COMPARATIVE STUDIES OF THREE APHELINID PARASITOIDS OF *TRIALEURODES VAPORARIORUM* (WESTWOOD) (HEMIPTERA: ALEYRODIDAE) WITH EMPHASIS ON *ERETMOCERUS EREMICUS* ROSE AND ZOLNEROWICH

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Abstract

This thesis investigates the effectiveness of *Eretmocerus eremicus* Rose and Zolnerowich (Hymenoptera: Aphelinidae) as a parasitoid of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae). This investigation was performed on glasshouse tomatoes, because this is most economically important glasshouse crop in New Zealand. The *Er. eremicus* strain used in this study has been recently identified in New Zealand and differs significantly from other strains of *Er. eremicus* found in Europe and America.

The parasitism and host-feeding of *Er. eremicus* were investigated and compared to two other whitefly parasitoids (*Encarsia formosa* and *Encarsia pergandiella*) to determine which is the most effective parasitoid of the greenhouse whitefly. The parasitism study was performed on tomato plant leaf cuttings and the host-feeding study on tomato plant leaf disks. The leaf materials used in these studies were infested with 2nd instar greenhouse whitefly nymphs. To replicate the range of temperatures encountered in a glasshouse, these studies were performed within temperature controlled rooms set to 15, 20, 25, and 30^{o}C.

The results indicated that at temperatures of 25 and 30^{o}C, both *Er. eremicus* and *En. formosa* performed better in terms of parasitism and host-feeding than *En. pergandiella*. *En. formosa* parasitised the highest average number of whitefly nymphs (26 nymphs), which was 19% higher than *Er. eremicus* (21 nymphs) and 42% higher than *En. pergandiella* (19 nymphs). *En. formosa* also killed a significantly higher average number of whitefly nymphs through host-feeding (8 nymphs), which was 13% greater than *Er. eremicus* (7 nymphs) and 25% greater than *En. pergandiella* (6 nymphs). Furthermore, *En. formosa* also had a significantly longer average longevity (6 days), which was 17% greater than the *Er. eremicus* and *En. pergandiella* (both 5 days). At 30 and 20^{o}C, *En. formosa* had a higher parasitism than *Er. eremicus* and at 30^{o}C also a higher level of host-feeding. However the difference between these two parasitoids was small overall. *En. pergandiella* only displayed a high level of parasitisation at 15 and 20^{o}C, indicating it has adapted to cool temperatures, in New

Zealand, and is unlikely to be beneficial as a biological control agent in glasshouses - except in winter.

Two further studies were performed on *Er. eremicus* to determine the effect of adult parasitoid age on levels of parsatism and preference for specific greenhouse whitefly nymph instars. These studies found that the highest levels of parasitism occur in the first 5 days after the adult parasitoid emerges and a clear preference for early instar nymphs (1^{st}, 2^{nd}, and 3^{rd} instars).

These results of the studies presented in this thesis do not indicate any advantage in developing *Er. eremicus* as a biological control agent of greenhouse whitefly in tomato glasshouses in New Zealand. *En. formosa* had a significantly higher level of parasitisation and host-feeding with a wider temperature tolerance and greater longevity. Since *En. formosa* is already used as a biological control agent in New Zealand tomato glasshouses, this study shows no benefit in replacing it with *Er. eremicus*.

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Chapter One
General Introduction

The greenhouse whitefly (*Trialeurodes vaporariorum*) (Westwood) (Hemiptera: Aleyrodidae) is an economically important pest insect of covered crops in New Zealand (Martin, 1996). The control of greenhouse whitefly relies upon chemical treatments and/or biological control (Martin, 1996). Biological control agents are the natural enemies of pest insects that control their abundance in the wild (through predation, parasitism and infection) and have been shown to also effectively control target pest insects within crops (Gullan & Cranston, 2006). One of the most popular and important biological control agents for the control of greenhouse whitefly in glasshouses is the parasitic wasp *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae). In New Zealand, growers have had varied success using *En. formosa* to control greenhouse whitefly in glasshouses and there is a demand for more biological control agents to complement or replace this parasitoid (Peter Workman, pers comm., 2008). Growers are adopting biological control to produce crops with low chemical residues, in demand by consumers (Albert et al., 1999) and prevent the development of resistance to chemical treatments by pest insects (Costello et al., 1992).

This thesis explores the potential benefit of developing a New Zealand strain of *Eretmocerus eremicus* (Rose and Zolnerowich) (Hymenoptera: Aphelinidae) as a biological control agent of the greenhouse whitefly in New Zealand tomato glasshouses. *Er. eremicus* has only recently been identified in New Zealand, although it has likely been present in the country for at least ten years (Workman et al., 2008). The research presented in this thesis aims to determine the potential benefit of developing this strain of *Er. eremicus* as a biological control agent in New Zealand. Producing a new biological control requires an extensive investment of resources (Conlomg & Mugoya, 1996) and many trials to test their effectiveness within a crop (van Lenteren, 2005). This study aims to indicate if this investment is worthwhile.

Studies have shown that parasitism and host-feeding are the most important actions of a parasitoid to understand in determining its effectiveness in controlling a population of whitefly (Hoddle & Driesche, 1999; van Lenteren & Noldus, 1990). Parasitism occurs when an adult parasitoid deposits an egg into or underneath a whitefly nymph using its ovipositor. The developing parasitoid larva burrows into its host and feeds upon its tissues, timing its own development to co-inside with the development of its host (Hoddle, 1997). During host-feeding the adult parasitoid also uses its ovipositor to puncture a hole in the cuticle of a whitefly nymph, from which it will then feed upon the haemolymph extruded from the wound (Kassis & Michelakis, 1993) to obtain nutrients for prolonged longevity and maturation of eggs (Tumlinson et al., 1993). Both parasitism and host-feeding ultimately kill the whitefly host.

Within this thesis are presented experiments on the host-feeding and parasitism efficacy of *Er. eremicus, En. formosa* and *En. pergandiella;* all parasitoids that are found within tomato glasshouses in Auckland, New Zealand and contribute towards control of greenhouse whitefly. *En. formosa* is a well known biological control agent of greenhouse whitefly in New Zealand and was introduced into the country for that purpose in 1933 (Martin, 1999). *En. formosa* was included in the trial because this parasitoid is presently mass reared for use as a biological control agent of greenhouse whitefly in New Zealand (Workman & Pedley, 2007) and acts as a good 'yard stick' to assess the performance of other parasitoids against. *En. pergandiella* (Howard) is a hyper-parasitoid which is occasionally encountered within crops parasitizing greenhouse whitefly, but has been little studied (Marotto, 2007). Because *En. pergandiella* can also become naturally established within glasshouses in New Zealand (Workman & Pedley, 2007) and contribute towards control of whitefly, it has been included in this study to see if there is also value in pursuing the development of this parasitoid as a biological control agent. The comparison between these parasitoids aims to determine if *Er. eremicus* has a suitable level of parasitism and host-feeding for use as a biological control agent of greenhouse whitefly in New Zealand.

The parasitism efficacy and host-feeding experiments were performed on tomato leaf material, because this is the primary glasshouse crop within New Zealand in

which parasitoids are used for biological control of greenhouse whitefly. Parasitism efficacy was tested on tomato leaf cuttings, a technique developed from trials of different methods that would maintain healthy whitefly nymphs and leaf material over the lifespan of an adult parasitoid. Other researchers have favoured clip cages (Greenberg et al., 2002), but trials with this technique resulted in build up of honey-dew within the chambers and impaired results. Host-feeding was tested on tomato leaf disks, presented within vented, agar-based Petri dishes, which are commonly used in parasitoid experiments (Headrick et. al., 1995). Trials with this technique showed the environment within the dishes was easily fouled by honey-dew, but this was offset by being able to produce many replicates that were easy to handle. The Petri-dishes also kept the whitefly nymphs and leaf material in an acceptable level of health for the 10 days of the host-feeding experiment.

The parasitism and host-feeding performed by these three parasitoids are investigated at temperatures of 15, 20, 25, and 30^{o}C. These temperatures aim to simulate the range of temperatures encountered within glasshouses in New Zealand (John Thompson pers comm., 2008). These studies were performed in controlled temperature rooms at Plant & Food Research, in Auckland, on parasitoids and whitefly reared at Bioforce Ltd. All studies where performed on tomato leaf material, either on "Moneymaker" tomato plant leaf cuttings or leaf disks.

Two other studies were performed on *Er. eremicus,* to determine the effect of adult parasitoid age on parasitism and the preferred whitefly nymph instar stage for parasitism at 20^{o}C, on tomato leaf material infested with 2nd instar greenhouse whitefly nymphs. These studies take a closer look at aspects of *Er. eremicus* biology identified as being important for mass rearing a parasitoid (Conlong & Mugoya, 1996; Steven & Naranjo, 2001) and are also variables that may effect interpretation of the parasitism efficacy study. These studies were selected after consultation with entomologists at Plant & Food Research and Massey University and biological producers at Bioforce Ltd, to ensure that the information provided would be of most interest to the industries involved.

In summary, the main objectives of this study are to:

1. Compare the parasitism efficacy of *Er. eremicus, En. formosa* and *En. pergandiella*.

2. Compare the host-feeding efficacy of *Er. eremicus, En. formosa,* and *En. pergandiella*.

3. Determine the preferred greenhouse whitefly nymph instar stage for parasitisation by *Er. eremicus.*

4. Investigate the effect of adult age on parasitism of greenhouse whitefly by *Er. eremicus*.

5. To recommend the suitability of *Er. eremicus* as a biological control agent of greenhouse whitefly on covered crops in New Zealand.

The goal of these studies is to obtain a clear understanding of the role *Er. eremicus* might have has a biological control agent of greenhouse whitefly on glasshouse tomatoes in New Zealand. This information can then be conveyed to producers of biological controls in New Zealand, for them to weigh up the benefit of developing mass rearing techniques and facilities and to research institutes to add to their understanding of this parasitoid.

Chapter Two
Literature Review

2.01 Introduction

The aim of this literature review is to provide a context of how parasitoids are used as biological control agents within a glasshouse crop, by reviewing the environment within a glasshouse, some common glasshouse pests, how biological control agents are incorporated into Integrated Pest Management systems, challenges in making biological control agents work in crops, how parasitoids operate, and measures of parasitism efficacy.

Biological controls are the natural enemies of pest insect species that can be reared in sufficient numbers and can adapt to a crop environment to contribute to the control of pest insect species (Wiedenmann & Smith, 1997). Natural enemies such as predators, parasitic wasps and flies, as well as pathogens have long been studied for exploitation as biological control agents (Malais & Ravensberg, 2003). Many natural enemies are unsuitable for a covered crop environment because of a lack of sufficient food and shelter, an unsuitably hot or humid environment, or the crop plants have unsuitable leaf surfaces (Steven & Naranjo, 2001).

2.02 Protected Cropping Systems

Protective cropping systems are structures used in horticulture to protect crops from the elements (most notably wind and rain), provide support and to provide warmth or shade (Stanhill, 1980). Glasshouses are a common type of protected environment and are used to extend the growing seasons and / or provide out of season produce (Bertin et. al., 2000). With a high demand for out of season produce and large export markets developing, the production of fruit and vegetables within glasshouses is gaining popularity (Houter & Nederhoff, 2006;

Malais & Ravensberg, 2003). Many of these crops are also fragile and the enclosed environment of a glasshouse provides an easily controllable stable environment, where all factors from temperature, soil, humidity, light intensity, nutrient availability and plant shape can be directly controlled by the grower (Canas et al., 2007; Costello et al., 1992). The sheltered, clean environment and small plants also makes pruning and spraying practices easy. Unwittingly, the grower has also created the perfect environment, both stable and warm and with an abundant food source, for pest insects to quickly multiply (van Lenteren & Martin, 1999).

This review focuses upon glasshouse protected crops within New Zealand, to provide a context for this study on control of greenhouse whitefly with parasitoids on glasshouse tomatoes. Modern glasshouse designs in New Zealand have a steel beam frame with UV protecting glass panels (http://www.nzglasshouse.co.nz/). These glasshouses are automatically ventilated with roof ducts and heated with metal pipes carrying water heated from a diesel burner (John Thompson pers comm., 2008). The crops are most commonly fertilised through automated irrigation systems that provide an optimal mix of nutrients at a sustained level (John Thompson pers comm., 2008). The temperatures within New Zealand glasshouses vary with seasons, with heating during winter being conservative to save energy costs (Nederhoff & Houter, 2006). Summer temperatures range from 25-35^{o}C, with shade cloths and roof vents used to reduce temperatures during hot days (Nederhoff & Houter, 2006). In winter temperatures vary from 15-25^{o}C, with hot water pipes lying at ground level used to raise temperatures, especially during nights and mornings (Nederhoff & Houter, 2006). The most common problems in New Zealand glasshouses is damage to plants from intense ultra-violet radiation, which has been reduced in recent years with use of UV resistant glass (http://www.nzglasshouse.co.nz/), and from high soil temperatures in summer leading to development of fungus and the plant roots dying (Canas et al., 2007; Nederhoff, 2001).

New Zealand stakes a global reputation as a producer of high quality produce and is able to provide out of season produce to markets in Asia and Europe at

competitive prices (Saunders et al., 2003). In New Zealand, tomatoes are the most economically important crop grown within glasshouses. Other economically important crops are cucumbers, capsicums and cut flowers (http://www.freshvegetables.co.nz). In New Zealand, tomatoes are grown from seed within sterile punnets and then transferred to large plastic flower pots filled with commercial potting mix (Peter Workman pers comm., 2008). The tomato plants are grown within glasshouses and attached to string lines to support their growth. They are also connected to automated irrigating systems to dispense a consistent quantity of water and nutrients (Houter & Nederhoff, 2006). The main export market for New Zealand tomatoes is Australia and Japan (http://www.tomatoesnz.co.nz/) with produce also sold locally.

2.03 Pests of Protected Cropping Systems

Pest insects are recognised as insects that can occur in such abundance that they cause ecological, economical, or aesthetical damage to a natural system or man-made crop / environment (Gullan & Cranston, 2006). Insects often become pests because they have been introduced or disperse to an area which does not have natural enemies to control them (Costello et al. 1992) and they have become serious crop pests (van Lenteren et al., 1997). In northwestern Europe, 75% of the species of greenhouse pests (approximately 40 species) have been accidentally introduced into the region (van Lenteren et al., 1997). Islands, in particular, are vulnerable to foreign pest species because they have a smaller diversity within their insect fauna that could provide naturally occurring biological controls (Martin, 2005). In sub-tropical regions the damage caused by introduced pest insects is often reduced, due to a more diverse native insect fauna with more natural controls (Snapp et al., 2005).

There are a diverse range of pest insects that can become established within a protected crop, with the most common types being species of aphids, mites, whitefly, thrips and psyllids (van Lenteren, 2000). The control of pest insects is a significant cost to the production of crops in a protected system (Phatak, 1992).

Many of these pests now have global distribution, because of being introduced into countries on plants (Gilkeson, 1992). Many of the countries they have been introduced into have few natural enemies established to control them (van Lenteren, 2000).

Thrips feed on both the pollen and foliage of plants and their feeding results in withering of the leaves and loss of plant vigour (Piatkowski, 2008). Thrips commonly invade glasshouse environments and proliferate rapidly within this warm and sheltered environment with an abundant food source (van Lenteren, 2005). The most frequent species, causing the greatest damage, are *Frankliniella occidentalis* and *Thrips tabaci* (Snapp et al., 2005). Integrated pest management programmes have been developed in many countries to control thrips in glasshouses (Malais & Ravensberg, 2003). In glasshouse crops *Thrips tabaci* are controlled using prophylactic pesticide treatments, use of the predators *Amblyseius barkeri* and *Neoseiulus cucumeris*, crop rotations and selection of cultivars resistant to thrips feeding (Lindquist, 1991). Many thrip species have been introduced into New Zealand and they pose a significant and ongoing biosecurity threat. A pesticide-resistant glasshouse strain of the Western flower thrips (WFT) (*Frankliniella occidentalis*) was discovered in the Auckland region in 1992 on glasshouse capsicums (Teulon & Nielsen, 2005), which has been a major international pest of glasshouse crops (Malais & Ravensberg, 2003).

Thrips can be a major pest of tomatoes, because they can transmit Tomato Spotted Wilt Virus (TSWV) (van Lenteren et al. 1997). In New Zealand the onion thrips (*Thrips tabaci*) is the main vector of TSWV, but flower thrips (*Thrips obscuratus*) are also commonly found on tomatoes. In commercial glasshouses in New Zealand, thrips may be controlled by a predatory mite *Neoseiulus cucumeris* (Riley et al., 2010).

There are approximately 4,000 aphid species in the world and some of these species have become serious glasshouse pests (Heinz, 1998), as the climatic factors and plant condition are often optimal for their development and reproduction (Lindquist, 1991). There are two main groups of pest aphids: polyphagos species (*Myzus persicae* and *Macrosiphum euphorbiae)* that infest

mainly solanacaeae plants, but will also attack a wide range of plants and oligophagus species (*Nasonovia ribisingri*, *Hyperomyzus lactucae*, and *Acyrthosiphon lactucae)* that target specific plant species (Rabasse & van Steenis, 2002). There is an increasing demand amongst growers for biological control options against aphids due to insecticide resistance in several species (Sunderland et al., 1992) and to complement biological control strategies used for other pest insects (Snapp et al., 2005). Aphids are very well adapted to exploiting a new and temporary habitat by rapid population increase (Lindquist, 1991). Their structure is simplified to enable them to perform best in feeding and reproduction, with most of their nutrients directed to reproduction. However, they have retained their ability to walk and fly and winged morphs are specially produced when the populations need to disperse towards a new food source (Sunderland et al., 1992).

The green peach aphid (*Myzus persicae)* is a significant pest of greenhouse crops because of its wide host range, worldwide distribution, number of viral diseases it vectors, and difficulty of control (Cameron, 1996). Management of this species relies on understanding that the females do not have to mate in order to reproduce and produce live young which can result in population explosions of this aphid (Mansour, 1993). There are several biological control options established to control this aphid, including: green lacewings (*Chrysoperla carnea* and *Chrysopa rufilabris*), aphid midges (*Aphidoletes aphidimyza*), parasitic wasps (*Aphidius colemani and Aphidius matricariae*) and lady beetles (*Hippodamia convergens*) (Malais & Ravensberg, 2003).

Psyllids are another significant pest in glasshouse crops, which they can damage through feeding and transmitting plant pathogens (Wiedenmann & Smith, 1997). The feeding of psyllids results in deposit of honey dew, which can build up on fruit and leaves causing sooty mould to develop and spoil fruit (Liu & Trumble, 2005). Severely affected plants lose turgor; growth ceases and leaves absice (Phillips, 2009). Less severe infestations reduce vegetative growth and the initiation and formation of flower buds (Mehrnejad & Jalali, 2004). Psylids are also known to transmit many damaging pathogens (Gullan & Cranston, 2006). Management of psyllid populations within glasshouses is problematic, requiring

an integrated approach including: use of clean stock plants resistant to psyllid feeding and use of pesticides and biological control agents (van Lenteren & Manzaroli, 1999). The most effective biological controls have been parasitoids, such as the *Tamarixia radiata* used to control the Asian citrus psyllid, (*Diaphorina citri*) in America (Hall & Jenkins, 2009).

In New Zealand the potato/tomato psyllid (*Bactericera cockerelli*) was discovered in 2006 (Teulon et al., 2009). Damage to crops by this psyllid can be considerable, with a reported loss of 80% of a glasshouse tomato crop in California in 2001 and 50% in 2004 (MAF factsheet, 2009). This psyllid is a host to a bacterial pathogen (*Liberibacter Sp.*) that it spreads to tomato plants when feeding (Munyaneza et al., 2007) and causes diseases reducing the quality and yield of crops. The symptoms of the bacterial disease are commonly referred to as "psyllid yellows" and result in yellow curled leaves, stunted growth and small fruit (Phillips, 2009). No naturally occurring natural enemies have a significant impact on the control of this species in New Zealand and within two years it has spread too many tomato glasshouses, resulting in the closure of some export markets (Phillips, 2009). Parasitoids are being trialed as biological control agents to control this psyllid in New Zealand, incluing *Tamarixia triozae* a parasitoid imported from Mexico (Workman & Whiteman, 2009).

Whiteflies, in particular, represent a major economic cost to the production of covered crops (Malais & Ravensberg, 2003). *Bemisia tabaci, Bemisia argentifolii* and *Trialeurodes vaporariorum* are species of whitefly that have been self-introduced into many countries and cause significant disruption to crop production in protected cropping systems (van Lenteren & Martin, 1999). *Bemisia argentifolii* has also become a major problem in North America since the early 1980's, infesting first greenhouse crops and then commercial crops and ornamental plantings (Gill, 2000). It quickly spread throughout greenhouse production areas of North America and chemical treatments have lead to the rapid build-up of insecticide resistance (Matteoni, 1993).

2.04 Greenhouse Whitefly

The greenhouse whitefly (*Trialeurodes vaporariorum)* (Westwood) (Homoptera: Aleyrodidae) is an economically important pest in greenhouse vegetable crops worldwide (Michelakis, 1995) and in New Zealand (Workman & Davidson, 2007). Greenhouse whitefly were first reported in Europe, in UK greenhouses in 1856, and described that year by Westwood (Russell, 1977). It was thought to have been introduced on living plants from Mexico (Succop, 1997). This species is now cosmopolitan and one of the most prevalent pests of greenhouse crops worldwide (van Lenteren & Noldus, 1990).

Greenhouse whitefly attacks more than 500 species of food, fibre and ornamental plants causing crop losses that total to hundreds of millions of dollars (van Lenteren, 2005). Within glasshouses, tomato and cucumber crops are both fed upon by greenhouse whitefly (Malais & Ravensberg, 2003). Both adults and immature nymphs settle on the underside of leaves and feed upon the plant phloem using piercing-sucking mouthparts (McMahon & Lindquist, 1992). Excessive feeding by greenhouse whitefly leads to stunted plant growth and production of fewer and smaller tomatoes (Workman & Davidson, 2007). Whiteflies also excrete sticky and sugary honeydew, which collects on leaves and fruit creating a favourable environment for sooty mould growth (van Lenteren & Martin, 1999). The build-up of mould on leaves results in less photosynthesis and consequently less sugar for fruit development and ripening (Malais & Ravensberg, 2003). The build-up of mould on fruit requires extra handling time to wash the collected fruit (Cloyd, 1999; Costello et al., 1992).

The greenhouse whitefly was introduced into New Zealand at about 1933, on crop plants (Martin, 1999). Greenhouse whitefly is the commonest whitefly species found in New Zealand and occurs there on a wide range of solanaceous plants, such as tomatoes; cucurbits, such as cucumbers and pumpkins; legumes, such as beans and compositae, such as, the milky thistle (Smith, 2009). A strain of greenhouse whitefly has adapted to tamarillo, while others have adapted to feeding and breeding on weeds (Smith, 2009). The greenhouse whitefly may be

found all year round in greenhouse crops and outdoors in the warmer parts of the country (Martin, 2005). If left uncontrolled large populations can develop on greenhouse crops over a production season (Martin, 2005). High infestations of greenhouse whitefly can have a large impact on plant growth and yield (Smith, 2009). The greenhouse whitefly is a particularly important pest of tomato plants, which is New Zealand's largest greenhouse crop by area and value (Ferguson et al., 2007). Whitefly may be present in greenhouses throughout the year round, but most growers report that infestations are worst in summer, especially when it is hot and dry, or sunny (Cameron et al., 2009).

Fourteen other species of whiteflies occur in New Zealand, eight of which are indigenous and six introduced (Martin, 1999). The sweet potato whitefly *(Bemisia tabaci)* is the only other whitefly present in New Zealand of potential economic importance to covered crops in New Zealand (Gill, 2000). However, this whitefly is not found in significant enough numbers here to be a threat to covered crops (Workman & Davidson, 2007).

2.05 Control Options for Protected Crops

The control of pest insects using synthetic chemical sprays first developed in the 1950's, which together with the industrial revolution lead to large-scale production of monoculture crops (van Lenteren & Martin, 1999). The application of chemical sprays has traditionally been on fixed calendar dates, irrespective of the presence or abundance of pest insects within a crop (Phatak, 1992). The attraction of this approach is that the grower had a very simple control strategy and that chemical sprays were inexpensive to apply (van Lenteren et al. 1997). Chemical sprays continue to be the primary means of pest control employed in crop production; but increasingly, concerns over the chemical resistance in pest insects, destruction of non-target organisms (pollinators, natural enemies and soil arthropods) and demand from consumers for low chemical residue produce, has lead to interest in less toxic methods of pest control (Gullan & Cranston, 2005). In an effort to reduce the use of chemical sprays, growers are beginning to look

towards control options using natural enemies as biological controls and less toxic sprays, used only when needed (van Lenteren, 2005).

2.06 Integrated Pest Management Programmes

In an attempt to reduce chemical spray residues on crops and provide reliable and efficient management practices for growers, integrated pest management (IPM) programmes have been developed (Martin, 2005). This approach couples using biological controls with the selection of pest resistant cultivars, low-toxicity pesticides, and efficient crop husbandry techniques (van Lenteren, 2003; Zchori-Fein et al., 1994). Many interesting developments have emerged in this field, including manipulating the crop environment to suit the biological control agents and using complementary chemical control options (Martin, 2005). A key point in developing IPM strategies is that the management practices used are complementary to each other (Gullan & Cranston, 2005). Applying IPM effectively requires a detailed knowledge of the biology of both pest and beneficial insects, so that natural enemies are present in a crop environment at times when pest insects reach certain thresholds or are expected to disperse into that system, or that sprays are applied only at key times when they will be most effective in controlling pest insects and least disruptive to beneficial insects (Martin, 2005). A major focus in developing IPM strategies is to identify effective new biological control agents (Michelakis, 1995; Tauber et al., 2000).

The following list sets out a series of management practices for greenhouse whitefly which is typical of an integrated pest management programme (van Lenteren, 2003).

- Destruction of crop residue from all susceptible crops immediately after harvest.
- Removal of host weed plants of pest insects surrounding and within the crop environment.
- Planting of smooth leafed varieties that encourage dispersal of beneficial insects through the crop.

- Avoiding moisture stress in the crop, which reduces resilience to pathogens and damage from pests insect feeding.
- Avoiding the use of broad spectrum chemistry early that destroys predators and parasites.
- Sampling for presence and abundance of insect regularly and set threshold levels of pests to be reached before chemical treatments are applied.
- Delaying use of pyrethroid products for as long as possible.
- Adhering to Insecticide Resistance Management Strategy (IRMS).

Despite the advantages of using IPM programmes they have been slow to be adopted by growers (Steven & Naranjo, 2001). This has been because of the high cost of biological controls compared to insecticide alternatives (Cranshaw et al., 1996), the inconsistency in the supply of natural enemies and their quality (van Lenteren, 2003), and the lack of crop and pest specific management guidelines (Hoddle, 1997). Growers are also under pressure from export markets demanding high-quality produce, free of any blemish associated with pest insects (Phillips, 2009). Such standards favour a pest-free environment within a covered crop, which is not possible in a system where pest insects are controlled by a sustainable population of biological controls (Martin, 2005).

The development of more biological controls of greenhouse whitefly is seen as a primary objective for establishing sustainable IPM systems in New Zealand (Workman & Pedley, 2007). Biological control options in New Zealand are few because of the isolation of this landmass, which has resulted in a limited insect fauna (Martin, 1996). This makes crops in New Zealand susceptible to damage by introduced pest insects because there are few natural enemies to control them (Martin, 1996). A range of pest insects have been introduced into New Zealand and researchers struggle to keep ahead of these outbreaks and develop suitable biological controls (Workman & Davidson, 2009). In particular, the development of an effective biological control of the greenhouse whitefly on glasshouse tomatoes is seen by the horticulture industry in New Zealand as highly desirable (due to the high export revenue from this crop) to improve the

control of this pest using techniques which produce fruit with low chemical residues (Workman & Pedley, 2007).

2.07 Biological Controls

Using biological controls in horticulture aims to re-establish some of the natural enemies of pest insects, which would control their abundance in their natural habitat (Steven & Naranjo, 2001). Without biological control, the production of energy by plants would be a tiny fraction of what is currently produced (Kuack, 1995). These natural enemies are most often parasitoids or predators of the pest insects (van Lenteren & Manzaroli, 1999). Biological control is present in all ecosystems, both natural and manmade and is always active (van Lenteren, 2000).

Natural enemies occur in all production systems, from the backyard garden to the commercial field. They are adapted to the local environment and to the target pest, and their conservation is generally simple and cost-effective (van Lenteren, 2003). In natural ecosystems, a myriad of natural enemy species maintain plant-eating insects at low population densities (van Lenteren & Manzaroli, 1999). With relatively little effort parasitoids, lacewings, lady beetles and hover fly larvae can be encouraged into crops infested with aphid colonies (Phatak, 1992). Following periods of high humidity, fungus-infected adult flies are also often encountered (Steven & Naranjo, 2001).

Even in agro-ecosystems, many potential pests are held at non-damaging levels by natural enemies which occur naturally (Wiedenmann & Smith, 1997). DeBach and Rosen (1991) estimate that more than 90% of all agricultural pest species are under natural control. In many instances the importance of natural control does not become apparent until insecticide use is stopped or reduced (van Lenteren, 2003). At that time natural enemies become re-established within a crop and have noticeable effect on reducing numbers of pest insects (Wiedenmann & Smith, 1997). If an insecticide is needed, every effort should be

made to reduce its impact on the natural enemies present within a system (Tillman et al., 2004).

Altering a cropping system to augment or enhance the effectiveness of a natural enemy can be a cost-effective means to improve the effectiveness of natural enemies within a crop (Snapp et al., 2005). Mixed plantings and the provision of flowering borders can increase the diversity of habitats and provide shelter and alternative food sources (Sunderland et al., 1992). These techniques can be incorporated into home gardens and even small-scale commercial plantings, but are more difficult to accommodate in large-scale crop production (van Lenteren, 2003).

The lack of diversity within intensively farmed monoculture crops makes it hard for natural enemies to become established within them (van Lenteren, 2003). The environment within a crop can be manipulated to encourage local natural enemies to become established and contribute towards pest control (van Lenteren, 2000). Recent work in California has demonstrated that planting prune trees in grape vineyards provides an improved overwintering habitat or refuge for a key grape pest parasitoid (Johnson & Wilson, 1995). The prune trees harbor an alternate host for the parasitoid, which could previously overwinter only at great distances from most vineyards.

Researchers are increasingly realising the importance of proving refuges within a glasshouses crop to maintain populations of biological controls within them (Snapp et al., 2005). Techniques being used include planting refuge plants within the glasshouses that provide pollen as an alternative food source for mirids, ladybirds, and lacewings within a glasshouse (Wiedenmann & Smith, 1997). To prevent these refuges acting as harbours of pest insects and plant diseases. Phatak (1992) recommends that they are also rotated, preferably at times when there are sufficient numbers of biological controls within the main crop.

An important aspect of the habitat provided for biological controls is the type of surface on the leaves of the main crop within a glasshouse (van Lenteren, 2003).

Cultivars of many tomatoes and cucumbers have irritating tricomes on the underside of leaves, which agitate and deter many biological controls and reduce their effectiveness within a crop (Steven & Naranjo, 2001). Researchers are increasingly realising the importance of selecting crop cultivars with smooth leaf surfaces to encourage movement of biological controls amongst the crop plants (Snapp, 2005).

The theory of using biological controls within a crop is to provide a sustainable method of pest control which requires minimal use of chemical sprays and is strait forward for a grower to use (Van Lenteren, 2003). The application however, is complicated by the small number of biological controls available that can operate effectively within a glasshouse environment and are able to be mass reared (DeBach & Rosen, 1991), changing the practices of growers (Phatak, 1992) and the establishment of new pest species (Martin, 1996).

The range of biological controls available to growers is increasing as more natural enemies are discovered and rearing techniques for them developed (Cloyd, 1999; Conlong & Mugoya, 1996). Some common problems encountered in rearing biological control agents are the space requirements for mass rearing, labour involved and production of an economical food source (Ashfaq, 2004). New approaches to rearing techniques aim to minimize these difficulties through using artificial diets and mechanized production systems (van Lenteren, 2003; New, 2002).

A single biological control agent is usually inefficient at sustained control of pest insects and does not replicate a natural system where a combination of natural enemies occur (Sunderland et al., 1992). To replicate a natural system, researchers recommend releasing a variety of biological control agents into a crop, to target pest insects at a number of phases of their lifecycle (Wardlow, 1998). Commonly, the biological control agents selected are some combination of predatory mites, parasitoids and predatory bugs - such as mirids (Wiedenmann & Smith, 1997). Having a variety of biological control agents also acts as insurance against pest outbreaks, or if one biological control agents is affected by lack of food, pruning, spraying, or crop rotations (Albert et al., 1999).

2.08 Production of Biological Control Agents

Biological control agents are often identified by collecting natural enemies of a target pest species that are found migrating into glasshouses, or are found on plants infested by that pest species in gardens or natural environments (Workman & Pedley, 2007; McHugh, 1996). These natural enemies then need to be passed through a rigorous quarantine process, to ensure that no unwanted organisms (such as hyperparasitoids) are introduced (van Lenteren & Manzaroli, 1999).

Production and establishment of new biological control agents requires a detailed knowledge of their function on various crop types, response to insecticides, optimal diet, mass rearing methods, and their development within a glasshouse environment (Steven & Naranjo, 2001; Tauber et al., 2000; van Lenteren & Woets, 1998). Once a potential natural enemy is identified, research is then needed to identify its ability to act as a biological control agent of the pest species within a crop (Wiedenmann & Smith, 1997).

Common problems encountered in producing natural enemies are space requirements for mass rearing, labour involved and production of an economical food source (Ashfaq, 2004). Modern rearing techniques aim to manage these problems through use of artificial diets and mechanized production systems (Ashfaq, 2004). Tauber et al. (2000) describes the fundamentals of developing an efficient mass rearing system as:

- The use of inexpensive nutritious diets.
- Mechanized and space-efficient production systems.
- Reliable storage methods.
- Periodic evaluation of natural enemy quality.

There are a number of challenges facing a biological control agent within a glasshouse crop, including: a limited food supply, lack of shelter, adverse cultural practices, and use of insecticides (Albert et al., 1999). These problems

are further exasperated in an annually disturbed cropping system, which do not allow a population of natural enemies to remain established (Wiedenmann and Smith, 1997). Many potential biological control agents cannot adapt to monoculture crop environments, especially within glasshouses where the temperatures are higher than within the natural environment (van Lenteren & Manzaroli, 1999). Many crop plants also have leaf tricomes that irritate many insect species and deter their spread through that crop (Malais & Ravensberg, 2003).

2.09 Functional Responses

Many predators and parasitoids used as biological control agents are thought to respond to changes in prey density by altering their behaviour or adapting their reproductive activity (van Lenteren & Manzaroli, 1999). The response of predatory insects to changes in prey density is described by two terms, the functional response (changes in predator attack rate in response to variations in prey density) and the numerical response (changes in predator numbers with variation in prey density) (Solomon, 1949). Addressing the functional response of insect predators and parasitoids, in the biological control of pest insects, is thought by many researchers as being critical in understanding the predator/prey dynamics involved (Foglar et al., 1990).

A method for determining the functional response was described by van Alphen & Jervis (1996) as follows:

1. Individual insects to be confined in an arena/cage with different numbers of prey or hosts, for a fixed period of time.

2. At the end of the experiment, the natural enemies are removed and, in the case of predators, the number of prey killed is counted.

3. From the counts, a graph can then be plotted relating the number of prey attacked to the number offered.

4. The plot is then compared with the functional response curves.

There are four distinctive functional responses of predators to numbers of available prey (van Alphen & Jervis, 1996), described below.

- Type I: A linear rise in the number of prey eaten in response to increases in prey density, towards a maximum.

- Type II: The number of prey eaten decreases with increases in prey density, towards a maximum value (the response is curvilinear up to the asymptote, in contrast to the Type I response).

- Type III: The number of prey eaten decreases with increasing prey density (like Type II) and accelerates at decreasing prey density, producing a sigmoid response when graphed.

- Type IV: Where the response resembles the Type II response except that at higher densities it declines, producing a dome-shape response when graphed.

While the functional response of predators can be largely accounted for by changes in prey density, the functional response of parasitoids has been shown to be more complex. A review by Fernández-arhex & Corley (2003) investigated if there was a relationship between the type of functional response of parasitoids and their performance as biological control agents within a crop. This review revealed that only 32 out of 94 papers dealt experimentally with the functional response of parasitoids used in biological control, showing that the functional response of parasitoids is not given enough attention by researchers. Within most

papers the parasitoids responded quickly to low prey densities, with responsiveness decreasing with increasing prey numbers to a plateau, where the parasitoids were unresponsive to further increases. This plateau was thought to be due to high numbers of prey creating environmental conditions that are detrimental to parasitoids (such as sooty mould from honey deposits of whitefly) and because of biological limitations of the parasitoids (number of eggs available for parasitism). Fernández-arhex & Corley observed from their review that most parasitoid species have a type II response and that there was no clear relationship between the curve shape and their success as biological control agents. They concluded that other aspects of the parasitoids behaviour deserve more attention in order to understand and predict these insects' success as biological control agents.

A number of experiments have been performed to determine what other variables beside (or in addition to) host density affects the functional response of parasitoids. Jones et al. (2003) found that a quadratic equation that encompassed the functional response of parasitoids to both to temperature and prey density could account for 83 % of the variation in the parasitism. Foglar et al. (1990) found that egg limitations due to the time required for eggs to move from the ovarioles to the oviducts was the most important factor limiting parasitism, when there was no shortage of potential hosts available.

These studies show that many factors affect the functional response of parasitoids, including changes in host density, temperature and egg limitations. In addition to this the quality controls used in mass rearing the parasitoids (van Lenteren, 2003) the crop type (Steven & Naranjo, 2001) humidity (Jones et al., 2003) and presence of chemical treatments (Martin, 2005) can all be reasonably expected to affect the functional response of parasitoids.

Producers of natural enemies recognise the effect of prey density on parasitoids and suggest different release strategies. They recommend growers disperse parasitoids within a crop when whiteflies are at moderate densities, with spray favoured at higher densities (John Thompson pers comm., 2008). If densities become too high, it is thought to create unsuitable environmental conditions for a

parasitoid, due to build-up honey dew and sooty mould on leaf surfaces (Kassis & Michelakis, 1993). If densities of whitefly nymphs are too low, then whitefly nymphs are often parasitized multiple times, or killed by host feeding, with development of parasitoid larvae within their nymph hosts reduced by both actions (van Roermund & van Lenteren, 1995).

2.10 Release Strategies

Biological control agents have historically been introduced into countries to combat a pest insect species that have become established from introduced plants (Malais & Ravensberg, 2003). The practice of importing and releasing natural enemies to control an introduced (exotic) pest insects is called classical biological control (Gullan & Cranston, 2006). These biological controls have been introduced because the local insect fauna does not contain any effective natural enemies to control the introduced pest insect (van Lenteren, 2005).

There are many successful examples of biological controls being introduced into a country with some, like *En. formosa,* now present in most countries with established glasshouse industry (Malais & Ravensberg, 2003). One of the earliest successes of classical biological control was with the cottony cushion scale, a pest that was devastating the California citrus industry in the late 1800's example (Caltagirone & Doutt, 1989). A predatory insect (the vedalia beetle) and a parasitoid were introduced from Australia and within a few years the cottony cushion scale was completely controlled by these introduced natural enemies (Caltagirone & Doutt, 1989). Caution is needed when introducing a insect species into a country for classical biological control, to ensure it does not have a damaging effect on the native invertebrate population, especially with generalist predators (Gullan & Cranston, 2006).

Those biological control agents that have a very specific host range are least likely to have a damaging effect against the native insect fauna (Malais & Ravensberg, 2003). For this reason parasitoids with a specific host-range are

commonly selected for introduction into countries (Greathead & Greathead, 1992). Many effective biological controls are generalist predators, such as many mirids, lacewings, and ladybirds (Malais & Ravensberg, 2003). There is no way of predicting the impact these species will have on the native insect fauna and their introduction requires extensive host/prey testing to ensure they will not have a damaging effect (van Lenteren, 2005). Island nations, like New Zealand, are particularly vulnerable to introduction of foreign species, because of their characteristic species poor flora and fauna (Martin, 2005).

The method by which biological control agents are released into a crop, can have strong influence on the success of establishing biological control agents within a crop (van Lenteren & Bueno, 2003). The theoretical objective of using biological controls is to maintain a small population of natural enemies within a glasshouse to act as a buffer against keep pest insects at a low level and to supplement this population with further releases at times of the year when pest outbreaks are expected (Malais & Ravensberg, 2003; Phatak, 1992). To achieve this it is recommended to use the inoculative release method, where a small number of biological control agents are released into a crop at critical times of the season (Gullan & Cranston, 2006). Many of these original releases may then die, but their future progeny will hopefully persist within the crop and be available to control fresh incursions of pest insects (Albert et al., 1999; Debach & Rosen, 1991). An example of the inoculative method is the release of *Phytoseiulus persimilis* (a predatory mite) into glasshouses in spring in anticipation of a build up of two-spotted spider mite (*Tetranychus urticae*) on beans (van Lenteren & Martin, 1999). *P. persimilis* is often able to maintain a low density within bean crops and build in numbers quickly in response to increases in *T. urticae* numbers (Wardlow, 1998).

Another technique, the innundative method, is to swamp pest insects with large numbers of mass reared biological control agents, applied much like a biological pesticide (van Lenteren & Bueno, 2003). Lady beetles, lacewings, or parasitoids (such as *Trichogramma*) are frequently released in large numbers with the aim to reduce pest insects to very low densities (van Lenteren, 2000). Using this technique, the recommended release rates for *Trichogramma* in vegetable or

field crops can be as high as 200,000 parasitoids per acre, per week. Similarly, entomopathogenic nematodes are released at rates of millions and even billions per acre, for the control of soil-dwelling insect pests (Bai et al., 2009). The inundative approach is often favoured by growers who do not want any pest insects within their crop, but would prefer to use biological control techniques instead of chemical control (Feaster & Steinkraus, 1996).

Stringent export regulations promote the use of inundative releases of biological controls, because any sign of pest insect infestation will result in rejection of an entire consignment of produce (van Lenteren, 2000). The application of this type of biological control however, like calendar spraying, does not promote a sustainable population of the biological control within a crop (van Lenteren & Bueno, 2003). The intention is not to maintain a balance of pest and natural enemy within a crop which will provide long term control, but to have immediate and absolute removal of all pest insect species within a crop (Feaster & Steinkraus, 1996). The inability of many biological control agents to become established in glasshouses (due to a lack of habitat diversity and food reserves) has also favoured the inundative method (Steven & Naranjo, 2001).

2.11 Predators

There are a number of insect predators that have been used as biological control agents within glasshouse crops. Some of the most popular are species of green lacewing, predatory mites, lady beetles and mirids (Malais & Ravensberg, 2003). The main problem encountered with using predators as biological controls is with the space required, labour involved, and production of an economical food source to mass-rear them (Ashfaq, 2004). Modern rearing techniques aim to manage these problems through use of artificial diets and mechanized production systems (Tauber et. al., 2000). Many predators also do not adapt well to conditions within a monoculture glasshouse crop and will quickly disperse out of the glasshouse if appropriate food and shelter is not available (Wardlow, 1998).

There are currently three main types of predatory mites recognized (Braun et al., 1993). Type I mites are specialist feeders that can be cheaply mass reared, adapt to many glasshouse crops and will target specific prey species (Messelink et al., 2006). The disadvantage of Type I mites, is that when their target prey disappears from a glasshouse they cannot switch to other food resources to survive (Messelink et al., 2006). One of the most widely used predatory mites, *Phytoseiulus persimilis*, is a type I mite and is a very effective predator of two-spotted spider mites and related *Tetranychus* species (van Lenteren & Manzaroli, 1999). Type II Mites are also selective, but can also adapt to other prey species and also feed upon plant pollen. Because of this characteristic adaptability they make a good biological control agent for general prevention and management of pest insects in glasshouses. Some common examples are *Neoseiulus californicus*, *Galendromus occidentalis* and *Neoseiulus cucumeris*. Type III mites are "generalist" predatory mites and will feed on a wide variety of suitably sized prey species, in addition to plant sap and pollen (Braun et al., 1993). Many of these mites are difficult to rear in large numbers and do not often adapt well to a glasshouse, monoculture crop environment (van Lenteren & Manzaroli, 1999). One successful example is *Amblyseius swirskii*, which will prey upon a wide range of pest insects including thrips, 2-spotted mite, whitefly, psyllid and aphids (Bead & Walter, 1996). In New Zealand *Phytoseiulus persimilis* and *Neoseiulus cucumeris* are commercially available as biological control agents and a Type III mite (*Typhlodromalus limonicus*) is being investigated for commercial production (John Thompson pers comm., 2008).

The most common lacewing used for biological control is *Chrysoperla carnea,* which is available in most western countries except Australia and New Zealand (Malais & Ravensberg, 2003). Australia has a similar green lacewing *Mallada stigma*, which is also developed as a biological control agent. New Zealand does not have any related green lacewings, but does have two brown lacewings *Micromus tasmaniae* and *Drepanacra binocula* which both have potential use as biological control of pest insects in glasshouses (New, 2002). The larvae of green lacewings feed predominantly on aphids, but will also consume two-

spotted mite, whitefly and various scale insects. The adults feed on nectar and pollen (Malais & Ravensberg, 2003).

In North America many lady beetle species are commercially available for the biological control of a diverse range of pest insects, such as: *Harmonia axyridis* a generalist predator used for control of aphids and psyllids, *Cycloneda sanguinea* a biological control agent of both green and brown citrus aphids and *Cryptolaemus montrouzeri* for biological control of mealybugs (Wardlow, 1998; Habeck et al., 1990). No lady beetle is currently commercially available in New Zealand as a biological control agent, but *Cleobora mellyi* is being investigated as a biological control of psyllids (Workman & Pedley, 2007).

Predatory mirids are also occasionally used as biological control agents, but producers often have trouble with cannibalism in mass-reared colonies, their tendency to feed upon plant sap and pollen and in some instances transmit plant viruses (Arnoldi et al., 1992). In the western Mediterranean area native predatory mirid bugs (*Macrolophus caliginosus* and *Dicyphus tamaninii*) have been recorded naturally colonizing tomato plants in glasshouses and preying upon greenhouse whitefly (Castañé et al., 2004). Predatory mirids from New Zealand (*Macrolophus pygmaeus* and *Engytatus nicotianae*) have also been reported feeding upon greenhouse whitefly nymphs in laboratory studies (Workman & Pedley, 2007). *M. pygmaeus* is a used in Europe as a biological control agent of whitefly and green peach aphid and may have potential use for control of these pests in New Zealand (Eyles et al., 2008).

2.12 Entomopathogenic Nematodes

Entomopathogenic nematodes are receiving increasing interest for use as endoparasitic biological control agents and are commercially available in Europe and America (Liu et al., 2000). These nematodes live in the soil and are attracted to soil insects by their carbon dioxide exhalations (Malais & Ravensberg, 2003). They infect a wide variety of larval flies, moths and beetles and adult crickets and grasshoppers. They enter a host through body openings and multiply rapidly

within their host, which when it dies will release up to 100 000 times the number of nematodes that had originally infected it (Liu et al., 2000).

2.13 Entomopathogenic Fungi

Entomopathogenic fungi (EPF) are common in many glasshouse crops and are receiving increasing attention for use as biological control agents. Most EPF have life cycles which synchronise with insects host stages and function best in warm and moist conditions (Arnold & Lewis, 2005). These fungi attach to the external surfaces of insects in the form of microscopic spores, which then bore through the cuticle to infect heir host. The fungal cells then proliferate within their host, which they kill by releasing fungal toxins (Ekesi et al., 2005). EPF have potential to spread outside of the area in which they used and infect the surrounding environment, so risks associated with their use are being carefully monitored (Keller et al., 2003).

2.14 Parasitoids

Parasitoids are insects that lay an egg within a host insect, with the parasitoid larva feeding on body fluids of the host and eventually leaving the host to pupate or emerging as an adult (Malais & Ravensberg, 2003). Most beneficial insect parasitoids are wasps or flies. Parasitoids are often highly host-specific, because they have evolved to overcome the defences of a particular host insect (van Lenteren et al., 1997). Their host-specificity has made them popular biological control agents because they pose a low risk to non-target insect species (Gullan & Cranston, 2005). The larva of the parasitoid times its development to match the development of its host, to insure it does not kill the host pre-maturely. The parasitoid larva will often undertake most of its development and kill its host in the final stages of its host's development towards an adult (Malais & Ravensberg, 2003). The adult parasitoids are typically free-living and they consume high energy food such as honeydew, pollen and nectar to fuel their

search for potential hosts (Wardlow, 1998). The adult female parasitoids will also often feed upon and sometimes consume potential hosts, to provide nutrients to develop eggs. Insect parasitoids are generally easily to mass rear on host plants infested with a high number of host insects, which when parasitised are harvested for dispersal in a crop (van Lenteren et al., 1997).

2.15 Parasitism Efficacy of Parasitoids

The efficacy of a parasitoid is a measure of how many hosts a single female can parasitize (van Lenteren et al., 1997). The parasitism efficacy can be used to indicate the value of a parasitoid for use as a biological control agent. The more hosts are parasitised, the greater the impact that parasitoid will have in controlling numbers of its host (Vet et al., 1995).The upper limit to a parasitoids efficacy is the number of eggs held within its oviducts, or the number of eggs capable of developing to adult (Asplen et al, 2009).

Many of the eggs held by a female parasitoid are undeveloped and the female must feed upon some of its potential hosts to obtain the nutrients required to develop the eggs (Fransen & van Lenteren, 1993). In some species the female parasitoid emerges as an adult with its full complement of undeveloped eggs. Adult female *Er. eremicus* have approximately 54 eggs undeveloped eggs, which take between 4-8 days to mature and become available for parasitism (Asplen et al., 2009). The number of hosts *Er. eremicus* can parasitize then depends upon the availability of hosts for host-feeding, to obtain nutrients to mature undeveloped eggs (Bellamy et., 2004). Other parasitoids, such as *En. formosa,* are capable of developing new eggs during their adult phase and this parasitoid can lay up to 100 eggs (van Roermund & van Lenteren, 1995).

Other factors also play a role in limiting the number of hosts a parasitoid can parasitize. The primary factor is the ability of the parasitoid to locate suitable hosts (Wiedenmann & Smith, 1997). Within a glasshouse crop, potential hosts of a parasitoid used as a biological control agent are often widely distributed and at

low density, due to management practices by growers to reduce their numbers (van Roermund & van Lenteren, 1995). A parasitoid released in this environment may not be able to locate any host, which is often compensated for by releasing large numbers of the parasitoid throughout the crop (van Lenteren & Martin, 1999). Parasitoids are capable of flying short distances within a crop and are attracted to potential hosts by chemical cues from the plants, emitted in response to the feeding of insects (Vet et al., 1995). The parasitoid narrows in on hosts by using its antennae to detect further chemical cues. A parasitoid may also be inhibited from locating hosts by chemical insecticide treatments that interfere with their biology (Hoddle & Driesche, 1999).

Trials of a parasitoids efficacy are either performed on a large scale, to determine their effectiveness controlling a pest insect species when released as a biological control agent within a crop, or on a small scale, to determine the number of hosts a single parasitoid can parasitize (van Lenteren & Manzaroli, 1999). Large scale experiments involve releasing thousands of parasitoids and collecting leaf samples from the crop (van Lenteren, 2005). These leaves are placed within agar-based vented Petri dishes and the number of adult whitefly or parasitoids emerging from the whitefly nymphs counted (Bellamy et al., 2004). A high incidence of parasitoids emerging from hosts on these leaves indicates a relatively successful treatment (Fransen & van Lenteren, 1993). This technique can be used to compare the effectiveness of different parasitoids, to compare parasitoids to spray treatments, and compare parasitoid treatments to untreated crops (van Lenteren & Manzaroli, 1999). The effect from introducing parasitoids into a crop in controlling numbers of whitefly, is easier to observe at low whitefly density (Goolsby et al., 2005).

To determine the parasitism of an individual parasitoid, small-scale experiments are often performed within clip-cages. A single adult female is placed onto a leaf containing a known number of hosts and enclosed within the clip-cage (Greenberg et al., 2002). The number of hosts they are then able to parasitize is then recorded as their parasitism efficacy (van Roermund & van Lenetern, 1995). Small-scale experiments have also been conducted by placing a small treatment plant and parasitoid into a mesh cage, which allows the parasitoid to move more

freely over the plant in search of hosts (Headrick et al., 1995). Results from these experiments indicated the importance of keeping the number of hosts to a low number, to avoid fouling the environment from honey-dew deposits (Greenberg et al., 2002; Gerling, 1965).

2.16 Host-feeding by Parasitoids

In addition to parasitism of whitefly nymphs, many parasitoids also control whitefly populations by directly feeding upon them, called host-feeding. Host-feeding can have an important influence on the ability of a parasitoid to control populations of whitefly within glasshouses (van Lenteren & Martin, 1999). The most common method of host-feeding is by parasitoid puncturing the cuticle of a whitefly nymph with the ovipositor and feeding upon the haemolymph exuded from the wound. This process kills whitefly nymph or makes it unsuitable for parasitism (van Roermund & van Lenteren, 1995; Headrick, et. al., 1995). Adult parasitoids use the nutrients obtained from host-feeding to mature eggs (Collier & Hunter, 2001) and without a suitable number of hosts to feed upon are unable to develop their full complement of eggs (Wiedenmann & Smith, 1997). Adult parasitoids also consume honey dew excreted from whitefly and use the energy from this to increase adult longevity (De Barro et al., 2000).

Host-feeding has been of interest to researchers primarily because of the influence of the nutrients obtained on development of eggs and thus parasitism efficacy and also within the context of interactions between different parasitiod species. By host-feeding upon a nymph already parasitized, the developing parasitoid will be killed (Wiedenmann & Smith, 1997**).** Further, parasitoids have shown a bias in selecting parasitized nymphs to host-feed upon (Fransen & van Lenteren, 1993). This may be an activity that acts to prevent a parasitoid population becoming over-abundant and eliminating the host species (Asplen et al., 2009).

2.17 Host Stage Preference by Parasitoids

Most mass rearing systems rely upon providing parasitoids with a large number of whitefly nymphs on a broad-leaved plant that is able to grow quickly and support a large number of whitefly without dying (McMahon & Lindquist, 1994). The parasitized whiteflies are then stripped off the plants leaves before they emerge, dried and put onto tags to dispense amongst the plants of a glasshouse crop (Minkenberg & Santangelo, 1997). For this system to work effectively the whitefly nymphs must be presented to adult parasitoids for parasitism at the same age (so that they can be harvested at the same time) and at an appropriate developmental level to ensure the maximum numbers are parasitized (McMahon & Lindquist, 1994). By presenting these parasitoids with the preferred whitefly nymph stage a maximum yield of parasitized nymphs can be expected (Conlong & Mugoya, 1996). Determining the preference of a parasitoid for specific whitefly nymph instar stages is therefore of great importance in developing a mass rearing system.

Research has shown that every whitefly parasitoid has a particular preference for particular nymph instar stages for parasitism (van Lenteren et al., 1997). This preference is thought to be due to differences in developmental biology of the parasitoid larvae and to avoid competition (Rumei et al., 1993; van Lenteren et al., 1997).

Eretmocerus parasitoids prefer 2nd and 3rd instar whitefly nymphs for oviposition and lay one egg beneath each of these nymphs with their ovipositors (Liu & Stansly, 1996; Jones & Greenberg, 1998). *Eretmocerus* larvae then burrow into their hosts, where they become dormant until their hosts enter into their final developmental stage at 4th instar (Zolnerowich & Rose, 2008). It is during this stage that the parasitoid larva consumes its hosts and accelerates its own development, from a 1st instar larva to 3rd instar, in approximately 10 days (Malais & Ravensberg, 2003).

Many *Encarsia* species, however, have a preference for 3rd and 4th instar whitefly nymphs and lay one egg inside the whitefly nymph host by piercing the cuticle with their ovipositors (Shishehbor & Brennan, 1996).The larva of *Encarsia* sp. also time their development to co-inside with their host, to avoid prematurely killing it.

These differences in developmental strategies have little impact on when the adult parasitoid of both types emerges, with *Encarsia* sp. emerging 2-3 days earlier than *Eretmocerus* sp. (Malais & Ravensberg, 2003). Researchers have not noted any advantage to either developmental strategy in their effectiveness as biological controls in glasshouses (Hoddle & Driesche, 1999).

2.18 Fecundity of Parasitoids

The fecundity of a parasitoid is a measure of how many eggs it can produce as an adult. The extent to which parasitic wasps are limited by their egg supply is very important in understanding their potential as biological control agents. Egg reserves are dynamic, with most wasps maturing new eggs throughout their life (synovigeny) and many species reabsorbing eggs that are not used in oviposition (Rivero-lynch & Godfray, 1997). In most parasitoids studied, the majority of parasitism occurs within the first 48 hours following emergence from the host (van Lenteren, 2003; Rivero-lynch & Godfray, 1997) with the longevity of adult parasitoids and parasitism of further hosts being dependent on obtaining sufficient nutrients to support egg production (Asplen et. al., 2009).

This has an important implication on the handling and transport of parasitoids used for biological control in glasshouses. A producer of parasitoids must know how long they can use adult parasitoids for parasitism of whitefly nymphs on their host plants before they must be discarded (McMahon & Lindquist, 1994). These researchers have shown that newly emerged parasitoids that have an abundant food source to mature undeveloped eggs have the best chance of

parasitising the maximum number of whitefly nymphs available (Asplen et al., 2009).

2.19 Whitefly Parasitoids

Parasitoids are most commonly used biological control of whitefly (van Lenteren & Manzaroli, 1999). Parasitoid adults lay a single egg within or underneath an immature nymph of their hosts. Once the parasitoid larva emerges it begins to consume the host to sustain its own development. If the egg is laid beneath the host, the larva first burrows into the host (Asplen et al., 2009). Non-vital tissues of the host are consumed first by the developing parasitoid larvae, which will time its own development to its host, to avoid killing it prematurely (Gullan & Cranston, 2005).

Factors affecting the success of parasitoids in controlling populations of whiteflies include: the fitness of the mass reared colony, suitable climatic conditions within the crop, host plants type, and interference from pesticides (van Lenteren et al., 1997). Many insecticides used to control whitefly are also toxic to its parasitoids (Gorman et al., 2000; Cahill et al., 1996). Low toxicity soaps are often recommended by natural enemy produces to deter and kill greenhouse whitefly without leaving toxic residues which would be detrimental to parasitoids (Michelakis, 1995).

In New Zealand, the success of the parasitoid *Encarsia formosa* is undermined in winter by temperatures within the glasshouse below 20^{o}C (Workman & Davidson, 2007), which are needed for this parasitoids to fly and locate hosts (van Roermund & van Lenteren, 1995). This is thought to result in whitefly build up during winter months to levels which are too high for the parasitoid to control when warmer weather arrives in spring (John Thompson pers comm., 2008). There are also a large number of weeds in New Zealand that act as host plants to greenhouse whitefly and sources for it to re-infest glasshouses (Martin, 2005).

The cost associated with using parasitoids to control whitefly populations is considerably greater than the chemical insecticide alternative (van Lenteren, 2000). However, the application of insecticides to control whitefly negatively affects both yield and quality of produce and limits staff working time in the crop (DeBach & Rosen, 1991). Also, concern about the development of pesticide resistance in whiteflies (Liang & Liu, 2002; Martin, 1996; Cahill et al., 1996) as well as the need to reduce pesticide usage because of environmental considerations, has resulted in increased emphasis on developing cost-effective biological control strategies. Parasitoids will probably be the mainstay of biological control strategies in the future, if they can be produced at low cost (van Lenteren et al., 1997).

2.20 *Encarsia formosa*

Internationally, the biological control of the greenhouse whitefly has relied on *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae) (Fig. 2.1). This parasitoid was discovered in 1926 by an English tomato grower and identified by the entomologist Speyer (Kassis & Michelakis, 1993). It was first used for biological control of whitefly in 1972 (van Roermund & van Lenteren, 1995). Populations of *En. formosa* are almost entirely females due to the presence of *Wolbachia* spp. bacteria (Hoddle et al., 1998), with males rarely encountered. Females vigorously search leaf surface for signs of whitefly nymphs using their antennae. They prefer to oviposit in the third and fourth instar and pre-pupal nymphs of the greenhouse whitefly (van Roermund & van Lenteren, 1995). The black coloured parasitized whitefly pupae (Fig. 2.2) are clearly visible in the later stages of the parasitoid development within hosts. When fully developed, the adult parasitoid emerges by chewing a round exit hole on the dorsal surface of its host (Kassis & Michelakis, 1993) (Fig. 2.2). At 21°C, and with the third instar greenhouse whitefly as hosts, the time from oviposition to adult emergence is 25 days (Succop, 1997).

Fig. 2.1. Adult female *Encarsia formosa* (scale 35:1).

Fig. 2.2. Greenhouse whitefly nymphs parasitized by *En. formosa*, with circular exit hole visible in two nymphs (Scale 30:1).

En. formosa adults reared from greenhouse whitefly can lay five eggs per day and parasitize an average of 59 nymphs (Succop, 1997). They can mature new eggs during their adult lifespan and can lay as many as 80 eggs. The ability of *En. formosa* to mature eggs depends upon it obtaining sufficient nutrients from host-feeding and honey dew. With parasitism and host-feeding combined, one female adult will kill, on average, a total of 95 nymphs over a 12 day life expectancy (Succop, 1997). As the adult *En. formosa* ages, the rate of parasitism declines. This decline is reduced by the adult parasitoid obtaining sufficient nutrients from host-feeding and consumption of honeydew to maintain energy levels and mature eggs (van Roermund & van Lenteren, 1995).

In New Zealand, the control of greenhouse whitefly populations within glasshouses using *En. formosa* is erratic due to high rates of immigration of adult whiteflies into the glasshouse, few supplementary biological control agents being available, and interference with pesticides (Workman & Davidson, 2007; Martin, 1999). These researchers identified the need for other natural enemies of greenhouse whitefly to supplement *En. formosa* as a high priority for the development and success of IPM strategies. Two other greenhouse whitefly parasitoids, *Er. eremicus* and *En. pergandiella,* also occur in the Auckland, New Zealand and may be the best options for supporting or replacing *En. formosa* (Workman et al., 2008; Workman & Davidson, 2007; Workman & Pedley, 2007).

2.21 *Encarsia pergandiella*

Encarsia pergandiella (Howard) (Hymenoptera: Aphelinidae) (Fig. 2.3) is a hyper-parasitoid of the greenhouse whitefly, A hyper-parasitoid can parasitize both normal whitefly nymphs and those parasitized by other parasitoids. Females are produced from parasitism of unparasitized whitefly nymphs, while males from parasitism of parasitized nymphs (Marotto, 2007). *En. pergandiella* biology presents a logistical problem in attempting to mass rear this parasitoid

for release into glasshouses because the high density of parasitoids within a mass rearing system results in many hyper-parasitized whitefly nymphs and a crash in the *En. pergandiella* colony from the production of a lot of males (Workman & Pedley, 2007; Marotto, 2007). *En. pergandiella* also interferes with the biological control from other parasitoids due to hyper-parasitism with only male *En. pergandiella* produced (Martin, 1999). Due to these problems little effort has been put into understanding the potential of mass rearing *En. pergandiella* and using it for biological control of whitefly. Despite this it is the parasitoid most commonly encountered in the Auckland region on greenhouse whiteflies in greenhouses (Workman & Pedley, 2007).

Fig. 2.3. Adult female *Encarisa pergandiella,* parasitising a whitefly nymph already containing a larva of another parasitoid (Scale 30:1).

2.22 *Eretmocerus eremicus*

Eretmocerus eremicus (Rose and Zolnerowich) (Hymenoptera : Aphelinidae) is a tiny parasitic wasp (~1 mm in length) that is indigenous to the southern desert

areas of California and Arizona (Bellamy et al., 2004) and is an important parasitoid of whiteflies in these areas (Asplen et al., 2001). Female *Er. eremicus* females are pale lemon yellow with green eyes and three prominent residual red eyes on the top of its head. The name *Eretmocerus* is derived from Latin, meaning "oar-like," and refers to the shape of the "clubbed" female antennae. Male wasps have longer, elbowed antennae (Fig. 2.4), and are yellowish brown in colour (Bellamy et al., 2004). The wings of *Er. eremicus* are oval with hairy edges (Fig. 2.5).

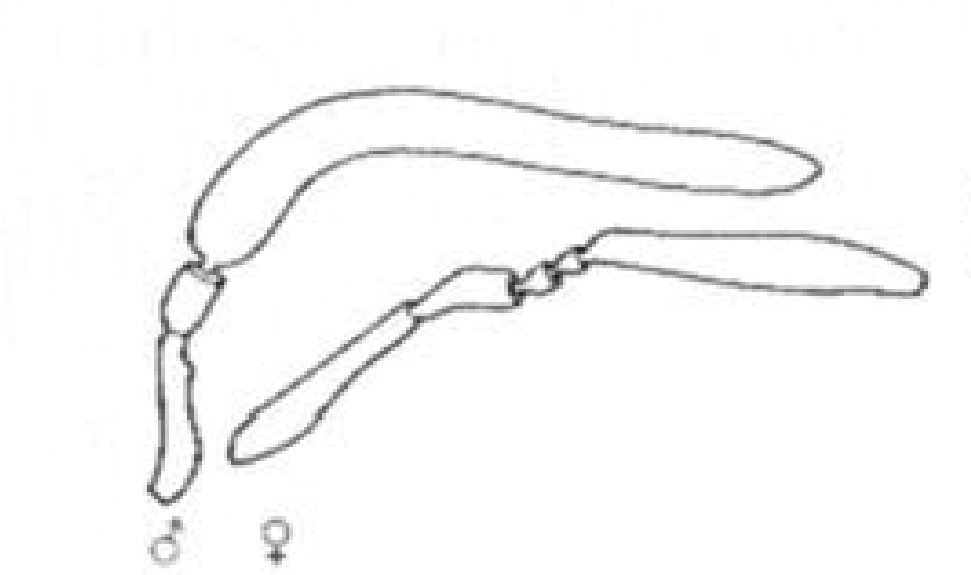

Fig. 2.4. Different antennae structure for male and female *Eretmocerus Sp*. (Malais & Ravensberg, 2003) (Scale 100:1).

Fig. 2.5. Adult female *Er. eremicus*, showing feathered wing structure in mid take-off (Scale 35:1).

Er. eremicus is used in Europe and America (Rose & Zolnerowich) for commercial production and release into glasshouses to control both greenhouse whitefly and sweetpotato whitefly. *Er. eremicus* is also reported to parasitize silverleaf whitefly *Bemisia argentifolii* and bandedwinged whitefly *Trialeurodes abutlonea* (Qiu et al., 2005). Hoelmer (2007) states that advantages of *Er. eremicus* over *En. formosa* as being: a tolerance to higher temperatures, preference for early instar nymph stages and ability to parasitize a wider variety of whiteflies. The two parasitoids are now often released together as a mix of parasitoids within glasshouses (Hoelmer, 2007).

The biology of *Er. eremicus* is dependent upon temperature. Temperatures below 15^0C will prevent it from flying (van Lenteren & Manzaroli, 1999), while the optimal temperature for rapid larval development and egg production is 25-29°C (Greenberg et al., 2002). In the greenhouse environment, the temperature should be manipulated to ensure that *Er. eremicus* will develop more quickly than its host (Collier & Hunter, 2001). In reality, however, the cost of heating

glasshouses means growers favour lower temperatures suited to whitefly development (van Lenteren & Martin, 1999; van Lenteren & Noldus, 1990).

Both male and female *Er. eremicus* develop as primary parasitoids of whiteflies (Powell & Bellows, 1992). Adult *Er. eremicus* detect potential whitefly nymph hosts by vigorously search all levels of a plant for chemical cues (Noldus & van Lenteren, 1990). When cues are detected the searching behaviour becomes more focus until a host is detected. The host is then probed with the sensitive antennae (Fig. 2.6) to determine suitability for parasitism. Females will oviposit under all immature whitefly stages, except eggs, but second instars may be preferred (Powell & Bellows, 1992).

Once a female *Er. eremicus* has selected a host it deposits a single egg underneath it using its ovipositor (Fig. 2.8) (Hoddle & Driesche, 1999). The egg hatches about 4 days after being laid (the exact time is dependent on temperature) and the newly emerged larva attaches its hook-like mouthparts to the underside of the whitefly nymph and chews a small hole into the whitefly (Powell & Bellows, 1992). After 3-4 days of chewing the parasitoid larva enters the host where it remains dormant until the whitefly pupates (van Lenteren & Martin, 1999). Once the whitefly pupal stage is reached, the wasp larva releases digestive enzymes and begins ingesting the semi-liquid body parts of the pupa (Hoddle & van Driesche, 1999). The wasp larva passes through three instars, requiring about 12 days to complete development within the cuticle of the host (Headrick et al., 1995). The larva is clearly visible within the cuticle of the host in the last days of its development (Fig. 2.9). Once the parasitoid larva has completed development, the adult parasitoid emerges by chewing a circular exit hole through the host's cuticle (Powell & Bellows, 1992).

The adult parasitoids have been recorded feeding on both hosts and honey dew deposits by hosts (Fig. 2.7) (van Lenteren & Martin, 1999). Asplen (2009) notes that the nutrients obtained from host-feeding are important for the development of immature eggs within the adult parasitoid. *Er. eremicus* emerges with its full complement of approximately 54 eggs during the first 2 days of adult life (Asplen et al., 2009; van Roermund & van Lenteren, 1995). The eggs are then

steadily absorbed up until 8 days following emergence if insufficient nutrients from host-feeding and honey dew are obtained (Asplen et al., 2009). The number of potential hosts it can parasitize is limited by the number of mature eggs it has available (Asplen et al., 2009).

Fig. 2.6. Adult female *Er. eremicus*, probing for signs of whitefly nymphs to parasitize (Scale 35:1).

Fig. 2.7. Adult female *Er. eremicus*, feeding upon honey dew deposits from whitefly nymphs (Scale 35:1).

Fig. 2.8. Adult female *Er. eremicus*, depositing an egg underneath a 2nd instar greenhouse whitefly nymph (Scale 30:1).

Fig. 2.9. Greenhouse whitefly nymph pupae completely consumed by larvae of *Er. eremicus*, which have pupated and are visible as a yellow grub within hosts (Scale 30:1).

A strain of *Er. eremicus* has recently been indentified in Auckland, New Zealand (Workman & Pedley, 2007) and has potential for the development as a biological control agent of greenhouse whitefly. This species may also occur in Australia, where it is named *Eretmocerus warrae* (Nauman & Schmidt) (De Barrow et. al., 2000). De Barrow (2000) found that *Er. warrae* only parasitized *T. vaporariorum* but not *B. tabaci,* unlike strains of *Er. eremicus* occurring in America and Europe. De Barrow (2000) also reported a high percentage of females within the population and a preference for 2nd instar whitefly nymphs. Early trials indicate the same characteristics also occur in the New Zealand strain of *Er. eremicus* (Workman & Pedley, 2007), except that the parasitism of *B. tabaci* has not been investigated.

The high percentage of females in the New Zealand strain of *Er. eremicus* is likely due to the infection with *Wolbachia,* a cytoplasmic bacterium that is responsible for many asexual parasitoid populations (De Barrow, 2000). The presence of this bacterium has also been linked with asexual and sexual

populations being unable to mate with each other, leading to genetic differences between populations exceeding that between separate species (Mohammad et al., 2004). The infection of a population of parasitiods with *Wolbachia* can however be removed through exposing females to antibiotics or high temperatures (>31°C) for two or more generations, allowing females to successfully produce male offspring (Succop, 1997). The differences in the New Zealand strain of *Er. eremicus* indicate that no assumptions should be made in applying how the American strain of *Er. eremicus* functions, and independent studies should be performed on all aspects of the biology of the New Zealand strain (De Barrow, 2000).

While the New Zealand strain of *Er. eremicus* has been discovered within glasshouse crops in New Zealand, parasitizing greenhouse whitefly (Workman & Pedley, 2007), its potential as biological control agent has not been investigated (Workman & Davidson, 2007). The effectiveness of a parasitoid (as a biological control agent) is determined by a measure of its parasitism efficacy, a measure of the number of hosts it is able to parasitize (van Lenteren, 2005).

2.23 Nomenclature of NZ *Eretmocerus eremicus*

The following is a summary of the history of *Er. eremicus* found in New Zealand (presented by Peter Workman of Plant & Food Research, in 2007).

- A yellow parasitoid was found in a greenhouse crop in the Auckland in 1997 and identified by Landcare Research as an *Eretmocerus* species.

- Three populations of a yellow parasitoid were found by Plant & Food Research in a survey of whitefly natural enemies in the Auckland area in 2006/07. Two populations were found on outdoor beans and one population within a crop of greenhouse tomatoes.

- Samples from the 2006/07 collection were sent to the Natural History Museum in London where it was identified morphologically as *Eretmocerus eremicus* (Rose and Zolnerowich).

- Samples of the 2006/07 collection were also supplied to Ian Scott, Plant & Food Research, Lincoln were it was identified by PCR sequencing as the Australian species *Eretmocerus warrae* (Nauman & Schmidt) (De Barrow et al., 2000).

- Samples of *Eretmocerus* that were collected in 1997 from the Landcare Arthropod collection and samples of the 2006/07 collection were sent to Schmit in Australia and they were determined morphologically to be the same species, *Er. eremicus*. Schmit was one of the authors of the paper that described *Er. warrae* and in 2008 regarded this species as synonymous with *Er. eremicus*.

- Research by Plant & Food Research scientists indicate that there are significant differences between the species of *Eretmocerus* present in New Zealand and the North American species of *Er. eremicus*, namely that it does not parasitize *Bemisia tabaci*, and has a higher percentage of females within a population (Workman & Pedley, 2007).

- Research by Ian Scott indicates that the species of *Eretmocerus* that is present in NZ is also molecularly different from the North American *Er. eremicus*. Until further work is done the species present in New Zealand is regarded as *Er. eremicus*.

- The colony at BioForce Ltd, used in this study, originated from the collections made in Auckland in 2006/07 and at this stage should be regarded as *Er. eremicus*.

2.24 Summary

There are many challenges facing a grower in controlling pest insect populations within a crop, including: invasion of foreign pest species, inadequate diversity and quality of biological controls available, and resistance of pest insects to chemical treatments. The growing demand by consumers for produce with low chemical residues and the increasing resistance of pest insects to chemical treatments has encouraged the use of biological controls by growers. However, there has been a slow uptake of biological control by growers within covered crops due to the relative recentness of these techniques and the lack of enough successful examples of it operating. Growers identify a larger variety of effective biological control agents as being the most important factor in developing pest management strategies**.** The most effective natural enemies for whiteflies have been parasitoids, which can be reared cheaply and released in large numbers into a glasshouse. Parasitoids are most effective when teamed up with other natural enemies that target earlier life stages of pest insects, such as with predatory mites that target eggs and 1st instar whitefly nymphs.

The success of biological control is also impaired by the lack of suitable food resources and shelter for biological controls within an intensively managed monoculture glasshouse crop. The adaptation of a glasshouse environment to better suit biological controls is a major stepping stone required for greater success of biological control strategies within covered crops. Ways to achieve this are by creating habitat refuges within a glasshouse with plants that provide pollen and invertebrate food sources. The provision of these habitats acts as a buffer against crop rotations, spray programmes and lack of adequate food sources within a glasshouse environment. To avoid build up of pest insect populations and plant diseases the refuge plants should also be rotated during the year with fresh plants, at a times when there are adequate numbers of biological control agents within the main crop.

These developments within management of glasshouse crops are still a long way coming and until these changes are made, and coupled with the provision of a more diverse range of biological control options from growers, the sustainable control of pest insects within a glasshouse environment is unlikely to be realized. This thesis aims to make a small contribution to the development of this field, by investigating the benefit of developing *Er. eremicus* as a new biological control agent of the greenhouse whitefly on glasshouses tomato in New Zealand.

Chapter Three
Materials and Methods

3.01 Preparation of Tomato Plants

For all experiments presented in this thesis, "Money Maker" tomato plants were used and grown from seed within a glasshouse at Auckland. Tomato plant seedlings were planted into 30cm diameter pots filled with commercial potting mix and set up in a glasshouse unit equipped with an automated irrigation system supplying dilute fertilizer.

Once the tomato plants were 40-50cm high they were transferred to a glasshouse unit containing tobacco plants infested with a high concentration of whitefly adults. The whitefly adults were blown off the tobacco plants with a hair dryer onto the tomato plants, which were left in this room for 4 hours. During this time the whitefly laid approximately 20 whitefly eggs/cm^2 upon the tomato plants leaves. The average of 10 counts of whitefly eggs, within a 1 cm^2 square on separate leaves, was used to determine the overall density of whitefly eggs on each plant. The whitefly adults were then blown off the tomato plants with a hair dryer. The tomato plants were then transferred to another glasshouse unit and connected back to the automated irrigation system. The plants were left in the glasshouse until the whitefly eggs had hatched and the nymphs developed to 2^{nd} instar stage.

3.02 Insect Colonies

The *En. formosa* and *Er. eremicus* parasitoids and greenhouse whitefly used for these experiments were obtained from Bioforce Ltd, a commercial producer of natural enemies in Auckland and were raised on tobacco plants within glasshouses. The *En. pergandiella* parasitoids were raised on tomato plants at

Plant & Food Research within a glasshouse. The *En. pergandiella* and *Er. eremicus* colonies were established from parasitized whiteflies collected from commercial tomato glasshouses in the Auckland region. The *En. formosa* and greenhouse whitefly colonies had been established for many years at Bioforce Ltd from an unknown source in Auckland, New Zealand.

The adult parasitoids used for experiments in this thesis were collected while still inside their hosts, by collecting parasitized 4th instar whitefly nymphs. These nymphs where washed off the tobacco or tomato plants, dried and placed within Petri dishes. These Petri dishes were then transferred to temperature controlled rooms at Plant & Food Research for use in the experiments. Only adult parasitoids which had emerged each morning (less than 10 hours old as adults) were used for the experiments. These adult parasitoids were placed within a controlled temperature room set to 10^{o}C (to immobilise the adults) and transferred to the experimental containers using a moistened fine-tipped paint brush.

3.03 Controlled Temperature Rooms

The controlled temperature rooms used for these studies were located at Plant & Food Research, Auckland. Three rooms were used for these studies, a 10^{o}C room where experimental containers were transferred to for moving parasitoids or make observation of host-feeding and two rooms which were initially set to 15 and 25^{o}C for the first set of parasitism efficacy and host-feeding experiments (in December, 2007) and later set to 20 and 30^{o}C for the second set of parasitism efficacy and host-feeding experiment (in January, 2008). These rooms were temperature controlled with air conditioners, which were accurate within a few degrees of the temperature they were set to. Illuminating the work bench, used to put experimental containers upon, were four 30 cm long, 200 Watt fluorescent lights (Fig. 3.1). The lights were automatically set to 16 hours of light and 8 hours of darkness photoperiod.

Fig 3.1. Set up of parasitism efficacy containers within controlled temperature rooms at Plant & Food Research, showing bench and fluorescent lighting. Scale 1:10.

3.04 Preparation of Leaf Cuttings

The tomato leaf "shoot" cuttings were made following the technique used in horticulture for the propagation of soft stemmed plants (Akoumianaki-Iannidou et al., 1999). A heel cutting is made by pulling down at the base of a shoot where it joins the main stem of the plant, exposing a large number of cambium cells along the heel of the cutting (Guifeng, 1993). From these cambium cells new roots will quickly develop. For this experiment excess leaf material was stripped away to acquire a surface area of leaf that was approximately 10 cm^2. These cuttings were selected from tomato plants infested with early 2^{nd} instar greenhouse whitefly nymphs. Each cutting had an average of 140 (range 80 - 180) 2^{nd} instar whitefly nymphs. This number would ensure that a single parasitoid female would not have a limiting number of potential hosts for both parasitism and host-feeding.

The base of each cutting was inserted through a 6mm diameter hole in the lid of a 45 mm diameter container filled with water (Fig. 3.2). A small role of Bluetac™ was then fitted around the stem of the cutting to create a tight seal on the lid of the container to secure the position of the cutting. This arrangement was then placed within a larger 115 mm diameter clear plastic container (Fig. 3.3). The lid of this container had an 80 mm diameter hole cut into it and covered with a sheet of tissue paper, which allowed good air circulation within the container to keep conditions healthy. The base of the cuttings developed adventitious roots after a few days of being immersed in water, which kept the leaf material fresh throughout the experiment (Fig. 3.4).

Fig. 3.2. Tomato leaf cutting immersed into container of water and sealed with Bluetac™. Scale 1:1.

Fig. 3.3. Vented container containing tomato leaf cutting infested with greenhouse whitefly, used for parasitism efficacy study. Scale 1:2.

Fig. 3.4. Tomato leaf cutting at end of parasitism efficacy experiment (approx 3 weeks), showing growth of adventitious roots from base of cutting. Scale 1:1.

3.05 Preparation of Leaf Disks

Leaf disks were prepared from tomato plants infested with 2nd instar whitefly nymphs. A disk was made by punching a 60 mm hole out of a tomato leaf. Each disk had between 50-80 (average 62) 2nd instar greenhouse whitefly nymphs. These disks were placed top-side downwards onto the base of an 80 mm diameter plastic Petri dish, filled with 5ml of 2% agar solution (Fig. 3.5). The agar solution kept the leaf material fresh, while placing the disk bottom-side upwards exposed the whitefly nymphs to the parasitoid introduced. The lid of the Petri dish had a 12mm hole punched into it to allow air to circulate and avoid build up of mould from the honey-dew excreted by the whitefly. This hole was covered by fine metal gauze to prevent escape of the parasitoid introduced (Fig. 3.6). The layout of dishes on the bench is shown in Fig. 3.7.

Fig. 3.5. A tomato plant leaf disk placed on agar in a Petri dish. Scale 1.5:1.

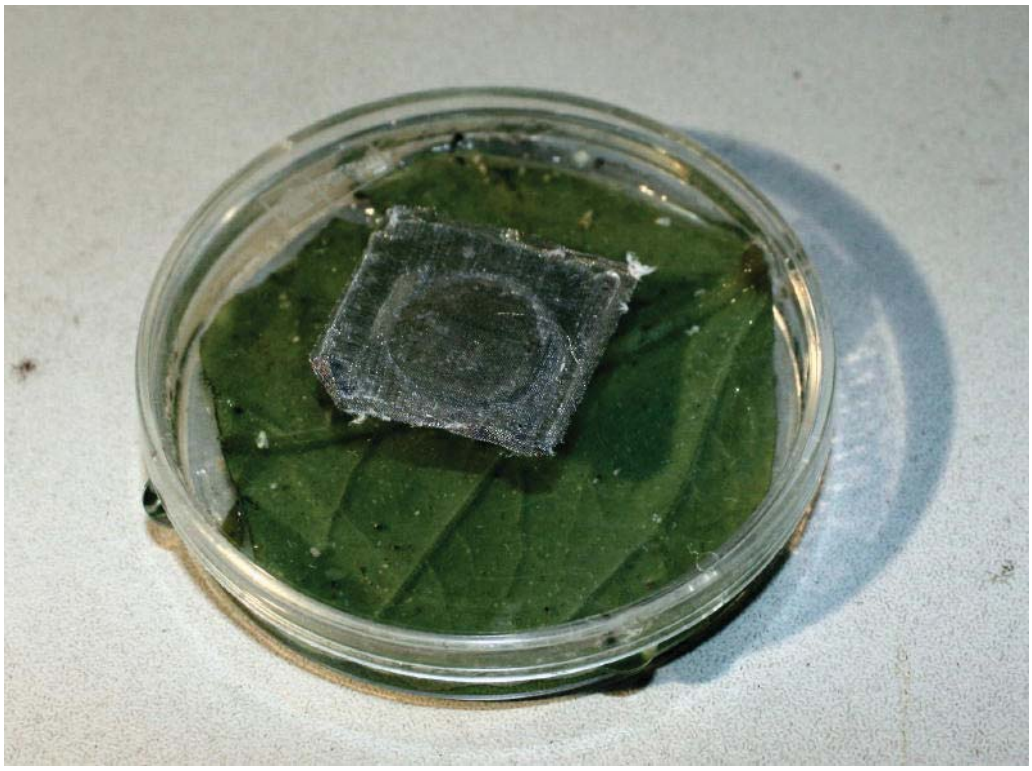

Fig. 3.6. Vented Petri dish setup with leaf disk. Scale 1.5:1

Fig. 3.7. Layout of Petri dishes in temperature controlled room at Plant & Food Research. Scale 1:10.

3.06 Parasitism Efficacy Study

Within this experiment the parasitism efficacy of *Er. eremicus*, *En. formosa* and *En. pergandiella* was recorded on tomato plant leaf cuttings infested with 2nd instar whitefly nymphs and enclosed with a vented plastic container. Eighty vented containers (Fig. 3.3) where set up within temperature rooms at Plant & Food Research (Fig. 3.1). This comprised of twenty replicates of each parasitoid (*En. formosa*, *En. pergandiella*, and *Er. eremicus*) and 20 controls with no parasitoid added. Controls were included to determine if the whitefly nymphs on the cuttings had been parasitized by any parasitoids while the tomato plants were kept in glasshouses prior to the experiment. Two controlled temperature rooms were used for the study and set to 15 and 25^{o}C in December 2007 and 20 and 30^{o}C in January, 2008. A single adult female parasitoid was released onto a cutting within each container and left within the container for its entire lifespan.

The parasitism efficacy was recorded as the average number of whitefly nymphs parasitized by 20 different adult female parasitoids of each species. This average of the total number of whitefly parasitized was then compared between the parasitoids, to determine which one was the most effective during the experiment, or which had the highest parasitism efficacy. The comparative parasitism efficacy of *Er. eremicus* will be used as an indication of its suitability for use as a biological control agent, in tomato glasshouses in New Zealand.

The effect of temperature on parasitism efficacy was also investigated, by repeating the study at 15, 20, 25 and 30^{o}C. This range of temperatures aimed to replicate the range of temperatures encountered within glasshouses in New Zealand. This range of temperatures will also indicate if *Er. eremicus* has a high level of parasitism (compared to the other two parasitoids) over a wide range of temperatures, or if there is a specific temperature range in which it performs best.

A two-way ANOVA in Minitab was used to determine if there was a significant effect from parasitoid type or temperature treatment on numbers of whitefly nymphs parasitized, followed by a Fisher's LSD test. This analysis was

performed on the number of whitefly nymphs parasitized from 20 replicates of each parasitoid type and 20 controls without any parasitoids added.

The number of whitefly nymphs parasitized by each parasitoid was recorded once all unparasitized whitefly had emerged as adults (parasitoids take between 4-8 days longer to emerge from their hosts than adult whitefly (van Lenteren, 2003) and the origional parasitoid had died. At this time the parasitoid larvae were clearly visible within the cuticle of their host and a visual analysis of nymphs under 10 x magnification was used to determine successful parasitism (Hoelmer, 2007). Using this technique black nymphs indicated parasitism by *En. formosa*, yellow nymphs parasitism by *Er. eremicus*, and brown nymphs parasitism by *En. pergandiella*. At this point all unparasitized whitefly had emerged as adults, removing the possibility of mistaking unparasitized for parasitized nymphs and all original parasitoids had died. These results were used in this study, but the containers were kept to observe emergence of adult parasitoid and verify identity of parasitoids.

3.07 Host-feeding Study

Within this experiment the host-feeding exhibited by *Er. eremicus*, *En. formosa* and *En. pergandiella* was recorded on tomato plant leaf disks infested 50-80 (average 62) 2nd instar whitefly nymphs (Fig. 3.5), within vented, agar based Petri dishes (Fig. 3.6) and compared at temperatures of 15, 20, 25 and 30^{o}C. The aim of this study was to investigate if parasitoid type and/or temperature had a significant effect on number of whitefly nymphs killed by host-feeding.

A two-way ANOVA in Minitab was used to determine if there was a significant effect from parasitoid type or temperature treatment on numbers of whitefly nymphs killed by host-feeding, followed by a Fisher's LSD test. This analysis was performed on the number of whitefly nymphs killed over a 10 day period, from 20 replicates of each parasitoid species and 20 controls without any parasitoids added.

The number of whitefly nymphs killed by host-feeding was recorded each day, for 10 days, by transferring the Petri dishes to a 10^{o}C room to prevent escape of adults. Host-feeding was recorded if the nymph's appeared shrunken when observed with a 10X eyepiece lens. The shrunken appearance is a result of a female parasitoid penetrating them with its ovipositor and feeding upon the haemolymph from the wound (Succop, 1997; Viggiani, 1984). Data was only collected for the first 10 days because by this point almost half the parasioids had died at the higher temperatures studied.

If this experiment indicates a high level of host-feeding from *Er. eremicus* (compared to the other two parasitoids) this will be used as an another indication of its suitability as a biological control agent of greenhouse whitefly in tomato glasshouses in New Zealand. The aim of performing the experiment at the range of temperatures noted is to determine the effect of temperature on the biology of *Er. eremicus* compared to other common whitefly parasitoids.

3.08 Longevity of Parasitoids

The longevity of three greenhouse whitefly parasitoids: *Er. eremicus, En. formosa* and *En. pergandiella* was investigated on tomato leaf disks infested with 2nd instar greenhouse whitefly nymphs, within vented, agar-based Petri dishes. This study was performed on twenty replicates of each parasitoid and repeated at 15, 20, 25 and 30^{o}C (within controlled temperature rooms at Plant & Food Research) to compare longevity between parasitoid species and temperature treatments.

The survival of all replicates was recorded each day, by transferring the Petri dishes to a 10^{o}C controlled temperature room at Plant & Food Research, which immobilised the adult and allowed them to be observed. Any build up of honey-dew on the lids of the Petri dishes was also wiped away at this time, to maintain a

healthy environment within them. These observations were repeated until the last parasitoid had died.

The average number of days (across 20 replicates) each parasitoid species survived was used to compare longevity between each parasitoid species. The significance of temperature treatment and parasitoid species on longevity was analysed in minitab using a two-way ANOVA, followed by a Fisher's LSD test.

3.09 Functional Response Study

The parasitism efficacy experiment had a variable density of 2nd instar whitefly nymphs upon the leaves (80-180 nymphs) with no preferred selection of a particular density within this range. This variability was due to the tomato plants used for the study being exposed to adult whitefly for 4 hours, during which time they laid approximately 20 whitefly eggs/cm^2 of tomato leaf. Large well formed leaves, with a quantity of nymphs within the range of 80-180 nymphs upon them, were then used for the parasitism efficacy experiment. If this study reveals a strong effect of host density on parasitism efficacy, this variable may prove more important than changes in temperature to the numbers of nymphs parasitized that was recorded.

This study investigates the effect of whitefly host density on parasitism efficacy. Data from the parasitism efficacy experiment (at all temperatures) where used in this study, with the data divided into groups representing leaf cuttings with 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, and 180 nymphs upon them. The average number of whitefly parasitised at each density of nymphs was compared, to determine the effect of whitefly density on parasitism efficacy. The smallest number of replicates amongst the different densities was three, so this same number of replicates was used for all the densities, to perform a meaningful comparison between them. In the instance that there were more than three replicates for a particular density, the first three replicates were selected. The

significance of whitefly density and parasitoid type on parasitism efficacy were analysed with a two-way ANOVA in minitab.

3.10 Host Stage Preference of *Eretmocerus eremicus*

The aim of this study is to investigate the preferred whitefly nymph instar stage for parasitism by *Er. eremicus*. The preference of New Zealand the strain of *Er. eremicus* for specific developmental stages of greenhouse whitefly on tomato plants has not been studied. The results of this study will improve the understanding of aspects of *Er. eremicus* biology important in its function as a biological control agent and for mass rearing this parasitoid. The results of this study can be presented to producers of natural enemies, if the parasitism efficacy and host-feeding studies indicate there is benefit in mass rearing this parasitoid for use as a biological control agent of greenhouse whitefly in tomato glasshouses in New Zealand.

The whitefly nymph instar stage best suited for parasitism by *Er. eremicus* was investigated in vented containers on tomato plant "shoot" cutting infested with either 1st, 2nd, 3rd, or 4th instar whitefly nymphs in a 20^{o}C controlled temperature room at Plant & Food Research. Each leaf cutting supported 80-180 (average 140) whitefly nymphs. Fifteen replicates of each instar stage were tested, by placing a single newly emerged *Er. eremicus* inside each container with a cutting. Each parasitoid was left within the experimental container for its entire lifespan.

All the containers were placed within a temperature controlled room at Plant & Food Research, set at 20^{o}C. The parasitoids were placed into each container by transferring the containers to a 10^{o}C room (a temperature that immobilized them). Every 24 hours each female was transferred into a new container with the same whitefly nymph instar stage, by briefly transferring the container back to the 10^{o}C room. This allowed the number of whitefly nymphs parasitized each day by a single female *Er. eremicus* to be recorded. Once the 2nd generation

parasitoids were close to emerging from their host shells and the original parasitoid had died, the number of parasitized whitefly nymphs was counted and compared between trials.

A 1-way ANOVA analysis was performed in Minitab, followed by a Fisher's LSD test, was performed on the data, to determine the significance of whitefly instar stage on the number of whitefly nymphs parasitized in 24 hours by a single adult *Er. eremicus* female.

3.11 Effect of Adult Age on Parasitism by *Eretmocerus eremicus*

This study investigates the effect of age on adult female *Er. eremicus* ability to parasitise 2nd instar greenhouse whitefly nymphs. This information can then be provided to producers of natural enemies if the studies on parasitism efficacy and host-feeding indicate that *Er. eremicus* has potential as a biological control agent of greenhouse whitefly. Natural enemy producers can then use this information in designing mass rearing systems to commercially produce this parasitoid. This study also has benefit in advancing the general understanding of the biology of the New Zealand strain of *Er. eremicus*. The effect of age on adult *Er. eremicus* is a variable that is not expected to effect the results of the parasitism efficacy study, because the parasitoids were left on the tomato leaf cuttings within the vented plastic containers for their entire lifespan.

Ten newly emerged female *Er. eremicus* were placed separately within 10 vented containers, containing tomato leaf cutting infested with 80 to 180 (average 140) 2nd instar whitefly nymphs, held in a 20^{o}C temperature controlled room at Plant & Food Research. Every 24 hours each female was transferred into a new container, with a fresh leaf cutting infested with a similar number of 2nd instar whitefly nymphs, until it died. This allowed the number of whitefly nymphs parasitized each day by a single female *Er. eremicus* to be recorded, for its entire lifespan. The average number of nymphs parasitized (between the 10 parasitoids) was recorded each day and used as a comparison between day treatments. The

significance of the effect of age on parasitism of whitefly nymphs was analysed in one-way ANOVA in mintab, followed by a Fisher's LSD test.

Chapter Four

Results

4.1 Parasitism Efficacy Study

Both parasitoid species and temperature treatments had significant effects on the numbers of parasitized whitefly nymphs by a single adult female *Er. eremicus*, *En. formosa* or *En. pergandiella* (P<0.01, ANOVA) (Fig. 4.1). The controls had a very low average number of parasitized nymphs (1-2 nymphs/treatment), indicating a low contamination and a strong effect on numbers of nymphs parasitized from introducing a parasitoid. The controls were not included in Fig. 4.1. These results indicate that all three parasitoid species achieved similar parasitism rates at 15°C while *En. formosa* performed significantly better than other two species at 20°C. At 25°C, both *En. formosa* and *Er. eremicus* parasitised similar number of nymphs while at 30°C *En. pergandiella* and *Er. eremicus* were not effective.

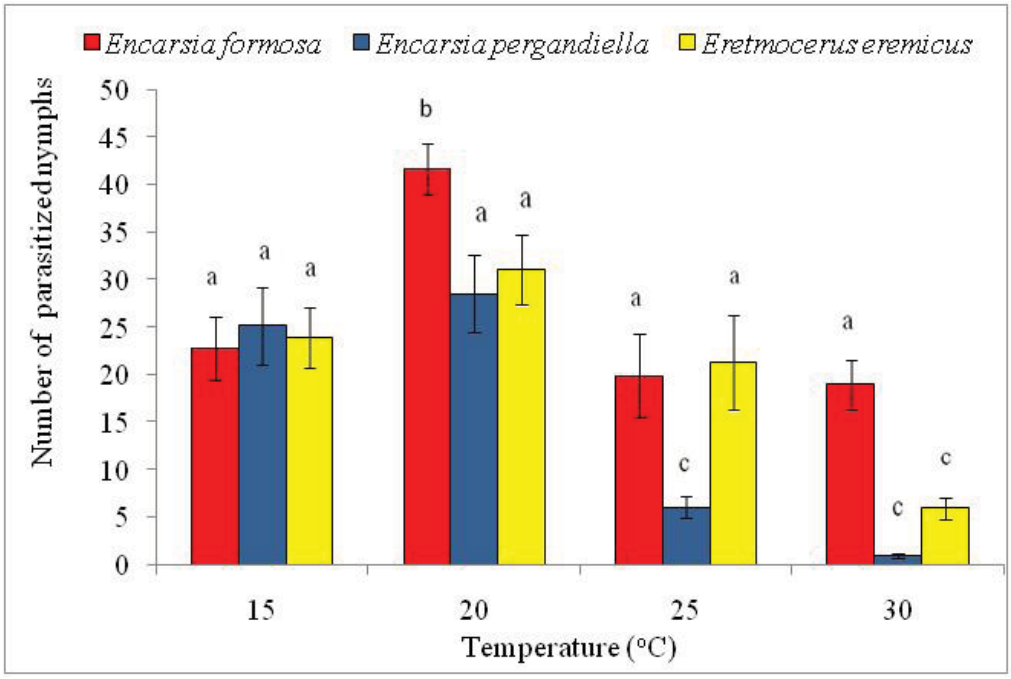

Fig. 4.1. The average (±SE) of the total number of whitefly nymphs parasitized by a single female *En. formosa*, *En. pergandiella*, or *Er. eremicus*. Bars with the different letters are significantly different (P<0.01, ANOVA).

4.2 Host-feeding Study

My results show that both parasitoid species and temperature treatments had significant effect on numbers of whitefly nymphs killed (P<0.01, ANOVA) (Fig. 4.2). At 15 and 20°C, all three species of parasitoids killed similarly low number of whitefly nymphs by feeding. However, at 25 and 30°C, *Er. eremicus* and *En. formosa* performed significantly better than *En. pergandiella*.

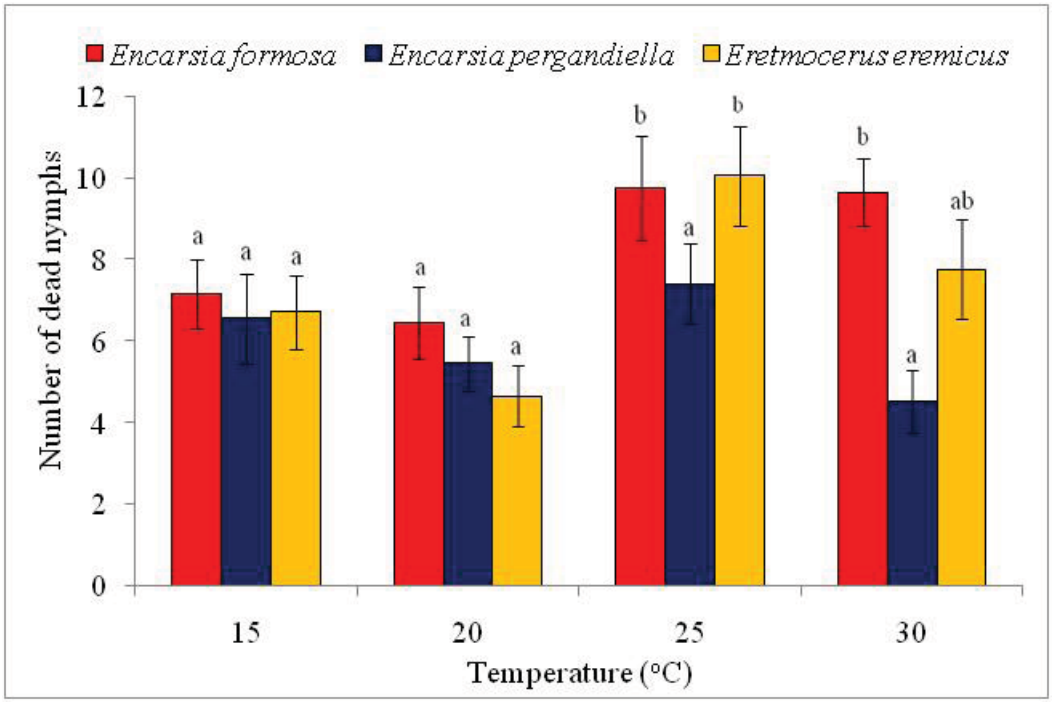

Fig. 4.2. The average (±SE) number of whitefly nymphs killed from host-feeding by a single female *En. formosa*, *En. pergandiella*, or *Er. eremicus*. Bars with different letters are significantly different (P<0.01, ANOVA).

4.3 Longevity of Parasitoids

My analysis indicates that there was a significant effect of parasitoid species and temperature treatments on longevity (P<0.01, ANOVA) (Fig. 4.3). *En. formosa* generally lived longer than the other two species at all temperature treatments.

The longevity of *Er. eremicus* was significantly shorter than *En. formosa* and *En. pergandiella* at 20°C.

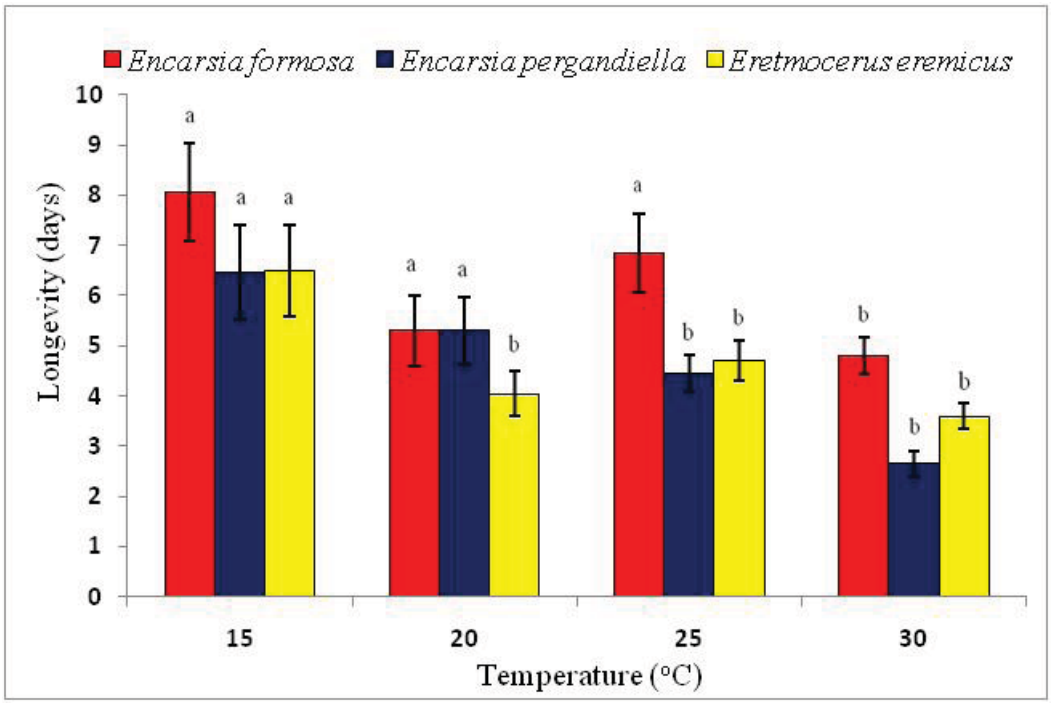

Fig. 4.3. The average (±SE) longevity of parasitoids (*En. formosa*, *En. pergandiella* and *Er. eremicus)* in days at temperatures of 15, 20, 25, and 30°C. Bars with different letters are significantly different (P<0.01, ANOVA).

4.4 Functional Response

My results show that the number of whitefly nymphs on a cutting did not have a significant effect on the number of whitefly nymphs parasitised (P>0.05, ANOVA) but that there was a significant effect caused by parasitoid species (P<0.01, ANOVA) (Fig. 4.4). *Er. eremicus* and *En. formosa* parasitised a similar number of nymphs across all the densities but parasitised a higher number of nymphs than *En. pergandiella*.

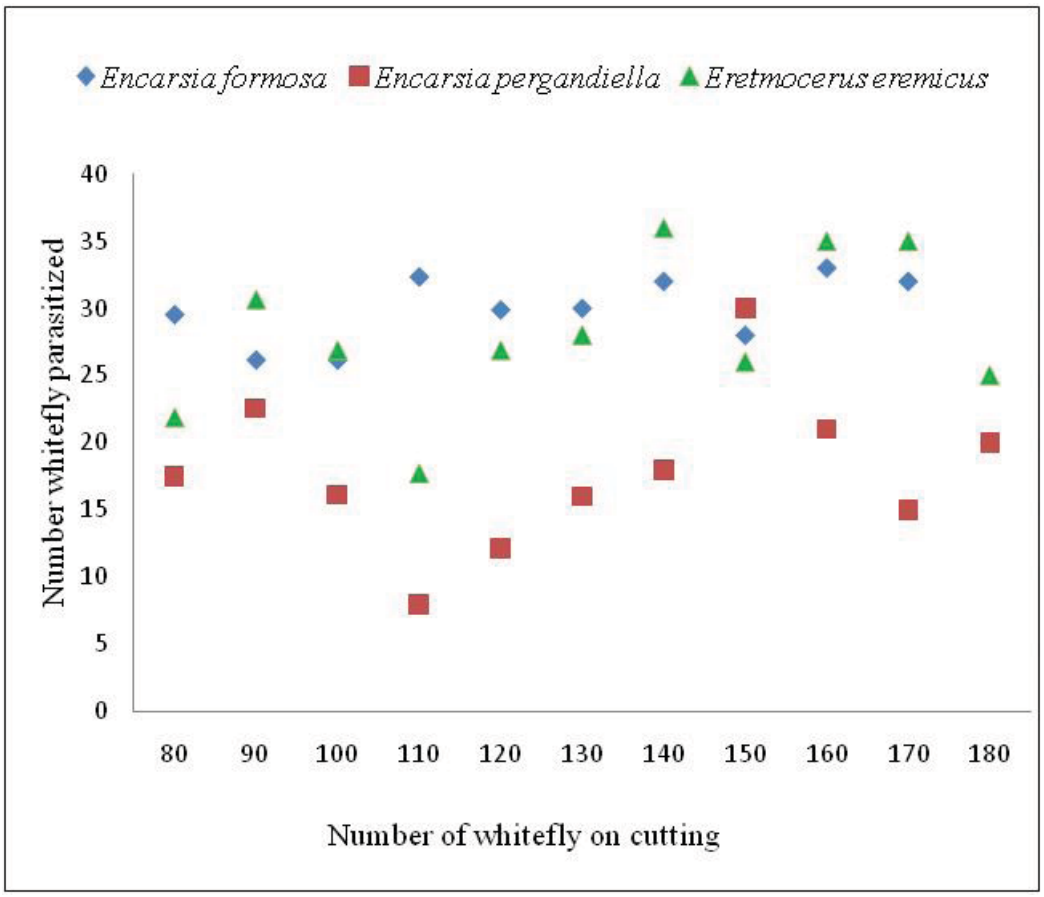

Fig. 4.4. The average (±SE) number of whitefly nymphs parasitized by a single female *En. formosa*, *En. pergandiella*, or *Er. eremicus* on tomato leaf cuttings with varying densities of whitefly nymphs.

4.5 Host Stage Preference of *Eretmocerus eremicus*

Female *Er. eremicus* significantly preferred the second and third instar to fourth instar nymphs for oviposition ($P<0.01$, ANOVA) (Fig. 4.5). Although the number of the first instar nymphs parasitised was lower than that of the second and third instar nymphs, the difference was not significant ($P>0.05$, ANOVA) (Fig. 4.5).

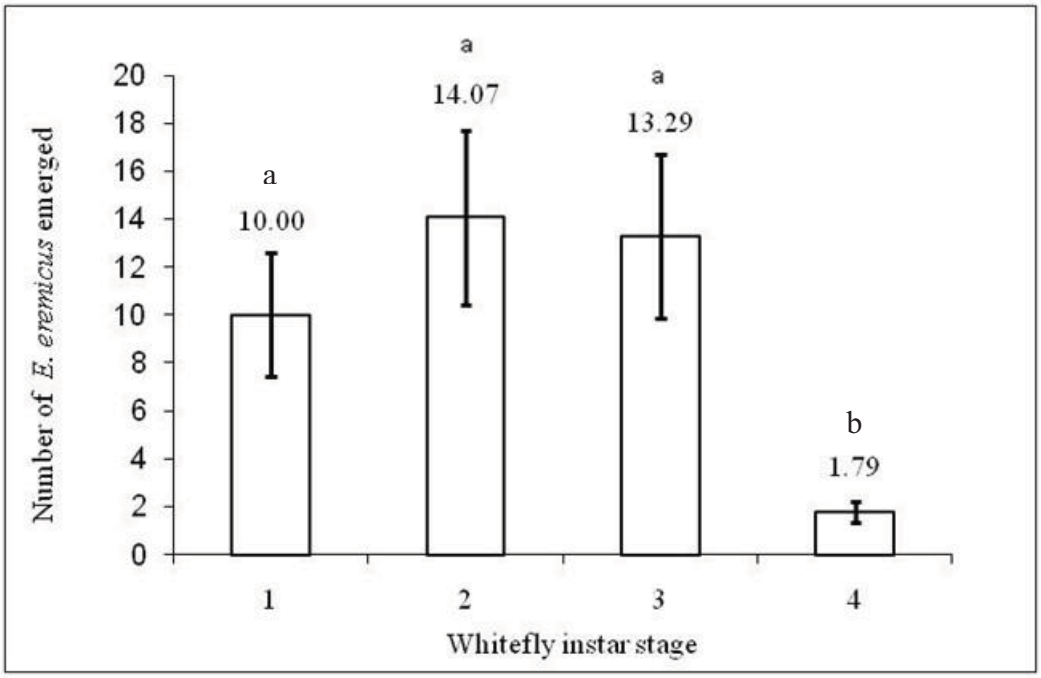

Fig. 4.5. Average (±SE) number of whitefly nymphs parasitized at four different developmental stages. Bars with different letters are significantly different (P<0.01, ANOVA).

4.6 Effect of Adult Age on Parasitism by *Eretmocerus eremicus*

My results indicate that daily oviposition rate by *Er. eremicus* females was significantly higher in the first 5 days following emergence than that by older ones, with a peak of daily oviposition occurring 2-3 days after emergence (P<0.01, ANOVA)(Fig. 4.6).

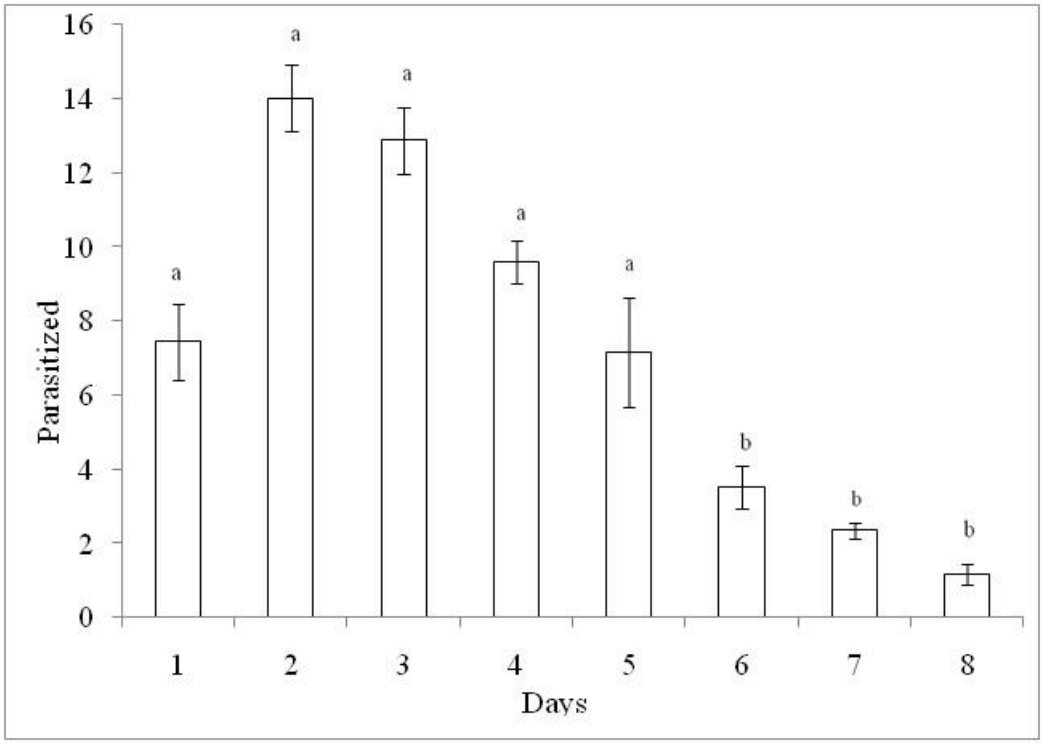

Fig. 4.6. Average (±SE) number of whitefly nymphs parasitized each day by a single *Er. eremicus* female. Bars with different letters are significantly different (P<0.01, ANOVA).

Chapter Five
Discussion & Conclusion

5.1 Aim of Thesis

The aim of this thesis was to investigate the potential benefit of developing a recently re-descovered parasitic wasp *Eretmocerus eremicus* as a biological control agent of the greenhouse whitefly. The greenhouse whitefly has a significant economic impact on production of tomatoes within glasshouses in New Zealand. The only biological control agent presently being used in another parasitic wasp *Encarisa formosa* and growers are looking for a greater range of biological control agents to improve the effectiveness of biological control techniques.

The main studies presented in this thesis compare the host-feeding, parasitism efficacy and longevity of three parasitoids of the greenhouse whitefly found in New Zealand (*Er. eremicus*, *En. formosa* and *Encarsia pergandiella*) at temperatures of 15, 20, 25, and 30^{o}C. This comparison aimed to determine the most effective parasitoid of the greenhouse whitefly on tomato plants in glasshouse conditions. Further studies were performed on the preferred whitefly nymph instar stage for parasitism and effect of adult age on parasitism of *Er. eremicus.* The additional studies aimed to obtain a better understanding of some important aspects of the biology of this parasitoid and to determine the influence of these factors on the experimental design of the previous experiments.

Performing the main studies at 15, 20, 25 and 30^{o}C aimed to replicate the range of temperatures commonly encountered within covered crops in New Zealand glasshouses and to indicate differences in tolerance to temperature between the parasitoids. Researchers have noted that *En. formosa* performs at a reduced level at lower temperatures (Rumei et al., 1993; Succop, 1997) while *Er. eremicus* has a tolerance to higher temperature than *En. formosa* (Hoddle & Driesche, 1999), likely due to its adaptation to the dry desert regions of Arizona where it

originates. The New Zealand strain of *Er. eremicus* however, could be better adapted to the cooler New Zealand conditions and may outperform *En. formosa* at the lower temperatures being studied.

5.2 Results of Studies

The results of the parasitism efficacy study show that *En. formosa* parasitised the highest average number of whitefly nymphs (26 nymphs), which was 19% higher than *Er. eremicus* (21 nymphs) and 42% higher than *En. pergandiella* (19 nymphs). *En. formosa* also killed a significantly higher average number of whitefly nymphs through host-feeding (8 nymphs), which was 13% greater than *Er. eremicus* (7 nymphs) and 25% greater than *En. pergandiella* (6 nymphs). Furthermore, *En. formosa* also had a significantly longer average longevity (6 days), which was 17% greater than the *Er. eremicus* and *En. pergandiella* (both 5 days).

Other researchers have reported a higher parasitism for both *En. formosa* [(approximately 59 nymphs (Succop, 1997)) and *Er. eremicus* [(approximately 54 eggs (Asplen et al., 2009)) than that reported in the parasitism efficacy study. The experimental design can have a marked influence on the results obtained from an experiment (van Lenteren, 2003). The main differences between the studies in this thesis and those in Asplen et al. (2009) is that Asplen carried out studies at the parasitoids optimal temperatures 22-26^{o}C, whereas the rate of parasaitism in this study was the average across temperatures of 15-30^{o}C. Also studies by Asplen et al. (2009) where within clip cages instead of on cuttings. Within clip cages the parasitoids are exposed to whitefly feeding upon a healthy mature plant, while the cuttings used in this study would not have provided such an optimum food resource for the developing whitefly nymphs. The quality of the whitefly nymph food source has been shown to have a marked effect on the number of parasitoids that successfully develop (Godfray, 1994).

The numbers of nymphs killed by host-feeding was also less than other studies, with Collier & Hunter (2001) reporting *En. formosa* as host-feeding on between 10-20 nymphs, *Er. eremicus* up to 30 nymphs and *Er. pergandiella* as 2 nymphs. Succop (1997) reports that from a combination of parasitism and host-feeding *En. formosa* can kill up to 95 whitefly nymphs during its lifetime. Environmental conditions (including humidity and temperature), health of the whitefly nymphs and health of the tomato leaf disk material are again most likely responsible for these differences. The leaf disk within a Petri dish, in particular, presents a restricted micro-habitat very different from that encountered by a parasitoid on a healthy plant. However, studies of host-feeding by parasitoids are commonly performed on leaf disks within vented Petri dishes (Succop, 1997; van Lenteren et al., 1997; Godfray, 1994). Ultimately however, the goal of the parasitism efficacy and host-feeding studies presented in this thesis was to compare the performance of the parasitoids, not provide an indication of their parasitism efficacy or host-feeding potential.

The data from the parasitism efficacy study was used in an analysis of the effect of whitefly nymph density on numbers of whitefly paralysed by the three parasitoids. This study found there was no significant effect from host density on numbers of whitefly parasitized, but that there was from parasitoid type. Other researchers have noted that the density of whitefly nymphs on leaf surfaces can have a meaningful influence on parasitism within glasshouses (Fernández-arhex & Corley, 2003) and in outdoor crops (Bellamy et al., 2004). The lack of an effect from whitefly density on parasitism in this study was probably due to leaf cuttings being selected that had a range of 80 to 180 nymphs upon them. Trials with the tomato leaf cutting method showed that this range of densities neither limited a parasitoid from having a suitable number of hosts to select from (this was determined by at least 20 adult whitefly emerging from the leaf cuttings) or too high a number that detrimental environmental conditions would occurr from mould growing on honeydew deposits and suppress the activities of a parasitoid. These results would suggest that with moderate densities of whitefly nymphs on leaf surfaces, parasitoids can function at their optimal level and are not significantly affected by the density of nymphs.

To obtain a clearer picture of aspects of the biology of *Er. eremicus* important for mass-rearing and which may have an effect on the experimental design of the parasitism efficacy and host feeding studies, the effect of adult age on parasitism efficacy and preference for specific whitefly nymph instar was investigated on *Er. eremicus*. The selection of particular hosts by adult parasitoids is of importance to producers of natural enemies, to ensure whitefly nymph hosts are presented to adult parasitoids at a suitable developmental stage (Conlong & Mugoya, 1996). Vet et al. (1995) notes that *Eretmocerus* sp. often have a strong preference for 2nd and 3rd instar stage whitefly nymphs, which is also the longest developmental phase for a whitefly (Russell, 1977). *Eretmocerus* sp. are also capable of parasitizing 1st instar nymphs because they lay an egg beneath their host, instead of inside it as with *Encarsia* sp. parasitoids (Kassis & Michelakis, 1993).

The results of the instar preference study showed a significant preference for 1st, 2nd and 3rd instars over 4th instar. There was also a greater average number of 2nd and 3rd instar nymphs parasitised than 1st instar nymphs. The adult age study showed that *Er. eremicus* parasitised a consistently high number of nymphs in the first 5 days following emergence at 20^{o}C, with parasitism falling off sharply thereafter and many parasitoids dying.

The preference for 2nd instar whitefly nymphs by *Er. eremicus* may have given it a slight advantage over *En. formosa* and *En. pergandiella* in the design of the parasitism efficacy study, that exposed all three parasitoids to 2nd instar nymphs. The 2nd instar nymphs would have taken 2-6 days (depending on temperature) to develop to the 3rd instar stage preferred by *Encarsia* sp. parasitoids (Hoddle et al., 1998; van Lenteren et al., 1997). Studies have shown that the optimal "window" of time for adult parasitoids to be most effective is the first five days following emergence (van Lenteren, 2003).

However, the longevity of parasitoids increases with decreasing temperature (Hua et al., 2003) and at lower temperatures they actively parasitize nymphs for a longer period **(**Rumei et al., 1993). The increased longevity results in a similar number of nymphs being parasitized between 15-30^{o}C, with the parasitoids

taking longer to parasitize this number of nymphs at the lower temperatures (Jones et al., 2003). Also, while 2nd instar nymphs are not preferred by *Encarsia* sp. parasitoids, studies have shown that when they are presented only with 2nd instar nymphs, the level of parasitism is no different to their levels of parasitism when 3rd or 4th instar nymphs were offered (Vet et al., 1980). Because a parasitoid has a prolonged optimal time for parasitisation at lower temperatures, the prolonged exposure of the *Encarsia* sp. parasitoids to 2nd instar nymphs (in the parasitism efficacy study) is not expected to significantly affect the comparison between the parasitoids. While these changes in the biology of the parasitoid (to match the development of their host) should result in a unbiased comparison between the parasitoids in the parasitism efficacy study, this experiment could have been designed to include a matrix of differently aged whitefly nymphs and parasitoids (Jones et al., 2003; Hua et al., 2003) to ensure no possibility of a bias within the results could occur.

The adult age study on *Er. eremicus* indicated that this parasitoid parasitizes most whitefly nymphs within the first five days, which is also supported by other studies (Asplen, 2009; van Lenteren, 2003). A technique is therefore required, when using this parasitoid as a biological control agent, for it to be release within a crop while it is still inside its host (van Lenteren & Manzaroli, 1999). This is so that the first few days of potential parasitization as an adult are not lost during transit to a crop. Despite the short active lifespan of adult parasitoids during study temperatures (mostly 20-25°C), at 10°C *En. formosa* has been recorded living up to 90 days (Rumei, 1993). Many adult parasitoids also overwinter within their native habitats (Steven & Naranjo, 2001). The longevity of adult parasitoids used as biological control agents might therefore be increased by cold storage before dispersal within a crop.

5.4 Experimental Design

Two experimental designs were used in this thesis. Host-feeding and longevity were investigated using tomato plant leaf disks placed upon a 2% agar solution

within vented Petri dishes. Parasitism efficacy and host density (of all three parasitoids) and instar preference and age effect (of *Er. eremicus*) were investigated on tomato leaf cuttings.

For the parasitism efficacy study and the studies on the biology of *Er. eremicus* an environment was required that would present adult parasitoids with an easily accessible source of hosts for their entire lifespan, without that environment becoming contaminated with mould from honey dew deposited by the whitefly nymphs feeding. Trials with different experimental designs indicated that presenting the parasitoids with fresh and healthy leaf material with enough hosts for both parasitization and host-feeding (but not so many that the environment becomes contaminated) was most important. This environment also needed to be suitably spacious and well ventilated to allow good air circulation and avoid mould growing. In addition, this environment had to be small enough to be replicated eighty times on a bench within a controlled temperature room at Plant & Food Research. The tomato leaf cuttings experimental design was created to present the parasitoids an environment that was both healthy and easily replicable.

Within the tomato leaf cutting study a parasitoid was presented with a small leaf (approximately $10cm^2$) infested with 2^{nd} instar whitefly nymphs. The leaf material was kept fresh by the base being immersed in a small container of water and this environment was kept well-aerated by the cuttings being placed within a larger vented plastic container. At the completion of the experiments these tomato shoot cuttings had well developed adventitious roots and the leaf material was still green (although stressed from whitefly feeding and accumulation of honey dew on leaf). When a selection of these cuttings were planted, most recovered quickly to grow into healthy tomato plants. Most other small-scale parasitism efficacy studies are performed within clip cages, but this design was found to result in the study environment becoming quickly fouled with honey dew deposits and did not allow sufficient air circulation to keep the parasitoids and whitefly in a healthy condition.

The agar based Petri dishes, with tomato plant leaf disks, were ideally suited for close observation of host-feeding damage to whitefly nymphs. Trials indicated that to obtain a good number of replicates for this study a small experimental set-up was required, that could be easily handled and the whitefly nymphs quickly counted (under 10X magnification) for signs of host-feeding damage. Recording of host-feeding were also only made for the first ten days, to minimise any influence on results from fouling of the environment (created by mould growing on honey dew deposits). Measures of longevity were also performed in the Petri dishes, but these results were only used for a comparison between the parasitoids, rather than an indication of their longevity potential. Petri dishes with leaf disks and clip cages on leaves are both commonly used experimental techniques for investigating the biology of parasitoids (Hanan et. al., 2009; Workman & Davidson, 2007; Hoddle & van Driesche, 1999).

5.5 Conclusion

The results of the studies presented in this thesis do not indicate any advantage in developing *Er. eremicus* as a biological control agent of greenhouse whitefly in tomato glasshouses in New Zealand. *En. formosa* had a significantly higher level of parasitisation and host-feeding with a wider temperature tolerance and greater longevity. Since *En. formosa* is already used as a biological control agent in New Zealand tomato glasshouses, this study shows no benefit in replacing it with *Er. eremicus*. *En. pergandiella* only displayed a high level of parasitisation at 15 and 20^{o}C, indicating it has adapted to cool temperatures, in New Zealand, and is unlikely to be beneficial as a biological control agent in glasshouses - except in winter.

There may be an advantage to developing *Er. eremicus* as a biological control agent of greenhouse whitefly to complement the biological control presently achieved by *En. formosa* alone. Overseas, both *En. formosa* and *Er. eremicus* are commonly released together (Hoddle & van Driesche, 1999). *Er. eremicus* is seen to complement *En. formosa* in a number of key areas: its tolerance to higher

temperatures, parasitism of earlier stage instar, and ability to parasitize *Bemisia tabaci* whitefly in addition to greenhouse whitefly. The New Zealand strain however, is quite different to that found overseas, and the same "amiable" partnership may not apply in New Zealand. Of particular importance is the high number of females in the New Zealand population (most likely a result on infection with *Wolbachia* bacteria), adaption to a cooler climate, and inability to parasitize *B. tabaci*.

There are some possible benefits to these differences. Firstly, the New Zealand *Er. eremicus* may be better performing during cooler parts of the year when *En. formosa* has been identified as "struggling" to control whitefly populations in glasshouses (Workman & Davidson, 2007), the high percentage of females in the population could also result in a high rate of parasitism within a crop environment (Ardeh, 2004) and the preference for early instar whitefly nymphs would enable a longer window for parasitism to occur when combined with *En. formosa*. There may also be benefit in releasing *Er. eremicus* during cooler parts of the year into glasshouses and *En. formosa* during warmer parts of the year.

Using multiple biological controls within the same crop does pose a few potential difficulties, with competition between parasitoids for hosts seen by some researchers as causing negative interference (Bogra et al., 2002). Greathead & Greathead (1992) however, noted that using multiple parasitoids together that target different instar stages ensures that a wider range of life stages of the pest are exposed to biological control. Getz & Mills (1996) also noted that the interference between different parasitoid species was not significantly different than between individuals of the same species. This was contradicted by Donnell & Hunter (2002), who noted a slight preference of the parasitoid *En. pergandiella* to parasitize whitefly nymphs already parasitized by another parasitoid, over unparasitized nymphs. It is likely however, that inter-species competition between multiple parasitoid species within a glasshouse would not outweigh the benefit of targeting a greater range of life stages of the target pest insect (Bogra et al., 2002; Godfray, 1994).

5.6 Recommendations

A better understanding of the relationship between the New Zealand strain of *Er. eremicus* and *En. formosa* within covered crops is required, to identify the role these parasitoids have when used in combination to control greenhouse whitefly in glasshouses. Some suggested studies would be to compare control of greenhouse whitefly population in identical glasshouses using each parasitoid separately, in combination and without either. Some tomato crops are grown in the Auckland area within small isolated glasshouses and offer the perfect conditions for trialling these studies.

Developing a mass rearing system for *Er. eremicus* will also require more research into the development of this parasitoid on hardy plants that have a large leaf surface area and can carry a heavy load of whitefly nymphs, which would be suitable for mass rearing techniques. Leaf surfaces and secretions also have an important influence on the function of parasitoids (Qiu et al., 2005). Minkenberg & Santangelo (1997) noted that *Er. eremicus* developed well on large leaved eggplants. Tobacco plants are also widely used in mass rearing of parasitoids (McMahon & Lindquist, 1994). The current method of dispensing parasitized whitefly nymphs onto sticky tags will also need to be reviewed, because *Er. eremicus* only emerges from the dorsal side of its host, unlike *En. formosa* which can turn within the nymph's skin and emerge from any direction (Hoddle & Driesche, 1999).

References

Albert, R., Schneller, H., & Sautter, H. (1999). Development of biological control in ornamentals. *Bulletin OILB SROP*, *16* (8), 1-5.

Akoumianaki-Iannidou, A., Kravari, E., & J. Chronopoulos, J. (1999). Propagation of *Polygala Myrtifolia* by Cuttings. *ISHS Acta Horticulturae, 54.*

Anis, A.I.M., & Brennan, P. (1982). Susceptibility of different populations of glasshouse whitefly (*Trialeurodes vaporariorum)* to a range of chemical insecticides. Faculty of General Agriculture University College of Dublin. *Research Report*, 55.

Ardeh, M.J. (2004). Whitefly control potential of *Eretmocerus* parasitoids with different reproductive modes. Ph.D. dissertation, Wageningen University.

Arnold, A.E., & Lewis, L.C. (2005). Ecology and evolution of fungal endophytes and their roles against insects. In F.E. Vega & M. Blackwell (Eds.), *Insect-Fungal Associations: Ecology and Evolution* (pp. 74-96). Oxford University Press.

Arnoldi, D., Stewart, R. K., & Boivin, G. (1992). Predatory mirids of the green apple aphid *Aphis pomi*, the two-spotted spider mite *Tetranychus urticae* and the European red mite *Panonychus ULMI* in apple orchards in Québec. *BioControl, 37*, 283-292.

Ashfaq, M. (2004). Advances in Mass Rearing of *Chrysoperla carnea* (Stephen) (Neuroptera: Chrysopidae). *South Pacific Study, 24 (*2), 47-53.

Askew, R.R. & Shaw, M.R. (1986). Parasitoid communities: their size, structure and development. In J. Waage & D. Greathead (Eds), *Insect Parasitoids* (pp. 22-234). London Academic Press.

Asplen, M.K., Hardin, J.A., Byrne, D.N. (2009). The relationship between pre-oviposition flight behaviour and reproductive timing in whitefly parasitoids *Physiological Entomology, 34*, 350-358.

Asplen, M.K., Bellamy, D. E., David N., & Byrne, D. N. (2001). Eggs of *Eretmocerus eremicus*, a whitefly parasitoid. Vegetable Report: University of Arizona, College of Agriculture. [Online] Available: http://ag.arizona.edu/pubs/crop/az1252/ [Retrieved: June 15 2009]

Bader, A.E., Heinza, K.M., Whartona, R.A., & Bográna, C.E. (2006). Assessment of interspecific interactions among parasitoids on the outcome of inoculative biological control of leafminers attacking chrysanthemum. *Biological Control, 39*, 441-452.

Bai, X., Adam, B.J., Ciche, T.A., Clifton, S., Spiet, J., Sternberg, P.W., Gaugler, R., Hogenhout, S.A., Wilson, R.K., & Grewal, P.S. (2009). Transcriptomic analysis of the entomopathogenic nematode *Heterorhabditis bacteriophora*. *BMC Genomics, 10*, 205-210.

Bead, J. & Walter, D.E. (1996). Australian mites of the genera *Paraphytoseius Swirski* and *Shechter* and *Paraamblyseius Muma* (Acarina: Phytoseiidae) *Australian Journal of Entomology, 35*, 235–241.

Be'langer, R.R. (2001). Biological control in greenhouse systems. *Annual Review of Phytopathology, 39*, 103-133.

Bellamy, D. E., Asplen, M. K., & Byrne, D. N. (2004). Impact of *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) on open-field *Bemisia tabaci* (Hemiptera: Aleyrodidae) populations. *Biological Control, 29*, 227-234.

Bertin, N., Guichard, S., Leonardi, C., Longuenesse, J.J., Langlois, D., & Naves, B. (2000). Seasonal evolution of the quality of fresh glasshouse tomatoes under

Mediterranean conditions, as affected by air vapour pressure deficit and plant fruit load. *Annals of Botany, 85*, 741-750.

Bogra, C.E., Heinz, K.M., & Ciomperlik, M.A. (2002). Interspecific competition among insect parasitoids: Field Experiments with whiteflies as hosts in cotton. *Ecology, 83*, 653–668.

Braun, A.R., Mesa, N.C., Cuellar, M.E., Melo, E.L. & Moraes, G.J. (1993). Biosystematics of *phytoseiid* mites (Acari: Phytoseiidae) associated with cassava. *Experimental and Applied Acarology, 17*, 205-213.

Cahill, M., Gorman, K., Day, S., Denholm, I., Elbert, A., & Nauen, R. (1996). Baseline determination and detection of resistance to imidacloprid in *Bemisia tabaci* (Homoptera: Aleyrodidae). *Bulletin of Entomomological Research, 86*, 343–349.

Caltagirone, L.E., & Doutt, R.L. (1989). The history of the vedalia beetle importation to California and its impact on the development of biological Control. *Annual Review of Entomology, 34*, 1-16.

Canas, L., Ling, P., & Frantz, J. (2007). Effect of CO_2 enrichment, light, and nutrients on plant-herbivore performance and impact on pest management practices. *Ohio State Integrated Pest Management Grants Program Reports,* 66-71.

Cameron, P.J, Walker, G.P., Hodson, A.J., Kale, A.J., & Herman, T.J.B. (2009). Trends in IPM and insecticide use in processing tomatoes in New Zealand. *Crop Protection 28,* 421-427.

Cameron, P.J. (1996). Green peach aphid resistance management strategy. In G.W. Bourdot & D.M. Suckling (Eds.), *Pesticide Resistance: Prevention and Management* (pp. 207-209). Lincoln, New Zealand: New Zealand Plant Protection Society.

Castañé, C., Alomara, O., Goulab, M. & Gabarraa, R. (2004). Colonization of tomato greenhouses by the predatory mirid bugs *Macrolophus caliginosus* and *Dicyphus tamaninii. Biological Control, 30,* 591-597.

Cloyd, R. A. (1999). Know your friends: *Delphastus pusillus*: whitefly predator. *Midwest Biological Control News*, *October,* 3.

Collier, T.R., Hunter, M.S. (2001). Lethal interference competition in the whitefly parasitoids *Eretmocerus eremicus* and *Encarsia sophia*. *Oecologia, 129,* 1432-1939.

Conlong, D. E., & Mugoya, C.F. (1996). Rearing beneficial insects for biological control purposes in resource poor areas of Africa. *Entomophaga, 41*, 505-512.

Costello, R. A., Elliot, D., Gilkenson, D., Gillespie, D. (1992). Integrated control of greenhouse pests. *Ministry of Agriculture, Fisheries and Food Report.*

Cranshaw, W., Sclar, D. C., & Cooper, D. (1996). A review of 1994 pricing and marketing by suppliers of organisms for biological control of arthropods in the United States. *Biological Control, 6,* 291–296.

Cronin, J.T., Strong, D.R., (1999). Dispersal-dependent oviposition and the aggregation of parasitism. *American Naturalist, 154*, 23-36.

De Bach, P., & Rosen, D. (1991). *Biological Control by Natural Enemies*. Cambridge University, England.

De Bach, P. (1964). Biological Control of Insect Pests and Weeds. In J.C. van Lenteren (Ed.), *Quality Control and Production of Biological Control Agents: Theory and Testing Procedures* (pp. 327-337). Wallingford, UK: CABI Publishing.

De Barrow, P. J., De Driver, F., Naumann, I. D., Clarke, G. M., Schmidt, S., & Curran, J. (2000). Descriptions of three species of *Eretmocerus* Haldeman

(Hymenoptera: Aphelinidae) parasitising *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) in Australia based on morphological and molecular data. *Australian Journal of Entomology, 39*, 259–269.

Donnell, D.M., & Hunter, M. (2002). Developmental rates of two congeneric parasitoids, *Encarsia formosa* and *E. pergandiella* (Hymenoptera: Aphelinidae), utilising different egg provisioning stratergies. *Journal of Insect Physiology, 48*, 487-493.

Elhag, E.A., & Horn, D.J. (1983). Resistance of greenhouse whitefly (Homoptera: Aleyrodidae) to insecticides in selected Ohio greenhouses. *Journal of Economic Entomology, 76*, 945-948.

Ellers, J., van Alphe, J.J.M & Sevenster, J.G. (1998). A field study of size-fitness relationships in the parasitoid *Asobara tabida*. *Journal of Animal Ecology, 67*, 318-324.

Ekesi, S., Shah, P.A., Clark, S.J., & Pell, J.K. (2005). Conservation biological control with the fungal pathogen *Pandora neoaphidis*: implications of aphid species, host plant and predator foraging. *Agriculture and Forest Entomology, 7*, 21-30.

Eyles, A.C., Marais, T. & George, S. (2008). First New Zealand record of the genus *Macrolophus* Fieber, 1858 (Hemiptera: Miridae: Bryocorinae: Dicyphini): *Macrolophus pygmaeus* (Rambur, 1839), a beneficial predacious insect. *Zootaxa, 1779,* 33-37.

Feaster, M, A., & Steinkraus, D.C. (1996). Inundative biological control of *Helicoverpa zea* (Lepidoptera: Noctuidae) with the entomopathogenic nematode *Steinernema riobravis (Rhabditida: Steinernematidae)*. *Biological Control, 7*, 38-43.

Ferguson, C. M., Moeed, A., Barratt, B. & Kean, J. M. (2007). *BCANZ—Biological Control Agents introduced to New Zealand*. [Online] Available www.b3nz.org/bcanz/index.php [Retrieved: September 20, 2009].

Fernández-arhex, V., & Corley, J.C. (2003). The functional response of parasitoids and its implications for biological control. *Biocontrol Science and Technology, 13*, 403-413.

Foglar, H., Malausa, J.C., Wajnberg, E., & Holling, C.S. (1990). The functional response and preference of *Macrolophus caliginosus (Heteroptera: Miridae*) for two of its prey *Myzus persicae* and *Tetranychus urticae*. *Entomophaga, 35*, 465–474.

Fonseca, C.R., Prado, P.I., Almeida-Neto, M., Kubota, U., & Lewinsohn, T.M., (2005). Flower-heads, herbivores, and their parasitoids: food web structure along a fertility gradient. *Ecological Entomology, 30,* 36–46.

Fransen, J., & van Lenteren, J. (1993). Host selection and survival of the parasitoid *Encarsia formosa* on greenhouse whitefly, *Trialeurodes vaporariorum*, in the presence of hosts infected with the fungus *Aschersonia aleyrodis*. *Entomologia Experimetalis et Applicata, 69*, 239-249.

Getz, W. M., & Mills, N. J. (1996). Host-parasitoid coexistence and egg-limited encounter rates. *American Naturalist, 148,* 301-315.

Gerling, D. (1965). Studies with whitefly parasites of Southern California II. *Eretmocerus californicus* Howard (Hymenoptera: Aphelinidae). *Canadian Entomologist, 98,* 1316-1329.

Gill, S. (2000). Pest control: whitefly control for cut flower growers. *The Cut Flower Quarterly, 12*, 26-30.

Gilkeson, L. A. (1992). A pest-by-pest IPM primer. *American Vegetable Grower, 46,* 48-52.

Godfray, H.C.J, & Shimada, M., (1999). Parasitoids as model organisms for ecologists. *Research on Population Ecology, 41*, 3-10.

Godfray, H.C.J. (1994). *Parasitoids: Behavioural and Evolutionary Biology*. Princeton, New Jersey: Princeton University Press.

Goolsby, J.A., De Barrow, P.J., Kirk, A.A., Sutherst, R.W., Canas, L., Ciomperlik, M.A., Ellsworth, Gould, J.R., Hartley, D.M., Hoelmer, K.A., Naranjo, S.E., Rose, M., & Roltsch, W.J.M., (2005). Post-release evaluation of biological control of *Bemisia tabaci* biotype "B" in the USA and the development of predictive tools to guide introductions for other countries. *Biological Control, 32,* 70-77.

Gorman, K., Devine, G.J., & Denholm, I. (2000). Status of pesticide resistance in UK populations of glasshouse whitefly, *Trialeurodes vaporariorum*, and the two-spotted spider mite, *Tetranychus urticae*. *The BCPC Conference: Pests and Diseases, 1,* 459-464.

Greathead, D. J. & Greathead, A. (1992). Biological control of insect pests by insect parasitoids and predators: the BIOCAT database. *Biocontrol News and Information, 13*, 61-68.

Greenberg, S. M., Jones, W. A., & Liu C, T.-X. (2002). Interactions Among Two Species of Eretmocerus (Hymenoptera: Aphelinidae), Two Species of Whiteflies (Homoptera: Aleyrodidae), and Tomato. *Environmental Entomology, 31*, 397-402.

Grud, N. (2005). Impact of environmental factors in production quality of greenhouse vegetables for fresh consumption. *Critical Reviews in Plant Science*, *24*, 227-247.

Guifeng, L. (1993). The occurrence and development of the adventitious roots forming green shoot cutting of *Larix olgensis*. *Journal of Forestry Research, 4*, 64-69.

Gullan, P.J., & Cranston, P.S. (2005). *The Insects: An Outline of Entomology*. Australia: Blackwell Publishing.

Habeck, D.H., Bennett, F.D., & Frank, J.H. (1990). Classical biological control in the Southern United States. *Southern Cooperative Series Bulletin, 355*, 197-210.

Hall, D. G, & Jenkins, A. (2009). Abundance of Asian citrus psyllid on yellow sticky traps in Florida, Puerto Rico, and Texas citrus groves. *Southwestern Entomologist, 34*, 1-11.

Hanan, A., He, X.Z., Shakeel, M. & Wang, Q. (2009). Diurnal rhythms of emergence, host feeding and oviposition of *Eretmocerus warrae* (*Hymenoptera: Aphelinidae). New Zealand Plant Protection, 62,* 156-160.

Hawkins, B.A., Shaw, M.R. & Askew, R.R. (1992). Relations among assemblage size, host speciatization and climatic variability in North American parasitoid communities. *American Naturalist, 139,* 5-79.

Headrick, D. H., Bellows, T. S., & Perring, T. M. (1995). Behaviours of *Eretmocerus* sp. nr. *californicus* (Hymenoptera: Aphelinidae) attacking *Bemisia argentifolii* (Homoptera: Aleyrodidae) on sweet potato. *Environmental Entomology, 24,* 412-422.

Heinz, K.M. (1998). Dispersal and dispersion of aphids and selected natural enemies in spatially subdivided greenhouse environments. *Environmental Entomology, 27*, 1029-1038.

Heinz, K.M., & Nelson, J.M. (1996). Interspecific interactions among natural enemies of *Bemisiain*: an innundative biological control program. *Biological Control, 6,* 384-393.

Heinz, K., & Parrella, M. (1994). Poinsettia (*Euphorbia pulcherrima*) cultivar mediated differences in performance of five natural enemies of *Bemisia argentifolii* (Homoptera: Ayeyrodidae). *Biological Control, 4,* 305-318.

Hoddle, M.S., & van Driesche, R.G. (1999). Evaluation of *Eretmocerus eremicus* and *Encarsia formosa* (Hymenoptera: Aphelinidae) Beltsville strain in commercial greenhouses for biological control of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on coloured poinsettia plants. *Florida Entomologist, 82*, 556-569.

Hoddle, M.S., van Driesche, R.G., & Sanderson J.P. (1998) Biology and use of the Whitefly parasitoid *Encarsia formosa*. *Annual Review of Enomology, 43*, 645-669.

Hoddle, M.S. (1997). The strength of biological control in the battle against invasive pests: a reply. *Conservation Biology, 18*, 61-64.

Hoelmer, K.A., Schuster, D.J., & Ciomperlik, M.A. (2008). Indigenous parasitoids of Bemisia in the United States and potential for non-target impacts of exotic parasitoid introductions. In J. Gould, K. Hoelmer & J. Goolsby (Eds.), *Classical Biological Control of Bemisia tabaci in the United States: a Review of Interagency Research and Implementation, 4 (*pp. 307-324). The Netherlands: Springer, Dordrecht.

Hoelmer, K.A. (2007). Field cage evaluation of introduced Eretmocerus species (Hymenoptera: Aphelinidae) against *Bemisia tabaci* strain B (Homoptera: Aleyrodidae) on Cantaloupe. *Biological Control, 43,* 156-162.

Houter & Nederhoff. (2007). Plant management for generative or vegetative steering. *Grower, 62* (5), 42-44.

Houter & Nederhoff. (2006). Using greenhouse temperature for plant control. *Grower, 61* (10), 49-52.

Hua, J.S., Gelmana, D.B., & Blackburn, M.B. (2003). Age-specific interaction between the parasitoid, *Encarsia formosa* and its host, the silverleaf whitefly, *Bemisia tabaci* (Strain B). *Journal of Insect Science, 28*, 1-10.

Johnson, M.W., & Wilson, L.T. (1995). Integrated pest management: contribution of biological control to its implementation. *Biological Control in the Western United States*. Oakland Publishers. University of California.

Jones, D.B., Giles, K.L., Berberet, R.C., Royer, T.A., Elliot, N.C., & Payton, M.E. (2003). Functional responses of an introduced parasitoid and an indigenous parasitoid on greenbug at four temperatures. *Environmental Entomology, 32*, 425-432.

Kassis, G., & Michelakis, S. (1993). The effectiveness of biological control of greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Homoptera, Aleyrodidae) by *Encarsia formosa* (Hymenoptera: Aphelinidae). *Journal of Applied Entomology, 116*, 298-302.

Keller, S., Kessler, P., & Schweizer, C. (2003). Distribution of insect pathogenic soil fungi in Switzerland. *BioControl, 48*, 307-319.

Kent, A. D., & Triplett, E. W. (2002). Microbial communities and their interactions in soil and rhizosphere ecosystems. *Annual Review of Microbiology, 56*, 211-236.

Liang, G., & Liu, T. (2002). Repellency of a kaolin particle film, surround, and a mineral oil, sunspray oil, to silverleaf whitefly (Homoptera: Aleyrodidae) on melon in the laboratory. *Journal of Economic Entomology, 95*, 317-324.

Liu, D., Poinar, G.O., & Berry, R.E. (2000). Control of insect pests with entomopathogenic nematodes: the impact of molecular biology and phylogenetic reconstruction. *Annual Review of Entomology, 45*, 287-306.

Liu, D., & Trumble, J.T. (2005). Interactions of plant resistance and insecticides on the development and survival of *Bactericerca cockerelli* [Sulc] (Homoptera: Psyllidae). *Crop Protection, 24*, 111–117.

Lindquist, R. (1991). A guide to aphid control. *GrowerTalks, October*, 75.

Liu, T-X., & Stansly, P. A. (1996). Oviposition, development and survivorship of *Encarsia pergandiella* adults (Hymenoptera: Aphelinidae) in four instars of *Bemisia argentifolii* (Homoptera: Aleyrodidae). *Entomological Society of America, 89*, 96–102.

Madden, J. L., & Mensah, R. K. (1990) Integrated control of pests of boronia crops. In J.L. Madden & R.K. Mensah (Eds.), *The Biology, Ecology and Intergrated Pest Management of Ctenarytaina thysanura in Tasmania.* Hobart, Tasmania: University of Tasmania, Deptartment of Agricultural Science.

MAF Spreadsheet: Potato/Tomato Psyllid *Bactericera cockerelli*. [Online] Available:http://www.biosecurity.govt.nz/pests/potato-tomato-psyllid [Retrieved: December 20, 2009]

McHugh, J. (1996). Monitoring - the first line of defence. *Greenhouse Grower, February,* 66.

McMahon, R.W., & Lindquist, R.R. (1994). Comparison of commercially and locally produced *Encarsia formosa* Gahan on the control of sweetpotato whitefly on poinsettias. *HortTechnology, 4*, 295-298.

McMahon, R.W., & Lindquist, R.R. (1992). Comparison of biological and chemical control of greenhouse whitefly on poinsettia stock plants. *HortTechnology, 2*, 457-460.

Malais, M.H., & Ravensberg, W.J. (2003). *Knowing and Recognizing: the Biology of Glasshouse Pests and Their Natural Enemies*. The Netherlands: Reed Business Information.

Mansour, F. A. (1993). Natural enemies and seasonal abundance of the blackmargined aphid (*Monellia caryella*) in pecan orchards in Israel. *Journal of Phytoparasitica, 21*, 329-332.

Matteoni, J. (1993). Chemical effects on greenhouse biological control agents. *Grower Talks, August,* 81-85.

Marotto, E.D. (2007). Impacts of *Encarsia pergandiella* on Whitefly Biological Control in Glasshouses. A M.Sc. thesis. California Polytechnic State University.

Martin, N. A. (2005). Whitefly insecticide resistance management and prevention strategy. In N.A Martin, R.M. Beresford, & K.C Harrington (Eds.), *Pesticide Resistance: Prevention and Management Strategies* (pp. 90-102). Hastings, New Zealand: New Zealand Plant Protection Society.

Martin, N.A. (1999). Natural enemies of whitefly and their biology. *Crop & Food Research, Broadsheet No. 92.*

Martin, N.A. (1996). Whitefly resistance management strategy. In G.W. Bourdot & D.M. Suckling (Eds.), *Pesticide Resistance: Prevention and Management*. (pp. 194-203). Lincoln, New Zealand: New Zealand Plant Protection Society.

Martin, N.A. (1989). Greenhouse Tomatoes - A survey of pest and disease control. *DSIR Plant Protection Bulletin, No. 1,* 42.

Mehrnejad, M. R., Jalali, M. A. (2004). Life history parameters of the coccinellid beetle, *Oenopia conglobata contaminata*, an important predator of the common pistachio psylla, *Agonoscena pistaciae (Hemiptera: Psylloidea)*. *Biocontrol Science and Technology, 14,* 701-711.

Memmot, J., Godfray, H.C.J., & Gauld, I.D. (1994). The structure of a tropical host-parasitoid community. *Journal of Animal Ecology, 63*, 521-540.

Messelink, G.J, Steenpaal, S.E.F., & Ramakers, P.M. (2006). Evaluation of *phytoseiid* predators for control of western flower thrips on greenhouse cucumber. *BioControl, 51*, 753-768.

Michelakis, S. (1995). The integrated control of the whitefly *Trialeurodes vaporarium* Westwood (Homoptera Aleyrodidae) and the quality of the vegetable crops. *Acta Horticulture, 379*, 587-591.

Minkenberg, O., & Rick Santangelo, R. (1997). Mass rearing of exotic *Eretmocerus*. University of Arizona, Department of Entomology. [Online] Available:http://ag.arizona.edu/crops/cotton/azbwg/azbwgpa2.html [Retrieved: January 05 2009]

Mohammad, J. A., Peter, W. J., Antoon, J. M., Loomans J. C., & van Lenteren. (2004). Inter- and intraspecific effects of volatile and nonvolatile sex pheromones on males mating ebhavior and hybridization in *Eretmocerus mundus* and *E. eremicus* (Hymenoptera: Aphelinidae). *Journal of Insect Behavior, 17*, 745-759.

Munyaneza, J.E., Crosslin, J. M., & Upton, J. (2007). Association of *Bactericera cockerelli* (Homoptera: Psyllidae) with "Zebra Chip," a new potato disease in Southwestern United States and Mexico. *Journal of Economic Entomology, 100*, 656-663.

Nederhoff, E., & Houter, B. (2006). Cost and benefit of an energy screen in a glasshouse in New Zealand. *Grower, 61* (1), 38-40.

Nederhoff, E. (2001). Biological control of root diseases especially with *Trichoderma*. *Grower, 56* (5), 24-25.

New, T.P. (2002). Prospects for extending the use of Australian lacewings in biological control. *Acta Zoologica Academiae Scientiarum Hungaricae, 48*, 209-216.

Nomikou, M., Sabelis, M.W., & Janssen, A. (2009). Pollen subsidies promote whitefly control through the numerical response of predatory mites. *BioControl, 55*, 253-260.

Perry, E. J. (2008). UC IPM Pest Management Guidelines. UC ANR Publication3437.[Online]Available:http://www.ipm.uiuc.edu/greenhouse/insects/greenhouse_whitefly/ [Retrieved July 2009]

Phatak, S. C. (1992). An integrated sustainable vegetable production system**.** *Horticulture Scientist, 27,* 738-741.

Philip, M.M., & Orr, D.B. (2008). Operational considerations for augmentation of *Trichogramma exiguum (Hymenoptera: Trichogrammatidae)* for suppression of *Rhyacionia frustrana (Lepidoptera: Tortricidae)* in *Pinus taeda* plantations. *Journal of Economic Entomology, 101*, 421-429.

Phillips, V. (2009). Vigilance needed as search for psyllid remedy continues. The Nelson Mail, 11/12/2009. [Online] Available: http://www.stuff.co.nz/nelson-mail/features/home-and-garden/3152435/Vigilance-needed-as-search-for-psyllid-remedy-continues [Retrieved: December 20, 2009].

Piatkowski, J. (2008). Integrated thrips control on glasshouse cucumbers. *EPPO Bulletin, 22*, 489-492.

Powell, D. A., & Bellows, T. S. (1992) Development and reproduction of two populations of *Eretmocerus* species (Hymenoptera: Aphelinidae) on *Bemisia tabaci* (Hymenoptera: Aphelinidae). *Environmental Entomology, 21*, 651-658.

Prakesh H. K., & Lumsden, R. D. (1998). Biopesticides: Use and Delivery. *Methods in Biotechnology, 5*, 103-116.

Qiu, B.L., De Barro, P.J., Xu, C-X., & Ren, S-X. (2006). Effect of temperature on the life history of *Encarsia bimaculata* (Hymenoptera: Aphelinidae), a

parasitoid of *Bermisia tabaci* (Hemiptera: Aleyrodidae). *European Journal of Entomology, 103*, 787-792.

Qiu, B.L, De Barro, P.J., & Ren, S.X. (2005). Development, survivorship and reproduction of *Eretmocerus sp.* nr furuhashii (Hymenoptera: Aphelinidae) parasitizing Bemesia tabaci (Hemiptera: Aleyrodidae) on glabrous and non-glabrous host plants. *Bulletin of Entomological Research, 95*, 313-319.

Rabasse, J., & van Steenis, M.J. (2002). *Integrated Pest and Disease Management in Greenhouse Crops*. Netherlands: Springer.

Riley, D., McPherson, R., & Wells, L. (2010). Thrips vectors of TSWV. [Online] Available:http://www.tomatospottedwiltinfo.org/ [Retrieved: November 5, 2010].

Rivero-lynch, A.P., & Godfray, H.C.J. (1997). The dynamics of egg production, oviposition and resorption in a parasitoid wasp. *Functional Ecology, 11*, 184-188.

Rose, M., & Zolnerowich., G. (1997). *Eretmocerus haldeman* (Hymenoptera: Aphelinidae) in the U.S., with descriptions of new species attacking *Bemisia* (*tabaci* complex) (Homoptera: Aleyrodidae). *Proceedings of the Entomological Society of Washington, 99*, 1-27.

Rumei, X., huo, C., & van Lenteren, J.C. (1993). The parasite-host relationship between *Encarsia formosa* Gahan (Hum., Aphelinidae) and *Trialeurodes vaporariorum* (Westwood) (Homoptera, Aleyrodidae). *Journal of Applied Entomolgy, 116*, 199-211.

Russell, L. M. (1977). Hosts and distribution of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Homoptera: Aleyrodidae). *USDA Cooperative Plant Pest Report, 2,* 449-458.

Sahayaraj, K., & Namasivayam, K.R. (2008). Mass production of entomopathogenic fungi using agricultural products and by product. *African Journal of Biotechnology*, *7*, 1907-1910.

Saunders, C., Cagatay, S., & Kaye-Blake, W. (2003). Economic Impacts on New Zealand of GM Crops. *Lincoln University Research Report*, *261*.

Schellhorn, N. A., Kuhman, T.R., Olson, A.C., & Ives, A.R. (2002). Competition between native and introduced parasitoids of aphids: Nontarget effects and biological control. *Ecology, 83*, 2745-2757.

Schmidt, S., & Polaszek, A. (2007). *Encarsia* or *Encarsiella*? – redefining generic limits based on morphological and molecular evidence (Hymenoptera, Aphelinidae). *Systematic Entomology, 32*, 81–94.

Schneller, H., & Sautter, H. (1999). Development of biological control in ornamentals. *WPRS bulletin*, *16*, 1-5.

Shah, P.A., & Pell, J.K. (2003). Entomopathogenic fungi as biological control agents. *Applied Microbiology and Biotechnology, 61*, 413-23.

Shishehbor, P., & Brennan, P.A. (1996). Parasitism of the castor whitefly, *Trialeurodesricini ricini* (Homoptera: Aleyrodidae) by *Encarsia formosa* (Hymenoptera: Aphelinidae): bionomics in relation to temperature. *Bulletin of Entomological Research, 86*, 67-72.

Solomon, M. E. (1949). The natural control of animal populations. *Journal of Animal Ecology, 18*, 1-45.

Stanhill, G. (1980). The energy cost of protected cropping: A comparison of six systems of tomato production. *Journal of Agricultural Engineering Research, 25*, 145-154.

Steven, E., & Naranjo, S. E. (2001). *Conservation and Evaluation of Natural Enemies in IPM Systems for Bemisia tabaci*. US Department of Agriculture: Elsevier Science Ltd.

Stephens, M. J., France, C. M., Wratten, S. D., & Frampton, C. (1998). Enhancing biological control of leafrollers (Lepidoptera: Tortricidae) by sowing buckwheat (*Fagopyrum esculentum*) in an Orchard. *Biocontrol Science and Technology, 8*, 47-558.

Snapp, S., Swinton, S.M., Labarta, R., Mutch, D., Black, J.R., Leep, R., Nyiraneza, J., & O'Neil, K. (2005). Evaluating cover crops for benefits, costs and performance within cropping system niches. *Agronomy Journal, 97*, 322-332.

Smith, P.E. (2009). Whitefly: identification and biology in New Zealand greenhouse tomato crops. *Ministry of Agriculture and Forestry, Factsheet 1*.

Succop, B. (1997). The Parasitism of Greenhouse Whitefly, *Trialeurodes vaporariorum*, by the Parasitic Wasp, *Encarsia formosa*: *A Biological Control in Greenhouses.* Insect Behaviour Review Articles. [Online] Available: http://www.colostate.edu/Depts/Entomology/courses/en507/papers_1997/succop.html [Retrieved: August 20 2009]

Sunderland, K., Chambers, R.J., Helyer, N.L., & Sopp, P.I. (1992). Integrated pest management of greenhouse crops in Northern Europe: Aphids. In J. Janick (Ed.), *Horticultural Reviews, Vol. 13*. (pp. 23-30). New York, NY: John Wiley and Sons, Inc.

Tauber, M.J., Tauber, C.A., Daane, K.M., & Hagen, K.S. (2000). Commercialization of predators: recent lessons from green lacewings (Neuroptera: Chrysopidae: Chrysoperla). *American Entomologist, 46*, 26-38.

Tillman, G., Schomberg, H., Phatak, K.S., Mullinix, B., Lachnicht, S., Timper, P., & Olson, D. (2004). Influence of cover crops on insect pests and predators in conservation tillage cotton. *Journal of Economic Entomology, 97*, 1217-1232.

Teulon, D.A.J., Workman, P.J., Thomas, K.L., & Nielsen, M.C. (2009). *Bactericera Cockerelli*: Incursion, dispersal and current distribution on vegetable crops in New Zealand. *New Zealand Plant Protection, 62*, 136-144.

Teulon, D.A.J., & Nielsen, M.C. (2005). Distribution of Western (glasshouse strain) and intonsa flower thrips in New Zealand. *New Zealand Plant Protection, 58*, 208-212.

Tripp, K., & Peet, M. (1993). New use for CO_2: Slowing whiteflies. American *Vegetable Grower, November,* 43-44.

Tumlinson, J. H., Lewis, W. J. & Vet, L. E. M. (1993). How parasitic wasps find their hosts. *Scientific American, 268*, 100-106.

van Lenteren, J.C. (2005) IOBC Internet Book of Biological Control. Version 1.

van Lenteren, J.C. (2003). *Quality Control of Production of Biological Control Agents: Theory and Testing Procedures*. Wallingford, UK: CABI Publishing.

van Lenteren, J.C., & Bueno, V.H.P. (2003). Augmentative biological control of arthropods in Latin America. *BioControl, 48*, 123-139.

van Lenteren, J.C. (2000). A greenhouse without pesticides: factor or fantasy? *Crop Protection, 19*, 375-384.

van Lenteren, J.C., & Manzaroli, G. (1999). Evaluation and use of predators and parasitoids for Biological Control of pests in greenhouses. In J.C. van Lenteren (Ed.), *Integrated Pest and Disease Management in Greenhouse Crops.* (pp. 183-201). Dordrecht: Kluwer Publishers.

van Lenteren, J.C., & Martin, A.A. (1999). Biological control of whiteflies. In J.C. van Lenteren (Ed.), *Integrated Pest and Disease Management in Greenhouse Crops*. (pp. 202-216). Dordrecht: Kluwer Publishers.

van Lenteren, J.C., Drost, Y.C., van Roermund, H.J.W., & Posthuma-Doodeman, C.J.A.M. (1997). Aphelinid parasitoids as sustainable biological control agents in greenhouses. *Journal of Applied Entomology, 121,* 473-485.

van Alphen, J.J.M., & Jervis, M.A., (1996). Foraging behavior. In M.J.N. Kidd (Ed.), *Insect Natural Enemies: Practical Approaches to Their Study and Evaluation* (pp. 1–62). New York: Chapman & Hall.

van Lenteren, J. C., & Noldus, L. P. (1990). Whitefly-plant relationships behavioral and ecological aspects. In D. Gerling, *Whiteflies: Their Bionomics, Pest Status and Management* (pp 47-89). Hants, UK: Intercept Andover.

van Lenteren, J.C., & Woets, J. (1988). Biological and integrated pest control in greenhouses. *Annual Review of Entomololgy, 33*, 239-269.

Van Roermund H.J.W., & van Lenteren J.C. (1995). Foraging behavior of the whitefly parasitoid *Encarsia formosa* on tomato leaflets. *Entomologia Experimentalis et Applicata, 76*, 13-324.

Vet, L.E.M., Lewis, W.J., & Cardé, R.T. (1995). Parasitoid foraging and learning. In R.T. Cardé & W.J. Bell (Eds.), *Chemical Ecology of Insects* (pp. 65–101). New York: Chapman & Hall.

Vet, L.E.M., van Lenteren, J.C., & Woets, J. (1980). The parasite-host relationship between *Encarsia formosa* and *Trialeurodes vaporariorum*. A review of the biological control of the greenhouse whitefly with suggestions for future research. *Zeitschrift für Angewandte Entomologie, 90*, 26-51.

Viggiani, G. (1984). Bionomics of the Aphelinidae. *Annual Review of Entomology, 29*, 257-276.

Wardlow, L. R. (1998). IPM in ornamentals: A guide to biocontrol. *GrowerTalks, September*, 78-82.

Wiedenmann, R. N., & Smith, J. W. (1997), Attributes of the natural enemies in ephemeral crop habitats. *Biological Control, 10*, 16-22.

Workman, P.J., & Whiteman, S.A. (2009) Importing *Tamarixia triozae* into containment in New Zealand. *New Zealand Plant Protection*, *62*, 136-144.

Workman, P., & Davidson, M. (2007). Potential biological control agents for greenhouse pests in New Zealand. *Crop & Food Confidential Report, No. 1920.* Christchurch, New Zealand: New Zealand Institute for Crop & Food Research.

Workman, P., Scott, I., & Drayton, G. (2008). *Eretmocerus warrae*: a new whitefly parasitoid found in New Zealand. *New Zealand Plant Protection, 61*, 386-389.

Workman, P., & Pedley, R. (2007). New natural enemies for greenhouse pests. *New Zealand Grower, October*, 54.

Zabel, A., Manojlovic, B., Stankovic, S., Rajkovic, S., & Kostic, M. (2001). Control of the Whitefly *Trialeurodes vaporariorum* Westw. (Homoptera, Aleyrodidae) on tomato by the new insecticide Acetamiprid. *Journal of Pest Science, 74*, 52-56.

Zar, J.H. (1999). *Biostatistical Analysis*. New Jersey: Prentice Hall.

Zchori-Fein, E., Roush, R.T., & Sanderson, J.P. (1994). Potential for integration of biological and chemical control of greenhouse whitefly (Homoptera: Aleyrodidae) using *Encarsia formosa* (Hymenoptera: Aphelinidae) and Abamectin. *Biological Control, 23*, 1277-1282.

Zolnerowich, G., & Rose, M. (2008). The genus *Eretmocerus*. In J. Gould, K. Hoelmer & J. Goolsby (Eds.), *Classical Biological Control of Bemisia tabaci in the United States - a Review of Interagency Research and Implementation* (pp. 89-109). The Netherlands: Springer.

Zolnerowich, G., & Rose, M. (2004). *Eretmocerus rui* n. sp. (Hymenoptera: Chalcidoidea: Aphelinidae), an exotic natural enemy of *Bemisia* (*tabaci* group) (Homoptera: Aleyrodidae) released in Florida. *Florida Entomologist, 87*, 283.