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Life Histories and Host Interaction Dynamics of Parasitoids Used for Biological Control of Giant Whitefly (*Aleurodicus dugesii*) Cockerell (Hemiptera: Aleyrodidae)

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by

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Dedication

To all the people in my life who are no longer here to see me achieve my dreams.

ABSTRACT OF THE DISSERTATION

Life Histories and Host Interaction Dynamics of Parasitoids Used for Biological Control of Giant Whitefly (*Aleurodicus dugesii*) Cockerell (Hemiptera: Aleyrodidae)

by

Erich Nicholas Schoeller

Doctor of Philosophy, Graduate Program in Entomology
University of California, Riverside, March 2018
Dr. Richard A. Redak, Chairperson

Whether interactions among biocontrol agents limits their ability to control shared prey has been one of the most important and controversial questions surrounding biological control. In California multiple parasitoid wasp species have been introduced to control the invasive giant whitefly *Aleurodicus dugesii* Cockerell (Hemiptera: Aleyrodidae). The overall goal of this dissertation was to determine aspects of these species' basic biologies and investigate factors contributing to the efficacy of these parasitoids. Findings of these studies will provide a better understanding of how parasitoid community complexity affects biological control and may enhance biological control of *A. dugesii* in California and elsewhere.

First the effects of temperature on survival and development of *A. dugesii* were assessed. Starting at 10°C development rate of *A. dugesii* increased until an optimum of 29°C was reached, then development ceased at 30°C. Using these data developmental degree days were calculated for *A. dugesii*.

Second, host stage preferences of the three parasitoids were determined. All host nymphal stages were accepted by *I. affinis* for oviposition, while only the 2nd–4th and 3rd–4th instars were accepted by *E. noyesi* and *E. krauteri* respectively. Host stage preferences overlapped considerably between species.

Third, the effects of *A. dugesii* nymphal wax production on *I. affinis* and *E. noyesi* efficacy was determined. Wax production decreased parasitoid effectiveness, with *I. affinis* being more negatively impacted by wax than *E. noyesi.* Wax was found to serve as an effective defense against parasitism.

Finally, the effects of climate and season on population dynamics of species in this system were assessed. Population densities of *A. dugesii* were found to not differ across climate types in southern California. Total and *E. noyesi* parasitism rates also did not differ across climate types. Parasitism rates of *I. affinis* were highest in inland climate sites. Parasitism by *E. noyesi* and *I. affinis* was high throughout the year, except during the early spring. The contribution of *E. krauteri* to *A. dugesii* biological control appears to be negligible.

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Chapter 1

Introduction

1.1 The Rational Basis for the Study of Biological Control

One of the fundamental theories in ecosystem ecology states that overall ecosystem functioning and resource utilization should increase as biodiversity increases (Schwartz et al., 2000; Naeem and Wright, 2003). A significant proportion of the interest in how consumer biodiversity affects resource utilization has been fueled by the field of classical biological control (Crowder and Jabbour, 2014; Letourneau et al., 2015). Here the introduction and permanent establishment of exotic natural enemy communities are performed with the intent of achieving permanent control of a target pest species (DeBach and Rosen, 1991). As public opinion shifts away from the use of chemical insecticides (Doutt and Smith, 1971; Paoletti and Pimentel, 2000) and insect species become more resistant to these chemicals (Sun and Sun, 1994; Nauen and Denholm, 2005; Tabashnik et al., 2014), biological control has become an increasingly pursued option for pest management. Cost benefit analyses of biological control programs have shown that these programs are highly justified (De Clercq et al., 2011), and in the United States alone the value of pest control services provided by natural enemies have been estimated to be at least $4.5 billion annually (Losey and Vaughan, 2006). The financial and ecological consequences of these programs make their preservation and advancement a high priority for a variety of stakeholders.

Despite the human influence behind classical biological control programs and the financial assets at stake, the dynamics of these systems remain ecologically driven processes. These dynamics are often complex and may involve multiple trophic levels each containing multiple species making their theoretical study a worthwhile endeavor. A great deal of the theoretical advancement of the fields of trophic and population ecology, as well as invasion biology has been derived from the study of biological control systems during the last century (Fagen et al., 2002; Pearse and Altermatt, 2013). Due to the complexity of these systems however, a great deal of research is still needed to enhance our ability to predict the outcome of biological control programs and manipulate the interactions of species involved in a meaningful way.

1.2 Coexistence in Parasitoid Communities

An intense debate surrounding whether herbivore suppression is enhanced by greater natural enemy diversity exists (Straub and Snyder, 2008; Tylianakis and Romo, 2010; Griffin et al., 2013). This debate is fueled by the fact that while herbivore suppression often correlates positively with natural enemy diversity, antagonistic interactions between natural enemies exist that may disrupt prey suppression (Rosenheim, 1995; Letourneau et al., 2009). Many studies interested in examining the effects of natural enemy diversity on herbivore control using biological control agents have focused primarily on parasitoids (insect parasites that kill their host to complete development), because multiple species of parasitoids frequently attack a single host species (Price, 1971; Hawkins, 1990; Polis, 1991; Hawkins and Mills, 1996; Polis and Strong, 1996). The relatively narrow diet breadth observed in parasitoid communities has

led to the prediction that intense resource competition should exist in these communities, and that these competitive interactions will ultimately lead to the exclusion of inferior competitors (Gause, 1934; Nicholson and Bailey, 1935; Allesina and Levine, 2011; Kramer and Drake, 2014).

Many models with varying assumptions and conclusions have been developed to examine the prediction that competitively inferior parasitoid species should be driven from a system (May and Hassell, 1981; Hogarth and Diamond, 1984; Kakehashi et al., 1984; Comins et al., 1992; Briggs, 1993; Briggs et al., 1993; Hochberg, 1996; Klopfer and Ives, 1997; Pedersen and Mills, 2004). Despite the variable conclusions of these models, most with support from empirical studies have shown that coexistence between competing parasitoid species is possible given that these parasitoids have evolved mechanisms (e.g. modes of niche separation) to diffuse the effects of competition. Some of the mechanisms shown to promote parasitoid coexistence suggested by various theoretical models are summarized in **Table 1.1**.

Table 1.1. Parasitoid coexistence mechanisms suggested by different theoretical models.

Mechamism(s) Proposed	Literature Source(s)
Differential aggregation within uniform environments.	May and Hassell, 1981; Hogarth and Diamond, 1984; Kakehashi et al., 1984
Differential dispersal rates within patchy environments.	Solé et al., 1992; Comins and Hassell, 1996
Differential partitioning of host and parasitoid habitats.	Snyder et al., 2005
Differences in survival rates/longevity.	Hogarth and Diamond, 1984; Bonsall et al., 2002
Differences in searching efficiency and fecundity.	Hochberg, 1996; Pedersen and Mills, 2004
Differences in development.	Hackett-Jones et al., 2009
Differential preferences for host stages with variable development times.	Briggs et al., 1993
Limited overlap in host stage use.	Bonsall et al., 2002; Hackett-Jones et al., 2009
Differential competitive ability under multiparasitism conditions.	Castillo and Velasco-Hernández, 2003
Differences in phenology.	Hackett-Jones et al., 2009

One of the most significant conclusions from models which suggest parasitoid coexistence is possible is that coexistence is mediated in these systems when intraspecific competitive interactions are stronger than interspecific competitive interactions (Briggs et al., 1993; Chesson, 2000; Finke and Snyder, 2008). This can be put into theoretical context when considering the basic Lotka-Volterra competition model with absolute competition coefficients (a):

$$\frac{dN_i}{dt} = r_i(1 - \alpha_{ii}N_i - \alpha_{ij}N_j),\ i = 1,2,\ j \neq i.$$

If $ajj > aii$ (i.e. intraspecific competition between species j is greater than intraspecific competition between species i); then species j cannot competitively exclude species i if the effect that species j has on itself is more than the effect that species j has on species i (Chesson, 2000). In other words if strong intraspecific competition occurs in both species i and species j then even if interspecific competition is occurs, self-limiting density-dependence will drive the overall system dynamics and lead to stable coexistence (MacArthur and Levins, 1967).

Understanding the factors facilitating parasitoid coexistence is an important question in ecology (Lawton and Hassell, 1984; Hawkins, 2000). The various coexistence mechanisms proposed by different models and the difficulties encountered when attempting to incorporate parameters which create more realistic models suggests that experimental and empirical approaches may be best at answering some of the remaining questions surrounding parasitoid coexistence (Myers et al., 1989).

1.3 Competitive Interactions Between Parasitoids

Competition between parasitoids takes two broad forms; interactions between adults searching for hosts (extrinsic) and between larvae developing on a shared host (intrinsic) (Harvey et al., 2013). Unlike predators which remove their prey from the system, individual hosts attacked by parasitoids can be encountered multiple times. Females of some solitary parasitoids occasionally inject more than a single egg into the host (superparasitism) or may lay eggs into hosts previously parasitized by another species (multiparasitism) creating the potential for intraspecific and interspecific intrinsic competition to occur respectively. The rejection or acceptance of hosts parasitized by conspecifics and heterospecifics has been referred to as intraspecific and interspecific host discrimination respectively (Salt, 1961; Mackauer, 1990).

Host discrimination is a form of extrinsic competition, and may lead to the occurrence of intrinsic competition depending on whether acceptance of a previously parasitized host occurs (van Alphen and Visser, 1990). While some parasitoid species appear to be capable of discriminating between hosts that have been previously parasitized by conspecifics and/or heterospecifics (McBrien and Mackauer, 1990; Ivens et al., 2009), others are not (Cronin and Strong, 1993). Interspecific host discrimination has been reported less frequently than intraspecific discrimination (Godfray, 1994), and the ability to recognize hosts parasitized by heterospecifics may be greater in closely related taxa (Vet et al., 1984).

The evolution of discrimination and avoidance of conspecifics is believed to be less likely than that of heterospecifics, because mechanisms to prevent superparasitism

are predicted to evolve first followed by the ability of competing parasitoid species to discriminate and avoid the marks produced by heterospecifics (Bakker et al., 1985).

In most hosts parasitized by multiple solitary endoparasitoids, lethal interference competition where one wasp larvae kills others inside the host occurs (Collier and Hunter, 2001). Research examining lethal interference competition suggests which individuals survive is dependent on the life history strategy of each species, and so the outcome should vary between competitors in a predictable manner when these traits are known (Yamamoto et al., 2007). Various mechanisms have been identified as influencing the outcome of lethal interference competition and include: physical attack using mandibles (Vinson, 1972; McBrien and Mackauer, 1990; Tian et al., 2008), physiological suppression via toxins (Strand and Vinson, 1984; Hagver, 1988), and asphyxiation (Fisher, 1963). The effects of these various processes may be further influenced by the order of oviposition, the time interval between the two oviposition events, and by the developmental rate of the two competitors (most influential in cases of multiparasitism) (Mackauer, 1990).

After successfully surviving a super- or multiparasitism event, the emerged adult has often been shown to suffer indirect fitness-related costs associated with the elimination of supernumerary larval competitors. It is predicted then that parasitoid females that are egg limited or do not gain any reproductive success by super- or multiparasitizing should avoid these scenarios if the cost of supernumerary parasitism is high enough to offset any loss in reproductive success due to host limitations (Bakker et al., 1985; Mackauer, 1990). Two of the most common negative fitness costs associated

with lethal interference competition are reduced larval survival and a reduction in the size of adult parasitoids. Adult size plays a major role in individual fitness, since in many parasitoids species it is correlated with mating success (Nicol and Mackauer, 1999; Ueno, 1999) and is often closely linked to female fecundity leading to a negative effect on per capita parasitoid fitness. Due to the potential negative and positive effects of supernumerary parasitism events on individual fitness it is important to understand the potential outcomes of these interactions when attempting to examine mechanisms of parasitoid coexistence for a given system.

In addition to superparasitism and multiparasitism, another common form of intrinsic competition is hyperparasitism. Hyperparasitic species lay their eggs within the larvae or pupae of conspecific or heterospecific parasitoid species and during this process the primary parasitoid larvae within the host is consumed. It has been argued that hyperparasitism due to the outcome of the interaction can be considered a form of intraguild predation (Briggs and Collier, 2001). Both hyperparasitism and intraguild predation is ubiquitous in nature (Sullivan, 1987; van Veen et al., 2006). Recent work by Amarasekare (2006, 2007) and Kidd and Amarasekare (2012) have applied intraguild predation theory (Rosenheim et al., 1995; Amarasekare, 2000) as a means of testing mechanisms of parasitoid coexistence under hyparasitism scenarios. Classical predation theory suggests that there should be a trade-off between intraguild predation and resource competition at intermediate levels of host resource productivity (Holt and Polis, 1997; Křivan and Diehl, 2005). This trade-off can occur between competing parasitoids if the inferior resource competitor can gain a competitive advantage by parasitizing a superior

resource competitor, thus gaining an additional source of resources. As stated however, this trade-off should only occur at intermediate levels of host productivity. At low levels the superior resource competitor is predicted to competitively displace the intraguild predator due to the effects of scramble competition, while at high host productivity the combined effects of predation and resource competition should lead to the competitive exclusion of the superior resource competitor (Mylius et al., 2001). While this trade-off may explain coexistence in parasitoid communities where super- and multiparasitism occur (i.e. many communities), the fact that host populations (productivity) fluctuate greatly both spatially and temporally suggests that other mechanisms must also exist if coexistence is going to occur between parasitoid species for any ecologically relevant length of time (Polis et al., 1989; Arim and Marquet, 2004).

In the past, a negative focus has been placed on the effects of lethal interference competition in mediating coexistence between parasitoids (Frago, 2016). For example, it has been suggested and high levels of supernumerary parasitism events can lead to failure of biological control programs due to increased larval parasitoid mortality rates and subsequent decreases in population levels (DeBach and Rosen, 1991; Xu et al. 2013). More recent theory however, suggests that lethal interference competition may actually promote parasitoid coexistence and lead to greater levels of pest suppression. The act of prior parasitism may serve as a niche partitioning mechanism, and models developed by Bonsall et al. (2002) suggest that parasitoid coexistence is possible when an inclusive niche is formed, due to differential abilities of competing parasitoids to use hosts previously parasitized by heterospecifics. Coexistence however, is only possible under

this scenario (one species cannot use hosts parasitized by heterospecifics) if the excluded parasitoid gains competitive superiority via other means such as behavioral and/or ecological trade-offs.

One of the primary areas of focus for new research examining parasitoid coexistence are the effects of habitat structure and quality (Didham et al., 1996; Tscharntke et al., 2002; László and Tóthmérész, 2013). It is clear from the literature that insect natural enemies possess varying spatial strategies, and certain habitats may affect natural enemies' ability to locate hosts or aggregate around prey sources differently (With et al., 2002).

Studies have shown that natural enemies such as parasitoids may be more sensitive to differences in spatial scale (Thies et al., 2003) and respond to differences at smaller scales than herbivores (Brewer et al., 2008). For example, strong competition within parasitoid guilds may cause niche partitioning at scales as small as within the plant canopy (Wieber et al., 1995; Harvey et al., 2014). Understanding how parasitoid interactions differ under varying habitats and the scale at which these interactions are affected is crucial developing effective biological control programs (Thies and Tscharntke, 1999).

1.4 Parasitoid Competition and Biological Control Success

Whether interspecific and intraspecific interactions among biological control agents affects community structure (May and Hassell, 1981; Luck, 1990; Rosenheim et al., 1995; Bonsall and Hassell, 1999) or limits the ability of biological control agents to control shared hosts has been one of the most debated issues surrounding classical

biological control (Turnbull and Chant, 1961; Ehler and Hall, 1982; Greathead and Greathead, 1992; Denoth et al., 2002). Competitive displacement among introduced parasitoids is not an uncommon phenomenon (Bess et al., 1961; Ehler and Hall, 1982), and displacement of native parasitoids by introduced parasitoids has also been shown to occur (Sorribas et al., 2010). Even when introduced parasitoids coexist in their native range, biotic and abiotic differences in their areas of introduction may provide a competitive advantage to one species over another (DeBach et al., 1978; LeBrun et al., 2009). Without a thorough understanding of such multi-species interactions, the approach of using multiple parasitoids to control pest species has led to many failures of biological control programs (Stilling, 1993).

Although theory predicts that ecosystem functioning should increase as biodiversity increases (Hawkins, 1993), the alternative and somewhat controversial hypothesis suggests that a few or even a single species within a system may account for the vast majority of the ecosystem services (Myers et al., 1989; Huston, 1997; Cardinale et al., 2006). This hypothesis has been a point of particular contention among biological control practitioners when debating the selection process of parasitoid species for use in biological control programs. Some researchers have argued that the identification and introduction of a single superior parasitoid species (the one capable of achieving lowest pest densities) is more practical than the release of multiple species, because there is the possibility that the superior resource competitor may be excluded or its efficacy compromised via interspecific competitive interactions (Turnbull and Chant, 1961; Watt, 1965; Turnbull, 1967; Ehler, 1982, 1985). Looking beyond the direct consequences of

these species on the target system, Hoelmer and Kirk (2005) also argue that releasing multiple species that may contain inefficient biological control agents is a waste of researchers' time and resources and may ultimately cost stakeholders money due to delaying the onset of a successful control program. In modern biological control programs the requirements that the host specificities of all candidate species be examined, due to concerns of non-target effects on native species may ultimately make it prohibitive to even consider all potential parasitoid species.

Other researchers have taken a more pragmatic stance and argue that the identification of the "best" species is impractical (van den Bosch, 1968; Huffaker et al., 1971) and that multiple species should be released given comparative levels of combined control. Supporting this view, the model developed by May and Hassel (1981) predicts lower pest equilibrium via the combined action of multiple parasitoids compared to that of a single superior parasitoid. They argue that "the reduced depression of the host equilibrium will in general be small and would not warrant the very considerable effort to rank all candidate species during preliminary screenings."

A major point of discussion regarding the release of multiple parasitoid species is whether releasing heteronomous hyperparasitoids is detrimental to biological control (Del Bene and Landi, 1991; Mills and Gutierrez, 1996; Williams, 1996; Gabarra et al., 1999; Zang et al., 2011). Heteronomous parasitoid species have a unique reproductive strategy where female eggs are deposited into a primary host (e.g. the target pest species) and male eggs are laid hyperparasitically on immatures of conspecific females or on the immatures of other parasitoid species. The majority of heteronomous species occur in the

aphelinid genera *Encarsia*, *Coccophagus*, *Coccobius*, and *Coccophagoides* (Hunter and Woolley, 2001). The primary concern with the release of heteronomous hyperparasitoids as biological control agents is that if they utilize other parasitoid species of the target pest they may ultimately reduce overall levels of pest control (Reitz and Trumble, 2002; Van Lenteren et al., 2006; Nofemela, 2013).

Supporting this concern is empirical evidence of biological control disruption by hyperparasitoids. Onillon et al. (1994) found that the presence of the facultative hyperparasitoid *Encarsia pergandiella* Howard reduced parasitism of the whitefly *Bemisia tabaci* (Gennadius) by 14% under greenhouse conditions. This finding was partially supported by Bográn et al. (2002) who found only limited differences in suppression of *Bemisia argentifolii* Bellows & Perring when exposed to variable combinations of *E. pergandiella* and the primary parasitoids *Eretmocerus mundus* and *Encarsia formosa* Gahan over single species treatments. Only under low host densities did they observe that the presence of *E. pergandiella* resulted in lower than expected levels of host suppression compared to *E. formosa* alone.

Despite the evidence provided above, successful biological involving heteronomous hyperparasitoids has also been documented on several occasions (Ehler, 1979; Thompson et al., 1987, Heinz and Nelson, 1996; Hunter et al., 2002; Zang et al., 2011). A significant body of research has been devoted to trying to explain the conditions where inclusion of a hyperparasitoid does not disrupt biological control. The model developed by Mills and Gutierrez (1996) shows that if hyperparasitoids only parasitize conspecifics then in combination with traditional parasitoids, hyperparasitoids may

achieve lower levels of pest suppression. This is possible if hyperparasitism rates are relatively low in order to prevent male-biased sex ratios that may limit the hyperparasitoid's reproductive capacity. If the hyperparasitoid species attacks both conspecifics and heterospecifics at equal rates however, disruption of pest control is likely to occur if the hyperparasitoid is an inferior resource competitor. Further work by Schreiber et al. (2001) suggests that a hyperparasitoid and primary parasitoid can coexist as biological control agents if the primary parasitoid suppresses the host to a lower equilibrium density. Disruption of biological control is prevented in this case when obligate hyperparasitoids are introduced, since the population equilibrium of the pest remains unchanged. It is clear from the literature that different predictions regarding the applicability of releasing hyperparasitoids can only be evaluated if the biology and behavior of the species in question are well understood.

Attempts to increase the success rate of parasitoids being used as biological control agents has led to a steady increase in empirical studies aiming to elucidate the form, function, and outcome of interactions between multiple parasitoids, so that these interactions can be manipulated in a predicted manner (Godfray and Shimada, 1999). Programs intended for use in agricultural settings offer a particular incentive to community ecologists to study the effects of parasitoid competition due to the economic importance of these systems and environmental impacts of pesticide use that these biological control agents aim to replace (Bográn et al., 2002). In addition to the questions still fueling the debate surrounding the merits and risks associated with the introduction of multiple parasitoid species for use in biological control programs, many unanswered

questions still remain seeking to elucidate the ways in which competitive interactions structure parasitoid communities. For example, very little is still known about the extent that parasitoids compete for hosts in nature, how these competitive interactions vary spatially and temporally, and how this variation in competitive pressure affects the population dynamics a shared host (Harvey et al., 2013). Given the varying outcomes of multiple parasitoid introductions, differing predictions regarding the importance of biodiversity, and valid arguments on both sides regarding the practice of implementing biological control programs there is still no clear answer to this debate.

1.5 The Giant Whitefly *Aleurodicus dugesii*

In order to contribute to the debate surrounding the release of multiple natural enemy species for use in biological control programs and to increase our ability to predict the outcome of these releases, I examined the dynamics of a relatively understudied biological control program against the giant whitefly *Aleurodicus dugesii* Cockerell (Hemiptera: Aleyrodidae: Aleurodicinae) in southern California. Classical biological control against *A. dugesii* has been ongoing for over 20 years in the United States, yet *A. dugesii* is still appears to be problematic in parts of its introduced range. Currently little is known about the biology and behavior of *A. dugesii* and its biological control agents, so this system offers a unique opportunity to examine the factors contributing to its control. Specifically, I will be focusing on the intra- and interspecific interactions between the parasitoid species used as biological control agents and how these interactions affect control of *A. dugesii*.

In this section I provide a historical overview of the biological control program against *A. dugesii*. I also provide an overview of what little was known about the biology of *A. dugesii* and its parasitoids prior to the start of this research.

Identification

The giant whitefly as its name suggests, is a comparatively large whitefly species measuring up to 4 mm in length. The wings and body of adult *A. dugesii* are covered in a fine white powder and the wings have a mottled grey pattern (Figure 1.1). Adult and nymphs of *A. dugesii* are highly gregarious and it is not uncommon to see over a thousand individuals on the underside of a heavily infested leaf. Due to its large size, *A. dugesii* is a relatively poor flier and will drop from the host rather than take to the wing and remains motionless when disturbed. Male and female *A. dugesii* are distinguished by the presence of claspers in males and by the presence of conspicuous wax bundles on the abdomen of females during the ovipositional phase.

Figure 1.1 The giant whitefly *Aleurodicus dugesii* Cockerell (Hemiptera: Aleyrodidae: Aleurodicinae) (right) in comparison to the smaller greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (left).

Distribution

Giant whitefly was first discovered in the United States in Texas in 1991. It has since become established in California in 1992 (Gill, 1992), Arizona, Florida and Louisiana in 1996 (Nguyen and Hamon, 2002; Hodges, 2004), and Hawaii in 2002 (Hue et al., 2004). The native range of *A. dugesii* is likely Central and Southern Mexico where it was originally collected (Cockerell, 1896; Sampson and Drews, 1941). It has also been found in Belize, Costa Rica, El Salvador, Guatemala, Nicaragua, Pakistan, and Venezuela (Lasalle et al., 1997; Evans, 2008). In 2007 *A. dugesii* was collected for the first time in Asia from Java, Indonesia and like the spiraling whitefly *Aleurodicus disperses* (Russell) it is expected to thrive (Muniappan et al., 2009).

In California *A. dugesii* was first discovered near Balboa Park in San Diego in 1992. Since its discovery, its range has slowly been expanding northward along the coastline and into adjacent counties (Figure 1.2). In 2004 *A. dugesii* was discovered for the first time in northern California in Sacramento County and has since spread to adjacent counties. This large gap in its introduced range is likely due to the transport of infested plant material. As of 2008 *A. dugesii* has been confirmed in 16 counties in California and has likely become established elsewhere.

Hosts

Giant whitefly is a highly polyphagous species with a host range encompassing at least 77 genera in 47 plant families (**Table 1.1**) (Zolnerowich and Rose, 1996; Bellows and Meisenbacher, 2000; Bellows et al., 2002; Hodges, 2004; Heu et al., 2004; Evans, 2008). The preferred hosts of *A. dugesii* appear to be woody dicotyledonous plants, and in California *A. dugesii* can be frequently be found attacking *Hibiscus* sp., *Strelitzia* sp.,

Bauhinia variegata (L.), and *Aralia* sp. (Bellows and Meisenbacher, 2000). I have observed that less preferred host species adjacent to preferred hosts appear to be only colonized after the suitable leaves on the preferred host have been completely colonized by adults.

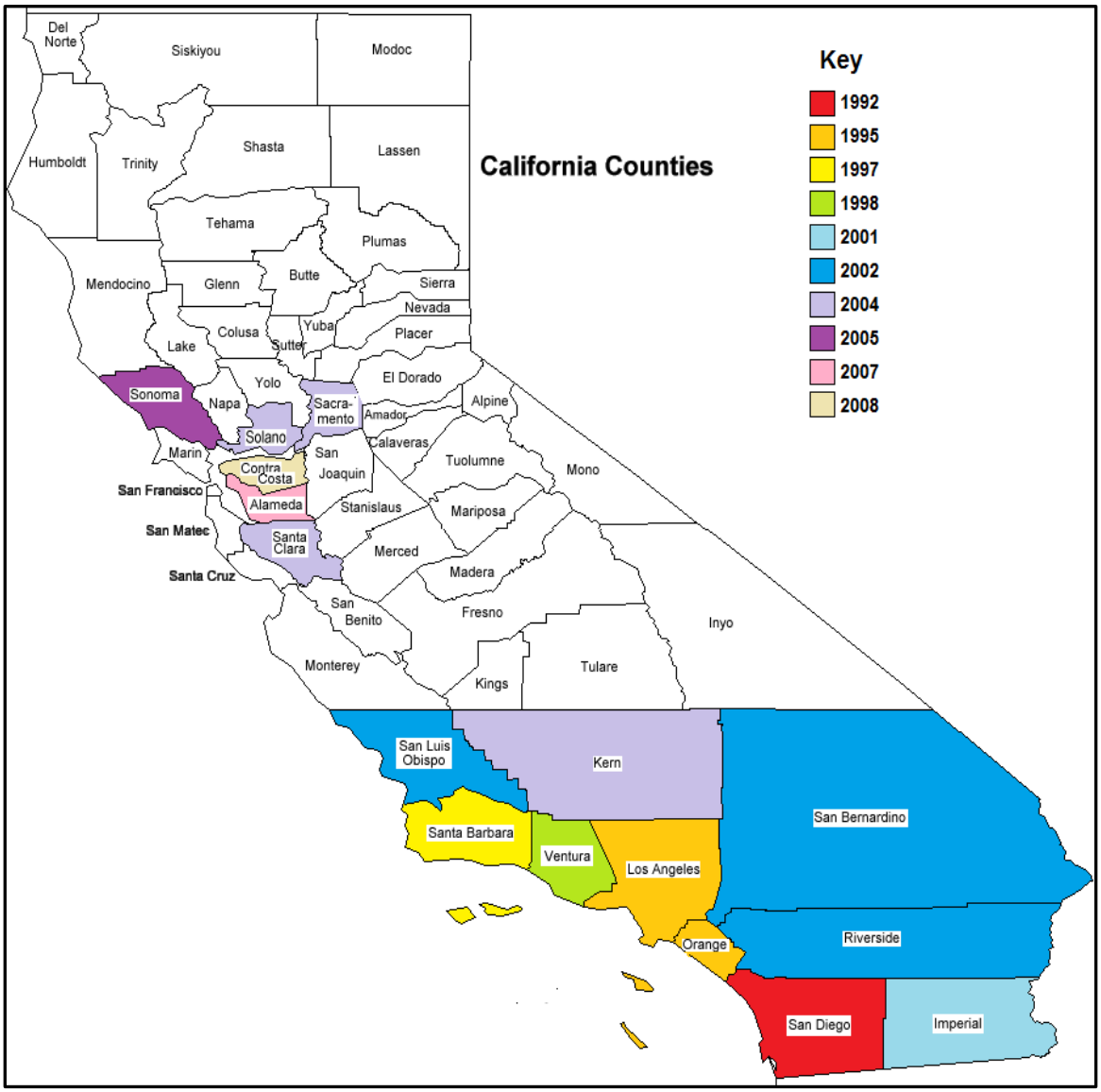

Figure 1.2 The reported distribution of giant whitefly *Aleurodicus dugesii* in California. Distribution data obtained from the California Department of Food and Agriculture's Plant Pest and Disease Reports (1992-2009).

Table 1.2 Partial list of *Aleurodicus dugesii* host plants.

Family	Hosts	Family	Hosts
Acanthaceae	*Eranthemum pulchellum*	**Lauraceae**	*Cinnamomum* sp.
Altingiaceae	*Liquidambar styraciflua*		*Persea Americana*
Anacardiaceae	*Schinus terebinthifolius*	**Malvaceae**	*Anoda* sp.
Annonaceae	*Annona* sp.		*Bombax* sp.
Apocynaceae	*Hoya* sp.		*Gossypium hirsutum*
	Mandevilla sp.		*Hibiscus mutabilis*
	Plumeria sp.		*Hibiscus rosa-sinensis*
Araliaceae	*Aralia* sp.		*Hibiscus syriacus*
	Hedera helix		*Urena lobata*
	Schefflera sp.	**Moraceae**	*Ficus nitida*
Araceae	*Colocasia* sp.		*Morus alba*
	Philodendron sp.	**Musaceae**	*Musa* sp.
	Spathiphyllum floribundum	**Myrtaceae**	*Eucalyptus* sp.
Arecaceae	*Cocos nucifera*		*Psidium* sp.
	Howea forsteriana		*Syzygium* sp.
Asteraceae	*Baccharis trinervis*	**Nyctaginaceae**	*Bougainvillea variegata*
	Osteospermum sp.	**Nymphaeaceae**	Water Lilly
Bignoniaceae	*Verbesina virginica*	**Oleaceae**	*Jasminum* sp.
Berberidaceae	*Pyrostegia venusta*	**Onagraceae**	*Fuchsia* sp.
Berberidaceae	*Begonia* sp.	**Orchidaceae**	*Calanthe* sp.
Buxaceae	*Nandina domestica*		*Phalaenopsis* sp.
Cannaceae	*Buxus japonica*	**Passifloraceae**	*Passiflora* sp.
Chrysobalanaceae	*Canna* sp.		*Turnera ulmifolia*
Convolvulaceae	*Chrysobalanus icaco*	**Pittosporaceae**	*Pittosporum* sp.
Cucurbitaceae	Morning Glory	**Rosaceae**	*Prunus* sp.
Cyperaceae	*Cucurbita* sp.	**Rutaceae**	*Citrus* sp.
Euphorbiaceae	*Cyperus papyrus*		*Murraya paniculata*
	Bishofia javanica	**Salicaceae**	*Salix* sp.
	Euphorbia pulcherrima		*Xylosma compacta*
Fabaceae	*Ricinus communis*		*Xylosma congestum*
	Acacia longifolia	**Sapindaceae**	*Acer negundo*
	Acacia salign	**Scrophulariaceae**	*Myoporum* sp.
	Bauhinia galpinni	**Solanaceae**	*Cestrum nocturnum*
	Bauhinia variegata		*Solandra hartwegii*
	Phaseolus vulgaris		*Solanum melongena*
	Calliandra sp.	**Sterculiaceae**	*Brachychiton* sp.
Geraniaceae	*Erythrina* sp.	**Strelitziaceae**	*Strelitzia nicolai*
Heliconiaceae	*Pelargonium* sp.		*Strelitzia reginae*
Iridaceae	*Heliconia* sp.	**Tropaeolaceae**	*Tropaeolum* sp.
Lamiaceae	*Gladiolus* sp.	**Verbenaceae**	*Citharexylum spinosum*
	Callicarpa Americana		*Clerodendrum speciosissimum*
	Plectranthus sp.		*Lantana* sp.
	Solenostemon scutellarioides	**Xanthorrhoeaceae**	*Hemerocallis* sp.
	Vitex lucens	**Zingiberaceae**	*Zingiber officinale*

Damage

Adult and nymphs of *A. dugesii* feed on the fluids of their host using needle-like mouthparts that they insert into the phloem tissue of the leaves. Feeding by *A. dugesii* deprives the host of water and nutrients and at high infestation levels can lead to severe leaf senescence and abscission, followed by plant dieback and even death. Additionally, feeding by *A. dugesii* promotes the development of sooty molds (e.g., *Capnodium* sp.), which use the sugary excrement produced by the nymphs and adults called honeydew as a substrate. Detrimental effects of sooty molds on plants include reduced rates of photosynthesis and gas exchange (Brink and Hewitt, 1992).

In addition to physical damage to the host, 2nd through 4th instars of *A. dugesii* produce copious amounts of wax filaments, giving infested leaves a "bearded" appearance (Figure 1.3). In the wild these filaments can attain lengths of up to 5 cm, but in greenhouses and areas shielded from wind these filaments can reach astounding lengths of > 25 cm (Hodges, 2004). Not surprisingly, the aesthetic value of plants is negatively affected by the presence of these wax filaments.

Development

Aleurodicus dugesii has six life stages, including an egg, mobile 1st instar "crawler" stage, three sessile nymphal stages, and an adult stage (Figure 1.4). Approximately 48 hours post-adult eclosion, female *A. dugesii* begin producing wax from their three abdominal wax glands (Nelson et al., 1999). This wax is applied to the underside of leaves in concentric circle patterns where the eggs are then deposited. After 6-7 days post-oviposition the eggs hatch and the mobile 1st instar locates a suitable spot

inside the circular wax deposit to attach and begin feeding. The developmental period of *A. dugesii* varies by temperature (see **Chapter 2**), and due to its relative size its developmental time is longer than most whitefly species.

1.6 Parasitoids of Giant Whitefly

More than 30 genera of parasitic Hymenoptera are known to attack whiteflies. These wasps belong primarily to 6 families within the Chalcidoidea and Platygastroidea; including the Aphelinidae, Eulophidae, Pteromalidae, Signiphoridae, Encyrtidae, and Platygastridae. Successful biological control against many whitefly species has been achieved with the aid of species within these groups (van Lenteren and Martin, 1999; Pickett and Pitcairn, 1999; Gerling et al., 2001; Heraty et al., 2008).

In the United States at least five species of chalcidoid wasps have been observed attacking *A. dugesii*. In this section I provide an overview of the history of these species as biological control agents of *A. dugesii* and what was known about their biologies at the onset of this research.

Entedononecremnus krauteri

The first parasitoid discovered attacking *A. dugesii* was *Entedononecremnus. krauteri* Zolnerowich & Rose (Hymenoptera: Eulophidae) (Zolnerowich and Rose, 1996) (Figure 1.5). This species was found on *A. dugesii* infesting *Hibiscus syriacus* L. in Comfort, Texas by entomologist Peter Krauter on October 10, 1995 (Figure 1.3). Collected individuals were maintained at the laboratory of Mike Rose at Texas A&M University, and once established in the laboratory approximately 3500 individuals of *E. krauteri* were sent to San Diego County in late 1995 for release. This species was also

released that same year in San Antonio, Fort Worth, and Houston, Texas. In the summer of 1997 about 500 *E. krauteri* were released in Seminole, Indian River, St. Lucie and Volusia Counties in Florida to help control *A. dugesii* populations there (Nguyen and Hamon, 2002). The native range of *E. krauteri* is unknown; however specimens have been collected from Texas, Mexico, Costa Rica, and El Salvador (Hansson and LaSalle, 2003).

Little is known about the life history of *E. krauteri*. The only reported host of *E. krauteri* is *A. dugesii*. Hansson and LaSalle (2003) however, report it from unidentified Aleyrodidae in Mexico and El Salvador. *Entedononecremnus krauteri* is an arrhenotokous, solitary endoparasitoid and anecdotal evidence suggests it specializes on 4th instar *A. dugesii*. Zolnerowich and Rose (1996) mention that in the field this species was male biased, but under laboratory conditions the sex ratio was found to be 5.6:1 female: male. It has also been observed that unlike other parasitoid species attacking *A. dugesii*, *E. krauteri* parasitizes nymphs through the upper surface of the leaf (Hodges, 2004). This may be a behavioral mechanism to avoid the waxy filaments produced by the nymphs.

Encarsia noyesi and *Idioporus affinis*

In California during the late 1990's it became apparent that there was a need for additional parasitoids to help control populations of *A. dugesii*. In May, 1997 entomologists Mark Hoddle and David Headrick from the University of California-Riverside went on an expedition to Guadalajara, Mexico to search for additional *A. dugesii* parasitoids (Bellows and Meisenbacher, 2000). During this expedition two

Figure 1.3 The *Hibiscus syriacus* located in Comfort, Texas that provided the source population of *Entedononecremnus krauteri* for biological control of *Aleurodicus dugesii* in other parts of the United States. Photo Credit: Peter Krauter, October, 10 1995.

parasitoids *Idioporus affinis* LaSalle & Polaszek (Hymenoptera: Pteromalidae) and *Encarsia noyesi* Hayat (=*Encarsiella*) (Hymenoptera: Aphelinidae) were found attacking *A. dugesii* on *Hibiscus* (Figure 1.4). These wasps were brought back to the University of California-Riverside and laboratory colonies were established. Once laboratory colonies were established field releases were initiated in San Diego and Orange Counties in 1997 and 1998 for *I. affinis* and *E. noyesi* respectively. These parasitoids were later released in additional counties, and by May 2000 a total of 187,315 individuals of these two species had been released. Follow-up surveys at field release sites suggest that these parasitoids were having an impact on *A. dugessi* populations (Bellows and Meisenbacher, 2000). In June, 1998 approximately 100 *E. noyesi* were released in Volusia and Indian River

Counties in Florida from California to help control populations of *A. dugesii* (Nguyen and Hamon, 2002).

Encarsia noyesi (Figure 1.5) is a cosmopolitan species that has been utilized to control whitefly pests in multiple countries. In addition to giant whitefly, *E. noyesi* is currently being investigated as a biological control agent for rugose spiraling whitefly *Aleurodicus rugioperculatus* Martin in Florida (Taravati et al., 2013) and *Aleurodicus chirripoensis* Martin in Costa Rica (Sánchez and Laprade, 2013). The native range of *E. noyesi* appears to be the Caribbean, and was originally described from Trinidad and Tobago (Hyat, 1983). In addition to the U.S. and Mexico, *E. noyesi* has been reported from Anguilla, Antigua, Barbados, Bermuda, Costa Rica, Grenada, Peru, and St. Vincent (Polaszek and Hayat, 1992). Other reported hosts of *E. noyesi* include *Aleurodicus cocois* (Curtis), *A. dispersus*, *Aleurodicus maritimus* Hempel, and *Aleurodicus floccosus* (Maskell), (Polaszek and Hayat, 1992; Blanco-Metzler and Laprade, 1998; Noyes, 2013).

While not unusual for the genus *Encarsia* (Walter, 1983; Hunter and Woolley, 2001), within the Chalcidoidea the heteronomous autoparasitic life history exhibited by *E. noyesi* is uncommon. The heteronomous life history of *E. noyesi* has been observed by Boughton et al. (2015) in laboratory studies and in the field during this study as well (see **Chapter 5**).

The genus *Idioporus* is monotypic and very little is known about the general life history of *I. affinis* (Figure 1.5). In addition to Mexico and the U.S., *I. affinis* is reported from Guatemala, El Salvador, and Costa Rica (LaSalle et al., 1997). The only known host of *I. affinis* is *A. dugesii*. In Hawaii in 2002 *I. affinis* was discovered attacking *A. dugesii*

on *Hibiscus*. It is believed that these two species arrived in Hawaii at approximately the same time via infested plant material originating from California. In Hawaii *I. affinis* has been observed affecting *A. dugesii* populations (Heu et al., 2004).

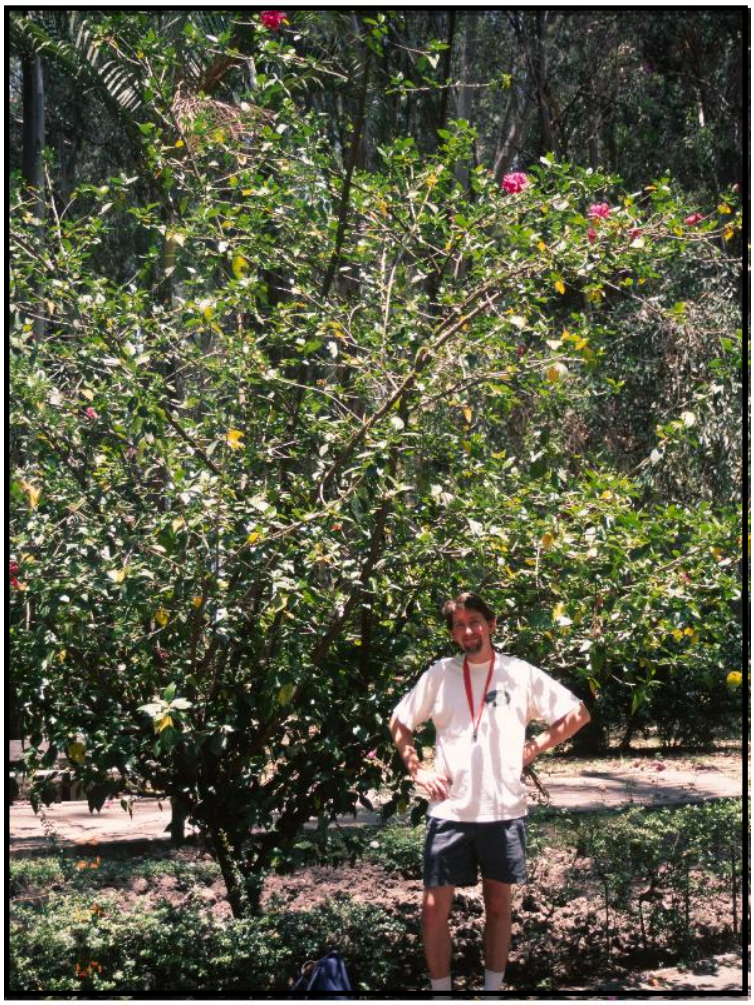

Figure 1.4 The *Hibiscus rosa-sinensis* located in Guadalajara, Mexico that provided the source populations of *Encarsia noyesi* and *Idioporus affinis* for biological control of *Aleurodicus dugesii* in the United States. Photo Credit: Mark Hoddle, May 5, 1997.

Encarsia hispida

The only parasitoid species found attacking *A. dugesii* that was already established in the U.S. at the time of *A. dugesii*'s initial invasion was the *Encarsia*

Figure 1.5 Parasitoids released as biological control agents against *Aleurodicus dugesii*. From left to right: *Entedononecremnus krauteri* (Hymenoptera: Eulophidae), *Encarsia noyesi* (Hymenoptera: Aphelinidae), and *Idioporus affinis* (Hymenoptera: Pteromalidae). Photo Credit: Jack Kelly Clark.

hispida. This species was discovered attacking *A. dugesii* by John Heraty (UCR) in San Diego in 2002. This species appears to prefer early instars of *A. dugesii* and likely complements other parasitoids in this complex. The current range of *E. hispida* is large, due to either its importation for control of various whitefly pests or via accidental introductions.

The original range of *E. hispida* appears to be neotropical, where it was originally described from Santa Fe, Argentina in 1947 (De Santis, 1948). Additional reported countries for *E. hispida* include: Barbados, Brazil, Canary Islands, Chile, Colombia, Dominican Republic, Ecuador, France, French Polynesia, Guadeloupe, Guatemala, Honduras, Italy, Jamaica, Madeira, Mexico, The Netherlands, Peru, Puerto Rico, South Africa, Spain, and Venezuela (Polaszek et al., 1992; Polaszek et al., 2004).

Compared to the other parasitoids attacking *A. dugesii*, *E. hispida* has a wide host range and has been reported attacking *A. dispersus*, *Aleurodicus floccissimus* (Martin *et*

al.) *Aleuroglandulus subtilis* Bondar, *Aleurothrixus aepim* Goeldi, *A. floccosus*, *Aleurothrixus porteri* Quaintance & Baker, *Aleurotrachelus rhamnicola* (Goux), *Aleurotrachelus socialis* Bondar, *Aleurotrachelus trachoides* (Back), *Aleyrodes proletella* (L.), *Aleyrodes singularis* Danzig, *Aleyrodes spiraeoides* Quaintance, the *Bemisia tabaci* Gennadius complex, *Bemisia tuberculata* Bondar, *Crenidorsum aroidephagus* Martin and Aguiar, *Dialeurodes* sp., *Lipaleyrodes* sp., *Metaleurodicus minimus* (Quaintance), *Parabemisia myricae* (Kuwana), *Siphoninus phillyreae* (Haliday), *Tetraleurodes acaciae*, *Trialeurodes abutilonea* (Quaintance), *Trialeurodes floridensis* (Quaintance), *Trialeurodes ricini* (Misra), *T. vaporariorum*, and *Trialeurodes variabilis* (Quaintance) (Hernández-Suárez et al., 2003; Oliveira et al., 2003; Polaszek et al., 2004).

Throughout the work of this dissertation I did not observe *E. hispida* activity on *A. dugesii* populations despite collecting host material from dozens of sites across southern California over a six year period. It is unclear if this species is rare or has been competitively displaced by the introduced parasitoid species. Regardless, the relative importance of this species as a natural enemy of *A. dugesii* is highly suspect.

Encarsia guadeloupae

Although not currently recorded attacking *A. dugesii* in California the wasp *E. guadeloupae* has been found attacking *A. dugesii* in Hawaii, where it was imported from Trinidad to control *A. dispersus* in the early 1980's (Heu et al., 2004). It has also been reported in Florida attacking the invasive rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin (Taravati et al., 2013), but it unclear whether it is attacking *A.*

dugesii there as well. In February 2011 this species was found attacking *A. dugesii* in Indonesia (Muniappan, 2011).

In addition to parts of the U.S. and Indonesia, the range of *E. guadeloupae* includes: Guadeloupe, India, Mexico, The Canary Islands, Benin, The Philippines, Thailand, Nevis, Micronesia, and Papua New Guinea (Evans, 2008; Myartseva, 2007). In India and The Canary Islands it was purposely introduced to control *A. dispersus* and *A. floccissimus* (Nijhof et al., 2000). Other reported hosts of *E. guadeloupae* include: *B. tabaci* (Schmidt et al., 2001) and *T. vaporariorum* (Viggiani, 1993).

Due to either the rarity or absence of *E. hispida* and *E. guadeloupae* in southern California, they were not included in the studies performed in this dissertation. Instead I focused my attention on the other three parasitoid species.

1.7 Objectives of Dissertation

It is becoming apparent that taking natural enemy interactions into consideration when designing biological control programs is critical for ensuring their long-term success (Ehler and Hall, 1982; Hoelmer and Kirk, 2005; Straub et al., 2008). To incorporate this information into control-program design, we must first gain a better understanding of factors mediating natural enemy interactions. Data obtained from this study will increase our understanding of the dynamics of multiple natural enemy interactions and allow us to contribute to the debate surrounding the validity of the use of multiple natural enemies in biological control programs.

The primary goal of this dissertation is to document the occurrence and dynamics of interactions between multiple biological control agents. Specifically, to elucidate how

environmental factors and species' life histories mediate these interactions and how these interactions might affect the control of a shared host under a biological control setting. This information can be used in tandem with the background knowledge of a given system to make educated predictions on the outcomes of specific natural enemy release strategies. The secondary goal of this dissertation is to provide the foundatational knowledge necessary on the dynamics of the interactions between species in this system to enhance biological control of *A. dugesii*.

The specific objectives for this project are:

1) To create developmental and degree day models for *A. dugesii* (Chapters 2).
2) To determine the host-stage preferences for all three parasitoids (Chapter 3).
3) To examine the impact of host defenses on parasitoid efficacy (Chapter 4)
4) To determine the spatial and temporal occurrence of species in this study system under different field conditions (Chapter 5).

1.8 Literature Cited

Allesina, S., Levine, JM. 2011. A competitive network theory of species diversity. Proc. Natl. Acad. Sci. USA. 108:5638–5642.

van Alphen, JJM., Visser, ME. 1990. Superparasitism as an adaptive strategy for insect parasitoids. Ann. Rev. Entomol. 35:59–79.

Amarasekare, P. 2000. Coexistence of competing parasitoids on a patchily distributed host: local vs. spatial mechanisms. Ecology. 81:1286–1296.

Amarasekare, P. 2006. Productivity, dispersal and the coexistence of intraguild predators and prey. J. Theor. Biol. 243:121–133.

Amarasekare, P. 2007. Species coexistence in communities with intraguild predation: the roles of trade-offs and temporal variation. Ecology. 88:2720–2728.

Arim, M., Marquet, PA. 2004. Intraguild predation: a widespread interaction related to species biology. Ecol. Lett. 7:557–564.

Bakker, K., van Alphen, JJM., van Batenburg, FHD., van Der Hoeven, N., Nell, HW., van Strien-van Liempt, WTFH., Turlings, TCJ. 1985. Function of host discrimination and superparasitism in parasitoids. Oecologia. 67:572–576.

Bellows, TS, Jr., Meisenbacher, C. 2000. Biological control of giant whitefly, *Aleurodicus dugesii*, in California. California Conference on Biological Control II, The Historic Mission Inn Riverside, California, USA, 11-12 July, 2000, pp. 113–116.

Bellows, TS, Jr., Kabashima, JN., Robb, KL. 2002. Giant Whitefly. UC ANR Publication 7400. Available online at: http://www.ipm.ucdavis.edu/PDF/PESTNOTES /pngiantwhitefly.pdf

Bess, HA., van den Bosch, R., Haramoto, FH. 1961. Fruit fly parasites and their activities in Hawaii. Hawaii Entomol. Soc. 17:367–378.

Blanco-Metzler, H., Laprade, S. 1998. Enemigos naturales de la mosca Blanca *Aleurodicus dispersus* Russell (Homoptera: Aleyrodidae): parasitoides y depredadores. Agron. Mesoam. 9:41–44.

Bográn, CE., Heinz, KM., Ciomperlik, MA. 2002. Interspecific competition among insect parasitoids: field experiments with whiteflies as hosts in cotton. Ecology. 83:653 –668.

Bonsall, MB., Hassell, MP. 1999. Parasitoid mediated effects: apparent competition and the persistence of host-parasitoid assemblages. Res. Popul. Ecol. 41:59–68.

Bonsall, MB., French, DR., Hassell, MP. 2002. Metapopulation structures affect persistence of predator- prey interactions. J. Anim. Ecol. 71:1075–1084.

Boughton, AJ., Mendez, MA., Francis, AW., Smith, TR., Osborne, LS., Mannion, CM. 2015. Host stage suitability and impact of *Encarsia noyesi* (Hymenoptera: Aphelinidae) on the invasive rugose spiraling whitefly, *Aleurodicus rugioperculatus* (Hemiptera: Aleyrodidae), in Florida. Biol. Control. 88:61–67.

Brewer, MJ., Noma, T., Elliott, NC., Kravchenko, AN., Hild, AL. 2008. A landscape view of cereal aphid parasitoid dynamics reveals sensitivity to farm- and region-scale vegetation structure. Eur. J. Entomol. 105:503–511.

Briggs, CJ. 1993. Competition among parasitoid species on a stage-structured host and its effect on host suppression. Am. Nat. 143:372–397.

Briggs, CJ., Collier, TR. 2001. Autoparasitism, interference and pest-parasitoid population dynamics. Theo. Pop. Biol. 60:33–57.

Briggs, CJ., Nisbet, RM., Murdoch, WW. 1993. Coexistence of competing parasitoid species on a host with a variable life cycle. Theor. Popul. Biol. 44:341–373.

Brink, T., Hewitt, PH. 1992. The relationship between the white powdery scale, *Cribrolecanium andersoni* (Hemiptera: Coccidae) and sooty mould and the effect on photosynthetic rates of citrus. Fruits. 47:413–417.

Cardinale, BJ., Duffy, D., Wright, JE., Downing, JP., Sankaran, A.L., Srivastava, M. et al. 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature. 443:989–992.

Castillo, D., Velasco-Hernandez, JX. 2003. Coexistence in a competitive parasitoid-host system. J. Theor. Biol. 221:61–77.

Chesson, P. 2000. Mechanisms of maintenance of species diversity. Annu. Rev. Ecol. Syst. 31:343–66.

Cockerell, TDA. 1896. A Mexican Aleurodicus. Can. Entomol. 28:302.

Collier, TR., Hunter, MS. 2001. Interference competition between whitefly parasitoids, *Eretmocerus eremicus*, and *Encarsia transvena*. Oecologia. 129:147–154.

Comins, HN., Hassell, MP. 1996. Persistence of multispecies host–parasitoid interactions in spatially distributed models with local dispersal. J. Theor. Biol. 183:19–28.

Comins, HN., Hassell, MP., May, RM. 1992. The spatial dynamics of host-parasitoid systems. J. Anim. Ecol. 61:735–748.

Cronin, JT., Strong, DR. 1993. Superparasitism and mutual interference in the egg parasitoid *Anagrus delicates*. Ecol. Entomol. 18:293–302.

Crowder, DW, Jabbour, R. 201) Relationships between biodiversity and biological control in agroecosystems: current status and future challenges. Biol. Control. 75:8–17.

DeBach, P., Rosen, D. 1991. Biological control by natural enemies. Cambridge University Press, Cambridge, UK.

DeBach, P., Hendrickson, RM., Rose, M. 1978. Competitive displacement: extinction of the yellow scale, *Aonidiella citrina* (Coq.) (Homoptera: Diaspididae), by its

ecological homologue, the California red scale, *Aonidiella aurantii* (Mask.) in southern California. Hilgardia. 46:1–35.

Del Bene, G., Landi, S. 1991. Biological pest control in glasshouse ornamental crops in Tuscany. Bull. IOBC/WPRS. 14:13–21.

De Clercq, P., Mason, PG., Babendreier, D. 2011. Benefits and risks of exotic biological control agents. BioControl. 56:681–698.

Denoth, M., Frid, L., Myers, JH. 2002. Multiple agents in biological control: improving the odds? Biol. Con. 24:20–30.

De Santis, L. 1948. Adiciones a la fauna Argentina de Afelínidos (Hymenoptera: Chalcidoidea). Notas Mus. La Plata. Buenos Aires, 13:43–48.

Didham, RK., Ghazoul, J., Stork, NE., Davis, AJ. 1996. Insects in fragmented forests: a functional approach. Trends Ecol. Evolu. 11:255-260.

Doutt, RL., Smith, RF. 1971. The pesticide syndrome – diagnosis and suggested prophylaxis. *In*: Huffaker, CB. (Ed.), Biological Control, Plenum Press, New York, New York. pp. 3–15.

Ehler, LE. 1979. Utility of secondary facultative parasites in biological control. Environ. Entomol. 8:829–832.

Ehler, LE. 1985. Species-dependent mortality in a parasite guild and its relevance to biological control. Environ. Ent. 14:1–6.

Ehler, LE., Hall, RW. 1982. Evidence for competitive exclusion of introduced natural enemies in biological control. Environ. Entomol. 11:1–4.

Evans GA (2008) The whiteflies (Hemiptera: Aleyrodidae) of the world and their host plants and natural enemies. Available online at: http://keys.lucidcentral.org/ keys/v3/whitefly/PDF_PwP%20ETC/world-whitefly-catalog-Evans.pdf

Fagan, WF., Lewis, MA., Neubert, MG., Van Den Driessche, P. 2002. Invasion theory and biological control. Ecol. Lett. 5:148–157.

Finke, DL., Snyder, WE. 2008. Niche partitioning increases resource exploitation by diverse communities. Science 321:1488–1490.

Fisher, RC. 1963. Oxygen requirements and the physiological suppression of supernumerary insect parasitoids. J. Exp. Biol. 40:531–540.

Frago, E. 2016. Interactions between parasitoids and higher order natural enemies: intraguild predation and hyperparasitoids. Curr. Opin. Insect Sci. 14:81–86.

Gabarra, R., Arnó, J., Alomar, O., Albajes, R. 1999. Naturally occurring populations of *Encarsia pergandiella* (Hymenoptera: Aphelinidae) in tomato greenhouses. Bull. IOBC/WPRS. 22:85–88.

Gause, GF. 1934. The Struggle for Existence. Williams & Wilkins, Baltimore. 163 pp.

Gerling, D., Alomar, O., Arno, J. 2001. Biological control of *Bemisia tabaci* using predators and parasitoids Crop Prot. 20:779–799.

Gill, RJ. 1992. Giant whitefly. In: California Plant Pest and Disease Report. Calif. Dept. Food & Agric. 11:78–81.

Godfray, HCJ. 1994. Parasitoids: Behavioral and Evolutionary Ecology. Princeton University Press, Princeton, NJ.

Godfray, HCJ., Shimada, M. 1999, Parasitoids: a model system to answer questions in behavioral, evolutionary and population ecology. Res. Popul. Ecol. 41:3–10.

Greathead, DJ., Greathead, AH. 1992. Biological control of insect pests by insect parasitoids and predators: the BIOCAT database. Biocontrol News Inform. 13:61–68.

Griffin, JN., Byrnes, JEK., Cardinale BJ. 2013 Effects of predator richness on prey suppression: a meta-analysis. Ecology. 94:2180–2187.

Hackett-Jones, E., Cobbold, CA., White, A. 2009. Coexistence of multiple parasitoids on a single host due to differences in parasitoid phenology. Theor. Ecol. 2:19–31.

Hagver, EB. 1988. Multiparasitism of the green pea aphid, *Myzus persicae*: competition in the egg stage between *Aphidius matricariae* and *Ephedrus cerasicola*. Entomol. Exp. Appl. 47:275–282.

Hansson, C., LaSalle, J. 2003. Revision of the Neotropical species of the tribe Euderomphalini (Hymenoptera:Eulophidae). J. Nat. Hist. 37:697–778.

Harvey, JA., Poelman, EH., Tanaka, T. 2013. Intrinsic inter- and intraspecific competition in parasitoid wasps. Annu. Rev. Entomol. 58:333–351.

Harvey, JA., Snaas, H., Malcicka, M., Visser, B., Bezemer, TM. 2014. Small-scale spatial resource partitioning in a hyperparasitoid community. Arthropod Plant Interact. 8:393–401.

Hawkins, BA. 1993. Parasitoid species richness, host mortality, and biological control. Am. Naturalist. 141:634–641.

Hawkins, BA. 2000. Species coexistence in parasitoid communities: does competition matter? *In*: Hochberg, ME., Ives, AR. (Eds.) Parasitoid population biology. Princeton University Press, Princeton, New Jersey, pp. 198–213.

Hawkins, BA., Mills, NJ. 1996. Variability in parasitoid community structure. J. Anim. Ecol. 65: 501–516.

Hawkins, BA., Askew, RR., Shaw, MR. 1990. Influences of host feeding: Niche and foodplant type on generalist and specialist parasitoids. Ecol. Entomol. 15:275–280.

Heinz, KM., Nelson, JM. 1996. Interspecific interactions among natural enemies of *Bemisia* in an inundative biological control program. Biol. Control. 6:384–393.

Heraty, JM., Polaszek, A., Schauff, ME. 2008. Systematics and biology of *Encarsia*. *In*: Gould, J., Hoelmer, K., Goolsby, J. (Eds.), Classical Biological Control of *Bemisia tabaci* in the United States – A Review of Interagency Research and Implementation, vol. 4. Springer, The Netherlands, pp. 71–87.

Hernández-Suárez, E., Carnero, A., Aguiar, A., Prinsloo, G., LaSalle, J., Polaszek, A. 2003. Parasitoids of whiteflies (Hymenoptera: Aphelinidae, Eulophidae, Platygastridae; Hemiptera: Aleyrodidae) from the Macaronesian archipelagos of the Canary Islands, Madeira, and the Azores. Syst. Biodivers. 1:55–108.

Hochberg, ME. 1996. An integrative paradigm of monophagous parasitoid dynamics. Oikos. 77:556–560.

Hodges, G. 2004. Giant Whitefly, *Aleurodicus dugesii* Cockerell, in Florida. Pest Alert, Division of Plant Industry, Fla. Dept. Agr. Cons. Serv. Available online at: http://www.freshfromflorida.com/content/download/68178/1612763/Pest_Alert_-_Aleurodicus_dugesii,_Giant_whitefly.pdf

Hoelmer, KA., Kirk, AA. 2005. Selecting arthropod biological control agents against arthropod pests: can the science be improved to decrease the risk of releasing ineffective agents? Biol. Control. 34:255–264.

Hogarth, WL., Diamond, P. 1984. Interspecific competition in larvae between entomophagous parasitoids. Am. Nat. 124:552–560.

Holt, RD., Polis, GA. 1997. A theoretical framework for intraguild predation, Amer. Nat. 149:745–764.

Heu, RA., Nagamine, NT., Kumashiro, BR., Watanabe, TM. 2004. Giant whitefly, *Aleurodicus dugesii* Cockerell (Homoptera: Aleyrodidae). Hawaii Dept. Agr. New Pest Advis. No. 02-04. June 2004 (revision). Available online at: https://hdoa.hawaii.gov/pi/files/2013/01/npa02-04-giantwf.pdf

Huffaker, CB., Messenger, PS., DeBach, P. 1971. The natural enemy component in natural control and the theory of biological control. *In*: Huffaker, CB. (Ed.) Biological Control, Academic Press, NewYork, USA.

Hunter, MS., Woolley, JB. 2001. Evolutionary and behavioral ecology of heteronomous aphelinid parasitoids. Annu. Rev. Entomol. 46:251–290.

Hunter, MS., Collier, TR., Kelly, SE. 2002. Does an autoparasitoid disrupt host suppression provided by a primary parasitoid? Ecology. 83:1459–1469.

Huston, MA. 1997. Hidden treatments in ecological experiments: evaluating the ecosystem function of biodiversity. Oecologia, 110:449–460.

Hayat, M. 1983. The genera of Aphelinidae (Hymenoptera) of the World. Syst. Entomol. 8:63–102.

Ivens, AB., Shuker, DM., Beukeboom, LW., Pen, I. 2009. Host acceptance and sex allocation of *Nasonia* wasps in response to conspecifics and heterospecifics. Proc. R. Soc. Lond. B. 276:3663–3669.

Kakehashi, M., Suzuki, Y., Iwasa, Y. 1984. Niche overlap of parasitoids in host-parasitoid host systems: its consequence to single versus multiple introduction controversy in biological control. J. Appl. Ecol. 21:115–131.

Kidd, D., Amarasekare, P. 2012. The role of transient dynamics in biological pest control: insights from a host–parasitoid community. J. Anim. Ecol. 81:47–57.

Klopfer, ED., Ives, AR. 1997. Aggregation and the coexistence of competing parasitoid species. Theor. Popul. Biol. 52:167–178.

Kramer, AM., Drake, JM. 2014. Time to competitive exclusion. Ecosphere 5:1–16.

Křivan, V., Diehl, S. 2005. Adaptive omnivory and species coexistence in tri-trophic food webs. Theor. Popul. Biol. 67:85–99.

Lasalle, J., Polaszek, A., Noyes, JS., Zolnerowich, G. 1997. A new whitefly parasitoid (Hymenoptera: Pteromalidae: Eunotinae), with comments on its placement, and implications for classification of Chalcidoidea with particular reference to the Eriaporinae (Hymenoptera: Aphelinidae). Syst. Entomol. 22:131–150.

László, Z., Tóthmérész, B. 2013. Landscape and local effects on multiparasitoid coexistence. Insect Cons. Div. 6:354–364.

Lawton, JH., Hassell, MP. 1984. Interspecific competition in insects. *In*: Huffaker, CB., Rabb, RL. (Eds.) Ecological Entomology. Wiley, New York, pp. 451–495.

LeBrun, EG., Plowes, RM., Gilbert, LE. 2009. Indirect competition facilitates widespread displacement of one naturalized parasitoid of imported fire ants by another. Ecology. 90:1184–1194.

Letourneau, DK., Jedlicka, JA., Bothwell, SG., Moreno, CR. 2009. Effects of natural enemy biodiversity on the suppression of arthropod herbivores in terrestrial ecosystems. Annu. Rev. Ecol. Evol. Syst. 40:573–592.

Letourneau, DK., Ando, AW., Jedlicka, JA., Narwani, A., Barbier, E. 2015. Simple-but-sound methods for estimating the value of changes in biodiversity for biological pest control in agriculture. Ecol. Econ. 120:215–225.

Losey, JE., Vaughan, M. 2006 The economic value of ecological services provided by insects. Bioscience 56:311–323.

Luck, RF. 1990. Evaluation of natural enemies for biological control: a behavioral approach. Trends Ecol. and Evolut. 5:196–199.

Macarthur, R., Levins, R. 1967. The limiting similarity, convergence, and divergence of coexisting species. Amer. Nat. 101:377–385.

Mackauer, M. 1990. Host discrimination and larval competition in solitary endoparasitoids. *In*: Mackauer, M., Ehler, LE., and Roland J. (Eds.), Critical Issues in Biological Control, Intercept, Andover, Hants, pp. 41–62.

May, RM., Hassell, MP. 1981. The dynamics of multiparasitoid-host interactions. Am. Nat. 117:234–261.

McBrien, H., Mackauer, M. 1990. Heterospecific larval competition and host discrimination in two species of aphid parasitoids: *Aphidius ervi* and *Aphidius smithi*. Entomol. Exp. Appl. 56:145–154.

Mills, NJ., Gutierrez, AP. 1996. Prospective modelling in biological control: an analysis of the dynamics of heteronomous hyperparasitism in a cotton–whitefly–parasitoid system. J. Appl. Ecol. 33:1379–1394.

Muniappan, R. 2011. The giant whitefly and its natural enemies. IAPPS Newsletter No. 10, October 2011. Available online at: https://www.plantprotection.org/Portals /0/documents /Newsletters/2011/NewsOct11.pdf

Muniappan, R., Shepard, BM., Watson, GW., *et al*. 2009. New records of invasive insects (Hemiptera: Sternorrhyncha) in southern Asia and West Africa. J. Agr. Urban Entomol. 26:167–174.

Myartseva, SN. 2007. Species of Genus *Encarsia* Förster (Hymenoptera: Aphelinidae) – Parasitoids of Whiteflies (Hemiptera: Aleyrodidae) Associated with *Psidium guajava* L. in Mexico, with Key and Description of New Species. Biosystematica. 1:7–19.

Myers, JH., Higgins, C., Kovacs, E. 1989. How many species are necessary for the biological control of insects? Environ. Entomol. 18:541–547.

Mylius, S., Klumpers, K., de Roos, A., Persson, L. 2001. Impact of intraguild predation and stage structure on simple communities along a productivity gradient. Am. Nat. 158:259–276.

Naeem, S., Wright, JP. 2003. Disentangling biodiversity effects on ecosystem functioning: deriving solutions to a seemingly insurmountable problem. Ecol. Lett. 6:567–579.

Nauen, R., Denholm, I. 2005. Resistance of insect pests to neonicotinoid insecticides: current status and future prospects. Arch. Insect Biochem. Physiol. 58:200–215.

Nelson, DR., Fatland, CL., Buckner, JS., Freeman, TP. 1999. External lipids of adults of the giant whitefly, *Aleurodicus dugesii*. Comp. Biochem. Physiol. B. 123:137–145.

Nicholson, AJ., Bailey, VA. 1935. The balance of animal populations, Part I. Proc. Zool. Soc. Lond. 551–598.

Nicol, CMY., Mackauer, M. 1999. The scaling of body size and mass in a host parasitoid association: influence of host species and stage. Entomol. Exp. Appl. 90:83–92.

Nijhof, BW., Oudman, L., Torres, R., Garrido, C. 2000. The introduction of *Encarsia guadeloupae* (Hymenoptera, Aphelinidae) for control of *Aleurodicus dispersus* and *Lecanoideus floccissimus* (Homoptera, Aleyrodidae) on Tenerife. Proc. Exp. Appl. Entomol. 11:41–47.

Nofemela, RS. 2013. The effect of obligate hyperparasitoids on biological control: differential vulnerability of primary parasitoids to hyperparasitism can mitigate trophic cascades. Biol. Cont. 65:218–224.

Noyes, JS. 2013. Universal Chalcidoidea Database. Worldwide Web electronic publication. Available online at: http://www.nhm.ac.uk/entomology/chalcidoids/index.html

Nguyen, R., Hamon, AB. 2002. Biological control of giant whitefly, *Aleurodicus dugesii* Cockerell, in Florida. Fla. Dept. Agr. Cons. Serv. Available online at: http://www.nhm.ac.uk/resources/research-curation/projects/chalcidoids/pdf_X/NguyenHa2002.pdf.

Oliveira, MRV., Amancio, E., Laumann, RA., Gomes, LO. 2003. Natural enemies of *Bemisia tabaci* (Gennadius) B biotype and *Trialeurodes vaporariorum* (Westwood) (Hemíptera: Aleyrodidae) in Brasília, Brazil. Neotrop. Entomol. 32:151–154.

Onillon, JC., Polaszek, A., Coquempot, C., Maignet, P. 1994. Premiers observation sur l'entomofaune parasitaire infeodee a *Bemisia tabaci* (Homoptera, Aleurodidae) dans le sud-est de la France. IOBC-WPRS Bull. 17:67–69.

Paoletti, MG., Pimentel, D. 2000. Environmental risks of pesticides versus genetic engineering for agricultural pest control. J. Agric. Environ. Ethics. 12:279–303.

Pearse, IS., Altermatt, F. 2013. Predicting novel trophic interactions in a non-native world. Ecol. Lett. 16:1088–1094.

Pedersen, BS., Mills, NJ. 2004. Single vs. multiple introduction in biological control: The roles of parasitoid efficiency, antagonism and niche overlap. J. Appl. Ecol. 41:973–984.

Pickett, CH., Pitcairn, MJ., 1999. Classical biological control of ash whitefly: factors contributing to its success in California. BioControl 44:143–158.

Polazsek, A., Hayat, M. 1992. A revision of the genera *Dirphys* Howard and *Encarsiella* Hayat (Hymenoptera: Aphelinidae). Syst. Entomol. 17:181–197.

Polaszek, A., Manzari, S., Quicke, DLJ. 2004. Morphological and molecular taxonomic analysis of the *Encarsia meritoria* species-complex (Hymenoptera: Aphelinidae), parasitoids of whiteflies (Hemiptera: Aleyrodidae) of economic importance. Zool. Scr. 33:403–421.

Polis, GA., Strong, D. 1996. Food web complexity and community dynamics. Am. Nat. 147:813–846.

Polis, G A., Myers, CA., Holt, RD. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. Annu. Rev. Ecol. Syst. 20:297–330.

Price, PW. 1971. Niche breadth and dominance of parasitic insects sharing the same host species. Ecology. 52:587–596.

Reitz, SR., Trumble, JT. 2002. Competitive displacement among insects and arachnids. Annu. Rev. Entomol. 47:435–465.

Rosenheim, JA., Kaya, HK., Ehler, LE., Marois, JJ., Jaffe, BA. 1995. Intraguild predation among biological control agents: theory and evidence. Biol. Control. 5:303–335.

Salt, G. 1961. Competition among insect parasitoids. Symp. Soc. Exp. Biol. 15:93–97.

Sampson, WW., Drews, EA. 1941. Fauna Mexicana IV. A review of the Aleyrodidae of Mexico (Insecta, Homoptera). An. Esc. Cienc. Biol. Mex. 2:143–189.

Sánchez, CG., Laprade, SC. 2013. *Aleurodicus chirripoensis* Martin, 2008 (Sternorrhyncha: Aleyrodidae): una nueva plaga de mosca blanca que afecta al banano en Costa Rica. Dugesiana 20:89–91.

Schmidt, S., Naumann, ID., Barro, PJ. 2001, *Encarsia* species (Hymenoptera: Aphelinidae) of Australia and the Pacific Islands attacking *Bemisia tabaci* and *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae): a pictorial key and descriptions of four new species. Bull. Entomol. Res. 91:369–387.

Schreiber, SJ., Mills, NJ., Gutierrez, AP. 2001. Host-limited dynamics of autoparasitoids. J. Theor. Biol. 212:141–153.

Schwartz, MW., Brigham, CA., Hoeksema, JD., Lyons, KG., Mills, MH., van Mantgem, PJ. 2000. Linking biodiversity to ecosystem function: implications for conservation ecology. Oecologia. 122:297–305.

Snyder, RE., Borer, ET., Chesson, P. 2005. Examining the relative importance of spatial and nonspatial coexistence mechanisms. Am. Nat. 166:E75–E94.

Solé, RV., Bascompte, J., Valls, J. 1992. Stability and complexity of spatially extended 2-species competition. J. Theor. Biol. 159:469–480.

Sorribas, J., Rodriguez, R., Garcia-Mari, F. 2010. Parasitoid competitive displacement and coexistence in citrus agroecosystems: linking species distribution with climate. Ecol. Appl. 20:1101–1103.

Stiling, P. 1993. Why do natural enemies fail in classical biological control programs? Am. Entomol. 39:31–37.

Strand, MR., Vinson, SB. 1984. Facultative hyperparasitism by the egg parasitoid *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae). Ann. Entomol. Soc. Am. 77:679–686.

Straub, CS., Snyder, WE. 2008. Increasing enemy biodiversity strengthens herbivore suppression on two plant species. Ecology. 89:1605–1615.

Straub, CS., Finke, DL., Snyder, WE. 2008. Are the conservation of natural enemy biodiversity and biological control compatible goals? Biol. Control. 45:225–237.

Sun, YP., Sun, YQ. 1994. Insect resistance to insecticides and dynamics of insect toxicology. Entomol. Sin. 1:217–241.

Tabashnik, BE., Mota-Sanchez, D., Whalon, ME., Hollingworth, RM., Carrière, Y. 2014. Defining terms for proactive management of resistance to Bt crops and pesticides. J. Econ. Entomol. 107:496–507.

Taravati, S., Mannion, C., Glenn, H., Osborne, L. 2013. Natural enemies of rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin (Insecta: Hemiptera: Aleyrodidae) in the south Florida landscape. ENY-870. University of Florida, IFAS Extension. Available online at: http://edis.ifas.ufl.edu/pdffiles/IN/IN100400.pdf

Thies, C., Tscharntke, T. 1999. Landscape structure and biological control in agroecosystems. Science 285:893–895.

Thies, C., Steffan-Dewenter, I., Tscharntke, T. 2003. Effects of landscape context on herbivory and parasitism at different spatial scales. Oikos. 101:18–25.

Thompson, CR., Cornell, JA., Sailer, RI. 1987. Interactions of parasites and a hyperparasite in biological control of citrus blackfly, *Aleurocanthus woglumi* (Homoptera: Aleyrodidae), in Florida. Environ. Entomol. 16:140–144.

Tian, SP., Zhang, JH., Yan, YH., Wang, CZ. 2008. Interspecific competition between the ichneumonid *Campoletis chlorideae* and the braconid *Microplitis mediator* in their host *Helicoverpa armigera*. Entomol. Exp. Appl. 127:10–19.

Tscharntke, T., Steffan-Dewenter, I., Kruess, A., Thies, C. 2002. Contribution of small habitat fragments to conservation of insect communities of grassland–cropland landscapes. Ecol. Appl. 12:354–363.

Turnbull, AL. 1967. Population dynamics of exotic insects. Bull. Entomol. Soc. Am. 13:333–337.

Turnbull, AL., Chant, PA. 1961. The practice and theory of biological control of insects in Canada. Can. J. Zool. 89:697–753.

Tylianakis, JM., Romo, CM. 2010. Natural enemy diversity and biological control: Making sense of the context-dependency. Basic Appl. Ecol. 11:657–668.

Ueno, T. 1999. Host-feeding and acceptance by a parasitic wasp (Hymenoptera: Ichneumonidae) as influenced by egg load and experience in a patch. Evol. Ecol. 13:33–44.

van den Bosch, R. 1968. Comments on population dynamics of exotic insects. Bull. Entomol. Soc. Am. 14:112–115.

van Lenteren, JC., Martin, NA. 1999. Biological control of whiteflies. *In*: Albajes, R., Gullino, ML., van Lenteren, JC., Elad, Y. (Eds.), Integrated Pest and Disease Management in Greenhouse Crops, Kluwer, Dordrecht, The Netherlands, pp. 202–216.

van Lenteren, JC., Bale, JS., Bigler, F., Hokkanen, HMT., Loomans, AJM. 2006. Assessing risks of releasing exotic biological control agents of arthropod pests. Annu. Rev. Entomol. 51:609–634.

van Veen, FJF., Morris, RJ., Godfray, HCJ. 2006. Apparent competition, quantitative food webs, and the structure of phytophagous insect communities. Annu. Rev. Entomol. 51:187–208.

Vet, LEM., Meyer, M., Bakker, K., van Alphen, JJM. 1984. Intra- and interspecific host discrimination in *Asobara* (Hymenoptera), larval endo-parasitoids of Drosophilidae: comparison between closely related and less closely related species. Anim. Behav. 32:871–874.

Viggiani, G. 1993. New species of *Encarsia* Förster (Hymenoptera: Aphelinidae), parasitoids of whiteflies, from Hawaii and Yemen. Redia. 76:121–127.

Vinson, SB. 1972. Competition and host discrimination between 2 species of tobacco budworm parasitoids. Ann. Entomol. Soc. Am. 65:229–246.

Walter, GH. 1983. Differences in host relationships between male and female heteronomous parasitoids (Aphelinidae: Chalcidoidea): a review of host location, oviposition and pre-imaginal physiology and morphology. J. Entomol. Soc. S. Afr. 46:261–282.

Watt, KEF. 1965. Community stability and the strategy of biological control. Can. Entomol. 97:887–895.

Wieber, AM., Cook, SP., Webb, RE., Tatman, KM., Reardon, RC. 1995. Niche partitioning by four *Gelis* spp. (Hymenoptera: Ichneumonidae) hyperparastoids of the primary Gypsy moth parasitoid *Cotesia melanoscela* (Hymenoptera: Braconidae). Ann. Entomol. Soc. Am. 88:427–433.

Williams, T. 1996. Invasion and displacement of experimental populations of a conventional parasitoid by a heteronomous hyperparasitoid. Biocontrol Sci. Tech. 6:603–618.

With, KA., Pavuk, DM., Worchuck, JL., Oates, RK., Fisher, JL. 2002. Threshold effects of landscape structure on biological control in agroecosystems. Ecol. Appl. 12:52–65.

Xu, HY., Yang, NW., Wan, FH. 2013. Competitive interactions between parasitoids provide new insight into host suppression. PLoS ONE. 8:e82003.

Yamamoto, D., Henderson, R., Corley, LS., Iwabuchi, K. 2007. Intrinsic, inter-specific competition between egg, egg-larval, and larval parasitoids of plusiine loopers. Ecol. Entomol. 32:221–228.

Zang, LS., Liu, T-X., Wan, FH. 2011. Reevaluation of the value of autoparasitoids in biological control. PLOS ONE. 6:e20324.

Zolnerowich, G., Rose, M. 1996. A new species of *Entedononecremnus* (Hymenoptera: Chalcidoidea: Eulophidae) parasitic on the giant whitefly, *Aleurodicus dugesii* Cockerell (Homoptera: Aleyrodidae). P. Entomol. Soc. Wash. 98:369–373.

Chapter 2

Temperature-Dependent Development and Survival of Giant Whitefly at Constant Temperatures.

2.1 Introduction

Temperature plays a major role in determining the distribution, abundance, and phenology of insects (Bowler and Terblanche, 2008) by impacting survivorship and fitness (Amarasekare and Savage, 2012). Extreme temperatures, especially high temperatures, have been shown to induce high mortality and fitness loss in insects (Hoffman et al., 2013; Dallas and Ross-Gillespie, 2015). It is widely recognized that taking temperature into consideration is critical for developing effective management strategies for insects (Shi et al., 2011).

Development rate (the reciprocal of development time) is a metric frequently used to assess the effects of temperature on insect physiology. The simple linear regression model has traditionally been used to describe this relationship due to its ease of calculation and widespread application for calculating the themal units (degree-days) required for development. The linear model assumes that development rate is linear across the full range of temperatures encountered by insects, which is often not the case. Insects as well as many other ectotherms have been shown to exhibit a nonlinear response of development in respect to temperature (Huey and Stevenson, 1979). Insect

development rates typically decline as an upper developmental threshold is reached, and thus the linear model has difficulties estimating development at temperature extremes and may limit the linear model's usefulness for forecasting pest populations (Shi et al., 2011). Many nonlinear models have been developed that address the shortcomings of the linear model by accurately estimating development rates across the entire temperature range (Logan et al., 1976; Sharpe and DeMichele, 1977; Schoolfield et al., 1981; Lactin et al., 1995; Briére et al., 1999; Shi et al., 2011). There appears to be no model, however, that best describes this relationship across all insect taxa, thus appropriate models must be tested on a case by case basis.

The giant whitefly *Aleurodicus dugesii* Cockerell (Hemiptera: Aleyrodidae) is a pest of many economically important food and ornamental crops (Bellows et al., 2002). Giant whitefly's native range is Central and Southern Mexico (Sampson and Drews, 1941), and it was discovered in the U.S. in Texas in 1991. Since its introduction, *A. dugesii* has become established in California, Arizona, Florida, Louisiana, and Hawaii (Gill, 1992; Nguyen and Hamon, 2002; Hodges, 2004; Hue et al., 2004). In addition to the U.S., *A. dugesii* has also been found in Belize, Costa Rica, El Salvador, Guatemala, Nicaragua, Pakistan, Venezuela, and Indonesia (Lasalle et al., 1997; Evans, 2008, Muniappan et al., 2009).

Current management strategies for *A. dugesii* utilize an integrative pest management approach consisting of physical control practices such as leaf removal or washing infested plants; cultural control practices such as exposing at risk or infested plants to direct sunlight (Bellows et al., 2002); and classical biological control using non-

native parasitoids (Bellows and Meisenbacher, 2000). Chemical control strategies are not readily utilized due to fears of disrupting biological control. While current *A. dugesii* population densities are reportedly lower than seen during the first few years post-invasion (CH. Pickett, unpublished), overall control of *A. dugesii* appears to be much less than observed for other invasive whiteflies in California such as ash whitefly *Siphoninus phillyreae* (Haliday) (Jetter et al., 1997), sweet potato whitefly *Bemisia argentifolii* (= *B. tabaci* biotype B) Bellows & Perring (Goolsby et al., 2005) and Woolly whitefly *Aleurothrixus floccosus* (Maskell) (DeBach and Rose, 1976).

The objective of this study was to examine the effects of sex and temperature on development of *A. dugesii* stadia by constructing temperature-dependent developmental and degree-day models. Additionally, the effect of temperature on survival to adulthood was investigated. Little is known about the biology of *A. dugesii* besides some basic information on its host range and life cycle (Bellows and Meisenbacher, 2000; Bellows et al., 2002). A better understanding of the effect of temperature on the life history of *A. dugesii* may allow us to better predict areas at risk of *A. dugesii* invasion and predict its seasonal occurrence to optimally utilize control strategies. Development of specific stadia was examined to provide the foundational knowledge for future studies examining biological control of *A. dugesii* using parasitoids. Differences in the development times between sexes across temperatures is of interest due to the potential consequences that this difference may have on the operational sex ratio (OSR), the number of sexually active males to receptive females (Emlen and Oring, 1977), and its potential effects on reproductive success and fitness of *A. dugesii*.

2.2 Materials and Methods

Plant and Insect Material

Whiteflies used to establish laboratory colonies were collected from infested *Hibiscus rosa-sinensis* L. located in Santa Ana, California (N33°46.051', W117°53.143'). Adults still attached to leaves were placed into plastic containers over ice and transported to the laboratory and were utilized within 72 h of collection. A mixed sex group of 30-50 individuals were confined to leaves for 48 h to infest host plants. Plants used to rear *A. dugesii* were created using woody cuttings taken from a single large *H. rosa-sinensis* 'White Wings'. Cuttings were taken from straight branches and prepared so that they were 10-18 cm in length with 0.4-1.2 cm diameters. The bottoms of the cuttings were cut at a 45º angle below a leaf node and the tops were cut cleanly across 0.5 cm above another node. Approximately 1/3 the length of each cutting was dipped into Hormex® rooting powder No. 8 (Brooker Chemical Co., Chatsworth, CA) prior to being placed into a 10 cm pots containing UC Soil Mix Type 3 (Matkin and Chandler 1957; http://agops.ucr.edu/soil/). Cuttings were placed into an environmentally controlled room with artificial light (14:10 L:D photoperiod, 28 ± 2 ºC, ambient RH) and watered every other day. Plants were approximately 3 months old at the start of the experiment.

Temperature-Dependent Development of Giant Whitefly

To test the effects of nymphal stage, sex, and temperature on development rate, the abaxial surfaces of 6-month old *H. rosa-sinensis* leaves were infested by placing 10–15 72-h old mated females from the laboratory colony in small mesh bags enclosing the leaves for 12 h to ensure stage uniformity. Each plant had only a single leaf infested.

Initially only 10 leaves (200 individuals) were infested per temperature treatment, but additional leaves were infested (up to 14 total per treatment) as needed in treatments with high observed mortality in order to achieve at least 25 nymphal replicates per sex reaching adulthood. Females had been held with males for at least 48 h prior to the start of the experiment to ensure mating. After 12 h, leaves were examined and the density of eggs manipulated so that 20 eggs remained on each leaf. This density facilitated the process of tracking individuals and decreased the likelihood of leaves prematurely abscising due to feeding activity. A schematic was drawn to record the position of each egg on the leaf surface. Plants were placed into environmental chambers and held at constant temperatures of either 10, 15, 20, 25, 28, 30, or 35 ± 0.2°C (16L:8D photoperiod, 75±10% RH). Each temperature treatment was conducted in 4-6 different environmental chambers to control for chamber effects. Development was observed at 24 h intervals between 15:00 – 18:00 hours and the stage of each individual was recorded. Stadia transition was determined via observable changes in size between each nymphal stage. Stadia duration was determined for each individual using the first day a particular stage was observed as time zero until the day the subsequent developmental stage was observed with a full 24 h added in duration from the previous day's observation.

Once all crawlers had emerged and settled, the schematic of their position on the leaf was redrawn, since their egg of origin could not be determined. Due to the inability to accurately determine crawler identity until they had settled on the leaf, the duration of the crawler stage for each individual was estimated by calculating the mean duration (days) of crawler presence prior to the first day that all crawlers had emerged. The first day that

all crawlers had emerged was counted as day one and the mean duration of crawler presence (days) prior to this point was applied as a positive correction factor for the total duration of the crawler stage for each individual. Upon adult emergence the sex of each individual was determined and number of days required for complete development and duration for all life stages recorded. Developmental data were not separated by sex for eggs, as their sex could not be determined. The fraction of individuals successfully reaching the adult stage was analyzed as described below, but for development rate only individuals that reached the adult stage were considered for analysis.

The survival of *A. dugesii* under different temperatures was monitored during developmental trials described above. During each observation period the status (alive or dead/missing) was recorded for each individual. Dead individuals appeared discolored (yellow/brown) and mortality was confirmed under high magnification (250x) and light intensity by the lack of hemolymph flow and muscle contractions. Survival was monitored from the time of egg oviposition until the time to death or adult eclosion. Dead nymphs or emerged adults were removed during each observation period.

Data Analysis

The relationship between developmental rate r = (1/d) and temperature was estimated using both linear and nonlinear models. The three nonlinear models examined included a modification Logan model (**Model 1**) (Logan et al., 1976) developed by Lactin et al. (1995) (hereafter the Lactin-2 model), the Brière-1 (**Model 2**) and Brière-2 (**Model 3**) models (Brière et al., 1999). These models have proven to be useful in accurately estimating insect development across a broad range of temperatures, and are

improvements upon earlier models in the literature due to both their increased ease of calculation compared to other available models (e.g. Sharpe and DeMichele, 1977; Schoolfield et al., 1981), and elimination of redundant parameters.

$$r(T) = e^{\rho T} - e^{[\rho T_{max} - (T_{max} - T)/\Delta]} + \lambda \quad \textbf{[1]}$$

$$r(T) = aT(T - T_{min})(T_{max} - T)^{\frac{1}{2}} \quad \textbf{[2]}$$

$$r(T) = aT(T - T_{min})(T_{max} - T)^{\frac{1}{m}} \quad \textbf{[3]}$$

In the Lactin-2 model r(T) is developmental rate at each temperature, T_{max} is the upper developmental threshold, and ρ, Δ, and λ are fitted parameters. The value of ρ represents the rate of increase to the optimal temperature (T_{opt}), and Δ represents the width of the high temperature boundary layer ($\Delta = T_{max} - T_{opt}$) (Logan et al., 1976). The value of λ proposed by Lactin et al. (1995) forces the curve to intersect the abscissa in order to estimate the lower developmental temperature threshold (T_{min}).

In the Brière-1 and Brière-2 models T and T_{max} are the same as in the Lactin-2 model, T_{min} is the lower developmental threshold, and *a* is a fitted parameter. The Brière-2 model adds an additional parameter m, which is a shaping parameter and gives spread to the curve in order to describe the capacity of an insect to survive at high temperatures (Brière et al., 1999).

Linear regression analysis was performed using PROC REG and nonlinear regression analyses using PROC NLIN in SAS (SAS institute, 2011). Curves were fitted by iterative nonlinear regression based on the Marquardt algorithm on mean

developmental rates. The lower and upper temperature thresholds were estimated graphically and computed by a simulation method where the curves intersects the abscissa at suboptimal and superoptimal temperatures [r(T) = 0], respectively. The optimal temperatures were determined in the same manner, where the developmental rates in the curves reached a maximum value. Unlike the Lactin-2 model, the Brière-1 and Brière-2 models estimate both T_{max} and T_{min} and allows for the direct calculation of T_{opt} using the following equation for the value of x where $dr(T) / dx = 0$:

$$T_{opt} = \frac{[2mT_{max} + (m+1)T_{min}] + \sqrt{4m^2T_{max}^2 + (m+1)^2T_{min}^2 - 4m^2T_{min}T_{max}}}{4m+2}$$

The performance of each development model was assessed using the adjusted coefficient of determination (R^2_{adj}) and the residual sum of squares (RSS) (Roy et al., 2002). Since the value of R^2 automatically increases as the number of explanatory variables increase in a model, R^2_{adj} was used to account for this and allow direct comparison between models with varying number of parameters. Higher values of R^2_{adj} and lower values of RSS indicate a superior fit of the model to the data. The equation for R^2 is defined as:

$$R^2 = 1 - (\text{Sum of Squares}_{(\text{Residual})} / \text{Sumof Squares}_{(\text{Total})})$$

Calculation of R^2 for nonlinear models cannot be calculated with the above equation, since nonlinear models typically have non-identifiable intercepts (Freund and Littell, 1986). A related parameter can be calculated, which closely corresponds to R^2 and is referred to as Psuedo R^2. The equation for Psuedo R^2 is defined as:

$$\text{Psuedo } R^2 = 1 - (\text{Sum of Squares }_{(\text{Residual})} / \text{ Corrected Sumof Squares }_{(\text{Total})})$$

The Corrected Sum of Squares (Total) can be obtained in SAS using the PROC MEANS procedure. Using this Pseudo-R^2 value, the value of R^2_{adj} (Kvålseth, 1985) can then be calculated. The equation for R^2_{adj} is defined as:

$$R^2_{adj} = 1 - (1 - \text{Psuedo } R^2)[(n-1)/(n-p-1)]$$

Where n is the sample size and p is the number of model parameters estimated. The Akaike information criterion (AIC) was used as additional goodness-of-fit measures of the mathematical models tested. Lower AIC values indicate superior fits of the models to the data (Burnham and Anderson 2002). The equation for AIC is defined as:

$$AIC = n\ln(RSS/n) + 2p$$

Where n is the number of treatments and p is the number of model parameters estimated.

To calculate degree days required for development the linear model was utilized, which is defined by the equation:

$$r(T) = a + bT$$

Where r(T) is the development rate (1/d), T is the temperature, and a and b are estimates of the y-intercept and slope respectively (Sokal and Rohlf, 2012). In this study the y-axis is development rate and the x-axis is temperature. The linear model was utilized, since it is the only model which can calculate the thermal constant (K), which is the amount of thermal energy (degree-days) required to complete development. The thermal constant is defined as:

$$K = \frac{1}{b}$$

Additionally, the lower developmental threshold (T_{min}) can be estimated with the linear model with the following equation:

$$T_{min} = -\frac{a}{b}$$

The developmental data points for 30°C (eggs) and 28°C (all other stages) which deviated from a straight line were excluded in order to accurately calculate the linear regression (Campbell et al., 1974).

The duration of each stadia and complete development were compared between gender treatments and across temperature treatments using separate Kruskal-Wallis tests followed by Dunn's multiple comparison tests using PROC NPAR1WAY in SAS (Zar, 1996, Elliott and Hynan, 2011; SAS institute, 2011). Data were corrected for heteroscedasticity using optimal λ Box-Cox transformations determined using PROC TRANSREG in SAS prior to performing Kruskall-Wallis tests.

The homogeneity of *A. dugesii* survival curves among different temperatures was examined using the Kaplan-Meier method with the PROC LIFETEST procedure in SAS. Statistical differences in *A. dugesii* survival were determined based on the log-rank statistic and multiple comparisons were performed with the Sidák correction. Adult longevity could not be followed post-eclosion, so adult emergence times were treated as censored data. Mean survival of specific life stages were calculated on a per leaf basis (n = 10 to 14).

2.3 Results

Successful *A. dugesii* emergence occurred between 15 and 30°C, while complete development occurs between 15°C and 28°C (**Table 2.1**). Egg development was monitored for 150 days at 10°C, at which time no eclosion had occurred and observations were ceased. There was a significant effect of temperature observed for the egg ($F = 13389.00_{4,772}$; $P < 0.0001$), 1st instar ($F = 603.04_{3,390}$; $P < 0.0001$), 2nd instar ($F = 1173.17_{3,390}$; $P < 0.0001$), 3rd instar ($F = 575.42_{3,390}$; $P < 0.0001$), 4th instar ($F = 422.92_{3,390}$; $P < 0.0001$), and the egg-adult ($F = 2293.57_{3,390}$; $P < 0.0001$) mean stadia durations. Development rates increased with increasing temperature until 30°C for eggs and 28°C for the nymphal stadia then began to decline. Egg and nymphal stadia durations were approximately three to four times longer at 15°C compared to 25°C. Complete development time (± SEM) ranged from 27.22 ± 0.30 d at 25°C to 88.19 ± 1.27 d at 15°C for males, and 29.19 ± 0.18 d at 25°C to 94.67 ± 0.82 d at 25°C for females (**Table 2.1**). Male *A. dugesii* developed faster than females at all temperatures tested, with as much as a 6.5 d difference in development time between sexes observed at 15°C.

As temperature deviated from the thermal optimum (~29°C, see **Table 2.2**), an increase in the variation of eclosion times were observed. The greatest variation in eclosion times occurred at 15°C ($s^2 = 1.527$, range = 22-27 d) followed by 20°C ($s^2 = 0.7067$, range = 10-14 d), 30°C ($s^2 = 0.3724$, range = 6-8 d), 25°C ($s^2 = 0.2942$, range = 7-8 d), and 28°C ($s^2 = 0.2226$, range = 6-8 d). This variation persisted through subsequent developmental stages ultimately leading to increased variation in adult emergence.

Table 2.1 Mean ± SEM duration (days) of *Aleurodicus dugesii* development at seven constant temperature under laboratory conditions. Temperatures: ± 0.2°C; RH: 65 ± 10%; Photoperiod: 16:8 L:D.

Temp (°C)	Eggs (n)	1st Instar	2nd Instar	3rd Instar	4th Instar	Egg - Adult
				Males		
10	–	–	–	–	–	–
15	25.41 ± 0.10 (157)a	13.94 ± 0.43 (26)a	10.31 ± 0.23a	11.35 ± 0.29a	23.50 ± 0.74a	88.19 ± 1.27a
20	11.71 ± 0.08 (101)b	6.86 ± 0.40 (30)b	3.77 ± 0.18b	4.50 ± 0.12b	10.93 ± 0.27b	41.93 ± 0.77b
25	7.47 ± 0.04 (228)c	4.02 ± 0.14 (36)c	2.64 ± 0.11c	2.89 ± 0.18c	6.19 ± 0.23c	27.22 ± 0.30c
28	6.28 ± 0.03 (194)d	3.81 ± 0.12 (32)c	2.19 ± 0.14c	3.56 ± 0.31c	10.66 ± 0.82b	30.47 ± 0.88c
30	7.11 ± 0.06 (97)c	–	–	–	–	–
35	–	–	–	–	–	–
				Females		
10		–	–	–	–	–
15		17.66 ± 0.41 (58)a	11.05 ± 0.20a	11.66 ± 0.21a	25.29 ± 0.48a	94.67 ± 0.82a
20		7.75 ± 0.31 (37)b	4.22 ± 0.15b	5.27 ± 0.23b	12.89 ± 0.53b	45.68 ± 0.91b
25		4.97 ± 0.13 (121)c	2.84 ± 0.07c	3.12 ± 0.08c	6.76 ± 0.16c	29.19 ± 0.18c
28		4.61 ± 0.18 (54)c	2.28 ± 0.11d	4.11 ± 0.25d	15.13 ± 0.78b	36.33 ± 0.93d
30		–	–	–	–	–
35		–	–	–	–	–

Means in columns followed by same letter are not significantly different within sexes using Dunn's Test (p-value ≤ 0.05).

The relationship between development rate and temperature described by the three nonlinear models are depicted in **Figure 2.1** and parameter estimates are presented in **Table 2.2**. Thermal optimum estimation for complete development ranged between 25.25 and 26.40°C. The thermal minimum and maximum for complete development were estimated between 9.99-10.40°C and 30.00-35.11°C respectively. The Lactin-2 model overestimated the thermal maximum compared to the Brière-1 and 2 models as seen by other authors (e.g. Nielsen et al., 2008); however, the simulated thermal maximum was close to the observed values.

All three models fit the data well, but the best fit model (i.e. the model which provided the highest R^2_{adj} and lowest AIC values) varied by stage and sex (**Table 2.3**). Using the RSS and R^2_{adj} values the Lactin-2 model provided the best overall for of the data for both sexes; however, AIC values were marginally smaller with the Brière-1 model for some life stages. After removal of temperature points that deviated from a linear relationship, the linear model fit these data well for the immature and complete developmental periods **(Table 2.3**). Although the linear model generally provided the highest RSS and R^2_{adj} values, its use is impractical due to the incapability of the linear model to estimate development across all relevant temperatures.

The linear equations for each *A. dugesii* life stage and sex are presented in **Table 2.4**. The lower developmental thresholds estimated for each developmental stage ranged

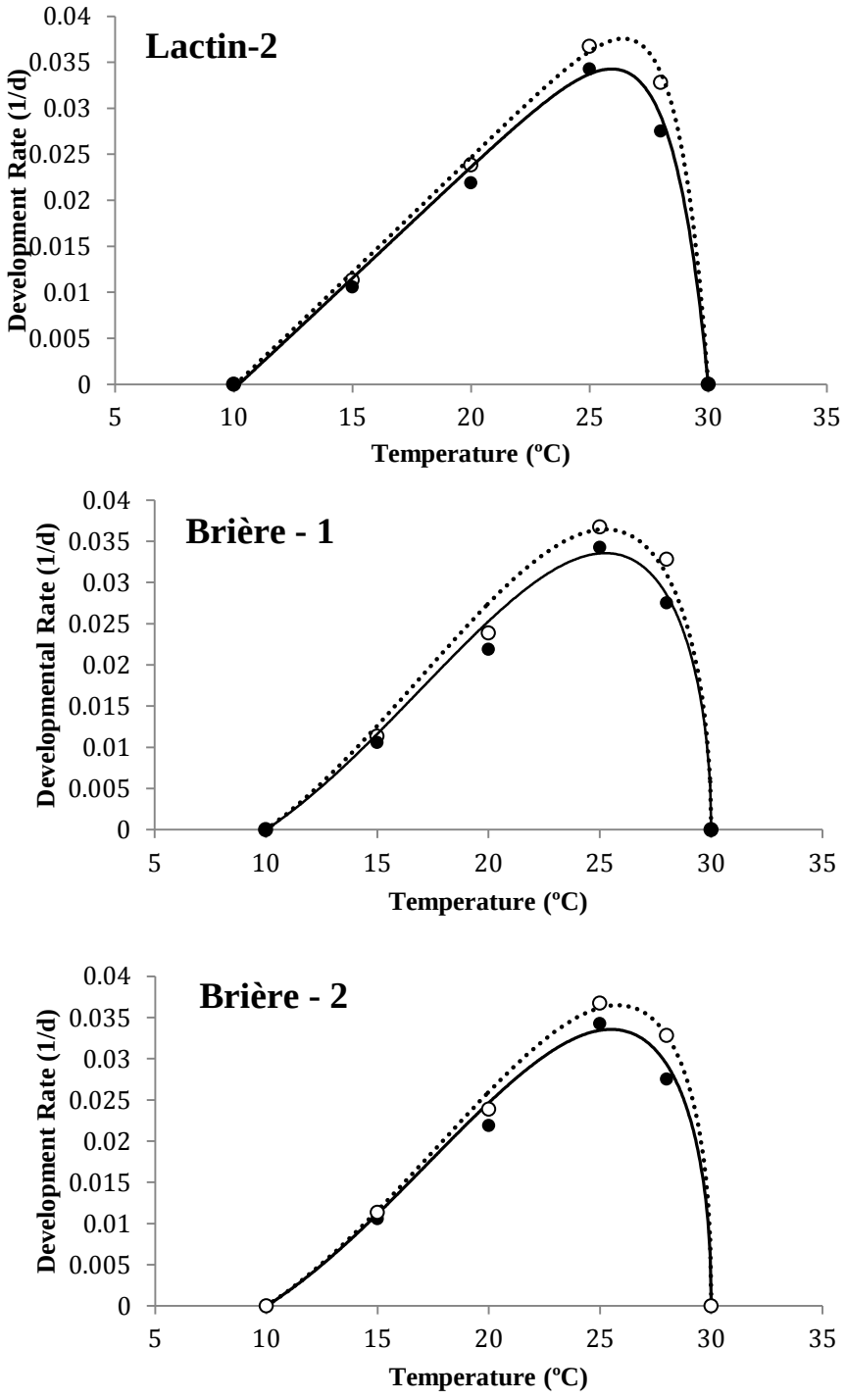

Figure 2.1 Constant temperature-dependent development of *Aleurodicus dugesii* using three nonlinear models for females (black circles) and males (white circles), with predicted development shown by solid and dashed lines respectively.

Table 2.2 Parameter estimates (± SEM) for three nonlinear developmental rate models for *Aleurodicus dugesii*.

Models	Para-meters	Stage Egg	Sex	1st Instar	2nd Instar	3rd Instar	4th Instar	Adult
Lactin-2	ρ (x10^{-2})	0.853 (0.0081)	Male	1.350 (0.0220)	2.000 (0.0414)	1.890 (0.0068)	0.964 (0.0310)	0.240 (0.0027)
			Female	1.180 (0.0226)	1.910 (0.0365)	1.840 (0.0605)	1.030 (0.0444)	0.234 (0.0029)
	Δ	2.9326 (0.0696)	Male	0.9385 (0.0713)	0.1276 (0.0156)	1.5092 (0.1730)	1.9215 (0.1492)	1.3327 (0.0422)
			Female	0.9032 (0.0831)	0.1221 (0.0126)	1.7427 (0.1679)	2.6318 (0.2198)	1.6456 (0.0498)
	λ	- 1.0939 (0.0017	Male	- 1.1462 (0.0045)	- 1.2259 (0.0121)	- 1.2139 (0.0139)	- 1.1038 (0.0050)	- 1.0245 (0.0004)
			Female	- 1.1287 (0.0053)	- 1.2213 (0.0053)	- 1.2120 (0.0132)	- 1.1150 (0.0068)	- 1.0242 (0.0004)
	$T_{max\ (Model)}$[a]	40.016 (0.1066)	Male	31.374 (0.0989)	30.143 (0.0025)	31.813 (0.1896)	33.426 (0.2361)	34.096 (0.1217)
			Female	31.432 (0.1277)	30.143 (0.0024)	32.156 (0.1910)	34.575 (0.3225)	35.112 (0.1443)
	$T_{max\ (Simulated)}$[a]	34.95	Male	29.95	30.00	29.95	29.95	29.95
			Female	29.95	29.95	29.95	29.95	29.95
	$T_{min\ (Simulated)}$[a]	10.55	Male	10.15	10.20	10.30	10.25	10.10
			Female	10.25	10.50	10.50	10.60	10.25
	$T_{opt\ (Simulated)}$[a]	28.90	Male	27.20	29.40	26.30	25.60	26.40
			Female	27.30	29.40	25.95	24.80	25.95
Brière-1	a (x10^{-4})	1.14 (0.0254)	Male	3.14 (0.0441)	5.46 (0.1400)	4.35 (0.1100)	1.76 (0.0313)	0.430 (0.0032)
			Female	2.64 (0.0350)	4.93 (0.0984)	4.00 (0.0703)	1.63 (0.0268)	0.400 (0.0022)
	T_{max}	35.00 (0.000)	Male	30.00 (0.000)	30.00 (0.000)	30.00 (0.000)	30.00 (0.000)	30.00 (0.000)
			Female	30.00 (0.000)	30.00 (0.000)	30.00 (0.000)	30.00 (0.000)	30.00 (0.000)
	T_{min}	9.94 (0.1002)	Male	9.99 (0.0000)	9.99 (0.0000)	9.99 (0.0000)	9.99 (0.0000)	9.99 (0.0000)
			Female	9.99 (0.0000)	9.99 (0.0000)	9.99 (0.0000)	9.99 (0.0000)	9.99 (0.0000)
	T_{opt}[b]	29.19	Male	25.25	25.25	25.25	25.25	25.25
			Female	25.25	25.25	25.25	25.25	25.25
Brière-2	a (x10^{-4})	1.07 (0.0185)	Male	3.81 (0.0893)	7.04 (0.2700)	4.92 (0.2600)	1.77 (0.0794)	0.480 (0.0074)
			Female	3.31 (0.0915)	6.73 (0.2200)	4.38 (0.2200)	1.36 (0.0709)	0.420 (0.0069)
	m	1.886 (0.0291)	Male	2.680 (0.1027)	2.996 (0.2026)	2.930 (0.1833)	2.026 (0.1105)	2.329 (0.0511)
			Female	2.849 (0.1407)	3.359 (0.2241)	2.269 (0.1590)	1.613 (0.0809)	2.140 (0.0467)
	T_{max}	35.00 (0.000)	Male	30.00 (0.000)	30.00 (0.000)	30.00 (0.000)	30.00 (0.000)	30.00 (0.000)
			Female	30.00 (0.000)	30.00 (0.000)	30.00 (0.000)	30.00 (0.000)	30.00 (0.000)
	T_{min}	9.99 (0.000)	Male	9.99 (0.0000)	9.99 (0.0000)	9.99 (0.0000)	9.99 (0.0000)	9.99 (0.0000)
			Female	9.99 (0.0000)	9.99 (0.0000)	9.99 (0.0000)	9.99 (0.0000)	9.99 (0.0000)
	T_{opt}[b]	28.931	Male	26.25	26.59	26.52	25.29	25.79
			Female	26.44	26.91	25.72	24.39	25.49

[a] The value of T_{max} estimated by the Lactin-2 model was not the temperature at which r(T) = 0; thus we differentiated between the model estimate T_{max} (Model) and the values obtained directly via simulations using the Marquardt method T_{max} (Simulated).

[b] The value of T_{opt} for the Briere-1 model was obtained using the equation for the value of x at dy/dx = 0.

from 10.01°C to 10.49°C, which were similar to those estimated by the three nonlinear models (**Table 2.2**). The estimated thermal degree days (DD) required for development were calculated as 109.65 for eggs; 231.71 and 245.33 for the total duration of the nymphal stage for males and females respectively; and 408.16 and 434.78 for complete development of males and females respectively (**Table 2.4**).

Temperature had a significant effect on *A. dugesii* survivorship (X^2 = 568.672, df = 4, P < 0.001) (**Figure 2.2**). Multiple comparisons indicated significant differences between temperatures, except between 15 and 20°C (X^2 = 1.188, P = 0.960), 15 and 25°C (X^2 = 0.165, P = 1.000), or 20 and 25°C (X^2 = 0.395, P = 0.999). Over 95% of eggs eclosed between 25 and 30°C, with the lowest successful eclosion rate of 78.5% observed at 15°C (**Table 2.5**). Across all viable temperatures examined, 1st instars exhibited the lowest survival rate, ranging from only 2% at 30°C to 85% at 25°C. At 30°C 1st instar mortality was high with only 5% present on the leaf at 24 h post-eclosion (**Figure 2.2**). Unlike 1st instars, survival of the 2nd through 4th instars was high and exceeded 85% between 15 and 28°C (**Table 2.5**). Egg to adult survival reached a peak of 67% at 20°C and then steadily declined to approximately 43% at 15 and 30°C.

Table 2.5 Survival (± SEM) of *Aleurodicus dugesii* life stages at seven temperatures.

Stage	Total Eggs	Eggs	1st Instar	2nd Instar	3rd Instar	4th Instar	Egg - Adult
		Survival (%) (n)	Survival (%) (n)	Survival (%) (n)	Survival (%) (n)	Survival (%) (n)	Survival (%) (n)
Temp (°C)							
10	100	0.00 (0)	–	–	–	–	–
15	200	78.50 ± 6.10 (157)	77.41 ± 7.48 (116)	90.78 ± 4.41 (104)	92.34 ± 3.04 (96)	87.92 ± 3.07 (84)	42.00 ± 4.29 (84)
20	120	84.17 ± 3.52 (101)	84.15 ± 5.27 (73)*	96.11 ± 2.42 (70)	99.75 ± 1.25 (69)	96.85 ± 1.97 (67)	67.00 ± 7.00 (67)
25	240	95.42 ± 1.82 (228)	85.24 ± 4.16 (194)	90.41 ± 2.80 (175)	91.46 ± 2.98 (162)	94.86 ± 1.89 (157)	65.42 ± 5.20 (157)
28	200	96.86 ± 1.69 (194)	63.67 ± 5.84 (124)	91.29 ± 3.21 (114)	86.08 ± 3.37 (100)	85.08 ± 5.07 (86)	43.00 ± 6.38 (86)
30	100	97.00 ± 1.22 (97)	2.00 ± 2.00 (2)	0.00 (0)	–	–	–
35	100	0.00 (0)	–	–	–	–	–

*A leaf was lost after all eggs emerged, so number surviving of subsequent life stages were based off 100 initial eggs at 20°C.

Table 2.3 Goodness-of-fit criteria of the linear and nonlinear models to developmental rate data for *Aleurodicus dugesii*.

Models	Performance Metric	Life Stage	Egg	Sex	1st Instar	2nd Instar	3rd Instar	4th Instar	Adult
Linear*	R^2_{adj}		0.983	Male	0.930	0.920	0.785	0.932	0.991
				Female	0.860	0.871	0.752	0.854	0.986
	RSS		0.027	Male	0.148	0.429	1.193	0.058	0.000
				Female	0.443	1.239	2.406	0.246	0.001
	AIC		-15.880	Male	-9.189	-4.934	-0.838	-12.916	-33.0465
				Female	-4.805	-0.688	1.967	-7.148	-29.264
Lactin-2	R^2_{adj}		0.975	Male	0.944	0.826	0.785	0.873	0.982
				Female	0.885	0.805	0.782	0.821	0.973
	RSS		0.089	Male	0.219	2.501	1.928	0.167	0.001
				Female	0.603	4.207	2.950	0.394	0.003
	AIC		-22.485	Male	-11.852	2.750	1.188	-13.507	-42.669
				Female	-5.786	5.870	3.741	-8.333	-37.955
Brière-1	R^2_{adj}		0.975	Male	0.911	0.754	0.774	0.865	0.973
				Female	0.855	0.722	0.774	0.799	0.970
	RSS		0.090	Male	0.350	3.550	2.031	0.177	0.002
				Female	0.760	6.002	3.059	0.445	0.003
	AIC		-24.477	Male	-11.044	2.850	-0.500	-15.150	-42.252
				Female	-6.398	6.002	1.959	-9.615	-39.275
Brière-2	R^2_{adj}		0.976	Male	0.929	0.788	0.778	0.865	0.977
				Female	0.875	0.767	0.776	0.806	0.971
	RSS		0.088	Male	0.278	3.053	1.9845	0.177	0.002
				Female	0.656	5.032	3.0317	0.427	0.003
	AIC		-22.587	Male	-10.431	3.945	1.362	-13.150	-41.340
				Female	-5.278	6.945	3.904	-7.858	-37.428

Higher values of R^2_{adj} reveal a better fit to the model to the data.

Lower values of RSS (Residual Sum of Squares) and the Akaike information criterion (AIC) indicate a better fit of the model to the data.

*The linear model was was only fit to part of the data ranging from 10-28°C.

Table 2.4 Lower developmental threshold (°C) and thermal constant (DD) estimated from the linear regression for *Aleurodicus dugesii*.

Life Stage	Sex	Regression Equation	Lower Developmental Threshold (°C)	Thermal Constant (K) Degree Days
Egg		0.00912x – 0.09472	10.39	109.649
1st Instar	Male	0.01596x – 0.15979	10.01	62.657
	Female	0.01449x – 0.14944	10.31	69.013
2nd Instar	Male	0.02696x – 0.27389	10.16	37.092
	Female	0.02546x – 0.26441	10.39	39.277
3rd Instar	Male	0.02529x – 0.26005	10.28	39.541
	Female	0.02377x – 0.24899	10.47	42.070
4th Instar	Male	0.01082x – 0.11082	10.24	92.421
	Female	0.01053x – 0.11044	10.49	94.967
Adult	Male	0.00245x – 0.02463	10.05	408.163
	Female	0.00230x – 0.02331	10.13	434.783

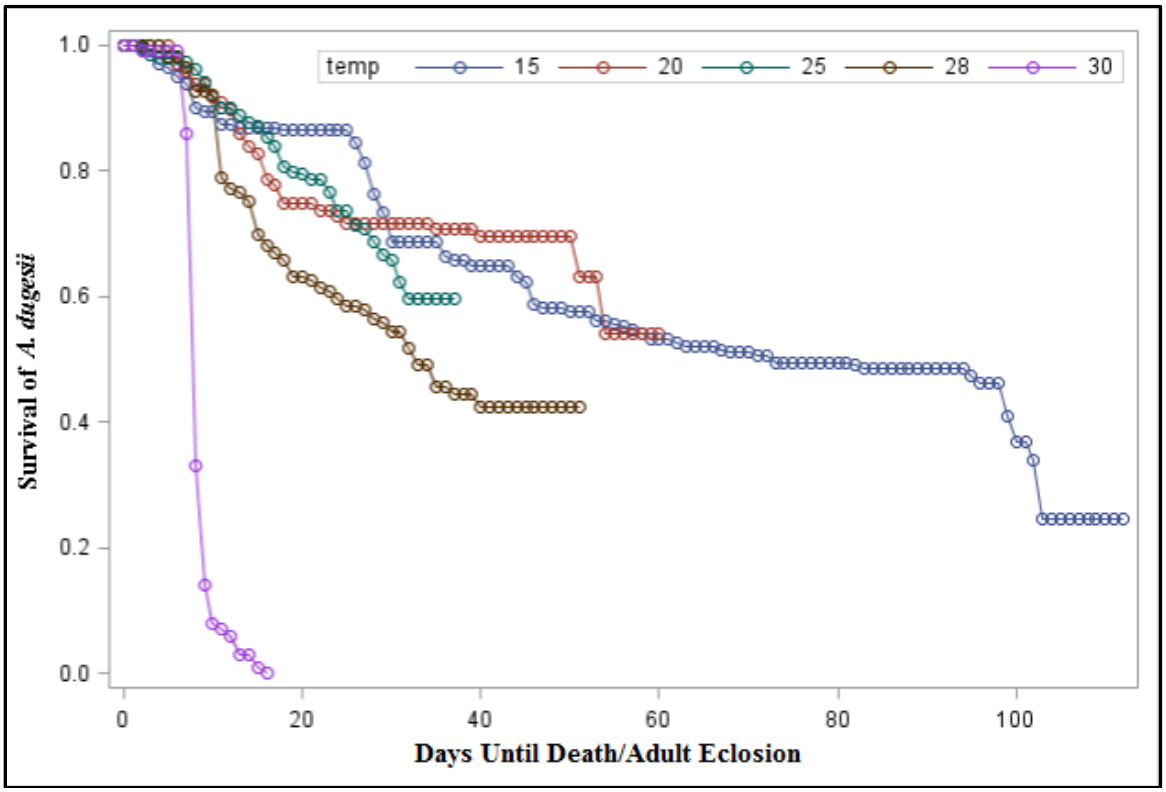

Figure 2.2 Age-specific survival curves from egg to either death or adult eclosion estimated (using the Kaplan-Meier method) of *Aluerodicus dugesii* at five constant temperatures.

2.4 Discussion

Temperature is one of the most critical habitat factors and frequent stressors that organisms encounter (Danks, 2007; Fisher et al., 2010), and ectotherms are particularly sensitive to temperature stress (Deutsch et al., 2008; Amarasekare and Savage, 2012; Paaijmans et al., 2013). A future increase in both mean temperatures and the occurrence of extreme temperature events is predicted due to the consequences of anthropogenic climate change (Easterling et al., 2000; Meehl and Tebaldi, 2004). Understanding the effects of temperature on ectotherms' behavioral, physiological, and molecular traits is critical for their management and conservation in current and future climate conditions (Deutsch et al., 2008; Kingsolver et al., 2013).

The thermal maximum observed for *A. dugesii* may explain its current geographic distribution and population expansion patterns in California. Non-coastal areas of southern California typically experience temperatures above 40°C making it difficult for *A. dugesii* to establish long-term populations. In these areas, *A. dugesii* can sometimes persist for long periods on smaller hosts well-shaded by adjacent structures, plants, or the basal portions of large hosts due to self-shading. With rising global temperatures and expected greater temperature variability, the current range of *A. dugesii* may be even more constrained to cooler areas. The observed optimal development temperature of 29°C being so close to the thermal maximum (30°C) observed for *A. dugesii* also suggests that it may be particularly sensitive to temperatures changes. These predictions are supported by field observations of inconsistencies in the presence of *A. dugesii* populations in inland sites compared to coastal sites in California (see **Chapter 5**)

particularly during the summer months during which temperatures frequently exceed the upper developmental threshold.

Increased duration and variation in *A. dugesii* development times observed at suboptimal temperatures may have significant impacts on predator-prey interactions (Costa and Kishida, 2015). For example, the observed increase in overall development times of different host stages at suboptimal temperatures may benefit some natural enemy species by lengthening the duration of the availability of susceptible host stages (He et al., 2005). The increased variance in development time may also synergize with increased developmental duration to further lengthen the overall time a specific stage is present in the field. The increased mortality rates observed at suboptimal temperatures however, may counteract the benefits of increased availability of suitable host stages on parasitoids' host acquisition success by reducing the total abundance and increasing the patchiness of *A. dugesii* in the field. Given that temperature is one of the primary factors influencing foraging performance of natural enemies as well (Fournier and Boivin, 2000; Amat et al., 2006), it is difficult to predict what effect increased host-availability has on predator-prey dynamics in this system.

The goodness-of-fits of the three nonlinear models observed in this study were similar and did not indicate a clear "best" overall model unless looking at a particular stage or sex. Because of this, using the Brière-1 model for *A. dugesii* temperature-dependent developmental modelling is recommended. The rationale for selecting the Brière-1 model is its ease of calculation compared to the Lactin-2 and Brière-2 models due to having fewer parameters (Brière et al., 1999) and the Brière-1 model allows for the

direct calculation of T_{opt} instead of graphically estimating this value as required by the Lactin-2 model.

The lowest survival rates observed in the crawler stage can be attributed primarily to individuals falling off the leaf during the process of searching the leaf for a permanent spot to settle and feed (pers. obs. EN. Schoeller). High mortality was also observed during the molting process into the next instar where nymphs had a greater chance to fall from the leaf or die due to abiotic conditions during the formation of a new cuticle. This seemed less likely during each subsequent molt as nymphs greatly increased in size. Finally, there appeared to be increased mortality during the adult emergence phase at high temperatures if individuals took too long to eclose and begin to feed.

The difference observed in development times between sexes was greatest at low temperatures. The 10 day lag in female emergence at 15°C may significantly affect the OSR of *A. dugesii* populations exposed to these conditions by leaving males without suitable mates for long periods of time, and males may even die before ever mating. The OSR can have significant effects on insect reproduction and fitness (Gao and Kang, 2006; Chuche and Thiéry, 2012) and potential changes in population dynamics driven by changes in OSR in this species requires further investigation.

Thermal tolerance and survivorship patterns observed for *A. dugesii* is similar to those reported for related species such as Spiraling Whitefly (*A. dispersus*) Russell, which was observed to have a development range of 12-32°C and high mortality at suboptimal temperatures (Cherry, 1979; Wen et al., 1994). Unlike *A. dugesii*, the range of *A. dispersus* has been limited to the southern tip of Florida due to its apparent inability to

cope with low temperatures (Cherry, 1979). A much more recent invader to the U.S., Rugose Spiraling Whitefly, *A. rugioperculatus* Martin, has been established in Florida since 2009. This species has caused similar problems to those experienced with *A. dugesii* (Francis et al., 2016). At the time of this study, very little is also known about the developmental biology of *A. rugioperculatus*, and the results presented here may provide a foundation for managing *A. rugioperculatus* as well as other invasive *Aleurodicus* species in the future.

The increased attention paid to physiologically based approaches in population modeling highlights the importance of these types of data for developing pest control strategies (Hodkinson, 1999). Results from this study provide a foundation for understanding the biology of *A. dugesii*, which can be used to enhance control strategies. For ornamental plants moving susceptible host species away from shaded areas such as structures or larger adjacent plants may lead to an increased temperature within the canopy. In full-sun *A. dugesii* populations appear to be restricted to the lower portion of the canopy of infested plants (pers. obs. EN. Schoeller), potentially facilitating population management. Regular pruning of ornamentals may also achieve the same desired effect by increasing interior canopy temperatures. In agricultural settings decreasing crop densities may result in increased canopy temperatures due to reduced transpiration resulting from lower soil moisture content (Tormann, 1986; Yang et al., 2014), which may potentially restrict the total infested surface area of the crop facilitating pest control. Given the overall high mortality rates for *A. dugesii* observed at suboptimal temperatures, these control tactics may be most effective in regions where temperatures frequently

exceed the upper lethal limit observed. It has yet to be determined how temperature affects natural enemies of *A. dugesii* and future work must be done to determine its impact prior to revising current biological control programs.

2.5 Literature Cited

Amat, I., Castelo, M., Desouhant, E., Bernstein, C. 2006. The influence of temperature and host availability on the host exploitation strategies of sexual and asexual parasitic wasps of the same species. Oecologia 148:153–161.

Bellows, TS., Jr., Kabashima, JN., Robb, KL. 2002. Giant Whitefly. UCANR Publ. 7400.

Bellows, TS., Jr., Meisenbacher, C. 2000. Biological control of giant whitefly, *Aleurodicus dugesii*, in California. California Conference on Biological Control II, The Historic Mission Inn Riverside, California, USA, 11-12 July, 2000, pp. 113–116.

Bowler, K., Terblanche, JS. 2008. Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? Biol. Rev. Camb. Philos. 83:339–355.

Burnham, KP., Anderson, DR. 2004. Multimodel inference—understanding AIC and BIC in model selection. Sociol. Methods Res. 33:261–304.

Briére, JF., Pracos, P., Le Roux, AY., Pierre, JS. 1999. A novel rate model of temperature-dependent development for arthropods. Environ. Entomol. 28:22–29.

Campbell, A., Frazier, BD., Gilbert, N., Gutierrez, AP., MacKauer, M. 1974. Temperature requirements of some aphids and their parasites. J. Appl. Ecol. 11:431–438.

Cherry, RH. 1979. Temperature tolerance of three whitefly species found in Florida. Environ. Entomol. 8:1150–1152.

Chuche, J., Thiéry, D. 2012. Egg incubation temperature differently affects female and male hatching dynamics and larval fitness in a leafhopper. Ecol. Evol. 2:732–739.

Costa, Z., Kishida, O. 2015. Nonadditive impacts of temperature and basal resource availability on predator–prey interactions and phenotypes. Oecologia. 178:1215–1225.

Dallas, HF., Ross-Gillespie, V. 2015. Sublethal effects of temperature on freshwater organisms, with special reference to aquatic insects. Water SA. 41:712–726.

Danks, HV. 2007. The elements of seasonal adaptations in insects. Can. Entomol. 139:1–44.

DeBach, P., Rose, M. 1976. Biological control of woolly whitefly. Calif. Agric. 30:4–7.

Deutsch, CA., Tewksbury, JJ., Huey, RB., Sheldon, KS., Ghalambor, CK., et al. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proc. Natl. Acad. Sci. USA. 105:6668–6672.

Easterling, DR., Meehl, GA., Parmesan, C., Changnon, SA., Karl, TR., Mearns, LO. 2000. Climate extremes: observations, modeling, and impacts. Science. 289:2068–2074.

Elliott. AC., Hynan, LS. 2011. A SAS® macro implementation of a multiple comparison post hoc test for a Kruskal-Wallis analysis. Comput. Meth. Prog. Bio. 102:75–80.

Emlen, ST., Oring, LW. 1977. Ecology, sexual selection, and the evolution of mating systems. Science. 197:215–223.

Evans, GA. 2008. The whiteflies (Hemiptera: Aleyrodidae) of the world and their host plants and natural enemies. Available online at: http://www.sel.barc.usda.gov: 8080/1WF/World-Whitefly-Catalog.pdf.

Fischer, K., Dierks, A., Franke, K., Geister, TL., Liszka, M., Winter, S., Pflicke, C. 2010. Environmental effects on temperature stress resistance in the tropical butterfly *Bicyclus Anynana*. PLoS ONE. 5:e15284.

Fournier, F., Boivin, G. 2000. Comparative dispersal of *Trichogramma evanescens* and *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) in relation to environmental conditions. Environ. Entomol. 29:55–63.

Francis, AW., Stocks, IC., Smith, TR., Boughton, AJ., Mannion, CM., Osborne, LS. 2016. Host plants and natural enemies of rugose spiraling whitefly (Hemiptera: Aleyrodidae) in Florida. Fla. Entomol. 99:150–153.

Freund, RJ., Littell, RC. 1986. SAS System for Regression, 1986 Edition, Cary, NC: SAS Institute Inc.

Gao, Y., Kang, L. 2006. Operational sex ratio and alternative reproductive behaviours in Chinese bushcricket, *Gampsocleis gratiosa*. Ethology. 112:325–331.

Gill, RJ. 1992. Giant whitefly. In: California Plant Pest and Disease Report. Calif. Dept. Food & Agric. 11:78–81.

Goolsby, JA., De Barro, P., Kirk, AA., Sutherst, R., Ciomperlik, M., Ellsworth, P., Gould, J., Hoelmer, K., Naranjo, S., Rose, M., Roltsch, W., Ruiz, R., Pickett, C., Vacek, D. 2005. Post-release evaluation of the biological control of *Bemisia tabaci* biotype "B" in the USA and the development of predictive tools to guide introductions for other countries. Biol. Control. 32:70–77.

He, XZ., Wang, Q., Teulon, DAJ. 2005. Host stage preference and reproductive fitness of *Aphidius eadyi* (Hymenoptera: Aphidiidae) on *Acyrthosiphon pisum* (Hemiptera: Aphididae). New Zeal. J. Agr. Res. 48:157–163.

Hodges, G. 2004. Giant Whitefly, *Aleurodicus dugesii* Cockerell, in Florida. Pest Alert, Division of Plant Industry, Florida Department of Agriculture and Consumer Service. Available online at: http://www.freshfromflorida.com/pi/pest-alerts/aleurodicus-dugesii.html

Hodkinson, ID. 1999. Species response to global environmental change or why ecophysiological models are important: a reply to Davis *et al*. J. Animal Ecol. 68:1259–1262.

Hoffmann, AA., Chown, SL., Clusella-Trullas, S. 2013. Upper thermal limits in terrestrial ectotherms: how constrained are they? Funct. Ecol. 27:934–949.

Heu, RA., Nagamine, NT., Kumashiro, BR., Watanabe, TM. 2004. Giant whitefly, *Aleurodicus dugesii* Cockerell (Homoptera: Aleyrodidae). Hawaii Dept. Agr. New Pest Advis. No. 02-04. June 2004 (revision). Available online at: http://hawaii.gov/hdoa/pi/ppc/npa-1/npa02-04-giantwf.pdf.

Huey, RB., Stevenson, RD. 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. Am. Zool. 19:357–366.

Jetter, K., Klonksy, K., Pickett, CH. 1997. A cost/benefit analysis of the ash whitefly biological control program in California. J. Arboric. 23:65–72.

Kingsolver, JG., Diamond, SE., Buckley, LB. 2013. Heat stress and the fitness consequences of climate change for terrestrial ectotherms. Funct. Ecol. 27:1415–1423.

Kvålseth, TO. 1985. Cautionary note about R^2. Am. Stat. 39:279–285.

Lactin, DJ., Holliday, NJ., Johnson, DL., Craigen, R. 1995. Improved rate model of temperature-dependent development by arthropods. Environ. Entomol. 24:68–75.

Lasalle, J., Polaszek, A., Noyes, JS., Zolnerowich, G. 1997. A new whitefly parasitoid (Hymenoptera: Pteromalidae: Eunotinae), with comments on its placement, and implications for classification of Chalcidoidea with particular reference to the Eriaporinae (Hymenoptera: Aphelinidae). Syst. Entomol. 22:131–150.

Logan, JA., Wollkind, DJ., Hoyt, SC., Tanigoshi, LK. 1976. An analytic model for description of temperature dependent rate phenomena in arthropods. Environ. Entomol. 5:1133–1140.

Matkin, OA., Chandler, RA. 1957. The U.C. soil mixes. *In*: Baker, KF. (Ed.) The U.C. System for producing healthy container-grown plants through the use of clean soil, clean stock, and sanitation. Calif. Agr. Exp. Sta. Serv. Manual 23, Berkeley, CA. pp. 68-85.

Meehl, GA., Tebaldi, C. 2004. More intense, more frequent, and longer lasting heat waves in the 21st century. Science. 305:994–997.

Muniappan, R., Shepard, BM., Watson, GW., et al. 2009. New records of invasive insects (Hemiptera: Sternorrhyncha) in southern Asia and West Africa. J. Agr. Urban Entomol. 26:167–174.

Nielsen, AL., Hamilton, GC., Matadha, D. 2008. Developmental rate estimation and life table analysis for *Halyomorpha halys* (Hemiptera: Pentatomidae). Environ. Entomol. 27:348–355.

Nguyen, R., Hamon, AB. 2002. Biological control of giant whitefly, *Aleurodicus dugesii* Cockerell, in Florida. Fla. Dept. Agr. Cons. Serv. Available online at: http://www.doacs.state.fl.us/pi/methods/giant-whitefly-bc.html.

Paaijmans, KP., Heinig, RL., Seliga, RA., Blanford, JI., Blanford, S., Murdock, CC., Thomas, MB. 2013. Temperature variation makes ectotherms more sensitive to climate change. Glob. Change Biol. 19:2373–2380.

Roy, M., Brodeur, J., Cloutier, C. 2002. Relationship between temperature and developmental rate of *Stethorus punctillum* (Coleoptera: Coccinellidae) and its prey *Tetranychus mcdanieli* (Acarina: Tetranychidae). Environ. Entomol. 31:177–187.

Sampson, WW., Drews, EA. 1941. Fauna Mexicana IV. A Review of the Aleyrodidae of Mexico (Insecta, Homoptera). An. Esc. Cienc. Biol. Mex. 2:143–189.

Schoolfield, RM., Sharpe, PJH., Magnuson, CE. 1981. Nonlinear regression of biological temperature-dependent rate models based on absolute reaction-rate theory. J. Theor. Biol. 88:719–731.

Shi, P., Ge, F., Sun, Y., Chen, C. 2011. A simple model for describing the effect of temperature on insect developmental rate. J. Asia Pac. Entomol. 14:15–20.

Sharpe, PJH., Demichele, DW. 1977. Reaction kinetics of poikilotherm development. J. Theor. Biol. 64:649–670.

Sokal, RR., Rohlf, FJ. 2012. Biometry: The Principles and Practice of Statistics in Biological Research, 4th ed. Freeman, New York, NY.

Tormann, H. 1986. Canopy temperature as a plant water stress indicator for nectarines. S. Afr. J. Plant Soil. 3:110–114.

Wen, H-C., Hsu, T-C., Chen, C-N. 1994. Effects of temperature on the development, adult longevity, activity and oviposition of the spiralling whitefly, *Aleurodicus dispersus* Russell (Homoptera: Aleyrodidae). Chin. J. Entomol. 14:163–172.

Yang, G-Z., Luo, X-J., Nie, Y-C., Zhang, X-L. 2014. Effects of plant density on yield and canopy micro environment in hybrid cotton. J. Int. Agric. 13:2154–2163.

Zar, JH. 1996. Biostatistical Analysis 3rd Edition. Prentice Hall, Englewood Cliffs, NJ.

Chapter 3

Host Stage Preferences of Parasitoids of the Giant Whitefly *Aleurodicus dugesii* (Hemiptera: Aleyrodidae).

3.1 Introduction

Multiple species of parasitoids are frequently observed attacking a single host species (Price, 1971; Hawkins and Mills, 1996; Derocles et al., 2015), and an individual host developmental stage may be utilized by multiple parasitoids (Price, 1972; Karamaouna and Copland, 2000; Forbes et al., 2010). Competitive interactions between parasitoid species are largely driven by resource competition (Godfray, 1994; Quicke, 1997; Harvey et al., 2014). Theory predicts that competitive exclusion can occur in systems where densities of unparasitized hosts are suppressed below a threshold where population growth of competitors occurs (Briggs, 1993). This prediction has been validated in some parasitoid-host systems, such as the California red scale *Aonidiella aurantii* and its parasitoids *Aphitis lignanensis* and *Aphitis melinus* (Murdoch et al., 1996). Despite theoretical work and evidence of competitive exclusion in the field, long-term coexistence between parasitoid species attacking a single host has also been shown to occur given that competition-dampening mechanisms exist; such as spatial (Rossi et al., 2006; Garcia-Medel et al., 2007; Harvey et al., 2014) and temporal (Wieber et al.,

1995) partitioning of hosts, or fine scale partitioning of stadia within developmental stages (Yu et al., 1990).

In the United States, three exotic parasitoid wasps were introduced in the mid-1990's as part of a biological control program against the invasive giant whitefly *Aleurodicus dugesii* Cockerell (Hemiptera: Aleyrodidae) (Bellows et al., 2002). These include *Encarsia noyesi* Hayat (Hymenoptera: Aphelinidae), *Idioporous affinis* LaSalle & Polaszek (Hymenoptera: Pteromalidae), and *Entedononecremnus krauteri* (Hymenoptera: Eulophidae). The source population of *E. krauteri* originated from a single location in Comfort, Texas in 1995, and it was later distributed to other parts of the U.S. through mass rearing efforts (Zolnerowich and Rose, 1996). Source populations of *I. affinis* and *E. noyesi* were collected from the same locality in Guadalajara, Mexico in 1997 and subsequently mass reared for release in areas invaded by *A. dugesii* across the U.S (Bellows and Meisenbacher, 2000). Despite over 20 years of coexistence in the field, variation in the ability of these parasitoids to control *A. dugesii* populations at manageable levels and differences in the spatial and temporal composition of parasitoid communities in southern California has been observed (per. obs. EN Schoeller). These observations have raised questions regarding the factors underlying the source(s) of this variation in order to find potential methods to enhance ongoing biological control efforts against *A. dugesii*. All three parasitoid species in this study system are solitary endoparasitoids that parasitize the nymphal stages of *A. dugesii*, so examining the role of interspecific resource competition was a logical starting point to this investigation.

Laboratory host specificity and preference tests have proven to be a critical tool for understanding the dynamics of parasitoid interactions (Sands and Van Driesche, 2000; Mansfield and Mills, 2004; Murray et al., 2010) and can assist in selecting suitable biological control agents for release (Mackauer, 1990; Bográn and Heinz, 2002; Irvin and Hoddle, 2005; Waterworth et al., 2015). The goal of this study was to examine the host stage preferences for each of the three parasitoid species in this system. These data will provide insight into the ability of each parasitoid to control densities of *A. dugesii* and may help predict the outcomes of interspecific competitive interactions. Given the observations in the field, I hypothesized that host use overlap exists between parasitoid species in this system and that parasitoids exhibit similar host stage preferences.

3.2 Materials and Methods

Host stage preferences of *I. affinis*, *E. noyesi*, and *E. krauteri* were examined using choice and no-choice tests. No-choice tests consisted of parasitoids exposed to a single *A. dugesii* nymphal stage. Paired-choice tests allowed parasitoids to choose their most preferred host stage from different pairwise combinations of the four nymphal stages. Treatments in paired-choice tests consisted of parasitoids exposed to all possible pairwise combinations of two instars (1^{st} versus 2^{nd}, 1^{st} versus 3^{rd}, 1^{st} versus 4^{th}, 2^{nd} versus 3^{rd}, 2^{nd} versus 4^{th}, and 3^{rd} versus 4^{th}). Simultaneous-choice tests consisted of all four instars offered to parasitoids at once. For each parasitoid species, 20 trials were performed for each host stage(s) treatment in paired-choice, simultaneous-choice, and no-choice tests.

Single stage cohorts for no-choice tests were prepared by infesting the abaxial surfaces of 6-month old *Hibiscus rosa-sinensis* leaves with adult whitefly from laboratory colonies by placing 10–15, 72-h old, mated females in small mesh bags enclosing the leaves for 6 h to ensure stage uniformity. Infested plants were placed into environmental chambers and maintained at 25°C ± 0.5°C (16L:8D photoperiod, 75±10% RH). The whitefly nymphs were checked daily until a sufficient number had reached the desired developmental stage. The density of the nymphs was then manipulated so that each leaf contained the desired number of individuals of each developmental stage.

Leaves containing multiple stadia used in choice tests were prepared by initiating single stage cohorts as described above, and then unsettled crawlers from donor leaves were transferred onto the experimental leaf with a fine camel hair brush. Crawler transfer was initiated at various times post-initial cohort establishment, so that the desired combination of developmental stages was achieved. In choice tests, 10 individuals of each instar were offered to parasitoids for the two combination treatments, while in the simultaneous treatment five individuals of each instar were offered.

Host stage preference behavior of female parasitoids was observed under a dissecting microscope at 120x magnification. Observational arenas for both choice and no-choice tests consisted of modified Munger cells (Morse, et al., 1986) with 3.2 cm diameter arenas placed over the leaf area containing the nymphs. For *E. noyesi* and *I. affinis* arenas were placed over the abaxial leaf surface where these species forage. To observe *E. krauteri* host selection behavior, arenas were placed over the adaxial leaf surface where the nymphs were located below, as *E. krauteri* parasitizes *A. dugesii*

nymphs through the leaf surface. The position of each nymph on the leaf was hand-drawn on paper and then assigned a unique identification number to assist in tracking parasitism rates. A small ink dot was placed on the upper leaf surface near the location of nymphs on the underside of the leaf to record their location in *E. krauteri* trials. Pilot trials showed that ink dots did not influence the searching behavior of females.

Single mated female parasitoids (48–72 h old) were introduced into the arena using a 100 µl micropipette tip inserted into holes positioned on opposite sides of the Munger cells. The hole used to introduce females was rotated between each trial to help control for directional bias. Females were allowed to forage for 1 h and observations were made between 13:00 and 17:00 hours daily. Each time a female encountered a host, the time, identity (unique number), and whether or not it was accepted or rejected for parasitism was recorded. The female was watched carefully and the time she disengaged the host after either rejection or oviposition was recorded.

Pilot trials were conducted to assess the general suitability of each nymphal stage for parasitism for each parasitoid species prior to running experimental trials. Trials containing nymphal stages suitable for parasitism were discarded if females did not parasitize any hosts within the first 10 minutes of observations or if there was more than 30 minutes of inactivity to control for unmotivated females. Trials containing hosts unsuitable for parasitism as suggested by the pilot trials presented either singly in no-choice tests or paired with other unsuitable stages in paired-choice tests were allowed to run for the full duration in order to observe potentially rare parasitism events. Although parasitism was not directly verified via nymphal dissection after the completion of trials,

preliminary tests showed that over 95% of nymphs were parasitized when oviposition lasted for more than 20 seconds, so all nymphs were considered parasitized if accepted by a female.

Parasitism rates are not always an effective measure of preference (Murray et al. 2010), so multiple metrics were assessed when possible to assess each parasitoid species' innate host preference. In paired-choice and no-choice tests stage-specific parasitism rates were calculated. In choice tests where all stages were offered simultaneously relative preferences and differences in the frequency of the first stage attacked were examined. In no-choice tests stage-specific handling times were compared in addition to parasitism rates.

Data Analysis

All statistical analyses were performed using SAS ver. 9.4 (SAS Institute Inc. 2013) and a significance level of $\alpha = 0.05$ was used for all analyses. Parasitism rates were calculated as the total number of unique nymphs parasitized divided by the total number available of a particular development stage over the 1 h observation period. Prior to analyzing no-choice tests, data were arcsine square-root transformed in order to meet the underlying assumptions of normality and equality of variances. The 1st instar treatment for *E. noyesi* and 1st and 2nd instar treatments for *E. krauteri* were dropped from the no-choice analyses due to no parasitism being observed for these stages. One-way ANOVA were performed on the *E. noyesi* parasitism rate data, followed by a post-hoc test to compare differences in treatment means. Data transformation did not remedy issues with heteroscedasticity with the *I. affinis* data, so Welch's ANOVA was performed followed

by a Games-Howell post-hoc test. A two sample t-test was performed for *E. krauteri* no-choice test data, due to only the 3rd and 4th instars being parasitized.

Parasitism rates in paired-choice tests were analyzed on the mean difference between each host stage combination using paired t-tests. The Wilcoxon signed-rank test was used for paired-choice test data when the assumption of normality of the distribution of paired differences was not met. Data from the *E. krauteri* 1st vs. 2nd instar paired-choice test was not analyzed as no parasitism was observed in either of these treatments.

For choice tests with all four *A. dugesii* instars offered simultaneously, relative host stage preferences were calculated using the ratio of the number of individuals parasitized for each stage divided by the total number of each stage present (Lockwood, 1998; Hougardy and Mills, 2008). These values were then standardized so that their sum totaled one (Lockwood, 1998). The null hypothesis was that no host stage preference existed, represented by expected relative expected preferences of 0.25 for each host stage. The proportional preferences were analyzed using Hotelling's T^2 and 95% confidence intervals were calculated around the mean proportional preferences using the PROC IML procedure. To control for experiment-wise error a Bonferroni correction was applied to the confidence intervals. Confidence intervals which contained zero indicated that there was no preference exhibited by female parasitoids between stages in that comparison (Lockwood, 1998). Differences in the first host stage parasitized were analyzed in the simultaneous choice tests using Fisher's exact tests. An equal probability of choice was assumed for the four hosts with an expected frequency of 5 individuals chosen first for each stage.

No-choice tests were used to examine differences in handling times between stages as we were only interested in differences in innate handling times exhibited between stages and not how handling times for a specific nymphal stage may differ in response to the presence of another stage. Handling times were compared between stages for each parasitoid species using a generalized linear model with handling time as the dependent variable and host stage, outcome (accepted or rejected for parasitism), and a stage*outcome interaction term as the explanatory variables. The interaction term was included to examine whether there was a larger than expected change in handling times when hosts were accepted or rejected for parasitism. Data were transformed using the natural logarithm prior to analyses to meet normality and variance homogeneity assumptions.

3.3 Results

Stage First Parasitized

Results from first stage parasitized observations during the simultaneous-choice tests showed clear differences in the frequencies of the first stage attacked according to Fisher's Exact Tests. A total of 4, 13, 2, and 1 individual(s) of the 4^{th}, 3^{rd}, 2^{nd}, and 1^{st} instars respectively were parasitized first by *I. affinis* and the observed frequencies differed significantly from the null hypothesis of equal probability of parasitism (X^2 = 18.0, df = 3, P < 0.0001). The observed frequency of first parasitism by *E. noyesi* also differed significantly from an equal probability of parasitism (X^2 = 45.2, df = 3, P < 0.0001), with 18, 2, 0, and 0 individual(s) of the 4^{th}, 3^{rd}, 2^{nd}, and 1^{st} instars respectively being parasitized first. As observed with the other two parasitoid species, the frequency

of first stage parasitized differed significantly from an equal probability of parasitism (X^2 = 39.6, df = 3, P < 0.0001) for *E. krauteri* with 17, 3, 0, and 0 individual(s) of the 4th, 3rd, 2nd, and 1st instars respectively parasitized first. These results support the findings of the preference tests indicating preference for the 4th instar by *E. noyesi* and *E. krauteri*, and for the 3rd instar by *I. affinis.*

Parasitism Rates and Relative Preferences

Choice Tests: *Idioporus affinis*

All four *A. dugesii* nymphal stages were successfully parasitized by *I. affinis.* There was a significant effect of stage on parasitism rates exhibited by *I. affinis* in paired-choice tests (**Figure 3.1a**). The combined findings of the paired-choice tests suggested that *I. affinis* exhibited a preference hierarchy for specific stages. The results suggest that the 3rd instar is the most preferred stage for parasitism (3rd vs. 1st: t = 6.92, P < 0.0001; 3rd vs. 2nd: t = 3.21, P = 0.0046; 3rd vs. 4th: t = 3.03, P = 0.0069), followed by the 4th instar (4th vs. 2nd: t = 5.35, P < 0.0001; 4th vs. 1st: t = 2.42, P = 0.026), 2nd instar (2nd vs. 1st: t = 3.38, P = 0.0032), and the 1st instar being least preferred.

Choice tests where all *A. dugesii* nymphal stages were offered simultaneously produced different results to those observed in the paired choice tests (**Figure 3.2a**). A significant difference was observed in the relative preferences of the different *A. dugesii* nymphal stages exhibited by *I. affinis* (T^2 = 153.74, $F_{3,17}$ = 45.85, P < 0.0001). The 95% Bonferonni confidence intervals showed differences in relative stage preferences, with the 1st instar being the least preferred and the 3rd instar being more preferred than the 2nd

instar. No differences in relative preferences between the 3rd and 4th or 2nd and 4th instars were observed.

Choice Tests: *Encarsia noyesi*

Paired-choice tests showed a significant effect of *A. dugesii* nymphal stage on *E. noyesi* parasitism (**Figure 3.1b**). Results suggested a preference hierarchy, with the 4th instar being most preferred (4th vs. 3rd: $t = 2.41$, $P = 0.0263$; 4th vs. 2nd: $z = 3.95$, $P < 0.0001$; 4th vs. 1st: $t = 15.54$, $P < 0.0001$), followed by the 3rd (3rd vs. 2nd $t = 7.17$, $P < 0.0001$; 3rd vs. 1st $t = 16.58$, $P < 0.0001$) and 2nd instars (2nd vs. 1st $t = 12.22$, $P < 0.0001$). Unlike *I. affinis*, the 1st instar of *A. dugesii* was not parasitized by *E. noyesi* making it the least preferred stage.

Simultaneous-choice tests showed very similar results to the paired-choice tests (**Figure 3.2b**). Simultaneous-choice tests found a significant difference in the relative preferences of the different *A. dugesii* nymphal stages exhibited by *E. noyesi* ($T^2 = 65.49$, $F_{3,17} = 19.53$, $P < 0.0001$). Again, the 4th instar appeared to be the most preferred stage of *E. noyesi* followed by the 3rd, 2nd, and 1st instar as indicated by the 95% Bonferonni confidence intervals.

Choice Tests: *Entedononecremnus krauteri*

There was a significant effect of stage on *E. krauteri* parasitism rates observed in paired-choice tests (**Figure 3.1c**). The 4th instar appeared to be the most preferred stage (4th vs. 3rd: $t = 5.88$, $P < 0.0001$; 4th vs. 2nd: $t = 15.25$, $P < 0.0001$; 4th vs. 1st: $t = 15.25$, $P < 0.0001$) followed by the 3rd instar (3rd vs. 2nd $z = 3.46$, $P < 0.0001$; 3rd vs. 1st $z = 3.46$, $P < 0.0001$). The 1st and 2nd instars were not parasitized.

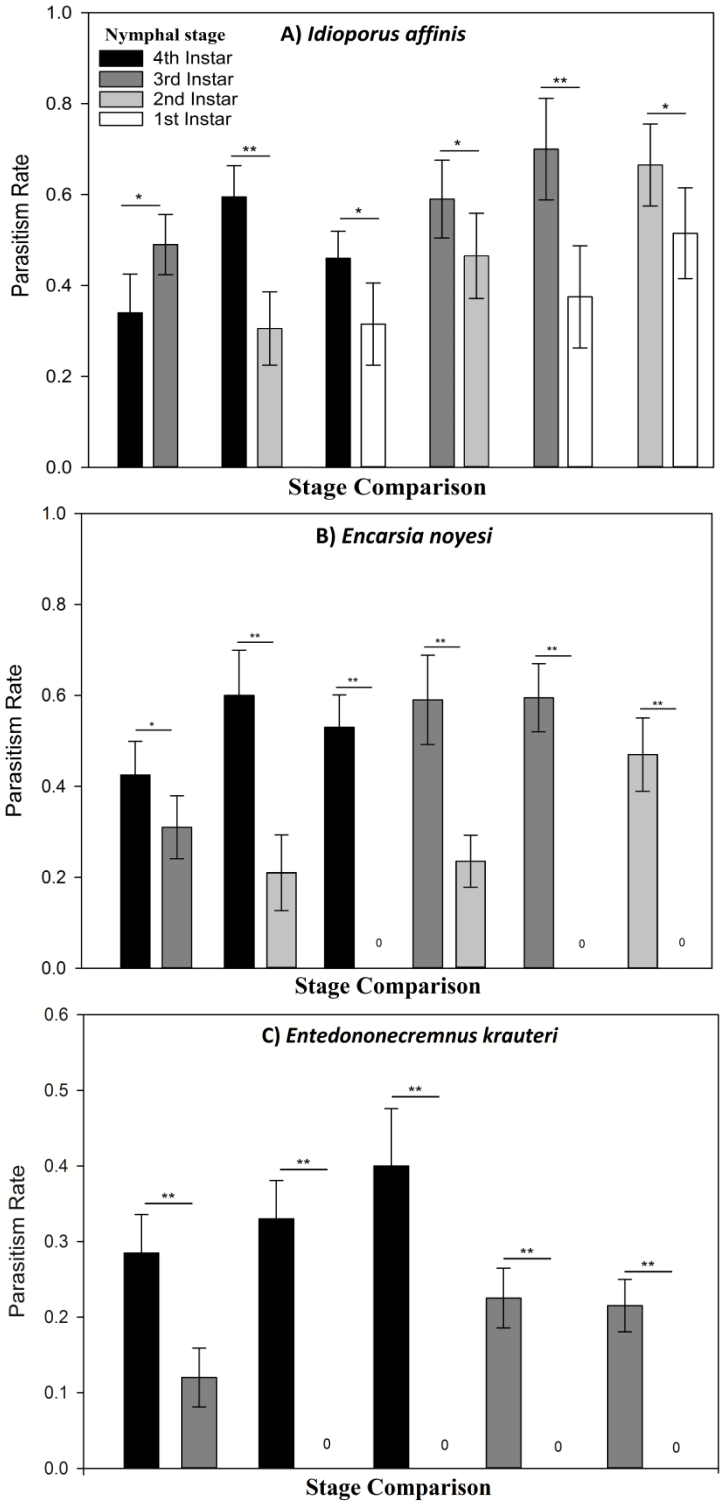

Figure 3.1 Mean (± 95% CI) parasitism rates of (**A**) *Idioporus affinis*, (**B**) *Encarsia noyesi*, and (**C**) *Entedononecremnus krauteri* in paired-choice tests of different *Aleurodicus dugesii* nymphal stages. Paired bars in each graph with an "*" indicate a significant difference at the $P \leq 0.05$ level and "**" indicates significance at $P < 0.0001$ in paired t-tests.

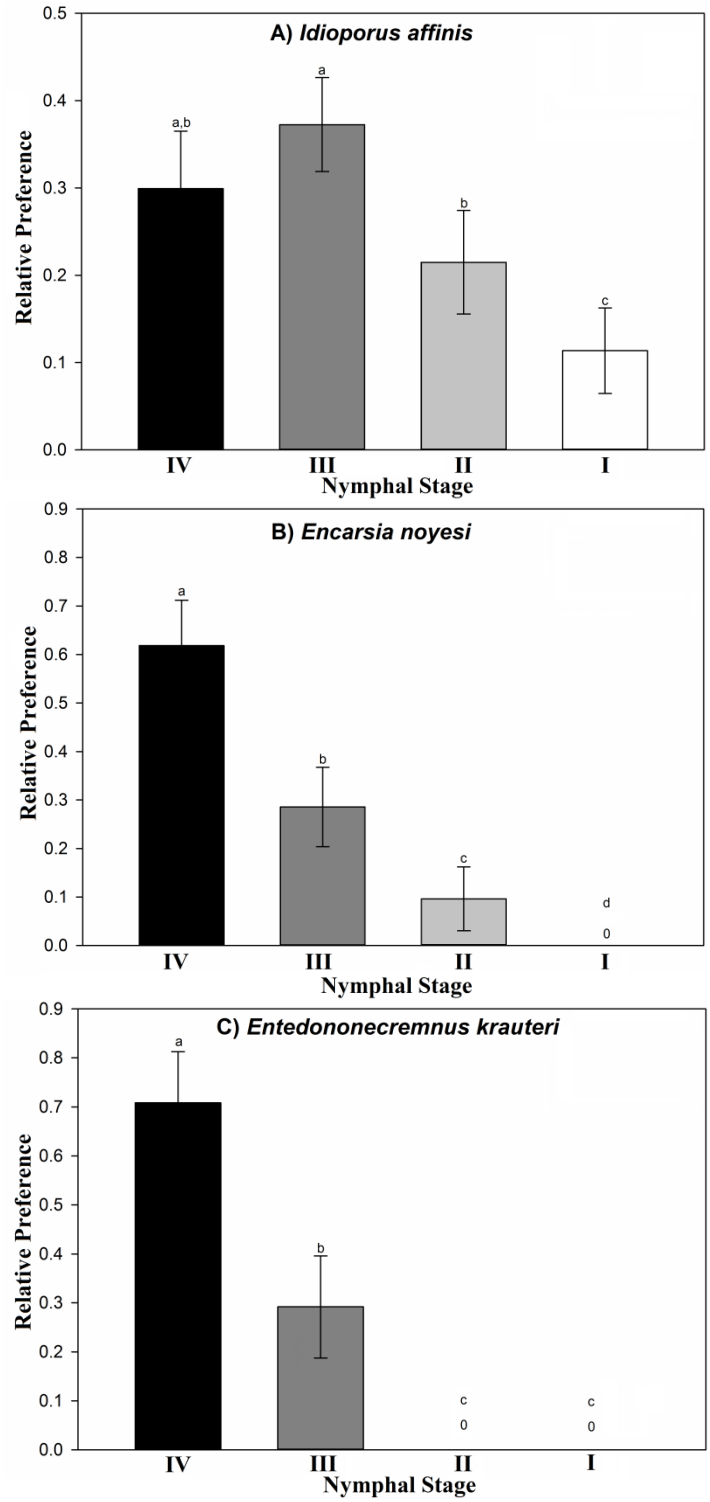

Figure 3.2 Mean (± 95% CI) relative stage preferences of (**A**) *Idioporus affinis*, (**B**) *Encarsia noyesi*, and (**C**) *Entedononecremnus krauteri* in choice tests with all *Aleurodicus dugesii* nymphal stages offered simultaneously. Bars in each graph followed by the same letter indicate no significant differences (Hotelling's T^2, $\alpha = 0.05$).

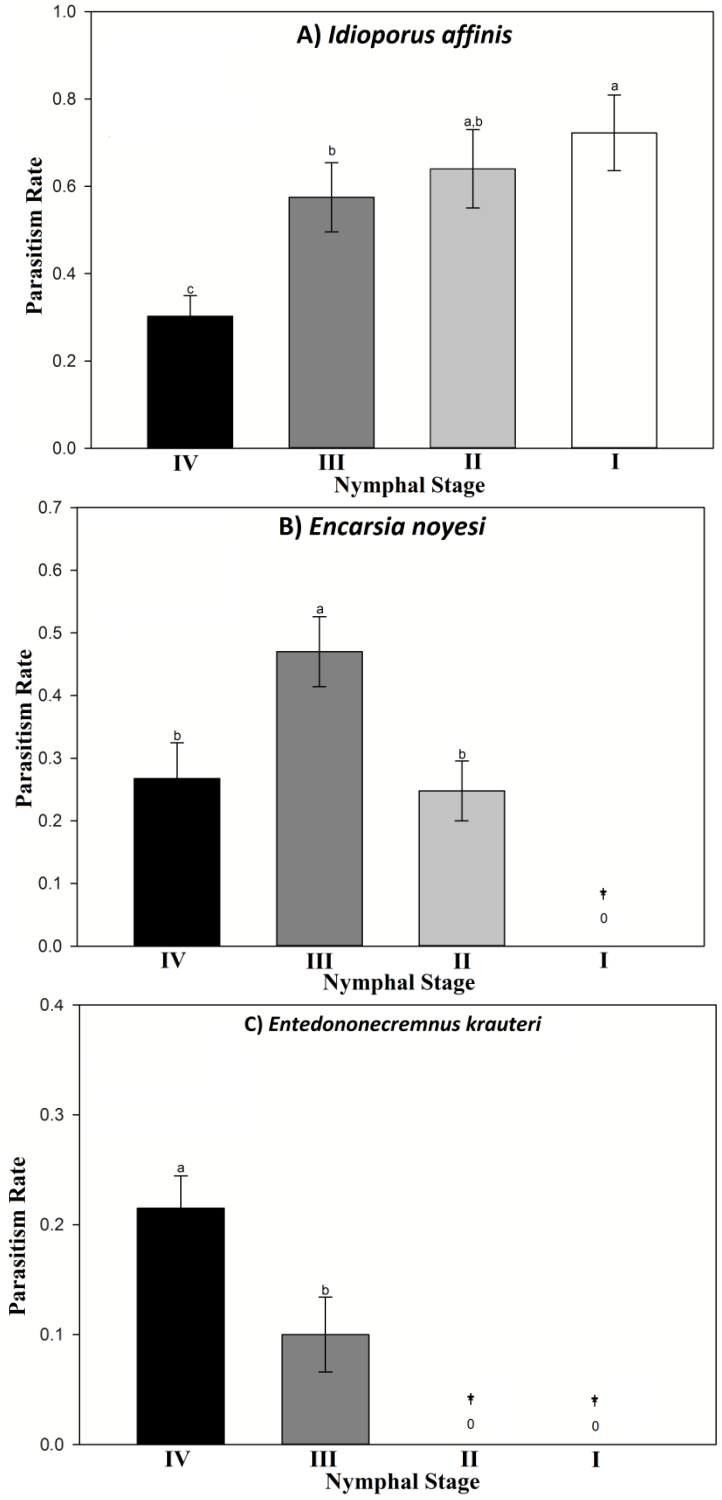

Figure 3.3 Mean (± 95% CI) parasitism rates of (**A**) *Idioporus affinis*, (**B**) *Encarsia noyesi*, and (**C**) *Entedononecremnus krauteri* in no-choice tests of different *Aleurodicus dugesii* nymphal stadia. Bars in each graph followed by the same letter indicate no significant differences (ANOVA, $\alpha = 0.05$). († Denotes instars that were no included in analyses due to treatments having zero variance).

Simultaneous-choice tests showed similar results to the paired choice tests (**Figure 3.2c**). A significant difference was observed in the relative preferences of the different *A. dugesii* nymphal stages exhibited by *E. krauteri* ($T^2 = 20.20$, $F_{3,17} = 6.03$, $P < 0.0055$). The 95% Bonferroni confidence intervals indicated that the highest relative preference for the 4th instar followed by the 3rd instar, and the 1st and 2nd instars not preferred at all.

No-Choice Tests: *Idioporus affinis*

The results from no-choice trails differed considerably from the results from the choice trials for *I. affinis* (**Figure 3.3a**). A significant effect of host stage on parasitism rate was observed (Welch's $F = 35.14$, $P < 0.0001$). Post-hoc analyses indicated a different host stage preference hierarchy than observed in the choice tests, with the 1st (4th vs. 1st: $P < 0.0001$; 3rd vs. 1st: $P < 0.0001$; 2nd vs. 1st: $P = 0.459$), and 2nd instars (4th vs. 2nd: $P < 0.0001$; 3rd vs. 2nd: $P = 0.642$) preferred the most, followed by the 3rd instar (4th vs. 3rd: $P < 0.0001$) and the 4th instar being least preferred.

No-Choice Tests: *Encarsia noyesi*

A significant effect of host stage on parasitism was observed ($F = 22.17$, $P < 0.0001$). Tukey's HSD test indicated that there were significant differences in parasitism rates between the 2nd and 3rd ($P < 0.0001$) as well as the 3rd and 4th instars ($P < 0.0001$), but not between the 2nd and 4th instars ($P = 0.8261$) (**Figure 3.3b**). While not explicitly tested, due to the absence of parasitism data for the 1st instar it is clear that this stage is preferred the least of the four nymphal stages. These results differ from the paired-choice

and simultaneous-choice trials, where the 4th instar appears to be the most preferred stage instead of the 3rd instar.

No-Choice Tests: *Entedononecremnus krauteri*

The results of the no-choice tests were similar to those observed in choice tests for *E. krauteri* (**Figure 3.3c**). No-choice tests showed a significant difference in parasitism rates between the 4^{th} and 3^{rd} instars ($t = 5.35$, $P < 0.0001$), with the 4^{th} instar being preferred over the 3^{rd} instar. As also seen in the choice tests, neither the 1^{st} nor the 2^{nd} instar was parasitized by *E. krauteri* in the no-choice tests.

Handling Times

Idioporus affinis

A significant effect of host stage on *I. affinis* handling times for both accepted ($F_{3,76} = 110.14$, $P < 0.0001$) and rejected ($F_{3,51} = 3.52$, $P = 0.0214$) hosts was observed. A trend for longer handling times was observed as instar age increased, with mean handling times increasing from 32 s for 1^{st} instars to 261 s for 4^{th} instars when hosts were accepted (**Figure 3.4a**). Mean handling times for rejected hosts showed a similar trend with handling times increasing from 6 s for 1^{st} instars to 22 s for 3^{rd} instars. A significant interaction effect between host stage and encounter outcome on handling times was observed for *I. affinis* ($F = 11.51$, $df = 3$, $P < 0.0001$). This indicated that the decrease in handling time observed between accepted and rejected hosts were not proportional across instars. This was apparent for the 4th instar, which *I. affinis* rejected as quickly as the 3^{rd} and 2^{nd} instars despite exhibiting the longest handling time for this stage when accepted for parasitism (**Figure 3.4a**).

Encarsia noyesi

A significant effect of host stage on handling times exhibited by *E. noyesi* was observed for accepted ($F_{2,57}$ = 23.07, P < 0.0001) but not rejected ($F_{2,42}$ = 1.82, P = 0.174) hosts. Unlike *I. affinis*, *E. noyesi* did not exhibit as strong of a trend for longer handling times as instar age increased (**Figure 3.4b**). Mean handling times of accepted hosts were 166, 151, and 314 s for the 2nd, 3rd, and 4th instars respectively. Mean handling times for rejected hosts were 12, 21, and 17 s for 2nd, 3rd, and 4th instars respectively. A significant interaction effect between host stage and encounter outcome on handling times was observed for *E. noyesi* (F = 4.43, df = 2, P = 0.014). Handling times observed for the 3rd instar between accepted and rejected individuals for parasitism appeared to not exhibit the same linear decline as observed for the other instars (**Figure 3.4b**).

Entedononecremnus krauteri

A significant effect of host stage on handling times exhibited by *E. krauteri* was observed for accepted ($F_{1,33}$ = 152.77, P < 0.0001) and rejected ($F_{1,17}$ = 26.43, P < 0.0001) hosts. Similar to *I. affinis*, *E. krauteri* exhibited longer handling times for late instars (**Figure 3.4c**). Mean handling times of accepted hosts were 194 s and 289 s for the 3rd and 4th instars respectively. Mean handling times for rejected hosts were 10 s and 18 s for the 3rd and 4th instars respectively. A significant interaction effect between host stage and encounter outcome on handling times was observed for *E. krauteri* (F = 6.94, df = 1, P = 0.0112). A larger decrease in handling times by *E. krauteri* when individuals were rejected for parasitism was observed for the 3rd instar compared to the 4th instar (**Figure 3.4c**).

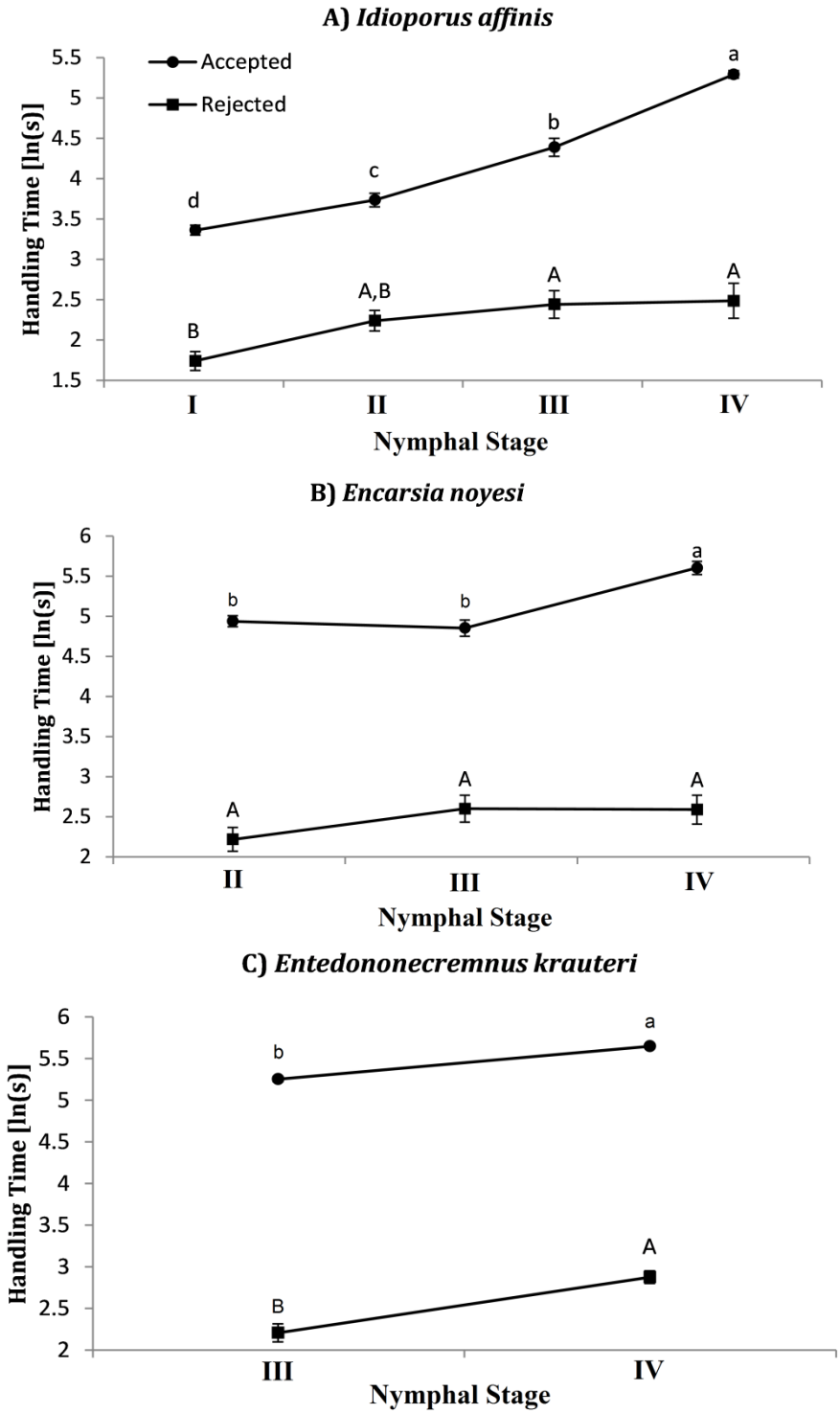

Figure 3.4 Mean (±SE) handling times ln(s) of *Idioporus affinis* (A), *Encarsia noyesi* (B), and *Entedononecremnus krauteri* (C) for *Aleurodicus dugesii* nymphal stadia accepted and rejected for parasitism. Within accepted/rejected treatments stages with different letters indicate a significant difference ($\alpha = 0.05$) in handling times.

3.4 Discussion

The literature has shown that the release of multiple species of biological control agents can have a positive, negative, or neutral effect on prey suppression, and that the outcome is often context dependent (Straub et al., 2008; Tylianakis and Romo, 2010). When functional redundancy exists between species (e.g. shared feeding niches) the outcome of releasing multiple biological control agents more likely leads to negative or neutral effects on prey suppression due to interspecific competition negating the overall positive effects of natural enemy diversity (Casula et al., 2006). Partitioning of the host resource can lead to niche complementarity between natural enemy species, but it is often difficult to predict the degree of niche complementary required to enhance prey suppression, as both natural enemy and prey-specific traits can influence the trophic interactions between biological control agents (Wilby et al., 2005). Continued research is needed to further develop the theoretical basis for multi-predator effects on the outcome of biological control programs.

The results from this study support the hypothesis that host use overlap exists between parasitoid species in this system, but the hypothesis that parasitoids exhibit similar preferences for *A. dugesii* nymphal stages was only partially supported. While host use overlap was observed, there were differences in preference hierarchies between parasitoid species. The broadest host use breadth was observed for *I. affinis*, which successfully utilized all four *A. dugesii* nymphal stages. The narrowest host use breadth was observed for *E. krauteri*, which only parasitized the 3rd and 4th instars. *Encarsia noyesi* exhibited an intermediate host use breadth parasitizing all stages except the 1st

instar. All three parasitoids exhibited the highest preference for either the 3rd or 4th instar of *A. dugesii*.

As seen frequently in other host preference studies (Babendreier et al., 2005) a general consensus in the results between choice and no-choice tests was observed for each parasitoid species; however there were some inconsistencies. The conflicting results for host stage preferences of *I. affinis,* and to a lesser extent *E. noyesi,* observed for no-choice tests compared to choice tests may be due to differences in the number of hosts encountered during the observational period. For example, in no-choice trails *I. affinis* encountered an average (± SE) of 27.4 ± 2.7, 32.1 ± 3.3, 25.9 ± 2.9, and 9.4 ± 0.8 individuals/hr for the 1st, 2nd, 3rd, and 4th instars respectively resulting in the increased level of parasitism observed for the younger instars. These differences are likely driven by shorter handling times observed by *I. affinis* for younger instars (up to an 8x difference between 1st and 4th instars) allowing for more individuals to be encountered during the observation period. I hypothesize that the increased handling time observed for older instars may be related to thickness of the dorsal cuticle, making it take longer for females to penetrate the cuticle and deposit eggs. The ovipositors of *E. noyesi* and *E. krauteri* are long, at least ½ the length of the gaster, while the ovipositor of *I. affinis* is very small and approximately ¼ the length of the gaster (Lasalle et al., 1997). The small ovipositor of *I. affinis* may explain the more apparent increase in handling times for older instars than observed for *E. noyesi* and *E. krauteri* if this hypothesis is valid.

Given the host use breadths and preference hierarchies observed, in the absence of other potential factors mediating competition, I predict that *I. affinis* may be under the

least competitive pressure for hosts in this system due to its ability to utilize earlier stages of *A. dugesii* than *E. noyesi* and *E. krauteri*. Parasitoids that can utilize earlier host stages than competitors have been shown to possess a competitive advantage in resource competition (Murdoch et al., 1996; Wang et al., 2003; Teder et al., 2013) unless previously attacked host stages can be used by other species (Briggs, 1993). With the exception of *E. noyesi* (discussed below), use of previously parasitized hosts by *I. affinis* and *E. krauteri* does not appear to occur in this system (per. obs. EN Schoeller). In addition to the broader host use exhibited by *I. affinis*, the trend for higher absolute parasitism rates and shorter handling times observed for this species further supports the hypothesis that this species may be the most important biological control agent in this system. Alternatively, *E. krauteri* may be under the strongest competitive pressure of the three species due to its narrow observed host use breadth and strong preference for the 4th instar, which may subject it to strong effects of exploitative competition. These predictions are supported by observations of *E. krauteri* populations in the field, which suggest *E. krauteri* is uncommon and if present parasitism rates are low (see **Chapter 6**).

Unlike *E. noyesi*, which is polyphagous (Polazsek and Hayat, 1992), *E. krauteri* and *I. affinis* are only reported from *A. dugesii* and are likely resource specialists (Zolnerowich and Rose, 1996; Lasalle et al., 1997). The host-use strategy of *E. krauteri* may be one that attempts to utilize late instars that have escaped parasitism by *I. affinis*. Whether *E. krauteri* can discriminate between hosts previously parasitized by *I. affinis* would be a necessary behavioral adaptation for this strategy to succeed, and research providing evidence that *E. krauteri* can detect hosts previously parasitized would help

test the validity of this hypothesis. Future research examining if the presence of *E. noyesi* affects overall host-use efficiency achieved by *I. affinis* and *E. krauteri* may also provide insight into how other generalist competitors across the introduced range of *A. dugesii*'s may impact its control.

As it forages on the upper leaf surface without direct contact with hosts until the act of oviposition, one of the major questions remaining that pertains to resource utilization in this system is how *E. krauteri* locates and discriminates between hosts. It is unlikely that host chemical cues indicating the position of nymphs on the underside of leaves exist on the upper leaf surface, and *E. krauteri* was not observed to indiscriminately probe leaves looking for hosts. We suggest that *E. krauteri* utilizes vibrotaxis like many other parasitioids (e.g. leafminer parasitoids) whose hosts reside beneath a substrate to facilitate host location (Meyhöfer and Casas, 1999). As observed for other parasitoid species, such as *Psyttalia concolor* (Szépligeti) (Hymenoptera: Braconidae) (Canale and Loni, 2006), *E. krauteri* may have difficulty locating earlier instars using vibrotaxis and this may have resulted in the lower parasitism rates observed for these stages rather than differences in preference per se.

Unlike *I. affinis* and *E. krauteri*, which are primary parasitoids, *E. noyesi* is a heteronomous hyperparasitoid (Boughton et al., 2015) where females develop as primary parasitoids and males develop hyperparasitically on the pupal stage of conspecific females or males and females of heterospecifics. The pupal stage of the three parasitoids in this system does not occur until late into the 4th instar of *A. dugesii*. The observed preference for the 4th instar exhibited by *E. noyesi* may be linked to having a higher

probability of encountering hosts suitable for both male and female production. The hyperparasitic life history of *E. noyesi*, which allows it to use previously parasitized hosts for male production, may give it a large competitive advantage over *E. krauteri* and *I. affinis* (Hunter and Woolley, 2001). Future research investigating host use in this system should focus on the effects of prior parasitism on host preference and acceptance for each parasitoid species.

3.5 Literature Cited

Babendreier, D., Bigler, F., Kuhlman, U. 2005. Methods used to assess non-target effects of invertebrate biological control agents of arthropod pests. BioControl. 50:821–870.

Bellows, TS, Jr., Meisenbacher, C. 2000. Biological control of giant whitefly, *Aleurodicus dugesii*, in California. California Conference on Biological Control II, The Historic Mission Inn Riverside, California, USA, 11-12 July, 2000, pp. 113–116.

Bellows, TS, Jr., Kabashima, JN., Robb, KL. 2002. Giant Whitefly. UC ANR Publication 7400. Available online at: http://www.ipm.ucdavis.edu/PDF/PESTNOTES /pngiantwhitefly.pdf

Bográn, CE., Heinz, KM. 2002. Host selection by the heteronomous hyperparasitoid *Encarsia pergandiella* multiple-choice tests using *Bemisia argentifolii* as primary host. Entomol. Exp. Appl. 103:11–21.

Boughton, AJ., Mendez, MA., Francis, AW., Smith, TR., Osborne, LS., Mannion, CM. 2015. Host stage suitability and impact of *Encarsia noyesi* (Hymenoptera: Aphelinidae) on the invasive rugose spiraling whitefly, *Aleurodicus rugioperculatus* (Hemiptera: Aleyrodidae), in Florida. Biol. Control. 88:61–67.

Briggs, CJ. 1993. Competition among parasitoid species on a stage-structured host and its effect on host suppression. Am. Nat. 141:372–397.

Canale, A., Loni, A. 2006. Host location and acceptance in *Psyttalia concolor*: role of host instar. Bull. Insectol. 59:7–10.

Casula, P., Wilby, A., Thomas, MB. 2006. Understanding biodiversity effects on prey in multi-enemy systems. Ecol. Lett. 9:995–1004.

Collier, TR., Hunter, MS. 2001. Interference competition between whitefly parasitoids, *Eretmocerus eremicus*, and *Encarsia transvena*. Oecologia. 129:147–154.

Derocles, SAP., Evans, DM., Nichols, PC., Aifionn Evans, S., Lunt, DH. 2015. Determining plant – leaf miner – parasitoid interactions: a DNA barcoding Approach. PloS ONE. 10:e0117872.

Forbes, AA., Hood, GR., Feder, JL. 2010. Geographic ranges and host breadths of parasitoid wasps associated with the *Rhagoletis pomonella* (Diptera: Tephritidae) species complex. Ann. Entomol. Soc. Am. 103:908–915.

Garcia-Medel, D., Sivinski, J., Diaz-Fleischer, F., Ramirez-Romano, R., Aluja, M. 2007. Foraging behavior by six fruit fly parasitoids (Hymenoptera: Braconidae) released as a single- or as multiple species cohorts in field cages: influence of fruit location and host density. Biol. Cont. 43:12–22.

Godfray, H. 1994. Parasitoids: Behavorial and Evolutionary Ecology. Princeton, NJ: Princeton University Press.

Harvey, JA., Snaas, H., Malcicka, M., Visser, B., Bezemer, TM. 2014. Small-scale spatial resource partitioning in a hyperparasitoid community. Arthropod Plant Interact. 8:393–401.

Hawkins, B.A., Mills, NJ. 1996. Variability in parasitoid community structure. J. Anim. Ecol. 65:501–516.

Hougardy, E., Mills, NJ. 2008. Comparative life history and parasitism of a new colour morph of the walnut aphid in California. Agric. For. Entomol. 10:137–146.

Hunter, MS., Woolley, JB. 2001 Evolution and behavioral ecology of heteronomous aphelinid parasitoids. A. Rev. Entomol. 46:251–290.

Irvin, NA., Hoddle, MS. 2005. The competitive ability of three mymarid egg parasitoids (*Gonatocerus* spp.) for glassy-winged sharpshooter (*Homalodisca coagulata*) eggs. Biol. Control. 34:204–214.

Jervis, MA., Hawkins, BA., Kidd, NAC. 1996. The usefulness of destructive host feeding parasitoids in classical biological control: Theory and observation conflict. Ecol. Entomol. 21:41–46.

Karamaouna, F., Copland, MJW. 2000. Oviposition behaviour, influence of experience on host size selection, and niche overlap of the solitary *Leptomastix epona* and the gregarious *Pseudaphycus flavidulus*, two parasitoids of the mealybug *Pseudococcus viburni*. Entomol. Exp. Appl. 97:301–308.

Lasalle, J., Polaszek, A., Noyes, JS., Zolnerowich, G. 1997. A new whitefly parasitoid (Hymenoptera: Pteromalidae: Eunotinae), with comments on its placement, and implications for classification of Chalcidoidea with particular reference to the Eriaporinae (Hymenoptera: Aphelinidae). Syst. Entomol. 22:131–150.

Lockwood, JR., III. 1998. On the statistical analysis of multiple-choice feeding preference experiments. Oecologia 116:475–481.

Mackauer, M. 1990. Host discrimination and larval competition in solitary endoparasitoids. *In*: Mackauer, M., Ehler, LE., Roland, J. (Eds.). Critical Issues in Biological Control. Intercept, Andover.

Mansfield, S., Mills, NJ. 2004. A comparison of methodologies for the assessment of host preference of the gregarious egg parasitoid *Trichogramma platneri*. Biol. Control. 29:332–340.

Meyhöfer, R., Casas, J. 1999 Vibratory stimuli in host location by parasitic wasps. J. Insect Physiol. 45:967–971.

Morse, JG., Bellows, TS. Jr., Iwata, Y. 1986. Techniques for evaluating residual toxicity of pesticides to motile insects. J. Econ. Entomol.79:281–283.

Murdoch, WW., Briggs, C., Nisbet, RM. 1996. Competitive displacement and biological control in parasitoids: a model. Am. Nat. 148:807–826.

Murray, TJ., Withers, TM., Mansfield, S. 2010. Choice versus no-choice test interpretation and the role of biology and behaviour in parasitoid host specificity tests. Biol. Control. 52:153–159.

Polazsek, A., Hayat, M. 1992. A revision of the genera *Dirphys* Howard and *Encarsiella* Hayat (Hymenoptera: Aphelinidae). Syst. Entomol. 17:181–197.

Price, PW. 1971. Niche breath and dominance of parasitic insects sharing the same host species. Ecology. 52:587–596.

Price, PW. 1972. Parasitoids utilizing the same host: adaptive nature of differences in size and form. Ecology. 53:190–195.

Quicke, DL. 1997. Parasitic Wasps. London: Chapman & Hall.

Rossi, AM., Murray, M., Hughes, K., Kotowski, M., Moon, DC., Stiling, P. 2006. Non-random distribution among a guild of parasitoids: implications for community structure and host survival. Ecol. Entomol. 31:557–563.

Sands, DPA., Van Driesche, RG. 2000. Evaluating the host range of agents for biological control of arthropods: rationale, methodology and interpretation. *In*: Van Driesche, RG., Heard, T., McClay, AS., Reardon, R. (Eds.) Host-specificity testing of exotic arthropod biological control agents: the biological basis for improvement in safety. FHTET-99- 1, Morgantown, West Virginia.

Teder, T., Tammaru, T., Kaasik, A. 2013. Exploitative competition and coexistence in a parasitoid assemblage. Popul. Ecol. 55:77–86.

Tylianakis, JM., Romo, CM. 2010. Natural enemy diversity and biological control: making sense of the context-dependency. Basic Appl. Ecol.11:657–668.

Wang, XG., Messing, RH., Bautista, RC. 2003. Competitive superiority of early acting species: a case study of opiine fruit fly parasitoids. Biocontrol Sci. Technol. 13:391–402.

Waterworth, RA., Robinson, LJ., Redak, RA., Morse, JG. 2015. Evaluating the performance and preference of *Aphytis melinus* (Hymenoptera: Aphelinidae) on an exotic species, *Acutaspis albopicta* (Hemiptera: Diaspididae), and *Aonidiella aurantii* (Hemiptera: Diaspididae). Environ. Entomol. 45:83–93.

Wieber, AM., Cook, SP., Webb, RE., Tatman, KM., Reardon, RC. 1995. Niche partitioning by four *Gelis* spp. (Hymenoptera: Ichneumonidae) hyperparastoids of the primary Gypsy moth parasitoid *Cotesia melanoscela* (Hymenoptera: Braconidae). Ann. Entomol. Soc. Am. 88:427–433.

Wilby, A., Villareal, SC., Lan, LP., Heong, KL., Thomas, MB. 2005. Functional benefits of predator species diversity depend on prey identity. Ecol. Entomol. 30:497–501.

Yu, DS., Luck, RF., Murdoch, WW. 1990. Competition, resource partitioning and coexistence of an endoparasitoid *Encarsia perniciosi* and an ectoparasitoid *Aphytis melinus* of the California red scale. Ecol. Entomol. 15:469–480.

Zolnerowich, G., Rose, M. 1996. A new species of *Entedononecremnus* (Hymenoptera: Chalcidoidea: Eulophidae) parasitic on the giant whitefly, *Aleurodicus dugesii* Cockerell (Homoptera: Aleyrodidae). P. Entomol. Soc. Wash. 98:369–373

Chapter 4

Host-produced Wax Affects the Searching Behavior and Efficacy of Parasitoids of the Giant Whitefly.

4.1 Introduction

Wax production by insects is widespread and diverse in both form and function (Blomquist and Bagnères, 2010). Functions of insect wax include: maintenance of internal water levels (Ramsay, 1935; Locke, 1965; Hadley, 1981); protection from UV radiation (Pope and Hinton, 1977; Hadley, 1994); epicuticular immunity against microorganisms (Koidsumi, 1957; St. Leger, 1991; Ortiz-Urquiza and Keyhani, 2013); chemical communication (Carlson et al., 1971; Howard and Blomquist, 2005), and protection from the soiling effects of honeydew (Gullan and Kosztarab, 1997; Smith, 1999; Pike et al., 2002).

One of the most intriguing functions of insect wax is its ability to mediate interactions between insect herbivores and their natural enemies. In many situations these waxes appear to serve as a defense against natural enemy attack and, depending on the circumstances, the mode of defense provided has been observed as primary (i.e. one that offers pre-emptive protection) or secondary (i.e. one that offers protection during detection/attack) (Edmunds, 1974). An example of a primary defensive function of insect wax is visual camouflage against natural enemy detection (Moss et al., 2006). Examples

of secondary defensive attributes of insect wax include the physical prevention of feeding by interfering with the proper functioning of natural enemy mouthparts (Eisner, 1994; Völkl and Vohland, 1996; Liereand and Perfecto, 2008), acting as a physical barrier against natural enemy attack (Mueller et al., 1992; Agarwala and Yasuda, 2001), chemical repellency of natural enemies (Schwartzberg et al., 2010), and prevention of natural enemy aggression via chemical mimicry of non-prey (Howard et al., 1990; Liepert and Dettner, 1996).

While wax production can benefit the producer, it can also benefit the natural enemies of the herbivore. For example, some natural enemies harvest wax from their prey in order to camouflage themselves from their prey or to protect against intraguild predation (Eisner et al., 1978; Eisner and Siberglied, 1988; Mason et al., 1991). Prey-produced wax can also assist in host seeking behaviors of natural enemies by serving as arrestment cues (van den Meiracker et al., 1990) and ovipositional stimulants (Takabayashi and Takahashi, 1985).

One of the most striking examples of wax production in insects is exhibited by the giant whitefly *Aleurodicus dugesii* Cockerell (Hemiptera: Aleyrodidae). Giant whitefly is an invasive species introduced into the United States from Mexico; it was first discovered in the United States in 1991 (Hodges, 2004). To date, it is established in at least six states including Texas, California, Arizona, Florida, Louisiana, and Hawaii (Gill, 1992; Nguyen and Hamon, 2002; Hue et al., 2004). Giant whitefly has a broad host range encompassing at least 77 plant genera in 47 families (Bellows et al., 2002; Evans, 2008). Feeding by giant whitefly adults and nymphs deprives their host plants of water and

nutrients and, at high infestation levels, can lead to leaf senescence or abscission followed by plant dieback and even death (Bellows et al., 2002). In addition to physical damage to the host plant, giant whitefly adults and nymphs produce copious amounts of wax. This wax affects the aesthetic value of infested plants making giant whitefly a particular nuisance in urban settings.

While the wax produced by adults is noticeable, it is the wax produced by the nymphs which is truly striking. Giant whitefly possesses four nymphal stages; a mobile first instar "crawler" and three sessile stages. The bulk of wax production by nymphs occurs during the 4th instar. Nymphs appear to produce two major forms of wax; long filaments which are produced from two separate rows of five pores each located on the dorsum, and short curls which are produced from numerous dorsal-lateral pores (**Figure 4.1**) (Nelson et al., 2000). Wax filament production by the nymphs gives afflicted leaves a "bearded" appearance, and under natural conditions these wax filaments can attain lengths of 5 - 20 cm depending on wind conditions (Hodges, 2004).

Little is known on the function of the wax produced by giant whitefly nymphs; however, some have speculated that it may serve as a defense against predators and parasitoids (Nelson et al., 1999). This is of concern as three parasitoid species have been introduced as part of a biological control program against giant whitefly in the United States. They include *Encarsia noyesi* Hayat (Hymenoptera: Aphelinidae), *Idioporous affinis* LaSalle & Polaszek (Hymenoptera: Pteromalidae), and *Entedononecremnus krauteri* Zolnerowich & Rose (Hymenoptera: Eulophidae). Both *E. noyesi* and *I. affinis* forage for hosts on the underside of leaves amongst the nymphs and come into direct

contact with their wax. In contrast, *E. krauteri* has the unusual behavior of foraging on the adaxial leaf surface and parasitizes nymphs through the leaf. All three parasitoid species in this system are solitary primary endoparasitoids, with exception of male *E. noyesi* which are produced as hyperparasitoids on immatures of conspecific females (Boughton et al., 2015) or on immature male and female *I. affinis* and *E. krauteri* (pers. obs. EN Schoeller). These parasitoids are koinobionts and emerge during the late "pupal" fourth instar of *A. dugesii* regardless of the instar initially parasitized. No studies have been done examining the reproductive biology of these species, however *I. affinis* and *E. noyesi* both appear to be synovigenic and possess a clutch of mature eggs two to three days post-eclosion (pers. obs. EN Schoeller). The reproductive biology of *E. krauteri* is still unknown. Adults of all three parasitoid species feed on the honeydew excreted by *A. dugesii*, but only *E. noyesi* has been observed host-feeding on nymphs (pers. obs. EN Schoeller).

The primary objective of this study was to test the hypothesis that wax production by giant whitefly nymphs provides a successful defense against parasitoids. This was achieved by performing a comparative study of the foraging behaviors and parasitism of *E. noyesi* and *I. affinis* in the presence or absence of giant whitefly wax.

Figure 4.1 Wax filament bundles (arrow) produced by abdominal plates on an adult female *Aluerodicus dugesii* top) and wax production by 4th instar *Aluerodicus dugesii* (bottom).

4.2 Materials and Methods

Plant, Host, and Parasitoid Material

Plants used to rear giant whitefly were prepared using woody cuttings taken from a single large *Hibiscus rosa-sinensis* 'White Wings'. Cuttings were placed into a 10 cm pots containing UC Soil Mix Type 3 (Matkin and Chandler, 1957; http://agops.ucr.edu/soil/) and grown in environmentally controlled rooms with artificial light (16:8 light:dark photoperiod, 28 ± 2 ºC, and ambient RH). Plants were watered every other day and fertilized once a month with a water soluble fertilizer (Growmore®, 20N-10P-20K; Gardena, CA). Plants were ready for use five months post-propagation.

Individuals of *A. dugesii*, *E. noyesi* and *I. affinis* used in this study were collected from infested *H. rosa-sinensis* located in Oceanside, CA (33°10'44.96"N; 117°22'15.77"). Infested leaves were brought back to the laboratory and placed into 14 cm diameter ventilated petri dishes. Adult *A. dugesii* and parasitoids on the leaves at the time of collection were immediately removed by aspirating them off of plant material. Adult *A. dugesii* were saved for subsequent use and parasitoids discarded as their ages were unknown. Parasitoid emergence from developing whiteflies was monitored daily and newly emerged parasitoids were placed into 2.5 ml glass vials containing moist cotton and a small piece of honey-soaked tissue (Kimwipes®, Kimberly-Clark Co., Neenah, WI) as an energy source. A total of 10 males and 30 females of each parasitoid species were placed into each vial and then vials were placed into environmental chambers (14:10 L: D photoperiod, 25 ± 0.5 ºC, 70 ± 10% RH) to allow parasitoids to mate for 48-72 h prior to use.

Effect of Whitefly Wax on Parasitism

Infestation of plants with *A. dugesii* was performed by covering individual uninfested leaves still attached to the plants with 7.6 cm x 12.7 cm fine mesh drawstring bags. Fifteen to thirty unsexed adult whiteflies were place into each bag for a 6 h oviposition period thus restricting differences in whitefly progeny age. Whitefly infested plants were placed into environmental chambers (16:8 light:dark photoperiod, 25 ± 0.5 ºC, 75 ± 10% RH) and held for 20 days, at which time the majority of nymphs had reached the 4th instar. At 20 days post-infestation, earlier instars still present on the leaves were removed and the density of the remaining 4th instars manipulated such that 20 individuals remained. The position of each nymph on the leaf was recorded by taking a photograph and then each nymph was assigned a unique identification number to assist in tracking parasitism rates (**Figure 4.2**).

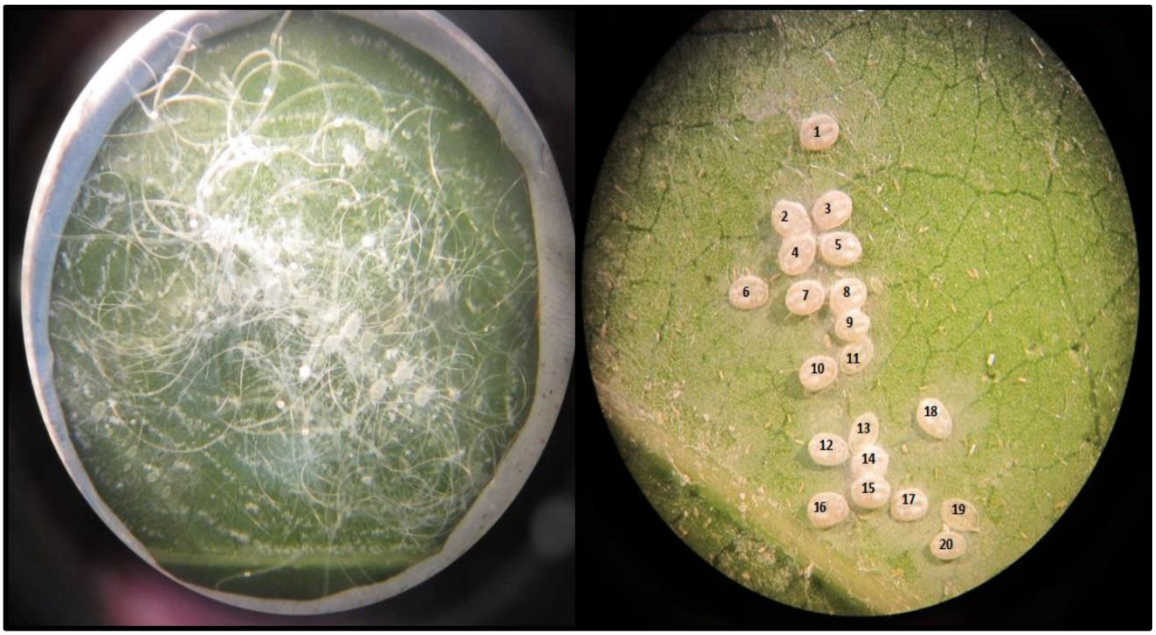

Figure 4.2 Experimental design for parasitoid efficacy tests; with wax treatment (left) and waxless treatment (right) consisting of 20 *Aluerodicus dugesii* nymphs.

To investigate the effects of the whitefly wax on the percentage of hosts parasitized by the two parasitoid species two treatments were prepared. A wax treatment (control) consisted of host plant patches with wax left untouched, while a no wax treatment consisted of host patches with wax blown away vigorously using an aspirator to deliver an airstream until no wax (both adult and nymphal) remained (Figure 5.3). The removal of wax did not damage the nymphs or alter their position on the leaves. A control of wax reapplication after removal was not necessary due to the sessile lifestyle of the nymphs making it unlikely their behaviors would change due to the removal of wax. Observational arenas consisted of a modified Munger cell (Morse et al., 1986) with a 3.2 cm diameter placed over the leaf area containing the nymphs. Munger cells were placed on the abaxial leaf surface to allow natural foraging behavior by *E. noyesi* and *I. affinis*. The space between the Munger cell and leaf was sealed with a ring of Blu-tak® (Bostik Ltd., Stafford, UK) to prevent parasitoid escape.

Pilot trials suggested that female parasitoids encountered hosts infrequently during wax-treatment trials. It was unknown if this was due to the presence of wax or lack of motivation to oviposit. To ensure female parasitoids were consistently selected with a high degree of motivation, immediately prior to use, females were exposed to wax-free nymphs and their behavior observed. Females that exhibited a motivation to oviposit were removed before they were successful and transferred to experimental arenas.

A single mated *I. affinis* or *E. noyesi* female (48–72 h old) was introduced into the experimental arena using a 100 µl micropipette tip into one of two holes located on the sides of the cells. Trials began after a one-minute acclimation period and females were

allowed to forage for 1 h. Foraging behavior of female parasitoids was observed under a dissecting microscope at 120x magnification. During each host encounter, the time of encounter, identity (unique number assigned to each nymph), and host accepted was recorded. Trials were paused if females walked off the leaf and resumed once they returned. If females failed to return to the leaf within 15 minutes the trial was stopped and the data discarded. Observations were made between 13:00 and 17:00 hours daily. A total of 20 replicates consisting of a single female foraging bout were performed for each parasitoid species and wax treatment combination. These data were used to calculate the percent parasitism (dependent variable) for each parasitoid species (treatment 1, two levels) and wax condition (treatment 2, two levels).

Effect of Whitefly Wax on Parasitoid Host-Searching Behavior

In addition to examining effects of wax on percent parasitism, differences in parasitoid host selection behavior were compared between wax treatments for each species. To facilitate behavioral analyses, females were simultaneously recorded during parasitism trials using a Zarbeco ZC-203 2.0 megapixel video camera mounted on a Mycrolyte Hi Mag® Standard Zoom digital video imaging system (EmCal Scientific Inc., San Diego, CA) for subsequent analysis.

For each trial the frequency, duration, and sequence of each behavioral event was recorded. The behaviors of interest were:

(1) *Walking*: The wasp moves at a relatively constant speed with its antennae held tightly together above its head. Walking may include brief (< 1s) *grooming* behavior, but the wasp does not stop moving to do so.

(2) *Searching*: The wasp asynchronously touches (at a slower frequency than *drumming*) its antennae, which are held wildly apart, to the leaf surface while either moving or remaining still.

(3) *Resting*: The wasp remains motionless with its antenna held tightly above its head.

(4) *Feeding*: After detecting honeydew with its antennae the wasp lowers its head and drinks or chews the honeydew. The antennae are held tightly above the head.

(5) *Grooming*: The wasp uses its prothoracic legs to clean its head, antenna, and mesothoracic legs. It then runs its legs through its mouth. The mesothroacic legs are used to clean the wings, and the metathroacic legs are rubbed together to clean themselves. *Grooming* behavior also happened during *ovipositing* and was not scored when it happened simultaneously.

(6) *Host Encounter*: While *searching* the antennal club comes into contact with the host body and the wasp immediately initiates *drumming* behavior.

(7) *Drumming*: The wasp rapidly and asynchronously touches the surface of the host's body with its antennae, which are held closely together, and the wasp moves towards the center of the host.

(8) *Ovipositing*: The wasp stops *drumming* and assumes a species-specific oviposition stance. *Encarsia noyesi* downturns its abdomen and pierces the top of the host with its ovipositor at a 90° angle with its antennae

downturned; *Idioporus affinis* downturns its abdomen and pierces its ovipositor into the host's side with its antennae downturned.

(9) *Disengagement*: The wasp raises its abdomen to remove its ovipositor from the host and raises its antennae back tightly to its head as observed in the *resting* state.

In this experiment data were used to calculate the proportion of time spent searching (dependent variable 1) and grooming (dependent variable 2) for each parasitoid species (treatment 1, two levels) and wax condition (treatment 2, two levels).

Data Analysis

Percent parasitism [(number of nymphs parasitized/total number of nymphs) x 100] was calculated for each wax treatment and parasitoid species combination. A generalized linear model with a negative binomial distribution was performed to test for effects of wax treatment, parasitoid species, and their interaction on percent parasitism using the PROC GENMOD procedure in SAS version 9.4 (SAS Institute Inc., 2013). The negative binomial distribution was selected due to frequency of zeros present in the data and evidence of overdispersion in the Poisson model (Pearson's $X^2/df = 3.719, \neq 1$). The lower Akaike information criteria values obtained for the negative binomial models from goodness-of-fit analyses confirmed an overall better fit of the data over the Poisson model. When significant treatment effects were observed, post-hoc analyses were performed for all four species*wax pairwise comparisons using the Bonferroni adjustment.

A two-way multivariate analysis of variance (MANOVA) was performed to examine the effects of wax treatment and parasitoid species on the time allocated (% of time allocated during the 1h observation period) to specific behaviors recorded. To prevent dependency issues arising as a result of including all behaviors representing 100% time allocation, only time spent searching and grooming were analyzed. When the two-way MANOVA was found to be significant, individual ANOVAs were performed followed by multiple comparison tests on each species*wax combination using the least squared means with a Bonferroni adjustment to identify significant differences between treatment groups.

4.3 Results

Effects of Wax on Parasitism

There was a significant effect of species (χ^2 = 4.21; df = 1; P < 0.0402), wax presence (χ^2 = 147.25; df = 1; P < 0.0001), and an interaction between wax presence and parasitoid species (χ^2 = 85.38; df = 1; P < 0.0001) on observed parasitism (**Figure 4.3**). Multiple comparison tests indicated that the percentage of hosts parasitized differed significantly for all four of the treatment combinations except percent parasitism between *E. noyesi* and *I. affinis* in the waxless treatment (z = 2.05; P = 0.2412). While both species exhibited reduced parasitism in the presence of wax, *I. affinis* appeared to be more affected by wax than *E. noyesi*. Parasitism of *I. affinis* were reduced from 30-fold, while *E. noyesi* parasitism was reduced 2-fold due to the presence of wax (**Figure 4.3**). The disproportionate effect of wax on percent parasitism between species appeared to be driven primarily by changes in *I. affinis* behavior.

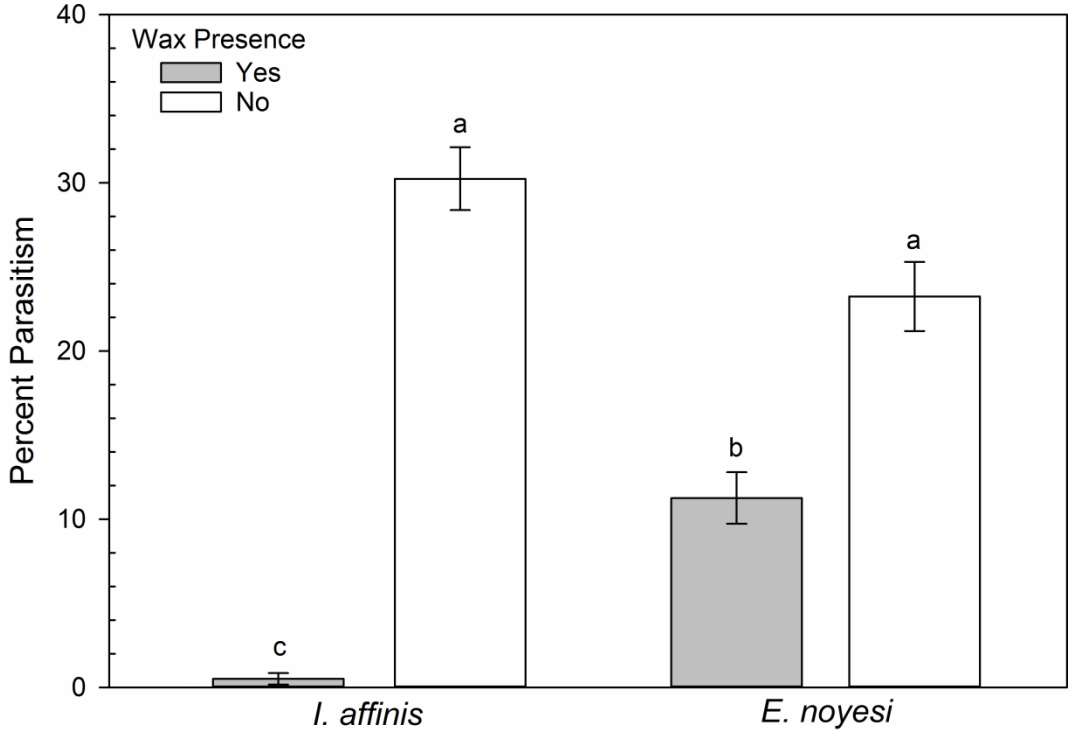

Figure 4.3 The effects of the presence and absence of *Aluerodicus dugesii* nymphal wax on *Encarsia noyesi* and *Idioporus affinis* parasitism. Error bars are standard errors of the means and different letters indicate a significant difference between treatments (α = 0.05).

The two-way MANOVA indicated an overall interaction between parasitoid species and wax presence on the proportion of time spent grooming and searching ($F_{2,75}$ = 11.05; P < 0.0001) (**Figure 4.4**). Looking at each behavior individually there was a significant effect of wax presence ($F_{3,76}$ = 119.09; P < 0.0001) and interaction ($F_{3,76}$ = 11.02; P = 0.0014) observed on the proportion of time spent grooming, but no main effect of species ($F_{3,76}$ = 0.81; P = 0.3695). There was a marginally significant effect of species ($F_{3,76}$ = 3.98; P = 0.0496), and significant effect of wax presence ($F_{3,76}$ = 4.97; P = 0.0288) and interaction ($F_{3,76}$ = 4.78; P = 0.0319) on proportion of time spent searching.

Both *I. affinis* and *E. noyesi* spent more time grooming in the presence of wax according to multiple comparison tests (*I. affinis*: $t = 10.06$, $P < 0.0001$; *E. noyesi*: $t = 5.36$, $P < 0.0001$), and the time allocated to grooming in the presence of wax differed between species ($t = 2.99$, $P = 0.023$). However, there was no difference in the proportion of time spent grooming between species with wax absent ($t = 1.71$, $P = 0.5494$). The presence of wax increased the proportion of time spent grooming by *E. noyesi* almost 2-fold and by *I. affinis* 3-fold (**Figure 4.4**).

The proportion of time spent searching differed significantly between species in the presence and of wax ($t = 2.96$, $P = 0.0249$). There was no difference between the proportion of time spent searching between species in the absence of wax ($t = 0.14$, $P = 1.000$) and no difference in searching time exhibited by *E. noyesi* in the presence or absence of wax ($t = 0.03$, $P = 1.000$). The presence of wax did not increase the proportion of time spent searching by *E. noyesi*, but increased the time spent searching by *I. affinis* 1.5-fold (**Figure 4.4**). The significant interaction observed between parasitoid species and wax presence suggests that the magnitude of the effect of wax presence on the proportion of time spent searching and grooming was stronger for *I. affinis* than *E. noyesi*.

4.4 Discussion

The results from this study further highlight the importance of considering the influence of host characteristics in mediating parasitoid-prey interactions. The differences in the magnitude of wax effects observed on parasitism and foraging behaviors between *E. noyesi* and *I. affinis* was unexpected due to the fact both species forage amongst the

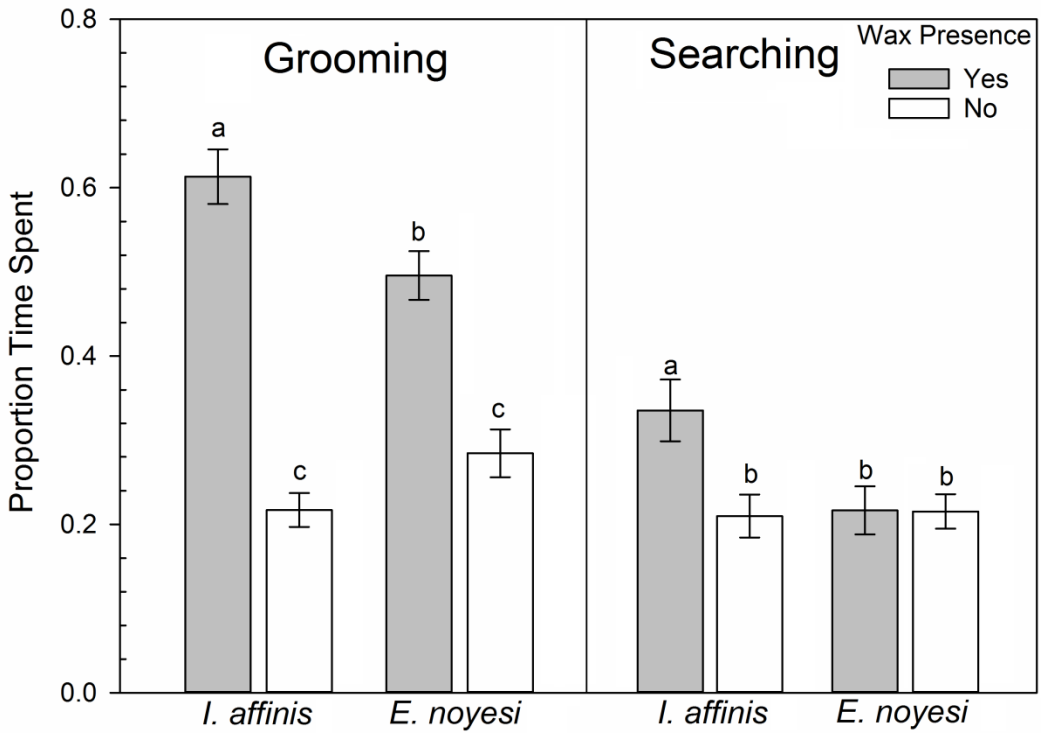

Figure 4.4 The effects of the presence and absence of *Aluerodicus dugesii* nymphal wax on *Encarsia noyesi* and *Idioporus affinis* foraging behaviors. Error bars are standard errors of the means and different letters indicate a significant difference between treatments within behaviors ($\alpha = 0.05$).

Figure 4.5 The accumulation of wax particles produced by *Aluerodicus dugesii* nymphs on the body of a female *Idioporus affinis* after foraging.

nymphs. Not apparent from these data was the fact that *I. affinis* appeared to be unable to effectively remove wax particles while grooming. While *E. noyesi* remained mostly wax-free after grooming bouts, *I. affinis* often remained covered with wax particles during experimental trials (**Figure 4.5**), and this may have contributed to its poor efficacy in the presence of wax. The difference in the magnitude of the effect of wax presence on the time spent grooming and searching exhibited between parasitoid species was likely due to the buildup of wax particles on the body of *I. affinis* impeding its movement as it searched for hosts.

Without possessing behavioral differences or life history traits that may provide it an advantage in resource acquisition over the other two parasitoids, these results would suggest that *I. affinis* may be the least effective biological control agent. Differences in host preferences between parasitoid species however, may help dampen the negative effects of this wax due to the substantial differences in wax production by different instars. This hypothesis appears to have merit for *I. affinis*, which prefers earlier instars that produce little amounts of wax (Schoeller and Redak, 2017 in prep.). Although we did not test the effects of wax on *E. krauteri* due to its rarity in the field at the time of this study, we expect that the presence of wax would have no effect on its parasitism rate due to its unique behavior of foraging on the adaxial leaf surface. This behavior may have evolved to allow *E. krauteri* to achieve higher parasitism, since it prefers to parasitize the 4^{th} instar (Schoeller and Redak, 2017 in prep.), the stage that produces the greatest amount of wax. However, there may be tradeoffs associated with foraging on the upper leaf surface, such as greater exposure to predators, negative environmental conditions, or

reduced searching efficiency. Thus, given differences between parasitoid species in host stage preferences and ability to overcome nymphal wax the combined effects of the three parasitoids may achieve higher levels of host suppression than any species alone.

It is possible that pilot trials created experimental bias due to exposure of wax-free nymphs causing females used in wax trials to alter their searching behaviors to reflect the absence of wax, however we believe this to be unlikely due to the short exposure period of females to these conditions and the fact females were never allowed to oviposit into these hosts. Additionally, if an altered searching behavior due to wax presence is a real phenomenon and these changes occurred, we believe it is likely that females would readjust their searching behaviors in response to the presence of wax at the start of the experimental trials and the majority of searching behavior data would reflect wax effects. Nonetheless, results from wax trials should be interpreted with this potential bias in mind.

The results suggest that wax production by *A. dugesii* nymphs functions primarily as a secondary defense against parasitoids. While the attractiveness of *A. dugesii* wax was not empirically tested in this study, both *I. affinis* and *E. noyesi* were observed anttennating the wax present on leaves and focusing their foraging on areas of leaves where wax was present. This may increase the risk of parasitism to some degree by aggregating parasitoids to the location of the nymphs, however the fact that the wax particles were able to inhibit movement of parasitoids may be sufficient counteract this effect. These observations suggest that *A. dugesii* wax production may only provide a weak primary defense against parasitoids. Despite the unclear role of *A. dugesii* nymphal

wax as a primary defense, it was clear that wax served as a strong secondary defense by inhibiting parasitoid movement when nymphs were encountered by providing a physical barrier against parasitoid oviposition attempts.

To date only one other study which has examined the potential defensive properties of whitefly wax. Guershon and Gerling (1994) looked at the defensive properties of wax produced by the whitefly *Aleyrodes singularis* Danzig against two parasitoids, *Encarsia inaron* (Walker) and *Encarsia sophia* (= *E. transvena*) (Girault & Dodd). The nymphs of *A. singularis* create "exuvial pyramids" over their bodies in addition to wax which is supplemented by adults. Similar to the findings of this study, the authors found that nymphal wax increased the duration of parasitoid foraging behaviors and that there were differences in the strength of wax effects on the two parasitoid species' efficacies (Guershon and Gerling, 1994).

In addition to the potential of wax serving as a defense against natural enemies, during the process of blowing away wax during experiments we observed that some nymphs would expel honeydew during contact with the airflow. Additionally, during foraging when parasitoids came into direct contact, nymphs would also expel honeydew. Honeydew expulsion during the wax removal procedure did not fall back onto the leaf, so there was no apparent difference in the amount of honeydew on the leaf at the start of trails, which may have altered parasitoid foraging activities between treatments. On occasion during parasitism events honeydew would adhere to the anterior surface of the parasitoids and force them to disengage from the act of oviposition to groom. Nymphs of *A. dugesii* may be able to sense a potential natural enemy using tactile or vibrational

stimuli and this honeydew excreting behavior may provide an additional form of defense, however this hypothesis needs to be tested experimentally. Many species of whiteflies, in addition to other sessile taxa such as mealybugs and scale insects form mutualisms with ants that provide a defense against natural enemies in return for access to honeydew resources (Delabei, 2001; Styrsky and Eubanks, 2007). Honeydew expulsion in response to the presence of parasitoids in addition to inhibiting the movement of parasitoids in this system may also serve to aggregate ants to the location of natural enemies.

Taking into consideration the variable impact of host defenses on different natural enemy species is critical for selecting the most suitable species for biological control. Future work should focus on examining how wax production mediates the interspecific competitive interactions of parasitoids in this system due to the differential impact on species' efficacies observed. Given that *E. noyesi* and *I. affinis* are capable of coexisting on *A. dugesii* for long periods of time; this would imply that *I. affinis* possesses a behavioral or biological mechanism to compete with *E. noyesi* despite the effects of host defenses. Studies examining these parasitoid's efficacies when given the opportunity to select less defended host stages may also provide a more realistic picture of dynamics in the field since host patches typically have a mixture of different host stages available at any given point in time.

4.5 Literature Cited

Agarwala, BK., Yasuda, H. 2001. Larval interactions in aphidophagous predators: effectiveness of wax cover as defence shield of *Scymnus* larvae against predation from syrphids. Entomol. Exp. Appl. 100:101–107.

Bellows, TS., Jr., Kabashima, JN., Robb, KL. 2002. Giant Whitefly. UC ANR Publ. 7400.

Blomquist, GJ., Bagnères, A-G. 2010. Insect Hydrocarbons: Biology, Biochemistry and Chemical Ecology. Cambridge University Press.

Buckner, JS., Hagen, MC., Nelson, DR. 1999. The composition of the cuticular lipids from nymphs and exuviae of the silverleaf whitefly, *Bemisia argentifolii*. Comp. Biochem. Physiol. 124B:201–207.

Byrne, DN., Hadley, NF. 1988. Particulate surface waxes of whiteflies: morphology composition and waxing behavior. Physiol. Entomol. 13:267–276.

Carlson, DA., Mayer, MS., Silhacek, DL., James, JD., Beroza, M., Bierl, BA. 1971. Sex attractant pheromone of the housefly: Isolation, identification, and synthesis. Science. 174:76–77.

Delabie, JHC. 2001. Trophobiosis between Formicidae and Hemiptera (Sternorrhyncha and Auchenorrhyncha): an overview. Neotrop. Entomol. 30:501–516.

Edmunds, M. 1974. Defence in Animals: A Survey of Anti-predator Defences. Longmans, London.

Eisner, T. 1994. Integumental slime and wax secretion: defensive adaptations of sawfly larvae. J. Chem. Ecol. 20:2743–2749.

Eisner, T., Silberglied, RE. 1988. A chrysopid larva thatcloaks itself in mealybug wax. Psyche. 95:15–19.

Evans, GA. 2008. The whiteflies (Hemiptera: Aleyrodidae) of the world and their host plants and natural enemies. Available online at: http://www.sel.barc.usda.gov:8080/1WF/World-Whitefly-Catalog.pdf.

Gill, RJ. 1992. Giant whitefly. In: California Plant Pest and Disease Report. Calif. Dept. Food Agric. 11:78–81.

Guershon, M., Gerling, D. 1994. Defense of a sessile host against parasitoids: *Aleyrodes singularis* versus *Encarsia* spp. Norwegian J. Agric. Sci. Supp. 16:255–260.

Gullan, PJ., Kosztarab, M. 1997. Adaptations in scale insects. Ann. Rev. Entomol. 42:23–50.

Hadley, NF. 1981. Cuticular lipids of terrestrial plants and arthropods: a comparison of their structure, composition, and waterproofing function. Biol. Rev. 56:23–47.

Hadley, NF. 1994. Water relations of terrestrial arthropods. Academic Press, San Diego.

Hodges, G. 2004. Giant Whitefly, *Aleurodicus dugesii* Cockerell, in Florida. Pest Alert, Division of Plant Industry, Florida Department of Agriculture and Consumer Service. Available online at: http://www.freshfromflorida.com/content/download/68178/1612763/Pest_Alert_-_Aleurodicus_dugesii,_Giant_whitefly.pdf (Last Accessed: December 2017).

Howard, RW., Blomquist, GJ. 2005. Ecological, behavioral, and biochemical aspects of insect hydrocarbons. Ann. Rev. Entomol. 50:371–393.

Howard, RW., Stanley-Samuelson, DW., Akre, RD. 1990. Biosynthesis and chemical mimicry of cuticular hydrocarbons from the obligate predator, *Microdon albicomatus* Novak (Diptera: Syrphidae) and its ant prey, *Myrmica incompleta* Provancher (Hymenoptera: Formicidae). J. Kansas Entomol. Soc. 63:437–443.

Heu, RA., Nagamine, NT., Kumashiro, BR., Watanabe, TM. 2004. Giant whitefly, *Aleurodicus dugesii* Cockerell (Homoptera: Aleyrodidae). Hawaii Dept. Agr. New Pest Advis. No. 02-04. June 2004 (revision). Available online at: http://hawaii.gov/hdoa/pi/ppc/npa-1/npa02-04-giantwf.pdf.

Koidsumi, K. 1957. Antifungal action of cuticular lipids in insects. J. Insect Physiol. 1:40–51.

Liere, H., Perfecto, I. 2008. Cheating on a mutualism: indirect benefits of ant attendance to a coccidophagous coccinellid. Environ. Entomol. 37:143–149.

Liepert, C., Dettner, K. 1996. Role of cuticular hydrocarbons of aphid parasitoids in their relationship to aphid-attending ants. J. Chem. Ecol. 22:695–707.

Locke, M. 1965. Permeability of insect cuticle to water and lipids. Science. 147:295–298.

Mason, RT., Fales, HM., Eisner, M., Eisner, T. 1991. Wax of a whitefly and its utilization by a chrysopid larva. Naturwissenschaften. 78:28–30.

Matkin, OA., Chandler, RA. 1957. The U.C. soil mixes. *In*: Baker, KF. (Ed.), The U.C. System for producing healthy container-grown plants through the use of clean soil, clean stock, and sanitation. Calif Ag Exp Sta Serv Manual 23, Berkeley, CA. pp. 68–85.

Morse, JG., Bellows, TS., Jr., Iwata, Y. 1986. Techniques for evaluating residual toxicity of pesticides to motile insects. J. Econ. Entomol.79:281–283.

Moss, R., Jackson, RR., Pollard, SD. 2006. Mask of wax: secretions of wax conceal aphids from detection by spider's eyes. New Zeal. J. Zool. 33:215–220.

Mueller, TF., Blommers, LHM., Mols, PJM. 1992. Woolly apple aphid (*Eriosoma lanigerum* Hausm., Hom., Aphidae) parasitism by *Aphelinus mali* Hal. (Hym., Aphelinidae) in relation to host stage and host colony size, shape and location. J. Appl. Entomol. 114:143–154.

Neal, JW., Jr., Leonhardt, BA., Brown, JK., Bentz, JA., Devilbiss, ED. 1994. Cuticular lipids of greenhouse whitefly and sweetpotato whitefly type A and B (Homoptera: Aleyrodidae) pupal exuviae on the same hosts. Ann. Entomol. Soc. Am. 87:609–618.

Nelson, DR., Fatland, CL., Buckner, JS., Freeman, TP. 1999. External lipids of adults of the giant whitefly, *Aleurodicus dugesii*. Comp. Biochem. Physiol. B. 123:137–145.

Nelson, DR., Freeman, TP., Buckner, JS. 2000. Waxes and lipids associated with the external waxy structures of nymphs and pupae of the giant whitefly, *Aleurodicus dugesii*. Comp. Biochem. Physio. B. 125:265–278.

Nguyen, R., Hamon, AB. 2002. Biological control of giant whitefly, *Aleurodicus dugesii* Cockerell, in Florida. Fla. Dept. Agr. Cons. Serv. Available online at Available at: http://www.nhm.ac.uk/resources/researchcuration/projects/chalcidoids/pdf_X/NguyenHa2002.pdf

Ortiz-Urquiza, A., Keyhani, NO. 2013. Action on the surface: Entomopathogenic fungi versus the insect cuticle. Insects. 4:357–374.

Pike, N., Richard, D., Foster, W., Mahadevan, L. 2002. How aphids lose their marbles. Proc. R. Soc. B. 269:1211–1215.

Pope, RD., Hinton, HE. 1977. A preliminary survey of ultraviolet reflectance in beetles. Biol. J. Linn. Soc. 9:331–348.

Ramsay, JA. 1935. The evaporation of water from the cockroach. J. Exp. Biol. 12:373–383.

Schwartzberg, EG., Haynes, KF., Johnson, DW., Brown, GC. 2010. Wax structures of *Scymnus louisianae* attenuate aggression from aphid-tending ants. Environ. Entomol. 39:1309–1314.

Smith, RG. 1999. Wax glands, wax production and the functional significance of wax use in three aphid species (Homoptera: Aphididae). J. Nat. Hist. 33:513–530.

St. Leger, RJ. 1991. Integument as a barrier to microbial infections. *In*: Binnington, K., Retnakaran , A. (Eds.), Physiology of the Insect Epidermis. CSIRO Pub. Victoria, Australia. pp. 284–306.

Styrsky, JD., Eubanks, MD. 2007. Ecological consequences of interactions between ants and honeydew-producing insects. Proc. R. Soc. B. Biol. Sci. 274, 151–164.

Takabayashi, J., Takahashi, S. 1985. Host selection behavior of *Anicetus beneficus* Ishii & Yasumatsu (Hymenoptera: Encyrtidae). III. Presence of oviposition stimulants in the scale insect of the genus *Ceroplastes*. App. Entomol. Zool. 20:173–178.

Van den Meiracker, RAF., Hammond, WNO., van Alphen, JJM. 1990. The role of kairomones in prey finding by *Diomus* sp. and *Exochomus* sp., two coccinellid predators of the cassava mealybug, *Phenacoccus manihoti*. Entomol. Exp. Appl. 56:209–217.

Völkl, W., Vohland, K. 1996. Wax covers in larvae of two *Scymnus* species: do they enhance coccinellid larval survival. Oecologia. 107:498–503.

Chapter 5

Effects of Temperature on Phenology and Biological Control of Giant Whitefly *Aleurodicus dugesii* (Hemiptera: Aleyrodidae) in Southern California.

5.1 Introduction

The impact of climactic conditions on the dynamics of host-parasitoid interactions and interactions between multiple parasitoid species is of interest due to the frequent use of parasitoids as biological control agents (Waage and Hassell, 1982; Greathead and Greathead, 1992). Climate is an important regulator of the distribution and abundance of insects (Huffaker et al., 1971; Price, 1987; Crozier, 2004), and the effects of weather conditions (Quezada and DeBach 1973; Risch, 1987; Fink and Völkl, 1995; Bourchier and Smith, 1996) and climate as a whole (Stilling, 1990; Sorribas et al., 2010) have been shown to impact the success of parasitoids. Sensitivity to changes in climactic conditions is predicted to be highest at the upper trophic levels (Voigt et al., 2003), and parasitoids may be particularly sensitive to climate conditions (Hance et al., 2007; Walther, 2010; Wetherington et al., 2017).

Divergence in the pattern of response to climate conditions can disrupt predator-prey population synchrony (van Nouhuys and Lei, 2004; Kiritani, 2006), and high levels of variation in climate conditions can further increase the risk of asynchrony (Stireman et al., 2005; Wetherington et al., 2017). Tightly-coupled population dynamics with their

host is critical for the success of parasitoids (Hance et al., 2007), and climate-driven phenological asynchrony may directly impact the abundance and distribution of parasitoid populations (Stenseth and Mysterud, 2002; Voigt et al., 2003; van Nouhuys and Lei, 2004). Variation in the degree of phenological synchrony between parasitoids and their hosts due to factors such as differences in thermal tolerances have been shown to elicit various consequences, such as leading to fluctuations in population densities (van Nouhuys and Lei, 2004), influencing population persistence (Godfray et al., 1994), or altering community structure (Hawkins and Sheehan, 1994; Briggs and Latto, 1996).

Climate-driven effects, particularly phenological asynchrony between parasitoid and host populations, have been identified as one of the primary causes of biological control program failures (Debach and Hagen, 1964; Hågvar, 1991; Stiling, 1993). Phenological asynchrony however, may benefit parasitoids in some systems by stabilizing host-parasitoid population dynamics (Godfray et al., 1994), promoting long-term population persistence. This stability may lead to higher relative host population equilibrium in some cases, which for biological control purposes is not desirable (Briggs et al., 1999).

In classical biological control programs parasitoids are imported into the invaded range of their host to reduce its population density below an economic threshold. If climate conditions differ between the parasitoid's native and introduced range these differences may have an impact on host-parasitoid interactions across the entire invaded range (Simberloff, 2012). If the target pest species occupies a broad geographic range consisting of multiple climates, pest control may be suppressed in certain areas where

climate conditions negatively impact parasitoid efficacy (Huffaker et al., 1971; Huffaker and Gutierrez, 1990; Rochat and Gutierrez, 2001). Climate-induced changes may result in population outbreaks of the target pest, which may be due in part to the population growth potential of parasitoids being reduced (Samways et al., 1999; Thomson et al., 2010). For example, unsuitable conditions may negatively impact important parasitism-dependent processes such as host location ability or the ability to overcome the host's immune responses (Hérard et al., 1988; Blumberg, 1991; Thomas and Blanford, 2003; van Baaren et al., 2005; Bannerman et al., 2011). The phenological synchrony between parasitoid and host populations may also become uncoupled under climate conditions in the invaded range (Harrington et al., 1999; Jeffs and Lewis, 2013). However, in some cases the climate conditions in the invaded range may be more suitable for parasitoids, resulting in increased levels of parasitism (Virtanen and Neuvonen, 1999). Compared to insect herbivore-plant interactions, we still know very little about the effects of climate on parasitoid-host interactions (Baffoe et al., 2012). Understanding how climate affects parasitoid-host interactions is critical for making accurate predictions on the outcome of classical biological control programs.

Classical biological control programs frequently utilize multiple species of parasitoids to control a target pest (Turnbull and Chant, 1961; Ehler and Hall, 1982; Hoelmer and Kirk, 2005). In systems where the combined effects of multiple parasitoid species achieve greater levels of biological control than any species alone, promoting parasitoid coexistence is an important objective for biological control practitioners. One way in which long-term coexistence between parasitoids has been shown to occur is via

temporal partitioning of the host resource (Amarasekare, 2007; Sorribas et al., 2010). This temporal partitioning is frequently attributed to differences in parasitoid species' thermal tolerances (Amarasekare, 2007) and may result in the formation of a temporal refuge, during which time interspecific competition is relaxed (Hassel, 2000). Even when introduced parasitoids coexist in their native range, abiotic differences in their areas of introduction may provide a competitive advantage to one species over another (DeBach et al., 1978; LeBrun et al., 2009) leading to competitive exclusion. In addition to understanding climactic effects on parasitoid-host interactions, understanding the effects of climate on parasitoid interspecific interactions is also critically important for developing and managing classical biological control programs.

In the United States three exotic parasitoid wasps have been introduced as biological control agents for the giant whitefly *Aleurodicus dugesii* Cockerell (Hemiptera: Aleyrodidae) (Bellows et al., 2002). These include *Encarsia noyesi* Hayat (Hymenoptera: Aphelinidae), *Idioporous affinis* LaSalle & Polaszek (Hymenoptera: Pteromalidae), and *Entedononecremnus krauteri* (Hymenoptera: Eulophidae). Giant whitefly was first discovered in the United States in Texas in 1991 and has become established in other states including California in 1992 (Gill, 1992), Arizona, Florida and Louisiana in 1996 (Nguyen and Hamon, 2002; Hodges, 2004), and Hawaii in 2002 (Hue et al., 2004). The native range of *A. dugesii* is likely Central and Southern Mexico, but it has also been found in Belize, Costa Rica, El Salvador, Guatemala, Nicaragua, Pakistan, Venezuela, and Indonesia (Lasalle et al., 1997; Evans, 2008; Muniappan et al, 2009). In California, *A. dugesii* was first discovered in San Diego in 1992 and then slowly began

expanding northward along the coastline and into adjacent counties. In 2004 *A. dugesii* was discovered for the first time in northern California in Sacramento County. Anecdotal observations of *A. dugesii* and parasitoid populations in the field suggest that population densities vary considerably across California and that the degree of biological control achieved by parasitoids also varies (per obs. EN Schoeller). These observations have prompted an investigation into the potential underlying abiotic and biotic factors involved in this variation.

In southern California a variety of climate conditions exist, ranging from coastal conditions with cool summers and warm winters to interior conditions with hot summers and cool winters. These climate conditions occur within a relatively small geographic range, making this portion of the invaded range of *A. dugesii* an ideal location to investigate how population dynamics of *A. dugesii* is influenced by local conditions and how these conditions affect the degree of biological control achieved. The objectives of this study were to determine:

(1) How *A. dugesii* population densities vary by climate and time of year, and

(2) How climate and time of year affect parasitism rates of *E. noyesi*, *I. affinis*, and *E. krauteri*.

I predicted that *A. dugesii* population densities would be highest at coastal sites and decline as populations move further inland, given that unlike inland and intermediate climates in southern California, historical coastal temperature extremes infrequently exceed the thermal developmental maximum observed for *A. dugesii* (see **Chapter 2**). Alternatively, I predicted that parasitism rates would be highest at inland sites, as

parasitism rates have been shown to increase with increasing temperatures (Jalali et al., 2005; Péré et al. 2013; Romo and Tylianakis, 2013).

5.2 Materials and Methods

Field Sites

A year-long study during 2015-2016 was conducted in southern California in order to examine the effects of temperature on the seasonal phenology of *A. dugesii* and its parasitoids as well as parasitism rates. An attempt was made to include equal numbers of coastal (USDA Hardiness Zone 10b; Sunset Climate Zone 24), intermediate (USDA Hardiness Zone 10a; Sunset Climate Zones 20–22), and inland (USDA Hardiness Zones 9a/9b; Sunset Climate Zones 18–19) sites; however, months of searching yielded fewer suitable inland and intermediate sites (**Figure 5.1**). A total of nine sites (**Table 5.1**) were selected for surveying. There were negligible differences in day length between sites due to latitudinal differences (There was a 118 km difference in latitude between the northernmost and southernmost sites).

Study sites were located on private properties to ensure study plants were not trimmed or treated with insecticides during the study period. Each site contained a single *Hibiscus rosa-sinensis* (L.) (1.5 – 3.4 m tall) infested with *A. dugesii*. At each site an Onset® HOBO data logger was installed to record daily temperature fluctuations (highs/lows) and means. Temperature data were recorded hourly throughout the duration of the study period. We observed that the heaviest whitefly infestations occurred in shaded areas at the bottom of plants, so data loggers were placed at 15% of each plant's total canopy height (Figure 6.2) to record the temperature in these areas.

Table 5.1 Field site's climate type, GPS coordinates, and elevation.

Site Name	Region	Location	Latitude (ddm)	Longitutde (ddm)	Elevation (m)
FV	Coastal	Fountain Valley	N33°41.773'	W117°57.455'	6.2
CA	Coastal	Carlsbad	N33°05.791'	W117°18.108'	16.2
HC	Coastal	Harbor City	N33°47.695'	W118°18.272'	32.8
TO	Coastal	Torrance	N33°49.801'	W118°22.325'	41.9
CO	Coastal	Corona del Mar	N33°36.094'	W117°51.895'	59.1
SA	Intermediate	Santa Ana	N33°46.051'	W117°53.143'	37.0
GL	Intermediate	Glendale	N34°08.863'	W118°14.028'	187.4
ES	Inland	Escondido	N33°08.200'	W117°03.835'	201.1
RI	Inland	Riverside	N33°55.153'	W117°21.045'	443.0

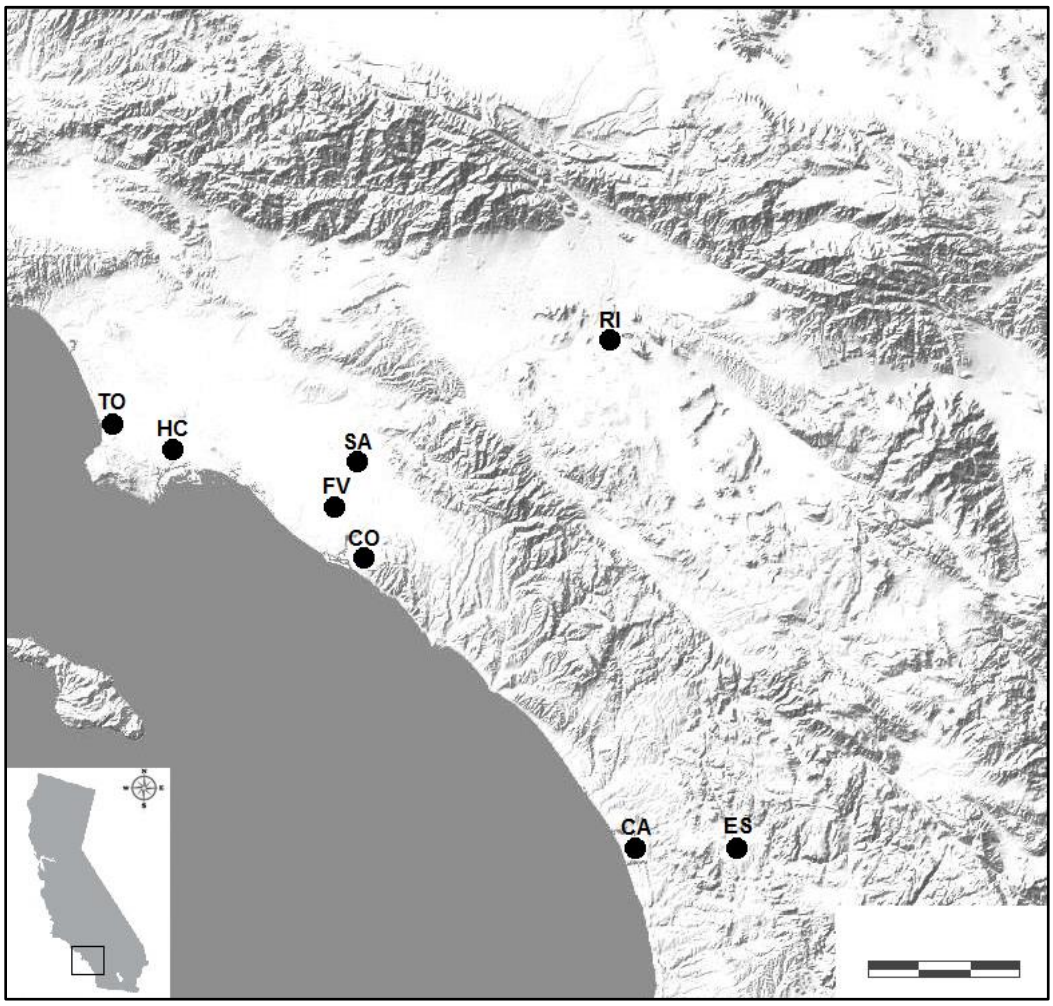

Figure 5.1 Study site locations. Markers indicate site's geographic locations within southern California.

Monitoring Seasonal Activity of Insects on Plants

Starting in July 2015 sites were sampled every two weeks until July 2016 for a total of 26 censuses. Plants were divided vertically into three quadrants (**Figure 5.2**) and branches randomly chosen for sampling using a random number generator to find percentage height and radial position around the plant (out of 360°, with 0° to the north). Plant height was measured every four months to account for new growth. Sampling by cardinal direction was not used as potential directional effects were obscured due to plant proximity to structures such as fences or houses. Two of the first six leaves from the tip of each branch were sampled, which had maximum widths of at least 3 cm. This procedure was repeated until a total of five branches (10 leaves/quadrant) had been sampled from each quadrant. Leaves were put individually into labelled petri dishes and placed into a paper bag in an ice-filled cooler to be transported back to the laboratory. Leaves were stored at 15°C for up to one week before being processed.

Densities of *A. dugesii* developmental stages [eggs, nymphs (I-IV instars), and adults] and adult parasitoids was estimated on a per leaf area basis in cm^2 (Pickett et al., 1996). Densities of insects were only estimated from the abaxial surface of leaves. Occasionally *A. dugesii* nymphs will fall from the upper canopy and affix to the adaxial leaf surface of lower canopy leaves, but the number of these individuals are typically few. Due to noticeable differences in leaf morphology between cultivars of *H. rosa-sinensis* present in this study, separate regression equations used to estimate leaf area were obtained for each study plant. The regression equations were obtained by plotting total leaf area against the product of a leaf's width x length measurements. Total leaf area

(lamina area in cm^2) was measured from scanned leaves using the software ImageJ (National Institutes of Health, Bethesda, MD, USA). This technique has proven to be reliable for estimating total leaf area across a variety of plant taxa (O'Neal et al., 2002). Regression equations were constructed using a total of 50 leaves per cultivar. The generated r^2 values from these equations ranged 0.985 - 0.997.

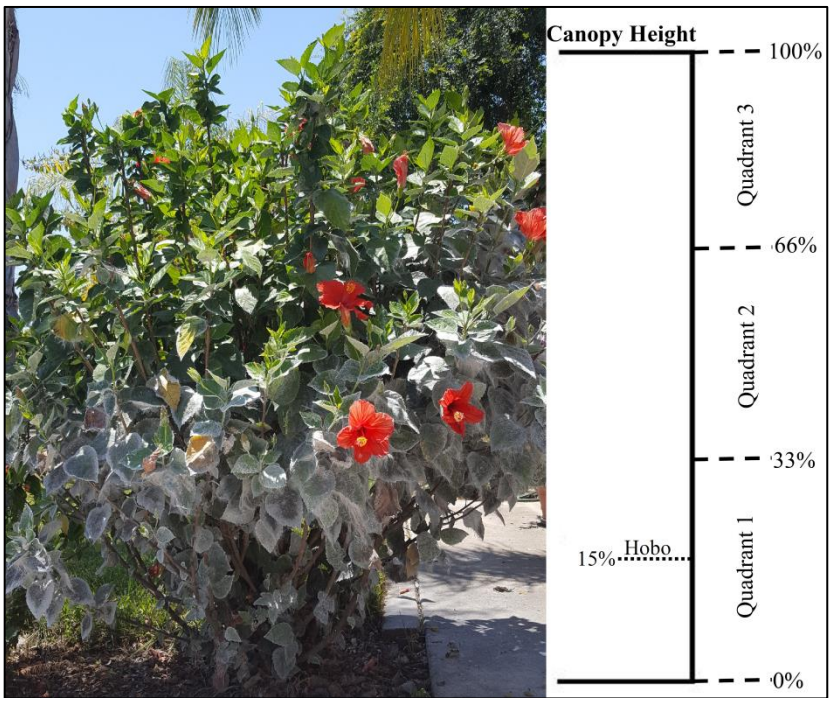

Figure 5.2 Illustration of sampling methodology for experimental plants. Canopies were divided into three quadrants based on percentage height. Plants were sampled three times by removing leaves from the tips of five selected branches. Two leaves were removed per branch.

Estimating Giant Whitefly Parasitism Incidence

The proportion of *A. dugesii* nymphs parasitized by each parasitoid species was estimated from the non-feeding late 4th instar "pupal" of *A. dugesii*, as early 4th instars typically died before parasitoids or adult whitefly emerged after leaves were collected. The proportion of *A. dugesii* nymphs parasitized was estimated from leaves containing a

minimum of 15 nymphs, and up to 50 nymphs were examined per leaf. Nymphs were observed starting from the leaf mid-vein at the petiole and selected at an increasing distance from this point. Nymphs were examined under a stereomicroscope at 60x magnification and divided into four categories: (1) nymphs with parasitoid emergence holes (parasitized and immediate ID possible), (2) nymphs with t-shaped splits (emerged adult whitefly), (3) "darkened" nymphs (clearly parasitized and species ID possible at a later date), and (4) nymphs with no evidence of parasitism. Nymphs in the first two categories were counted, identified, and removed from the leaf. The remaining nymphs were placed into an environmental chamber (25°C, 60-75% RH, 14:10 L:D photoperiod) and held for up to two weeks to allow parasitoids and adult whiteflies to emerge. These individuals were then identified and added to the initial parasitism counts. Total parasitism rates were calculated across the three parasitoid species and species-specific parasitism rates were calculated after excluding nymphs that contained unidentifiable parasitoid emergence holes.

Data Analysis

To prevent the confounding effects of temperature and site identity due to each temperature point corresponding to a single site per sampling interval, observed temperature was not included in the whitefly density and parasitism models. Instead, the observed mean daily temperature (dependent variable) at each site was modeled separately. Site was included in the model as a random predictor nested within climate type. Day of the month from which each temperature was recorded during the experiment was used as a random repeated measure to calculate monthly averages. Sampling month

was also included as a fixed predictor. There were 13 sample month (two Julys), because the experiment started in July, 2015 and ended in July 2016. Data were checked for normality assumptions prior to being analyzed using the PROC MIXED procedure in SAS. If the model results showed that the observed temperatures differed significantly between climate types and month then any significant climate and sampling interval effects on observed densities or parasitism rates could be reasonably attributed to in part to temperature differences.

To assess the effects of climate, sampling interval, and parasitism on total population densities of *A. dugesii*, a generalized linear mixed model (GLMM) was utilized with a Poisson error distribution and log link function. Climate type (Coastal, Intermediate, and Inland) was included as a categorical fixed predictor. Models included site (1–9) as a random effect to allow for non-independence of multiple samples per site. Sampling interval (2–26) nested within season was treated as a repeated measure. The first sampling interval was dropped from the analyses in both GLMMs (see below) due to lacking data for predictors requiring information from the previous sample interval. To improve the interpretability of the results, intervals were grouped within seasons using solstice/equinox dates as an unbiased cutoff between intervals. Spring consisted of intervals 19–24, summer consisted of intervals 1–5/25–26, fall consisted of intervals 6–11, and winter consisted of intervals 12–18. Mean total parasitism rate for each site from the prior sampling interval was included in the model as a continuous fixed effect to account for potential lag effects of parasitism on observed whitefly density. Whitefly densities were square-root transformed prior to analysis.

The effects of climate and sampling interval on species-specific primary parasitism and total primary parasitism rates were assessed using a GLMM with a binomial error distribution and logit link function. Model factors used included those listed above in addition to including total *A. dugesii* nymphal densities from the previous sampling interval as a covariate to account for potential lagged density-dependent effects on proportion of hosts parasitized. Total density of nymphs was used to model total parasitism rate, but rather than using the total density of *A. dugesii* nymphs as a covariate to model species-specific parasitism rates, the total density of nymphs which are susceptible to parasitism by each parasitoid species was utilized (see **Chapter 3**). These densities consisted of all nymphal stages for *I. affinis*, all nymphal stages except the 1st instar for *E. noyesi*, and only the 3rd and 4th instars for *E. krauteri*. All GLMM analyses were performed using the PROC GLIMMIX procedure in SAS (SAS institute, 2011).

The maximal models were determined in each case by including all the variables of interest and progressively removing non-significant interaction terms followed by main effects from the model. This was done until no further improvement to the model fit was achieved, as measured by the Akaike Information Criterion (AIC). During the model fitting process, models were fit using maximum likelihood and the final model was fit using restricted maximum likelihood (Bolker et al., 2009). Each maximal model was checked for evidence of overdispersion relative to the reference model, by examining the ratio of the mean deviance over degrees of freedom. When significant overdispersion was observed (i.e. φ much smaller or larger than 1), a multiplicative scale parameter was added, calculated from the ratio of the Pearson statistic to the degrees of freedom, to the

variance function in the model. When significant effects were observed, multiple comparison post-hoc tests were performed using the Tukey-Kramer adjustment to control the family-wise error rate.

5.3 Results

Effect of Climate Type and Month on Mean Daily Temperatures

There was a significant effect of month ($F_{12,17}$ = 194.77; P < 0.0001), climate type ($F_{2,6}$ = 14.07; P = 0.0054), and a month*climate interaction ($F_{24,72}$ = 2.07; P = 0.0095) on observed mean daily temperatures between sites. Seasonal patterns in the mean daily temperatures showed that coastal sites were characterized by cool summers and warm winters, while inland sites were characterized by hot summers and comparatively cooler winters than coastal sites (**Figure 5.3**). The intermediate climate type site's temperature patterns were more variable, and matched more closely to inland sites in the summer months and coastal sites in the winter months (**Figure 5.3**). The significant month*climate interaction appeared to be due to a larger proportional increase in temperatures at inland and intermediate sites during the summer months (**Figure 5.4**).

The average daily temperatures within each sample month ranged 9.97–26.17°C for inland sites, 10.83–25.83°C for intermediate sites, and 11.35–23.98°C for coastal sites during the study period. The average of the main daily temperatures across the entire study period was 19.25 (± SEM throughout the text) 0.20°C for inland, 18.54 ± 0.09°C for coastal, and 19.54 ± 0.18°C for intermediate sites. Differences in mean monthly temperatures between sites within climates types were greatest during the summer months (sample intervals 1–5, 25–26) (**Figures 5.3, 5.4**).

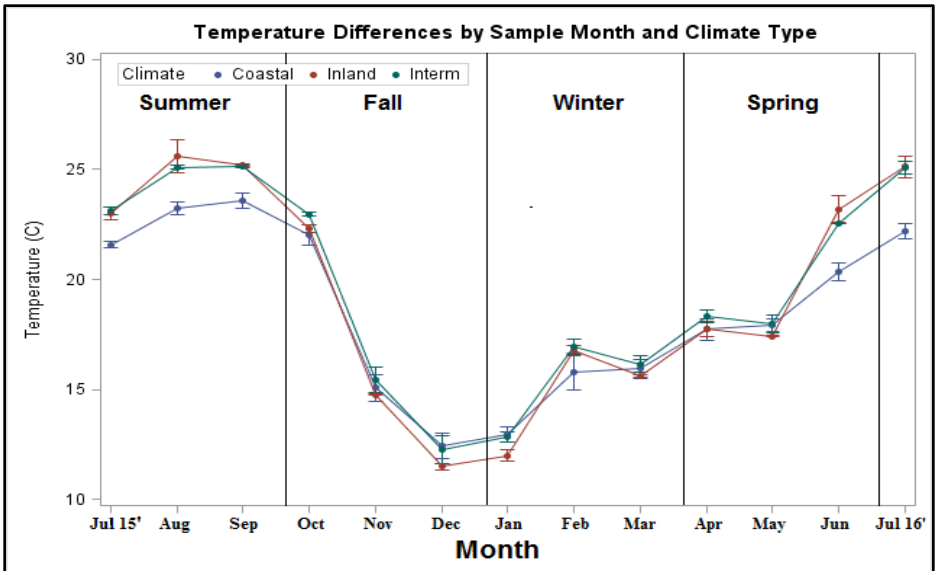

Figure 5.3 Temperature profiles of study sites belonging to either the coastal, intermediate, or inland climate types in Southern California throughout the 2015 to 2016 study period. Data points correspond to mean monthly temperatures (± SEM).

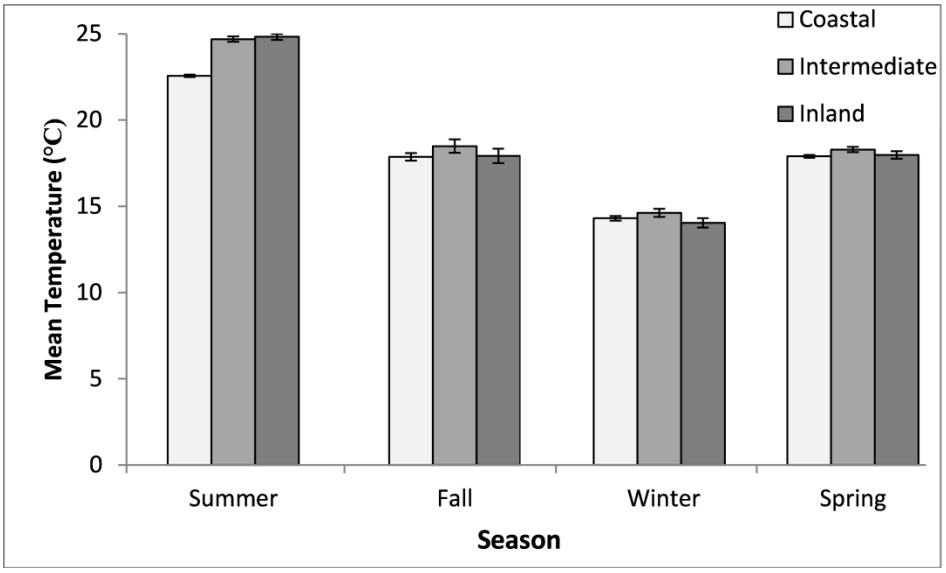

Figure 5.4 Temperature profiles of study sites belonging to either the coastal, intermediate, or inland climate types in Southern California throughout the 2015 to 2016 study period. Data points correspond to mean seasonal temperatures (± SEM).

Effects of Climate, Season, and Parasitism on *A. dugesii* Densities

Results from the whitefly density model indicated there was a significant climate*season interaction effect ($F_{6,131}$ = 2.57; P = 0.0157) (**Figure 5.5**), but no main effect of climate ($F_{2,131}$ = 0.33; P = 0.7215) on observed total *A. dugesii* population densities. The significant interaction effect between climate type and season appeared to be due in part to a greater decrease in *A. dugesii* populations at inland sites during the summer than observed for coastal and intermediate climates and a larger decrease in costal densities in the winter (**Figure 5.5**).

The model indicated that there was a significant main effect of season ($F_{3,131}$ = 30.84; P <0.0001) on observed total *A. dugesii* population densities. Post-hoc multiple comparison tests indicated that total population densities of *A. dugesii* was highest in the spring and summer and lowest during the winter (**Figure 5.5**). The model also indicated that the total parasitism rate from the previous sampling period was a significant covariate (Estimate = -0.601 ± 0.184) for observed total *A. dugesii* population densities in the subsequent sampling period ($F_{1,131}$ = 10.68; P = 0.0014) (**Figure 5.6**).

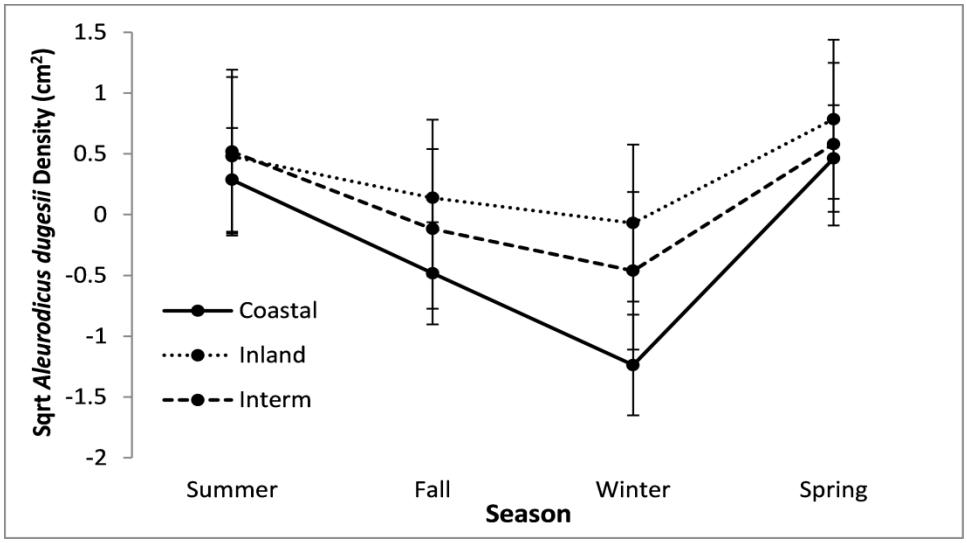

Figure 5.5 Square root of total *A. dugesii* population densities within study sites over the duration of the study period between different climate types within seasons. Values shown are least squared means (± SEM).

Effect of Climate and Season on Total Parasitism Rates

The results from the total parasitism model showed that there was a significant effect of season ($F_{3,137}$ = 14.40; P < 0.0001) on total parasitism rates observed (**Figure 5.7**). The model also indicated that there was no significant main effect of climate ($F_{2,137}$ = 0.71; P = 0.4957) or a climate*season interaction ($F_{6,131}$ = 1.68; P = 0.1305) on observed total parasitism rates, so these terms were not included in the maximal model. Unlike the significant effect of total parasitism rate from the previous sample interval on observed whitefly densities, the mean site total nymphal density from the previous sample interval was not a significant covariate for total parasitism rates in the subsequent sampling interval ($F_{1,137}$ = 2.91; P = 0.0905).

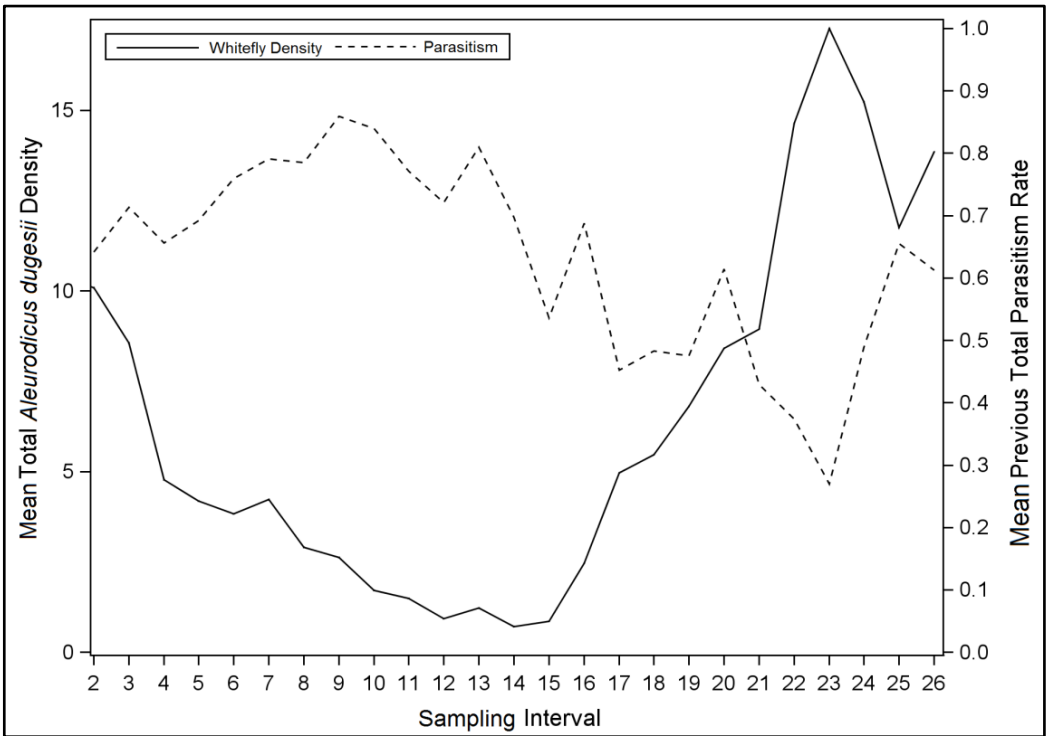

Figure 5.6 Plot of mean total *A. dugesii* population densities against the mean total parasitism rate from the previous sampling interval.

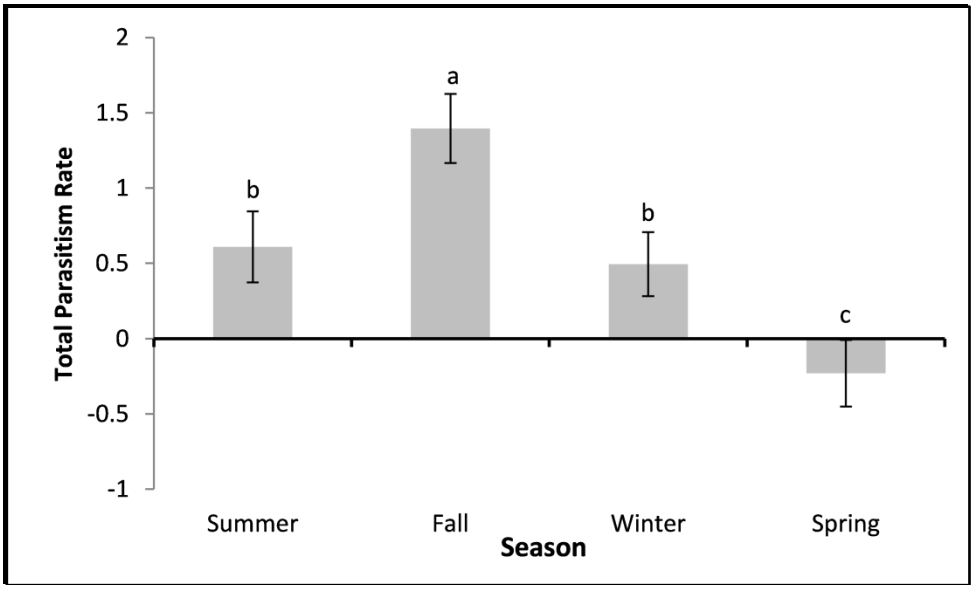

Figure 5.7 Total parasitism rates within study sites over the duration of the study period between seasons. Values shown are least squared means (± SEM).

Total *A. dugesii* parasitism rates across the entire study period were high, averaging 64.5 ± 1.1%. Multiple comparison tests showed that there was a significant difference in total parasitism rates between seasons, except between the summer and winter (t = 0.51; df = 137; P = 0.9570) (**Figure 5.7**). Total parasitism was highest in the fall, averaging 81.0 ± 1.4%. After reaching their fall peak, parasitism steadily declined throughout the winter until rates reached a low of 46.2 ± 2.6% in the spring, after which total parasitism rates exhibited a rapid increase into the summer months (**Figure 5.6**).

Effect of Climate and Season on Species-Specific Parasitism Rates

Idioporus affinis Parasitism Rates

Results from the *I. affinis* parasitism rate model indicated that there was no significant climate*season interaction ($F_{6,131}$ = 1.28; P = 0.2709) on observed parasitism rates. The mean site total nymphal density from the previous sample interval was also

shown to not be a significant covariate for *I. affinis* parasitism rates in the subsequent sampling interval ($F_{6,137}$ = 0.42; P = 0.5201). There was however, a significant main effect of climate ($F_{2,137}$ = 3.37; P = 0.0372) (**Figure 5.8**) and main effect of season ($F_{6,131}$ = 6.51; P = 0.0004) (**Figure 5.9**) on observed *I. affinis* parasitism rates.

Parasitism rates for *I. affinis* were overall the highest of the three parasitoid species, averaging 36.6 ± 1.1% throughout the study period. Multiple comparison tests indicated *I. affinis* parasitism rates were highest in inland sites, and did not differ between coastal and intermediate sites (t = 1.87; df = 137; P = 0.1505) (**Figure 5.8**). Inland *I. affinis* parasitism rates were consistently higher throughout the study period averaging 48.4 ± 1.8%, compared to 32.2 ± 1.5% and 25.8 ± 2.8% for coastal and intermediate sites respectively. Seasonal trends showed that *I. affinis* parasitism rates were relatively consistent across seasons (37–43%), except for a large observed decrease the spring where mean parasitism rates were 22.2 ± 2.1% (**Figure 5.9**).

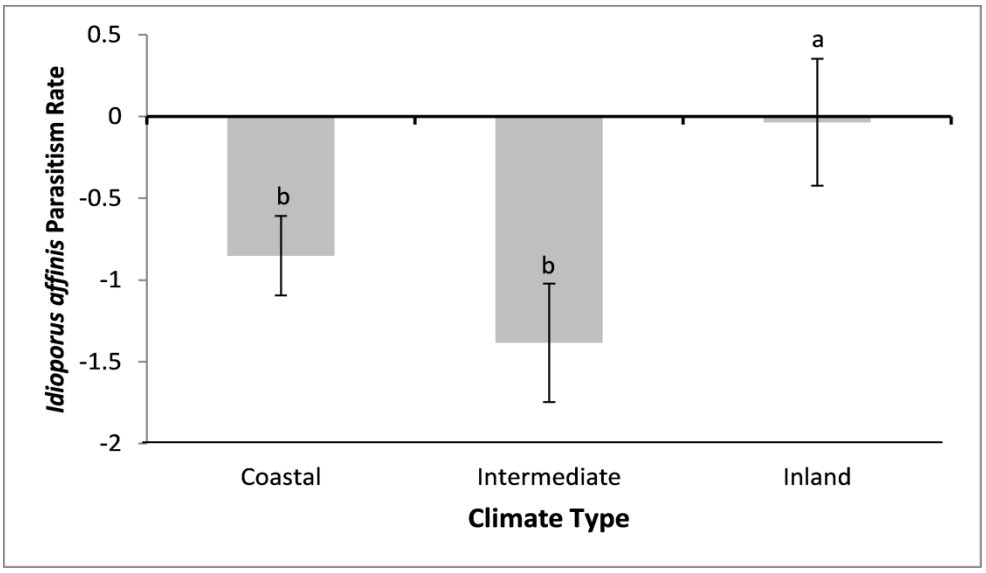

Figure 5.8 *Idioporus affinis* parasitism rates within study sites over the duration of the study period between climate types. Values shown are least squared means (± SEM).

Encarsia noyesi Primary Parasitism Rates

The *E. noyesi* primary parasitism rate model indicated that there was no significant climate*season interaction ($F_{6,118}$ = 1.67; P = 0.1346) or main effect of climate ($F_{2,137}$ = 0.66; P = 0.5206) on observed primary parasitism rates. As seen with the other parasitism rate models, the *E. noyesi* primary parasitism rate model indicated that there was a significant main effect of season ($F_{3,137}$ = 3.35; P = 0.0209) on observed primary parasitism rates (**Figure. 5.10)**. Similar to the other parasitism rate models, mean site 2nd –4th instar *A. dugesii* nymphal densities from the previous sample period was not a significant covariate for *E. noyesi* primary parasitism in the next sample period ($F_{1,137}$ = 2.23; P = 0.1376).

Primary parasitism rates for *E. noyesi* were the second highest of the three parasitoid species and averaged 26.7 ± 1.0% across the study period. Post-hoc analyses of seasonal trends showed that *E. noyesi* parasitism rates were relatively consistent across seasons (22–25%), except for the fall where mean parasitism rates were 35.8 ± 2.2%. Unlike *I. affinis* parasitism rates, primary parasitism rates of *E. noyesi* did not exhibit a significant spring decline, and remained consistent moving from the winter and into summer months (**Figure 5.10**).

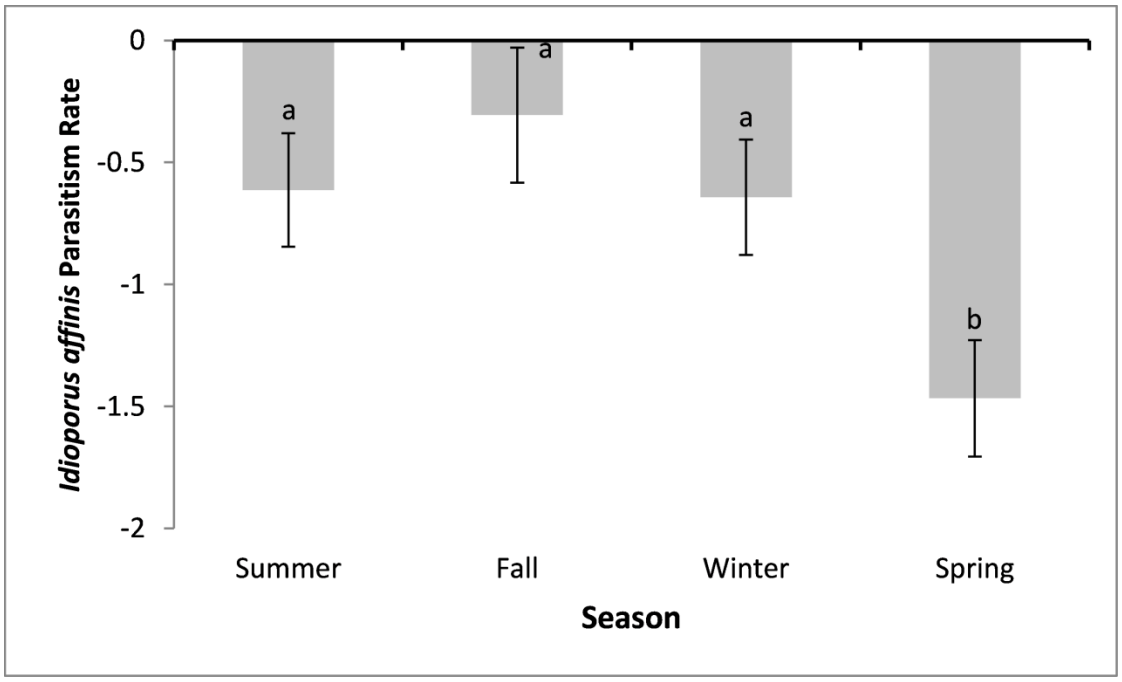

Figure 5.9 *Idioporus affinis* parasitism rates within study sites over the duration of the study period between seasons. Values shown are least squared means (± SEM).

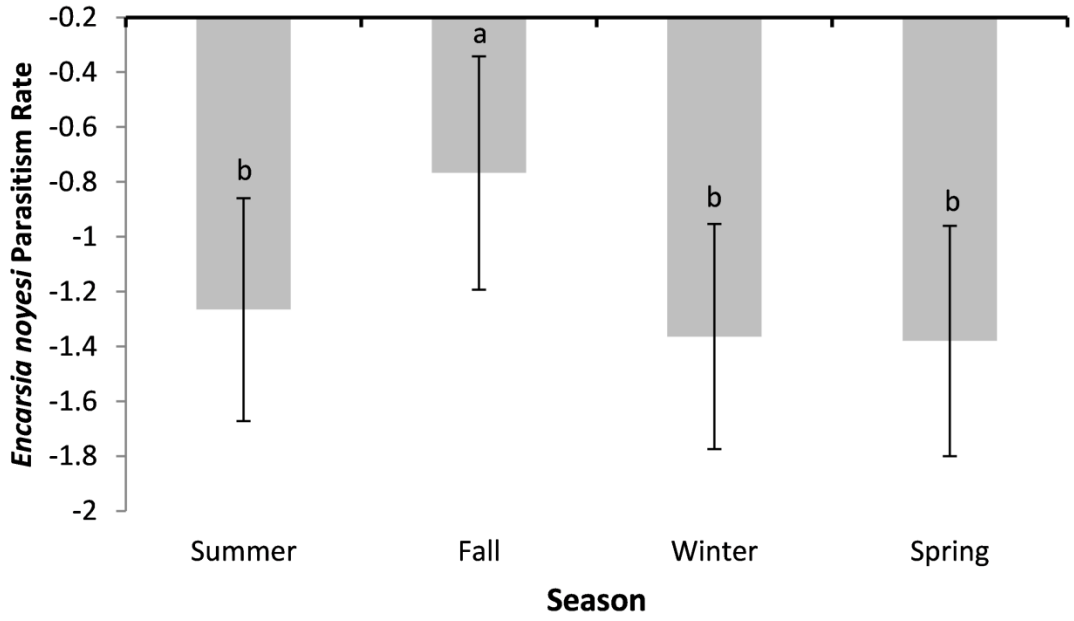

Figure 5.10 *Encarsia noyesi* parasitism rates within study sites over the duration of the study period between seasons. Values shown are least squared means (± SEM).

Entedononecremnus krauteri Parasitism Rates

Parasitism rates of *E. krauteri* were very low and averaged 1.4 ± 0.0% during the study period. Due to how infrequently *E. krauteri* parasitism events were observed and how variable parasitism events were across the study period (**Figure 5.11**), there was insufficient data points to obtain a working model for this species. Field observations of *E. krauteri* parasitism suggested that parasitism rates may be highest in the late summer and fall where average parasitism rates ranged 2.0–2.5% compared to less than 0.05% parasitism rates observed in the spring and winter respectively. Field observations also suggested that *E. krauteri* parasitism rates were highest in coastal sites where average parasitism during the study period was 2.08 ± 0.01%, compared to 0.059 ± 0.02% and 0.702 ± 0.54% average parasitism rates observed at intermediate and inland sites respectively.

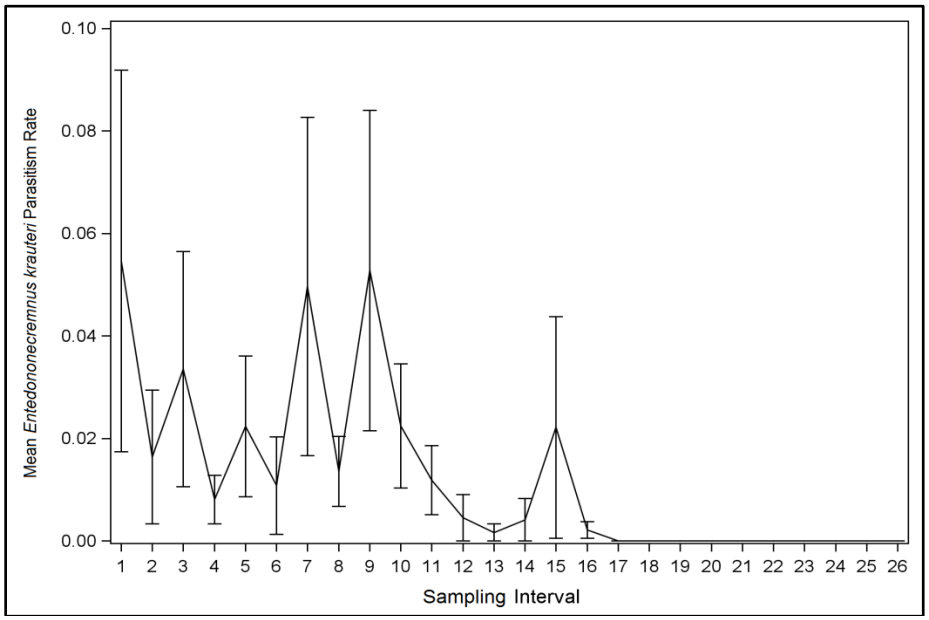

Figure 5.11 *Entedononecremnus krauteri* parasitism rates within study sites over the duration of the study period. Values shown are means (± SEM).

5.4 Discussion

Contrary to the prediction that *A. dugesii* population densities would be highest at coastal sites and would decline as whitefly populations moved further inland, results indicated there were no differences in *A. dugesii* population densities between climates. Higher extreme summer temperatures at inland sites were observed, with inland sites experiencing mean daily maximum temperatures during the summer of 32.2°C, compared to 30.9°C and 27.8°C for intermediate and coastal sites respectively. The upper developmental threshold determined for *A. dugesii* under laboratory conditions is 30°C (see **Chapter 2**), and population decline during periods exceeding this temperature was consistent with high mortality rates observed for variety of whitefly species at temperatures greater than 30°C (Cherry, 1979; Curnutte et al., 2014). Given that even short periods of heat stress exposure can inflict high mortality in whiteflies (Cui et al., 2008), summer temperatures may result in population die-offs at inland climates in southern California. The climate by season interaction observed for total *A. dugesii* population densities (**Figure 5.5**), which indicated a larger decrease in *A. dugesii* densities at inland sites during the spring to summer transition is consistent with this prediction.

Despite the potential negative effects of extreme summer temperatures on *A. dugesii* survival, during the majority of sample months mean daily temperatures at inland and intermediate sites were consistently closer to the thermal developmental optimum of 26°C observed for *A. dugesii* (see **Chapter 2**). This was particularly true for the spring and summer where significantly higher overall *A. dugesii* population densities were

observed. Elevated temperatures have been shown to increase the reproductive rates in whiteflies (Curnutte et al., 2014), and the combined effects of lower temperature extremes observed at coastal sites, but higher mean daily temperatures observed at intermediate and inland sites may explain the lack of population density differences between climates observed for *A. dugesii* in southern California.

Results indicating no overall climate effect on total parasitism and *E. noyesi* parasitism rates were also not aligned with my initial predictions. Parasitoids may (Campbell et al., 1974) or may not (Karban, 1998) exhibit higher thermal tolerances than their hosts, so it is unclear if parasitoid fitness in this system will be negatively impacted more or less by intermediate and inland site conditions than *A. dugesii*. Studies examining the thermal developmental maxima for the parasitoids in this system are needed to determine if mean daily maximum temperatures at intermediate or inland sites exceed their tolerance limits. If this is the case for *E. noyesi*, but not for *I. affinis,* then potentially higher mortality rates at inland and intermediate sites negate the predicted benefits of higher temperature on parasitism rates resulting in the lack of observed difference in *E. noyesi* parasitism between climates.

Idioporus affinis parasitism rates were highest at inland sites, which supports my initial prediction. It is unclear if higher levels of *I. affinis* parasitism at inland sites is due to more favorable environmental conditions enhancing *I. affinis* fitness, or due to competitive release with *E. noyesi* and *E. krauteri*. Higher temperatures at inland conditions may enhance some parasitism related processes, such as increasing parasitoid searching speeds (Mason and Hopper, 1997), but may inhibit others such as increasing

host handling times (Jalali et al., 2005; Wu et al., 2011) or enhancing the host's immune response (Blumberg and DeBach, 1981; Fellowes et al., 1999). Under ambient laboratory conditions, *I. affinis* was observed to possess shorter handling times and exhibited higher absolute parasitism rates than *E. noyesi* and *E. krauteri* (see **Chapter 4**). Thus, the higher temperatures observed at inland and intermediate sites may provide *I. affinis* with a greater competitive advantage within these climates. This hypothesis requires further testing of differences in these parasitoid species' foraging efficiencies under different temperatures.

An unexpected finding was the relative scarcity of *E. krauteri* in the field and little apparent contribution it provides to *A. dugesii* biological control. Both the flight phenology and parasitism rate data suggest that *E. krauteri* may be either a late-summer to fall active species or that conditions during this time of year provide weakened competition with *I. affinis* and *E. noyesi*, allowing its activity to be detected. A variety of anecdotal evidence suggests that *E. krauteri* is both a seasonal species and poor resource competitor. Field observations in southern California made by the authors during five years prior to the start of this study only observed *E. krauteri* activity during October through December. The original collection date for *E. krauteri* in the U.S. in 1995 was also in October (Zolnerowich and Rose, 1996), suggesting that *E. krauteri* may be a seasonal species. Additionally, population densities of *E. krauteri* were reportedly high in the U.S. with hundreds of adults observed foraging on plants infested with *A. dugesii* prior to the introduction of *E. noyesi* and *I. affinis* (per. comm. CH. Pickett & P. Krauter), which suggests that the *E. krauteri* is being competitively displaced. These observations

are supported by observations of *E. krauteri* laboratory colonies, which are capable of reaching high numbers of individuals in the absence of competitors (per. obs. EN Schoeller).

The large spike in *A. dugesii* population densities in the spring coincided with both rising temperatures and the lowest level of parasitism rates observed. High pupal mortality during winter months has been observed in California for other parasitoid species (DeBach et al., 1955) resulting in loss of biological control. Other forms of control may be necessary during the early part of spring when *A. dugesii* populations regain activity to prevent populations from reaching high levels until parasitoid populations rebound. From a biological control perspective, it remains unclear which climate type in southern California offers the greatest potential for control of *A. dugesii* populations. The predicted lower population growth rate of *A. dugesii* at coastal sites potentially facilitating its control in these areas may be offset by stronger competition between *I. affinis* and *E. noyesi*, which may favor *E. noyesi* the perceived inferior control agent. Under inland and intermediate climate conditions, where *A. dugesii* population growth is expected to be high except during summer conditions, *I. affinis* may be successfully regulating *A. dugesii* populations reaching higher levels than observed in the other climate types due to its increased performance. As global temperatures continue to rise (Karl and Trenbeth, 2003; Wuebbles et al., 2014), biological control under inland climate conditions may be enhanced as long as temperature increases do not negatively impact *I. affinis* performance. In order to obtain a clearer picture of the various processes impacting control of *A. dugesii* across southern California climates, studies are needed

that exclude each parasitoid from the system in order to assess the interaction between parasitoid competition and abiotic conditions on the level of biological control achieved.

5.5 Literature Cited

Amarasekare, P. 2007. Species coexistence in communities with intraguild predation: the roles of trade-offs and temporal variation. Ecology. 88:2720–2728.

Baffoe, K., Dalin, P., Nordlander, G., Stenberg, JA. 2012. Importance of temperature for the performance and biocontrol efficiency of the parasitoid *Perilitus brevicollis* (Hymenoptera: braconidae) on *Salix*. BioControl 57:611–618

Bannerman, JA., Gillespie, DR., Roitberg, BD. 2011. The impacts of extreme and fluctuating temperatures on trait-mediated indirect aphid–parasitoid interactions. Ecol. Entomol. 36:490–498.

Bellows, TS., Jr., Kabashima, JN., Robb, KL. 2002. Giant Whitefly. UC ANR Publ. 7400.

Blumberg, D. 1991. Seasonal variations in the encapsulation of eggs of the encyrtid parasitoid *Metaphycus stanleyi* by the pyriform scale, *Protopulvinaria pyriformis*. Entomol. Exp. Appl. 58:231–237.

Blumberg, D., DeBach, P. 1981. Effects of temperature and host age upon the encapsulation of *Metaphycus stanleyi* and *Metaphycus helvolus* eggs by brown soft scale *Coccus hesperidium*. J. Invertebr. Pathol. 37:73–79.

Bolker, BM., Brooks, ME., Clark, CJ., Geange, SW., Poulsen, JR., et al. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24:127–135.

Bourchier, RS., Smith, SM. 1996. Influence of environmental conditions and parasitoid quality on field performance of *Trichogramma minutum*. Entomol. Exp. Appl. 80:461–468.

Briggs, CJ., Latto, J. 1996. The window of vulnerability and its effect on relative parasitoid abundance. Ecol.l Entomol. 21:128–140.

Briggs, CJ., Murdoch, WW., Nisbet, RM. 1999. Recent developments in theory for biological control of insect pests by parasitoids. *In*: Hawkins, BA., Cornell, HV.

(Eds.) Theoretical Approaches to Biological Control, Cambridge University Press. Cambridge, UK.

Campbell, A., Frazer, BD., Gilbert, N., Gutierrez, AP., Mackauer, M. 1974. Temperature requirements of some aphids and their parasites. J. Appl. Ecol. 11:431–438.

Cherry, RH. 1979. Temperature tolerance of three whitefly species found in Florida. Environ. Entomol. 8:1150-1152.

Crozier, L. 2004. Warmer winters drive butterfly range expansion by increasing survivorship. Ecology. 85:231–41.

Cui, XH., Wan, FH., Xie, M., Liu, TX. 2008. Effects of heat shock on survival and reproduction of two whitefly species, *Trialeurodes vaporariorum* and *Bemisia tabaci* biotype B. J. Insect Sci. 24:1–10.

Curnutte, LB., Simmons, AM., Abd-Rabou, S. 2014. Climate Change and *Bemisia tabaci* (Hemiptera: Aleyrodidae): Impacts of Temperature and Carbon Dioxide on Life History. Ann. Entomol. Soc. Am. 107:933–943.

DeBach, P., Fisher, TW., Landi, J. 1955. Some effects of meteorological factors on all stages of *Aphytis lingnanensis*, a parasite of the California red scale. Ecology 36:743–753.

DeBach, P., Hagen, KS. 1964. Manipulation of entomophagous species. *In*: DeBach, P. (Ed.) Biological Control of Insect Pests and Weeds, Chapman and Hall Ltd, London, UK.

DeBach, P., Hendrickson, RM., Rose, M. 1978.Competitive displacement: extinction of the yellow scale, *Aonidiella citrina* (Coq.) (Homoptera: Diaspididae), by its ecological homologue, the California red scale, *Aonidiella aurantii* (Mask.) in southern California. Hilgardia 46:1–35.

Ehler, LE., Hall, RW. 1982. Evidence for competitive exclusion of introduced natural enemies in biological control. Environ. Entomol. 11:1–4.

Evans GA (2008) The whiteflies (Hemiptera: Aleyrodidae) of the world and their host plants and natural enemies. Available online at: http://keys.lucidcentral.org/keys/v3/whitefly/PDF_PwP%20ETC/world-whitefly-catalog-Evans.pdf

Fellowes, MDE., Kraaijeveld, AR., Godfray, HCJ. 1999. Cross-resistance following artificial selection for increased defense against parasitoids in *Drosophila melanogaster*. Evolution. 53:966–972.

Fink, U., Völkl, W. 1995. The effect of abiotic factors on foraging and oviposition success of the aphid parasitoid, *Aphidius rosae*. Oecologia 103:371–378.

Gill, RJ. 1992. Giant whitefly. California Plant Pest and Disease Report. Calif. Dept. Food Agric. 11:78–81.

Godfray, HCJ., Hassell, MP., Holt, RD. 1994. The population dynamic consequences of phenological asynchrony between parasitoids and their hosts. J. Animal Ecol. 63:1–10.

Greathead, DJ., Greathead, AH. 1992. Biological control of insect pests by insect parasitoids and predators: the BIOCAT database. Biocontrol News Inform. 13:61–68.

Hågvar, EB. 1991. Ecological problems in the establishment of introduced predators and parasites for biological control. Acta Entomol. Bohemoslov. 88: 1–11.

Hance, T., van Baaren, J., Vernon, P., Boivin, G. 2007 Impact of extreme temperatures on parasitoids in a climate change perspective. Annu. Rev. Entomol. 52, 107–126.

Harrington, R., Woiwod, I., Sparks, T. 1999. Climate change and trophic interactions. Trends Ecol. Evol. 14:146–150.

Hassell, MP. 2000. The spatial and temporal dynamics of host–parasitoid interactions. Oxford University Press, Oxford UK.

Hawkins, BA., Sheehan, W. 1994. Parasitoid Community Ecology. Oxford University Press, Oxford.

Hérard, F., Keller, MA., Lewis, WJ., Tumlinson, JH. 1988. Beneficial arthropod behaviour mediated by airborne semiochemicals. III. Influence of age and experience on flight chamber responses of *Microplitis demolitor* Wilkinson. J. Chem. Ecol. 14:1583–1596.

Hodges, G. 2004. Giant Whitefly, *Aleurodicus dugesii* Cockerell, in Florida. Pest Alert, Division of Plant Industry, Fla. Dept. Agr. Cons. Serv. Available online at: http://www.freshfromflorida.com/content/download/68178/1612763/Pest_Alert_-_Aleurodicus_dugesii,_Giant_whitefly.pdf

Hoelmer, KA., Kirk, AA. 2005. Selecting arthropod biological control agents against arthropod pests: can the science be improved to decrease the risk of releasing ineffective agents? Biol. Control. 34:255–264.

Heu, RA., Nagamine, NT., Kumashiro, BR., Watanabe, TM. 2004. Giant whitefly, *Aleurodicus dugesii* Cockerell (Homoptera: Aleyrodidae). Hawaii Dept. Agr. New Pest Advis. No. 02-04. June 2004 (revision). Available online at: http://hawaii.gov/hdoa/pi/ppc/npa-1/npa02-04-giantwf.pdf.

Huffaker, CB., Gutierrez, AO. 1990. Evaluation of efficiency of natural enemies in biological control. *In*: Rosen, D. (Ed.), World Crop Pest Volume 4B: Armored Scale Insects: Their Biology, Natural Enemies and Control, Elsevier, Amsterdam.

Huffaker, CB., Messenger, PS., DeBach, P. 1971. The natural enemy component in natural control and the theory of biological control. *In*: Huffaker, CB. (Ed.) Biological Control. Academic Press, New York.

Hunter, MS., Kelly, SE. 1998. Hyperparasitism by an exotic autoparasitoid: secondary host selection and the window of conspecific and native heterospecific hosts. Entomol. Exp. Appl. 89:249–259.

Jalali, SK., Venkatesan, T., Murthy, KS., Biswas, SR., Lalitha, Y. 2005. Influence of temperature and host density on functional response of *Telenomus remus* Nixon, an egg parasitoid of *Spodoptera litura* Fabricius. Entomon, 30:193–199.

Jeffs, CT., Lewis, OT. 2013. Effects of climate warming on hostparasitoid interactions. Ecol. Entomol. 38:209–218.

Karban, R. 1998. Caterpillar basking behavior and nonlethal parasitism by tachinid flies. J. Insect Behav. 11:713–23.

Karl, TR., Trenbeth, KE. 2003. Modern global climate change. Science. 302:1719–1723.

Kiritani, K. 2006. Predicting impacts of global warming on population dynamics and distribution of arthropods in Japan. Popul. Ecol. 48:5–12.

Lasalle, J., Polaszek, A., Noyes, JS., Zolnerowich, G. 1997. A new whitefly parasitoid (Hymenoptera: Pteromalidae: Eunotinae), with comments on its placement, and implications for classification of Chalcidoidea with particular reference to the Eriaporinae (Hymenoptera: Aphelinidae). Syst. Entomol. 22:131–150.

Lebrun, EG., Plowes, RM., Gilbert, LE. 2009. Indirect competition facilitates widespread displacement of one naturalized parasitoid of imported fire ants by another. Ecology 90:1184–1194.

Mason, PG., Hopper, KR. 1997. Temperature dependence in locomotion of the parasitoid *Aphelinus asychis* (Hymenoptera: Aphelinidae) from geographical regions with different climates. Environ. Entomol. 26:1416–1423.

Muniappan, R., Shepard, BM., Watson, GW., *et al*. 2009. New records of invasive insects (Hemiptera: Sternorrhyncha) in southern Asia and West Africa. J. Agr. Urban Entomol. 26:167–174.

Nguyen, R., Hamon, AB. 2002. Biological control of giant whitefly, *Aleurodicus dugesii* Cockerell, in Florida. Fla. Dept. Agr. Cons. Serv. Available online at Available at: http://www.nhm.ac.uk/resources/researchcuration/projects/chalcidoids/pdf_X/NguyenHa2002.pdf

O'Neal, ME., Landis, DA., Isaacs, R. 2002. An inexpensive, accurate method for measuring leaf area and defoliation through digital image analysis. J. Econ. Entomol. 95:1190–1194.

Pickett, CH., Ball, JC., Casanave, KC., Klonsky, KM., Jetter, KM., Bezark, LG., Schoenig, SE. 1996. Establishment of the ash whitefly parasitoid *Encarsia inaron* (Walker) and its economic benefit to ornamental street trees in California. Biol. Control. 6:260–272.

Péré, C., Jactel, H., Kenis, M. 2013. Response of insect parasitism to elevation depends on host and parasitoid life-history strategies. Biol. Lett. 9:20130028.

Price, PW. 1987. The role of natural enemies in insect populations. *In*: Barbosa, P., Schultz, JC. (Eds.) Insect Outbreaks, Academic Press, San Diego, USA.

Quezada, JR., DeBach, P. 1973. Bioecological and population studies of the cottony scale, *Icerya purchase* Mask. and its natural enemies, *Rodolia cardinalis* Mul. and *Cryptochaetum iceryae* Wil., in southern California. Hilgardia. 41:631–688.

Risch SJ. 1987. Agricultural ecology and insect outbreaks. *In*: Barbosa, P., Schultz, JC. (Eds.) Insect Outbreaks, Academic Press, San Diego, USA.

Rochat, J., Gutierrez, AP. 2001. Weather-mediated regulation of olive scale by two parasitoids. J. Anim. Ecol. 70:476–490.

Romo, CM., Tylianakis, JM. 2013. Elevated temperature and drought interact to reduce parasitoid effectiveness in suppressing hosts. PLoS One. 8:e58136.

Samways, MJ., Osborn, R., Hastings, H., Hattingh, V. 1999. Global climate change and accuracy of prediction of species' geographical ranges: establishment success of introduced ladybirds (Coccinellidae, *Chilocorus* spp.) worldwide. J. Biogeography. 26:795–812.

Simberloff, D. 2012. Risks of biological control for conservation purposes. Biocontrol. 57:263–276.

Sorribas, J., Rodriguez, R., Garcia-Marí, F. 2010. Parasitoid competitive displacement and coexistence in citrus agroecosystems: linking species distribution with climate. Ecol. Appl. 20:1101–1113.

Stenseth, NC., Mysterud, A. 2002 Climate, changing phenology, and other life history traits: nonlinearity and match-mismatch to the environment. Proc. Natl. Acad. Sci. USA. 99:13379–13381.

Stiling, P. 1990. Calculating the establishment rates of parasitoids in classical biological control. Am. Entomol. 36:225–230.

Stiling, P. 1993. Why do natural enemies fail in classical biological control programs? Am. Entomol. 39:31–37.

Stireman, JO., Dyer, LA., Janzen, DH., Singer, MS., Lill, JT., Marquis, RJ., et al. 2005. Climate unpredictability and parasitism of caterpillars: Implications of global warming. Proc Natl. Acad. Sci. USA. 102:17384–17387.

Thomas, MB., Blanford, S. 2003. Thermal biology in insect-parasite interactions. Trends Ecol. Evol. 18:344–350.

Thomson, LJ., Macfadyen, S., Hoffmann, AA. 2010. Predicting the effects of climate change on natural enemies of agricultural pests. Biol. Control. 52:296–306.

Turnbull, AL., Chant, PA. 1961. The practice and theory of biological control of insects in Canada. Can. J. Zool. 89:697–753.

van Baaren, J., Outreman, Y., Boivin, G. 2005. Effect of low temperature exposure on oviposition behavior and patch exploitation strategy in parasitic wasps. Anim. Behav. 70:153–163.

van Nouhuys, S., Lei, G. 2004. Parasitoid-host metapopulation dynamics: the causes and consequences of phenological asynchrony. J. Anim. Ecol. 73:526–35.

Virtanen, T., Neuvonen, S. 1999. Performance of moth larvae on birch in relation to altitude, climate, host quality and parasitoids. Oecologia. 120:92–101.

Voigt, W., Perner, J., Davis, AJ., Eggers, T., Schumacher, J, et al. 2003. Trophic levels are differentially sensitive to climate. Ecology. 84:2444–2453.

Waage, JK., Hassell, MP. 1982. Parasitoids as biological control agents-a fundamental approach. Parasitology. 84:241–268.

Walther, G.-R. 2010. Community and ecosystem responses to recent climate change. Philos. Trans. R. Soc. Lond. B Biol. Sci. 365:2019–2024.

Wetherington, MT., Jennings, DE., Shrewsbury, PM., Duan, JJ. 2017. Climate variation alters the synchrony of host–parasitoid interactions. Ecol. Evol. 7:8578–8587.

Wu, GM., Barrette, M., Boivin, G., Brodeur, J., Giraldeau, LA., Hance, T. 2011. Temperature influences the handling efficiency of an aphid parasitoid through body size-mediated effects. Environ. Entomol. 40:737–742.

Wuebbles, D., Meehl, G., Hayhoe, K., Karl, T. R., Kunkel, K., Santer, B., Sun, L. 2014. CMIP5 climate model analyses: Climate extremes in the United States. Bull. Am. Meteorol. Soc. 95:571–583.

Zolnerowich, G., Rose, M. 1996. A new species of *Entedononecremnus* (Hymenoptera: Chalcidoidea: Eulophidae) parasitic on the giant whitefly, *Aleurodicus dugesii* Cockerell (Homoptera: Aleyrodidae). P. Entomol. Soc. Wash. 98:369–373.

Chapter 6

Concluding Remarks

6.1 Additional Remarks

The broad objective of this dissertation was to determine aspects of the basic biologies of *A. dugesii* and its parasitoids in order to better understand the dynamics of this system and assess the potential for *A. dugesii* biological control. I sought to identify factors mediating competition between parasitoids in order to better understand how various parasitoid life history and behavioral traits determine the outcome of competition. The findings of this dissertation will enable researchers to better predict the outcome of multiple parasitoid species introductions for biological control programs by providing additional context for trait- and behaviorally-mediated parasitoid interspecific competitive interactions.

Chapter 2

Both global temperatures and the frequency of extreme weather events are predicted to increase by the year 2100 (Kunkel et al., 1999; Easterling et al., 2000; Karl and Trenbeth, 2003). Climate change induced increases in global temperatures and events such as extreme droughts are predicted to greatly impact insect population dynamics and interspecific interactions (Hance et al., 2007; Tylianakis et al., 2008; Kiritani, 2013; Romo and Tylianakis, 2013). My findings showed that given the upper thermal physiological limit of *A. dugesii*, that mortality levels may increase in parts of southern

California (e.g. inland climates) as global temperatures continue to rise. Rising global temperatures however, may allow the northward expansion of *A. dugesii* populations. It remains unclear whether parasitoids in this system will respond to these changes in a similar way.

Chapter 3

I found that while there was overlap in host stage use between parasitoid species in this system, that stage preferences were not identical. The long-term prospects for parasitoid species coexistence in this system appears promising, at least for *E. noyesi* and *I. affinis*, but the host preference studies indicated a dubious future for *E. krauteri* given its narrow host use.

Given that these parasitoids did not co-occur historically in the native range of *A. dugesii*, the conditions under which host use patterns and stage preferences may have evolved is still a mystery. It's unclear how long *E. noyesi* has existed in sympatry with the source populations of *E. krauteri* and *I. affinis*. The native range of *E. noyesi* appears to be Trinidad (Hayat, 1983), but it was exported to other Caribbean Islands such as St. Vincent and Barbados in 1950 (Cock et al., 1985) as well as Brazil in 1966 (Kamath, 1979) as a biological control agent for coconut whitefly *Aleurodicus cocois* (Curtis). How *E. noyesi* arrived in Mexico where source populations were collected for biological control of *A. dugesii*, or whether populations existed there without human intervention is unclear. It may be possible to trace the origin of Mexican populations of *E. noyesi* using microsatellite marker techniques (Stouthamer, 2006; Margaritopoulos et al., 2009). This information may prove useful for interpreting host use strategies in this system, because it

is unknown whether current host use patterns by *I. affinis* and *E. krauteri* reflect competition with *E. noyesi*. This is because potential shifts in host use by parasitoids can occur rapidly in the presence of competitors (Bográn et al. 2002).

During host preference trials host feeding behavior (consumption of hemolymph) by *E. noyesi* was observed, but not by *I. affinis* or *E. krauteri*. This behavior was rarely observed during trials as wasps were well-fed prior to the start of experiments. Further investigation showed that starved individuals of *E. noyesi* spent considerable amounts of time host-feeding. All three parasitoid species fed on honeydew attached to leaves, but only *I. affinis* was observed antennating the vasiform orifice of nymphs waiting for honeydew excretion when in a starved state. Host feeding (lethal interference competition) can provide an advantage in resource competition by directly removing competitors from the system (Collier and Hunter, 2001). The fact that *E. noyesi* host feeds may provide it with an additional competitive advantage over the other two parasitoid species. Host feeding as an additional source of host morality has been viewed as a positive attribute in biological control agents (Jervis et al. 1996)., and how host feeding in this system relates to parasitoid competition and host suppression capabilities needs to be investigated further.

Chapter 4

Among insects, the hemipteran suborder Sternorrhyncha is particularly notable for its prolific lipid production (Smith, 1999; Blomquist and Bagnères, 2010). In these groups, lipid production appears to be most common in sessile taxa. For example, while most aphids (Hemiptera: Aphididae) are mobile feeders and produce little wax, species

like the woolly apple aphid *Eriosoma lanigerum* (Hausm.) are comparatively sessile and produce copious amounts (Mueller et al., 1992). Although the strength of this trend has not been examined, lipid production may be one of the few secondary defense mechanisms available for sessile insects such as *A. dugesii* against natural enemies.

My findings in this system are consistent with the literature showing that wax production is an effective form of defense for a variety of insect taxa. For example, Mueller et al. (1992) found that the defensive strength of wax produced the woolly apple aphid *Eriosoma lanigerum* (Hausm.) was correlated with colony size and larger colonies (dense wax) experienced lower parasitism rates by the aphelinid *Aphelinus mali* (Haldeman). Moss et al. (2006) found that significantly more individuals of *E. lanigerum* were stalked by the salticid spider *Marpissa marina* Goyen that had their wax coating left intact compared to individuals that had their wax experimentally removed. In addition to visual camouflage provided by wax, chemical camouflage against predation by wax presence also occurs as seen for the wax-producing coccinellid larvae *Scymnus louisianae* J. Chapin against attack by the cornfield ant *Lasius neoniger* Emery (Schwartzberg et al., 2010). A lot more research is needed on the impact of host defenses on the efficacy of biological control agents given the ubiquity of these defenses in nature.

Chapter 5

The invasion process of non-native species is typically described by three distinct phases: arrival, establishment, and spread (Kolar and Lodge, 2001; Jeschke and Strayer, 2006; Liebhold and Tobin, 2008). In classical biological control programs the arrival phase of species is facilitated by humans, leaving the most difficult hurdle of achieving

successful biological control the initial establishment of control agents (Hall and Ehler, 1979; Julien et al., 1984; Stiling, 1990, 1993; Denoth et al., 2002). Establishment failures are often attributed to mismatches in climactic tolerances of parasitoid species between their native and introduced ranges (Bartlett and van den Bosch, 1964; Beirne, 1975; Hopper et al., 1993; Murphy and Kay, 2000).

Selecting suitable biological control agents for release is a key step in classical biological control programs. In some cases multiple candidate natural enemy species exist and may occupy a wide geographic distribution within their native range encompassing many climactic conditions (van Driesche et al., 2008). It is important to consider the environmental tolerances of different candidate species, as well the potential existence of locally adapted "biotypes" when selecting control agents as these choices may greatly impact the level of pest control achieved (Sands and Harley, 1981; Armstrong and Wratten, 1996). In this system no efforts were undertaken to survey and collect the most suitable populations for release in the United States and all individuals of each parasitoid species originates from a single source population. Given this fact, parasitoid populations may be maladapted to the various local conditions where they have released in the United States and genetically constrained preventing local adaptation from occurring.

Climate matching is a process by which candidate natural enemies are selected from areas with climates most similar to the intended release site, with the working hypothesis that these individuals will be pre-adapted to handle similar environmental stress. Climate matching has been shown can greatly facilitate the process of natural

enemy establishment (Cameron et al., 1993; Hawkins and Cornell, 1994). Prospective climate matching between the native range and areas of introduction of natural enemies has proven to be an important technique in both determining the success of biological control programs and reducing the time investment involved in searching for candidate natural enemies (DeBach, 1964; Wilson and Huffaker, 1976; Hoelmer and Kirk, 2005, van Driesche et al., 2008). It may be a worthwhile endeavor to locate other source populations of these parasitoid species in climates in their native range that most closely match that of southern California and other problem areas such as Florida to enhance biological control of *A. dugesii*.

After a few months of collecting and examining samples from the field during the phenology experiment, it became apparent that *E. noyesi* was a heteronomous hyperparasitoid (Hunter and Kelly, 1998), where females develop as primary parasitoids on *A. dugesii* and males develop on pupae of conspecific females or on the pupae of *I. affinis* and *E. krauteri.* This life history was confirmed by another research group near the conclusion of my study (Boughton et al., 2015). Beginning in November 2015, *E. noyesi* hyperparasitism rates were recorded until the end of the study. Hyperparasitism events by *E. noyesi* are easily distinguishable visually compared to primary parasitism events of *I. affinis* and *E. krauteri* (**Figure 6.1**). While conspecific hyperparasitism occurs frequently, it is difficult to identify with certainty as the male larvae typically destroy the female pupal remains. Because of this, only hyperparasitism events on *I. affinis* and *E. krauteri* were quantified. Dissections were performed to further confirm hyperparasitism events of nymphs with parasitoid emergence holes at the time of collection. Male *E. noyesi* that

emerged after nymphs were held in environmental chambers were matched with their host of origin to ensure all hyperparasitism events were accounted for.

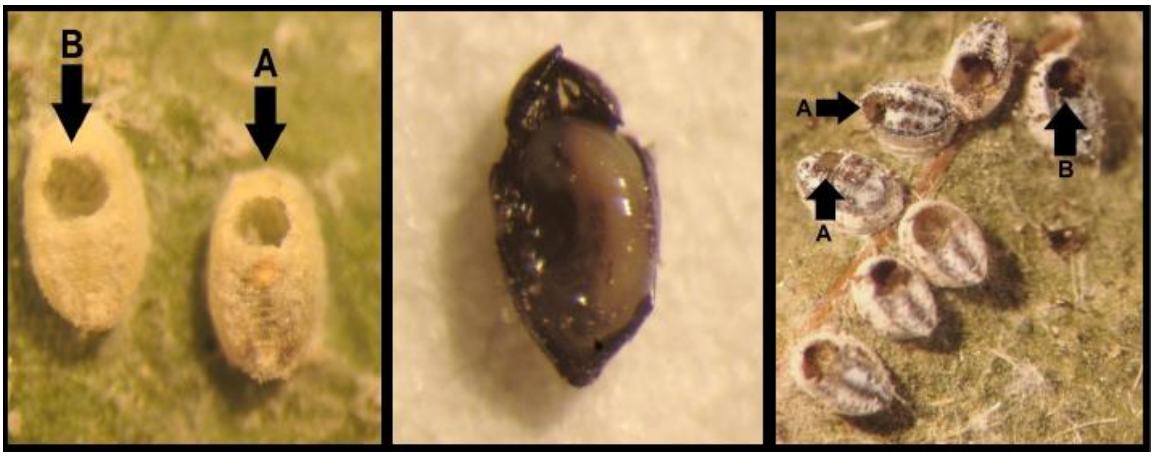

Figure 6.1 Visual evidence of *E. noyesi* hyperparasitism. Male *E. noyesi* emergence holes (**A**) and primary parasitoid emergence holes (**B**) and are shown. Pictured from left to right: *I. affinis* produces clear pupal exuvia and hyperparasitism events can be determined by the black pupal exuvia *E. noyesi* males leave behind after emergence; A male *E. noyesi* larva feeding on a female *E, noyesi* pupa; The much smaller and off-center *E. noyesi* male emergence holes compared to the larger and centered *E. krauteri* emergence holes.

Field observations indicated that total *E. noyesi* hyperparasitism rates on *I. affinis* and *E. krauteri* were highly variable and averaged 11.96 ± 1.1% across the study period (sample intervals 9–26, the time points after *E. noyesi* hyperparasitism was first recognized) (**Figure 6.2**). Mean observed *E. noyesi* hyperparasitism rates in inland sites were 7.0 ± 1.5%, which is approximately half the rates observed in coastal (17.2 ± 3.4%) and intermediate sites (14.1 ± 5.3%). The majority of *E. noyesi* hyperparasitism events were observed on *I. affinis* (n = 349 leaves) due to the rarity of *E. krauteri* in the field (n = 34 leaves). When present, hyperparasitism events on *E. krauteri* averaged 13.2 ± 5.1% compared to 12.3 ± 1.2% for *I. affinis.* Additional studies are necessary examining the ability of *E. noyesi* to recognize previously parasitized hosts and to determine whether a

preference exists for hosts parasitized by conspecifics or heterospecifics to better understand the potential consequences of hyperparasitism in this system.

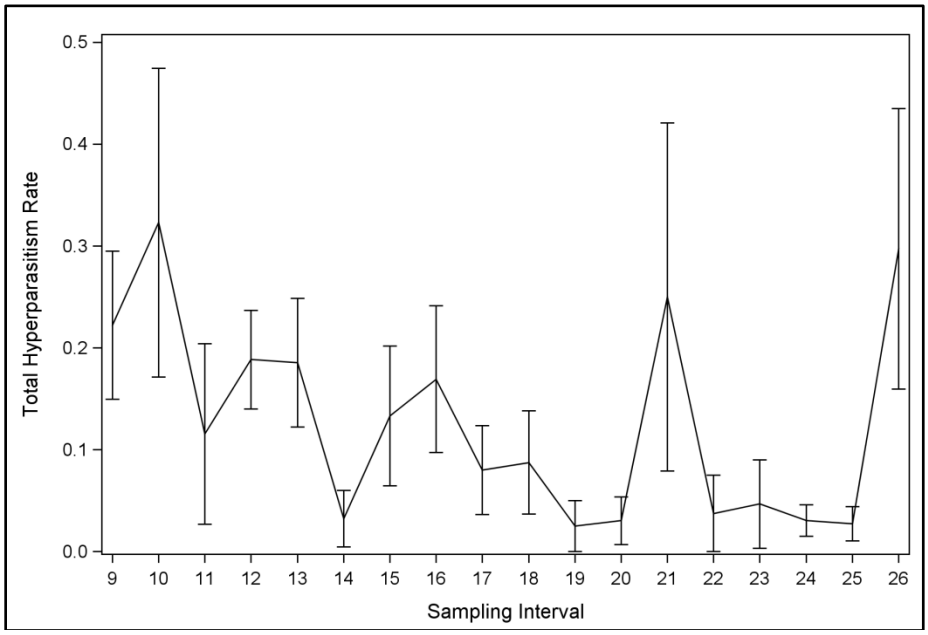

Figure 6.2 Total *Encarsia noyesi* hyperparasitism rates across study sites over the course of the sampling period. Values shown are means (± SEM).

6.2 Conclusions

The culmination of findings from this dissertation suggest that in the absence of competition *I. affinis* may be the superior *A. dugesii* biological control agent, but that *E. noyesi* is the superior competitor. The contribution of *E. krauteri* to *A. dugesii* biological control however, appears limited and my findings suggest that *E. krauteri* may be competitively excluded from the system over time. Not surprising, the outcome of the competitive interactions observed in this system appears context dependent, which is to be expected given that interspecific competitive outcomes between parasitoids depends on many variables.

We are only beginning to scratch the surface on the dynamics of this system and many questions still remain unanswered. One of the most important remaining questions is the degree of *A. dugesii* biological control that can be achieved by each parasitoid species. In the future, studies focusing on the contribution of each parasitoid species alone and in combination with the other species in this system on the level of *A. dugesii* control achieved should be a priority to confirm the findings of this study. This information is necessary in order to make informed recommendations for enhancing current biological control programs against *A. dugesii* or to establish new programs as the infested range of *A. dugesii* continues to expand.

6.3 Literature Cited

Armstrong, KF., Wratten, SD. 1996. The use of DNA analysis and the polymerase chain reaction in the study of introducedpests in New Zealand. *In*: Symondson, WOC., Liddell, JE. (Eds.), The Ecology of Agricultural Pests: Biochemical Approaches Chapman and Hall, London, UK.

Bartlett, BR., van den Bosch, R. 1964. Foreign exploration for beneficial organisms. *In*: DeBach, P. (Ed.), Biological Control of Insect Pests and Weeds. Reinhold Publishing, New York, USA.

Beirne, BP. 1975. Biological control attempts by introductions against insect pests in the field in Canada. Can. Entomol. 107:225–226.

Blomquist, G.J., Bagnères, A-G., 2010. Insect Hydrocarbons: Biology, Biochemistry and Chemical Ecology. Cambridge University Press.

Bográn, CE., Heinz, KM., Ciomperlik, MA. 2002. Interspecific competition among insect parasitoids: field experiments with whiteflies as hosts in cotton. Ecology. 83:653–668.

Boughton, AJ., Mendez, MA., Francis, AW., Smith, TR., Osborne, LS., Mannion, CM. 2015. Host stage suitability and impact of *Encarsia noyesi* (Hymenoptera: Aphelinidae) on the invasive rugose spiraling whitefly, *Aleurodicus rugioperculatus* (Hemiptera: Aleyrodidae), in Florida. Biol. Control. 88:61–67.

Cameron, PJ., Hill, RL., Bain, J.. Thomas, WP. 1993. Analysis of importations for biological control of insect pests and weeds in New Zealand. Biocontrol Sci. Technol. 3:387–404.

Cock, MJW. 1985. A review of biological control of pests in the Commonwealth Caribbean and Bermuda up to 1982. CIBC Technical Communication. 9. pp 218.

Collier, TR., Hunter, MS. 2001. Interference competition between whitefly parasitoids, *Eretmocerus eremicus*, and *Encarsia transvena*. Oecologia. 129:147–154.

DeBach, P. 1964. Biological Control of Insect Pests and Weeds. Chapman and Hall, London, UK.

Denoth, M., Frid, L., Myers, JH. 2002. Multiple agents in biological control: improving the odds? Biol. Control. 24:20–30.

Easterling, DR., Meehl, GA., Parmesan, C., Changnon, SA., Karl, TR., Mearns, LO. 2000. Climate extremes: observations, modeling, and impacts. Science. 289:2068–2074.

Hall, RW., Ehler, LE. 1979. Rate of establishment of natural enemies in classical biological control. Bull. Entomol. Soc. Am. 25:280–82.

Hance, T., van Baaren, J., Vernon, P., Boivin, G. 2007 Impact of extreme temperatures on parasitoids in a climate change perspective. Annu. Rev. Entomol. 52, 107–126.

Hawkins, BA., Cornell, HV. 1994. Maximum parasitism rates and successful biological control. Science. 266:1886–1890.

Hoelmer, KA., Kirk, AA. 2005. Selecting arthropod biological control agents against arthropod pests: can the science be improved to decrease the risk of releasing ineffective agents? Biol. Control. 34:255–264.

Hopper, KR., Roush, RT. 1993. Mate finding, dispersal, number released, and the success of biological control introductions. Ecol. Entomol. 18:321–331.

Hunter, MS., Kelly, SE. 1998. Hyperparasitism by an exotic autoparasitoid: secondary host selection and the window of vulnerability of conspecific and native heterospecific hosts. Entomol. Exp. Appl. 89:249–259.

Kamath, MK. 1979. A review of biological control of insect pests and noxious weeds in Fiji. Fiji Agric. J. 41:55–72.

Kolar, CS., Lodge, DM. 2001. Progress in invasion biology: predicting invaders. Trends Ecol. Evol. 16:199–204.

Kunkel, KE., Pielke, RAJ., Changnon, SA. 1999. Temporal fluctuations in weather and climate extremes that cause economic and human health impacts: a review. Bull. Am. Meteorol. Soc. 80:1077–1098.

Jeschke, JM., Strayer, DL. 2006. Determinants of vertebrate invasion success in Europe and North America. Glob. Change Biol. 12:1608–1619.

Jervis, MA., Hawkins, BA., Kidd, NAC. 1996. The usefulness of destructive host feeding parasitoids in classical biological control: Theory and observation conflict. Ecol. Entomol. 21:41–46.

Julien, MH., Kerr, JD., Chan RR. 1984. Biological control of weeds: an evaluation. Prot. Ecol. 7:3–25.

Karl, TR., Trenbeth, KE. 2003. Modern global climate change. Science. 302:1719–1723.

Kiritani, K. 2013. Different effects of climate change on the population dynamics of insects. Appl. Entomol. Zool. 48:97–104.

Liebhold, AM., Tobin, PC. 2008. Population ecology of insect invasions and their management. Annu. Rev. Entomol. 53:387–408.

Margaritopoulos, JT., Kasprowicz, L., Malloch, GL., Fenton, B. 2009. Tracking the global dispersal of a cosmopolitan insect pest, the peach potato aphid. BMC Ecology. 9:13.

Moss, R., Jackson, R.R., Pollard, S.D., 2006. Mask of wax: secretions of wax conceal aphids from detection by spider's eyes. New Zeal. J. Zool. 33, 215–220.

Mueller, T.F., Blommers, L.H.M., Mols, P.J.M., 1992. Woolly apple aphid (*Eriosoma lanigerum* Hausm., Hom., Aphidae) parasitism by *Aphelinus mali* Hal. (Hym., Aphelinidae) in relation to host stage and host colony size, shape and location. J. Appl. Entomol. 114, 143–154.

Murphy, BD., Kay, MK. 2000. *Paropsis charybdis* defoliation of *Eucalyptus* stands in New Zealand's Central North Island. N. Z. Plant Prot. 53:334–338.

Romo, CM., Tylianakis, JM. 2013. Elevated temperature and drought interact to reduce parasitoid effectiveness in suppressing hosts. PLoS One. 8:e58136.

Sands, DPA., Harley, KLS. 1981. Importance of geographic variation in agents selected for biological control of weeds. *In*: Delfosse, ES. (Ed.), Proceedings of the V International Symposium on the Biological Control of Weeds, 22-27 July 1980, Brisbane, Australia, CSIRO, Melbourne, pp. 81–89.

Smith, R G., 1999. Wax glands, wax production and the functional significance of wax use in three aphid species (Homoptera: Aphididae). J. Nat. Hist. 33:513–530.

Stiling, P. 1990. Calculating the establishment rates of parasitoids in classical biological control. Am. Entomol. 36:225–282.

Stiling, P. 1993. Why do natural enemies fail in classical biological control programs? Am. Entomol. 39:31–37.

Stouthamer, R. 2006. Molecular methods for the identification of biological control agents at the species and strain level. *In*: Bigler F., Babendreier, D., Kuhlmann, U. (Eds.), Environmental Impacts of Invertebrates for Biological Control of Arthropods: Methods and Risk Assessment. CABI Publishing, Wallingford, UK. pp. 187–201.

Schwartzberg, E.G., Haynes, K.F., Johnson, D.W., Brown, G.C., 2010. Wax structures of *Scymnus louisianae* attenuate aggression from aphid-tending ants. Environ. Entomol. 39, 1309–1314.

Tylianakis, J., Didham, RK., Bascompte, J., Wardle, DA. 2008. Global change and species interactions in terrestrial ecosystems. Ecol. Lett. 11:1351–1363.

van Driesche, R., Hoddle, M., Center, T. 2008. Control of Pests and Weeds by Natural Enemies: An Introduction to Biological Control. Blackwell, Malden, MA, USA.

Wilson, F., Huffaker, CB. 1976. The physiology, scope and importance of biological control. *In*: Huffaker, CH., Messenger, PS. (Eds.), Theory and Practice of Biological Control. Academic Press.