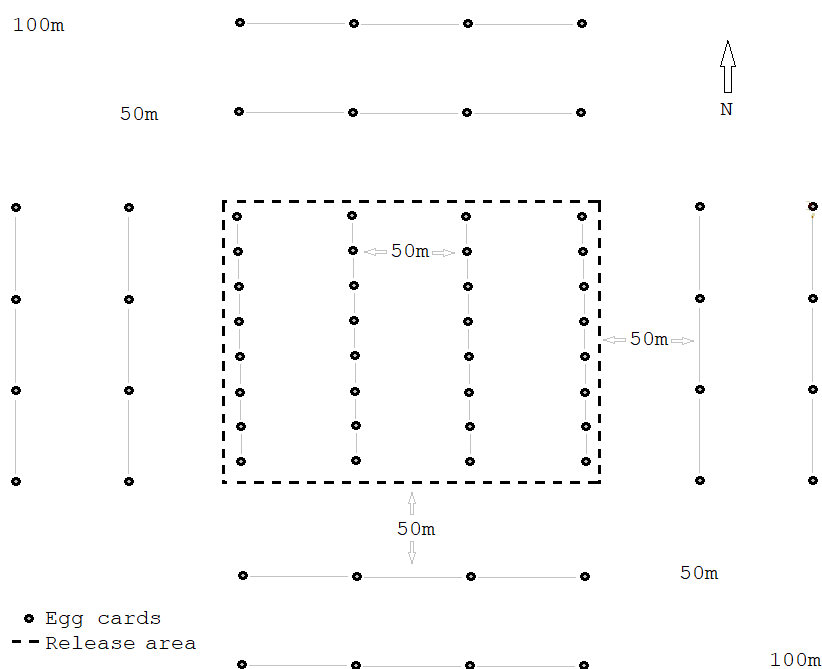


Figure 4.1 Placement of sentinel egg cards in crop.

4.2.3 Summer egg parasitoid abundance in adjoining crops

Three months after the spring releases of *T. pretiosum* in canola and wheat, neighbouring crops of either sorghum (five sites) or cotton (five sites) were monitored for egg parasitoid abundance using *Helicoverpa* sentinel egg cards in the same way as egg parasitoid activity had been monitored in the preceding spring. An additional two sites for each crop were monitored approximately 3–5 km from release sites. Forty egg cards were deployed in each crop on two sampling dates, 23–24 January and 25–26 February 2009.

In summer 2006–07 and 2007–08, five cotton crops and five sorghum crops were monitored for egg parasitoids on farms that later had releases or were close to farms with releases in spring 2008 (Appendices 6 to 8). At each crop, 30 egg cards were deployed. A comparison of average parasitism in these crops was made against four cotton and four sorghum crops that were within 50 m of the release crop and had 40 egg cards deployed.

Water-pan traps were also used to measure parasitoid abundance and detect male egg and other parasitoids that were not detected with sentinel egg cards. Six white plastic plates (diameter 21 cm) were half spray-painted yellow. Previous analysis by Carr (2009) had shown preferences for colour and in this experiment both colours were used on the one plate. In addition to crops, four forest sites (two sites <2 km from the release area and two sites >4 km away) and three grassland sites dominated by plains grass (*Austrostipa aristiglumis* L.) in the Breeza area were monitored using *Helicoverpa* sentinel egg cards and water pan traps along with cotton and sorghum crops to determine relative *Trichogramma* abundance.

4.2.4 Statistical analysis

Generalised linear mixed models (GLMM) were used to analyse egg-card parasitism in the release area using a logit link function and binomial error distribution. GLMM analyses were conducted separately for the mid-release survey and post-release survey, and only considered data from the twelve treated crops in which *T. pretiosum*

was released since parasitism in the six control crops was negligible. Release rate (high vs low) was treated as fixed effect and landscape (Piallaway-Breeza vs Caroonā–Quirindi) as a random effect. GLMMs with a logit link function and binomial error distribution were also used to compare the effect of distance (50 vs 100 m) and direction (N, E, S, W) and their interaction on egg-card parasitism across the 18 crops, treating crop (site) as the random effect. Parasitism rates in wheat and canola crops were determined by averaging the total of parasitised egg cards out of 40.

4.3 Results

4.3.1 Spring release survey in release area

Pre-release surveys in crops yielded few *Trichogramma* with only one *H. armigera* egg card parasitised in each of two wheat crops (one control and one high-release site). One parasitoid was identified as *Trichogrammatoidea bactrae* and the other parasitised egg did not hatch. These were found in the Caroonā–Quirindi landscape (Appendix 6). Since only two of 576 egg cards were parasitised across the 18 sites, we concluded that the background level of egg parasitoids in the study crops at the commencement of the spring fieldwork was negligible and the pre-release data were excluded from further analysis. No egg-card parasitism was detected in control crops in the mid-release survey and only two *H. armigera* egg cards were parasitised at the wheat control site in the post-release survey (Fig. 4.2), so background levels of egg parasitoids in control crops remained at negligible levels throughout the spring fieldwork. An egg parasitoid specimen recovered from the wheat control site in the post-release survey was a Trichogrammatid.

In the mid-release survey, the average parasitism of *H. armigera* egg cards in high and low treatments was 48% and 70%, respectively, which was not quite statistically significant ($F = 4.41$, $p = 0.065$). The impact of irrigation at treatment sites was negligible and the two landscapes, Piallaway-Breeza and Caroonā–Quirindi, had

similar parasitism rates (50% and 54%, respectively; Fig. 4.2). In the post-release survey, the average parasitism of egg cards in high and low treatments was 27% and 37%, respectively, and not significant ($F = 1.21$, $p = 0.314$). The impact of irrigation and landscape was again minor, but this time the effect of insecticide sprays was marked, reducing parasitism in high-rate canola site 8 from 47% to 19% after use of Pirimicarb™, and from 78% to 0% in high-rate canola site 9 after use of Alpha cypermethrin. In low-rate wheat site 4, egg card parasitism decreased from 38% to 6% after Alpha cypermethrin application (Fig. 4.2).

In the mid-release survey, average parasitism of *H. armigera* egg cards in high and low-release sites combined was higher in canola (57%) than wheat (35%), averaging 51% overall. Parasitism of egg cards peaked in the low-rate treatments at 88% in canola and at 78% in wheat (Fig. 4.2). In the post-release survey, the average parasitism of *H. armigera* egg cards in high and low-rate treatments combined was similar in wheat (33%) and canola (35%), averaging 34%.

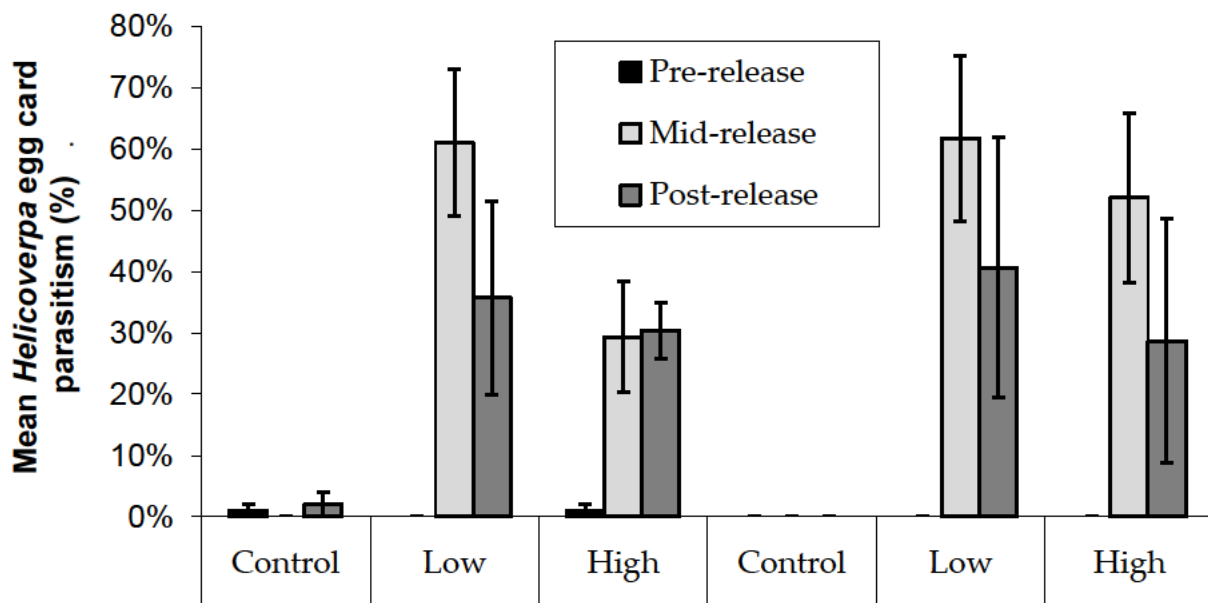


Figure 4.2. Percentage parasitism of 32 *H. armigera* egg cards placed in the *T. pretiosum* release area in the pre-release, mid-release and post-release surveys. The control is wheat and canola crops with no releases, and the low release rate is 30,000 wasps per hectare and high rate is 60,000 wasps per hectare.

4.3.2 Movement from release site in spring

Pre-release, only three (0.5%) of 576 egg cards were parasitised at 50 m or 100 m away from the release area in the 18 control and treatment crops sites, consistent with the negligible level of egg parasitoid activity in release areas prior to the commencement of the experiment (Table 4.1). By comparison, a total of 54 (9.4%) and 52 (9.0%) egg cards were parasitised at 50–100 m from the release area across the 18 crop sites in the mid-release and post-release surveys, respectively. In the mid-release survey, distance from release area significantly affected egg-card parasitism ($F = 24.22$, $p < 0.001$), with average parasitism of 16% of egg cards at 50 m declining to 2% at 100 m (Table 4.1). Direction marginally affected egg-card parasitism ($F = 2.39$, $p = 0.073$), with more parasitism east and west rather than north or south of release areas (Table 4.1); the interaction between distance and direction did not affect parasitism at all ($F = 1.32$, $p = 0.271$). During the mid-release surveys of wheat and canola crops in which *T. pretiosum* had been released, mean egg-card parasitism at 50 m from the release area was 21% and 28%, respectively, and 2% and 5% at 100 m, respectively.

Table 4.1. Average parasitism of egg cards during pre-release, mid-release and post-release surveys at 50 m and 100 m from the release area in each direction, averaged across all 18 crop sites.

Distance from release area	Cardinal direction from release area				Total
	N	E	S	W	
<i>Pre-release</i>					
50 m	0.0%	0.0%	0.0%	0.0%	0.0%
100 m	1.4%	1.4%	1.4%	0.0%	1.0%
<i>Mid-release</i>					
50 m	9.7%	18.1%	12.5%	25.0%	16.3%
100 m	4.2%	2.8%	0.0%	2.8%	2.4%
<i>Post-release</i>					
50 m	11.1%	18.1%	13.9%	12.5%	13.9%
100 m	0.0%	1.4%	11.1%	4.2%	4.2%

In post-release surveys, distance from the target area again significantly affected mean egg-card parasitism ($F = 8.45$, $p = 0.005$), with average parasitism declining from 14% at 50 m to 4% at 100 m from the release area (Table 4.1). Direction did not affect egg-card parasitism ($F = 1.16$, $p = 0.331$), but the interaction between direction and distance was marginally significant ($F = 2.55$, $p = 0.059$), with little difference in percentage parasitism at the two distances south of the release area but marked declines in parasitism with distance in other directions (Table 4.1). Mean egg-card parasitism in wheat and canola crops in which *T. pretiosum* was released was 18% and 23%, respectively, at 50 m from the release area, and 8% and 4%, respectively, at 100 m. The weather stations showed that prevailing winds were easterly during the release, perhaps explaining egg-card parasitism of 25% at 50 m west of release areas in the mid-release surveys (Table 4.1).

4.3.3 Summer survey

In the 40 *H. armigera* egg cards placed in each of the 21 sites in January and February in cotton, forest, grass, and sorghum, there was decreasing parasitism detected with increasing distance from the release sites (Fig. 4.3). This was more apparent in cotton (Fig. 4.4), and less pronounced in sorghum which had almost entirely *T. pretiosum* present, and there was limited parasitism of egg cards in the grass sites which had few *T. pretiosum* present and more species of other Trichogrammatids.

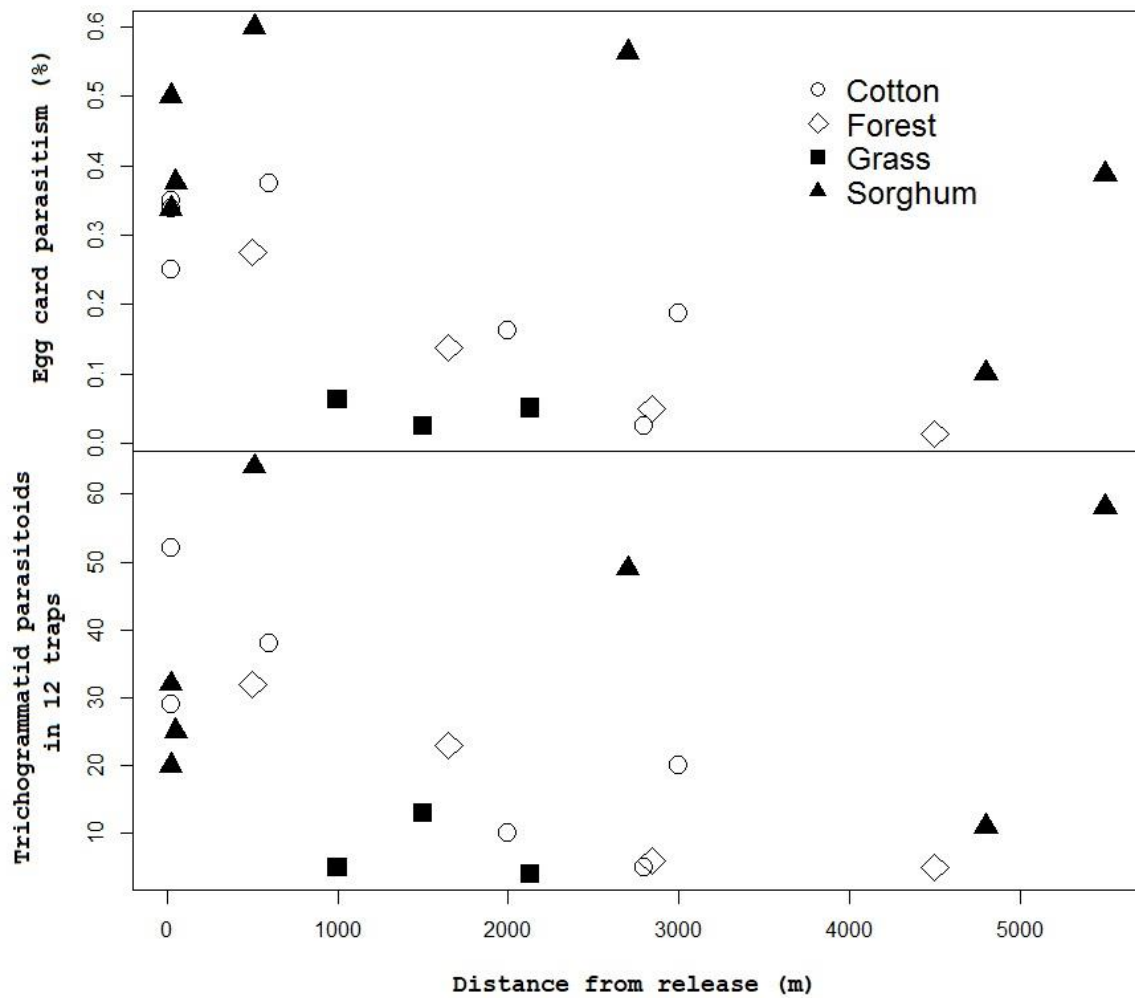


Figure 4.3 Percentage parasitism of 40 *H. armigera* egg cards and *Trichogramma* caught in yellow and white water pan traps in January and February 2009 (data pooled across both months) with increasing distance from the spring release sites

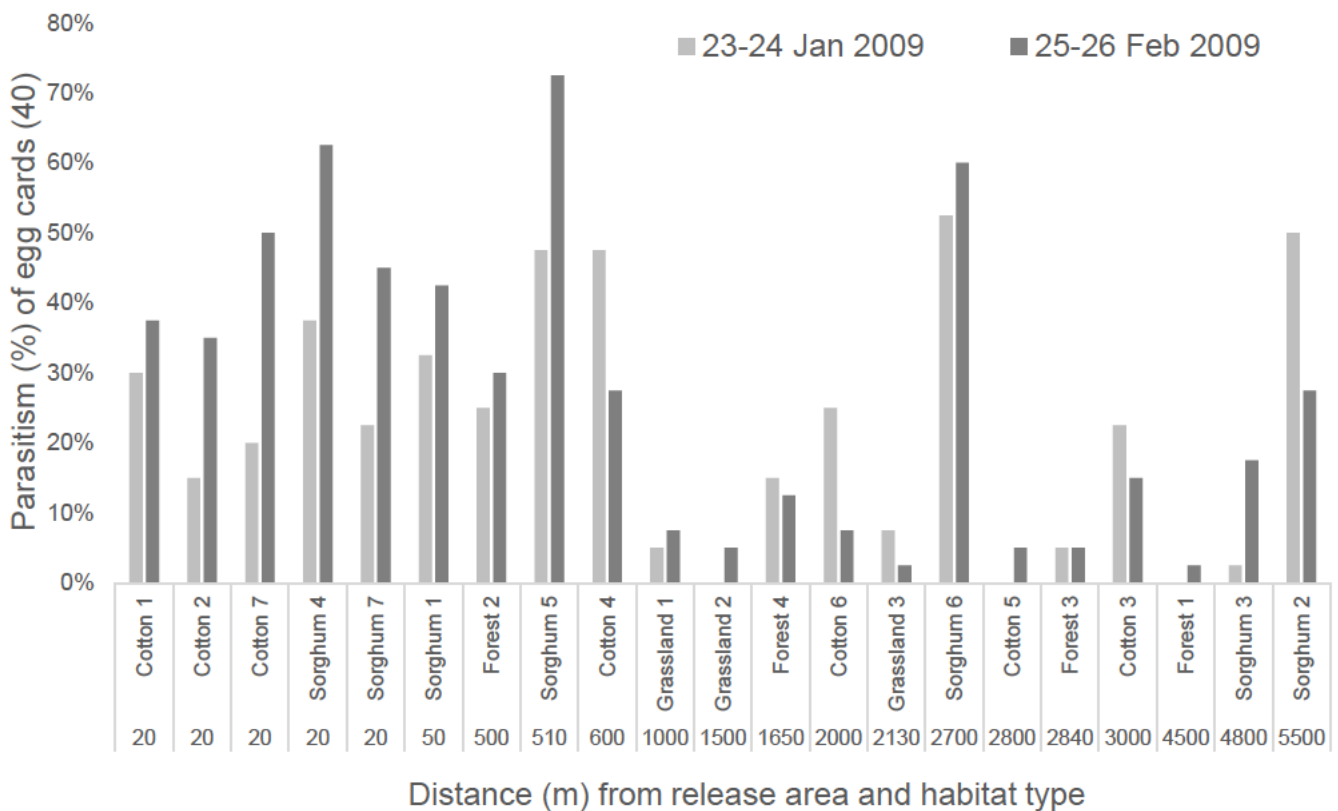


Figure 4.4. Percentage of 40 *H. armigera* egg cards parasitised by *T. pretiosum* in cotton in January and February surveys, relative to release site where close is <50m and far is >2000m.

Water traps yielded similar results to the egg cards (Table 4.1), with a correlation of 0.8702 between the two sets of results.

In January and February 2007, average natural parasitism as recorded by egg card parasitism over five cotton crops was 5.8% and 6.6%, respectively. In five sorghum crops, it was 1.2% and 4.0%, respectively. In January and February 2008, average egg card parasitism over 5 cotton crops was 4.7 % and 8.0 %. In five sorghum crops it was 11.3% and 7.3%, respectively. In January and February 2009, average egg card parasitism in four cotton crops within 50m of the spring release crops was 27% and 33.0%, and in five sorghum crops it was 38.5% and 56.5% (Fig 4.5).

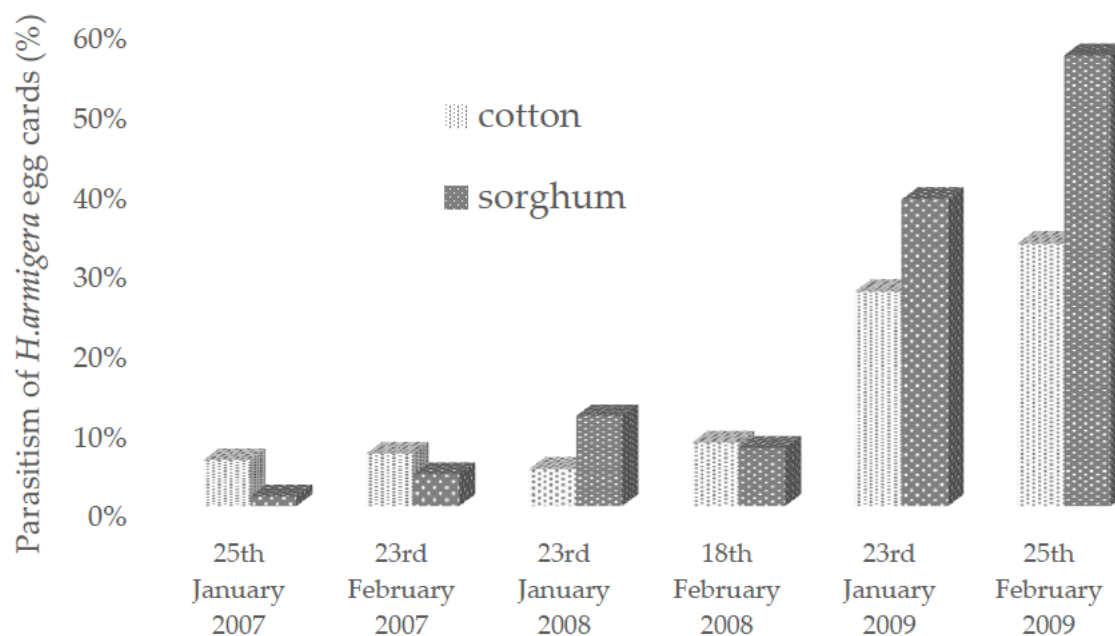


Figure 4.5. Comparison of *H. armigera* egg card parasitism from surveys conducted in 2007 and 2008 in the Breeza and Caroon–Quirindi landscapes measuring average natural parasitism in five crops the average parasitism of four cotton and four sorghum crops within 50m of the spring release crop sites.

4.4 Discussion

The main finding in this study was that *T. pretiosum* survive and parasitise *H. armigera* in wheat and canola, which act as a nursery crop for these parasitoids in spring, subsequently enhancing egg parasitoid abundance in cotton and sorghum crops in the subsequent summer season. We found that when releasing *T. pretiosum* in wheat and canola, movement outside of the release area into other areas of the crop within the season was substantial at 50m. We found quite good levels of parasitism (20-30%) at 50m in the release crops in both the mid-release and post-release surveys and negligible at 100 m which was a little above the background control levels.

The evenness of distribution of insect releases has been cited as an important factor influencing the effectiveness of parasitoid releases (Mills and Kuhlmann, 2000).

Trichogramma are not generally known to be skilled fliers, their between-plant

dispersal often being made through jumps or short flights (Hendricks, 1967; Yu *et al.*, 1984a). Wang & Shipp (2004) demonstrated in glasshouse tomatoes that *T. pretiosum* females travelled between 0.67 and 0.90 m in 4 days after emergence. The limited dispersal capacity of *T. pretiosum* after release was demonstrated in control of soybean noctuids, *Anticarsia gemmatalis* and *Pseudoplusia includens* (de Freitas Bueno *et al.*, 2012). Other species of Trichogrammatidae have greater dispersal ability (Pak and Oatman, 1982; Nafus and Schreiner, 1986; McDougall and Mills, 1997a; Wright *et al.*, 2001; Oztemiz *et al.*, 2009), with McDougall & Mills (1997a) concluding most of movement reports of *Trichogramma* are within a dispersal range of 20–70 m, which is consistent with our findings. The control sites during the releases in spring had little or no parasitoid activity (Fig. 4.1; Table 4.1). This meant that our measurements of egg parasitism should not have been influenced by background egg parasitism, and that all the parasitised eggs were parasitised by *T. pretiosum*. The use of host egg cards to measure movement is frequently used (McDougall and Mills, 1997b; Scholz, 2000; Davies, 2006; Bastos *et al.*, 2010), although it only measures parasitism rate and not parasitoid activity or male parasitoids, and there is variability in time due to age and the host-finding ability of *Trichogramma*. It may well be that other egg parasitoids do not favour *Helicoverpa* sentinel egg cards as much as *T. pretiosum* does (Scholz, 2003).

In this study, we used a lower density of egg cards to measure parasitism away from the release area than within the release area itself, which may have affected parasitism rates. However, the limited parasitism of egg cards 100m away from the release site in this study suggests that more egg cards would not necessarily have indicated further dispersal, especially at 100 m distance. Allen and Gonzalez (1974) demonstrated that the distance that *T. pretiosum* moved away from the release area was not normally distributed, abundance peaking at a distance of 21 m from the release point and subsequently declining. Fournier *et al.* (2005) showed that *T. pretiosum* behavioural traits increased interspecific variation in movement compared

to *T. evanescens*, as *T. pretiosum* tended to have less flight propensity but greater walking speed and foraging activity. In this study, the lack of movement of the first release generation and the subsequent progeny suggest that an approach where *T. pretiosum* is applied over the whole crop will be most successful, as has been found by studies in orchards (Yu *et al.*, 1984b; McDougall and Mills, 1997a). Of the two extremes, Heimpel and Asplen (2011) have suggested that minimal dispersal is less of an impediment to successful biological control than too much dispersal.

To establish populations, a critical density of female *Trichogramma* is required, Hopper and Roush (1993) demonstrated in theoretical reaction–diffusion modelling that parasitoid establishment was an increasing function of female dispersal rate (i.e. the more female dispersal the more females needed for establishment). In comparison to other *Trichogramma* species, *T. pretiosum* has been found to be less affected by wind direction in dispersal (Yu *et al.*, 1984a; McDougall and Mills, 1997a). In our study wind direction did not affect egg-card parasitism ($F = 1.16$, $p = 0.331$), but the interaction between direction and distance was marginally significant ($F = 2.55$ $p = 0.059$), with little difference in parasitism percentage at the two distances south of the release area but marked declines in parasitism with distance in other directions (Table 4.1).

The application of *Trichogramma* capsules in canola by hand in the later stages of crop development can be difficult because of the difficulty in walking along rows where the plants have become tangled. This could be resolved by planning some wider rows during planting, which some of our farms in the study had done because of their larger machinery and intent for latter chemical application. Other application methods such as aerial release of *T. pretiosum* in an oil solution applied over most of the crop warrant further investigation in large broadacre cropping systems to determine their economic effectiveness.

We released *T. pretiosum* over three 1-week periods to provide greater stability in population dynamics, and to account for repeated flights of *Helicoverpa* and the

associated oviposition. Releasing *Trichogramma* a few days before the first influx of seasonal *Helicoverpa* emergence improves the chance of *Trichogramma* finding hosts. After the first *T. pretiosum* release, a significant local emergence of *H. armigera* and some *H. punctigera* occurred in the area as anticipated from historical records, coinciding well with *T. pretiosum* emergence from the capsules in wheat and canola. The effectiveness of *T. pretiosum* in controlling *Helicoverpa* in canola is not well known. There has been little research on or collections of *Trichogramma* in canola, although studies of *Plutella xylostella*, a common pest in canola in southern Queensland, show that it is parasitised at significant rates by *T. pretiosum* (Liu *et al.*, 2004). Flowering canola is susceptible to *Helicoverpa*, which is a significant pest in northern NSW. Future inoculative releases covering the entirety of this crop could increase *Helicoverpa* control preventing larval hatch and reducing economic damage to this crop with minimal use of insecticide.

Trichogramma spp. are very sensitive to pesticides (Franz *et al.*, 1980) and, historically, release failures have largely been due to pesticide use after the *Trichogramma* wasps have been released (Lopez and Jones, 1985; Smith, 1996; Suh *et al.*, 2000a). The use of broad-spectrum insecticides, which are favoured for *Helicoverpa* control in canola, is a major obstacle in the use of *T. pretiosum* in the management of *Helicoverpa* in canola, demonstrated by our observation of major reductions in parasitism after chemical applications. The wheat crop W1C (Fig. 4.1 & Appendix 7) was sprayed for aphids after the mid-release sample using a synthetic pyrethroid (Lambda cyhalothrin), and our results show a dramatic drop in parasitism of egg cards, with just one egg card parasitised. This is consistent with the results of Suh *et al.* (2000a) for *T. exiguum* where they found Lambda cyhalothrin application severely affected emergence. Broad-spectrum organophosphate, pyrethroid, and carbamate insecticides are generally highly toxic to beneficial insects and *Trichogramma* is also sensitive to insecticides due to its small size. Hassan (1989) suggested that with organophosphates, there is a reduction of 70% in *Trichogramma* effectiveness, and

that although the population is not eliminated with one spray, they are unable to survive multiple chemical applications. Some studies have shown that *Trichogramma* is not affected by pesticides at the egg stage but others have shown that contact with pesticides in the egg stage can cause prolonged development time of immature stages and reduction in emergence, fecundity, parasitism capacity, adult longevity and mating likelihood (Franz *et al.*, 1980; Consoli *et al.*, 1998; Suh *et al.*, 2000a; Takada *et al.*, 2001; Hassan *et al.*, 2004). Long residual effects of chemicals have been observed in trichogrammatids (Llewellyn, 2000; Scholz, 2001), which should influence the timing of releases after chemical application; in the case of broad-spectrum insecticides, approximately 4 weeks should elapse prior to subsequent *Trichogramma* releases (Llewellyn, 2000).

In the spring release, egg card parasitism measurements showed a rapid increase in parasitism of up to 88% in canola, 1 week after the first release. This was similar in wheat with up to 78% of egg cards parasitised in the release area (Fig. 4.1).

Correspondingly, there was high parasitism in the subsequent generation (41% and 72%, respectively). This suggests that these crops had repeated moth egg-lays and perhaps greater host egg availability. According to Knipling and McGuire's (1968) theoretical models of *Trichogramma*, 80% parasitism is required for economic control of *Helicoverpa*. However, Hawkins and Cornell (1994) found that successful biological control was likely at parasitism rates above 32% in field situations. Our results suggest that it may be possible to achieve economic control in canola using *T. pretiosum*, and further studies to examine whole-crop coverage are warranted.

There is a lack of research around varying release rates of *Trichogramma* (Scholz and Parker, 2004) and their viability in different crops. This study had similar findings to research conducted by Scholz and Parker (2004) in releasing *T. pretiosum* in cotton and sorghum on the Darling Downs in Queensland, where the low rate of release of 30,000 wasps/ha was comparable in parasitism (or better than) the higher rate of 60,000 wasps/ha. The associated cost saving with lower release rates is attractive in

broad-acre cropping systems, particularly in the situation where there is little, to no egg parasitoid activity as found in spring on the Liverpool Plains. Where there are already reasonable *Trichogramma* populations for instance, Johnson (1985) found that smaller release rates of *T. pretiosum* of between 12,500 and 37,500 wasps/ha in cotton made no difference to the background parasitism rate of around 40%. The cost per hectare of 30,000 wasps is approximately \$26/ha which is comparable to Gemstar® (Nuclear Polyhedrosis Virus) at \$29/ha (excluding application costs). The lack of refuge sites for *Trichogramma* may be a factor in their low activity, but inoculation enhances the ability of egg parasitoids to build up numbers rapidly, and our results show that their populations can be more abundant in subsequent summer crops.

There was a prominent increase in *Trichogramma* activity in 2008–09 compared to the previous 2 years of surveys conducted on the Liverpool Plains measuring natural parasitism in cotton and sorghum (Fig. 4.5) near the spring release sites. Whilst 2006–07 was a drought year which may have affected *Trichogramma* populations, there was an increase in 2007–08, which was generally considered an average season.

Percentage parasitism has been used widely in measuring impact in the field. How this relates estimating actual populations requires comparison of different methods. The water traps produced highly correlated results of *Trichogramma* abundance to the *H. armigera* egg cards. This was not the case in previous summer surveys of abundance using yellow plates alone, but the use of half white and half yellow traps generated catches that reflected closely the levels of egg card parasitism. The backing of the egg cards was yellow, so the yellow in both the egg cards and the water traps may have had the same attractive effect. This could be a more efficient method of sampling *Trichogramma* than the use of egg cards. *T. pretiosum* was the dominant parasitoid in cotton and sorghum crops in the summer of 2009, but the water-pan catches had higher proportion of other trichogrammatids (*Trichogrammatoidea bactrae*, *Trichogramma australicum*, unknown *Trichogramma* spp.) and *Telenomus* spp. in the grassland and forest sites.

When we measured egg card parasitism in adjacent sorghum and cotton crops, only cotton demonstrated a decreasing parasitism with distance from the spring release site. Lopez and Morrison (1985) found high variability in parasitism of *Helicoverpa* in sorghum and cotton after early season *T. pretiosum* releases in a 3-year seasonal field study. In their study they indicated that insecticide usage around the release site was a significant factor accounting for this variation. In the far sorghum crop 6, a remarkably elevated level of egg card parasitism was recorded (60%). It is unknown if *T. pretiosum* has previously been released in this area, but maize is the predominant crop on the farm and the grower reported using very little chemical control in his crops. Habitat-wise there is a significant amount of non-crop vegetation surrounding the farm, more than the other crops surveyed, which may have increased population size along with the limited chemical use. Additionally, the highest rates of background parasitism in previous years were recorded on nearby properties in cotton, sorghum and native vegetation. It was difficult to find *Helicoverpa* eggs on wheat and canola, so other than the moth trapping (Chapter 2), the background population of *Helicoverpa* was not recorded, other than for historical records and consultant data, but it would be useful in future surveys to do this. High competition for hosts may have led *Trichogramma* to superparasitise such that the parasitoid larvae may have died before emergence, accounting for the higher release rate not being as effective.

In conclusion, our study suggests that low inoculative release rates of *Trichogramma* may be the most cost-effective method of utilising *Trichogramma* as part of an IPM strategy. Early-season low-density releases boosted the population of egg parasitoids to not only benefit the crops where wasps were released, but also the subsequent summer crops where additional insect pest control and economic benefit were likely. In this study, we did not concentrate on economic factors such as measuring damage or yield returns per wasp release, but this should be an important focus for future studies. Similarly, further research should investigate the potential to economically

control *Helicoverpa* in canola via egg parasitoid inoculation that covers the whole crop and is repeatedly applied including before flowering starts. Our study suggests that canola and wheat are effective nursery crops for *T. pretiosum* for balancing the ecology of egg parasitoids where populations are naturally low. We have shown that *T. pretiosum* causes significant *Helicoverpa* mortality when measured by host egg sentinel parasitism. The strong correlation between both the water pan trap samples and the sentinel egg cards suggest that percentage parasitism is a useful indicator of *Trichogramma* populations. However, moth eggs are subjected to significant mortality from many sources and this has been measured and documented for *Helicoverpa* in Australia crops (Kyi et al. 1991). How percentage parasitism translates into real reductions in *Helicoverpa* populations should also be investigated when evaluating effectiveness of the releases, but was beyond the scope of this study.

Given that low release rates of *T. pretiosum* via inoculation are effective, further refinement of whole-crop application and measurement of direct *Helicoverpa* mortality should be conducted. In addition, the economic impact should be measured to give farmers the confidence to supplement egg parasitoids annually (as a preventative measure) as well as utilising other parasitoid conservation methods for a sustainable IPM alternative to chemical applications alone.

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Chapter 5: Conclusions and synthesis

5.1 Introduction

The aim of this research was to assess the presence of *Trichogramma* in northern NSW broadacre field crops, determine their preferred habitats and consider how the potential of *Trichogramma*, in integrated pest management (IPM) of *Helicoverpa* can be optimised in Liverpool Plains cropping systems. Previously research into the distribution, abundance and diversity of *Trichogramma* in northern NSW and the impact of its parasitism of *Helicoverpa* in major crops was limited (Davies *et al.*, 2011b).

5.1.1 Aims and objectives

The preceding chapters in this thesis: (1) reviewed the literature and the success and failures of *Trichogramma* in IPM, and identified the areas that have previously limited the effectiveness of *Trichogramma* in IPM, which are applicable to broadacre cropping on the Liverpool Plains in north-western New South Wales; (2) determined the abundance, diversity and seasonal phenology of *Trichogramma* and other egg parasitoids of *Helicoverpa* in crop and non-crop vegetation, including summer landscapes with different proportions of native vegetation; (3) evaluated *Trichogramma* crop choice preferences in glasshouse trials, and quantified combinations of crops and plant parts to determine if there were differences in parasitism; (4) assessed the role of wheat and canola as nurseries for the population build-up of *Trichogramma* and determined effective rates of release and movement from release areas; (5) measured the persistence and impact of *T. pretiosum* in the summer cropping season after inoculative releases in spring nursery crops in crop and non-crop habitats. This chapter provides a summary and a synthesis of the main findings of the research and explains the theoretical and conceptual significance of the work. I present management recommendations for the conservation of natural

and released *Trichogramma* on the Liverpool Plains and present options for their incorporation into a regional IPM strategy. I discuss the limitations of this research and make recommendations for future research on *Trichogramma* in broad-acre crops for the control of *Helicoverpa*.

5.2 Summary of main findings

The aim of Chapter 1 was to review the considerable literature on *Trichogramma* and establish the reasons for the variable success of using *Trichogramma* in IPM and identify the impediments to its successful incorporation in IPM strategies. One of the main findings was the lack of use of natural enemies in modern IPM despite the early proposal of an ecosystem approach harmonising the use of chemical and biological control (Stern *et al.*, 1959). Whilst recent developments have widened the scope of IPM to include multiple tactics, the use of natural enemies in Australian crops has been limited. There seems to be a paucity of information about how to utilise natural enemies in broadacre crops effectively at a scale relevant to farmers (Macfadyen *et al.*, 2015). Whilst there are some studies documenting the failure of inundative control using *Trichogramma* to control *Helicoverpa* in broadacre crops, notably cotton as a stand-alone tactic (Davies, 2006), *Trichogramma* is more successfully integrated with other control tactics (Scholz, 2003; Davies *et al.*, 2011b). Natural pest control is not the only benefit of *Trichogramma*. Resistance management of insecticides and genetically modified Bt cotton are key reasons to optimise *Trichogramma* populations for IPM. Studies in broad-acre crops that assess natural predators and pests at a scale beyond a single field are limited in Australia, as well as movement of pest and beneficial insects at a landscape scale (Schellhorn *et al.*, 2014). On the Liverpool Plains, lack of knowledge of what crops and non-crop habitats *Trichogramma* occurs in and the range of species present has limited consideration of this parasitoid genus in IPM for farmers. In an interview of 15 farmers in the region (refer to Appendices 9 and 10), most (80%) said they monitored

for natural predators, mainly those of aphids, and 75% said their monitoring results influenced spraying decisions, most holding off spraying if the damage was below economic thresholds. Only three farmers considered wasps, but these were not *Trichogramma*. Of the farmers interviewed, 60% had controlled *Helicoverpa* in the last 5 years using chemical insecticides. The farmers in this survey had one or more of their crops surveyed for *Trichogramma* in the research described in this thesis, and most were aware of the potential utility of *Trichogramma* from reports in other farming regions. When asked what they would like out of this research, the main responses were the species identification (80%), overwintering (73%) and effectiveness of *Trichogramma* at keeping *Helicoverpa* below threshold (47%). Growers mostly said they would modify their practices to encourage *Trichogramma* if the research supported it (60%).

5.2.1 Landscape ecology of Trichogramma and other egg parasitoids on the Liverpool Plains, Northern NSW

In Chapter 3, *Trichogramma* was found in every major habitat except for chickpea. The species identified were *Trichogrammatoidea bactrae*, *Trichogramma pretiosum*, *T. australicum*, *Telenomus* species and unidentified species of Trichogrammatidae. In spring 2006, egg card parasitism was observed in faba bean (1%), linseed (2%) sunflowers (1%) and forest (2%). In spring 2007, egg card parasitism over was observed in faba bean (2%), linseed (1%), sunflower (1%), lucerne (4%), wheat (1%), forest (4%) and grassland (1%). The most abundant egg parasitoid in spring was *Trichogrammatoidea bactrae*. In 2007, egg card parasitism in summer habitats peaked in sorghum (27%), cotton (40%) maize (20%) and forests (4%). In 2008, egg card parasitism in summer peaked in sorghum (47%), cotton (67%), maize (43%) and forest (13%) habitats. In summer crops in 2007–09, *Trichogramma pretiosum* was the most abundant parasitoid.

To examine overwintering habitats, native vegetation was surveyed in 2007 and 2008. Before this study, it was unknown if *Trichogramma* and other egg parasitoids

over-wintered on the Liverpool Plains. There was low species abundance but a diversity of taxa in the forest habitats over winter. Unidentified trichogrammatids made up the bulk of specimens, but both *T. pretiosum* and *Trichogrammatoidea bactrae* parasitised *Helicoverpa* egg cards. One or more *Telenomus* spp. were widespread, and *Trichogramma australicum* had low abundance. The discovery of overwintering egg parasitoids has practical implications for the preservation of non-crop habitats in the landscape.

Two different landscapes on the Liverpool Plains were compared in summer 2006–07 and 2007–08, the Carroona–Quirindi landscape having a higher proportion of forested areas than the Piallaway–Breeza landscape. In summer 2006–07, seasonal abundance in water pan traps (WPT) and percentage egg card parasitism (ECP) in cotton (0.40 per WPT; 2.1% ECP) and sorghum (0.70 per WPT; 1.8% ECP) in the Carroona–Quirindi landscape was greater than in cotton (0.26 per WPT; 0.3% ECP) and sorghum (0.37 per WPT/0.6%) in the Piallaway–Breeza landscape. In 2007–08, WPT abundance per trap and percentage ECP in the Carroona–Quirindi landscape in cotton (0.83 per WPT; 5.4% ECP) and sorghum (1.05 per WPT; 5.2% ECP) was greater than in the Piallaway–Breeza landscape in cotton (0.25 per WPT/1.4% ECP) and sorghum (0.20 per WPT; 2.4%). The between-year difference was less in maize, but the Carroona–Quirindi landscape had higher parasitism of egg cards in 2007–08. In summer 2006–07, average egg parasitoid abundance in forest habitats decreased at the same time there was a significant increase in abundance and egg card parasitism in sorghum and cotton in the same landscape. Whilst the increase wasn't observed in subsequent years, the sudden decrease in egg parasitoids in native vegetation wasn't either. Both *Trichogrammatoidea bactrae* and species identified as Trichogrammatids decreased in forest habitats and increased in the cotton and sorghum habitats in the Carroona–Quirindi landscape. We can only speculate about their movement into surrounding crops.

5.2.2 Crop and plant-part preferences of *Trichogramma pretiosum* parasitising *Helicoverpa armigera* under glasshouse conditions

Chemicals contained in certain plants may attract and retain parasitoids in diversified cropping systems (Altieri *et al.*, 1981a; Nordlund *et al.*, 1984).

Trichogramma species are used in a range of pest and host systems and an understanding of their behaviour in crop choice is essential for optimising their use in biological control programmes (Bjorksten and Hoffmann, 1998; Gurr *et al.*, 2012). For example, biological control agents may not be able to manage pest insect populations successfully due to limited host-searching capacity (Murali-Baskaran *et al.*, 2017).

We separated winter/spring crops into group 1 (linseed, canola and wheat) and group 2 (lucerne, adzuki and faba bean) crops, and investigated a third group of summer crops (cotton, maize and sorghum). The mean percentage parasitism across the range of crops varied from 7% (adzuki) to 42% (canola and faba bean). The group 1 crop plants had similar mean percentage parasitism levels: linseed (40%), canola (42%) and wheat (37%). However, group 2 crop plants differed in percentage parasitism ($P \leq 0.001$): lucerne (26%), faba bean (42%) and adzuki (7%). The summer crop plants also differed in percentage parasitism ($P = 0.002$), with cotton (34%) being preferred over sorghum (19%) and maize (9%). There was no effect of crop combination (nested within crop) for any of the three groups of crops (group 1, $P = 0.896$; group 2, $P = 0.742$; group 3, $P = 0.124$). In this respect, our results contrasted with those of Scholz and Parker (2004) who found higher rates of parasitism when sorghum (42%) was intercropped with cotton (56%) in the field. The lack of significance of the crop combination effect (nested within crop) suggests that *T. pretiosum* will readily parasitise *Helicoverpa* eggs in canola, linseed or wheat regardless of the proximity of other crops in this group. Similarly, we found no evidence that parasitism would vary among group 2 or group 3 crops with proximity to other crops in each respective group.

The level of parasitism of *Helicoverpa* eggs was affected by position on the plant (i.e. plant part: flowers, leaves and stems) in each group. In group 1 (linseed, canola and wheat), parasitism was less among flowers (17%) than on leaves (44%) or stems (49%; $P < 0.001$). The interaction of crop and plant part in group 1 was almost significant ($P = 0.076$), with marginally higher parasitism among linseed flowers (29%) than wheat flowers (9%). In group 2 (lucerne, adzuki and faba bean), flowers (11%) were less favoured than leaves (20%) or stems (25%) ($P = 0.011$), but the interaction of crop and plant part was not significant ($P = 0.433$). *Trichogramma* was more successful on the leaves and stems of faba bean (39–45% parasitism) and lucerne (28–30%), but all parts of adzuki were less attractive for *T. pretiosum*, where low rates of parasitism were recorded (4–10%). In group 3, leaves (19%) and stems (19%) were again preferred over flowers (5%) ($P < 0.001$) with no interaction between crop and plant part ($P = 0.743$).

In conclusion, *T. pretiosum* parasitised eggs on most of the nine species of crop plant readily in cages in the glasshouse, favouring eggs attached to leaves and stems over flowers. This would suggest that when releasing *T. pretiosum* in the field for biological control, attaching capsules to leaves and stems will be preferable to flowers.

5.2.3 Inoculative release of a beneficial wasp egg parasitoid, Trichogramma pretiosum (Hymenoptera: Trichogrammatidae), to supplement natural wasp populations for control of Helicoverpa in northern New South Wales field crops

The main finding in this study was that *T. pretiosum* survived and parasitised *H. armigera* in wheat and canola, which acted as nursery crops for these parasitoids in spring, subsequently enhancing egg parasitoid abundance in nearby cotton and sorghum crops in the subsequent summer season. Naturally occurring populations of the egg parasitoid, *T. pretiosum*, have been identified on the Liverpool Plains, but reach peak activity when most of the summer crops have already reached maturity and are at levels below those considered effective for economic control. Whilst

Trichogramma has generally failed to suppress *Helicoverpa* when released in cotton-growing regions (Davies *et al.*, 2011c), the traditional means of inundative release may not be well suited to *Helicoverpa* control (King and Powell, 1992; Scholz and Parker, 2004). Inoculative releases are particularly useful where the parasitoid does not occur commonly, or at a time of the year when it is not abundant (Stehr, 1982) or parasitism levels are <30% (Llewellyn, 2006).

Pre-release surveys in crops yielded few *Trichogramma* with only one *H. armigera* egg card parasitised in each of two wheat crops (one control and one high-release site). One parasitoid was identified as *Trichogrammatoidea bactrae* and the other parasitised egg did not hatch. No egg-card parasitism was detected in control crops in the mid-release survey and only two *H. armigera* egg cards were parasitised at the wheat control site in the post-release survey.

In the mid-release survey, the average parasitism of *H. armigera* egg cards in high and low-release treatments was 48% and 70%, respectively, which was not quite statistically significant ($F = 4.41$, $P = 0.065$). Considering that the low rate of release (30,000 wasps/ha) was as, if not more, effective than the high rate, the associated cost saving with low release rates is attractive in broad-acre cropping systems, particularly where there is little or no egg parasitoid activity, as is the case in spring on the Liverpool Plains. In the post-release survey, the average parasitism of egg cards in high and low treatments was 27% and 37%, respectively, and not significantly different ($F = 1.21$, $P = 0.314$). In the mid-release survey, average parasitism of *H. armigera* egg cards in high and low-release sites combined was higher in canola (57%) than wheat (35%), averaging 51% overall. Parasitism of egg cards peaked in the low-rate treatments at 88% in canola and at 78% in wheat. In the post-release survey, the average parasitism of *H. armigera* egg cards in high and low-rate treatments combined was similar in wheat (33%) and canola (35%), averaging 34%. Flowering canola is susceptible to *Helicoverpa*, which is a significant pest in northern NSW. Future inoculative releases covering the entirety of this crop could

increase *Helicoverpa* control by preventing larval hatch and reducing economic damage with minimal use of insecticide.

When we released *T. pretiosum* in wheat and canola in spring, we found modest levels of parasitism in wheat and canola 50 m beyond the release areas in the release crops in the mid-release (21% and 28%, respectively) and post-release surveys (18% and 23%, respectively). Parasitism at 100 m from the release areas was low and not much greater than background control levels in either wheat or canola (2% and 5%, respectively, mid-release; 8% and 4%, respectively, post-release). In the mid-release survey, distance from release area significantly affected egg-card parasitism ($F = 24.22$, $P < 0.001$), with average parasitism of 16% of egg cards at 50 m (averaged over treatment and control sites) declining to 2% at 100 m. The interaction between distance and direction did not affect parasitism ($F = 1.32$, $P = 0.271$). Post-release, distance from the release area again affected mean egg-card parasitism ($F = 8.45$, $P = 0.005$), but direction was not significant ($F = 1.16$, $P = 0.331$).

There was a marked increase in *Trichogramma* activity (as measured by egg card parasitism in cotton and sorghum crops) in summer 2008–09 near the spring release sites, compared to the previous two summers on the Liverpool Plains. Decreased parasitism with increasing distance from the release sites was observed in cotton. There was little parasitism of egg cards in grassland sites, which had fewer *T. pretiosum* and more Trichogrammatids present.

In conclusion, our study suggests that low inoculative release rates of *Trichogramma* may be the most cost-effective method of utilising *Trichogramma* as part of an IPM strategy. Early-season low-density releases boosted the population of egg parasitoids to not only benefit the crops where wasps were released, but also the subsequent summer crops where additional insect pest control and economic benefit were likely.

5.2.4 Synthesis

This research found very low levels of *Helicoverpa* egg parasitoids on the Liverpool Plains in spring in crops (1-4% parasitism). Certain crops such as faba beans, linseed, chickpea and canola are most attractive to *Helicoverpa*, reaching thresholds that sometimes cause economic damage requiring insecticide application. *Helicoverpa* is not of major economic importance in other crops such as wheat, lucerne and sunflowers, as the plants are generally able to tolerate large infestations of *Helicoverpa* and still produce a reasonable yield. Several researchers have suggested that *Trichogramma* populations could be increased by inoculative releases where parasitism is non-existent or <20% (Walker, 1999; Scholz, 2000; Llewellyn, 2006). We found high levels of egg card parasitism in canola (up to 88%) and wheat (up to 78%) as a result of inoculative releases in spring. Our experimental aim was to build up *Trichogramma* populations and see if this increased summer populations by using these crops as nurseries. However, releases could also be released over a longer period in faba bean, linseed and canola to combat *Helicoverpa* hatch and prevent major economic damage in these crops. In our glasshouse experiments, *T. pretiosum* searched the leaves and stems of faba bean, linseed and canola to a similar degree, suggesting that releases in these crops would be effective and not affected by nearby crops. *Trichogramma* was not recorded in the field in chickpea, and glasshouse research demonstrated that adzuki bean was not a plant that *T. pretiosum* readily searched for *Helicoverpa* eggs. Viral and fungal biocide applications may be more applicable to control *Helicoverpa* in these crops. The increase in *T. pretiosum* in summer in cotton following spring inoculative release in canola and wheat was substantial near the release sites. Sorghum showed less of this trend, but the sorghum crops away from the release areas that skewed this result where in the Carroona–Quirindi landscape, which had higher levels of *Trichogramma* before the releases. Thus, the landscape with a greater proportion of native vegetation positively influenced abundance and impact of *Trichogramma*.

Spring inoculative releases not only improved *Helicoverpa* pest control in these crops but provided further ecosystem services in crops in the following season. Compared to synthetic insecticides, biological control agents are estimated to provide 5–10 times higher control of pest insects (Pimentel *et al.*, 1992). The majority of farmers in this study monitored for beneficial insects (80%) and their decision to spray insecticide was influenced by these numbers. By not using insecticides, other beneficial insects such as coccinellids, tachinids, syrphids and arachnids have a better chance of combating secondary pests such as aphids. The effect of insecticide spraying on crop costs, human health and the environment are additional costs of chemical control. *H. armigera* is resistant to most insecticide groups and this is particularly relevant for the Australian grains industry where the option of GM crops is not as widely available, and approved chemicals are being withdrawn due to the above concerns. Consumer choice is driving the way food is produced, as people become more aware of agricultural impacts on ecosystems. Ideally farms could be better planned to combat pests and encourage beneficial insects via conservation biological control, rather than supplementing populations. However, in the change to more diverse systems, this thesis has demonstrated that activities like inoculative releases can lessen the impact of one of the major moth pests of agriculture in Australia as part of a wider IPM management system.

5.3 Research limitations

Limitations and improvements to each of the chapters are summarised here. In the interview with the growers conducted about IPM and *Trichogramma* research (Appendix 10), a wider group of farmers could have been interviewed to extend their responses. Field results gained in surveys of *Trichogramma* and other egg parasitoids could have been presented to growers during interviews, providing them with more evidence on which to base their responses about their intention to adopt the research findings. The evaluation of the interview responses might have

benefited from comparing them to the opinions of farmers in southern Queensland, where many releases of *Trichogramma* have already occurred.

Direct movement of *Trichogramma* was not examined in field surveys of *Trichogramma* and other egg parasitoids (Chapter 2). Understanding landscape-scale effects on *Trichogramma* would benefit from movement studies. *Trichogramma* and its host, *Helicoverpa*, operate at widely different spatial scales, so their corresponding dispersal ranges need to be evaluated differently. The minute size and short generation time of *Trichogramma* add to the difficulty of determining movement of populations.

A survey of the very limited crops grown on the Liverpool Plains in July (e.g. lucerne) would have established if *Trichogramma* also utilises these crops. However, the lack of specimens caught in the habitats surveyed (0.01% of egg cards parasitised) indicates that other methods such as sticky traps may be useful for examining populations over an extended period. The identification of all parasitoids emerging from egg cards in the seasonal surveys would have provided better information about the species that parasitise *Helicoverpa*, in addition to the water pan traps. Species identification by molecular methods would have been better for identifying the Trichogrammatids that were not identified to species level by morphological characteristics. However, currently there is limited published research on molecular identification of Australian Trichogrammatids. Similarly, at the time of this study, use of percentage parasitism of egg cards was appropriate, but molecular approaches are now more advanced and could give more detailed information about species-specific parasitism than was possible at the time of this study. We attempted to separate crops and non-crop vegetation sites by at least 2 km, but surveys of aggregated crop types in different landscapes might reflect the variation in these habitats better. A simple analysis was used to determine the proportion of crop and non-crop habitat in the two landscapes selected. This could be improved upon. However, the complexities involved in the population processes

and population densities of natural enemies may not always be captured by the underlying drivers of biocontrol. The effects of weather, pesticides, individual plants and other factors influence habitat suitability. Determining the plant species in the native vegetation that *Trichogramma* was utilising would have been useful to determine the quality of native vegetation in relation to species abundance and potential impact.

Evaluation of *T. pretiosum* crop choice preferences, crop combinations and plant part preferences was expected to provide certainty of these effects. The appreciable difference between replicate one and two indicated that there was perhaps a problem with the quality of the *Trichogramma* used in the first replicate. Greater replication would have provided further elucidation of this matter. The choice to use a combination of three plants in cages was to determine if *T. pretiosum* would continue to search and stay longer on the same crop type to find host eggs. However, using two crop plant types in different combinations may have answered the question with more replicates of the combinations. The cage approach of combining plants to test for arrestment, retention or repellent factors, was small in scale and the same question could be examined in plots. The implicit choice test in the cages could have benefited from behavioural observations of *Trichogramma* movement, which might have revealed if *Trichogramma* spent as much time searching the flowers as the leaves or stems, in addition to measuring parasitism rate. Additional observations on arrestment or retention might have included the effects of herbivore damage, host density or other predators. The different rates of release of *T. pretiosum* in the field had similar levels of parasitism of egg cards, thus observation of *Trichogramma* behaviour in different densities but with the same number of host eggs might also shed light on whether parasitoid density is a factor in arrestment and retention or the decision to move to a different plant or crop plant.

Some of the crops selected to act as nurseries for *Trichogramma* population build-up in spring (Chapter 4) were affected by insecticide applications to control aphids.

Releases were conducted on commercial farms to ensure authentic conditions, but insecticide applications significantly reduced *T. pretiosum* egg card parasitism in some canola and wheat crops. By conducting several releases in two landscapes of 30–50-km extent each, we mitigated these effects. However, incorporating *T. pretiosum* releases with other IPM strategies to control aphids and other pests would be ideal.

Attempts to light traps and pheromone lures to estimate the arrival of *Helicoverpa* proved unreliable. Timing of the releases coincided well with *Helicoverpa* immigration and egg laying, which was determined from historical records.

However, seasonal variation in the date of the host arriving in crops is important in timing of releases. We were fortunate in this experiment, but additional methods are needed to predict *Helicoverpa* arrival. Three releases were considered a minimum for stabilising age cohorts, but arguably several more releases could be conducted over a longer period to ensure better build-up of parasitoid populations. Releases were conducted in the middle of crops of at least 20 ha in area and 2 km apart. This was to counter edge effects, interference from other releases and to measure parasitism at 50 m and 100 m from the release area. This approach could have made yield assessment difficult in release crops, which was beyond the scope of this project. However, the effects of IPM tactics on crop yield are an important factor for farmers to consider to help justify the outlay of time and money for releases.

Natural egg collection of *Helicoverpa* eggs in both wheat and canola was attempted but was time consuming and revealed very few eggs; none of these eggs were parasitised. The light traps captured a wide variety of moths and several other moths were observed in crops, but it was difficult to determine if these moths laid eggs in the crops. It would require extensive efforts to find host eggs and rear caterpillars to adulthood, as larval identification is often difficult. It is highly likely that *T. pretiosum* parasitised other benign moth species eggs that were laid in the crop, which increased its survival, and this warrants further investigation.

It was beyond the scope of this thesis to measure the direct effect of the inoculative release of parasitoids on *Helicoverpa* populations. Egg card parasitism is useful where there are limited host numbers in the field, enabling parasitoid activity to be monitored all year round regardless of host density. Another advantage is that sentinel egg host parasitism can be compared between different crop types across time and multiple habitats in agricultural landscapes in a standardised way.

However, percentage parasitism alone does not necessarily reflect impact on pest population growth. Additional factors incorporating immigration, emigration, reproduction rates and other mortality factors need to be considered. The results of egg card parasitism in this thesis need to be interpreted given this uncertainty.

The non-target effects of *T. pretiosum* releases could have been investigated but were not. However, surveys in crop and non-crop habitats in the first 2 years of research identified that *T. pretiosum* had already established in the region, thus evaluating the non-target effects of these populations and the impact of releases would be challenging. The monitoring of *Trichogramma* in summer maize after the spring inoculative release would have provided a comparison to releases made in spring on the Darling Downs and would have increased our understanding of *Trichogramma* dynamics in this crop. However, very little maize was grown in 2008–09 in the study region, and it was not possible to measure crops close to release fields or obtain sufficient replication needed to compare treatments and populations.

5.4 Theoretical and conceptual significance of the research

A 2-year field survey was conducted on the Liverpool Plains to determine the abundance, diversity and seasonal phenology of *Trichogramma* and other egg parasitoids of *Helicoverpa* in crop and non-crop vegetation (Chapter 2). In this survey the identified egg parasitoids were *Trichogrammatoidea bactrae*, *Trichogramma pretiosum*, *T. australicum*, *Telenomus* species and unidentified species of Trichogrammatidae. Scholz (2002) recommended that detailed surveys be conducted

on the Darling Downs to understand how *Trichogramma* overwinter. Previously it was unknown if *Trichogramma* and other egg parasitoids of *Helicoverpa* overwintered in northern NSW. This thesis research found all the identified egg parasitoids occurring in native vegetation in winter, albeit at very low levels.

Prior field surveys of natural egg parasitoid populations are an important prerequisite before attempting *Trichogramma* releases (Li, 1994; Smith, 1996). Several inundative releases of *Trichogramma* have reportedly failed due to lack of prior and follow-up work (King *et al.*, 1985; Davies, 2006). Pre-release evaluations of egg parasitoids are needed so that the species with the greatest pest management potential can be selected and evaluated as a biocontrol in the field (Scholz, 1990). In some cases, introduction of *Trichogramma* is made without consideration of natural populations. This occurred in northern Australia where *T. pretiosum* was introduced despite a high abundance of endemic populations of *T. australicum* and *Trichogrammatoidea flava* (Michael and Woods, 1978). Pak and Van Lenteren (1988) set out several criteria for the pre-release evaluation of a parasitoid: (1) parasitism levels and survival of parasitoids in the laboratory; (2) tolerance of climatic conditions in the release region; (3) host preference; (4) host selection; (5) host searching ability; (6) host acceptance; (7) good culture method, and (8) environmental risk.

Prior to the 1990s in Australia, investigations of native egg parasitoid species had rarely occurred. Scholz (1990) was the first to evaluate native egg parasitoids for the control of *Helicoverpa* in field crops, in particular, cotton. *Telenomus* spp. and *Trichogrammatoidea* species proved to be not amenable to mass rearing. *T. australicum* had problems with host (*Helicoverpa*) egg acceptance and oviposit singularly. We found similar species diversity on the Liverpool Plains to that found on the Darling Downs where the egg parasitoid community had already been evaluated by Scholz (1990). Thus, further exploration of *T. pretiosum* was pursued in this thesis.

Parasitism levels by *T. pretiosum* in different crop plants commonly grown on the Liverpool Plains were evaluated, as well as determining if combinations of crops and

different plant parts influenced parasitisation. Summer crops of maize, sorghum and cotton had previously been evaluated in the laboratory or glasshouse using *T. pretiosum* (Gonzalez *et al.*, 1970; Ables *et al.*, 1980; Scholz, 1990; Ruberson and Kring, 1993b; Saavedra *et al.*, 1997; Scholz, 2000; Davies, 2006; Llewellyn, 2006), although parasitisation of *Helicoverpa* eggs on different plant parts in combinations of these crops had not been evaluated together previously. Some studies have documented *T. pretiosum* parasitism in lucerne (Mensah, 1999; Hossain *et al.*, 2002; Scholz, 2003; Llewellyn, 2006). However, very few studies have evaluated *T. pretiosum* performance in canola, adzuki, linseed, wheat and faba bean, which are regularly grown on the Liverpool Plains in spring. All of these winter/spring crops except for adzuki bean were competently searched for *Helicoverpa* eggs by *T. pretiosum*. Leaves and stems of each crop tended to have similarly large levels of egg parasitism compared to flowers. The glasshouse research in Chapter 3 addressed the first six of the aforementioned pre-release evaluation criteria described by Pak and Van Lenteren (1988). Tolerance of the climatic conditions in the release region (criterion 2) was assumed because the cultured specimens came from a colony mixed with wild type specimens from southern Queensland, which has a similar climate to the Liverpool Plains, albeit slightly warmer. However, *T. pretiosum* readily parasitised *Helicoverpa* in the glasshouse experiment in Armidale in May, when a minimum of 13°C was recorded late in the experiment during replicate 2. The importance of temperature for selecting *Trichogramma* was investigated by Ramesh and Baskaran (1996) who examined 6-hour maximum temperature shocks on *T. chilonis*, *T. brasiliensis*, *T. japonicum* and *T. pretiosum*. The authors demonstrated that a single shock at 40°C was harmful to all species except *T. chilonis* and a single shock at 45°C was harmful to all species. Increased heatwaves due to climate change may change the climatic suitability of various cropping regions for *T. pretiosum* in the future. Scholz (2000) recommended that supplemental releases of *T. pretiosum* should be conducted in the Lockyer Valley during spring to determine if regional parasitism

levels could be increased. In a report by Walker (1999), very low levels of *Helicoverpa* egg parasitism was found in the lower Namoi in an investigation of natural predators in cotton agroecosystems. Walker (1999) highlighted the success of *Trichogramma* in northern Australia and southern Queensland and recommended that inoculative releases of *T. pretiosum* be conducted to see if populations could be established in northern NSW. Once the pre-evaluation criteria to select a suitable biological control candidate (*T. pretiosum*) were determined for the Liverpool Plains (Chapters 2 and 3), evaluation of a suitable nursery crop to build up populations was explored (Chapter 4). The detrimental effects of insecticide on *T. pretiosum* are well documented (Filho *et al.*, 2002; Bastos *et al.*, 2006b; Parra *et al.*, 2008; Khan and Ruberson, 2017) so a crop that is not usually sprayed for pests was best. The most ubiquitous crop in spring on the Liverpool Plains is wheat; although it has been known to harbour *Helicoverpa*, it is not considered a pest in this crop (Fitt, 1989). Another crop that stands out in the landscape is canola; although less grown, it is attractive to *Helicoverpa* and sometimes sprayed. There is very little published research of *Trichogramma* releases in these crops, but the glasshouse research (Chapter 3) found *T. pretiosum* readily searched these crops. There was no established rate of inoculative release of *Trichogramma* in these crops. Inoculative release rates were adapted from releases in vegetable crops (Llewellyn, 2000), maize (Scholz, 2000) and sorghum and cotton (Scholz and Parker, 2004). This study produced similar results to research conducted by Scholz and Parker (2004), who released *T. pretiosum* in cotton and sorghum on the Darling Downs in Queensland. They, too, found that the low rate of release of 30,000 wasps/ha was comparable in parasitism (or better than) the higher rate of 60,000 wasps/ha. Upon releasing *T. pretiosum* in wheat and canola, we found evidence of substantial movement 50 m beyond the release area into other parts of the crop during and after the release but little evidence at 100 m. This suggests that an approach where *T. pretiosum* is applied over the whole crop will be most successful, as has been found by studies in orchards (McDougall & Mills 1997a; Yu *et al.* 1984b). Of the two extremes, Heimpel

and Asplen (2011) suggested that minimal dispersal is less of an impediment to successful biological control than too much dispersal. To establish populations, as was our aim, a critical density of female *Trichogramma* is required, because Hopper and Roush (1993) found in theoretical reaction–diffusion modelling that parasitoid establishment is an increasing function of female dispersal rate (i.e. the more female dispersal, the more females needed for establishment).

There are substantial logistical problems associated with measuring the effects of landscape structure in parasitoid–host relationships (Menalled *et al.*, 1999a).

Although non-crop habitats can be a source of parasitoids in agroecosystems (Landis and Marino, 1999; Letourneau *et al.*, 2012; Macfadyen and Muller, 2013; Bianchi *et al.*, 2015), management practices such as the frequency of insecticide application can have a greater impact than resource availability (Jonsson *et al.*, 2012). This was apparent in the dramatic reduction in egg parasitism despite inoculative release of *T. pretiosum* in nursery crops, after insecticide application. Emerging evidence suggests that pest suppression is increased by proximity to native vegetation due to parasitism of crop pests (Gagic *et al.*, 2018). In this thesis, examination of two landscapes in the Liverpool Plains (Chapter 2) found increased abundance and impact of *Trichogramma* in the landscape with the higher proportion of native vegetation (Caroona–Quirindi). The reasons for this result need to be examined more closely and will enhance future biological control using *Trichogramma*.

5.5 Management recommendations

The research conducted in this thesis confirms that *Helicoverpa* egg parasitoids are not absent due to bioclimatic reasons on the Liverpool Plains. They are present in native vegetation habitats throughout the year, occur in most crops in very low levels in spring and reach peak activity in late summer crops. The lack of knowledge of this hitherto has meant that work incorporating these parasitoids into local IPM strategies has been limited. Natural enemies are an important ecosystem service for

pest suppression (Reid *et al.*, 2006b; Holland *et al.*, 2012), but are they underutilised in modern IPM programs (Kogan, 1998; Zalucki *et al.*, 2015). Strickland *et al.* (1996) highlighted the need for a key focus of IPM in future to maximise the use of natural enemies. However, the efficacy of natural predators needs to be quantified to be utilised efficiently (Fitt, 1989). Furthermore, several researchers have emphasised the need for assessing the impact of natural enemies on pest populations within the framework of pest-management programs (Furlong and Zalucki, 2010; Macfadyen *et al.*, 2015; Schellhorn *et al.*, 2015). Impact by *Trichogramma*, for example, can be measured by sentinel egg cards. Macfadyen *et al.* (2015) proposed that the measurement of impact is best approached by methods that address farmer needs in the pest management decisions that they face, thus affording practical recommendations for how to utilise natural enemies as an integral part of the management decision making. For instance, knowledge of the diversity and impact of *Trichogramma* in spring crops when *Helicoverpa* starts to impact on crops are important considerations. Landscape habitat manipulation can be achieved through establishing source habitats of parasitoids and other natural enemies (Schellhorn *et al.*, 2000a), provision of flowering resources (Wäckers, 2004) and augmentative releases. This study has shown that wheat and canola are potential nursery crops for the early establishment of *T. pretiosum* populations via inoculation in spring when *Helicoverpa* moths arrive on the Liverpool Plains. This has proven to be a successful method to build up populations in south-eastern Queensland on maize (Scholz, 2000), and was suggested by Walker (1999) as having future research potential in northern NSW.

Early theoretical models by Knipling and McGuire (1968) suggested that the threshold parasitism rate by *Trichogramma* required for control of a pest species was 80%. If this is not achieved in a single release, then multiple releases could be made to increase parasitism (Ravensberg and Berger, 1988). However studies by Hawkins and Cornell (1994) found effective parasitism likely at rates >30% for parasitoids in

their native range or >36% in exotic locations. Plečaš et al. (2014) found that control of aphids declined at parasitism rates of 22–24%. We found parasitism rates of egg cards of 30–62% mid-release (average 51%) and of 29–47% post-release (average 34%) in wheat and canola. This indicates that three releases are sufficient to potentially have a significant impact on *Helicoverpa* populations in these crops. Furthermore, in this study, increased populations of *T. pretiosum* persisted into the summer cropping season, significantly increasing egg card parasitism rates in cotton and sorghum crops.

Pimentel et al. (1992) estimated that biological pest control provides 5–10 times higher control of pest insects compared to conventional insecticides. By reducing the early-season numbers of *Helicoverpa* populations, there are less moths to infest valuable summer crops, and a reduced late-season population size may reduce abundance in the following year. If further actions are applied to control overwintering *Helicoverpa*, such as cultural controls (e.g. pupae busting), then subsequent populations could be further decreased.

In the Australian cotton industry, the development of chemical resistance by *H. armigera* forced the adoption of techniques other than chemical control alone. This included development of area-wide management groups in the 1990s and by 1999, researchers developed the first IPM guidelines (Downes et al., 2017). However, the development of genetically modified *Bt* cotton reduced *Helicoverpa* damage and populations in cotton landscapes. This was confirmed by a study by Wu et al. (2008) in China that showed increased areas of *Bt* cotton decreased *Helicoverpa* impact in other susceptible crops. The decrease in chemical application in cotton crops resulting from *Bt* crops has also increased natural predators in this crop. *T. pretiosum* actively searched cotton slightly more than maize and sorghum plants in our glasshouse study. Perović (2009) found that *T. pretiosum* had higher abundance in landscapes with a higher proportion of cotton crops. However, like insecticides, genetic modification faces recurring insect resistance requiring ever-increasing gene

additions. The waning of the effectiveness of *Bt* cotton results in farmers having to apply insecticides to control *Helicoverpa* in addition to the licencing fee for growing the genetically modified variety. This was experienced by farmers during this study with Bollgard II cotton. Essentially, the cost of developing these genetic technologies is passed on to the farmer with significant licencing fees. *Bt* cotton has major advantages including increasing natural predators such as *Trichogramma* because of reduced insecticide use and other aforementioned features. However, no one single technique can solve all pest problems. Whilst resistance management compliance involves trap crops and substantial monitoring of gene expression in *Helicoverpa*, the role of *Trichogramma* should also be a significant component in northern NSW. In cotton, the current 3Bt gene varieties are better at reducing resistance, but given that Ingard and Bollgard II developed resistance over time, a combination of tools is still warranted to reduce *Helicoverpa* resistance. This is especially relevant on the Liverpool Plains where there is mostly mixed cropping and *Trichogramma* offers benefits to limiting *Helicoverpa* resistance in host crops other than cotton. In the Australian grains industry, insecticide resistance is an ever increasing problem and there has recently been a renewed focus on IPM because of the severity of the resistance encountered in the field (Umina *et al.*, 2019). In north central USA, area-wide suppression of the European corn borer by Bt corn has led to limited resistance despite high selection pressure (Sappington, 2014). However, the western corn rootworm, another major pest of corn, has developed field resistance to Cry3Bb1 Bt corn (Sappington, 2014). This is why a multi-pronged approach to IPM is necessary rather than relying on one method alone. In other areas such as the Ord River Irrigation Area, *T. pretiosum* is already established and natural populations have reportedly reached parasitism levels of 97% in some cotton crops; thus, there is no need for seasonal inoculation or augmentation (Davies, 2006). In the Ord, *Trichogramma* are considered integral to the local insecticide resistance management strategy for continued and sustained *Bt* cotton production (Davies *et al.*, 2011b).

Scholz (2000) argued that *Trichogramma* is best thought of as part of an IPM program that increases the level of egg parasitism above naturally occurring levels. In this study, cotton crops closest to the release crops in spring had increased levels of egg card parasitism that declined significantly away from the release area. Thus boosting *T. pretiosum* populations in spring has added benefits for resistance management in the area, as well as reducing potential *Helicoverpa* damage. Unsprayed cotton may also be a summer refuge for *Trichogramma* populations, with egg card parasitism levels in our study reaching up to 62%. Maize and sorghum similarly had significant *Trichogramma* populations. Nursery crops are in important consideration as a relatively low-cost option to reduce *Helicoverpa* damage, but long-term sustainability in an ecologically focused pest management system would ideally be through modifications to cultural practices. The natural recruitment and persistence of beneficial insects is best achieved through habitat management, which increases overall plant and insect diversity (Michaud, 2018). In an ecological IPM approach, management is extended to not just pest control but other valued ecosystem services such as pollination, moisture retention, weed control and general soil health management.

Agricultural land is one of the main land uses on earth, it also contains most of the world's biodiversity. A recent comprehensive review by Sanchez-Bayo and Wyckhuys (2019) highlighted the world-wide decline of insect species and the possibility that 40% of the world's insect species over the next few decades may well become extinct. Consequently, pest species may increase due to lack of predators and parasitoids. Sanchez-Bayo and Wyckhuys (2019) found that the main drivers are pesticides and fertilisers, pathogens and introduced species, and climate change. Utilising more sustainable and ecological practices to safeguard vital ecosystem services is needed now more than ever.

In summary, IPM of *Helicoverpa* by egg parasitoids on the Liverpool Plains should take into consideration:

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- There is very low parasitism of *Helicoverpa* by egg parasitoids in most spring crops. Habitat manipulation may increase populations.
 - Egg parasitoids of *Helicoverpa* are present throughout the year in native vegetation. The range of taxa includes *Trichogrammatoidea bactrae*, *Trichogramma pretiosum*, *T. australicum*, *Telenomus* species and unidentified species of Trichogrammatidae. The potential for spray drift should be minimised in these habitats.
 - Inoculation of *T. pretiosum* boosts spring populations that persist into summer crops, in particular cotton. This has added benefits of reducing *Helicoverpa* in the landscape and should be considered as part of the local resistance management strategy.
 - Canola and wheat are potential nursery crops for *T. pretiosum*, and successful economic control of *Helicoverpa* in these crops may be achieved through whole-crop coverage by inoculative releases. Chemical applications to control other pests in these crops should be those that are relatively harmless to *Trichogramma*.
 - *Trichogramma* populations should be assessed by percentage parasitism of eggs in sorghum, maize and cotton, particularly in late January and February when populations reach their peak in these crops, when considering *Helicoverpa* control options.

5.6 Future research

- Investigate the effectiveness of inoculation of *T. pretiosum* in spring crops compared with trap crop refuges in *Helicoverpa* resistance management strategies.
- Assess the effects on yield by different levels of *Trichogramma* in spring crops such as canola, linseed, faba beans, sunflowers and wheat after inoculative release of *T. pretiosum*, and subsequent yield effects in summer crops.
- Determine the effectiveness of *T. pretiosum* in spring crops to control and prevent *Helicoverpa* damage and whether this can be achieved through whole-crop coverage by inoculative releases (in particular; canola, linseed, faba beans).
- Assess *Trichogramma* releases, in addition to coordinating with neighbours to control *Helicoverpa* via conservation of habitats that host *Trichogramma* on an area-wide management basis.
- Determine the effects of different grazing regimes in native vegetation on *Trichogramma* abundance and diversity.
- Determine the effect of *T. pretiosum* releases on native populations of egg parasitoids, and the non-target effects on other lepidopteran species.
- Identify plant types that *Trichogramma* may prefer to search, particularly in maintaining native vegetation habitats, but also field margins, roadsides, etc. Inter-row cropping and cover crops can be identified that are also likely to aid in increasing *Trichogramma* populations.
- Monitor the movement of *Trichogramma* from crop and non-crop habitats in the landscape.
- Investigate the effects of climate change on *Trichogramma* and *Helicoverpa* interactions in the landscape, and how they might be mitigated.
- Integrate a multiple-disciplinary approach to natural enemy utilisation in IPM by assessing impacts on multiple pests in the landscape.

- Use of molecular techniques and other digital tactics (for example mapping, sensing technologies, data analysis) to improve interpretation of the spatial distribution of egg parasitoids and other beneficial insects.

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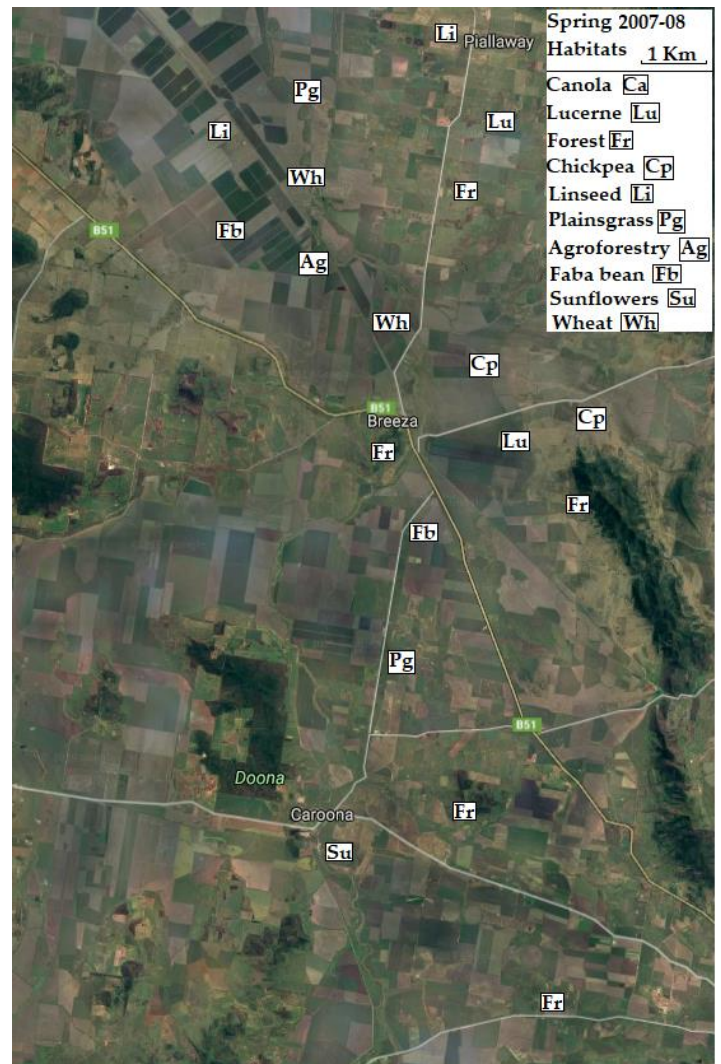
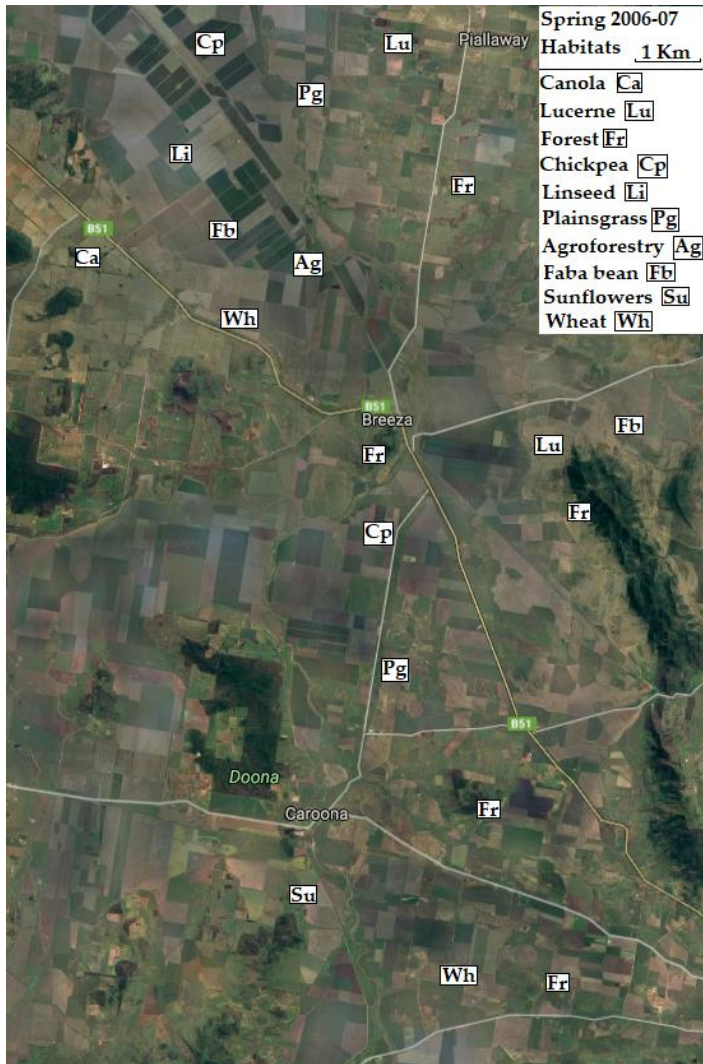
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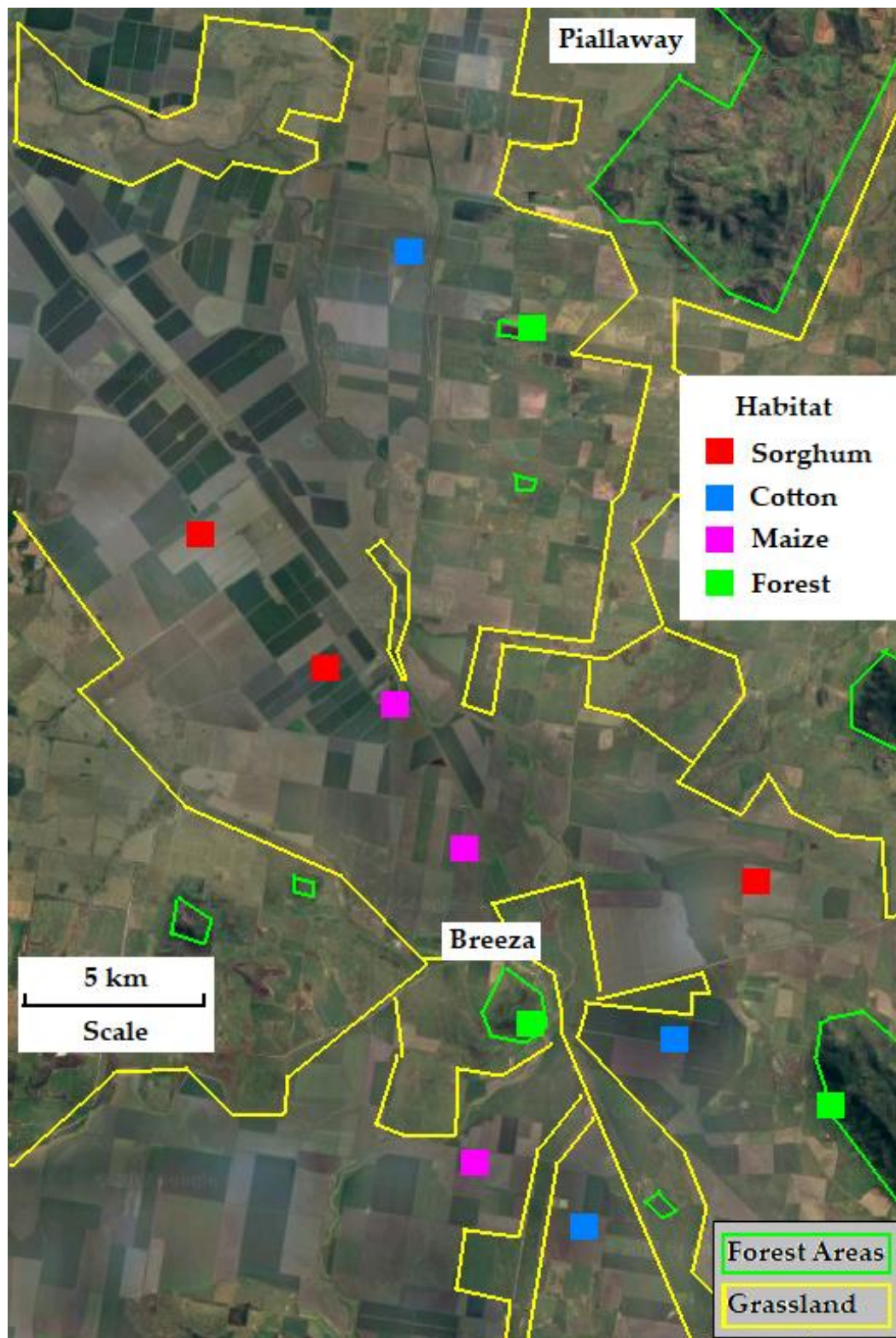
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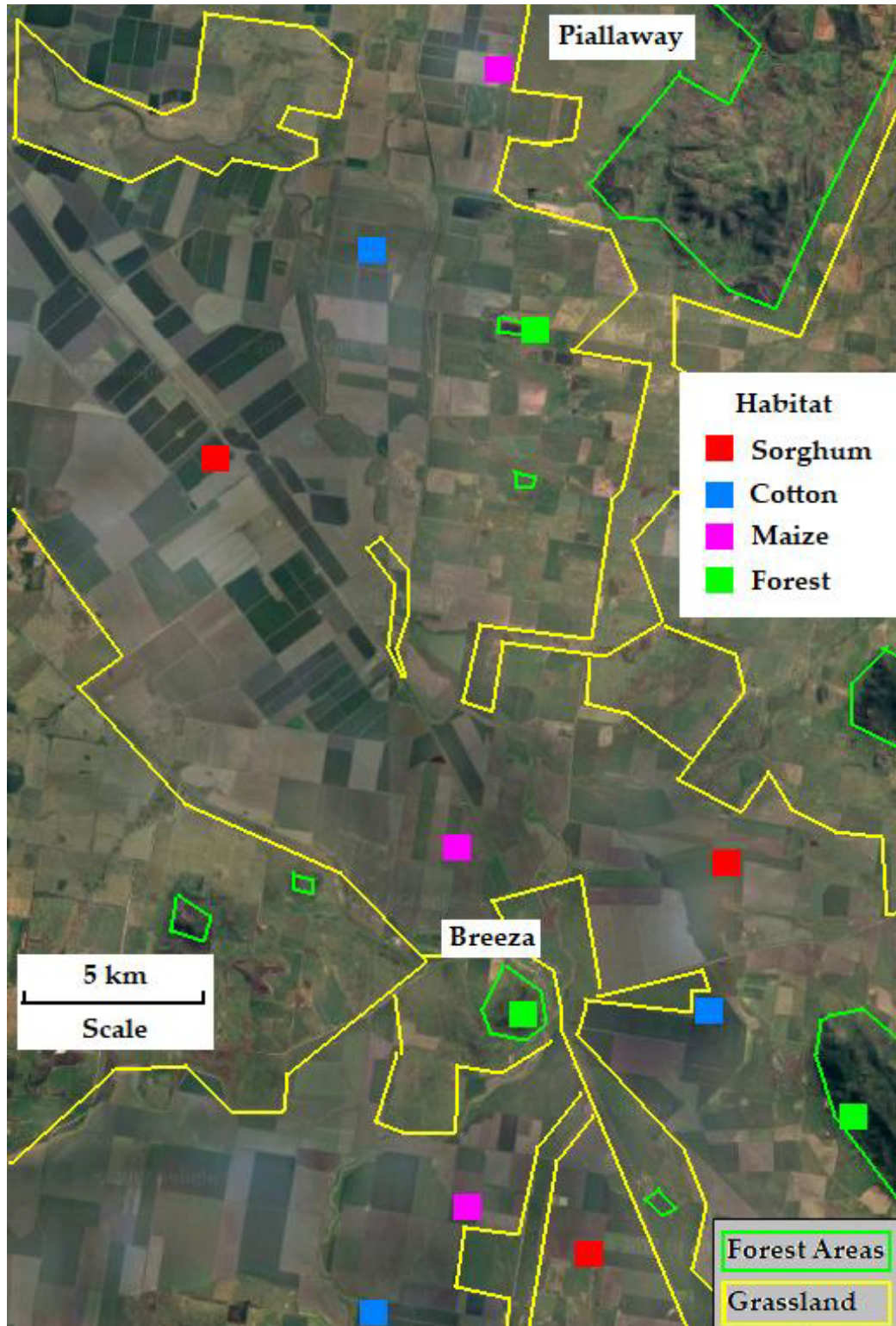
Appendix 1: Spring parasitoid field survey locations: 2006-07 and 2007-08



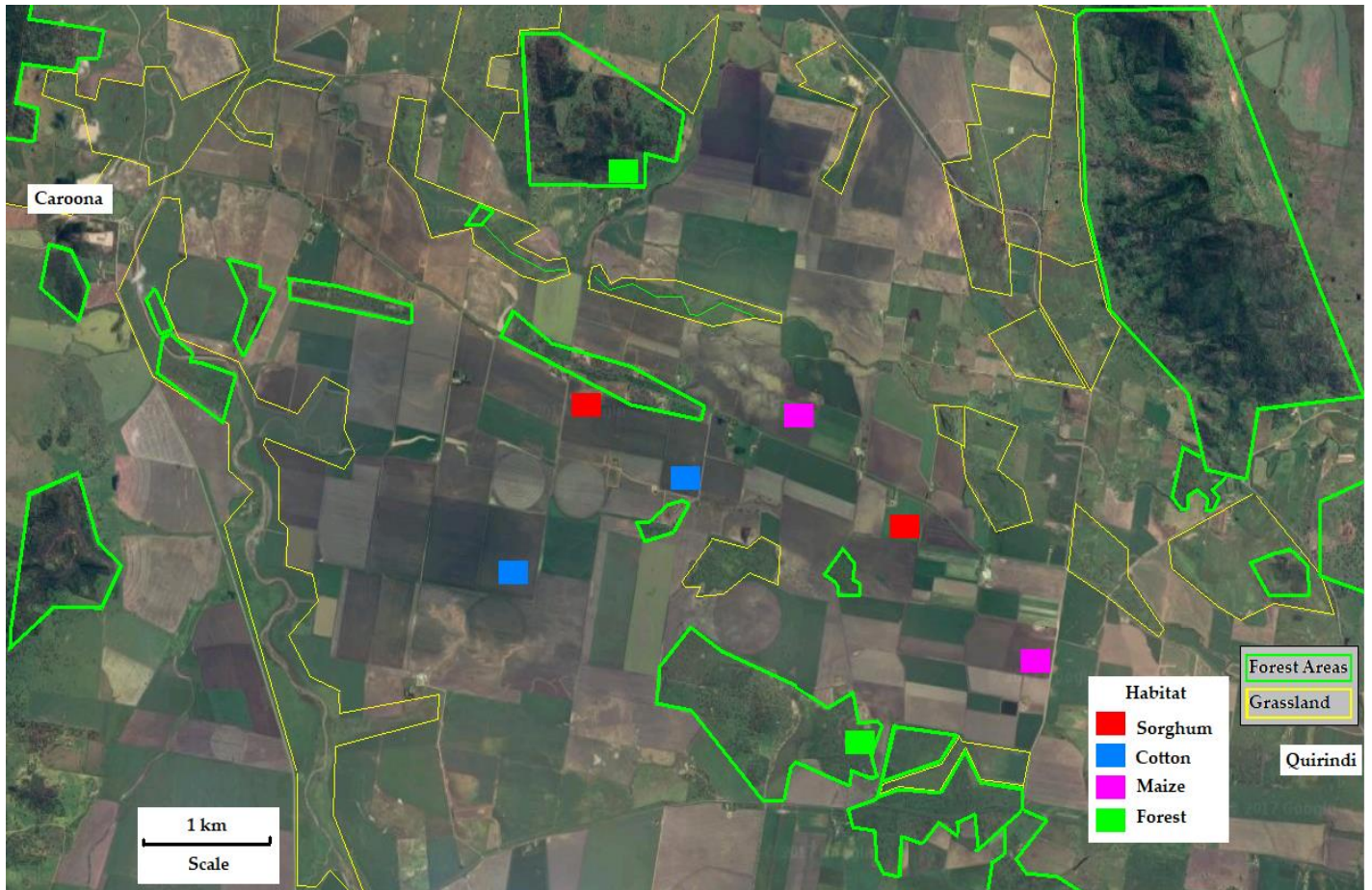
Appendix 2: Pialloway to Breeza, summer habitat surveys 2006-07



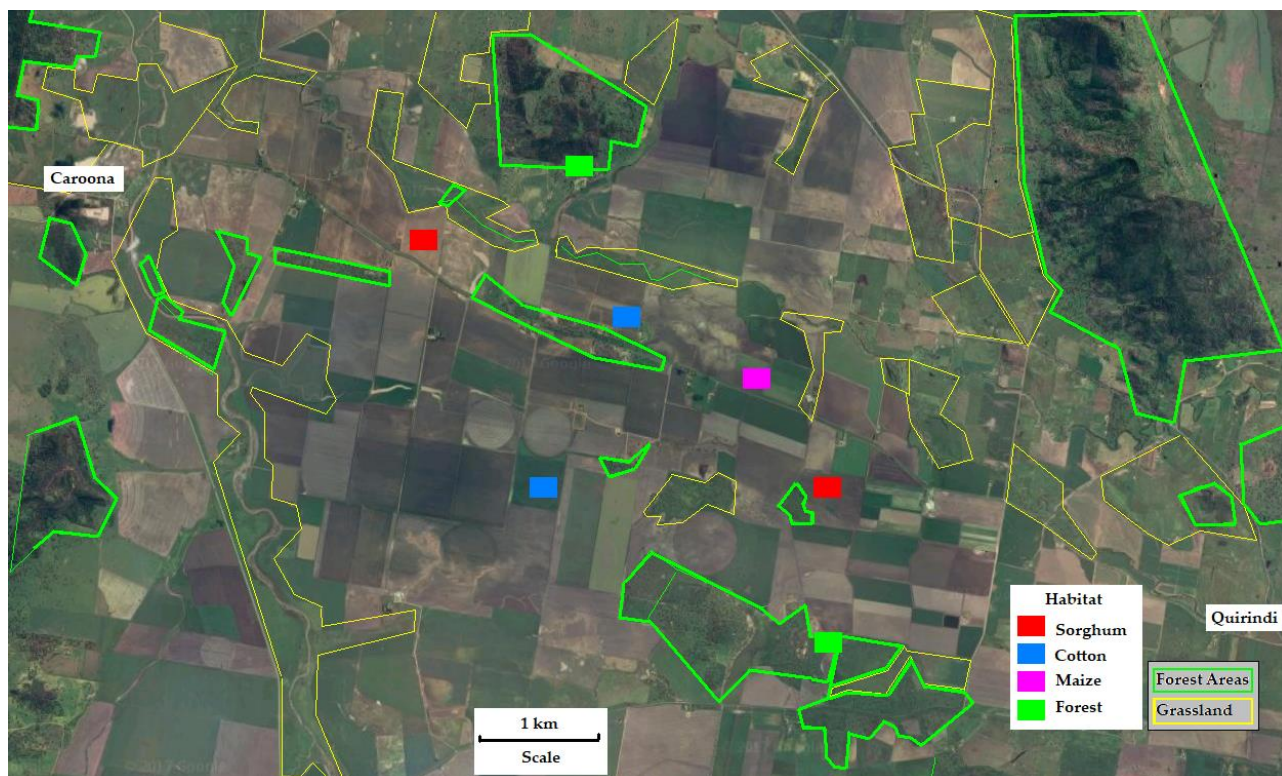
Appendix 3: Pialloway to Breeza, summer habitat surveys 2007-08



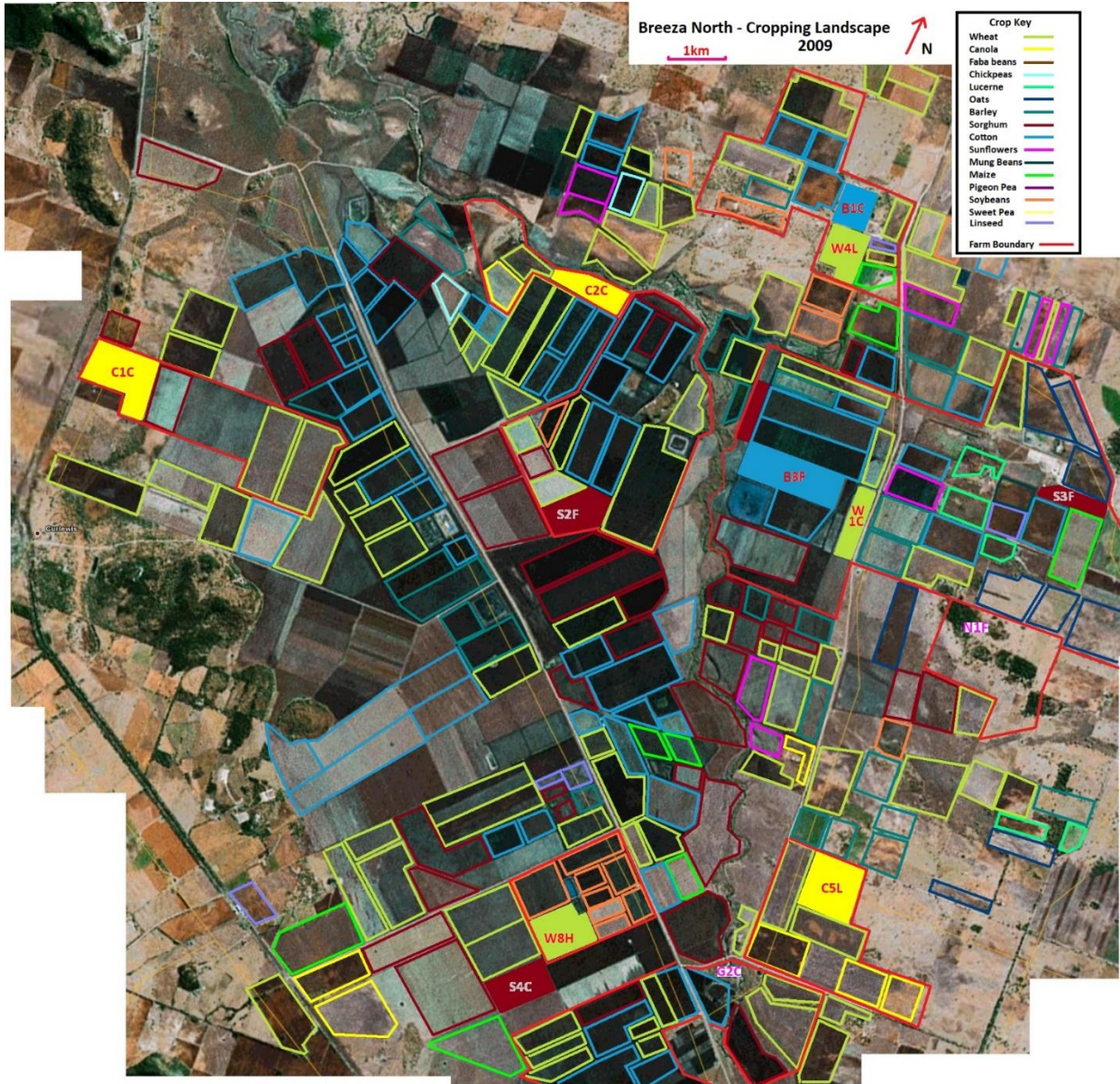
Appendix 4: Carroona to Quirindi summer habitat surveys 2006-07



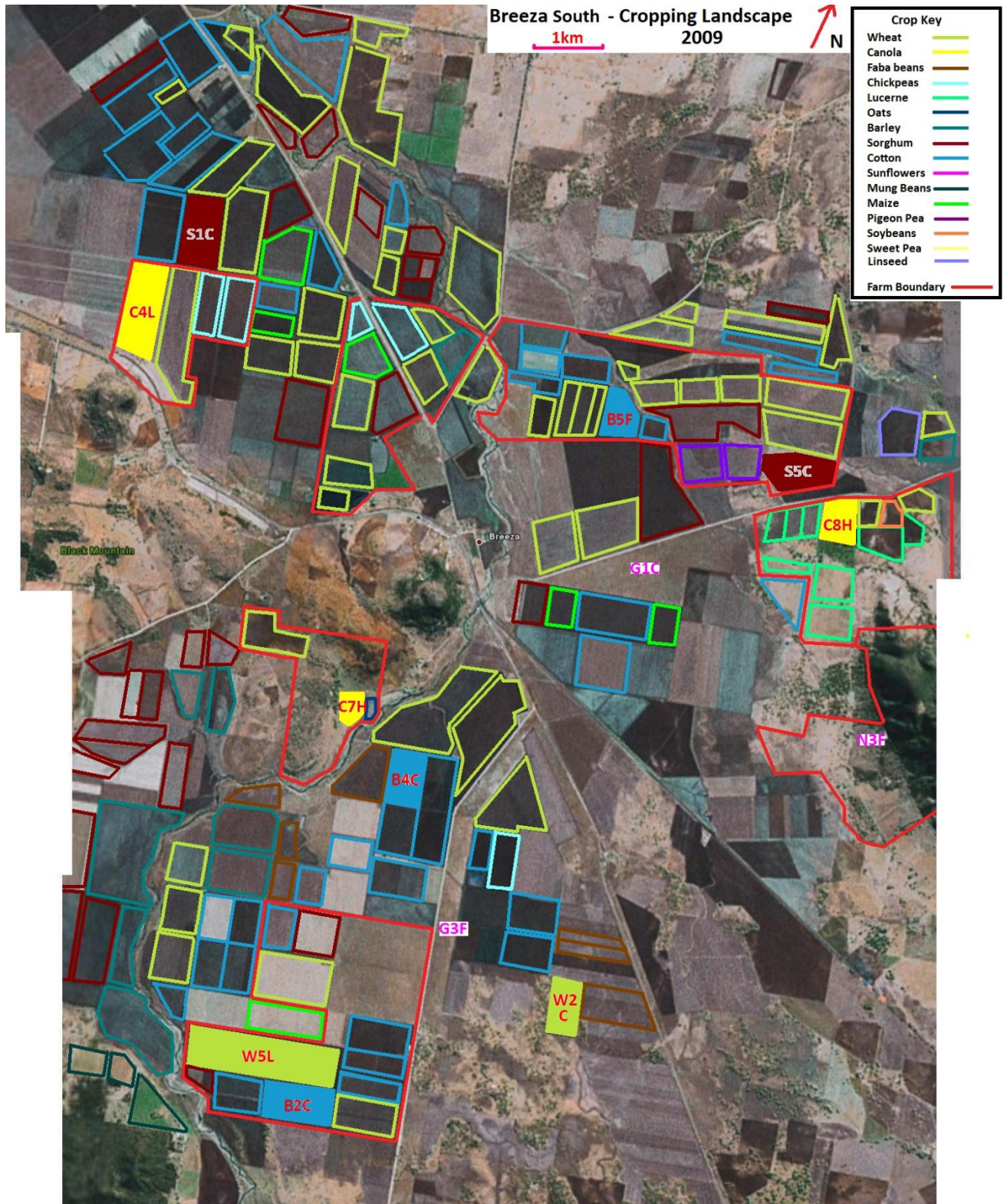
Appendix 5: Carroona to Quirindi summer habitat surveys 2007-08



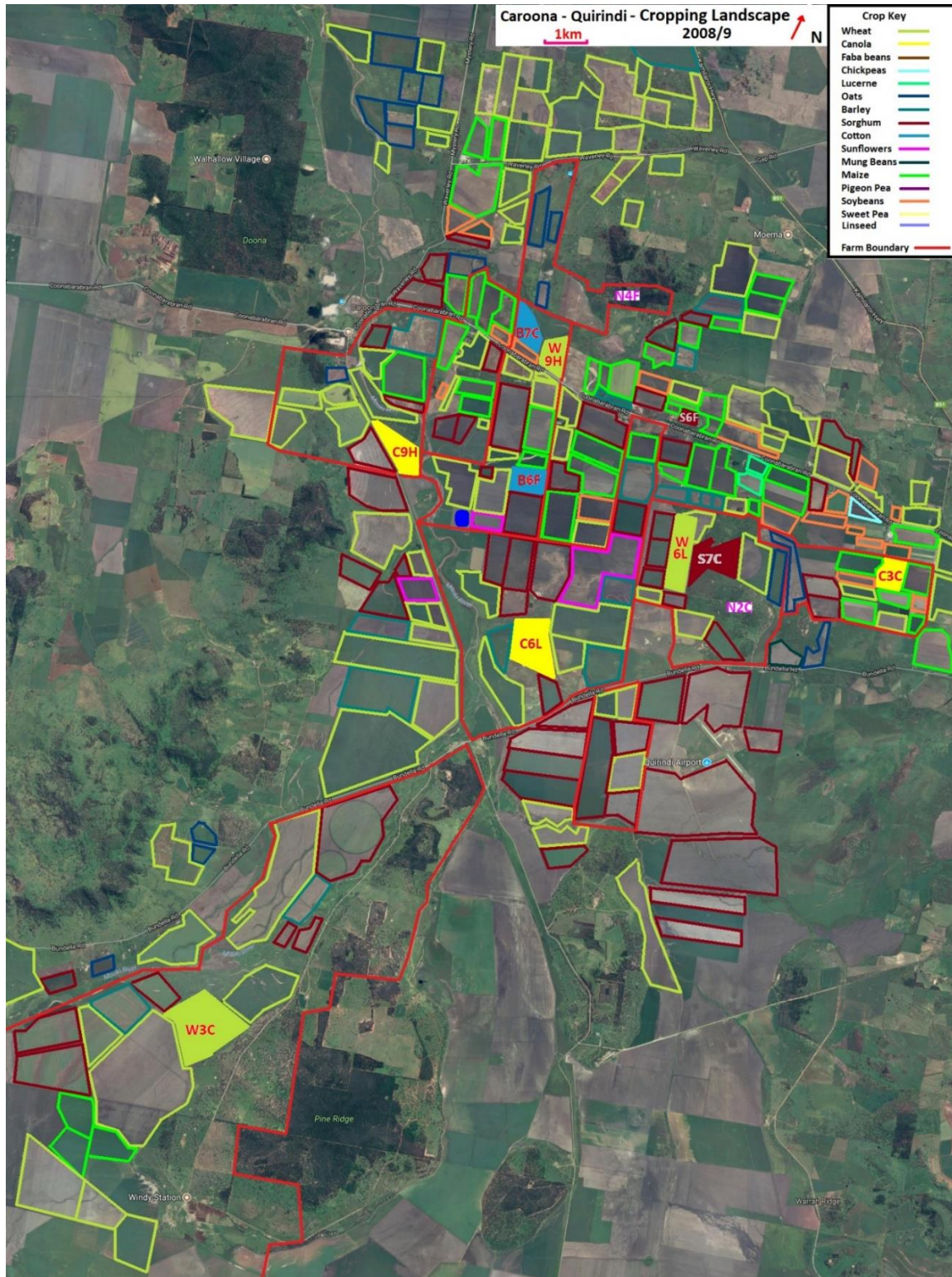
Appendix 6: Piallaway to Breeza cropping landscape 2008-09



Appendix 7: Breeza cropping landscape 2008-09



Appendix 8: Carroona to Quirindi, cropping landscape 2008-09



Appendix 9: Farmer Questionnaire

Details of chemical usage and IPM Breeza/Quirindi 2006-7

1) Property Name _____ 2) Date: _____

3) Name of farm owner/manager _____

4) Trial site crop details:

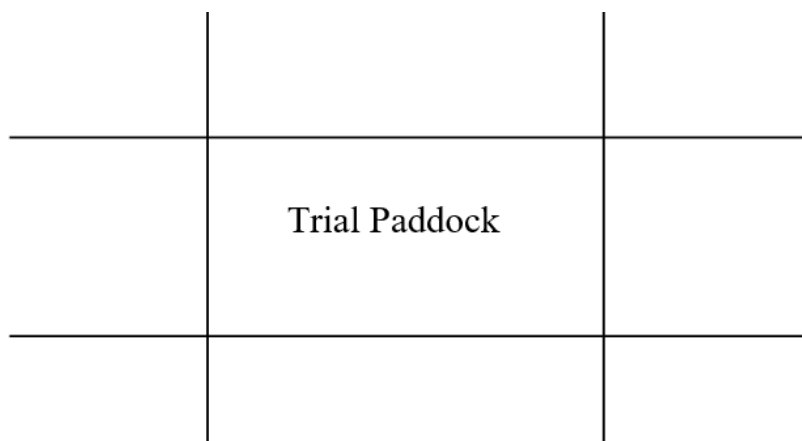
Crop: _____ Variety: _____ Planted: _____

Harvested: _____

5) What were the last 2 rotations in this paddock?

6) What other crops do you grow this season?

7) What are the surrounding crops to the trial site?



Chemical Usage

8) What chemicals were used in the trial site over the sampling period November 2006-March 2007 (including herbicides and fungicides)? (Attach notes if necessary)

9) Briefly what chemicals (insecticides only) were used in adjacent crops (list crops and dates of application) in the above sampling period for summer 2006/7 (provide only if convenient)

10) Did you spray for *Helicoverpa* or other pest moths this season or in the last 5 years in any crop (please state which pest/crop) on your property.

2003 _____

2004 _____

2005 _____

2006 _____

2007 _____

Integrated pest management (IPM) questionnaire

11) Do you monitor for insect predators? Yes / No

b. If so which ones?

c. Do predator numbers influence your spray decisions? Yes/ No

d. How?

12) What information would you like out of this *Trichogramma* research?

13) With this information, how would it influence your future decisions?

14) Any questions/ comments about this survey?

Thank you for your time

Christopher Carr

Appendix 10: Summary of the results of the farmer Questionnaire

Whilst the levels of *Trichogramma* had not been established on the Liverpool Plains prior to this study, farmers, especially cotton growers, were aware of the potential of *Trichogramma* from research in other areas of Australia. As part of my research about growers' use of insecticides in crops being monitored for egg parasitoids in this study, I interviewed 15 growers in a questionnaire in 2007-08. As part of the questionnaire I asked several questions about IPM and *Trichogramma* (Appendix 9). All the growers had mixed cropping and just over half (~60%) grew cotton. A similar number had controlled *Helicoverpa* in their crops in the previous 5 years. Most (~80%) said they or their consultant had monitored for insect predators (natural enemies). The social results are summarised in Fig. 1.2, with spiders (31%), hoverflies (19%) and lady beetles (31%) being the most commonly monitored natural enemies. Only three farmers considered wasps, but these were not *Trichogramma*.

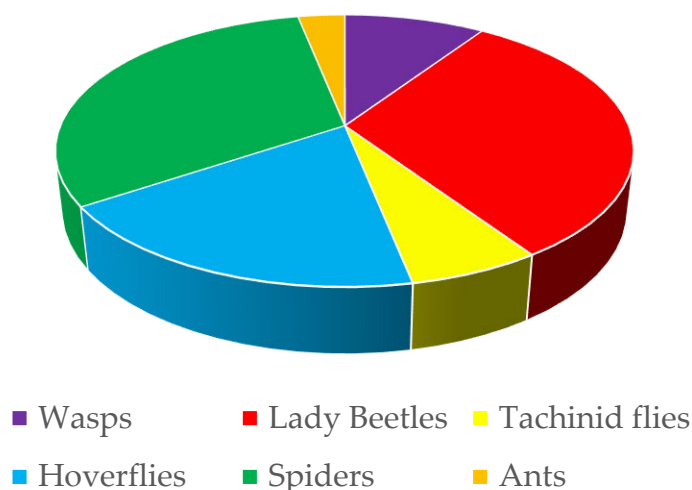


Figure A10a: The natural enemies monitored in field crops by 15 growers based on interviews on the Liverpool Plains in 2007-08

For the growers that monitored natural enemies, three-quarters said that their monitoring results influenced their spray decisions, with most holding off spraying if they thought pests were below economic thresholds and natural enemies were

perceived as keeping them at least partly under control. The most common pest at the time was aphids.

I discussed *Trichogramma* with growers at the start of the study (September 2006), but at the time of the interview in the second year (March 2008) I hadn't discussed any other research objectives. Each grower could give as many responses as they wanted. When I asked growers what they would like out of this research (Fig. 1.3), most wanted to know what species of *Trichogramma* there were (80%), if/where *Trichogramma* overwinter (73%), and how cost-effective they are against *Helicoverpa* at keeping *Helicoverpa* below threshold (47%). Growers also wanted to know about the *Trichogramma* life cycle (33%), if they occurred in non-crop habitats (27%), landscape effects and movement (13%), and how to encourage *Trichogramma* (13%). One grower also wanted to know if *Trichogramma* was more abundant in Bt cotton or conventional cotton, and if utilising *Trichogramma* could be used for farm carbon credits.

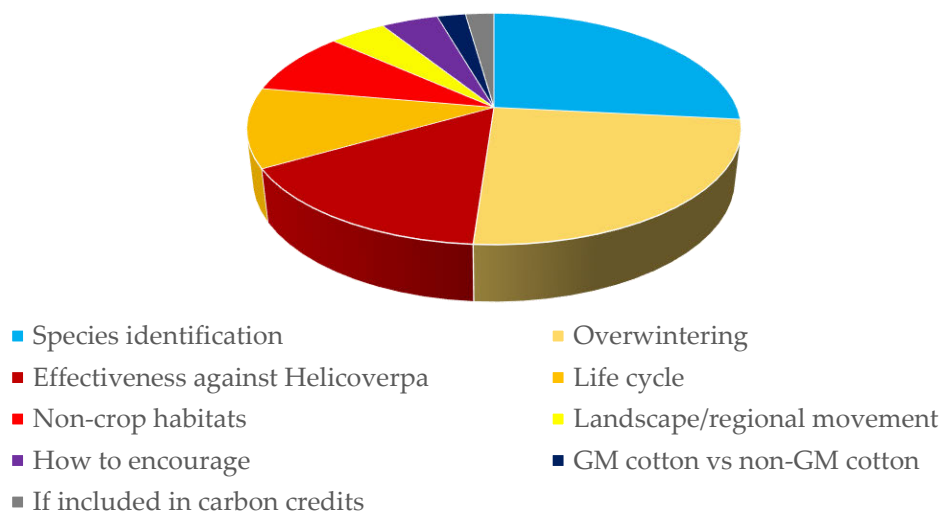


Figure A10b: The Information 15 growers whose crops were surveyed for *Trichogramma* would like from *Trichogramma* research on the Liverpool Plains in this study

Growers mostly said they would modify their practices to encourage *Trichogramma* if the research supported it (60%). Two said they would trial *Trichogramma* with their current practices, whilst three growers said they wouldn't do anything different.

Whilst not a comprehensive survey, the research information asked for by these growers is followed up in this thesis.