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ABIOTIC AND BIOTIC FACTORS AFFECTING LIGHT BROWN APPLE MOTH, *EPIPHYAS POSTVITTANA,* IN CALIFORNIA

by
Linda P. Buergi

A dissertation submitted in partial satisfaction of the
requirements for the degree of

Doctor of Philosophy
in
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in the
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University of California, Berkeley

Committee in charge:
Professor Nicholas J. Mills, Chair
Professor George K. Roderick
Professor Wayne P. Sousa

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ABSTRACT

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With the increase of globalization, the introduction of exotic species into new regions has become a worldwide threat for biodiversity and agricultural production. However, invasiveness of alien species depends on the extent to which abiotic and biotic factors affect the impact of exotic species in a new region. The Light Brown Apple Moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), is a leafroller native to southeastern Australia that was discovered in California in 2006. At the time of its discovery, little was known about how abiotic and biotic factors might limit its distribution and impact in California and North America. I therefore measured high and low temperature tolerance of LBAM in laboratory studies and found that it exhibited only moderate tolerance of extreme temperatures, which may limit its potential distribution in California and North America. I also monitored LBAM populations in the field and measured parasitism rates over a four year study period to determine the influence of of biotic resistance and to provide baseline population data for use in management decisions. I found that LBAM populations were cyclic or decreasing, had strongly overlapping generations and produced three generations annually in San Francisco and and four in Santa Cruz. The parasitoid assemblage of LBAM in California consisted of many species and parasitism rates were unusually high, but provided no evidence of an ability to regulate LBAM populations. I also found that low density LBAM populations did not exhibit demographic Allee effects, but instead populations of all sizes exhibited strong negative density dependence.

I also studied life history parameters of *Meteorus ictericus*, the most abundant parasitoid of LBAM in California, and found that it has a number of traits that could account for its dominance in the parasitoid assemblage. It was able to attack and develop in a wide range of host larval instars, had a preference for late larval instars, a female only lifestyle, a low generation time ratio in relation to LBAM, and similar temperature maxima and minima for development. However, *M. ictericus* exhibited an unusually low lifetime

fecundity, which could pose an important constraint on its potential to suppress LBAM populations.

Overall, the results of these studies have provided valuable insights that can be used to better understand the potential geographic distribution of LBAM, and to better inform management decisions. In addition, I suggest that the high level of resistance from resident parasitoids on LBAM in California, in combination with other pest management strategies, could prevent the widespread losses from agricultural crops that were originally anticipated in the United States.

Dedication

To my husband, for his love and support,
to my parents and brother, for their encouragement and for believing in me,
and to my grandpa, for getting me started on this path.

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INTRODUCTION

Invasive exotic species have been recognized as one of the major threats to biodiversity and ecosystem function (Mack et al., 2000; Ricciardi, 2007). Invasive plants, animals and microorganisms can alter ecosystem processes such as nutrient or water cycles, change disturbance regimes such as fire frequency and can drive native species to extinction by direct or indirect competition or by degrading their habitat (D'Antonio and Vitousek, 1992; Mack et al., 2000). In addition, invasive species cause serious economic costs from losses in agricultural production as well as from tactics used in their control (Pimentel et al., 2005). For example, it is estimated, that in the U.S. introduced pest insects are responsible for approximately $13 billion in crop losses annually. In addition to these losses, pesticides to deter them cost an addition $500 million, resulting in total economic damage of $13.5 billion per year (Pimentel et al., 2005). However, the economic and environmental impacts of an established exotic species can vary significantly (Ricciardi and Cohen, 2007) as only a small subset of those invaders that establish will proliferate and/or spread in the introduced range due to limitations imposed by biotic and abiotic factors (Kolar and Lodge, 2001; Richardson et al., 2000).

The Light Brown Apple Moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), is a leafroller native to southeastern Australia, that has established in New Zealand , Great Britain and Hawaii and was discovered in California in 2006 (Suckling and Brockerhoff, 2010). LBAM is a highly polyphagous pest with over 500 known host species in 363 genera and 121 families that include mostly dicotyledons, but also some monocotyledons, conifers and ferns (Brockerhoff et al., 2011). As a pest, LBAM is best known from fruit crops, including apples, pears, citrus, grapes and cane berries, and to a lesser extent from forestry, vegetable, and flower crops (Wearing et al., 1991). Larvae of this leafroller species feed within the protective shelter of leaves that they roll or spin together and cause damage to both foliage and the surface of fruit (Lo et al., 2000), which can lead to secondary disease development, causing rots in crops such as grapes (Bailey et al., 1996). After its discovery in 2006, an economic risk analysis for four major fruit crops (apple, grape, orange, and pear) in the mainland United States estimated the potential annual cost to be $105 million (Fowler et al., 2007). However, the estimated economic and ecological impact as well as the potential for control of LBAM as an exotic species in the U.S. depends on its estimated geographic range, its population density in the field as well as the extent of biotic resistance it experiences from resident natural enemies. In this dissertation I examine some of the key environmental conditions and biological interactions that may constrain the spread of LBAM in California. Using a mix of field and laboratory studies, I determine its high and low temperature tolerance, its seasonal phenology and abundance in the field, as well as the occurrence and impact of resident natural enemies in the field. In addition, I present information on life history traits of the most abundant resident parasitoid, *Meteorus ictericus*, and discuss its potential for use in conservation biological control.

The potential geographic range of an invasive organism depends on its capacity to withstand unfavorable environmental conditions, such as temperature extremes (Bale and Walters, 2001; Bowler and Terblanche, 2008; Tauber et al., 1985). LBAM does not have a winter diapause (Geier and Briese, 1981), the state of physiological inactivity entered by insects to endure harsh environmental conditions, and therefore cold temperatures are thought to be an important limiting factor for its potential range expansion. In Chapter 1, I examine two measurements of cold tolerance for LBAM: the supercooling point, the point at which body fluids spontaneously freeze when cooled below the melting point (Zachariassen, 1985), and the LT_{50}, the time at which 50% of the population is killed after an exposure at a given constant temperature (Watanabe, 2002). I compare the results of these two low temperature tolerance measures with those available for other related species with known distributions and discuss its implications for the potential geographic distribution of LBAM. Similarly, in Chapter 2 I determine the high temperature tolerance of LBAM by measuring LT_{50} values at ecologically relevant high temperatures. In a second experiment in Chapter 2, I quantify the change in LT_{50} values that results from a comparison of two different, but commonly used, endpoints: response to probing and ability to walk. Last, I evaluate the ecological significance of response to probing as a commonly used ad hoc endpoint, by following survivors through to adult emergence. I use the results of this study to determine how closely the commonly used ad hoc endpoints approximate the more labor intensive but ecologically relevant measure of adult emergence, and I compare the LT_{50} values with results for other related species with known distributions in the U.S. While in Chapter 1 and 2 I do not directly predict geographic distribution of LBAM based on these findings, my results can serve as key parameters for more in depth modeling studies that estimate the global or U.S. distribution of LBAM.

The potential for and cost of control of pest species, invasive or native, depends on population parameters of the species in the field such as seasonal or local abundance, voltinism and stage structure. In addition, pest management interventions are often based on applications of physiological time through prediction of the temperature-dependent phenology of a pest (Castle et al., 2005; Damos and Savopoulou-Soultani, 2010; Jones et al., 2010; Lopez et al., 2001). For LBAM, several of the control strategies proposed for use in California require knowledge about the seasonal distribution of vulnerable life stages, therefore requiring knowledge about LBAM phenology in relation to degree-days. In Chapter 3, I monitor abundance, stage structure and voltinism of LBAM populations at two locations in California. I discuss the results in comparison with results found for LBAM in its native Australia and the implications of our findings for management strategies.

The success of invasive species is often attributed to their escape from natural enemies as posited by the enemy release hypothesis (Darwin, 1859; Elton, 1958; Keane and Crawley, 2002; Mitchell and Power, 2003). It has been shown that parasitoid assemblages of species in their invasive range consist of fewer species, with a higher proportion of generalists, and lower parasitism rates compared with their native range (Keane and

Crawley, 2002). However, in some cases exotic species have been shown to be attacked by large parasitoid assemblages (Colautti et al., 2004; Godfray et al., 1995; Roy et al., 2011) and/or experience high levels of parasitism (Rose and DeBach, 1992), contributing to varying degrees to the control of these exotics. In Chapter 4, I therefore determine the richness, level of specialization and parasitism rates provided by the resident parasitoid assemblage of LBAM in California. I compare it with data available for the parasitoid assemblage of LBAM in its native range and to data for related native tortricid species in the Western U.S. to determine the potential for natural enemies to limit the invasiveness of LBAM in California, while at the same time providing a test for the enemy release hypothesis. However, while parasitoid richness and parasitism rates provide a measure of parasitoid diversity and impact, it does not test whether the parasitism observed is sufficient to impact LBAM population growth rate and to keep it from becoming invasive. In Chapter 5 I measure LBAM population growth rates and parasitism rates of four populations in California and analyze the effect of parasitism and population density on population growth rates. I discuss the relative importance of parasitism and other factors for the regulation of LBAM population growth rates and its implications for the invasiveness of LBAM.

During the course of this dissertation we found that 77% of total parasitism for LBAM in California was provided by the braconid endoparasitoid *Meteorus ictericus*. This parasitoid has only been reported once before in the U.S. (Madsen and Borden, 1949) and nothing is known about its biology and life history traits, leaving open the question of what makes this parasitoid so successful on LBAM. Many studies have focused on identifying factors that make parasitoids successful in controlling invaders (Kimberling, 2004; Mills, 2006). Two life-history traits found to correlate well with success in biological control introductions are fecundity (Lane et al., 1999) and generation time ratio (Kindlmann and Dixon, 2001). In Chapter 6, we study the life history traits of *M. ictericus*, including lifetime fecundity and development time, with the goal of identifying traits that make this parasitoid so successful on LBAM in California. The results of this chapter not only contributes to the search for characteristics that predict the success of parasitoids in biological control, but helps to evaluate the potential for enhancing biological control of LBAM by *M. ictericus*.

Estimating the invasion potential for LBAM in the U.S. requires better understanding of the interaction between LBAM and the biotic and abiotic factors it encounters in this newly invaded region. The six chapters in this dissertation deal with different research questions aiming to develop an understanding of factors limiting the success of LBAM in California and the U.S, ranging from basic laboratory studies on LBAM thermal tolerance and the biology of its main natural enemy, to field studies on population dynamics of LBAM and its resident parasitoids. In addition, components of this dissertation aim to contribute to a better understanding of broader research questions such as the enemy release hypothesis and the search for characteristics of successful biological control agents.

CHAPTER 1

COLD TOLERANCE OF THE OVERWINTERING LARVAL INSTARS OF LIGHT BROWN APPLE MOTH, *EPIPHYAS POSTVITTANA*

1.1 ABSTRACT

The light brown apple moth, *Epiphyas postvittana*, a leafroller native to southeastern Australia was discovered in California in 2006. The highly polyphagous nature of this pest adds to the importance of being able to predict the potential distribution of this invader across the North American continent. The spread of ectothermic species that lack winter diapause, such as *E. postvittana*, can be limited by their ability to tolerate cold temperature extremes. In this study we examined the cold hardiness of 4^{th} – 6^{th} instar *E. postvittana*, the only life stages known to overwinter in California, through a combination of supercooling point (SCP) and mortality at low temperatures. Our results showed that the mean SCP for *E. postvittana* ranged from -14.1 °C for 6^{th} instars to -16.0 °C for 4^{th} instars. Lethal time leading to 50% mortality (LT_{50}) for the three instars combined were 2.5 h at -10.5 °C, 41 h at -6.5 °C and 198 h at -0.9 °C. At 3 °C, the LT_{50} of 4^{th} instars was significantly lower at 775 h than that for 5^{th} and 6^{th} instars combined at 1029 h. The cold hardiness characteristics of later-instar *E. postvittana* larvae were comparable to those of pink bollworm, *Pectinophora gossypiella*, a diapausing invasive with a geographic distribution restricted to southern California. Slightly greater cold hardiness is shown by the indigenous non-diapausing leafroller *Argyrotaenia franciscana*, which is restricted to the Pacific Coast of North America. We therefore conclude that the moderate cold hardiness of *E. postvittana* will substantially limit its spread into northern temperate regions of North America.

1.2 INTRODUCTION

The light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), is an important horticultural and agricultural pest indigenous to southeastern Australia (Geier and Briese 1981, Suckling and Brockerhoff 2010). It has also been recorded from New Zealand, New Caledonia, Hawaii and the UK as a notable invasive species. Its recent discovery in California in 2006 has led to a comprehensive risk analysis, in which the potential annual cost in damage to four major crops (apples, pears, citrus, and grapes) in the U.S. has been estimated to be US$105 million (Fowler et al. 2009).

Epiphyas postvittana is highly polyphagous with over 500 known host species in 363 genera and 121 families that include mostly dicotyledons, but also some monocotyledons, conifers and ferns (Suckling and Brockerhoff 2010). As a pest, *E. postvittana* is best known from fruit crops, including apples, pears, citrus, grapes, and cane berries, and to a lesser extent from forestry, vegetable, and flower crops (Wearing et al. 1991). Larvae feed as leafrollers on leaves and occasionally on the surface of fruit by webbing a leaf to a fruit to create a protected shelter (Lo et al. 2000). This causes unsightly blemishes on fruit and can lead to secondary disease development, causing rots in crops such as grapes (Bailey et al. 1996). The zero tolerance of live larvae in exports significantly increases the economic impact of this pest.

In the Melbourne region of Australia *E. postvittana* has three distinct generations a year (Danthanarayana 1975) and no winter diapause (Geier and Briese 1981). Larval development is slowed, but remains continuous, under cool winter temperatures (Danthanarayana 1983). Similarly, LBAM has been found to have 2-3 generations in coastal Northern California (Bürgi and Mills, unpublished observations).

The potential geographic range of an invasive organism depends on its capacity to withstand unfavorable environmental conditions, such as temperature extremes (Tauber et al. 1986, Bale and Walters 2001, Bowler and Terblanche 2008), and to acquire an adequate thermal budget to complete its life cycle and reproduce (Hatherly et al. 2005). While thermal requirements for development and reproduction in *E. postvittana* have been investigated by Danthanarayana (1975) and Danthanarayana et al. (1995), survival limits at temperature extremes remain unknown. This is of particular concern as temperature extremes are more likely than thermal budgets to limit the potential distribution of ectothermic invaders that have no winter diapause or summer estivation, such as *E. postvittana*.

Cold tolerance can be assessed by a number of indices that are best considered in combination rather than separately (van Lenteren et al. 2006). Originally cold hardiness studies distinguished only between freeze tolerance and freeze avoidance, basing cold hardiness estimates on the supercooling point (SCP) alone. The SCP is defined as the temperature at which body fluids spontaneously freeze when cooled below the melting point (Zachariassen 1985). It generally represents the absolute lower lethal temperature

for freeze-intolerant individuals (Lee and Denlinger 1985, Lee et al. 1991). It is now widely recognized, however, that even in polar species, the SCP of acclimatized insects is below the lowest winter temperatures experienced (Bale 2002, Clark and Worland 2008). For the majority of insects inhabiting temperate climatic zones, the risk of chilling injury and death is thus greater than the risk of freezing injury and death (Bale 2002). Therefore, in addition to the SCP, lower lethal temperature (i.e., the temperature at which a given proportion of the population is killed after an exposure of fixed duration) and lethal time (i.e., the time at which a given proportion of the population is killed after an exposure at a constant temperature) are now used as indices of cold tolerance (Watanabe 2002). These cold hardiness parameters allow a more comprehensive classification system, as proposed by Bale (1996), where insects are categorized based on their tolerance of freezing and chilling temperatures.

The objective of the current study is to determine the cold tolerance of those life stages of *E. postvittana* that are known to overwinter in California, through measurement of the SCP and the lethal times leading to different levels of mortality when exposed to chilling temperatures above the SCP. This information can then be used to make better predictions about the potential distribution of this pest in the U.S. and other geographic regions where it may also become invasive in the future.

1.3 MATERIALS AND METHODS

Colony maintenance

Epiphyas postvittana larvae used in this study were from a colony that was initiated with larvae collected in Santa Cruz, CA in early 2007. The colony was maintained at 21 °C, 60-80% RH and 16:8 h (L:D). Larvae were reared on a bean-based diet developed by Cunningham (2007). Plastic cups (96 ml, Solo Cup Company, Highland Park, IL) were filled about one third full with the diet and 2-3 egg masses of 30-50 eggs were placed inside. Once larvae had pupated sets of 50 male and 50 female pupae were put into oviposition containers (946 ml transparent polypropylene deli containers, Fabri-Kal, Kalamanzoo, MI) and covered with gauze to await emergence, mating and oviposition. 10% honey water with 0.1% sorbic acid was provided to the adult moths through a 4cm cotton wick inserted into a slit lid of a 22 ml plastic cup (SOLO, Highland Park, IL) placed inside the oviposition containers. Adult females laid eggs into the grooves of the oviposition containers for about 1 week, before being transferred to a new container. Following Singh and Moore (1985), eggs were cut out from the oviposition containers and sterilized in a 5% formaldehyde solution for 20 min, soaked in water for another 20 min, and then left out to dry for 1 h before being placed in diet cups.

Experimental larvae were restricted to 4^{th}, 5^{th} and 6^{th} instars, the only life stages found during the winter period in four locations in coastal California where populations have

been sampled regularly for two years (Bürgi et al., unpublished observations). Instars were determined from head capsule widths of the larvae (Danthanarayana 1975).

Supercooling point

SCPs were measured using surface-contact thermometry, following the approach developed by Carrillo et al. (2004). We placed individual larvae into a longitudinal groove (1.5 x 0.3 x 0.3 cm) cut into 1 x 2 x 0.3 cm polystyrene rectangles, attached a copper-constantan thermocouple (thermocouple type: TT-T-36, Omega Engineering, Stamford, CT) to the larvae using high vacuum grease (Dow Corning, Midland, MI), and secured each arena by wrapping two layers of parafilm around the polystyrene rectangle. Insect-thermocouple arrangements were placed inside a 9 x 9 x 9 cm polystyrene cube, cut out of four glued-together (Glue-All, Elmer's, Columbus, OH) layers of R-Tech 2.5 x 60 x 121 cm polystyrene insulation boards (Insulfoam, Tacoma, WA), through a 4.5 cm long and 3.5 cm diameter tunnel cut in the center of one side. The hole was closed with a rubber stopper No. 6 (Fisher Scientific, Pittsburg, PA) and sealed with 4 strips of electrical tape. This arrangement was first placed at 15 ºC for 1h to achieve constant starting temperatures and was then transferred to a -25 ºC freezer (Isotemp Flammable-Materials Storage Freezer, Fisher Scientific, Pittsburgh, PA). The size of the polystyrene cube, starting temperature and freezer temperature permitted insects to cool at a rate of ≈ 0.5 °C min^{-1} (Carrillo et al. 2004). Larval body temperatures were recorded at 1 sec intervals with a CR 10 WP data logger (Campbell Scientific, Logan, UT). The SCP was determined as the lowest temperature reached before freezing, visualized by the emission of an exotherm (i.e., a small peak indicating heat release during the phase change, Lee et al. 1991). We ended each observation after 20 min at minimum freezer temperature.

Acclimated larvae were obtained by keeping them at 10 °C, 12:12 h (L:D), 70-85% RH for 7 days and starving them for the last 72 h before the experiment. Non-acclimated larvae were taken directly from the colony at 21 °C. The SCP of 20 acclimated and 20 non-acclimated 4^{th}, 5^{th} and 6^{th} instars was assessed, giving a total of 120 individual observations.

Mortality at low temperatures

The effect of longer-term exposure to low temperatures on *E. postvittana* larval survival was studied for the same three instars (4^{th}, 5^{th} and 6^{th}). For each instar we placed 10 larvae in a 96 ml plastic cup (Solo Cup Company, Highland Park, IL) and added a crumpled 8 x 26 cm piece of paper towel (Scott Single-Fold Towel, Kimberly-Clark Professional, Neenah, WI) to provide a suitable surface for the larvae and to prevent them from freezing to the sides of the cups. The larvae were not acclimated and taken straight from the colony. A set of 8 cups for each instar was assembled as a block, and 6 blocks of larvae were monitored on different dates in programmable growth chambers at 3 °C, -0.9

°C, -6.5 °C, and -10.5 (±1) °C. We selected these temperatures to represent a range that was below the threshold temperature for development of 7.5 °C (Danthanarayana 1975) and above the mean SCP of all three instars.

Our goal was to estimate larval mortality at successive time intervals following exposure to the cold temperatures. For each block of larvae two cups of each instar, representing a replicate, were removed at four different time intervals. The time intervals were altered between blocks to cumulatively represent a series of different periods of exposure to each cold temperature that spanned 0 - 0.95 or higher observed proportional mortality. The total number of replicates and time intervals varied between temperatures; 28 replicates for each instar over 10 different intervals at 3 °C, 26 replicates over 10 intervals at -0.9 °C, 23 replicates over 16 intervals at -6.5 °C, and 26 replicates over 11 intervals at -10.5 °C. A HOBO temperature logger (Onset Computer Corporation, Bourne, MA) was placed inside each growth chamber to monitor temperature fluctuations every 1min for the two lowest temperatures and every 5 min for the two highest temperatures. For the two highest temperatures there was no delay in larvae experiencing the specified temperatures, but for the two lowest temperatures, the HOBO data indicated that it took 50 min to reach -6.5 °C and 55 min to reach -10.5 °C. For these two colder temperatures exposure intervals were considered to start after the initial adjustment period was completed.

After the designated exposure interval, cups were removed from the growth chambers, placed at 21 °C, 60-80% RH, and 16:8 h (L:D), and mortality of larvae was assessed 24 h later. Death was defined as lack of mobility when individuals were probed with a pair of soft forceps (Carrillo et al. 2005).

Statistical analysis

All statistical analyses were carried out using R (R Development Core Team, version 2.10.0, 2009). Distributions of SCPs and their residuals were tested for normality using the Shapiro-Wilk W-test (Shapiro and Wilk 1965) in the stats package of R. We used a two-way ANOVA in the car package of R to analyze the effect of the three instars and two acclimation levels on the SCPs. To account for unequal replication, type III sums of squares were used with the ANOVA function because type I sums of squares are not appropriate for unbalanced designs (Fox 2002). Before running the analysis in the car package of R we set the contrasts to Helmert to insure orthogonality of model terms. The non-significant interaction term was dropped from the model and significant differences between means of each factor were separated using Tukey's honestly significant difference test at $\alpha = 0.05$.

Data from longer-term exposure of *E. postvittana* larvae to low temperatures were analyzed with generalized mixed models using the function lmer of the lme4 package of R (Bates et al. 2008). We used separate probit models with binomial error distributions for each temperature. Exposure interval and instar were considered fixed effects, while block

was considered a random effect to account for the possibility of variation among cohorts in the different blocks of observations (Barchia et al. 2003). Random variation was entered via effects on the intercept with fixed slope, and instar was nested within block. Significance of potential interactions between the two fixed factors, and potential differences between the individual instars, were assessed using model simplification and log likelihood ratio tests using χ^2 at an $\alpha = 0.05$ significance level. Models were checked for over- and under-dispersion, and where needed, were adjusted by using the quasibinomial family. Lethal times (LT_{50} and LT_{99}) and their 95% confidence intervals were calculated using Fieller's formula as described in Finney (1971, p78).

1.4 RESULTS

Supercooling point

SCPs for four of the six treatments (4^{th}, 5^{th} and 6^{th} instars non-accclimated and 4^{th} instar acclimated) followed a normal distribution ($P > 0.05$), and as variances for the two remaining treatments did not differ significantly among treatments we used ANOVA to test for differences among treatment means. The SCPs of acclimated larvae occurred at higher temperatures than those of non-acclimated larvae ($F_{1,115} = 9.79$, $P = 0.002$) and we found no significant interaction between instar and acclimation ($F_{2,113} = 0.011$, $P = 0.99$). There was significant variation among SCPs of the larval instars ($F_{2,115} = 4.86$, $P = 0.009$), with the lowest achieved by 4th instars and highest by 6th instars (Fig. 1).

Mortality at low temperatures

The sigmoidal increase in larval mortality with increasing exposure time at constant low temperature was well described by probit models for all four temperatures (Fig. 2). Potential interactions between exposure time and instar were not significant at any of the low temperatures (Table 1). At the three lowest temperatures there was no significant variation in larval mortality between instars (Table 1), but at 3 °C, 4^{th} instars suffered higher mortality than 5^{th} and 6^{th} instars ($\chi^2 = 21.84$, df = 1, $P < 0.001$). LT_{50} ranged from 2.5 h at -10.5 °C to 774 h for 4^{th} instars and 1028 h for 5^{th} and 6^{th} instars at 3 °C (Table 2). LT_{99} ranged from 5.07 h at -10.5 °C to 1863 h for 4^{th} instars and 2391 h for 5^{th} and 6^{th} instars at 3 °C (Table 2).

1.5 DISCUSSION

As a non-diapausing species, the ability of *E. postvittana* to tolerate cold temperatures has become of increasing importance for the prediction of its potential geographic distribution as it continues to invade new geographic regions, including its recent discovery

in the USA and detection in Sweden (Suckling and Brockerhoff 2010). Denlinger (1991) argues that while overwintering diapause frequently extends an insect's capacity to tolerate cold temperatures, cold hardening can be achieved independently of diapause by acclimation at a colder temperature (Fields et al. 1998) or shortened photoperiods (Horwath and Duman 1982). Examples of cold hardening among insects that lack an overwintering diapause include the mealworm *Tenebrio molitor* where the SCP changed from -8 to -15 °C after acclimation for 7 days at 5 °C and shortened daylength (Patterson and Duman 1978), and the leafminer *Liriomyza sativae* where acclimation at 5 °C significantly increased survival from 15 to 70% after 2 days at -5 °C (Chen and Kang 2005). Rapid cold hardening, an extremely fast acclimation response of non-overwintering life stages (Lee et al. 1987), can also be important as shown by a 90% increase in survival of *Musca domestica* when exposed to -7 °C for 2 h after 1.5 h of acclimation at 0 °C (Coulson and Bale 1990).

Consequently, we had expected that despite its lack of overwintering diapause *E. postvittana* would show increased supercooling capacity when acclimated at shorter days and cooler temperatures. In contrast, however, we found that after 7 days exposure at 10 °C, the SCPs of 4th to 6th instar *E. postvittana* were above those of non-acclimated individuals. Several other studies have also shown a lack of decrease in SCP when arthropods were exposed to acclimating temperatures. These include the grape moth *Lobesia botrana* (Andreadis et al. 2005), the stemborer *Sesamia nonagrioides* (Gillyboef et al. 1994), the parasitoid *Colpoclypeus florus* (Milonas and Savopoulou-Soultani 2005) and the phytoseiid spider mite predator *Typhlodromips montdorensis* (Hatherly et al. 2004). While no effect of acclimation on SCP was found in these studies, both Andreadis et al. (2005) and Hatherly et al. (2004) did show a significant decrease in mortality at low temperatures after acclimation. As suggested by Renault et al. (2002), SCP may be less suitable as an indicator of cold hardiness for insects that do not experience extreme cold during part of their life cycle. Thus acclimation could potentially increase the survivorship of *E. postvittana* at low temperatures above its SCP and might enhance our understanding of the cold hardiness of this species.

Differences in cold tolerance between instars are of greatest importance for non-diapausing insects, such as *E. postvittana*, since diapausing insects mostly overwinter in a specific life stage (Tauber and Tauber 1976). Non-diapausing insects overwinter in a range of different instars, and yearly differences in the stage structure of overwintering populations could greatly affect winter survival (Knight and Croft 1986). The significantly lower SCP of 4th instars compared to 6th instars from our experiment on *E. postvittana* larvae matches observations from another study (Watanabe and Tanaka 1997) in which younger instars had lower SCPs than later instars. In contrast, we found that mortality at low temperatures above the SCP did not differ between instars, except for the larvae at 3 °C, where 4th instars suffered greater mortality than 5th and 6thinstars. In this case, however, it could be argued that the mortality was determined by starvation tolerance

rather than cold tolerance, and that 4th instars had significantly lower fat reserves at the start of the experiment.

As later instar *E. postvittana* larvae suffered substantial mortality at cold temperatures above their SCP this would place them closer to chill-susceptible than to chill-tolerant according to Bale's (1996) classification system for cold hardiness. Relating laboratory estimates of cold hardiness for an invasive species to their potential for survivorship under field conditions in different geographic regions, however, can be problematic. One promising approach is to identify a specific cold hardiness index for a particular geographic region that correlates well with winter survival under local field conditions (Bale and Walters 2001). In the absence of such an index or for a more climatically variable region such as California, it can be informative to compare the laboratory estimates of cold hardiness for an invasive species to those of other similar species that have known geographic distributions in the region of interest. Comparison of LT_{50} values for *E. postvittana* larvae with those of the indigenous non-diapausing leafroller *Argyrotaenia franciscana* (= *citrana*), that is restricted to the Pacific Coast of North America (Landry et al. 1999), shows slightly greater survival at low temperatures for *A. franciscana* (Knight and Croft 1986). LT_{50} values for 3rd and 5th instar *A. franciscana* were 135 and 91 h at -5 °C respectively, compared to only 41 h for 4th to 6th instar *E. postvittana* at -6.5°C. Similarly, at -10 °C *A. franciscana* larvae survived 29 (3rd) and 16 h (5th) while *E. postvittana* larvae (4th to 6th) at -10.5 °C lived for only 2.5 h. *E. postvittana* also appears to share a similar level of cold hardiness to the pink bollworm *Pectinophora gossypiella*, an invasive species that overwinters in larval diapause and has a current distribution that is restricted to southern California (Gutierrez et al. 2006). *P. gossypiella* has an LT_{50} of 56.6 h at -6 °C (40.9 h for *E. postvittana* at -6.5 °C) and mean SCPs ranging from -14.0 to -16.4 °C (-12.5 to -16.0 °C for *E. postvittana*) for winter field collected larvae in Northern Greece (Kaltsa et al. 2006).

We conclude from this study that the moderate cold hardiness of *E. postvittana* larvae will substantially limit its spread into northern temperate regions of North America. The high levels of mortality at subzero temperatures observed in our laboratory studies are likely to limit its survival in places with temperatures that drop below zero for more than one week during winter. Similarly, in California, where *E. postvittana* was first discovered in 2006, its moderate cold hardiness is also expected to play a significant role in restricting its potential geographic distribution (Gutierrez et al. 2010).

1.6 TABLES

Table 1. Log likelihood ratio tests for the significance of the interaction between instar and exposure interval, and the significance of differences between instars for the proportional mortality of *Epiphyas postvittana* larvae exposed to constant low temperatures.

Temp °C	n	Interaction			Instar		
		$\chi 2$	df	P	$\chi 2$	df	P
3.0	84	3.81	2	0.15	24.00	2	<0.001
-0.9	78	0.27	2	0.87	3.42	2	0.18
-6.5	69	2.52	2	0.28	1.95	2	0.38
-10.5	78	4.88	2	0.09	4.12	2	0.13

Table 2. Estimated intercepts and slopes for the relationship between the proportional mortality of *Epiphyas postvittana* larvae and exposure interval (h) from probit regressions at four constant low temperatures, together with estimates of LT_{50} and LT_{99}. *** Statistically significant at $P < 0.001$.

Temp °C	df	Intercept (± SE)	Slope (± SE)	LT_{50} (95% CI)	LT_{99} (95% CI)
3.0 4th instar	6	-1.406 (± 0.008)***	0.0018 (± 0.00001)***	775 (767 - 783)	1864 (1844 - 1885)
3.0 5th/6th instar	6 9	-1.883 (± 0.013)***	0.0018 (± 0.00001)***	1029 (1020 - 1037)	2391 (2366 - 2417)
-0.9	5 6	-1.724 (± 0.005)***	0.0087 (± 0.00001)***	197.6 (196.7 – 198.4)	464.1 (463.1 – 465.1)
-6.5	5 3	-2.70 (± 0.01)***	0.0660 (± 0.0002)***	40.9 (40.6 - 41.1)	76.1 (75.7 - 76.5)
-10.5	5 6	-2.26 (± 0.01)***	0.905 (± 0.002)***	2.50 (2.48 - 2.52)	5.07 (5.05 - 5.09)

1.7 FIGURES

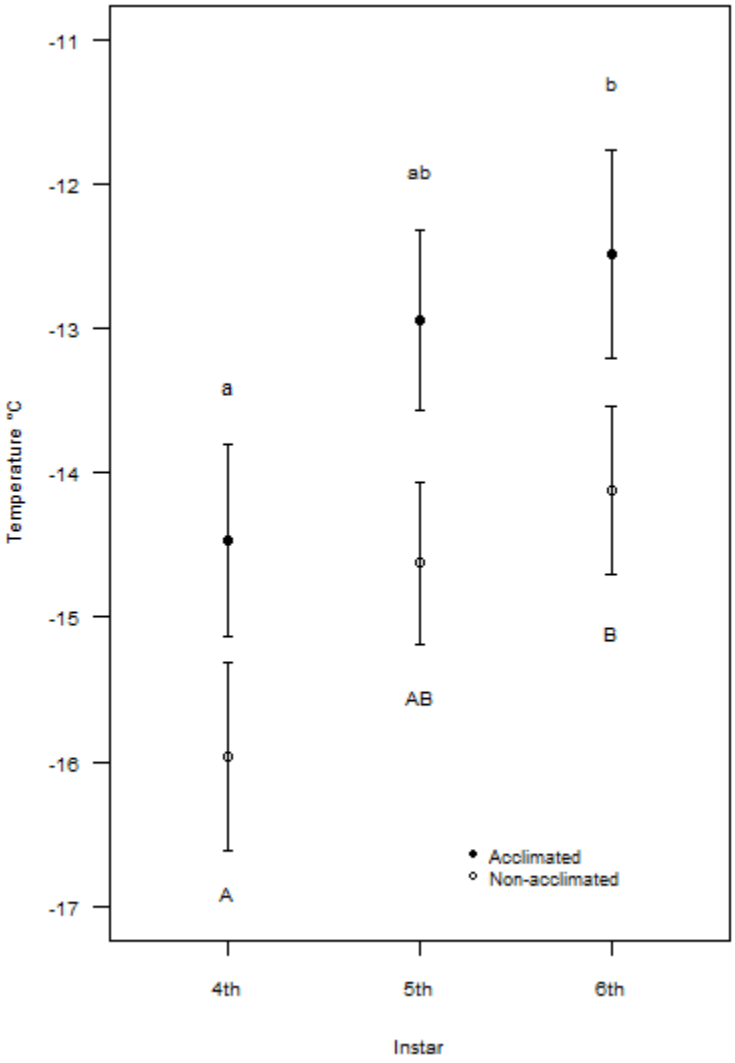

Figure 1. Mean supercooling points (± SE) of acclimated and non acclimated larvae for the overwintering instars of *Epiphyas postvittana*. Means followed by the same letters (lowercase for acclimated larvae, uppercase for non-acclimated larvae) are not significantly different ($P > 0.05$).

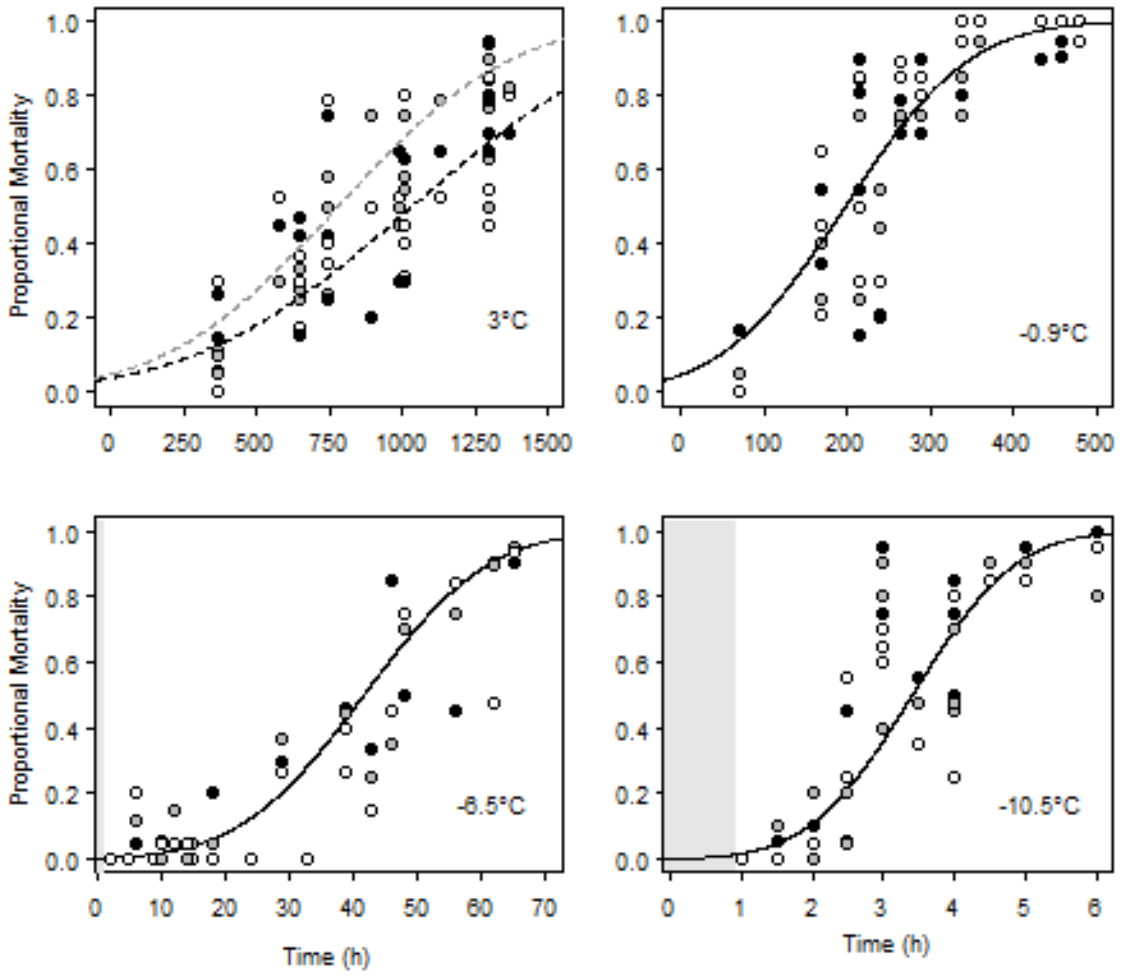

Figure 2. Proportional mortality of 4th (grey dots), 5th (black dots) and 6th (white dots) instar *Epiphyas postvittana* at four low temperatures in relation to exposure interval. Shaded areas at the two lowest temperatures indicate the periods during which larvae were reaching the set exposure temperatures. Sigmoid curves are from probit models fitted to all instars combined except 3 °C, where the response of 4th instar (grey dotted line) larvae was significantly different from that of 5th and 6th instar combined (black dotted line).

CHAPTER 2

ECOLOGICALLY RELEVANT MEASURES OF THE PHYSIOLOGICAL TOLERANCE OF LIGHT BROWN APPLE MOTH, *EPIPHYAS POSTVITTANA*, TO HIGH TEMPERATURE EXTREMES

2.1 ABSTRACT

Invasive ectothermic species are limited in their geographic range expansion primarily by their capacity to withstand temperature extremes. *Epiphyas postvittana* is a highly polyphagous invasive leafroller that was discovered in California in 2006. To predict its potential range and future response to climate change, high temperature tolerance of this species was determined for all life stages and larval instars. Using the static method to estimate high temperature tolerance with response to probing as an endpoint, the mean time leading to 50% mortality (LT_{50}) ranged from 45 - 187 h at 32.3 °C, 34 - 68 h at 36 °C, 11 - 21 h at 38 °C, and 1.2 - 5.6 h at 40.4 °C. There was no clear pattern in the relative tolerance of the life stages across the range of temperatures tested. For pupae and adults, gender did not influence the LT_{50} values at any of the temperatures tested. For the larval instars, LT_{50} values increased with increasing larval instar at the highest three temperatures while this trend was reversed for the lowest temperature (32.3 °C). An analysis of LT_{50} values obtained from acute responses to probing compared to subsequent survival to adult emergence, showed that chronic mortality severely affected all larval instars at three out of the four constant temperatures and resulted in 64-85% reduction in LT_{50} values. No difference in acute and chronic mortality was found for exposure of the egg stage to high temperatures. These findings have important implications for predicting thermal limits and range expansions of insect species, since upper thermal tolerance could readily be overestimated from the use of ad hoc rather than ecologically relevant endpoint measurements such as survival to adult emergence.

2.2 INTRODUCTION

Invasive alien species constitute a major threat to biodiversity, ecosystem function and agricultural production (Mack et al. 2000, Ricciardi 2007). Ectothermic invasive species are limited in their geographic range expansion primarily by their capacity to withstand unfavorable climatic conditions, such as temperature extremes (Bale and Walters, 2001; Somero 2005; Bowler and Terblanche, 2008; Gaston 2009), although biotic interactions can also pose further constraints (Davis et al. 1998; Van der Putten et al. 2010). Global climate change is predicted to raise average annual temperatures and produce more pronounced temperature extremes (Karl and Trenberth 2003, Easterling et al. 2000). Species that are able to survive a broad range of temperature conditions will not only have an advantage in range expansion, but will also be better able to persist during the more severe and frequent extreme temperature events brought about by global climate change. The potential for synergy between these two drivers of global change has been identified as a major concern for the future of both animal and plant communities (Millenium Ecosystem Assessment 2005, Van der Putten et al. 2010, Willis et al. 2010, Sandel and Dangremond 2012). Consequently, renewed attention has been given to thermal limits for a variety of taxa, as well as to the application of laboratory derived measures of physiological tolerance to the estimation of ecological responses of individual species in nature (Calosi et al. 2008, Chown et al. 2010, Helmuth et al. 2010, Hoffmann 2010, Sunday et al. 2011, Terblanche et al. 2011).

Two different approaches have been used for determining acute thermal tolerances of invertebrates (Lutterschmidt and Hutchison 1997). The dynamic method involves increasing the experimental temperature until a point of physiological failure is reached (Terblanche et al. 2007, 2011), representing the concept of a "Critical thermal maximum" (CT_{max}) (Cowles and Bogert 1944). For the static method on the other hand, temperature is typically held constant and exposure duration varied to yield an LT_{50}, the exposure time at which 50% of the population suffers mortality (Fry et al. 1942). Alternatively, exposure time can be kept constant while temperature is varied (e.g., Chidawanyika and Terblanche 2011). For the dynamic method, the two most commonly used endpoints associated with CT_{max} are "loss of righting response" (LRR) and the "sudden onset of muscular spasms" (OS) (Lutterschmidt and Hutchison 1997), and a wide range of variation exists in the ramping rates used for increasing the experimental temperatures. For the static method, mortality is also determined from a range of different endpoints, such as inability to walk, lack of response to probing or a light beam (Loeschcke et al. 1994, Feder et al. 1997), or ability to develop to the next stage in the life cycle (Krebs and Loeschcke 1999, Mahrhoof et al. 2003, Wang et al. 2004).

A major problem for comparisons across studies is the wide range of experimental designs and endpoint measurements that have been used within these two major frameworks (Hoffmann et al. 2003, Terblanche et al. 2011). In addition, it often remains unclear whether failure to express a particular endpoint measurement, using either

dynamic or static methods, relates to the overall performance or fitness of the experimental individuals. For the dynamic method an increasing number of studies have focused on comparative outcomes from the use of different ramping rates for single endpoint measurements (Terblanche et al. 2007, 2011, Mitchell and Hoffmann 2010). In contrast, for the static method, there have been few comparative studies particularly with respect to high temperature tolerance. To date, only one study has addressed the question of different endpoint measurements at high temperatures, and in this case, only for the egg stage of an insect (Yu et al. 2011). With the renewed focus on thermal tolerances of arthropods in the context of global change, there is a clear need for comparative studies that examine different endpoint measurements for a variety of life stages, and for studies that can relate acute endpoint measurements to the broader ecological consequences and fitness costs of exposure to high temperature extremes (Helmuth et al. 2010, Hoffmann 2010, Terblanche et al. 2011).

In this study, we use the static method with different endpoints to investigate the upper thermal tolerance of the Light Brown Apple Moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), a highly polyphagous leafroller pest that is native to south-eastern Australia and has successfully invaded New Zealand, Great Britain, Hawaii and California (Suckling and Brockerhoff 2010). A number of studies have investigated the upper thermal tolerance of *E. postvittana* in the context of post-harvest control of fruit infestations, using temperature in combination with other stress factors (Whiting et al. 1991, Lester et al. 1995, Beckett and Evans 1997, Alderson et al. 1998, Tabatabai et al. 2000). In contrast, only one study has been conducted on *E. postvittanna* at an ecologically relevant high temperature, and in this case only a single temperature (Whiting et al. 1995). In addition, the life stages most commonly monitored in the post-harvest control studies have been restricted to late instar larvae and pupae. Studies of the seasonal phenology of *E. postvittana* in Australia (Danthanarayana 1975), New Zealand (Wearing et al. 1991) and California (Bürgi et al. 2011) have shown that *E. postvittana* has strongly overlapping generations with all life stages and larval instars present throughout the warmer summer months. As life stages of *E. postvittana* are likely to show differential heat tolerance, as was found for cold tolerance in this species (Bürgi and Mills 2010) and for high temperature tolerance of several other insects (reviewed in Bowler and Terblanche 2008), the influence of high temperature extremes on the survivorship of *E. postvittana* needs to be considered for all life stages.

The objectives of this study are first, to determine the high temperature tolerance of all life stages and larval instars of *E. postvittana,* to provide baseline data that could be used to predict the potential for range expansion of this invasive species and its likely response to global climate change. As typical endpoints used for the dynamic ramping method to acute thermal tolerance are based on behavioral responses of the assayed organisms (Terblanche et al. 2011), we make use of the static method to allow us to test both the inactive life stages (eggs and pupae) as well as active life stages (larvae and adults) of *E. postvittana*. With the exception of the pioneering study of Davison (1969) on the heat

tolerance of *Calliphora erythrocephala* throughout its life cycle, to our knowledge, this is the only other study to include the complete range of life stages and larval instars of an insect at a series of ecologically relevant high temperatures allowing for detailed comparisons of the influence of time and temperature interactions on successive stages in the life cycle. A second objective is to quantify the change in LT_{50} values that results from a comparison of two different, but commonly used, ad-hoc endpoints; response to probing and ability to walk. A third objective is then to evaluate the ecological significance of response to probing, as a commonly used ad-hoc endpoint, by following survivors through to adult emergence.

2.3 MATERIALS AND METHODS

Colony maintenance

E. postvittana larvae used in this study were obtained from a colony that was initiated with larvae collected in Santa Cruz, CA in early 2007. The colony was maintained at 21 °C, 60–80% RH and a 16:8 h (L:D) photoperiod. Larvae were reared on a bean-based diet developed by Cunningham (2007). Plastic cups (96 ml, Solo Cup Company, Highland Park, IL) were filled about one third full with diet and 2–3 egg masses of 30–50 eggs were placed inside. Once larvae had pupated sets of 50 male and 50 female pupae were put into oviposition containers (946 ml transparent polypropylene deli containers, Fabri-Kal, Kalamanzoo, MI) and covered with gauze to await emergence, mating and oviposition. 10% honey water with 0.1% sorbic acid was provided to the adult moths in a 22 ml plastic cup (SOLO, Highland Park, IL) with a 4 cm cotton wick inserted through the lid and placed inside the oviposition containers. Adult females laid eggs into the grooves of the oviposition containers for about 1 week before being transferred to a new container. Following Singh et al. (1985), eggs were cut out from the oviposition containers and surface sterilized in a 5% formaldehyde solution for 20 min, soaked in water for another 20 min, and then left to dry for 1 h before being placed in diet cups.

Acute responses of all life stages to high temperature exposure - experiment 1

The effect of exposure to high temperatures on *E. postvittana* was studied for all life stages. For each of the six larval instars, we placed 10 larvae in a 15ml glass vial (2.9 x 9.4 mm, Fisher Scientific, Pittsburg, PA) together with a 0.5 x 0.5 x 0.5 cm piece of diet (see colony maintenance). Instars were determined from head capsule widths of the larvae (Danthanarayana 1975). The bottom of the vial was lined with a 7 x 7 cm piece of paper towel (Scott Single-Fold Towel, Kimberly-Clark Professional, Neenah, WI) to prevent drowning of smaller instars in condensation from the diet. The vial was sealed with a one-hole rubber stopper (No. 6, Fisher Scientific, Pittsburg, PA) with a 1 x 1cm piece of fine cloth glued over the hole on the inside of the vial to prevent larvae from escaping. On the

outside, a 0.6 x 7 cm aluminum pipe was inserted into the hole in the stopper to serve as a ventilation and decompression tube. The vial was weighed down with a stainless steel nut (5/8''-11, Ace Hardware, Berkeley, CA) placed over the aluminum ventilation tube. The experimental setup for egg, pupal and adult stages was the same minus the diet. Four to six day old, medium size egg masses (40-80 eggs) were counted before the start of the experiment, and 1 egg mass was used per vial. Pupal treatments contained 5 female and 5 male pupae per vial. For adult treatments the paper towel was replaced with a 1 x 1.5 cm piece of dental wick (TIDI Products, Neenah, WI), soaked in 10% honey solution, and each vial contained 5 female and 5 male moths that were 3 - 7 days of age.

Vials were submerged in a 12.5L water bath (Labline 18800, dual chamber, Imperial III, Labline, Melrose Park, IL) with the water level reaching approximately 2 cm above the rims of the glass vials. This arrangement prevented the water level from dropping below the rims of the vials due to overnight evaporation and ensured an even temperature distribution within the vial. Temperature was measured in a separate vial throughout each experiment with the external temperature cord of a HOBO temperature logger (Onset Computer Corporation, Bourne, MA) inserted through the pipe to record temperatures within a submerged vial in the water bath. Temperatures were not recorded inside the diet cube since larvae occurred only on the surface of the diet. Temperature was recorded once every minute. The constant temperatures monitored in this way averaged 32.3, 36.0, 38.0 and 40.4 °C. We selected these temperatures to represent a range that started just above the known upper developmental threshold of 31.3 °C for eggs (Danthanarayana 1975). Six replicate vials per instar or life stage were placed in the water bath at one time giving a total of 54 vials which were considered a block. This was repeated 7 times at each temperature. The exposure interval was altered between blocks to cumulatively represent a 0 - 100 % mortality range for each instar or life stage. After the designated exposure interval, vials were removed from the water bath, placed at 21 °C, 60-80% RH, and a 16:8h (L:D) photoperiod, and mortality of larvae was assessed after 24h. Larval death was estimated using two different ad-hoc endpoint measurements, lack of response when individuals were probed with a pair of soft forceps at all temperatures (Loeschcke et al. 1994) and inability to walk at 32.3 °C and 38 °C only (Bale et al. 2000). For adults, inability to walk was the only endpoint used at all temperatures. For egg and pupal stages success of hatch and emergence two weeks after exposure to each of the temperatures served as endpoints.

Ecological significance of acute responses to high temperature exposure - experiment 2

In a second experiment we assessed the ecological consequences of exposure to high temperature extremes by estimating, for each larval instar, the chronic mortality of those individuals that survived the initial exposure. In order to provide the experimental larvae with a similar level of high temperature exposure, the exposure interval for each instar at each temperature was set to the corresponding LT_{50} that had been estimated in

experiment 1 using lack of response to probing. Twelve vials of each larval instar were set up as in experiment 1 and exposed to each of the four temperatures. After exposure, the vials were transferred to 21 °C, 60-80% RH, and a 16:8h (L:D) photoperiod for 24 h and survivorship determined from response to probing. The surviving larvae from each vial were then placed in a 96 ml plastic cup with diet and the proportion of individuals reaching adulthood was recorded for each cup. As the observed mortality for an LT_{50} exposure interval at each temperature was not exactly 50% in experiment 2, the total number of surviving larvae assessed was not necessarily 60 (12 vials with 5 surviving larvae each), but ranged from 19 to 100 depending on instar and temperature. We accounted for control mortality by subjecting 10 vials of each instar to the same LT_{50} exposure intervals in a water bath set at 21.0 °C. The survivorship to adult emergence of the larvae that responded to probing after exposure at each of the experimental temperatures was then adjusted according to the corresponding survivorship of the controls for each instar using Abbott's correction (Abbott 1925), with $\text{survival}_{\text{corrected}} = 100*(\text{survival}_{\text{treatment}} / \text{survival}_{\text{control}})$.

Statistical analyses

All statistical analyses were carried out using R (R Development Core Team, version 2.10.0, 2009). Data were analyzed with generalized linear mixed models using the function lmer of the lme4 package (Bates and Maechler, 2009). A separate model was fitted for each temperature, using a probit link and a binomial error distribution. Exposure interval and instar were considered fixed effects, while block was considered a random effect to account for the possibility of variation among the cohorts of experimental individuals used in the different blocks of observations. Random variation was entered via effects on the intercept with fixed slope. Model significance was assessed through model reduction and performing log likelihood ratio tests. Lethal times (LT_{50} and LT_{90}) and their 95% confidence intervals were calculated using Fieller's formula as described in Finney (1971, p 78). Differences in larval instar LT_{50} values obtained from the two different endpoint measurements were assessed by fitting linear models for each of the two temperatures with instar and endpoint as factors, and their significance assessed by model reduction and log likelihood ratio tests. For pupae and adults, differences in estimated LT_{50} values between males and females were assessed by using lethal time ratios, as described by Robertson et al (2007). This test was chosen because it is more powerful for comparisons of lethal times than examination of the overlap in 95% confidence intervals (Wheeler et al. 2006). According to Robertson et al. (2007), two LT_{50} values are not significantly different if the 95% confidence interval (CI) of the LT_{50} ratio includes 1.

Eggs and larval instars that survived the high temperature exposure in experiment 2 were subsequently used to estimate survivorship to adult emergence using an adjusted proportional mortality rate that takes into account the additional mortality that occurred later in the life cycle. The adjusted proportional mortality rate was estimated by multiplying the assumed 0.5 survival rate from exposure by the Abbott-corrected mortality

rate observed for the period following exposure through to adult emergence, and adding this additional mortality to the assumed 0.5 mortality rate that results from an LT_{50} exposure interval. The intercept of the original linear probit model for each instar and for eggs from experiment 1 was then increased sufficiently to allow it to intersect the new adjusted proportional mortality rate at the original LT_{50} exposure time. New LT_{50} values (±95% CI), representing survivorship through to adult emergence for eggs and each larval instar, could then be calculated using Fieller's formula. Comparisons of LT_{50} values estimated from response to probing with those estimated from adult emergence for larval instars were assessed by fitting linear models with instar and endpoint measurement as factors and assessing significance at the $\alpha = 0.05$ level by model reduction and log likelihood ratio tests. For eggs, the difference between the acute and longer-term life cycle endpoints was assessed with lethal time ratios as before. Significances were only reported for the most parsimonious model.

2.4 RESULTS

Acute responses of all life stages to high temperature exposure

Mortality of *E. postvittana* life stages and larval instars increased with exposure interval at each of the four temperatures. Interaction terms between exposure interval and instar were significant at all four temperatures (32.3 °C: $\chi^2 = 58.3$, df = 8, $P < 0.001$, 36.0 °C: $\chi^2 = 50.2$, df= 8, $P < 0.001$, 38.0 °C: $\chi^2 = 49.3$, df = 8, $P < 0.001$, 40.4 °C: $\chi^2 = 185$, df = 8, $P < 0.001$) indicating that separate slopes as well as intercepts provided the best fit for each life stage or larval instar (Table 1). The resulting LT_{50} values (Figure 1) ranged from 45 h - 187 h for the lowest temperature (32.3 °C), to 1.2 h - 5.6 h for the highest temperature (40.4 °C). At the highest temperature, LT_{50} values increased linearly with larval instar, but this positive trend was reversed at the lowest temperature such that the youngest larval instar was the most tolerant. For LT_{50} values among the other life stages, pupae mostly ranked equal to or higher than eggs and adults in their level of tolerance at all high temperatures tested (Table 2). At the two highest temperatures, pupal LT_{50} values were as high or higher than those for larval instars, while at the lowest two temperatures LT_{50} values for all (36 °C) or some (32 °C) of the larval instars were higher than those for any of the other life stages (Figure 1). Thus, there was no evidence that any one life stage was consistently more heat tolerant than any other life stage, and no clear pattern of change was apparent for the relative tolerance of the life stages across the range of temperatures tested. In addition, for pupae and adults, gender did not influence the LT_{50} values at any of the temperatures tested (Supplementary Table 1).

Comparison of two ad-hoc endpoint measurements for response to high temperature exposure

At 38.0 °C, out of a total of 915 larvae that were able to respond to probing after exposure and were thus judged to have survived the acute exposure intervals, 97 were not able to walk. At 32.3 °C the corresponding number was 34 out of a total of 860 surviving larvae. At both temperatures, the LT_{50} values resulting from these two different ad hoc endpoint measurements were not significantly different (Figure 2A) as shown by the common fitted line at each temperature (minimal model significance, 32.3 °: $F_{1,10} = 277$, $P < 0.001$; 38.0 °C: $F_{1, 10} = 10.8$, $P = 0.008$).

Ecological significance of acute responses to high temperature exposure - experiment 2

In a separate experiment the life cycle consequences of the survivorship of eggs and larvae after high temperature exposure were estimated, using an exposure interval that corresponded to the estimated LT_{50} from experiment 1. A comparison of the LT_{50} values for adult emergence with those from response to probing (larvae) or hatch rate (eggs) showed different trends for the four temperatures (Figure 2B). At 40.4 and 38.0 °C, the intercepts of the LT_{50} values for larvae surviving to adult emergence were significantly lower, by 64% and 79% respectively, than for larvae responding to probing (40.4 °C: $F_{1,9} = 23.77$, $P < 0.001$; 38.0 °C: $F_{1,9} = 9.81$, $P = 0.01$; F and P values for model significance were obtained through model reduction and log likelihood ratio tests). At 36.0 °C, both the intercept and the slope of the LT_{50} values were significantly different, but crossed at mid instar ($F_{1,8} = 8.43$, $P = 0.02$). At 32.3 °C, the intercept of the LT_{50} values again was significantly lower by 85% for larvae surviving to adult emergence than for larvae responding to probing ($F_{1,9} = 22.27$, $P = 0.001$). The difference in slopes was marginally non-significant ($F_{1,8}=4.58$, $P=0.065$) and was therefore not retained in the model. For the egg stage there was no significant difference in LT_{50} values between hatch rate and survival to adult emergence at any of the four constant temperatures as determined by ratio tests (±95% CI); 41 °C: 1.095 (0.43-1.76); 38 °C: 1.054 (0.43-1.68); 36 °C: 1.076 (0.71-1.35), 32 °C: 1.48 (0.47-2.49).

2.5 DISCUSSION

Temperature tolerance has gained renewed attention both in the context of invasive species which face temperature extremes as key obstacles for invasion and range expansion (Lalouette et al. 2012; Nyamukondiwa and Terblanche, 2010; Preisser et al. 2008), as well as in the context of predicting species responses to more extreme temperatures brought about by global climate change (Kingsolver et al. 2011; Ma and Ma 2012). Our study investigating heat tolerance of all life stages of the invasive leafroller *E. postvittana* has shown that LT_{50} values ranged from 1.2 to 5.6 h at 40.4 °C for response to probing as an endpoint measurement and from 0.5 to 3.2 h at 40.4 °C when considering

adult emergence as an endpoint measurement. The relevance of these estimates is best considered in the context of similar estimates for other native and invasive leafrollers. For example, mortality of the adult stage of the globally invasive tortricid *Cydia pomonella* was around 20% after 2 h exposure at 41 °C (Chidawanyika and Terblanche, 2011), compared to 50% for *E. postvittana* adults after 2 h at 40.4 °C. Similarly, exposure of 5th instar *C. pomonella* larvae to 38 °C for 96 h resulted in only 5% mortality (Neven and Rehfield, 1995), whereas for 5th instar *E. postvittana* larvae, the LT_{50} was 13.9 h at 38 °C when taking adult emergence as the endpoint measurement. In contrast, Whiting et al. (1991) compared the tolerance of eggs and 1st, 3rd and 5th instar larvae of a New Zealand population of *E. postvittana* to that of five endemic leafroller species at 40 °C. Mean LT_{99} values for all four life stages of all five endemic species ranged from a minimum of 2.2 to 6.1 h, whereas those for *E. postvittana* were significantly higher and ranged from 7 to 21 h, an estimate that was also substantially higher than the LT_{90} values in our study at 40.4 °C (3.1- 7.6 h). Thus, heat tolerance of the invasive *E. postvittana* seems to rank lower than that of the globally invasive *C. pomonella*, but well above that of the endemic leafroller species in New Zealand. This suggests that the range expansion of *E. postvittana*, as an invasive species in the U.S., will be more restricted than that of *C. pomonella*. Its lower heat tolerance seems likely to play an important role in restricting its potential geographic distribution in California to near coastal areas, as predicted by Gutierrez et al. (2010). Similarly, a lower tolerance of temperature extremes provides valuable supporting evidence for the finding that maximum temperature in the warmest month contributed most to the species distribution models developed for *E. postvittana* by Lozier and Mills (2011).

The successive life stages of insects often occupy different habitats in which they experience different microclimates, and may be expected to be differentially impacted by climate change (Kingsolver et al. 2011). In contrast, the life stages of *E. postvittana* share a remarkably similar habitat (leafrolls for larval and pupal stages, and leaf surfaces for eggs and adults, Suckling and Brockerhoff 2010), and are unlikely to be able to avoid temperature extremes at particular stages in their life cycle. One advantage for *E. postvittana* in its ability to survive temperature extremes, however, is that it has unsynchronized generations with all life stages and larval instars present throughout the summer months (Bürgi et al. 2011). As the life stages and larval instars showed significant differences in heat tolerance the mixed stage structure could enhance survivorship of at least some life stages of the population during temperature extremes. Moreover, the results of our study showed no consistency as to which life stage or larval instar was most tolerant at each of the four high temperatures. At the two highest temperatures, pupal LT_{50} values were the greatest, whereas at the lower two temperatures larvae represented the most heat tolerant stage. Both egg and adult stages seemed to be either equally or less tolerant than the pupal stage. In comparison, Abdelghany et al. (2010) presented a list of high temperature tolerance for different life stages of insects and found that the egg stage

was reported to be most tolerant for four species, the larval stage for four species, and the pupal stage for only one species. Bowler and Terblanche (2008) similarly reviewed the effects of life stage on high temperature tolerance, concluding that high temperature limits generally declined with age and more advanced life stages. However, as shown in our study the order of the most to least heat tolerant stage can change depending on the temperatures tested, and thus general statements about the most or least heat tolerant stage of a species should be made with caution.

In our study we observed a distinct trend of increased temperature tolerance with increasing larval instar at 40.4 °C, and the reverse of this trend at 32.3 °C. Whiting et al. (1995) found in their study of 1st, 3rd and 5th instar larvae of *E. postvittana* at 40 °C, the same increase in LT_{99} with increasing larval instar. Higher temperature tolerance for older larvae over younger larvae has also been reported for *Tribolium confusum* (Boina and Subramanyam 2004) at six different temperatures between 46 -60 °C and for codling moth, *C. pomonella*, at six out of nine temperature and time combinations between 48 and 52 °C (Wang et al. 2004). No differences were found between L1 and L3 larvae of olive fruit fly, *Bactrocera olea* (Pappas et al. 2010) at a range of temperatures between 34 - 40 °C, and a higher temperature tolerance for younger over older larvae was shown for S*tegobium paniceum* at 42 °C (Abdelghani et al. 2010). The only other study to show a reversal in relative heat tolerance of larval instars at different temperatures is that of Mahroof et al. (2003), who found that old *Tribolium castaneum* larvae showed significantly higher LT_{99} values at lower temperatures (42 °C), a non-significant difference at 46 °C and a reversed order of tolerance for temperatures equal to and higher than 50 °C.

The lack of difference in LT_{50} values between females and males of *E. postvittana* at ecologically relevant high temperatures in our study has also been found for *Helicoverpa armigera* (Mironidis and Savopoulou-Soultani 2010), *Ceratitis capitata* and *C. rosa* (Nyamudkondiwa and Terblanche 2009) and for four desert species of *Drosophila* (Stratman and Markow 1998). In contrast, significant gender differences for high temperature tolerance have been documented for *Frankliniella occidentalis* (Li et al. 2011), *Bactrocera oleae* (Pappas et al. 2010), *Drosophila melanogaster* (Folk et al. 2006) and *Aphidius rhopalosiphi* and *A. avenae* (LeLann et al. 2011). For most insects, both sexes would be expected to share the same microhabitat and consequently to share the same high temperature tolerance. Thus, for those species that do show a gender-specific tolerance, they are also likely to exhibit heterogeneity in either habitat or behavior.

High temperatures can affect a wide range of cellular mechanisms and there has been much debate about which biochemical or physiological processes or combination of processes really limit performance at the organismal level (Angilletta 2009). Heat shock proteins (HSPs) are often expressed when insects are exposed to an environmental stress, such as high temperature, and are thought to provide protection by preventing aggregation or improper folding of proteins (Schlesinger 1990). While considered a generalized form of protection from high temperature extremes in insects, HSP expression has also been shown to vary significantly with life stage, exposure time and exposure temperature. For

example, Krebs et al. (1998) demonstrated that HSP70 levels varied significantly between young and old larvae and adults of *Drosophila buzzatii*. The effect of temperature and time on HSP synthesis has been shown by Mahroof et al. (2005) in a study of young instar larvae of *Tribolium castaneum*. When exposed for 30 min to 46, 54 and 58 °C, larvae produced 150%, 100% and 25% respectively of the HSP70 levels produced by larvae at a control temperature of 23 °C. In addition, compared to HSP70 levels in control larvae at 23 °C, larvae exposed to 40 °C did not differ from the controls after 1 h, but after 8 h peaked at approximately 210% with a subsequent decline back to control levels after 32 h.

For *E. postvittana* the importance of HSP production for high temperature tolerance has been demonstrated by Lester and Greenwood (1997), who found that increased production during acclimation at temperatures ranging from 28 – 40 °C correlated with decreased mortality during subsequent exposure to 43 °C. In our study, the duration of exposure times ranged from 8 h at 40.4 °C to 280 h at 32.3 °C. Therefore, on the one hand, larval instars of *E. postvittana* may have differed in how quickly their HSP production was initiated and how long its expression lasted. On the other hand, the protection provided by HSPs at low and medium temperatures may not have been complete, and thus the proximate cause of larval mortality may have differed between the high and low temperatures, resulting in the observed reversal of relative heat tolerance among instars.

The almost linear increase (at 40.4 °C) or decrease (at 32.3 °C) in LT_{50} with larval instar suggests that the surface to volume ratio of the larvae or body weight could also have played a role in their estimated heat tolerance. For example, LeLann et al. (2011) found a linear relationship between surface to volume ratio and high temperature tolerance for adults of two parasitoid species, while Terblanche et al. (2011) describe the logarithmic relationship between body weight and high temperature survival caused by starvation and/or desiccation. Exposure times in our study also fell well within the range necessary for starvation or dessication to have had a significant effect on survival (Terblanche et al. 2011). However, no obvious signs of desiccation were observed, as a result of the humidity provided by the block of diet in the experimental vials, and starvation could only have occurred if the larvae were unable to feed due to high temperatures. Thus the exact combination of mechanisms that led to the mortality of *E. postvittana* when exposed to high temperatures in our study currently remains unknown.

Finally, differences in LT_{50} values estimated for *E. postvittana* from the two ad-hoc endpoints, ability to walk and response to probing, were not significantly different due to the low number of larvae experiencing visible, but not acutely lethal, damage. However, for all larval instars, when comparing LT_{50} values estimated from survival to adult emergence to those from response to probing, the former were significantly lower for three out of the four high temperatures with reductions ranging from 64-85%. In contrast, for the egg stage no significant difference between hatch rate and survival to adult was found at any of the four constant temperatures. Similarly, Yu et al. (2011) found that LT_{99} values for eggs of the beetle *Lasioderma serricorne* did not change when they were estimated from hatch rate as endpoint compared to survival to adult emergence as endpoint. From an ecological

perspective, our findings suggest that a proportion of the field populations of *E. postvittana* would suffer acute mortality immediately after an extreme high temperature event, and additionally, the surviving proportion of the population would suffer chronic mortality later in their development. This has important implications for predicting the thermal limits of insect species, the potential for range expansion of invasive species, such as *E. postvittana*, and the responses of insects to climate change, since upper thermal tolerance could readily be overestimated from the use of ad hoc rather than ecologically relevant endpoint measurements. The question of extrapolation from comparative physiological studies to ecological relevance for field populations has received little attention so far in the context of global environmental change (Chown et al. 2010, Hoffmann 2010, Terblanche et al. 2011), but is clearly one that deserves much closer attention in the future. Certainly, more studies are needed before we can assess the general relevance of our findings for a broader range of invasive species, but our current findings caution against reliance on traditional ad hoc endpoints for ecological studies of the responses of insects to high temperature extremes.

2.6 TABLES

Table 1. Ratio (95% CI) and ratio test significance for the differences in LT_{50} values as estimated from response to probing for non-active life stages of *Epiphyas postvittana* at four constant high temperatures.

Temperature	Stage comparison	Ratio (95% CI)
40.4 °C	Pupa - egg	1.28 (0.89-1.66) NS
	Pupa -adult	2.91 (2.52-3.31) *
	Egg -adult	2.28 (1.98-2.59) *
38.0 °C	Pupa - egg	1.64 (1.19-2.08) *
	Pupa -adult	2.02 (1.50-2.54) *
	Egg -adult	1.23 (0.84-1.63) NS
36.0 °C	Pupa - egg	3.13 (2.48-3.78) *
	Pupa -adult	1.21 (0.70-1.73) NS
	Egg -adult	0.39 (-0.21-0.98) *
32.3 °C	Pupa - egg	3.28 (2.57-3.99) *
	Pupa -adult	2.23 (1.59-2.88) *
	Egg -adult	0.68 (-0.17-1.53) NS

NS not significant

* Statistical significance ($P < 0.05$)

Table 2. Estimated LT_{50} (95% CI) values for male and female pupae and adults of *Epiphyas postvittana* at four high temperatures as estimated from response to probing, with ratio (95% CI) and ratio test significance for adult and pupal gender differences at four constant high temperatures.

Temperature	Stage	Sex	LT_{50}	Ratio (95% CI)
40.4 °C	Adult	Female	1.91 (1.67-2.13)	0.97 (0.58-1.36) NS
	Adult	Male	1.97 (1.74-2.18)	
	Pupa	Female	5.71 (4.85-7.21)	1.04 (0.53-1.54) NS
	Pupa	Male	5.51 (4.86-6.50)	
38.0 °C	Adult	Female	10.68 (9.37-11.71)	1.02 (0.48-1.56) NS
	Adult	Male	10.48 (9.30-11.45)	
	Pupa	Female	21.06 (18.86-25.88)	0.97 (0.35-1.60) NS
	Pupa	Male	21.62 (19.50-25.98)	
36.0 °C	Adult	Female	34.82 (20.83-43.26)	0.98 (0.47-1.49) NS
	Adult	Male	35.49 (22.06-43.74)	
	Pupa	Female	43.04 (33.57-50.59)	0.93 (0.29-1.58) NS
	Pupa	Male	46.08 (36.81-53.46)	
32.3 °C	Adult	Female	42.35(-9.61-68.50)	0.89 (0.05-1.73) NS
	Adult	Male	47.41 (-5.39-73.92)	
	Pupa	Female	87.53 (4.32-117.53)	0.79 (0.29-1.30) NS
	Pupa	Male	110.62 (68.18-134.33)	

NS not significant

2.7 FIGURES

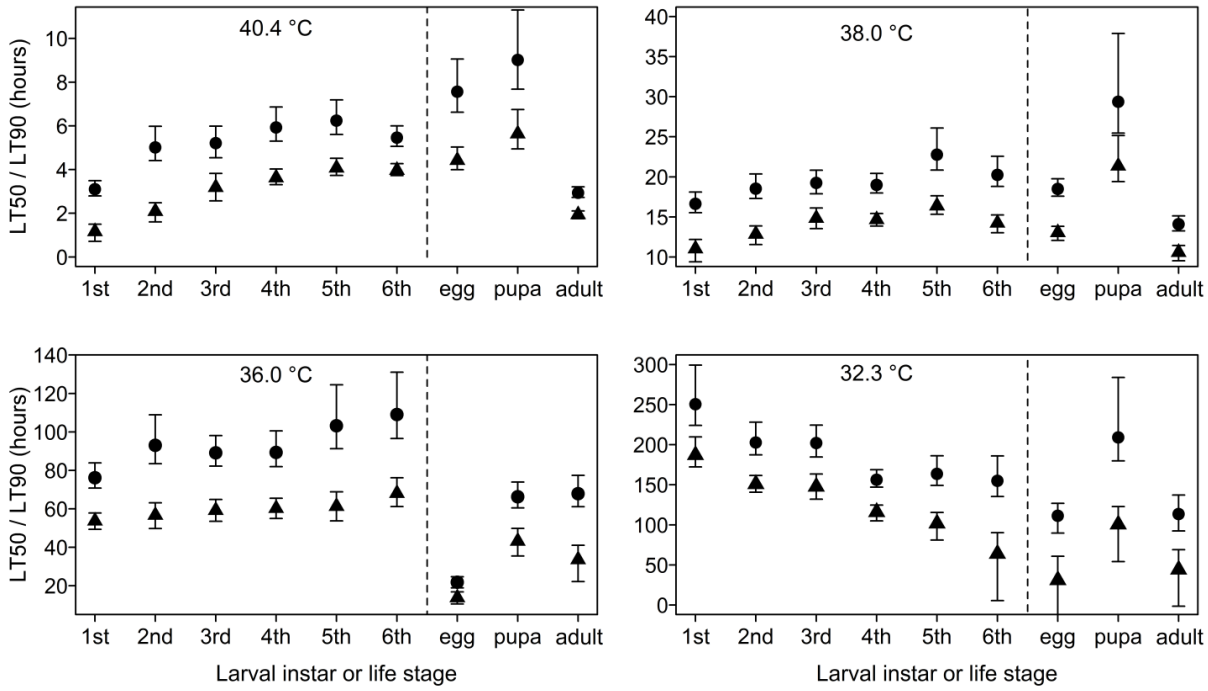

Figure 1. LT_{50} (95% CI) (triangles) and LT_{90} (95% CI) (dots) values for *Epiphyas postvittana* life stages and larval instars exposed for a series of time intervals to four constant high temperatures (40.4, 38.0, 36.0 and 32.3 °C) as estimated from response to probing. Estimates of LT values were obtained from probit models fitted to mortality data at each constant temperature separately with separate intercept and slope for each instar.

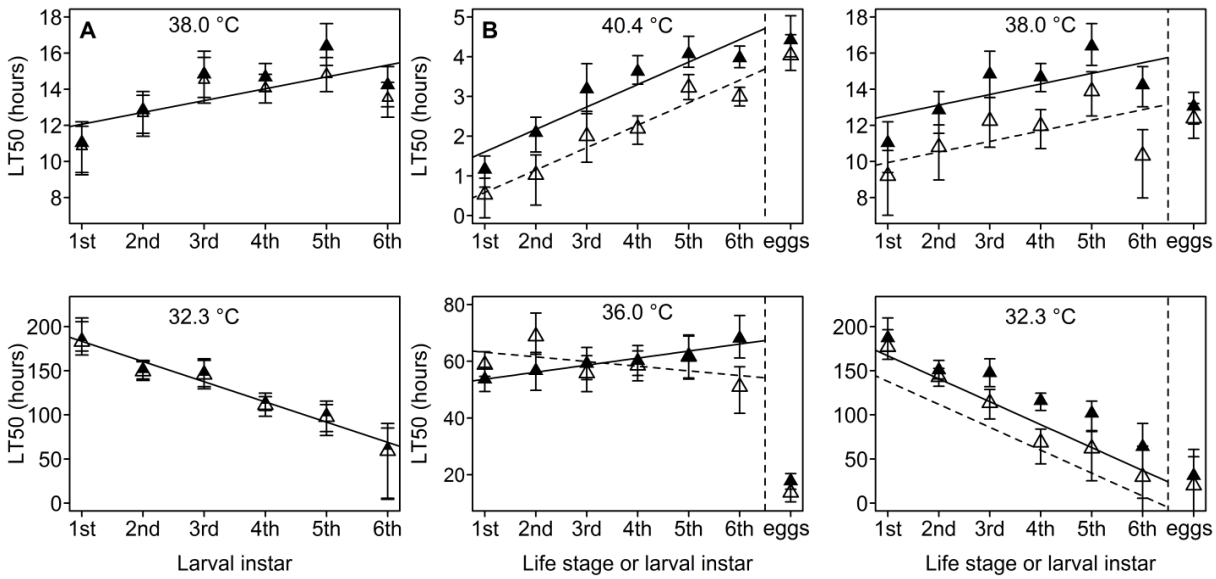

Figure 2. A comparison of estimated LT_{50} (95% CI) values from different endpoint assessments of heat tolerance in *Epiphyas postvittana*. (A) A comparison of response to probing (filled triangles) and inability to walk (open triangles) for larval instars at 38.0 °C and 32.3 °C with common fitted lines indicating lack of a significant difference between the two endpoint assessments [$y = 0.66\,x + 11.41$, $F_{1,10} = 10.82$, $P = 0.008$ at 38 °C; $y = -22.9\,x + 206$, $F_{1,10} = 277$, $P < 0.001$ at 32 °C]. (B) A comparison of response to probing or hatch rate (closed triangles) and survival to adult emergence (open triangles) for larval instars and eggs at four constant high temperatures with fitted lines indicating significant differences in either intercepts or slopes between the two endpoint assessments [$y_{probing} = 0.57x + 1.04$; $y_{adult\ emergence} = 0.57x + 0.02$, $F_{1,9} = 23.8$, $P < 0.001$ at 41 °C; $y_{probing} = 0.59x + 11.95$; $y_{adult\ emergence} = 0.59x + 9.35$, $F_{1,9} = 9.8$, $P = 0.01$ at 38 °C; $y_{probing:} = 2.48x + 51.19$; $y_{adult\ emergence} = -1.64x + 64.83$, $F_{1,8} = 8.43$, $P = 0.02$ at 36 °C; $y_{probing} = -26x + 164$, $y_{adult\ emergence} = -26x + 192$, $F_{1,9} = 22.3$, P = 0.001 for 32 °C]. All significances and *F* values obtained through log-likelihood ratio tests.

CHAPTER 3

ABUNDANCE, AGE STRUCTURE, AND VOLITINISM OF LIGHT BROWN APPLE MOTH POPULATIONS IN CALIFORNIA

3.1 ABSTRACT

The light brown apple moth, *Epiphyas postvittana* (Walker), is native to Australia and was first detected in California in 2006. In this study, we regularly sampled populations on *Leptospermum laevigatum* (Gaertn.) F.Muell. at two sites in San Francisco and on *Arctostaphylos densiflora* M.S. Baker at two sites in Santa Cruz over a two year period to monitor the abundance, age structure, and voltinism of this potential pest in relation to degree-days. Our results showed that larval abundance declined at two sites, cycled with peaks in mid summer at one site, and remained steady at one site. Generations overlapped at all four sites with the full range of larval instars being present for most of the year, although populations during the winter were predominantly mid to late instars. Accumulated degree-days predict an average of 3.27 and 4.58 generations per year in San Francisco and Santa Cruz respectively, which matched our observed peaks of late instar larvae in the field remarkably well. This new information on light brown apple moth phenology in coastal California will be invaluable for the development of effective monitoring and management strategies for this new invader in the studied region.

3.2 INTRODUCTION

The light brown apple moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), is an important horticultural and agricultural pest native to southeastern Australia. It has been accidentally introduced to New Caledonia, Hawaii, Great Britain and New Zealand and was discovered in California in 2006 (Suckling and Brockerhoff 2010). Larvae of this leafroller species feed within the protective shelter of leaves that they roll or spin together and cause damage to both foliage and the surface of fruit (Lo et al. 2000). They are highly polyphagous and feed on more than 500 known host species in 363 genera and 121 families (Suckling and Brockerhoff 2010), which aggravates the potential for damage to both agricultural commodities and to natural ecosystems, and increases the difficulty and/or cost of implementing eradication, quarantine, and other management strategies. A zero tolerance for live individuals of any life stage in exports further increases the potential economic impact of this pest.

Development of poikilothermic organisms such as plants and insects is temperature dependent with each organism requiring a specific measure of heat accumulation between lower and upper developmental thresholds to complete development (Cullen and Zalom 2000). As a result, their development rates are often expressed in day-degrees (DD), the amount of heat that accumulates between the two threshold temperatures during each 24-h period (Baskerville and Emin 1968, Zalom et al. 1983), with the heat accumulation required to complete a specific life stage or the full life cycle being referred to as the sum of effective temperatures (Jarosik et al. 2002) or thermal constant (Trudgill et al. 2005). Applications of physiological time are often used to guide the choice and timing of pest management interventions through prediction of the temperature-dependent phenology of a pest (Castle et al. 2005, Lopez et al. 2001, Damos and Savopoulou-Soultani 2010, Jones et al. 2010). For LBAM, several of the control strategies proposed for use in California require knowledge about the temporal distribution of vulnerable life stages therefore requiring knowledge about LBAM phenology in relation to degree days. Other critical parameters that aid management decisions include seasonal or yearly population fluctuations and age structure.

Currently, developmental parameters for LBAM such as upper and lower development thresholds and thermal constants are known only for a population from Victoria, Australia (Danthanarayana 1975, Danthanarayana et al. 1995). In Australia, McLellan (1973) also found that LBAM has no winter diapause, and that larval development slowed but remained continuous under cool winter temperatures.

The objective of this study was to sample field populations of LBAM in two different coastal areas of California to determine seasonal patterns of population abundance, age structure, and voltinism. Subsequently, we use these field observations with the temperature-dependent developmental parameters of Gutierrez et al. (2010) to test the predictability of the seasonal pattern of phenology of LBAM in California. Overall, these

findings will greatly contribute to choices and timing of LBAM management strategies in California.

3.3 MATERIALS AND METHODS

Sampling Sites

LBAM populations were monitored at two sites in Golden Gate Park, San Francisco county (37° 45' 59.12" N, 122° 29' 14.19" W referred to as SF1, and 37° 45' 54.31" N, 122° 30' 10.66" W referred to as SF2) and two urban sites in Santa Cruz county (36° 57' 21.28" N, 122° 2' 15.39" W referred to as SC1, and 36° 58' 49.05" N, 121° 54' 32.11" W referred to as SC2). At the SF1 site, LBAM was sampled on Australian tea tree (*Leptospermum laevigatum*). Plants ranged from 1-2m in height and had been planted only a few years prior to the beginning of the study in 2008. During the dry summer months this site was irrigated regularly. At the SF2 site, a population was sampled on Australian tea tree plants that were much older (50+ years) with heights of up to 3.5m. The site SC1 was located on a mulched road median in a suburban neighborhood bordering the Pacific Ocean. LBAM was sampled on ornamental manzanita plants (*Arctostaphylos densiflora*) that were up to 0.5m high and planted in small groups with patch diameters ranging from 2-4m. Manzanita was also sampled at site SC2 where plants were 1.5m high and formed a continuous 20m long hedge surrounding a large parking lot. These sites were selected for regular sampling because, as noted by Geier and Briese (1980a), in Australia extensive and virtually continuous infestations of LBAM are observed only in very specialized habitats such as suburban gardens and horticultural crops. These sites were thought to have had some of the longest established populations of LBAM in California based on initial numbers of adults collected in a pheromone-based trapping survey (USDA- APHIS 2011). The host plant species selected in each county were those that most consistently supported LBAM populations of sufficient abundance to allow destructive sampling. While LBAM was present on other host plant species in both counties, populations occurred only seasonally or were too small to allow effective sampling.

Abundance, Age Structure and Occupancy

Sites were visited at two-week intervals from April - October and at four-week intervals from November - March during the period from May 2008 to June 2010. Sampling at site SC2 did not start until January 2009 as it was a replacement for a previously sampled site where the LBAM population proved to be too low to be adequately monitored. To sample LBAM populations at the SF1 and SF2 sites, five minute counts of visible leafrolls were conducted on each of 22 Australian tea tree plants. At SF1, initially whole plants were counted, requiring approximately seven minutes to complete, but were switched to timed five minute counts on April 10, 2009. Similar five minute counts were conducted on

ornamental manzanita plants at the SC1 and SC2 sites. Site SC1 originally had 22 plants, but this was reduced to 16 plants after a severe pruning event in October 2008, while at site SC2 there were 15 plants.

At all four sites, 50 leafrolls were collected from additional non-sample plants on each sampling occasion. At SC1 we observed an obvious initial gradient in larval population densities and therefore collected 100 leafrolls from different plants throughout the sampling area. Leafrolls were brought back to the laboratory and carefully opened to determine occupancy by larvae and pupae, and the instars of the larvae present based on head capsule measurements (Danthanarayana 1975). Larvae were then pooled into young (1^{st} and 2^{nd}), medium (3^{rd} and 4^{th}) and late (5^{th} and 6^{th}) instar larvae to better represent age structure. Occupancy was determined as the number of individuals per 50 (or 100 for SC1) leafrolls and could exceed 50 (or 100 for SC1) due to double or triple occupancy of a leafroll. Larvae were reared to adults on a bean-based diet (Cunningham 2007) in 96ml plastic cups (Solo Cup Company, Highland Park, IL) under constant conditions at 21 °C, 70-85% RH and a 16:8 h L:D photoperiod. After emergence the identity of adults was checked using the keys of Gilligan and Epstein (2009). The timed leafroll counts were then multiplied by the proportional occupancy of the 50 (or 100 for SC1) leafrolls and divided by five or seven minutes to get a standardized LBAM count per minute for all sites.

Voltinism

Temperature data used for degree-day calculations were daily minimum and maximum temperatures recorded at the closest available National Oceanic and Atmospheric Administration (NOAA) weather stations. In San Francisco, the station (# 04-7767) is located at 37° 43’ 40’’ N and 122° 30’ 16’’ W at 2.4m elevation. Aerial distance between the weather station and SF1 was 4.53 km and 4.12 km for SF2. In Santa Cruz, the weather station (#04-7916) is located at 36° 58’ 01’’ N and 122° 00’ 39’’ W at 3m elevation. From the weather station aerial distance to SC1 was 3.41 km and 8.40 km to SC2. The climate at these coastal California sites can be described as Mediterranean with wet winters and dry summers, similar to the climate in southeastern Australia (Gutierrez et al. 2010).

Degree day calculations for each sample location were made using the single sine method with vertical threshold cutoff (Baskerville and Emin 1969). Developmental thresholds were based on reanalysis (Gutierrez et al. 2010) of data from laboratory studies in Australia (Danthanarayana 1975), resulting in a lower and upper threshold of 6.8 °C and 31.5 °C and a thermal constant of 646 *dd* representing the physiological time from egg to an adult female that has laid 50% of her eggs. We used temperature thresholds for the larval stages since these constitute the largest part of the thermal constant for a LBAM generation.

To determine the voltinism of the LBAM populations, the cumulative relative frequency of late instar (5^{th} and 6^{th}) larval counts were used over successive sample dates spanning 646 *dd* periods. Late instar larvae were selected to examine the voltinism of LBAM as these instars have a long duration and are thus well represented in the sampling data. Since LBAM does not have a synchronizing diapause stage in its seasonal activity, there is no clear biofix to represent the start of a particular generation. Thus distinct peaks in late instar larval abundance that occurred midway through the two year sampling period were selected to establish an initial generation and thus to reduce propagation of error over the full sampling period. The selected peaks were July 27, 2009 for SF1, June 30, 2009 for SF2, May 14, 2009 for SC1, and September 17, 2009 for SC2. As the relative frequency of late larval instars followed an approximately normal distribution, 323dd were both added to and subtracted from the selected peak date for each sample site. Subsequently 646 *dd* intervals were added successively on either side of this anchoring generation to cover the entire two year sampling period. When the end point of a 646 *dd* period occurred between sample dates, larval counts from the following sample date were allocated proportionally between the two consecutive 646 *dd* periods based on the degree-days belonging to each period. Curves were fitted to the cumulative relative frequency data using a generalized linear model with binomial error distribution and probit link in R (R Development Core Team, version 2.10.0, 2009). Underdispersion was corrected for by using a quasibinomial error distribution.

To further assess the suitability of the 646 *dd* generation estimate for LBAM generations in California, intervals of 646 *dd* were superimposed on the relative frequency of late instar larvae over the two year sampling period. Again, distinct peaks in the representation of late instar larvae were used as anchoring generations and 646 *dd* intervals were added to and subtracted from these peaks at each site. Separate anchoring peaks were established for each calendar year; July 2008 and 2009, and March/April 2010 for SF1 and SF2, and May/June each year for SC1 and SC2.

3.4 RESULTS

Abundance

The abundance of LBAM populations showed different trends over the two year period at each of the sites sampled. The population at SF1 showed a steady decline in abundance from an initial peak of 110 individuals per minute of search in June 2008 to less than 10 individuals per minute in 2010 (Fig. 1a). The population at SC2 showed a similar decline from a peak of 65 individuals per minute of search in May 2009 (Fig. 1d). In contrast, population abundance at the SF2 site remained steady at around 5-10 individuals per minute of search (Fig. 1b). Finally, at SC1 the LBAM population showed a cyclical trend

with peak densities of 40-60 individuals per minute in mid summer (Fig. 1c). At the latter three sites, populations tended to be highest between April and August.

Age Structure and Occupancy

The age structure of the LBAM populations clearly indicated overlapping generations at each of the sites (Fig. 2). Throughout the year all larval instars from early (1st and 2nd) to late (5th and 6th) were present although there were more distinct periods of late instar larval occurrence that provide an indication of the successive generations throughout the year. Similarly, occupancy levels, represented as the number of individuals per 50 or 100 leafrolls (Fig. 2), remained fairly stable throughout the year, fluctuating between 30-60%, with some distinct peaks reaching 80% and 140% around May at the SC2 and SC1 sites respectively. Numbers exceeded 50 or 100 due to multiple individuals per leafroll. In San Francisco LBAM entered the winter months predominantly as medium instar larvae (3rd and 4th) and at the end of February was still found as either medium (SF1) or as equal numbers of medium and late (5th and 6th) instar larvae (SF2). In Santa Cruz, LBAM started the winter months with about equal representation of medium and late instar larvae (except for the winter of 2009 at SC2) and always ended the winter as late instar larvae.

Voltinism

For SF1 and SF2 accumulated degree-days over the two years totaled 4226 (2178 *dd* from May 1, 2008 - May 1, 2009 and 2048 *dd* from May 1, 2009 – May 1, 2010), allowing for an average of 3.27 generations (3.37 in 2008 - 2009 and 3.17 in 2009 - 2010). The timing of these three generations is shown from the cumulative relative frequency distributions of late instar larvae (Fig. 3a,b), where the intervals between the mid points (0.5 cumulative relative frequency) of each distribution represent the development times for successive generations. The distance between the successive peaks in the relative frequency of late instar larvae match the estimated 646 *dd* for an LBAM generation reasonably well with the exception of the overwintering generation (Fig. 4a,b). At SF1 and SF2 it took an additional 318 and 102 *dd*, respectively, in 2008/2009 and 123 and 180 *dd* in 2009/2010 for completion of the overwintering generation. The peaks in the relative frequency of late instar larvae occurred in April - July - September in SF1 and March - July - September in SF2, with the spring generation emerging following the overwintering of mid larval instars.

In Santa Cruz county accumulated degree-days over the two years totaled 5917 (2949 *dd* from June 4, 2008 to June 4, 2009, and 2968 *dd* from June 4, 2009 to June 4, 2010), which allowed for 4.56 generations in 2008-09 and 4.59 generations in 2009-10 at these two sites. The cumulative relative frequencies show the timing of the four generations for both SC1 and SC2 (Fig. 3c,d). Similarly to the SF1 and SF2 sites, peaks

in the relative frequency of late instar larvae were well matched by the estimated 646 *dd* except for the overwintering generation which required an additional 115 and 392 *dd* for both years at SC1 and SC2, respectively (Fig. 4c,d). At both sites there appeared to be three peaks in the representation of late instar larvae, occurring in January - May - August at SC1, and in February - June - September at SC2. The August/September peaks were broader, however, and based on an estimated 646 *dd* per generation, they likely resulted from an overlap in the representation of late instar larvae from both the third and fourth generations.

3.5 DISCUSSION

Information about abundance, age structure and phenology of LBAM in California is crucial for control decisions for this new invasive species. During this two year study, year to year population densities exhibited either a decline or cyclic pattern, that is, having similar peak densities between years with population increases happening around late spring to summer. This suggests that a moderate level of biotic resistance exists to the local expansion of LBAM populations, although the observed trend must be interpreted with caution as a two year period is probably too short a time frame for monitoring consistent population trends. At the SF1 site, plant maturation could be an explanation for the population decline with the Australian tea tree plants having only recently been planted when sampling started in 2008, but having grown substantially to become less vigorous in their growth toward the end of the sampling period. In Australia, a similar decline of LBAM infestation was observed by Geier and Briese (1980a) from 1963-64 to 1965-66 on young apple trees, where they found a significant positive correlation between tree vigor (measured as trunk girth and canopy development) and infestation, linking the decline in LBAM abundance to tree maturity. Other possible explanations for the population decline that was observed at two of our sites include higher than expected parasitism rates (Bürgi and Mills, unpublished observations), and the impact of regular destructive sampling on neighboring plants at the same sites. However, further monitoring will be needed to document the longer-term trends in LBAM abundance.

Most interesting and consequential for LBAM control strategies was our observation that all larval stages are present throughout most of the year. This observation is also supported by USDA trap catch data for adult moths, showing fluctuating but uninterrupted catches throughout the year (USDA - APHIS 2011). This will likely limit or at least greatly complicate the timing of pest management practices that target certain life stages, since they will likely need to be implemented much of the year. Several factors could contribute to the more continuous presence of all larval instars through the year. Since LBAM has no diapause (MacLellan 1973) and continues to develop slowly throughout the winter months, there is no temperature or photoperiodic event that synchronizes the population and this allows overlapping generations to develop and persist. An overlap in generations can readily develop as a result of variance in development times within each generation which

becomes amplified over successive generations each year in the absence of a synchronizing event such as overwintering diapause. This trend could further be exacerbated by the differences in development rates of larvae feeding on different host plants (Tomkins 1989, Danthanarayana 1975), which may be particularly important for LBAM due to its extreme polyphagy. Also, variation in development rates can be caused by larval crowding as described by Danthanarayana (1982). Similar observations of overlapping generations and life stages were noted during the warmer summer months in both Australia (Danthanarayana 1975) and New Zealand (Wearing et al. 1991).

Accumulated degree-days and the succession of 646 *dd* periods through the year suggest that LBAM is capable of producing three generations per year in San Francisco county and four generations in Santa Cruz county. In San Francisco we recorded an average of 2113 *dd* per year and observed three generations of LBAM. Despite our use of a slightly lower thermal constant and lower threshold (Gutierrez et al 2010), three generations of LBAM were also found in Melbourne, Victoria where there are 2176 *dd* per year (Danthanarayana 1975), as well as in the Australian Capital Territory (MacLellan 1973) where Geier and Briese (1980a) recorded approximately 2100 *dd* per year. In contrast, we believe that there are four generations per year for populations of LBAM in Santa Cruz where weather stations recorded 2956 *dd* per year. Similarly, Green (1984) found that 2510 *dd* per year permitted four generations to be completed annually in Auckland, New Zealand.

Overall, our field observations suggest that the thermal constant for the generational development and thus the voltinism of LBAM in California fit the degree day predictions from Australia reasonably well. For all sites, peaks of proportional late instar larvae were clearly visible early in the year and showed a good accordance with the 646 *dd* predicted intervals. Yet 3rd and 4th generations of LBAM in San Francisco and Santa Cruz counties, respectively, were less clearly detectable from the sampling data (see Fig. 4). This could be due to the overlap of generations discussed above which would likely be most marked in late summer, resulting in a single combined peak in the relative frequency of late instar larvae of the third and fourth generations in Santa Cruz county. In addition, since these late summer generations in Santa Cruz county were shorter than 8 weeks, the sampling interval of two weeks may not have been sufficiently frequent to be able to separate the peaks for these two successive generations.

In general, some deviation in generation times is common, especially for multivoltine insects, where earlier or later generations in the year can differ in their degree day requirements. Lopez et al. (2001), for example, found that the first generation of the noctuid *Sesamia nonagrioides* takes much longer than the second, whereas Pitcairn et al. (1992) found that the first generation of *Cydia pomonella* develops much faster than the second and third generation. LBAM populations at all four sites showed increased degree day requirements during the colder winter months. Geier and Briese (1981) found that under constant temperatures LBAM significantly slowed its development when days were shortened from 14:10 h D:L to 8.5:15.5 h D:L, perhaps to avoid the potential for adult

emergence during winter. At Santa Cruz, the shortest and longest days are 9.38 daylight hours in December and 14.42 h in June, while in San Francisco the corresponding daylight hours are 9.33 in December and 14.47 in June (U.S. Naval Observatory 2011). These seasonal changes in daylength in California are very similar to those used experimentally by Geier and Briese (1981) providing a plausible explanation for the extended generation times observed for LBAM during the winter.

Finally, continued monitoring of LBAM phenology will be needed throughout the establishment, adaptation period, and potential spread of LBAM populations in California. Geier and Briese (1980b) found a remarkably high level of variability in the demographic characteristics of LBAM, including variation in the development rates of populations from different regions of Australia reared under uniform conditions in the laboratory. The duration of pre-imaginal development increased at a rate of 5.81 *dd* per degree of latitude for populations of LBAM in the southern hemisphere (Geier and Briese 1980b). Currently LBAM populations in California fit the estimated 646 *dd* generation time quite well. However, as this new invader continues its predicted northward spread (Lozier and Mills 2012) it is likely to experience a similar increase in development time. Combined with a reduction in cumulative degree-days this would lead to fewer generations per year, which in turn could affect its population dynamics.

3.6 FIGURES

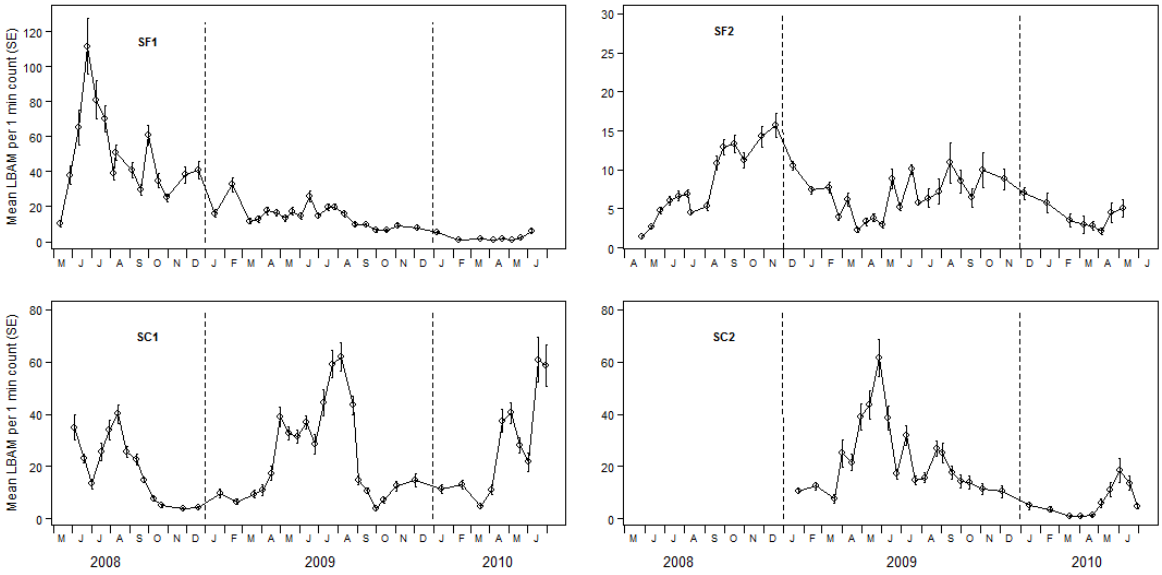

Figure. 1. Mean (± SE) LBAM individuals (larvae and/or pupae) per minute of search from regular sampling at two sites in San Francisco (a,b) and two sites in Santa Cruz (c,d) counties from May 2008 to June 2010.

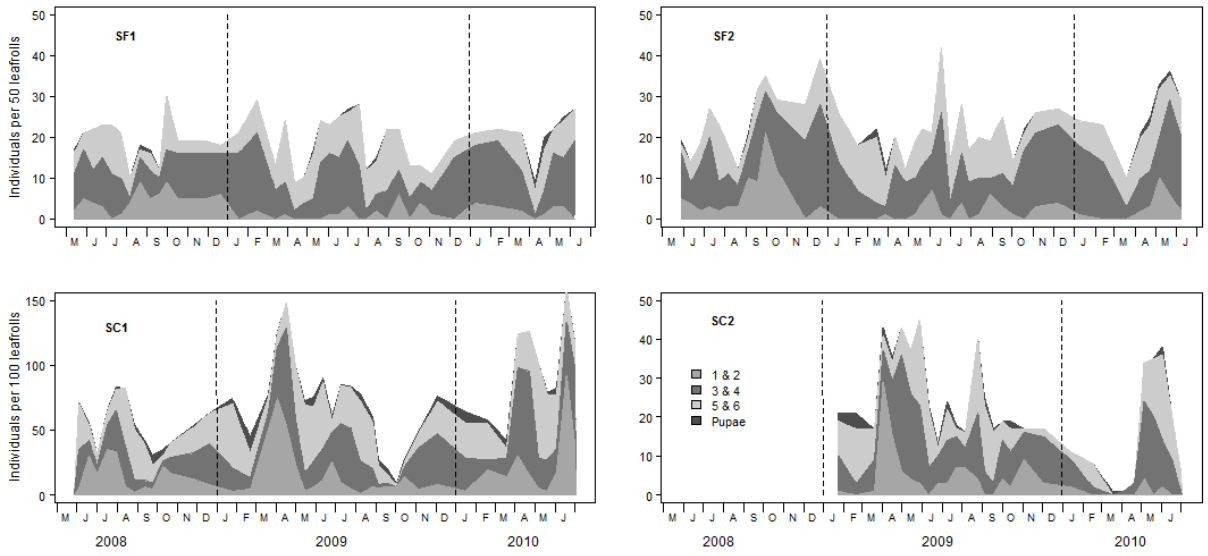

Figure 2. Occupancy and age structure of the LBAM populations sampled regularly at two sites in San Francisco (a,b) and two sites in Santa Cruz (c,d) counties from May 2008 to June 2010.

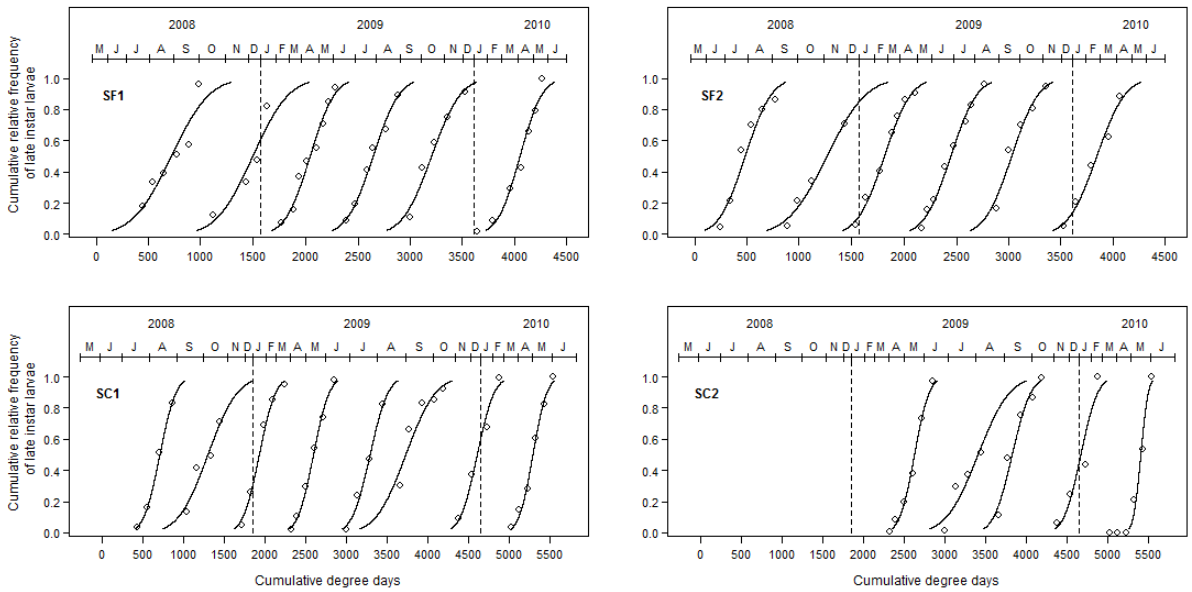

Figure 3. Cumulative relative frequency of late instar larvae showing voltinism of LBAM populations at two sites in San Francisco (a,b) and two sites in Santa Cruz (c,d) counties from 2008 to 2010. Circles represent sample dates and cumulative relative frequencies of late instar larvae. See Appendix 1 for parameters of the fitted curves.

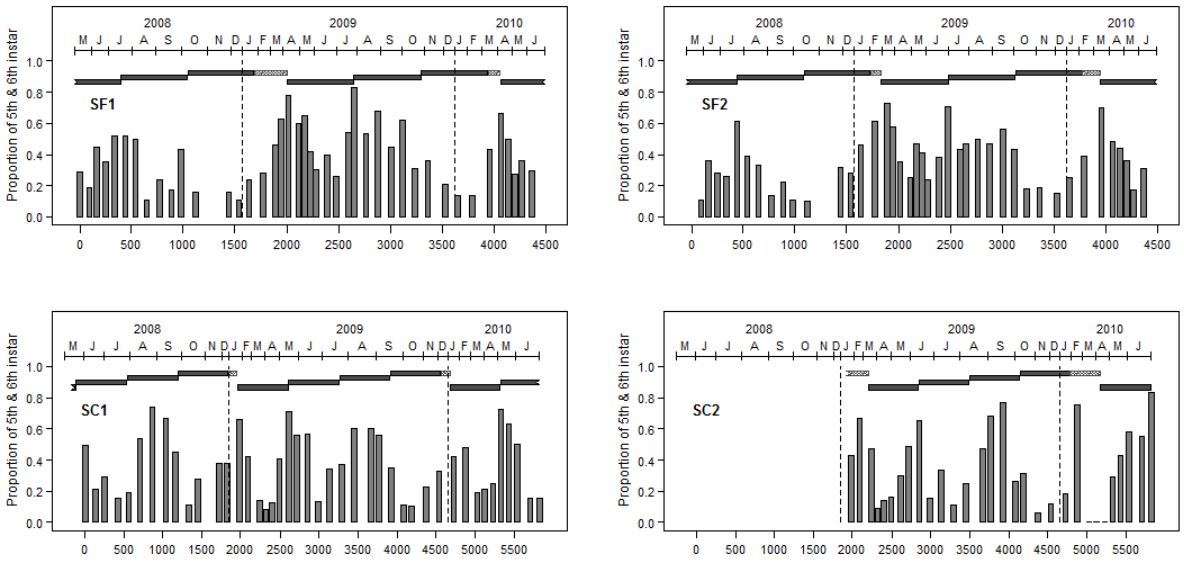

Figure 4. Voltinism of LBAM populations at two sites in San Francisco (a,b) and two sites in Santa Cruz (c,d) counties from 2008 to 2010, as represented by the relative frequency of late (5^{th} and 6^{th}) instar larvae (vertical bars) in relation to cumulative degree-days and degree day generation intervals (646 ± 4 *dd* horizontal bars). White areas in the horizontal bars are additional *dd* needed for completion of the winter generation (a-d).

CHAPTER 4

LACK OF ENEMY RELEASE FOR AN INVASIVE LEAFROLLER IN CALIFORNIA: TEMPORAL PATTERNS AND INFLUENCE OF HOST PLANT ORIGIN

4.1 ABSTRACT

The enemy release hypothesis posits that the success of invasive species can be attributed to their escape from natural enemies. Invading hosts are expected to encounter an enemy assemblage consisting of fewer species, with lower representation of specialists, and to experience less mortality as a result. In this study, we examined parasitism of the Light Brown Apple Moth (LBAM), *Epiphyas postvittana* (Walker), in California, an exotic leafroller that is native to southeastern Australia. From 2008 - 2011 we monitored parasitoid species richness, representation of specialist parasitoids, and parasitism rates of LBAM collected three times per year from four plant species of Australian origin and six plant species of non-Australian origin, at two locations in coastal California. We found the resident parasitoid assemblage of LBAM in California to be equally rich, to have a similar representation of specialists, and to inflict the same parasitism rates as in its native range. The two dominant parasitoids were *Meteorus ictericus* (Braconidae) and *Enytus eureka* (Ichneumonidae). Parasitoid species richness varied with season and plant origin and decreased slowly, but significantly, over the four year period. Parasitism rates were lowest in spring and highest on plants of Australian origin, but did not change with year. Hyperparasitism rates were higher on *E. eureka* (36.5%) compared with *M. ictericus* and other parasitoids combined (3.3%) and were highest on plants of Australian origin. We subsequently discuss the lack of both apparent and realized enemy release for LBAM in California and the unique finding that a shared plant origin enhanced the parasitism of this exotic leafroller by resident parasitoids.

4.2 INTRODUCTION

Invasive species are among the major threats to biodiversity, displacing native species, posing risks to endangered species, and altering ecosystem functioning (Ricciardi, 2007; Vitousek et al., 1996). The economic costs of invasive species incurred annually by the United States through direct damage and management expenses is estimated to be around 120 billion dollars (Pimentel et al., 2005). With such dramatic impacts on natural and agricultural ecosystems it is essential to understand the invasion process and the factors that can influence the invasiveness of alien species (Allendorf and Lundquist, 2003; Davis, 2009; Gurevitch et al., 2011).

One of the central concepts of invasion biology is the enemy release hypothesis (ERH) which posits that the success of invasive species can be attributed to their escape from natural enemies (Elton, 1958; Keane and Crawley, 2002; Mitchell et al., 2003; Liu and Stiling, 2006). Enemy release can be assessed by either comparing the enemy assemblage of a host species in an invaded region to that in its native region (biogeographical comparison), or by making comparisons of enemy assemblages on closely related native and invasive hosts in the same community (community comparison) (Colautti et al., 2004). Parasitoids, as a group of natural enemies, are insects that develop on (ectoparasitoids) or in (endoparasitoids) the bodies of other insects, with endoparasitoids in particular sharing a strong coevolutionary relationship with their hosts (Quicke, 1997). This, and the fact that they are often a major cause of mortality among phytophagous insect populations (Cornell and Hawkins, 1995), makes them effective model organisms for the study of enemy release among phytophagous insect hosts.

The ERH is based on the observation that in the introduced range exotic host species are often attacked by a reduced number of resident enemy species, representing an apparent enemy release, and that in most, but not all cases, apparent release is also accompanied by a reduction of the impact of resident enemies on exotic host species, providing evidence of realized enemy release (Colautti et al., 2004; Mitchell and Power, 2003; Torchin et al., 2003). From a biogeographical comparison of phytophagous insects as both natives and exotics by Cornell and Hawkins (1993), 68% of phytophagous insect species showed much greater parasitoid species richness as natives and 94% showed greater parasitism rates as natives, indicating that realized enemy release is somewhat more common among exotic phytophagous insects than apparent enemy release. Another aspect of the ERH is that enemy assemblages on exotic hosts are expected to have a greater representation of generalists than specialists (Keane and Crawley, 2002) as resident specialists take longer to acquire the phenological, behavioral, or ecological adaptations needed to successfully use a novel exotic host. The generalist versus specialist paradigm for parasitoid assemblages is often represented by the proportion of idiobionts versus koinobionts (Sheehan and Hawkins, 1991; Althoff, 2003). Idiobionts are parasitoids that typically kill or permanently paralyze their host at oviposition (Askew and Shaw, 1986; Haeselbarth, 1979). They do not to need to adapt as effectively to the specific physiology of

their hosts, which allows them to exploit a broader host range and to switch to novel hosts more easily. Koinobiont parasitoids, on the other hand, allow their hosts to continue to feed and develop after oviposition (Askew and Shaw, 1986; Haeselbarth, 1979). This latter mode of development is considered to be more intimate and to require a greater level of adaptation to a host than for idiobiont parasitoids, resulting in a smaller host range.

In the present study, we examined the parasitoid assemblage and parasitism rates of the light brown apple moth (LBAM) *Epiphyas postvittana* (Walker) as an invasive species in California. LBAM is a tortricid leafroller native to South Eastern Australia and was first discovered in California in 2006 (Brown et al., 2010). Larvae of this species are highly polyphagous and feed on 545 known plant species in 363 genera and 121 families (Brockerhoff et al., 2011). Highly polyphagous exotic insect herbivores, like LBAM, are likely to be found on a variety of host plants in an introduced region that will differ in both origin and coevolutionary history with the herbivore, and consequently, will generate differences in the composition and activity of associated resident parasitoids. Similarly, we might also expect differences in parasitoid composition and activity between seasons and years (Le Corff et al., 2000). Our specific objective in this study was to evaluate parasitism of LBAM in California by resident parasitoids and to test three predictions of the ERH: 1) that the parasitoid assemblage should be less species rich in California than in its native home, 2) that parasitism rates should be lower in California than in its native home, and 3) that the parasitoid assemblage associated with LBAM in California should primarily be represented by idiobiont species. In addition, we assessed the extent to which parasitoid species richness and parasitism rates varied with host plant origin, year and season. To our knowledge, this is the first study to investigate the effect of a shared origin between an exotic herbivore and its host plants on the species richness and impact of resident enemies at the third trophic level.

4.3 MATERIALS AND METHODS

Sampling locations, sites, and dates

LBAM populations were sampled at two locations, one in San Francisco county, referred to as SF, and another in Santa Cruz county, referred to as SC, with an aerial distance between them of 98 km. These two locations are thought to have had some of the longest established populations of LBAM in California based on initial numbers of adults collected in a pheromone-based trapping survey (USDA- APHIS 2011). The two locations differ climatically with SF accumulating an average of 2169 degree days centigrade annually between 2008-2011 with temperature maxima and minima of 35.6 and 9.4 °C, and corresponding values for SC of 2477 degree days centigrade with 38.3 and -2.2 °C, as measured at the nearest weather stations (Half Moon Bay, NCDC #3714, and De Laveaga, CIMIS #10) (UC IPM 2012).

At both locations, an extensive search for multiple sites was undertaken before the start of the project, resulting in 11 and 8 sites at SF and SC respectively. At the SF location, all sampling sites were located within Golden Gate Park, with a maximal aerial distance of 4.7 km between them. At SC, sampling sites were located in urban gardens, parking lots, or small county parks between Santa Cruz and Capitola, with a maximum aerial distance between them of 7.6 km. Sites consisted of a group of plants of the same species that supported LBAM populations that were consistent enough that they could be sampled at least 4 times during the study period. Seven different plant species were sampled at the SF location, with numbers of sites and plant origin (A for Australian and O for other) for each species included in parentheses: *Leptospermum laevigatum* (3; A), *Melaleuca linariifolia* (1; A), *Agonis flexuosa* (1; A), *Mersine africana* (2; O), *Choisya ternata* (1; O), *Hypericum calycinum* cv. 'Hidcote' (2; O) and *Correa alba* (1; A). At the SC location three different plant species were sampled: *Pittosporum tobira* (4; O), *Arctostaphylos densiflora* (3; O) and *Abelia x grandiflora* (1; O). To test for the effect of plant size on parasitoid species richness, the height of 10 random plants were measured at each site once at the end of the study period.

Egg masses of LBAM could not be found reliably and were not sampled for parasitism at any of the sites. As no parasitoids of tortricid hosts complete their development in the earlier larval instars (Mills, 1994), fourth to sixth instar larvae and pupae were sampled to allow maximum exposure of hosts to parasitism in the field before collection. Plants were sampled three times per year during periods that had been identified as having the highest proportional representation of 4-6th instar larvae in the age structure of LBAM populations that have 3-4 overlapping generations throughout the year in California (Bürgi et al., 2011). Sampling was conducted from summer 2008 through fall 2011 and the timing of the three sample periods varied slightly between the two locations, May 18-30 (spring), July 09-27 (summer), and September 19 to October 20 (fall) for SF, and May 16-21 (spring), July 28 to August 11 (summer), and September 25 to October 17 (fall) for SC.

Parasitoid species richness, parasitism rates and hyperparasitism

Forty LBAM leafrolls occupied by fourth to sixth instar larvae, pupae or parasitoid cocoons were collected at each site and brought back to the laboratory on each sampling occasion. Groups of 12-15 larvae of the same instar were placed in 96ml plastic cups (Solo Cup Company, Highland Park, IL) and were reared to adult on a bean-based diet (Cunningham 2007) under constant conditions at 21 °C, 70-85% RH and a 16:8 h (L:D) photoperiod. Larval instars of the field-collected LBAM were determined from head capsule measurements (Danthanarayana, 1975). Cups were checked weekly for LBAM pupae and parasitoid cocoons. Pupae were removed from cups and kept in 33ml plastic vials in groups of up to five and after emergence the identity of LBAM adults was checked using Gilligan and Epstein (2009). Parasitoid cocoons were kept separately in 38ml glass

vials to await adult emergence. Adult parasitoids were identified to genus before obtaining species determinations from a series of taxonomic specialists.

The number of parasitoid species reared from field-collected leafrolls was used as a measure of parasitoid species richness. Parasitism rates for primary parasitoids, i.e. parasitoids that directly attack LBAM life stages, were estimated from the number of parasitized LBAM hosts in relation to the number of occupied leafrolls collected on each occasion at each site. Dividing the number of parasitized hosts by the number of occupied leafrolls collected provides a conservative estimate of parasitism as there was consistent mortality of LBAM larvae (~30%) during laboratory rearing that could have differentially affected parasitized individuals.

As all hyperparasitoids, i.e. parasitoids that attack the primary parasitoids of LBAM, were found to be solitary species, hyperparasitism was estimated as the total number of hyperparasitoid individuals emerged divided by the total number of primary parasitoid cocoons collected.

Statistical analysis

All statistical analyses were conducted using R (R Development Core Team, version 2.15.0, 2012). For analyses of parasitoid species richness, parasitism rates and rates of hyperparasitism we used generalized linear mixed models with penalized quasi likelihood (glmmPQL in the package "MASS") and either a Poisson (counts) or binomial (rates) error structure. For all of the models, year, season and plant origin were included as explanatory variables. Year was treated as a continuous variable, while season (spring, summer, fall) and plant origin (Australian, Other) were considered factors. To account for repeated measures at the same sites, site was included as a random factor and was nested within location (SF, SC). Significance values reported for the explanatory variables year, season and plant origin were obtained from the null model after elimination of factors with significance levels of $\alpha > 0.05$. For categorical factors with more than two levels, non-significantly different levels were pooled. For the analysis of rates of hyperparasitism, the only interaction tested was the season by year interaction due to overparameterization of the model.

For the analysis of parasitoid species richness, sample size (number of parasitoids) varied considerably between replicates due to large fluctuations in parasitism rates between plant species, year and season. Species richness is known to exhibit a logarithmic relationship with sample size that can be approximated by a linear relationship at the lower end of the curve (Hawkins, 1994). Visual inspection of the species accumulation curve confirmed that a linear approximation was adequate and we therefore included sample size as a covariate in the analysis (Hawkins, 1994).

To further investigate the influence of variation among plant species on the accumulation of parasitoid species associated with LBAM in California, we randomly

resampled (with replacement) the combined data set for both locations to generate rarefaction curves for sample sizes of 50, 250 or 500 parasitoid individuals from all possible combinations of from 1 to 10 plant species (using pooled data for multiple sites). As the number of plant species combinations varied with level of plant species richness, the extent of resampling for levels with fewer combinations was increased to match the sampling effort of the level with the most combinations which was 252 (= 10!/[5!*(10-5)!]).

We also tested for the dependence of cumulative parasitoid richness over the entire sampling period at each site on plant size, and for the dependence of mean parasitism rates averaged over the entire study period at each site on plant size. We used generalized linear models with Poisson and binomial error structures, respectively, and quasi likelihood where necessary. In the model for cumulative parasitoid species richness we also included number of parasitoids collected as a covariate to account for the effect of sample size. Lastly, we tested the relationship between parasitism rates and parasitoid species richness, with data collected for each site at each sample date as replicates, using a generalized linear model with a binomial error structure, logit link and quasi likelihood. For all three models, model reduction and log likelihood ratio tests were used to assess significances at $\alpha = 0.05$.

4.4 RESULTS

For the period from summer 2008 through fall 2011 a total of 2285 (SF) and 3592 (SC) LBAM individuals were collected and 976 (SF) and 864 (SC) parasitoids were reared from them. The total number of parasitoid species reared from LBAM collected at both locations combined was 13, belonging to 4 families and 12 different genera (Table 1), of which eight were larval parasitoids, four were pupal parasitoids and one was a larval-pupal parasitoid. All of the parasitoids are native to North America, with the exception of *Meteorus ictericus* that originates from the Palearctic region. The biological traits of each parasitoid species, their relative abundance, and their representation among the different host instars collected are provided in Table 1.

Meteorus ictericus, a solitary larval endoparasitoid, was found to be the numerically dominant parasitoid at all sites sampled and was responsible for 71.2% of the total parasitism of LBAM. The second most abundant parasitoid, providing 16% of the total parasitism, was the solitary larval endoparasitoid *Enytus eureka*. *Pediobius ni*, a gregarious pupal parasitoid, made a significant contribution to parasitism at the SC location (10.5%), but was absent from the SF location, resulting in a 5% contribution to total parasitism. Ranked fourth in their relative contribution to total parasitism (2.3%) was a group of tachinid flies that consisted of three species, *Nemorilla pyste*, *Actia interrupta*, and one unidentified species; data on the relative contribution of each species were not collected. The fifth most important species was the larval-pupal parasitoid *Ischnus inquisitorius* accounting for 1.8 % of the total parasitism. Both the tachinids and *I. inquisitorius* were

more significant at the SC location (4.2% and 3.6 %) than at the SF location (0.6 and 0.2 %). Only three of the parasitoid species found at the SC location, *P. ni, Centeterus* sp., and *Hormius* sp., were absent from the SF location, whereas all of the parasitoid species found at the SF location also occurred at the SC location. The generalist contribution to the primary parasitoid assemblage, as represented by the proportion of idiobionts, was 0.36.

Parasitoid species richness

Parasitoid species richness was significantly higher on plants with Australian origin ($t = 2.18$, $P = 0.05$), significantly lower in spring compared with summer and fall combined ($t = 3.4$, $P = 0.001$) and showed a significant but slow decrease with year ($t = 2.26$, $P = 0.03$, Fig. 1). Sample size as a covariate was significant ($t = 3.46$, $P < 0.001$). Cumulative parasitoid species richness over the entire period did not show a significant relationship with plant size, which ranged from 0.36 to 3.18 m ($\chi^2 = 0.03$, df = 1, $P = 0.86$), but sample size as a covariate was significant ($\chi^2 = 4.13$, df = 1, $P = 0.04$).

Rarefaction curves for parasitoid species accumulation in relation to plant species richness produced logarithmic curves that showed the importance of plant species as well as sample size on parasitoid species richness (Fig. 2). The curves indicate that a single plant species generates a statistical expectation of around three to five parasitoid species with little influence of sample size. However, when more plant species are considered the expected parasitoid richness asymptotes at about 5 species for the smaller sample size of 50, at almost 9 species for a sample size of 250 parasitoids, and about 11 species for a sample size of 500 parasitoid species collected.

Parasitism rates

Parasitism rates per site ranged from 0 – 100% with an overall grand mean of 34.47 % (± 2.21). Parasitism rates were significantly higher on plants of Australian origin ($t = 2.44$, $P = 0.03$) and were significantly lower in spring compared with summer and fall combined ($t = 5.92$, $P < 0.001$,Fig. 3). There was no significant effect of year on parasitism rates ($t = 1.61$, $P = 0.11$). The relationship between mean parasitism rate and plant size was not significant ($F_{1,16} = 0.72$, $P = 0.41$), but the effect of parasitoid species richness on parasitism rates was significant ($\ln[y/1 - y] = 0.24\,x - 1.54$; $F_{1, 117} = 11.21$, $P = 0.001$), resulting in an estimated increase from 0.21 proportional parasitism for one parasitoid species to 0.47 for six parasitoid species.

Hyperparasitism

A total of 110 hyperparasitoid specimens were recovered from the collected cocoons consisting of 4 species and a group of unidentified Pteromalidae (Table 2). The dominant

hyperparasitoid species, with 78.2 % of the total hyperparasitism, was *Gelis* sp1, a species that has wingless females and winged males. The second most abundant hyperparasitoid species was *Itoplectis quadricingulata*, making up 10% of the total hyperparasitism. The hyperparasitoid species and their relative frequency among primary parasitoid species are provided in Table 2. The overall rate of hyperparasitism was 8.96 % (± 1.46) for all parasitoid cocoons collected and varied significantly with primary parasitoid species, being lower for *M. ictericus* and "Other" parasitoids combined than for *E. eureka* ($t = 5.24$, $P < 0.001$, Fig. 4). The effect of plant origin was also significant, with hyperparasitism on plants of Australian origin being significantly higher ($t = 2.43$, $P = 0.03$). We also found a marginally significant effect of season, with hyperparasitism in spring being significantly lower than in summer and fall combined ($t = 1.83$, $P = 0.07$), while the effect of year was not significant ($t = 1.17$, $P = 0.24$).

4.5 DISCUSSION

Parasitoid species richness and specificity

The enemy release hypothesis (ERH) predicts that parasitoid assemblages on exotic hosts should have a reduced species richness with a high representation of generalists, and limited parasitism pressure (Torchin et al., 2003; Colautti et al., 2004; Liu and Stiling, 2006; Roy et al., 2011). In our study, in which we sampled fourth through sixth instar larvae and pupae of LBAM in coastal California, we found 13 species of primary parasitoids and 5 species of hyperparasitoids. In two other studies of parasitism of LBAM in California (Chapter 5; Wang et al., 2012), an additional two egg parasitoids (*Trichogramma fasciatum* (Perk.) and *T. platneri* Nagarkatti), four larval parasitoids (*Apanteles* sp., *Agrypon clandestinum* Först., *Campoplex* sp., Tachinid sp2), one pupal parasitoid (*Brachymeria ovata* [Say]) and one hyperparasitoid (*Scambus* sp.) were found. This raises the total California parasitoid species to 20 primary and 6 hyperparasitoids. In southeastern Australia, the native range of LBAM, Paull and Austin (2006) found the parasitoid assemblage of host larvae and pupae to consist of a total of 17 primary parasitoids and 5 hyperparasitoids (plus 3 species that were undetermined). There was no overlap between parasitoid species found in our study and parasitoid species found on LBAM in Australia (Paull and Austin, 2006). In addition to this biogeographical comparison, a community comparison of the parasitoid assemblages of related native leafrollers in the western U.S. yielded similar results. In coastal California, Walker and Welter (2004) collected 227 larvae and pupae of *Argyrotaenia citrana* (Fern.) from apple orchards and recorded a total of 6 parasitoid species. From a survey in Washington state, LaGasa et al. (1999) reared 92 larvae and pupae of *Choristoneura rosaceana* (Harr.) recording 20 different parasitoid species. Another 4 year study in Washington state apple orchards showed that *Pandemis pyrusana* Kaerfott and *C. rosaceana* both supported a total of 6 different parasitoid species (Mullinix et al., 2011), whereas in British Columbia apple orchards these leafrollers supported 8 and

18 parasitoid species respectively (Vakenti et al., 2001). These biogeographical and community comparisons suggest that the parasitoid assemblage of LBAM in California is equally as rich as in its native range and as rich as the parasitoid assemblages from related native hosts in the western U.S., providing no evidence for apparent enemy release. For comparison, Cornell and Hawkins (1993) found that parasitoid species richness on exotic hosts was equal to or greater than that of the same species as native hosts in 28 out of 87 comparisons, which shows that a lack of apparent enemy release is not uncommon among exotic phytophagous insects. These types of comparisons should be made with caution, however, since the apparent species richness of a parasitoid assemblage is strongly dependent on sample size (Hawkins, 1994).

In our study, we found 5 idiobionts and 9 koinobionts in the parasitoid assemblage of LBAM in California, suggesting that only about a third (0.36) of the species were generalists. The corresponding parasitoid assemblage of LBAM in its native Australia consisted of 9 idiobionts and 10 koinobionts and thus a greater proportion (0.47) of generalist species (Paull and Austin, 2006). In contrast, however, for native leafrollers in the western U.S., Walker and Welter (2004) found that *A. citrana* was attacked exclusively by koinobionts and LaGasa et al. (1999) found the proportion of idiobionts in the parasitoid assemblage of *C. rosaceana* to be 0.15. While parasitoid species richness in invaded regions has been shown to reach levels similar to those in native regions within 10 years of introduction, Cornell and Hawkins (1993) estimated that the idiobiont to koinobiont ratio of an exotic host can take anywhere from 150 to 10,000 years to achieve the ratio represented in its native range. A potential factor contributing to the rapid accumulation of both parasitoid species richness and koinobiont species by LBAM in California could be the presence of many closely-related tortricids that act as a catalyst in the recruitment of more specialized parasitoids due to physiological and behavioral similarities between hosts (Harvey et al., 2012). Although the genus *Epiphyas* is restricted to Australia, California is home to numerous species in the tribe Archipini and a wealth of species within the subfamily Tortricinae (Powell, 1964) all of which show the same larval leafrolling behavior as LBAM and appear to be associated with a high representation of koinobiont parasitoids.

Our analysis of the influence of plant origin and temporal factors on variation in parasitism rates revealed that parasitoid species richness was higher on host plants of Australian origin compared with host plants from other origins (Fig 1). In general, little is known about the effect of plant origin on parasitoid species richness. However Engelkes and Mills (2013) found reduced performance of LBAM larvae on plants native to California compared with congeneric plants from other origins, some of which had been present in the native range of LBAM for at least four decades. If we assume that the Australian plants in the current study resulted in higher quality LBAM hosts than the plants from other origins, then parasitoid species richness would be correlated to host quality. One possible mechanism for such a linkage could be that parasitoids preferentially attacked the higher quality hosts on Australian plants. Alternatively, a shared history of LBAM with Australian plants could have led to a stronger herbivore-induced plant volatile response, and

consequently to differential attraction of parasitoid species between plants from different origins. Parasitoid species richness also revealed a significant, but slow decrease in parasitoid species richness between years over the course of the study (Fig. 1). Most of the rarer parasitoid species were late larval or pupal parasitoids that could have been competitively displaced by the earlier-attacking and numerically-dominant parasitoids, such as *E. eureka* and *M. ictericus*, as they became more efficient at using LBAM as a novel exotic host. We also found that parasitoid species richness was significantly lower in spring compared with summer and fall combined. As most native tortricid species in California are univoltine and active only in spring (Powell, 1964), LBAM may have become an important alternative host for multivoltine parasitoid species later in the season.

Furthermore, we conclude from our analysis of rarified estimates of parasitoid species richness, that increasing the number of plant species sampled would be expected to have a greater impact on observed parasitoid species richness than increasing sample size. This not only helps to identify the best sampling strategy for parasitoids of polyphagous insect hosts, but also confirms the hypothesis that polyphagy of phytophagous insects is often linked with a higher parasitoid species richness (Sheehan, 1994; Stireman and Singer, 2002), although an earlier study did not find such a relationship (Hawkins and Lawton, 1987).

The competition theory of Askew (1980) predicts an increase in parasitoid species richness with increasing size and architectural complexity of host plants, as the presence of more herbivores reduces competition among generalist parasitoids and permits greater species packing among parasitoid assemblages. However, we found no such relationship between parasitoid species richness (or parasitism rates) and plant size in our study. The absence of any relationship between parasitoid species richness and plant size in both this and other studies (Sheehan, 1991; Mills, 1993; Stireman and Singer, 2003) question the validity of competition theory in structuring parasitoid communities.

Parasitism rate

Parasitism rates for LBAM from our study in California averaged 34%. To obtain comparable estimates of parasitism from the life tables developed for LBAM in Australia by Danthanarayana (1983) we divided the number of individuals dying from parasitism in each life table by the number entering the second through fifth larval instar stage minus the number dying from removal or predation. The resulting parasitism rates from the two abandoned apple orchards in the Melbourne region ranged from 5 - 55% and from 8 – 58% per generation, with an overall average of 22 and 30%, respectively. Similarly, for a community based comparison of native leafrollers in the western U.S., parasitism rates averaged 33.4% (two most abundant parasitoid species only) for *A. citrana* in California (Walker and Welter, 2004), 26 and 31% for *P. pyrusana* and *C. rosaceana* respectively in Washington (Mullinix et al., 2011), and 28% for *C. rosaceana* and *P. pyrusana* combined in British Columbia (Cossentine et al., 2004). From this we conclude that LBAM in California

experiences slightly greater levels of parasitism than in its native region and similar levels of parasitism as native leafrollers in the western United States, and thus that there is no evidence of a realized enemy release for LBAM in California. For comparison, Cornell and Hawkins (1993) found that only 3 out of 52 exotic host species had the same or higher parasitism rates as in their native ranges, indicating that a lack of realized enemy release is much less common than a lack of apparent enemy release for exotic phytophagous insects.

Our analysis of the influence of plant origin and temporal factors on variation in parasitism rates revealed that parasitism was higher on host plants of Australian origin compared with host plants from other origins. While variation in parasitism on different plant species has been documented previously (Barbosa et al., 2001; Lill et al., 2002), to our knowledge, no such an effect on parasitism of a shared origin between an exotic herbivore and its host plants has been documented before. Based on the assumption that host quality of LBAM on plants of Australian origin would be higher (Engelkes and Mills, 2012), higher parasitism rates could have resulted from greater survivorship of parasitoids on hosts collected from Australian plants. Alternatively, the increased parasitism rates of LBAM on Australian plants could have been due to greater abundance of LBAM on plants of Australian origin, but as we did not estimate host densities at each of the sample sites this cannot be confirmed. We also found that parasitism rates were significantly lower in spring compared with summer and fall. While seasonal patterns of parasitism have been found in several other studies (Myers, 1981; DeLoach, 1983; West, 1985; Le Corff et al., 2000), in this case the pattern most likely reflects a dilution effect due to the availability of alternative native tortricid hosts which typically have only a single generation in spring (Powell, 1964).

There was also a significant relationship between parasitism rate and parasitoid species richness of the LBAM hosts collected in our study. Other studies of the linkage between natural enemy richness and biological control have either found similar positive relationships (Snyder and Tylianakis, 2012; Stireman and Singer, 2003; Tylianakis et al., 2006), found no relationship at all (Rodríguez and Hawkins, 2000; Straub and Snyder, 2006), or even found a negative relationship (Finke and Denno, 2004). The positive relationship found in our study suggests complementarity among the resident parasitoid species that have adopted LBAM as a suitable novel host and likely relates to the broad range of host life stages attacked and developmental strategies used by the individual species in this parasitoid assemblage.

Hyperparasitism

We recorded 6 hyperparasitoid species with an overall rate of hyperparasitism on field-collected primary parasitoid cocoons of 13.8%, while Paull and Austin (2006) listed 5 species as hyperparasitoids of LBAM in Australia. Rates of hyperparasitism in California varied significantly with plant origin, being higher on plants of Australian origin compared with plants from other origins, reflecting the same pattern observed for parasitism rates by

primary parasitoids. We also found that hyperparasitism of the two numerically-dominant larval endoparasitoids, *M. ictericus* and *E. eureka*, was inversely related to their abundance. Although *M. ictericus* was 10 times more abundant than *E. eureka* it was hyperparasitized 12 times less frequently. An important difference between these two primary parasitoids is that hosts of *M. ictericus* stay alive until well after parasitoid cocoon formation and spin an additional layer of silk webbing around themselves and the parasitoid cocoon. From observations made in other studies, both the added silk webbing (Tanaka and Ohsaki, 2006) and the presence of a live larva (Harvey et al., 2011) can deter hyperparasitism of primary parasitoid cocoons.

Conclusion

Cornell and Hawkins (1993) found that only 3 out of 52 phytophagous insect species showed both a lack of apparent and realized enemy release as exotics and all three were Lepidoptera, the phytophagous insect order that supports the highest levels of parasitoid species richness (Hawkins and Lawton, 1987). Our study provides evidence that LBAM adds a fourth species to this category. In general, however, the practice of biological control is based on the notion that populations of exotic invaders are not effectively suppressed by resident enemies, and we know of only one example of the successful biological control of an exotic pest by a native parasitoid species, the bayberry whitefly, *Parabemisia myricae* (Kuwana) in southern California (Rose and DeBach, 1992). Nonetheless, practitioners of biological weed control continue to avoid the use of phytophagous control agents from taxonomic groups that support rich parasitoid assemblages in an effort to ensure that they benefit from realized enemy release (Paynter et al., 2010). During its invasion of California LBAM has met considerable resistance rather than release from resident parasitoids and factors that might have facilitated this resistance include: 1) the occurrence of numerous native leafrollers that support a rich native parasitoid species pool in the invaded region (Powell, 1964), 2) specialization among leafroller parasitoids that is based on the host feeding niche rather than host species (Mills, 1992), and 3) overlapping host generations with all life stages present throughout the year allowing native parasitoids to avoid the limitations of temporal synchronization with suitable host life stages (Bürgi et al., 2011). Additionally, we found that plants from the same region of origin as LBAM further contributed to the lack of realized enemy release, although the generality of such a finding remains to be tested for a broader range of species.

4.6 TABLES

Table 1. The primary parasitoids of *E. postvittana* in California indicating life style (K: koinobiont, I: idiobiont), mode of parasitism (G: gregarious, S: solitary) relative frequency at each location, and representation from different host stages collected.

Order	Family	Species	Life style and mode of parasitism	Relative frequency (# collected) SF	Relative frequency (# collected) SC	Percent representation from host stages collected (4^{th}, 5^{th}, 6^{th}, pupa)
Hymenoptera	Braconidae	*Meteorus ictericus* Nees	K, S	69.4% (677)	73.3 % (633)	25.7%, 41.3%, 32.5%, 0%
Hymenoptera	Braconidae	*Hormius* sp.	I, G	0% (0)	0.1% (1)	0%, 0%, 0%, 0% (found only as cocoons)
Hymenoptera	Ichneumonidae	*Enytus eureka* (Ashmead)	K, S	25.5% (249)	5.1% (44)	93.8%, 0%, 6.3%, 0%
Hymenoptera	Ichneumonidae	*Glypta franciscana* Dasch	K, S	1.2% (12)	0.2% (1)	16.7%, 50%, 33.3%, 0%
Hymenoptera	Ichneumonidae	*Centeterus* sp.	I	0% (0)	0.2% (1)	0%, 0%, 0%, 100%
Hymenoptera	Ichneumonidae	*Coccygomimus hesperus* Townes	I, S	0.2% (2)	0.8% (7)	0%, 0%, 0%, 100%
Hymenoptera	Ichneumonidae	*Ischnus inquisitorius* (Mueller)	K, S	0.2% (2)	3.6% (31)	0%, 0%, 0%, 100%
Hymenoptera	Ichneumonidae	*Exochus nigripalpis* Thompson	K, S	0.6% (6)	0.6% (5)	0%, 0%, 16.7%, 83.3%
Hymenoptera	Ichneumonidae	*Itoplectis quadricingulata* (Provancher)	I, S			
Hymenoptera	Eulophidae	*Pediobius ni* Peck	I, G	0% (0)	10.5% (91)	0%, 0%, 0%, 100%
Diptera	Tachinidae	*Actia interrupta* Curran *Nemorilla pyste* (Walker) *Tachinid* sp1	K, S	0.6% (6)	4.2% (36)	0%, 23.1%, 61.5%, 15.4%

Table 2. The hyperparasitoids of *E. postvittana* in California indicating life style, relative frequency at each location and representation from different host stages collected.

Order	Family	Species	Relative frequency (# collected) SF	Relative frequency (# collected) SC	Percent representation from *Meteorus*, *Enytus*, Others
Hymenoptera	Ichneumonidae	*Itoplectis quadricingulata*	9.3% (9)	14.3% (2)	44.4%, 55.6%, 0%
Hymenoptera	Ichneumonidae	*Gelis* sp1	78.4% (76)	76.9% (10)	10.6%, 85.9%, 3.5%
Hymenoptera	Ichneumonidae	*Gelis* sp2	6.2% (6)	0% (0)	0%, 100%, 0%
Hymenoptera	Ichneumonidae	*Mesochorus sp.*	2.1% (2)	0% (0)	0%, 100%, 0%
Hymenoptera	Pteromalidae	Unidentified spp.	2.1% (2)	21.4% (3)	33.3%, 33.3%, 33.3%

4.7 FIGURES

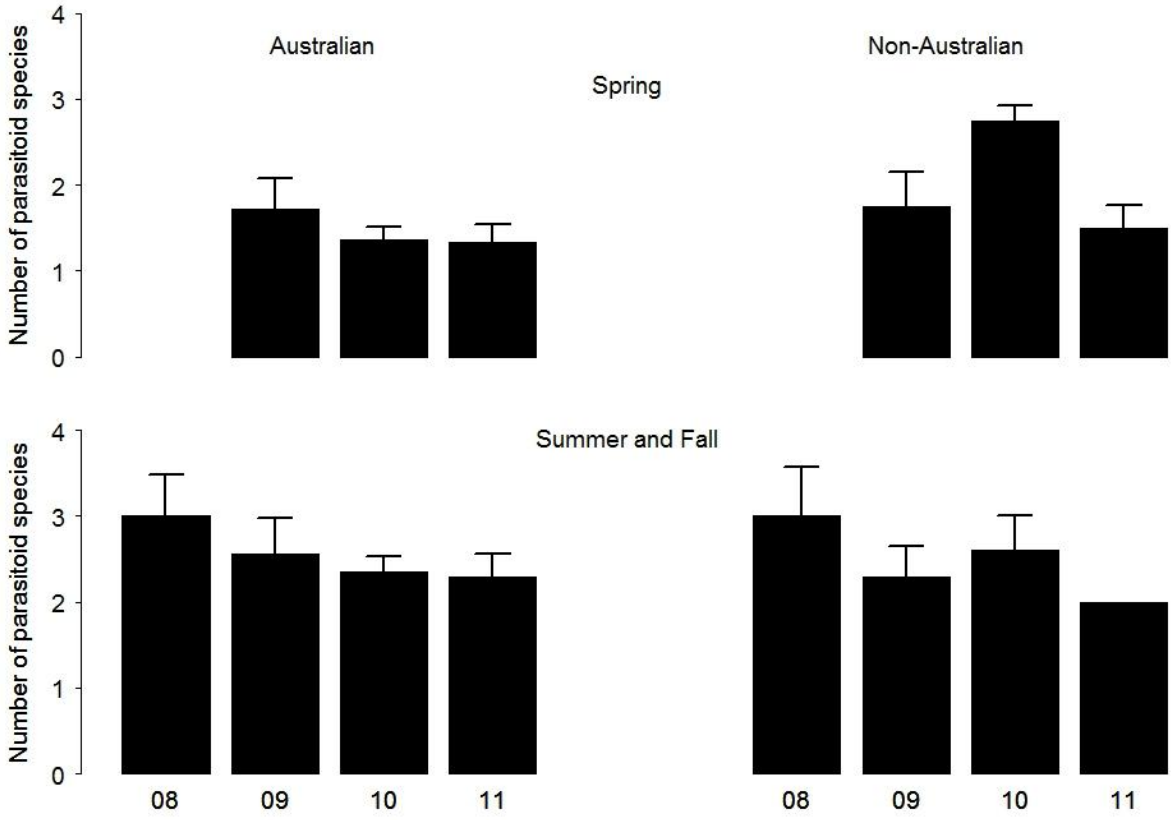

Figure 1. Mean parasitoid species richness (+SE) of *Epiphyas postvittana* in California showing significant differences for year, season and plant origin.

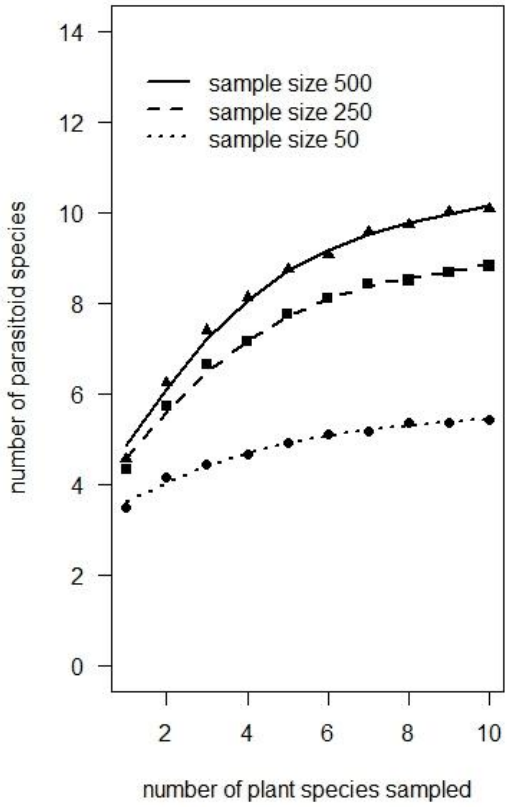

Figure 2. The predicted relationship between number of plant species sampled and number of parasitoid species encountered for different sample sizes of parasitoid individuals collected from *Epiphyas postvittana*.

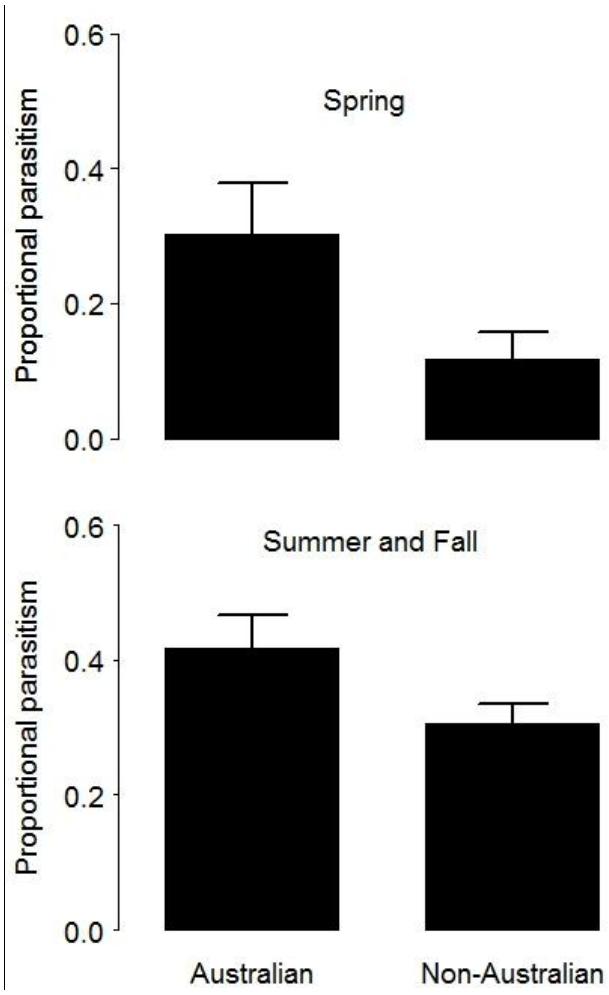

Figure 3. Mean parasitism rates (+SE) of *Epiphyas postvittana* in California showing significant differences for season and plant origin.

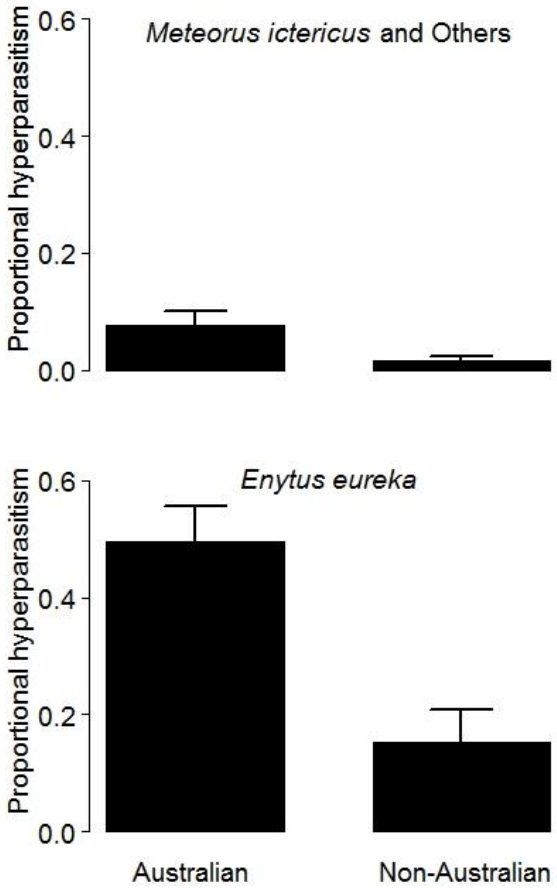

Figure 4. Mean proportional hyperparasitism (+SE) of field-collected cocoons of primary parasitoids of *Epiphyas postvittana* in California showing significant differences for primary parasitoid species and plant origin.

CHAPTER 5

ALLEE EFFECTS AND POPULATION REGULATION: A TEST FOR BIOTIC RESISTANCE AGAINST AN INVASIVE LEAFROLLER BY RESIDENT PARASITOIDS

5.1 ABSTRACT

Resident natural enemies can impact invasive species by causing Allee effects, leading to a reduction in establishment success of small founder populations, or by regulating or merely suppressing the abundance of established populations. The Light Brown Apple Moth (LBAM), *Epiphyas postivttana*, an invasive leafroller in California, has been found to be attacked by a large assemblage of resident parasitoids providing high rates of parasitism. Over a 4 year period, we measured the abundance and growth rates of four LBAM populations in California and determined parasitism rates. We found that at two of the sites, parasitism caused a component Allee effect, a reduction in individual survivorship at lower LBAM population densities, but that it did not translate into a demographic Allee effect, an impact on LBAM population growth rates at low densities. Instead, LBAM populations at all four sites exhibited strong negative density dependence. As we found no evidence for a negative relationship between LBAM population growth rates and parasitism rates, we concluded that resident parasitoids were unable to regulate LBAM populations in California. We did find, however, that parasitism rates were ocassionally high enough to suppress population growth rates below zero. We conclude that despite a lack of evidence for regulation or a demographic Allee effect, the impact of resident parasitoids on light brown apple moth populations is substantial and demonstrates significant biotic resistance against this new invader.

5.3 INTRODUCTION

With the increase of globalization, the introduction of exotic species into new regions has become a worldwide threat to biodiversity, ecosystem function and agricultural production (Mack et al., 2000; Ricciardi, 2007). Although the majority of introduced exotic species remain low in abundance and ecological or economic significance (Ricciardi and Cohen, 2007), some become invasive in the introduced region, exhibiting numerical dominance and a rapid rate of spread (Levine et al., 2004; Pysek & Richardson 2006). Numerous factors can influence the success of invasion, including propagule pressure, abiotic conditions, biotic traits of exotic species, and composition and structure of resident communities (Catford et al., 2009; Davis, 2009; Simberloff, 2009). Among these, two contrasting hypotheses are the enemy release hypothesis, which suggests that success can result from escape from natural enemies (Elton, 1958; Keane and Crawley, 2002; Liu and Stiling, 2006; Mitchell and Power, 2003), and the biotic resistance hypothesis, which suggests that predation from or competition with members of the recipient community can reduce the success of invasion (Elton, 1958; Maron and Vila, 2001; Carlsson et al., 2011; Dumont et al., 2011).

Enemy release and biotic resistance have been particularly well studied in the context of herbivory by vertebrates and insects on invasive plants (Colautti et al., 2004; Levine et al., 2004; Liu and Stiling, 2006; reviewed by Maron and Vilà, 2001), and of predation in aquatic and marine environments (Carlsson et al., 2011; Dumont et al., 2011; Torchin et al., 2003). In contrast, there have been few studies on invasive herbivores and their natural enemies. Moreover, biotic resistance and enemy release are commonly inferred from measurements of natural enemy richness and impact, i.e. numbers of natural enemy species and the mortality or damage they inflict on invasive populations (e.g., Mitchell et al., 2003; Torchin et al., 2003, Carlsson et al., 2011; reviewed by Levine et al., 2004). Yet, a higher richness of natural enemies does not necessarily translate to greater impact, and the net effect of mortality on population growth rates can vary significantly depending on life history strategy of the invader (Maron and Vilà, 2001) and how mortality at a particular life stage influences population growth (McEvoy and Coombs, 1999; Mills, 2005; Raghu et al., 2006). Studies of natural enemy impacts on invaders need a greater focus on population-level effects rather than individual responses (Halpern and Underwood, 2006), and only recently have matrix population models been used to integrate individual responses into population growth rates (Schutzenhofer et al., 2009). These population level studies provide the most unequivocal evidence for or against either the enemy release or biotic resistance hypothesis. However, such detailed or long-term studies are scarce and thus enemy release or resistance remain controversial hypotheses with short-term observational and experimental studies that both support and refute the importance of predation as a biotic interaction that influences the success of invasion.

For a natural enemy to play a decisive role in regulating the population growth rate of an invasive species, it must be responsible for inducing a negative density dependent effect

on the per capita growth rate of the invasive host population (Hassell, 2000; Murdoch et al., 2003; Seitz et al., 2001). In contrast, if smaller invasive populations suffer higher proportional mortality from natural enemies than larger populations, this can generate positive density dependence in which case the top-down effect is destabilizing rather than regulating. This decrease in fitness at lower population densities is known as an Allee effect (Allee, 1931; Courchamp et al., 2008; Kramer et al., 2009; Liebhold and Bascompte, 2003). Interest in Allee effects has surged in the last decade as a key aspect of invasion biology that can potentially limit the establishment or facilitate the eradication of new exotic species (Taylor and Hastings, 2005; Tobin et al., 2011). Allee effects can either be represented by reductions in individual fitness (component Allee effect), such as survival or mate finding, or in some cases by reductions in per capita population growth rate (demographic Allee effect, Stephens et al., 1999). In theory, demographic Allee effects have the potential to limit the establishment success of invaders (Drake and Lodge, 2006) or to reduce the risk and rate of spread of invaders (Lewis and Kareiva, 1993). In the absence of positive or negative density dependence, however, natural enemy-induced mortality can still play an important role in the dynamics of invasive species populations by suppressing its abundance to levels at which other factors can act to prevent population increases.

In the present study, over a four year period, we monitored the population size, parasitoid species richness and parasitism rates of Light Brown Apple Moth (LBAM), *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), as an invasive species in California. LBAM is a tortricid leafroller native to South Eastern Australia that was first discovered in California in 2006. Larvae of this species are highly polyphagous and feed on 545 known plant species in 363 genera and 121 families (Brockerhoff et al., 2011). In Chapter 4 we documented the parasitoid assemblage of LBAM in California and found that it did not differ from the assemblage in its native range with respect to richness, level of specialization, and parasitism rates. Thus, for LBAM in California, in the absence of any evidence for enemy release, there is the possibility for significant biotic resistance from the resident parasitoid community. Our specific objectives in this study were therefore to address the following three questions regarding LBAM populations in California: 1) does parasitism cause a component or demographic Allee effect? 2) is there any evidence of density dependence affecting population growth rates? 4) does parasitism influence population growth rates or suppress population growth?

5.4 MATERIALS AND METHODS

Sampling sites

LBAM populations were monitored at two sites in Golden Gate Park in San Francisco (37° 45' 59.12" N, 122° 29' 14.19" W referred to as SF1, and 37° 45' 54.31" N, 122° 30' 10.66" W referred to as SF2) and two urban sites in Santa Cruz (36° 57' 21.28" N, 122° 2' 15.39" W referred to as SC1, and 36° 58' 49.05" N, 121° 54' 32.11" W referred to as SC2). At

the SF1 site, LBAM was sampled on Australian tea tree (*Leptospermum laevigatum*) which had been planted only a few years prior to the beginning of the study in 2008, ranged from 1-2m in height, and were irrigated regularly in summer. At the SF2 site, a population was sampled on Australian tea tree plants that were much older (50+ years) with heights of up to 3.5m. LBAM was sampled from small groups (patch diameters ranging from 2-4m) of ornamental manzanita plants (*Arctostaphylos densiflora*) up to 0.5m high at the SC1 site, which was located on a mulched road median in a suburban neighborhood. Manzanita was also sampled at the SC2 site where plants were 1.5m high and formed continuous 20m long hedges surrounding large parking lots. These sites were selected for regular sampling because, as noted by Geier and Briese (1980), in Australia extensive and virtually continuous infestations of LBAM are observed only in very specialized habitats such as suburban gardens and horticultural crops. These sites were thought to have had some of the longest established populations of LBAM in California based on initial numbers of adults collected in a pheromone-based trapping survey (USDA-APHIS, 2012). The host plant species selected in each of the two locations were those that most consistently supported LBAM populations of sufficient abundance to allow destructive sampling. While LBAM was present on other host plant species in both locations, populations occurred only seasonally or were too small to allow effective sampling.

LBAM sampling and population growth rate

From May 2008 until June 2010, sites were visited at 2-wk intervals from April to October and at 4-wk intervals from November to March. From July 2010 until July 2012 sites were visited three (SF) and four (SC) times per year. These periods had been identified as having the highest proportional representation of 4-6th instar larvae in the age structure of LBAM populations that have three (SF) and four (SC) overlapping generations throughout the year in California (Bürgi et al., 2011). As no larval parasitoids of tortricid hosts complete their development in the earlier instars (Mills, 1994), sampling during times with highest proportional representation of 4th to 6th instar larvae allowed us to best estimate parasitism levels in the field. The timing of the sample periods varied slightly between years, with dates at SC sites being February 1-8 (early spring), May 10-17 (late spring), July 23 – August 19 (summer), September 23-28 (fall) and at SF sites March 29 – April 9 (spring), July 12 – August 10 (summer), October 5-14 (fall). Sampling at site SC1 was terminated in August 2011 when plants were removed, and did not start until January 2009 at site SC2 as it was a replacement for a previously sampled site where the LBAM population proved to be too low to be adequately monitored.

To sample LBAM populations, 5-min counts of visible leafrolls were conducted on each of 22 Australian tea tree plants at SF1 and SF2, and on 22 manzanita plants at SC1 and 15 manzanita plants at SC2. At SF1, initially the number of leafrolls on whole plants were counted, requiring approximately 7 min to complete, but the sampling was switched to

timed 5-min counts on 10 April 2009. In addition, the number of plants sampled at SC1 was reduced to 16 after a severe pruning event in October 2008.

At all four sites, 50 leafrolls were collected from additional non-sample plants on each sampling occasion. At SC1 we observed an obvious initial gradient in larval population densities and therefore collected 100 leafrolls from different plants throughout the sampling area. Leafrolls were brought back to the laboratory and carefully opened to determine occupancy by larvae and pupae. Proportional occupancy was determined as the number of individuals per leafroll (from 50 leafrolls, or 100 for SC1), which could exceed 1 at times because of double or triple occupancy of leafrolls. Larvae were reared to adults on a bean-based diet (Cunningham 2007) in 96ml plastic cups (Solo Cup Company, Highland Park, IL) under constant conditions at 21 °C, 70-85% RH and a 16:8 h L:D photoperiod. In July 2011, we changed the diet used to rear the LBAM larvae to *Pectinophora gossypiella* (Saunders) diet provided by the USDA - ARS Western Cotton Research Laboratory mass-rearing facilities located in Phoenix, Arizona (Bartlett and Wolf, 1985). The timed leafroll counts were then multiplied by the proportional occupancy and divided by 5 or 7 min to get a standardized LBAM count per minute as a measure of population density for each plant sampled at all sites.

For each sampling site, population growth rate on sampling date t was calculated as $\ln[N(t+650dd)/N(t)]$ (with N being the mean number of LBAM per minute), since one LBAM generation from egg to adult takes 650 degree days (dd), as found by Gutierrez et al. (2010) in a re-analysis of data from a laboratory study in Australia (Danthanarayana, 1975). When time (t+650dd) did not coincide with an actual sampling date, but rather fell between sampling dates, population abundance was estimated by linear interpolation using the two bracketing sampling dates.

Parasitoid species and parasitism

Cups with LBAM larvae were checked weekly for LBAM pupae and parasitoid cocoons. Pupae were removed from cups and kept in 33ml plastic vials in groups of up to 5 pupae. After emergence the identity of LBAM adults was checked using Gilligan and Epstein (2009). Parasitoid cocoons were kept separately in 38ml glass vials to await adult emergence. Adult parasitoids were identified to genus before obtaining species determinations from a series of taxonomic specialists. Parasitism rates (proportions) for primary parasitoids were estimated from the number of parasitized hosts divided by the number of host individuals collected on each occasion at each sampling site. Dividing the number of exiting parasitoids by the number of host individuals collected provides a conservative estimate of parasitism as there was consistent mortality (~30%) during laboratory rearing of host larvae that could have differentially affected parasitized individuals. As all hyperparasitoids were found to be solitary species, hyperparasitism rates (proportion) were estimated as the total number of hyperparasitoid individuals emerged divided by the total number of primary parasitoid cocoons collected. Frequency of

parasitism by a parasitoid species at each sampling site was estimated as the number of host individuals parasitized by a particular parasitoid species divided by the total number of hosts parasitized.

Data analysis

All statistical analyses were conducted using R statistical software (R Development Core Team, version 2.15.0, 2012). To assess whether parasitism showed evidence of a component Allee effect on LBAM survivorship, we tested for a positive relationship between survivorship from parasitism and LBAM population density separately for each sampling site, using a generalized additive mixed model (GAMM, package "mgcv") with binomial error distribution, logit link and non-parametric smooth terms, which allow for a non-linear relationship between the response and explanatory variables. Temporal pseudoreplication through repeated sampling was accounted for by including an exponentially decreasing correlation between replicates. Temporal distance between samples was determined by the number of degree days between them. Since GAMM models cannot produce log likelihood estimates, parameter significance is presented as values for non-significant and significant parameters obtained from the full and reduced models respectively.

As an assessment of evidence for demographic Allee effects and regulation of LBAM populations we examined the relationship between population growth rate and population density separately for each sampling site. A positive relationship at low densities would suggest a demographic Allee effect, while a negative relationship at higher densities would indicate regulation by density dependence. To assess whether parasitism rates influenced LBAM population growth rates, we tested for a negative relationship between population growth rate and parasitism rate. The effects of both parasitism and population density on LBAM population growth rates were tested simultaneously as explanatory variables in a single GAMM model with Gaussian error distribution and identity link.

To examine whether parasitism was sufficient to suppress population growth rates of LBAM at each of the sampling sites, observed parasitism rates were compared to those estimated to be necessary to reduce population growth rates of LBAM to zero from a stage-structured matrix model developed for LBAM by Mills (2008). Depending on their preferred host stages parasitized, parasitoids which contributed 5% or more to the frequency of parasitism were each assigned to one of the host life stage categories (L1, L2-L5, L6 and pupa) used for life table studies by Danthanarayana (1983) and for the analysis of life cycle vulnerability to parasitism by Mills (2008). Information on which life stage category a parasitoid species parasitized came from previous rearing observations (Chapter 4) and from the parasitoid literature (Mills, 1994, 1993). Parasitism rates observed for each of the life stage categories at each sample date were then compared directly with the rates estimated to be necessary to reduce population growth rates to zero.

5.5 RESULTS

LBAM densities, parasitoid species and parasitism

For the period from May 2008 to July 2012 a total of 6222 LBAM individuals were collected and 994 parasitoids reared from the four sites sampled. Maximum average LBAM densities per sampling date ranged from 111 LBAM counts per minute at SF1, to 60 and 61 at SC1 and SC2 and 16 at SF2 (Fig. 1). The total number of primary parasitoid species recorded was 16, and of hyperparasitoid species was six. The overall mean proportion of hosts parasitized was highest for the SF2 site at 0.35, lowest for the SC1 site at 0.03 and intermediate for SF1 (0.32) and SC2 (0.22). The dominant parasitoid in this study was *Meteorus ictericus*, accounting for 51% of total parasitism for all four sites combined, while the second most abundant parasitoid was *Enytus eureka*, which contributed 34% to the total parasitism. Rates of hyperparasitism were 0.31 (SF1), 0.23 (SF2), 0.00 (SC1) and 0.13 (SC2). Hyperparasitoid species were *Gelis* sp1, *Gelis* sp2, *Scambus* sp., *Mesochorus* sp. and unidentified spp, with *Gelis* sp1 being the most dominant hyperparasitoid accounting for 67% of the total hyperparasitism. Hyperparasitism of *M. ictericus* was 0.16 for all sites combined compared to 0.77 for *E. eureka* and 0.07 on other parasitoid species.

Component and demographic Allee effects

The relationship between survivorship from parasitism and LBAM population density at each sampling site was used to test for component Allee effects. These relationships were non-significant at the sites SF2 (edf = 1, df = 44, t = 0.62, P = 0.54) and SC1 (edf = 1, df = 46, t = 0.23, P = 0.82), but were significantly positive at the sites SF1 (edf = 1, df = 48, t = 2.04, P = 0.047) and SC2 (edf = 1, df = 37, t = 3.13, P = 0.003) (Fig. 2), with the latter thus being consistent with a component Allee effect.

To determine whether the component Allee effects for survivorship from parasitism would translate into demographic Allee effects, we tested for demographic Allee effects by examining the relationship between population growth rate and population density. There was a strong nonlinear decline in LBAM population growth rate with increasing population density that was highly significant at all four sampling sites (Fig 3, Table 2). The absence of a positive relationship at the lowest population densities confirmed the absence of a demographic Allee effect, while the negative relationship with increasing population density indicated strong density dependent regulation. At all sampling sites population growth rates for LBAM became negative at modest population densities and were estimated to be zero, representing the carrying capacity, at mean densities of 11.3, 6.8, 18.2 and 13.2 occupied leafrolls per minute for the sites SF1, SF2, SC1 and SC2 respectively.

Regulation or suppression of LBAM population growth by parasitism

To determine if the observed negative density dependent regulation could have been caused by parasitism, we examined the effect of parasitism on population growth rates at each sampling site. None of these relationships proved to be significant at any of the four sites (Fig. 4, Table 2), suggesting that LBAM population growth rates were not influenced by parasitism.

To further examine whether parasitism was ever sufficient to suppress LBAM growth rates to zero, parasitism rates at the different sites and sampling dates were compared with values estimated to be necessary to suppress population growth rates to zero. At SF1 and SF2 only one life stage category (L2-L5) of LBAM was occupied by parasitoid species that individually contributed more than 5% to the frequency of parasitism, while at SC1 and SC2 parasitoids were represented in two life stage categories (L2-L5 and pupa for SC1; L2-L5 and L6 for SC2). For the L2-L5 life stage category, the parasitism rate necessary to reduce the population growth rate of LBAM to zero was estimated to be 0.47 (Mills, 2008), a rate that was reached on 10 out of 50 sampling dates for SF2, on 8 out of 50 for SF1, 3 out of 39 for SC2, and 0 out of 48 for SC1 (Fig 1). Parasitism rates for pupae at SC1 and for L6 at SC2 were lower than those for L2-L5 at those sites (Table 1) and never reached the thresholds necessary to reduce population growth rates to zero.

5.6 DISCUSSION

From our study, we found that LBAM in California exhibited a parasitism-induced component Allee effect at two of the four sampling sites, which however, did not translate into a demographic Allee effect. Nonetheless, we found evidence for strong negative density dependence among LBAM populations at all four sampling sites, which could not have been caused by parasitoids, as parasitism did not have any influence on LBAM population growth rates. In addition, we found that parasitism rates from the species-rich parasitoid assemblage were high, confirming a lack of enemy release and indicating substantial biotic resistance, and that these parasitism rates occasionally reach levels estimated to be necessary for the reduction of population growth rates to zero (Mills, 2008).

The relationship between survivorship from parasitism and population density was not significant for two of the sites, but positive for the other two sites, suggesting that at least some populations of LBAM in California experience a decrease in individual fitness at lower densities representing a component Allee effect (Stephens et al., 1999). In the context of the different steps in the invasion process, if a component Allee effect from parasitism also becomes a demographic Allee effect, it has the potential to impact small founder populations, preventing them from becoming established (Drake and Lodge, 2006; Liebhold and Tobin, 2008). However, while it is fairly common for predators to induce component Allee effects (Angulo et al., 2007; Gascoigne and Lipcius, 2004; Kramer and Drake, 2010;

Kramer et al., 2009), they only occasionally translate into demographic Allee effects (Angulo et al., 2007) due to the complex interaction of density dependent and density independent factors acting on individual fitness (Gascoigne and Lipcius, 2004). Similarly, for LBAM in California the component Allee effect from parasitism did not translate into a demographic Allee effect, as population growth rates did not show any evidence of an increase at low population densities. In general, empirical evidence for demographic Allee effects, aside from some noteworthy examples (Davis et al., 2004; Johnson et al., 2006), is sparse (Gregory et al., 2010; Sibly et al., 2005) as founder populations experiencing an Allee effect are likely to decline or increase rapidly and experience large temporal variation in abundance (Stephens et al., 1999).

Surprisingly, we found that LBAM populations in California were influenced by negative density dependent growth rates at all four sampling sites, suggesting strong regulation. Since parasitism did not seem to have any influence on LBAM population growth rates, other factors, such as generalist predators (Berryman, 2002), food limitation or crowding (Rotem and Agrawal, 2003; Snell et al., 2001), or induced plant defenses (Karban and Baldwin, 1997; Kessler et al., 2012; Underwood, 2000), must have been responsible for the density dependent regulation. While generalist predators such as earwigs, spiders, and beetles are known to impact LBAM survival in its native range (Danthanarayana, 1983; Geier and Briese, 1980; MacLellan, 1973) and have been observed in its introduced range (Hogg et al., 2012), they are usually unable to generate strong density dependence due to a lack of aggregative or numerical responses to prey densities (Gascoigne and Lipcius, 2004; Symondson 2002), unless the prey species constitutes a major component of the total available prey (Symondson et al., 2002). Egg parasitism of LBAM by *Trichogramma fasciatum* and *T. platneri* was found to average 51 and 47% from March-November 2009 and 2010 at the SC1 site (Roltsch, 2010), and has been recorded to be as high as 84% in late summer (Wang et al., 2012). As 66% parasitism was estimated to be necessary at the egg stage to reduce LBAM population growth rates to zero (Mills, 2008), the potential for density dependence from egg parasitism deserves greater attention in the future. However, egg parasitism is typically rather lower earlier in the season, and more importantly, Roltsch et al. (2009, 2010) found that LBAM eggs on *L. laevigatum*, the plant monitored at the two SF sampling sites in our study, were never parasitized. We can therefore exclude egg parasitism as the main driver of the negative density dependence, at least for the two SF sites. Another factor often found to contribute to negative density dependence is crowding or food limitation (Rotem and Agrawal, 2003; Snell et al., 2001). However, in our study the density dependence was strongest at the lowest densities (less than 20 individuals per minute per plant). At these lowest densities, however, no obvious crowding or food limitation was observed in the field. This suggests that the most likely explanation for the negative density dependent growth rate at such low densities is induced plant defense, which has been shown to influence herbivore population growth rates both theoretically (Edelstein-Keshet and Rausher, 1989; Underwood, 1999; Abbott et al., 2008) and experimentally (Underwood and Rausher, 2002; Underwood, 2010). Similarly, Kaplan and Denno (2007) highlighted the importance of

indirect effects via induced defenses rather than direct reduction of resources via defoliation in mediating interspecific competition among insect herbivores. Induced resistance has been shown experimentally to lower plant quality with increasing herbivore density, leading to a reduction in population growth rates through increased development time (Underwood, 2010) and theoretically could reduce survivorship with increasing herbivore density (Abbott et al., 2008). Although none of these potential explanations were measured during our study of LBAM populations in California, induced plant defense and egg parasitism appear to be the most likely candidates and would benefit from further study.

While parasitism by resident parasitoids showed no evidence of being able to regulate LBAM populations in California, parasitism rates did occasionally reach high enough levels to be able to reduce population growth rates to zero (Mills, 2008). Parasitism by resident parasitoids was not only concentrated among L2-L5 larvae, the life stage at which the addition of mortality from parasitism has the greatest potential to reduce population growth rates to zero (Mills, 2008), but was also dominated by the two most frequent species, *M. ictericus* and *E. eureka*, that have been particularly effective in colonizing LBAM as an exotic herbivore in California (Chapter 4). For comparison, parasitism of LBAM at two sites in its native Australia reached 47% only once and twice respectively, as determined from parasitism rates estimated in Chapter 4 from the life table studies of Danthanarayana (1983). With high parasitism rates in both its native range and California, LBAM populations experience top down control that undoubtedly contributes to the suppression of their abundance.

We conclude that despite the lack of enemy release for LBAM in California, the biotic resistance provided by resident larval and pupal parasitoids did not exhibit the characteristics needed to regulate LBAM populations, although the levels of parasitism achieved were sufficient to reduce population growth rates to zero or to become negative at some locations in spring or late summer. To what extent biotic resistance from resident natural enemies can either prevent the successful establishment of invasive insect herbivores or limit their spread and impact as established invaders remains poorly known. Additional studies that focus on population level effects of biotic resistance in invasive populations will be needed to better understand the significance of such effects on the success or failure of exotic invasions. LBAM in California represents an unusual case of an exotic insect herbivore that has met far greater resistance from resident parasitoids than is typical for accidentally introduced insect herbivores. While this resistance appeared not to regulate LBAM abundance or prevent its successful establishment, it deserves to be investigated further along with egg parasitism and induced plant defense as factors potentially limiting the invasiveness of LBAM in North America.

5.7 TABLES

Table 1. Frequency of parasitism by the resident parasitoids of *Epiphyas postvittana* in California assigned to host life stage categories used for life table studies by Danthanarayana (1985) and for the analysis of life cycle vulnerability to parasitism by Mills (2008).

	SF1	SF2	SC1	SC2
L1				
L2-5	*Meteorus ictericus* (43.8%) *Enytus eureka* (49.7%)	*M. ictericus* (50.6%) *E. eureka* (43.7%)	*M. ictericus* (51.0%) *E. eureka* (13.7%)	*M. ictericus* (62.3%) *G. franciscana* (8.9%)
L6				*Hormius* sp. (12.6%) *Ischnus inquisitorius* (5.8%)
Pupa			*Pediobius ni* (11.7%) *C. hesperus* (7.8%)	
less than 5%	*Campoplex* sp. *Centeterus* sp. *Coccygomimus hesperus* *Exochus nigripalpus* *Glypta franciscana* *Hormius* sp. *Itoplectis quadricingulata* Unidentified tachinid sp.	*Apanteles* sp. *Campoplex* sp. *Centeterus* sp. *G. franciscana* *Hormius* sp.	*Brachymeria ovata* *Hormius* sp. *Nemorilla pyste* *Actia interrupta* (8.8%) Unidentified tachinid sp	*C. hesperus* *E. eureka* *E. nigripalpus* *P. ni*

Table 2. GAMM models for the relationship between population growth rates of *Epiphyas postvittana* at two sites in both San Francisco (SF) and Santa Cruz (SC), California and mean population density and parasitism rate, with estimated degrees of freedom (edf) for the smooth terms, degrees of freedom (df) and parameter significance.

Site	Predictor	edf	df	t	P
SF1	Density	7.52	46	7.52	< 0.001
	Parasitism	1.00	45	0.25	0.81
SF2	Density	2.23	44	5.24	< 0.001
	Parasitism	1.00	43	0.51	0.61
SC1	Density	3.15	45	3.47	0.001
	Parasitism	1.00	44	0.72	0.48
SC2	Density	3.77	36	3.69	< 0.001
	Parasitism	1.00	35	0.79	0.43

5.8 FIGURES

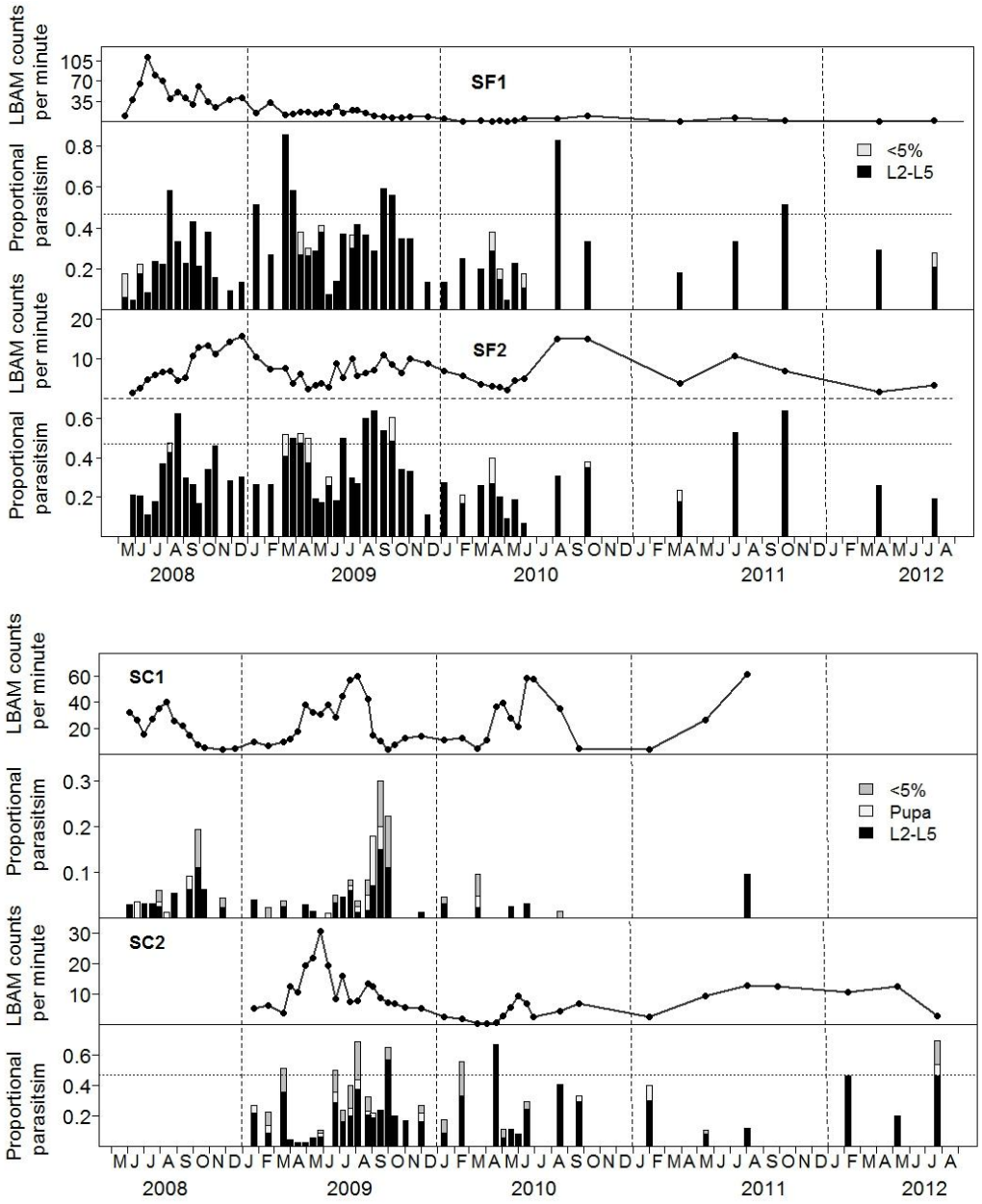

Figure 1. Population density of *Epiphyas postvittana* at two sites in both San Francisco (SF) and Santa Cruz (SC), California, represented by mean number of occupied leafrolls per minute per plant (upper line graph), and parasitism rates, represented either by host life stage category or for all parasitoid species contributing <5% to the total frequency of parasitism at each site (lower bar graph) from 2008 to 2012. The dotted horizontal line at a parasitism rate of 0.47 indicates the estimated level of parasitism of the L2-L5 larvae necessary to reduce population growth rates of *E. postvittana* to zero (Mills 2008).

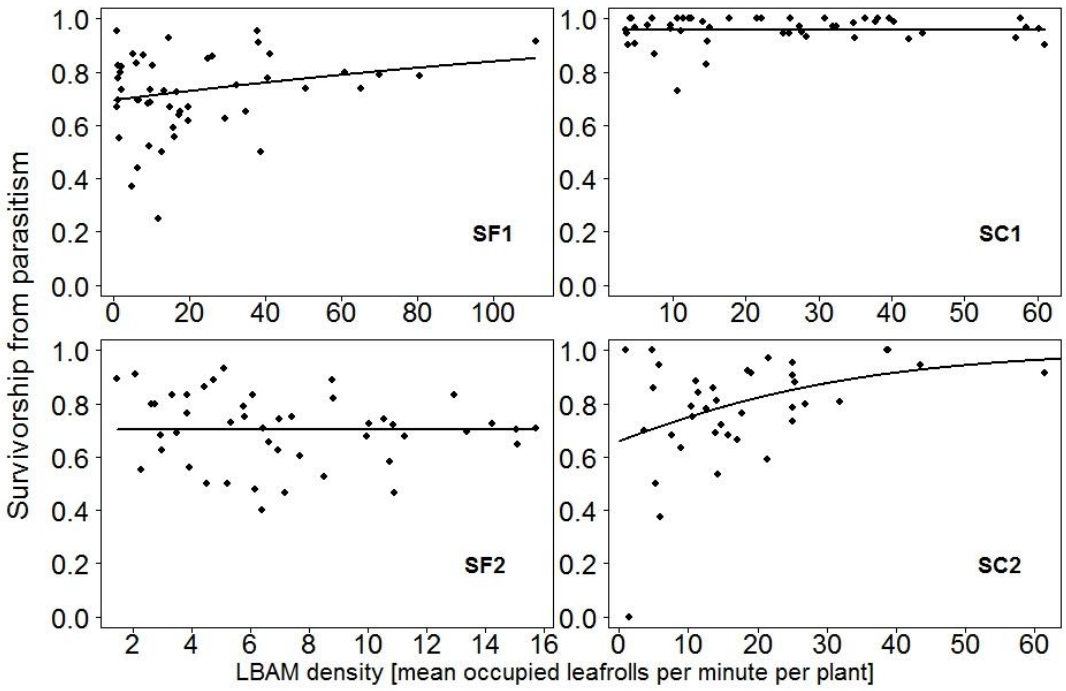

Figure 2. Relationship between survivorship from parasitism and population density of Epiphyas postvittana at two sites in both San Francisco (SF) and Santa Cruz (SC), California.

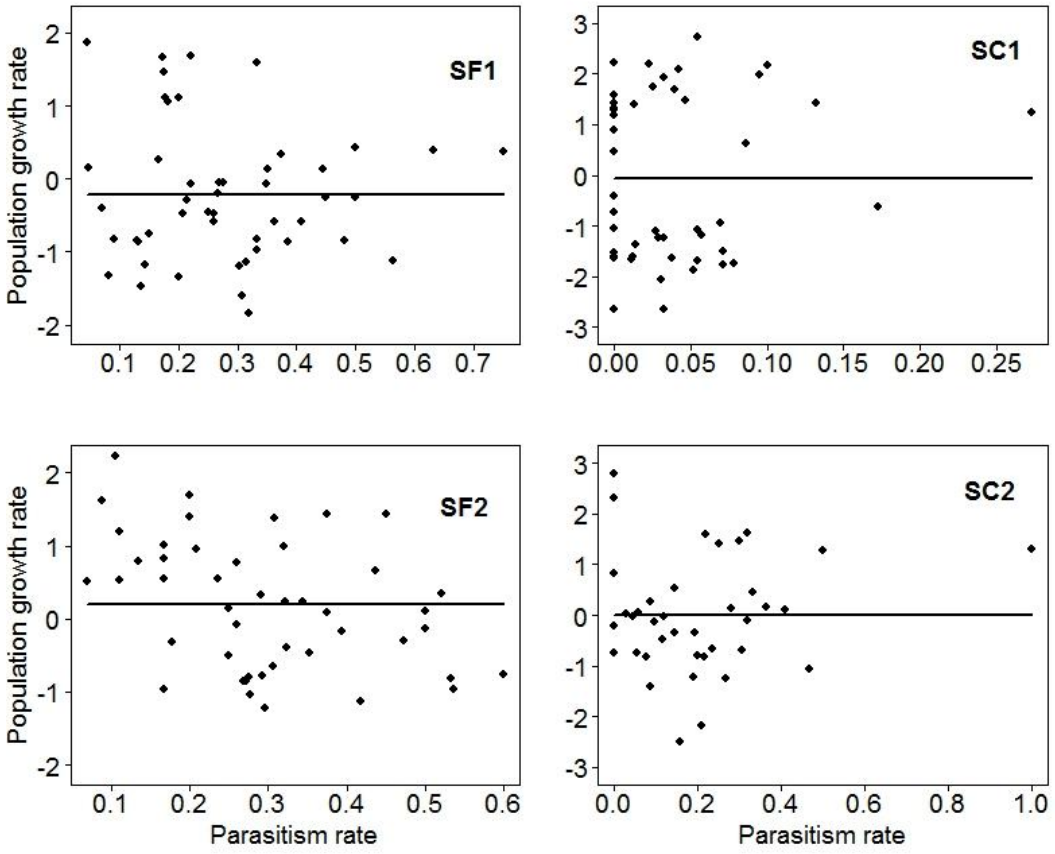

Figure 3. Relationship between population growth rates of Epiphyas postvittana (ln(Nt+650dd/Nt) at two sites in both San Francisco (SF) and Santa Cruz (SC), California and parasitism rates (see Table 3 for further details).

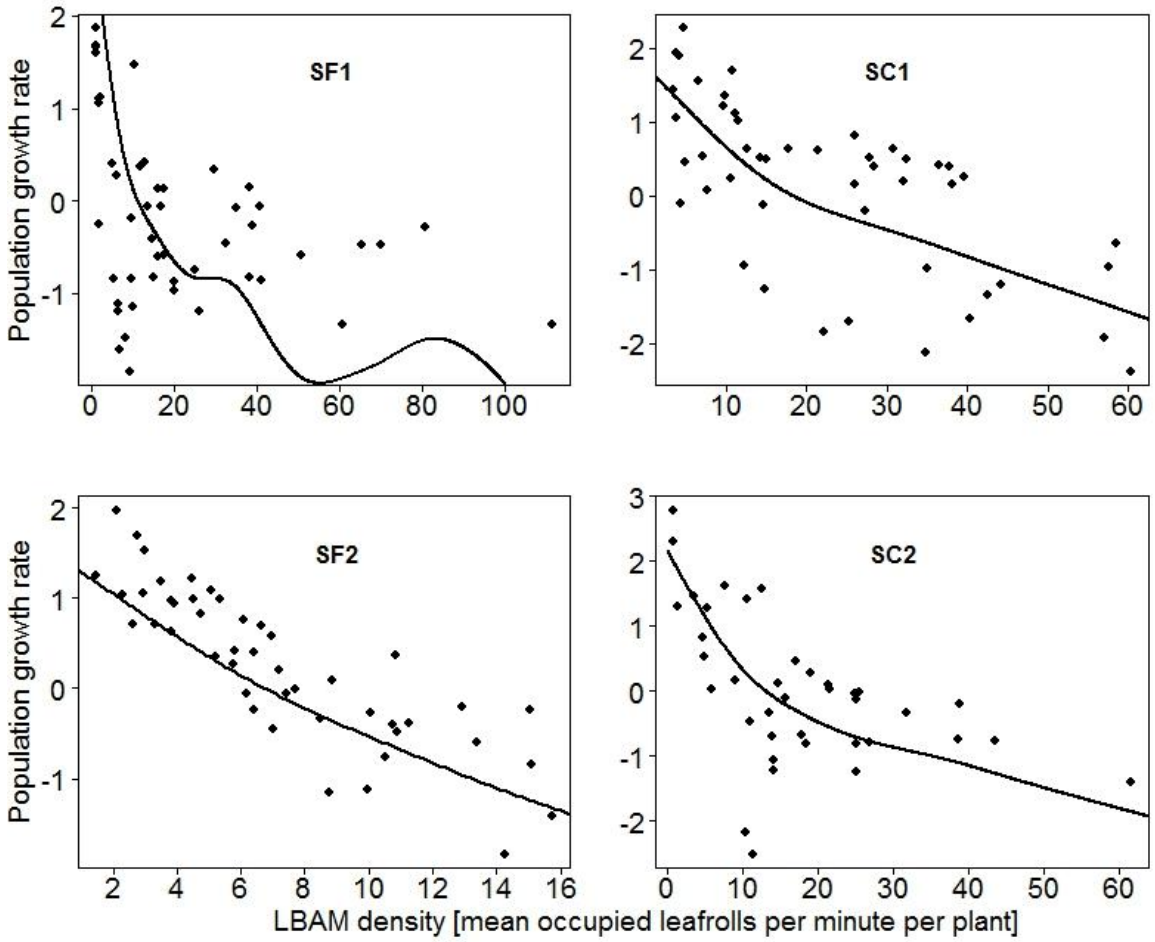

Figure 4. Relationship between population growth rates of *Epiphyas postvittana* ($\ln(N_{t+650dd}/N_t)$ at two sites in both San Francisco (SF) and Santa Cruz (SC), California and mean population density (N_t).

CHAPTER 6

DEVELOPMENTAL STRATEGY AND LIFE HISTORY TRAITS OF *METEORUS ICTERICUS*, PARASITOID OF THE LIGHT BROWN APPLE MOTH IN CALIFORNIA

6.1 ABSTRACT

The solitary endoparasitoid *Meteorus ictericus* has recently been found to be a dominant parasitoid of the invasive Light Brown Apple Moth (*Epiphyas postvittana*) in California. To better understand its current success and to evaluate its further potential as biological control agent, life history traits of this uniparental population of *M. ictericus* were studied. While choice preference experiments revealed a bias toward the attack of 6th instar hosts due to their greater mobility, no-choice tests showed that parasitism was equal on 4th through 6th instar hosts and only marginally lower on 3rd instar hosts. On smaller hosts, development time of the parasitoid was extended such that adult size was unaffected by host size at the time of oviposition. The fit of the Brière 1 model to temperature-dependent development time yielded an upper and lower development threshold of 5.94 and 30.06 °C. No relationship was found between parasitoid adult size and longevity. Lifetime fecundity was maximal at 22.7 °C with 33 cocoons produced, and showed a significant positive relationship with adult size. Lifetime fecundity increased when host larvae were renewed more frequently suggesting avoidance of the costs associated with self superparasitism. We discuss these findings in the context of the developmental and life-history strategies of hymenopteran parasitoids, and the potential of *M. ictericus* for biological control of *E. postvittana* in California.

6.2 INTRODUCTION

Meteorus ictericus (Nees) (Hymenoptera: Braconidae) is a solitary endoparasitoid of larval hosts in the family Tortricidae, with an occasional rearing from other lepidopteran species (Huddleston, 1983; Yu et al., 2005). Reports of this species predominantly stem from Europe, where it has been found as a minor parasitoid of several agricultural pest species such as *Syndemis musculana* (Hübn.) (Blommers et al., 1988), *Adoxophyes orana* F.v.R., *Archips rosana* (L.) and *Spilonota ocellana* (D. & S.) (Kienzle et al., 1997; Pluciennik and Olszak, 2010) and has been reared from forest pests such as *Ptycholomoides aeriferanus* (H. -S.) (Mills, 1993b). Despite its relative ubiquity in Europe, it has never been reported as the dominant parasitoid or as a significant biological control agent. Some less frequent reports come from Japan (Minamikawa, 1954; Maleque et al., 2010, Maeto, 1990) and Australia, where it is believed to have been introduced from Europe (Huddleston, 1983). In the U.S., *M. ictericus* has only been reported once, as a parasitoid of the eye-spotted bud moth (*S. ocellana*) in California, and parasitism levels were low (2.3%) but consisted almost exclusively (90%) of this species (Madsen and Borden, 1949). However, more recently, *M. ictericus* has been reported as the dominant parasitoid of the invasive Light Brown Apple Moth (LBAM), *Epiphyas postvittana* Walk. (Lepidoptera: Tortricidae) in California (Chapter 4; Wang et al., 2012).

LBAM is a highly polyphagous leafroller, native to southeastern Australia, and discovered in California in 2006 (Suckling and Brockerhoff, 2010). Extensive sampling (Chapter 4) in locations believed to be the original sites of LBAM establishment, and by Wang et al. (2012) in areas in which LBAM has more recently expanded its range showed that of the 16 parasitoids reared from this host, *M. ictericus* was the most consistent and abundant species. From these two studies *M. ictericus* represented either 72% of the total parasitism that averaged 33.6 % (Chapter 4) or 77% of the parasitism that averaged 35.5 % (Wang et al., 2012). In addition, *M. ictericus* was found on 39 of 70 LBAM-infested plant species sampled in 2011 (Wang et al., 2012) and on all 14 of the plant species sampled from 2008-2011 (Chapter 4).

As LBAM is a highly polyphagous pest that is well established on ornamentals in urban areas of coastal California and poses a significant threat to agricultural production (CDFA 2012), biological control is the most feasible approach to reduce its impact and spread as an invasive species. The discovery of *M. ictericus* as a resident parasitoid of LBAM in California and the unexpectedly high parasitism rates that it has achieved make it a promising candidate for use in conservation or augmentative biological control. Therefore, understanding the success of *M. ictericus* as a newly associated parasitoid of LBAM and identifying the life history and behavioral parameters that might have led to the high rates of parasitism observed is a crucial first step in enhancing the potential impact of this parasitoid. While several other species of *Meteorus* have been well studied (Grant and Shepard, 1984; Caballero et al., 1992; Fuester et al., 1993; Thiereau and Régnière, 1995; Bell et al., 2000), little is known about the life history and behavior of *M. ictericus*. In

addition, populations of *M. ictericus* reared from LBAM in California are uniparental, which has never before been reported for this species, although thelytoky is also known in the congeneric species *M. pulchricornis* (Wesmael) (Fuester et al. 1993).

In this study we focus on observations of the developmental strategy and life history traits of *M. ictericus* including host instar preference and suitability, effects of temperature on development rate, adult longevity, and age-specific fecundity, and consequences of imperfect host discrimination. These fundamental aspects of the biology of *M. ictericus* provide valuable background data for the development of new opportunities to use this parasitoid more extensively in the control of LBAM in California, and for estimation of the potential overlap of geographic ranges of this parasitoid with LBAM as the latter expands its range in the U.S. Lastly, identification of the traits that allow *M. ictericus* to be so successful as a resident parasitoid of LBAM in California could also contribute more generally to the search for predictive characteristics of successful biological control agents.

6.3 MATERIALS AND METHODS

LBAM colony

E. postvittana larvae used in this study were obtained from a colony that was initiated with larvae collected in Santa Cruz, CA in early 2007. The colony was maintained at 21 °C, 60–85% RH and a 16:8 h (L:D) photoperiod. Larvae were reared on a bean-based diet developed by Cunningham (2007). Plastic cups (96 ml, Solo Cup Company, Highland Park, IL) were filled about one third full with diet and 2–3 egg masses of 30–50 eggs were placed inside. Once larvae had pupated sets of 50 male and 50 female pupae were placed into oviposition containers (946 ml transparent polypropylene deli containers, Fabri-Kal, Kalamanzoo, MI) and covered with gauze to await emergence, mating and oviposition. 10% honey water with 0.1% sorbic acid was provided to the adult moths in a 22 ml plastic cup (SOLO, Highland Park, IL) with a 4 cm cotton wick inserted through the lid and placed inside the oviposition containers. Adult females laid eggs into the grooves of the oviposition containers for about 1 week before being transferred to a new container. Following Singh et al. (1985), eggs were cut out from the oviposition containers and surface sterilized in a 5% formaldehyde solution for 20 min, soaked in water for another 20 min, and then left to dry for 1 h before being placed in diet cups.

Meteorus ictericus colony

The parasitoids used in this study were obtained from a colony that was initiated with cocoons collected in Santa Cruz and San Francisco, CA, in early 2009. The colony was kept in sleeve cages (47.6 × 44.5 × 53.3 cm) with mesh sides and glass lids under constant conditions of 21 °C, 60-85% RH, and 16:8 h (L:D) photoperiod and was supplemented monthly with field-

collected parasitoids from the same locations. 10% honey water with 0.1 % sorbic acid was provided to the parasitoids through a 4 cm cotton wick inserted into a slit lid of a 22 ml plastic cup placed at the bottom of the cage and replenished as needed. Three *Plantago lanceolata* plants, previously infested with late instar LBAM larvae, were provided for oviposition and approximately 10 *M. ictericus* females were released in each cage with the LBAM-infested plants. After 10 days, parasitoids were transferred to a new cage with LBAM-infested plants and were continuously supplemented with newly emerging adult parasitoids.

Parasitoids used in experiments were obtained by exposing five 4th instar larvae to one *M. ictericus* female in a 9 cm diameter petri dish for 24 h with a streak of 50 % honey solution. After 24 h, the parasitoid female was released back into the colony cage and parasitized larvae were transferred into a 22 ml plastic cup with diet. Cups were checked daily for cocoon formation and newly formed cocoons were transferred to 4.66 ml glass vials (Fisher Scientific, Fair Lawn, NJ) where they were checked daily for parasitoid emergence. Newly emerged parasitoid females were provided with a 50 % honey solution and kept in the vial until used in the experiments. All experiments were carried out at 21 °C, 60-85% RH and 16:8 h (L:D) photoperiod unless noted otherwise. All statistical analyses were carried out using R (R Development Core Team, version 2.15.0, 2012).

Host instar preference

Arenas used for host instar preference consisted of two 9 cm diameter petri dish bottoms that were fitted together tightly with parafilm to ensure containment of 1st instar host larvae. The top petri dish had a 0.5 cm diameter hole for introduction of host larvae and parasitoids and was sealed with a piece of modeling clay. To test for innate preference the petri dish arenas were stocked with one individual of each host larval instar (L1 - L6) and a single naïve parasitoid female. Parasitoids were observed until their first attack on a host larva or for a maximum of 1.5 h, and the experiment was terminated immediately after the first attack. Time to attack was noted and the attacked larva was separated from the rest and placed in a 22 ml plastic cup with diet to verify the success of the attack as determined by cocoon formation. The experiment was repeated with different female parasitoids until 36 attacks had been observed.

For the innate preference test each larva within a petri dish was scored as either attacked (one) or not attacked (zero) and the results were analyzed using a generalized linear mixed model with binomial error structure and logit link. We included parasitoid female as a random effect to accurately reflect the lack of independence between the host larvae in the same petri dish (Mangeaud and Videla, 2005). Significant differences between host instars were assessed by stepwise pooling of the most similar instars and using log likelihood tests to determine significance between groups. To test for differences in success of attack (and/or parasitoid juvenile survivorship), we compared the ratio of number of cocoons formed to number of hosts attacked for each host instar. Finally,

differences in the time taken to attack each host larval instar were tested with a non-parametric Kruskal Wallis test due to highly unequal replicates.

In a separate set of no-choice tests the same petri dish arenas were used to expose five host larvae of the same instar to a naïve parasitoid female for 3 h. Exposed larvae were then reared in a 22 ml plastic cup with diet and parasitoid cocoon formation and host larval pupation were recorded. Number of replicate observations was 26, 27, 25, 30, 28 and 33 for 1st through 6th instar, respectively.

Each petri dish was used as a replicate with number of parasitoid cocoons recorded as successes and number of host pupae recorded as failures. The resulting successes and failures from each petri dish were analyzed using a generalized linear model with a binomial error distribution. Significant differences between instars were assessed by stepwise pooling of the most similar instars and using log likelihood tests to determine significance between groups.

Host instar suitability

To determine the effect of host larval instar on parasitoid size and development time one 3rd, 5th or 6th instar host larva was exposed individually to a naïve, 0 – 3 day old *M. ictericus* female for 24 h in a 4 cm diameter petri dish. To account for maternal effects, each female was subsequently used to parasitize one larva of each of the other two instars, with a maximum of 6 days between the first and last parasitism event. The order in which the larval instars were parasitized by each female was randomized. Nine of 20 females did not successfully parasitize all three host larval instars and in these cases a new, naïve female was used to parasitize the remaining instar(s). Immediately before exposure to parasitism, all host larvae were weighed and their head capsule widths were measured to verify instar. The parasitized host larvae were individually placed into 22 ml plastic cups with diet to record time to parasitoid cocoon formation as larval development time, and to re-measure head capsule width of the exited host larva. The cocoons were placed individually into glass vials and adult emergence was checked daily to measure cocoon development time and cocoon survivorship. Emerged parasitoids were frozen and their hind tibia lengths were recorded as a measure of adult size.

Due to mortality of experimental maternal parasitoids, the nested design was highly unbalanced and so it was necessary to analyse the data as if individual host larvae were independent replicates. The relationship between parasitoid larval development time (d_L) and host larval weight (w) at the time of parasitism (n = 61) was fitted with a power function model $d_L = aw\text{^}(b)$, where a and b are scale parameters. The model was fitted to the data using the nonlinear least squares procedure of the basic stats package in R and a log-likelihood ratio test with the null model (intercept only) was used to assess model significance. The influence of larval weight at the time of parasitism (n = 35) on both cocoon development time and adult size of the parasitoid progeny were also investigated

using linear models. Differences in cocoon survivorship of the parasitoid offspring produced from attack of different host larval instars was tested with a generalized linear model with binomial error structure and stepwise pooling of most similar instar levels using log likelihood tests to determine significance between groups.

Effect of temperature on juvenile development and adult longevity

Five 4th instar host larvae were exposed to individual female parasitoids in 9 cm diameter petri dishes with 50 % honey solution for 24 h after which the larvae were transferred to 22 ml plastic cups with diet. A series of 70 plastic cups of exposed host larvae were placed in constant temperature incubators at each of five mean temperatures, 12.3, 15.9, 22.7, 26.1 and 30.1 °C, and the number of parent females that produced offspring was 57, 61, 42, 44 and 0, respectively. Offspring cocoons were placed individually into glass vials, kept in the same incubator and checked daily to record adult emergence. Due to incubator malfunction the offspring from only 33, 33, 20, and 34 of the parent females respectively could be used to assess adult longevity. Adults in the longevity experiment were provided with a 50 % honey solution which was renewed sufficiently frequently to keep from drying out. Hind tibia lengths of a random subset of parasitoids were noted at the end of the experiments as a measure of adult female size.

We modeled the juvenile development rate of the parasitoid (d_J), the reciprocal of development time, from oviposition to adult emergence using the nonlinear Brière 1 model (Brière et al., 1999): $d_J = aT(T - T_0)(T_L - T)^{1/2}$, where T is temperature in degrees Celsius, a is a scale parameter, and T_0 and T_L are the lower and upper threshold temperatures for development, respectively. Development rate data from all five temperatures were used, including 30.1 °C where no development occurred and thus the measurement was set arbitrarily to the very small value of 0.0001. Parasitoid longevity (l) at the four different temperatures was modeled using the formula $l = a/\ln(b + T)$ where T is temperature in degrees Celsius, and a and b are scale parameters. The models were fitted to the data using the nonlinear least squares procedure of the basic stats package in R and model significance was obtained through log-likelihood ratio tests of the full and the null models (intercept only). Data for both models were averaged across parasitoid offspring from the same parental female to avoid pseudoreplication, since nonlinear least square models cannot make use of nested data. In addition, we analyzed the following relationships using a generalized linear mixed model with Gaussian error and identity link and parental parasitoid as a random factor to account for the non-independence of the offspring. From the development data, we tested the dependence of: 1) hind tibia length of the parasitoid offspring on rearing temperature, and 2) hind tibia length of the parasitoid offspring on parasitoid juvenile development time at each temperature separately, with the number of parental females being 46, 24, 20, and 21 at 12.3, 15.9, 22.7, and 26.1 °C, respectively. From the longevity data, we tested the dependence of adult longevity on hind tibia length

of the parasitoid offspring at each temperature separately with number of parental parasitoids being 23, 25, 19 and 34, respectively.

Effect of temperature on age-specific fecundity

Lifetime fecundity of *M. ictericus* was assessed at 12.3, 22.7 and 26.1°C with 21, 31 and 22 replicate females respectively. Parasitoids were reared on 4th instar LBAM larvae at each temperature as described in the development time experiment. On the day of adult emergence, the parasitoid females at 12.3 °C were exposed to 20 4th instar host larvae in a 96 ml plastic cup and provided with 50 % honey solution. Host larvae and honey were replaced every 48 h except on the first day, when new larvae were provided after 24 h to more accurately determine the preoviposition period. Following exposure to a parasitoid female, the plastic cups with host larvae were filled with diet and kept at 21 °C to record parasitoid cocoon formation. The experiments at 22.7 and 26.1 °C were conducted at a later time, and the number of hosts provided per plastic cup was reduced to 12, which still represented an excess of hosts. We also changed the diet used to rear the host larvae after parasitism to *Pectinophora gossypiella* (Saunders) diet provided by the USDA - ARS Western Cotton Research Laboratory mass-rearing facilities located in Phoenix, Arizona (Bartlett and Wolf, 1985). In addition, after each parasitoid died their hind tibia length was measured.

The number of parasitoid cocoons obtained over each 48 h interval was divided by two to estimate daily fecundity. Daily fecundity of the parasitoids (*mx*) was described using the Bieri et al. (1983) model, *mx* = *ax*/*b*^(*x*), where *x* is the age of the females in days and *a* and *b* are fitted constants. Separate models were fitted for each temperature and the significance of model parameters was checked by model reduction and log likelihood ratio tests. Only the most parsimonious models with $\alpha \leq 0.05$ are reported. Survivorship curves at the three temperatures were created by dividing the number of surviving females at each age by the total number of parasitoids at the beginning of the experiment. The dependence of lifetime fecundity on parasitoid hind tibia length was tested with data from 22.7 (n = 31) and 26.1 °C (n = 20) only (no adult size data were available for 12.3 °C) using a generalized linear model with Poisson error structure and temperature included as a factor.

Effect of frequency of renewal of host larvae on reproduction

To determine whether the frequency of renewal of host larvae would affect the number of offspring produced by *M. ictericus* we exposed groups of 4th instar host larvae for different time intervals to individual naïve 4 - 5 day old parasitoid females in 14.6 × 3.8 × 10.8 cm plexiglass sandwich boxes. The treatments were either one group of 20 larvae exposed for 48 h (n = 22), two sequential groups of 10 larvae exposed for 24 h each (n =

21), or four sequential groups of 5 larvae exposed for 12 h each (n = 21). To control for potential effects of host density on reproduction, we also repeated the same time exposure intervals with a consistent group size of 20 host larvae (thus two sequential groups of 20 larvae for 24 h (n = 22) and four sequential groups of 20 larvae for 12 h (n = 21)). Throughout the experiments parasitoids were provided with 50 % honey solution. After the 48 h experiment, exposed larvae were placed in 96 ml plastic cups with diet to record parasitoid cocoon formation.

The effects of frequency of renewal of host larvae and host larval density on the total number of cocoons produced by each female parasitoid in 48 h was analyzed using a generalized linear model with a Poisson error structure. Host larval density (5, 10 and 20 larvae) and number of host batches (1, 2 and 4) were included as continuous explanatory variables. Overdispersion was accounted for by using a quasi-Poisson family with a normal error distribution and significance was estimated by model reduction as before.

6.4 RESULTS

Host instar preference

Innate preference of parasitoid females differed significantly between host instars (χ^2 = 22.06, df = 5, P < 0.001, Table 1), with 6th instar larvae being attacked significantly more frequently than 2nd - 5th instar larvae combined (χ^2 = 12.39, df = 1, P < 0.001). First instar larvae were only attacked once which differed significantly from the number of attacks on 2nd - 5th instar larvae combined (χ^2 = 4.50, df = 1, P = 0.03). Overall, only 47.2 % of the attacks resulted in cocoon formation with no significant differences between host larval instars (χ^2 = 2.83, df = 5, P = 0.73). The low level of successful parasitism was likely due to strong host larval defense behavior; backward wriggling was observed for all larval instars and spitting and biting was observed for older larvae. Time to attack did not differ with instar chosen (χ^2 = 5.82, df = 5, P = 0.32).

In the no-choice tests, the proportion of hosts parasitized differed significantly between larval instars (χ^2 = 23.06, df = 5, P < 0.001, Fig. 1). First and 2nd instar hosts combined had significantly lower parasitism rates than 3rd through 6th instar hosts combined (χ^2 = 17.39, df = 1, P < 0.001). Parasitism on 3rd instar hosts was marginally lower than for 4th through 6th instars (χ^2 = 3.79, df = 1, P = 0.05) and marginally higher than for 1st and 2nd instars (χ^2 = 3.58, df = 1, P = 0.06).

Host instar suitability

Parasitoid larval development time was significantly reduced in host larvae that were larger at the time of oviposition, as expressed by fresh weight ($F_{1, 59}$ = 36.54, P < 0.001; Fig. 2A). Parasitoid cocoon development time, however, did not show a significant linear

relationship with host weight at the time of oviposition (R^2 = 0.03; P = 0.31). Similarly, there was no significant effect of host weight at the time of oviposition on the hind tibia length of the emerging parasitoid (R^2 < 0.01, P = 0.84, Fig. 2B). Parasitoid cocoon survivorship differed with host larval instar at the time of oviposition, with individuals from 3rd instar hosts showing reduced survival compared to those from 5th and 6th instar hosts combined (χ^2 = 5.56, df = 1, P = 0.02). At the time of cocoon formation, all parasitized 3rd instar host larvae had reached the 5th instar, only 2 of the 11 parasitized 5th instar larvae had molted to the 6th instar, and all 6th instar larvae remained in the same instar.

Effect of temperature on juvenile development and adult longevity

The fit of the Brière 1 model for the dependence of development rates of *M. ictericus* on temperature was significant ($F_{2, 263}$ = 3692, P < 0.001; Fig. 3A) and yielded estimates of upper and lower thresholds for development of 30.06 °C and 5.94 °C. The maximum development rate of 0.056 day $^{-1}$ occurred at 24.7 °C and no parasitoid development was observed at 30.1 °C. Hind tibia length of the parasitoid offspring decreased significantly with increasing rearing temperature (χ^2 = 27.8, df = 1, P < 0.001, intercept: 1.8 ± 0.02, slope = -0.007 ± 0.001). Separate analyses of the relationships between hind tibia length of the parasitoid and development time, however, were non-significant at all four temperatures (12.3 °C, χ^2 = 0.36, df = 1, P = 0.55; 15.9 °C, χ^2 = 2.64, df = 1, P = 0.10; 22.7 °C, χ^2 = 0.06, df = 1, P = 0.80; 26.1 °C, χ^2 = 2.5, df = 1, P = 0.11).

The longevity of the parasitoid offspring showed a significant nonlinear decline with temperature ($F_{1, 118}$ =52.39, P < 0.001; Fig. 3B). The effect of hind tibia length on adult longevity was non-significant at all temperatures (12.3 °C, χ^2 = 0.19, df = 1, P = 0.66; 15.9 °C, χ^2 = 0.03, df = 1, P = 0.87; 22.7 °C, χ^2 = 0.30, df = 1, P = 0.59; 26.1 °C, χ^2 = 0.00, df = 1, P = 1.00).

Temperature and age-specific fecundity

Total lifetime fecundity of *M. ictericus* was 12.90 (± 1.49) cocoons at 12.3 °C, 32.54 (± 3.87) cocoons at 22.7 °C, and 17.91 (± 2.94) cocoons at 26.1 °C. At 12.3 °C daily fecundity peaked at 14 days with a maximum of 0.3 eggs day^{-1}, at 22.7 °C on day 10 with 1.8 eggs day^{-1}, and at 26.1 °C on day 9 with 1.4 eggs day^{-1} (Fig. 4). Age-specific survivorship of ovipositing females fell below 0.5 on day 52 at 12.3 °C, on day 27 at 22.7 °C, and on day 10 at 26.1 °C (Fig. 4).

For 22.7 and 26.1 °C we found a very variable but significant relationship between lifetime fecundity and female hind tibia length ($F_{2,47}$ = 111.2, P < 0.001) with separate intercepts for the two temperatures ($F_{1, 47}$ = 41.47, P < 0.001, Fig. 5). Two data points for parasitoids of extremely small size (1.28 mm) that seemed to be driving these relationships were excluded from the analysis, although their inclusion made little difference to the

significance of the slope ($F_{2, 49}$ = 167.3, P < 0.001) and of temperature as factor ($F_{1, 49}$ = 31.5, P > 0.001).

Effect of frequency of renewal of host larvae on reproduction

Offering *M. ictericus* females 20 host larvae at a time compared to 5 or 10 host larvae did not significantly increase the total number of parasitoid cocoons produced over a 48 h period ($F_{1, 105}$ = 0.85, P = 0.36). The different numbers of host larvae per batch were subsequently pooled and the frequency of renewal of the host larvae (number of batches in a 48 h period) did lead to a significant increase in the number of cocoons produced ($F_{1, 105}$ = 10.29, P = 0.002, Fig. 6).

6.5 DISCUSSION

Our study has shown that in choice tests of innate host instar preference, *M. ictericus* attacked 6th instar LBAM larvae significantly more often than any of the other host larval instars. However, in the no-choice host instar preference experiment, *M. ictericus* showed no difference in parasitism rates between 4th through 6th instars, and only a marginally significant difference for 3rd instar. These contrasting results suggest that the greater size and mobility of 6th instar larvae in the choice tests may have biased the apparent innate preference of the parasitoid since *M. ictericus* did not reject any of the host larvae that it encountered, but rather readily attacked them all. This is further supported by the fact that 6th instar larvae were no more suitable for parasitoid development than 5th instar larvae as shown from the flattening out of the relationship between development time and host larval size at the time of oviposition (Fig. 2), and the absence of any other benefits of a larger host with respect to offspring size or cocoon to adult survival. Other species of the genus *Meteorus* differ considerably in their preference for or ability to develop in different host instars. Some preferentially attack and successfully develop in early to mid instar hosts (*M. dichomeridis* Wilk., Katiyar et al., 2001, *M. pendulus* (= *gyrator* [Thun.]), Bell et al., 2000; Smethurst et al., 2004, *M. pulchricornis* [uniparental], Fuester et al., 1993, Liu and Li, 2008) or only mid instar hosts (*M. pulchricornis* [uniparental], Chhagan et al., 2008; Liu and Li, 2006), while others prefer mid to late instar hosts (*M. rubens* [Nees], Caballero et al., 1992) or equally prefer early to late instar hosts (*M. trachynotus* Vier., Hébert and Cloutier, 1990, *M. autographae* Mues., Grant and Shepard, 1984). In general, hemolymph-feeding endoparasitoids, such as *Meteorus*, are able to develop in a broader range of host instars than tissue-feeding endoparasitoids because they do not need to consume the entire host to be able to successfully complete their development and egress (Harvey et al., 1999, 2000).

In contrast to idiobiont parasitoids, that use a predictable static resource by preventing further growth and development of their hosts at the time of attack, koinobiont

parasitoids use an unpredictable resource by allowing their hosts to continue to develop and grow after parasitoid oviposition. Harvey (2005) recognized three different developmental strategies among koinobiont parasitoids in response to different sized hosts at the time of oviposition. One strategy favors a constant adult size (and consequently reproduction) at the expense of development time (type C), a second favors constant larval development time (and consequently survivorship) at the expense of adult size (type B), and the third represents a trade-off between these two traits (type A). For *M. ictericus*, adult size was independent of host size at the time of oviposition, and this was achieved by larval development time being greatly extended when smaller hosts were attacked than when larger hosts were attacked, corresponding to the type C developmental strategy of Harvey (2005). However, a type C strategy is not typical of other haemolymph feeding parasitoids. For example, *Microplitis demolitor* Wilk. (Harvey et al., 2000), *Hyposoter fugitivus* (Say) and *Hyposoter exigua* (Vier.) when developing on a larger host species (Harvey and Strand, 2002) exhibited a type B strategy, in which development time was not extended in smaller hosts with the consequence that adult size was reduced. In contrast, *Microplitis croceipes* (Cress.), *M. trachynotus* and *H. exigua* developing on smaller host species (Harvey and Strand, 2002) exhibited a type A strategy, in which development time was extended in smaller hosts, as observed for *M. ictericus*, but in this case the increased development time was unable to fully compensate for a reduction in adult size. These developmental strategies of koinobiont parasitoids have also been shown to correlate well with differences in host feeding niches, where mortality risk relates to the potential for delayed development (Harvey and Strand, 2002). The greater protection experienced by parasitoids of concealed hosts allows them to sacrifice larval development time in order to maintain adult size (type C strategy), whereas the higher mortality risk for parasitoids of exophytic hosts favors rapid larval development time at the expense of adult size (type B strategy). LBAM is a semi-concealed host feeding within shelters made of leaves that are either folded or bunched together, offering some degree of protection, and the type C developmental strategy of *M. ictericus* appears to match that of parasitoids of concealed hosts (Harvey and Strand, 2002). In contrast, however, studies on other *Meteorus* species parasitizing either exposed hosts, such as *Spodoptera exigua* (Hübn.) (Liu and Li, 2006) and *Lacanobia oleracea* (L.) (Bell et al., 2003), or semi-concealed hosts, such as *Choristoneura fumiferana* Clem. (Hébert and Cloutier, 1990), found that parasitism of smaller host larvae led to reduced adult size despite an increase in development time (type A strategy). Whether a trade-off between adult size and development time, as observed for these other *Meteorus* species, represents a distinct development strategy or an inability of the parasitoids to achieve a larger size when attacking small individuals of lower quality hosts, deserves further attention.

The significant increase in number of hosts parasitized by *M. ictericus* when host larvae were renewed more frequently corresponded well with the hypothesis that the consequences of self-superparasitism should lead parasitoids with an imperfect ability to recognize previously parasitized hosts to lay only a limited number of eggs in one host patch (Rosenheim and Mangel, 1994). The potential for wastage of eggs through self-

superparasitism may be of particular importance for parasitoids, such as *M. ictericus*, that have a low lifetime fecundity. Superparasitism has frequently been reported within the genus *Meteorus*, *M. dichomeridis* laying an average of 4.3 eggs per host (Katiyar et al., 2001), *M. trachynotus* laying a maximum of 3.7 eggs per host (Thiereau and Régnière, 1995) and *M. pulchricornis* superparasitizing 13% of its hosts (Fuester et al., 1993). Since we did not examine superparasitism in this study, we do not know whether *M. ictericus* is able to discriminate between healthy and previously parasitized hosts, but since parasitism increased when hosts were replaced more frequently this result is consistent with avoidance of the costs of self-superparasitism.

Meteorus ictericus developing on 4th instar LBAM larvae had a lower and upper development threshold temperature of 5.9 and 30.0 °C, while the corresponding thresholds for LBAM were 6.8 and 31.3 °C as measured by Danthanarayana (1975) and reanalyzed by Gutierrez et al. (2010). The very similar developmental maxima and minima of *M. ictericus* and LBAM indicate that this parasitoid is likely to be able to follow its new host as the latter expands its range in California and other parts of the western region of the U.S. More generally, the potential of *M. ictericus* as a biological control agent for LBAM in California is also likely to be influenced by other life history traits, such as generation time ratio (Kindlmann and Dixon, 2001), lifetime fecundity (Lane et al., 1999), and thelytoky (Stouthamer, 2003). At a constant 20 °C we found that *M. ictericus* had a thermal requirement of 294 degree days (DD) to develop from egg to adult, while the corresponding requirement for LBAM is 594 DD (Danthanarayana, 1975; Gutierrez et al., 2010), resulting in a generation time ratio of 0.49. Such a low generation time ratio is of particular interest as this has been argued to be among the most important traits associated with the success of natural enemies in biological control (Kindlmann and Dixon, 1999, 2001; Kimberling, 2004; Mills, 2006; Murdoch et al., 2006).

At a temperature of 22.7 °C, we found *M. ictericus* to have an average lifetime fecundity of only 33 offspring over a life-span of 26 days. Such a low lifetime fecundity could pose an important constraint on the biological control potential of a parasitoid. For example, Lane et al. (1999) found a positive relationship between parasitoid fecundity and the success of biological control against Lepidoptera, and no examples of success among parasitoids that have a fecundity of 100 or less. For comparison, the fecundity of uniparental populations of *M. pulchricornis* shows considerable variation, with two studies finding high (150 – 194) offspring production (Fuester et al., 1993; Wu et al., 2008), while others found much lower (21 – 80) offspring production (Chhagan et al., 2008; Harvey et al., 2010). A similar level of variation in fecundity has also been observed among populations of biparental *Meteorus* species; 50 offspring for *M. dichomeridis* (Katiyar et al., 2001), 78 offspring for *Meteorus pendulus* (= *gyrator*) (Müller) (Bell et al., 2000), and 150 offspring for *M. pendulus* (=*communis*) (Costamagna and Landis, 2004). Thus there appears to be no clear linkage between fecundity and uniparental versus biparental reproduction among *Meteorus* parasitoids. Rather, we suggest that, as these *Meteorus* species attack a range of different hosts that vary considerably in body size and defense mechanisms, host

quality is likely to have varied among these examples and could have been responsible for much of the variation in offspring production.

Thelytoky, the production of female only offspring, has also been argued to be a beneficial trait in the context of biological control (Stouthamer, 2003). Uniparental parasitoids can avoid mating-related Allee effects in situations with low and patchy host populations, may achieve greater suppression of host densities than bisexual parasitoids, and have the potential to realize a faster rate of population increase for a comparable level of egg production. Thus thelytoky in the California population of *M. ictericus* may have allowed it to more rapidly colonize the small and patchy founder populations of LBAM. However, it remains unclear whether thelytoky could lead to a faster rate of population increase for *M. ictericus* as the number of hosts attacked must be constrained by its low lifetime fecundity.

These separate life history traits for *M. ictericus* must also be considered in the context of the evolution of parasitoid life history strategies (Jervis et al., 2008; Jervis and Ferns, 2011). According to the "balanced mortality hypothesis" of Price (1973), the average fecundity of a parasitoid should balance its expected juvenile mortality, and should therefore be greater for larval koinobionts that attack earlier versus later host larval instars. As *M. ictericus* shows a preference for 4th to 6th instar hosts this may partly account for its low fecundity, since these larger hosts support more rapid development with reduced risk of mortality. Moreover, in seeking evidence for a fast-slow continuum of life history traits among parasitoids, Blackburn (1991a) found that slow species with low fecundity, such as *M. ictericus*, also tend to have larger eggs, slower oviposition rates and longer preoviposition periods. While egg size of *M. ictericus* remains unknown, it does have a slow oviposition rate of less than two eggs per day, but does not have a preoviposition period (Fig. 4). It does, however, exhibit a broad 'parasite window', the period of the life cycle during which a host remains susceptible to attack, which Blackburn (1991b) found to be strongly correlated with low fecundity in parasitoids. Thus, we conclude that *M. ictericus* exhibits several characteristics that place it at the slow end of the continuum of life history traits among hymenopteran parasitoids (Blackburn, 1991a). In contrast, however, many other leafroller parasitoids fall more clearly into the fast end of the continuum, such as *M. trachynotus* as a parasitoid of *C. fumiferana* (Thiereau and Régnière, 1995); *Apophua simplicipes* (Cress.) as a parasitoid of *Choristoneura rosaceana* (Harris) (Cossentine et al., 2004), and *Dolichogenidea tasmanica* (Cam.) as a parasitoid of LBAM in Australia (Berndt and Wratten, 2005).

Overall, we conclude that despite its low fecundity, *M. ictericus* exhibits several traits that may have enabled it to quickly build its populations on *E. postvittana* in California and to become the numerically dominant member of the resident parasitoid assemblage (Chapter 4). These traits include a low generation time ratio, thelytoky, and a broad parasite window (ability to successfully parasitize four of the six host larval instars) that combines with the strongly overlapping generations that LBAM exhibits in California (Bürgi et al., 2011). Also, at least from a theoretical perspective, parasitoids with lower fecundity,

such as *M. ictericus*, can suppress host populations successfully provided that they have a low generation time ratio (0.5), efficient search, and an aggregated distribution of attacks (Mills, 2006). Thus together with the overlapping temperature thresholds for development of host and parasitoid, this combination of life history traits for *M. ictericus* suggest that it could contribute to the control of this new invader over most of its potential geographic distribution in California and the western U.S.

6.6 TABLES

Table 1. Innate preference of *M. ictericus* for host larval instars of *E. postvittana* in a choice test with one individual of each instar (n = 36)

Host larval instar	**L1**	**L2**	**L3**	**L4**	**L5**	**L6**
No. attacked	1	3	5	6	6	15
No. cocoons formed	0	2	2	4	2	7
Mean time to attack [min] (± SE)	13.0 (± 0.0)	60.3 (± 15.5)	40.8 (± 16.3)	30.4 (± 16.6)	31.2 (± 8.1)	51.7 (± 7.3)

6.7 FIGURES

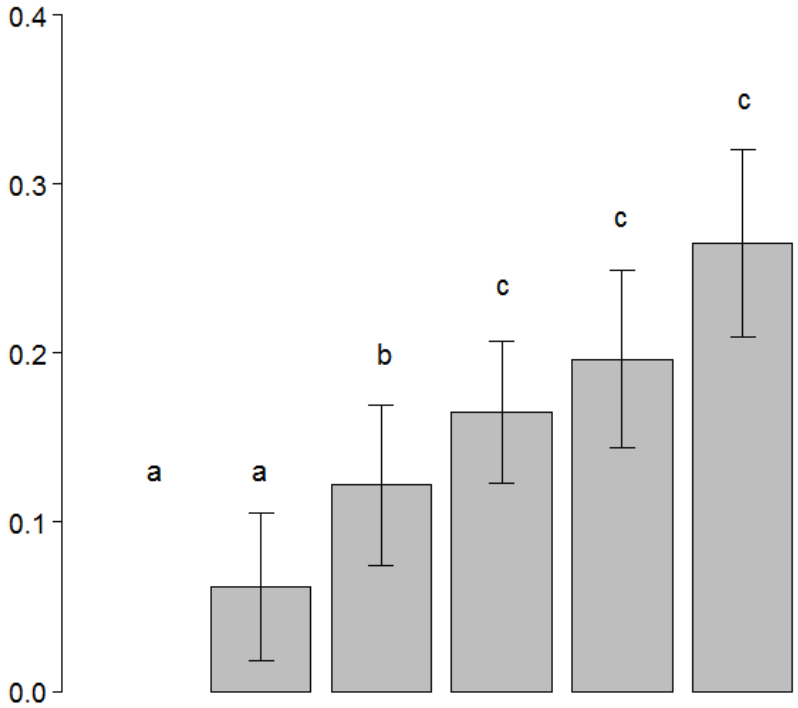

Figure 1. Preference of *M. ictericus* for different host larval instars of *E. postvittana* in no-choice tests with five individuals of each instar exposed to one naïve parasitoid female for 3 h. Bars with separate letters are significantly different at $\alpha < 0.05$.

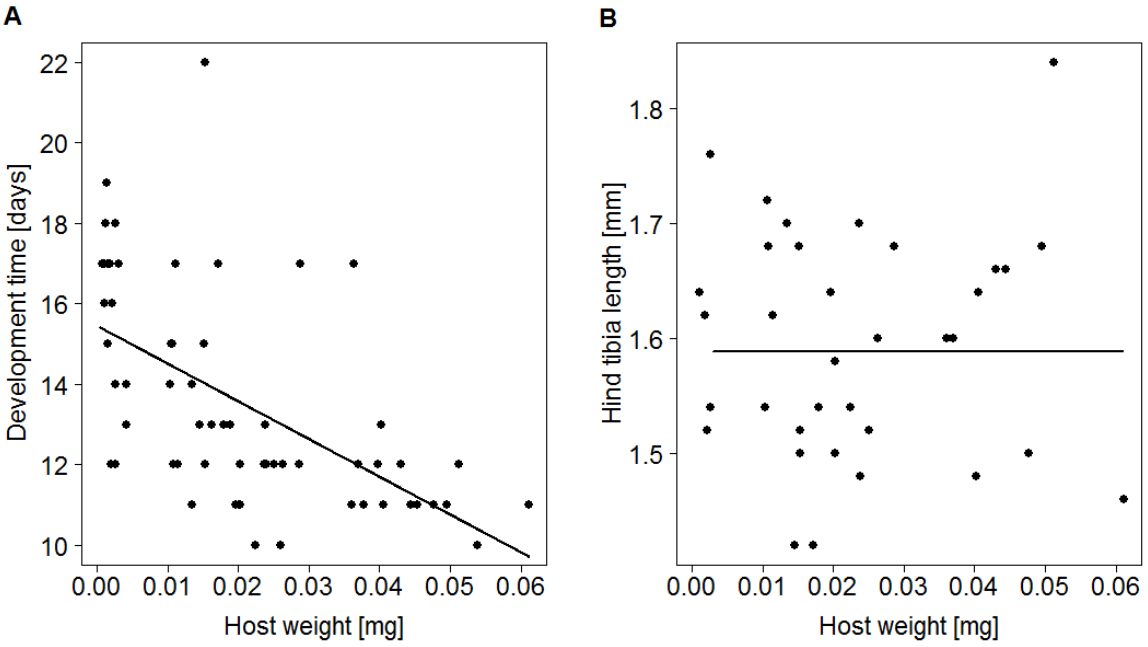

Figure 2. Influence of host larval weight at the time of oviposition on (A) parasitoid development time from oviposition to cocoon formation with fitted power function model [$d = aw^{\wedge}(b)$; a = 8.87 (±0.65) b = -0.09 (±0.02)] (grey circles: 3rd instar hosts, white circles: 5th instar hosts, black circles: 6th instar hosts), and (B) hind tibia length of emerging parasitoids [y = 1.59 (± 0.02)].

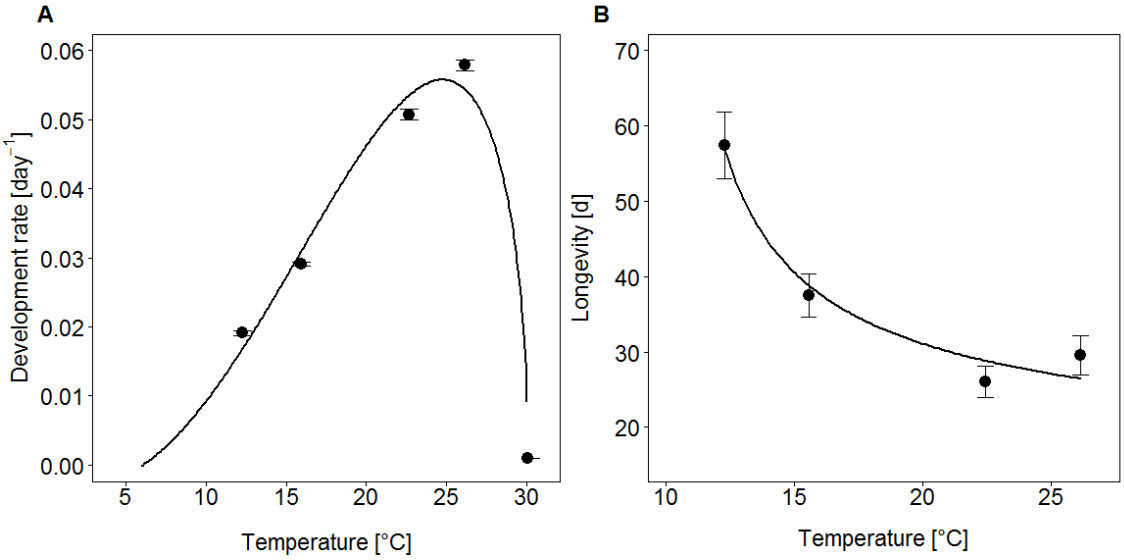

Figure 3. (A) Mean juvenile development rate (d_J ± SE) from oviposition to adult emergence for *M. ictericus* in relation to temperature with a fitted curve for the Brière 1 model [$d_J = aT(T - T_0)(T_L - T)^{1/2}$; a = 5.13 * 10^{-5} (± 9.5 *10^{-7}), T_0 = 6.08 (± 0.24), T_L = 30.06 (± 6.7 * 10^{-4})]. (B) Mean adult longevity (l ± SE) for *M. ictericus* in relation to temperature with a fitted curve for the model [$l = a/\ln(b + T)$; a = 75.9 (± 6.2), b = -8.5 (± 0.5)].

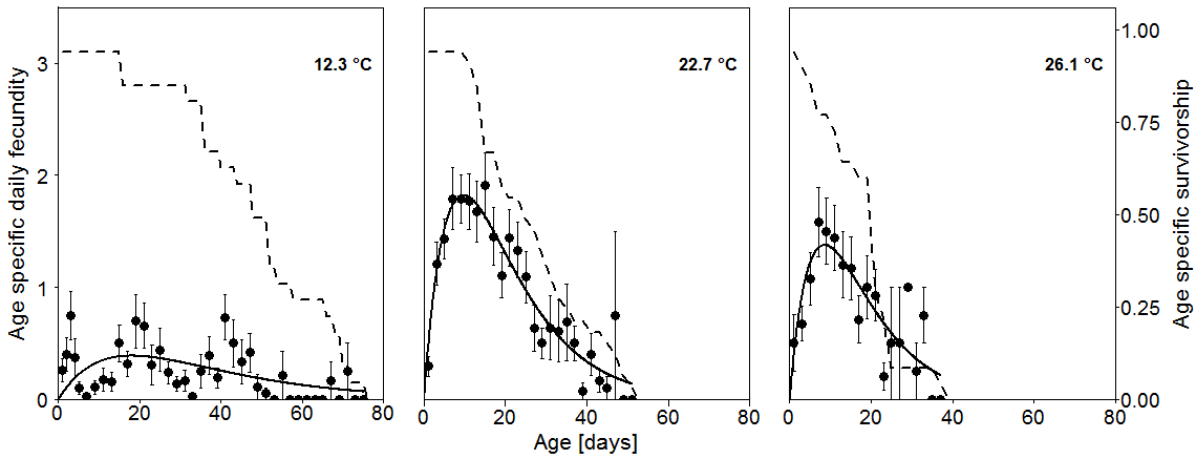

Figure 4. Age-specific fecundity (*mx*, mean ±SE) and survivorship of *M. ictericus* at three different temperatures. Fitted curves for age-specific fecundity are from the Bieri model [*mx* = *ax*/*b*^(*x*)]. Parameter estimates were: 12.3 °C: *a* = 0.03 (± 0.02), *b* = 1.04 (± 0.01), 22.7 °C: *a* = 5.1 (± 0.42), *b* = 1.11 (± 0.006), and 26.1 °C: *a* = 4.4 (± 0.66), *b* = 1.12 (± 0.01). All parameters were significant at the P < 0.001 level.

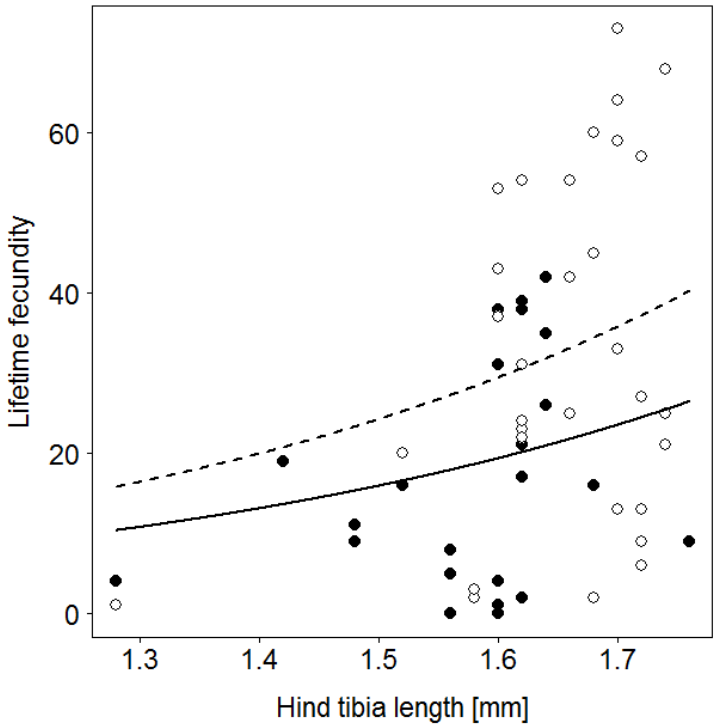

Figure 5. Relationship between lifetime fecundity and hind tibia length for M. ictericus at 22.7 (white circles, dotted line; y = 49.34x –48.55) and 26.1 °C (black circles, full line; y = 49.34x – 59.34), excluding the two smallest parasitoid females (1.28mm hind tibia length).

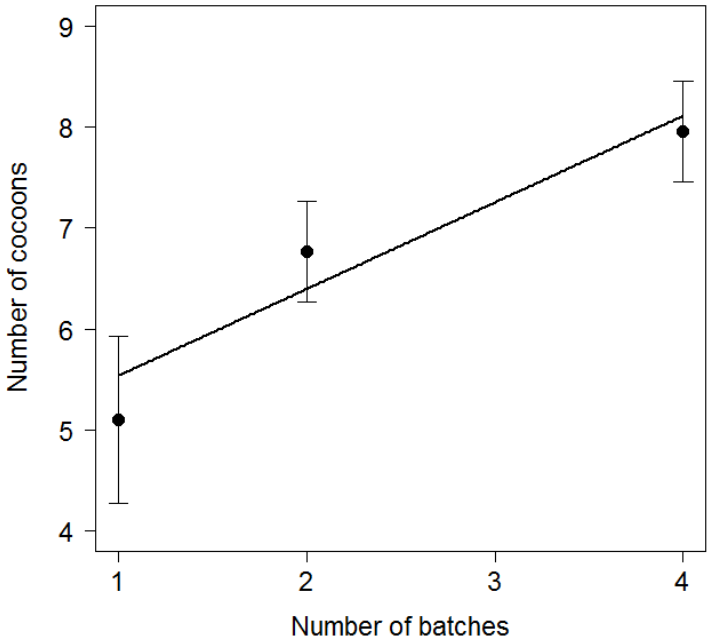

Figure 6. Effect of frequency of renewal (number of batches) of host larvae over a 48 h period on total number of cocoons produced by M. ictericus (y = 0.86x + 4.70). The different treatments were: one batch of 20 larvae exposed for 48 h, two batches of 10 or 20 larvae exposed for 24 h each, and four batches of 5 or 20 larvae exposed for 12 h each.

CONCLUSION

The light brown apple moth (LBAM) *Epiphyas postvittana* was discovered as an exotic species in California in 2006. Its controversial classification as a quarantine pest, in part due to its high polyphagy (Brockerhoff et al., 2011) and its predicted geographic distribution that extended throughout most of the southern half of the country (Fowler et al., 2009), brought considerable attention to this new invader. However, little was known about the thermal biology of this pest from its native home in Australia, where it experiences only moderately cold winter temperatures, but may be limited in its distribution by the impacts of high temperatures. In addition, while monitoring programs for adult moths were launched immediately in California (Fowler et al., 2009), no additional studies were being conducted on immature stages to determine their abundance or factors affecting their persistence. In view of this, for my dissertation, I focused on how abiotic factors, such as temperature, and biotic factors, such as parasitoids, would affect the distribution and abundance of LBAM in California.

In Chapter 1, I examined the cold temperature tolerance of the late larval instars of LBAM, the stages of the life cycle in which it overwinters in California, and found only moderate cold hardiness and high levels of mortality at temperatures below freezing. Its low tolerance of cold temperatures will likely prevent its spread into northern temperate regions of North America and limit its survival in regions with temperatures that drop below zero for more than one week during winter. As LBAM does not have a winter diapause (Geier and Briese, 1981), the state of physiological inactivity entered by insects to endure harsh environmental conditions, cold temperature tolerance was initially thought to be the main factor that would likely limit its geographic distribution. However, in Chapter 2, I examined the high temperature tolerance of LBAM and found that all life stages exhibited only moderate tolerance. I argue that its inability to tolerate higher temperatures is likely to restrict its potential geographic distribution in California to near coastal areas. This compares well with predictions from the species distribution models developed by Lozier and Mills (2011), which showed that maximum temperature in the warmest month was the most important variable influencing the distribution of LBAM in its native range and that the potential geographic distribution of LBAM in the mainland United States is likely to be much more limited than initial predicted (Fowler et al., 2009).

In Chapter 2, I also showed that the two different ad-hoc endpoint measurements used in determining lethal limits for LBAM, response to probing and ability to walk, yielded similar results. However, both measurements yielded results that significantly overestimated high temperature tolerance compared with the more ecologically relevant endpoint measurement of survival to adult emergence. With increasing interest in invasive species and climate change greater emphasis will be placed on the need for experimental studies of temperature tolerance, and yet my observations suggest that the use of simple ad-hoc endpoints measurements could be misleading. However, more studies will be needed to assess the generality of this finding for a broader range of species and to

address how to extrapolate most effectively from comparative physiological studies to ecological relevance for field populations. In addition, further research will be needed on issues such as microclimates that species actually experience and their behavioral adaptations to avoid extremes of temperature.

The impact and invasiveness of an exotic species depends – among other things - on the number of generations a species is able to complete per year. In addition, both voltinism and age structure of an exotic pest can influence the suitability and timing of different management options. In Chapter 3, I found that LBAM populations in San Francisco and Santa Cruz exhibited either a declining or cyclic pattern of abundance, with three and four generations per year in San Francisco and Santa Cruz respectively. This compares well with the three generations found for LBAM in South East Australia (Danthanarayana, 1975) and the four generations found in New Zealand (Green, 1984), where it has been established since 1890 (Mo et al., 2006). Most interesting and consequential for control strategies was my finding in Chapter 3 that all larval stages of LBAM are present throughout most of the year. The absence of discrete generations of LBAM in California with distinct and predictable adult flight periods could hamper management options such as mating disruption and sterile insect technology, but at the same time could enhance the ability of resident parasitoids that lack synchronization with its life cycle to adopt this invader as a suitable host.

In Chapter 4 I examined the parasitoid assemblage of LBAM in California and found that it is attacked by a large number of different resident parasitoid species that provide unusually high rates of parasitism. Comparing my findings with data available from Australia (Paull and Austin, 2006), I found that the parasitoid assemblage of LBAM in California was equally rich, had a higher degree of specialization, and provided similar levels of parasitism. This is somewhat unusual for a newly introduced species, as they are typically thought to experience a release from natural enemies in the invaded region Keane and Crawley, 2002). I hypothesized that a combination of factors may have led to this rapid and extensive adoption of LBAM by resident parasitoids: 1) overlapping host generations (Buergi et al., 2011), 2) parasitoid specialization at the level of host feeding niche rather than species (Mills, 1992), and 3) a rich community of confamilial hosts that support a large parasitoid species pool in California (Powell, 1964). For future studies, it would be interesting to estimate the abundance and parasitism of native leafrollers that occur within the region in which LBAM has become established to determine whether increased parasitoid populations, supported by the presence of LBAM, are having a negative impact on native leafroller communities (apparent competition). In Chapter 4, I also found that parasitism rates and parasitoid species richness varied significantly between seasons, years and origin of host plant species, with parasitism rates and parasitoid species richness being higher on plants with Australian origin. While I hypothesize that plants new to LBAM may have novel defenses that lead to decreased host quality and hence reduced parasitism, identifying the proximate mechanisms leading to this pattern would be of great importance

for understanding trophic interactions in a world of increasing globalization and accidental species introductions.

While parasitism rates encountered for LBAM in California were high and undoubtedly contribute to the suppression of established populations, I found in Chapter 5 that parasitism did not regulate LBAM population growth rates. I also determined that parasitism did not induce a demographic Allee effect, a reduction in population growth rates at smaller host densities, which can potentially lead to a reduction in the success of local establishment by eliminating founder host populations. Instead, I found that LBAM experienced strong negative density dependence, which I argue is most likely caused by induced plant defenses. Future studies should experimentally test whether induced plant defenses can account for the negative density dependence observed among LBAM populations, and should also evaluate the potential role played by egg parasitism in the suppression of LBAM populations.

In Chapters 4 and 5 I showed that approximately 75% of the parasitism of LBAM in California was attributable to *Meteorus ictericus*, a braconid larval endoparasitoid. As little is known of its life history traits, in Chapter 6, I examined this parasitoid to determine why it has been so successful in parasitizing LBAM in California and to evaluate its potential for use in conservation biological control. I identified traits favorable for biological control, such as its ability to attack and develop in a large range of larval instars, its preference for late larval instars, and its female only lifestyle. I also found other favorable traits including a low generation time ratio for *M. ictericus* on LBAM, and that both species share similar developmental maxima and minima. However, *M. ictericus* exhibited an unusually low lifetime fecundity, which could pose an important constraint on its biological control potential. It would be interesting to determine whether the low fecundity resulted from poor quality of LBAM larvae as hosts for *M. ictericus* or whether a low egg load and/or slow egg maturation rate were the cause of the low daily and lifetime fecundity in this parasitoid. Nonetheless, I concluded that the combination of life history traits for *M. ictericus* indicate that it could contribute to the suppression of LBAM populations throughout its potential geographic distribution in the United States.

Overall, the results obtained from the studies carried out for this dissertation have provided valuable insights that can be used to better understand the potential geographic distribution of LBAM, and to more effectively select and implement management decisions to minimize its environmental impact in the United States. In addition, I have been able to document the very unusual, but highly successful impact of resident parasitoids on LBAM populations in California. Parasitism of LBAM, in combination with other localized pest management strategies, seems likely to prevent widespread losses from agricultural crops that was anticipated when it was first discovered in the United States.

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APPENDIX

Table A1. Intercepts (±SE) and slopes (±SE) for probit functions fitted to mortality data of individual larval instars and life stages of *Epiphyas postvittana* at four constant high temperatures, as estimated from response to probing. Probit functions at each temperature are fitted from the same generalized linear mixed model with blocks as random effects, indicating significance of fitted parameters from 0.

Temperature (°C)	Stage	Intercept (±SE)	Slope (±SE)
40.4 °C	L1	-0.77 (±0.22)***	0.66 (±0.06) ***
	L2	-0.91 (±0.22) ***	0.44 (±0.04) ***
	L3	-2.02 (±0.24)***	0.63 (±0.05) ***
	L4	-2.03 (±0.24) ***	0.56 (±0.05) ***
	L5	-2.41 (±0.25)***	0.59 (±0.05) ***
	L6	-3.42 (±0.29) ***	0.86 (±0.06) ***
	Eggs	-1.81 (±0.20) ***	0.41 (±0.02) ***
	Pupae	-2.15 (±0.23) ***	0.38 (±0.03) ***
	Adults	-2.49 (±0.31) ***	1.29 (±0.12) ***
38.0 °C	L1	-2.53 (±0.31) ***	0.23 (±0.02) ***
	L2	-2.91 (±0.32) ***	0.23 (±0.02) ***
	L3	-4.32 (±0.43) ***	0.29 (±0.03) ***
	L4	-4.35 (±0.42) ***	0.30 (±0.02) ***
	L5	-3.29 (±0.34) ***	0.20 (±0.02) ***
	L6	-3.04 (±0.36) ***	0.21 (±0.02) ***
	Eggs	-3.07 (±0.22) ***	0.24 (±0.01) ***
	Pupae	-3.41 (±0.39) ***	0.16 (±0.02) ***
	Adults	-3.86 (±0.43) ***	0.36 (±0.03) ***
36.0 °C	L1	-3.11 (±0.30) ***	0.057 (±0.005) ***
	L2	-2.07 (±0.24) ***	0.035 (±0.004) ***
	L3	-2.60 (±0.27) ***	0.043 (±0.004) ***
	L4	-2.71 (±0.27) ***	0.044 (±0.004) ***
	L5	-1.94 (±0.23) ***	0.031 (±0.003) ***
	L6	-2.19 (±0.23) ***	0.031 (±0.002) ***
	Eggs	0.05(±0.22) NS	0.048 (±0.006) ***
	Pupae	-2.58 (±0.35) ***	0.058 (±0.006) ***
	Adults	-1.31 (±0.24) ***	0.037 (±0.004) ***

32.3 °C	L1	-3.78 (±0.41) ***	0.020 (±0.002) ***
	L2	-3.84 (±0.44) ***	0.025 (±0.003) ***
	L3	-3.60 (±0.41) ***	0.024 (±0.003) ***
	L4	-3.68 (±0.47) ***	0.032 (±0.003) ***
	L5	-2.11 (±0.33) ***	0.021 (±0.002) ***
	L6	-0.90 (±0.27)***	0.014 (±0.002) ***
	Eggs	-0.49 (±0.22)*	0.016 (±0.001) ***
	Pupae	-1.17 (±0.27)***	0.012 (±0.002) ***
	Adults	-0.84 (±0.26)**	0.019 (±0.003) ***

Table A2: Parameter estimates (±SE) for the generalized linear models fitted to cumulative relative frequency data of late instar larvae in Figure 3. The 50% cumulative relative frequencies (± SE) represent the median of larval counts within a 646 *dd* interval.

Site	Curve #	Intercept ± SE	Slope ± SE	50% cumulative relative frequency (*dd*) ± SE
SF1	1	- 3.09 ± 0.75 *	0.0034 ± 0.0008 *	895 ± 43
	2	- 6.14 ± 1.92	0.0037 ± 0.0012	1675 ± 52
	3	-11.98 ± 1.05 ***	0.0054 ± 0.0005 ***	2224 ± 13
	4	-14.29 ± 0.92 ***	0.0050 ± 0.0003 ***	2826 ± 10
	5	-15.45 ± 2.19 **	0.0046 ± 0.0006 **	3386 ± 23
	6	-25.31 ± 3.40 ***	0.0060 ± 0.0008 ***	4231 ± 20
SF2	1	- 3.34 ± 0.58 **	0.0050 ± 0.0008 **	660 ± 26
	2	- 4.87 ± 0.69 *	0.0034 ± 0.0005 *	1442 ± 38
	3	- 9.83 ± 0.59 ***	0.0049 ± 0.0003 ***	1992 ± 10
	4	-13.20 ± 0.80 ***	0.0050 ± 0.0003 ***	2627 ± 11
	5	-15.90 ± 2.19 **	0.0050 ± 0.0007 **	3209 ± 21
	6	-18.76 ± 2.04 **	0.0047 ± 0.0005 **	4024 ± 20
SC1	1	- 6.31 ± 0.23 **	0.0064 ± 0.0002 **	981 ± 05
	2	- 5.52 ± 1.31	0.0035 ± 0.0009	1570 ± 38
	3	-13.95 ± 1.52 **	0.0063 ± 0.0007 **	2201± 17
	4	-18.66 ± 1.37 ***	0.0065 ± 0.0005 ***	2865 ± 11
	5	-20.51 ± 2.18 *	0.0058 ± 0.0006 *	3560 ± 16
	6	-13.86 ± 3.33 *	0.0034 ± 0.0008 *	3998 ± 52
	7	-29.61 ± 4.92 *	0.0061 ± 0.0010 *	4875 ± 27
	8	-41.04 ± 3.46 ***	0.0074 ± 0.0006 ***	5558 ± 11
SC2	1	-20.35 ± 1.95 ***	0.0070 ± 0.0007 ***	2897 ± 14
	2	-12.03 ± 4.21	0.0033 ± 0.0012	3676 ± 80
	3	-22.81 ± 3.98 *	0.0056 ± 0.0010 *	4095 ± 28
	4	-30.15 ± 9.59	0.0061 ± 0.0019	4938 ± 50
	5	-77.93 ± 11.19 **	0.0137 ± 0.0020 **	5673 ± 11

* Statistically significant at P< 0.05, ** P<0.01, *** P<0.001