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Biotic and abiotic effects on pest management and community structure in agricultural systems

By

Sara E. Emery

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Environmental Science, Policy, and Management

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Nicholas J. Mills, Chair

Professor Mary Power

Professor Neil Tsutsui

Spring 2019

Abstract

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University of California, Berkeley

Professor Nicholas J. Mills, Chair

This dissertation focuses on three different aspects of the biotic and abiotic effects on pest management and community structure in agricultural systems, at a local level for a specific insect pest, at a landscape level for an insect community and in mesocosm experiments to examine the effects of predation on indirect interactions. Agricultural crops and pastures cover approximately 38% of the available land surface globally, and the intensity of agricultural production has continually increased over the past 300 years. The intensification of management practices in agricultural ecosystems, from pesticide applications to simplified rotations, have had wide-ranging consequences, from a rise in secondary pests to negative effects on native species and the efficacy of biological control from generalist natural enemy assemblages.

The walnut husk fly, *Rhagoletis completa* (Diptera: Tephritidae), is a secondary pest that has become more problematic in California walnut orchards with the decline in use of organophosphates, and a seasonal emergence pattern that is not well understood. To more effectively manage *R. completa* the factors that affect the timing of adult emergence were examined using both field data and mechanistic laboratory studies. A phenology model was developed for *R. completa* using a large historic data set to more accurately predict the timing of the flight period and optimize management decisions. The relationship between cumulative trap catch and degree-day accumulation for *R. completa* in California was modeled to predict thermal requirements for the start and mid-point of the flight period. Latitude, walnut cultivar leaf-out time, orchard age and year all contributed significantly to explain deviations from model predictions. The negative relationship between year and accumulated degree days at the start of the flight period corroborates grower observations and concerns that *R. completa* is emerging earlier in the season than it was 18 years ago.

A series of laboratory studies were conducted to further examine the effects of incubation temperature, pre-chill and chill durations, latitude, cultivar and size on the post-diapause development of *R. completa* puparia. The thermal requirement and developmental thresholds for adult emergence were estimated. Patterns based on sex were also examined in relation to puparial weight and post-diapause development. Percent adult emergence declined at both higher and lower incubation temperatures. Chill and pre-chill duration affected the thermal requirement, survival and synchronization of emergence for *R. completa*, with climate change implications for the future. Latitude and plant host species also affect the thermal requirement. The effects of these environmental factors on the post-diapause development of *R. completa* are discussed in relation to observations from other *Rhagoletis* species.

Insect pests and natural enemies respond to land-use intensity at varying scales. As agricultural production has intensified on the local scale over the last century, landscapes have also become increasingly homogenized. The effects of land-use intensity at both landscape and local scales on pests, generalist predators and sorghum yield were studied in Uruguay. Greater landscape intensity increased densities of pest species and provided evidence that they may interact indirectly through apparent commensalism leading to a decrease in the abundance of the generalist coccinellid community. In contrast to many field studies that have evaluated effects of local intensity on organic agriculture, in this study a multi-year rotational system was compared to continuous agriculture. Crop-grazing rotational systems resulted in lower coccinellid species richness, winter crop cultivation caused a higher proportion of non-native coccinellid individuals, and insecticide-use increased pest density. The use of path analysis provided a robust approach to examine the effects of land-use intensification on insect communities in a lesser known multi-year management system and an under-studied geographic region.

Generalist predators are increasingly recognized as important contributors to the reliability of conservation biological control. In addition, indirect interactions between prey species sharing a common predator are increasingly recognized as drivers of community dynamics. Consideration of how indirect interactions between prey species affect biological control potential is critical. Cage experiments with *Acyrtosiphon pisum* and *Aphis gossypii* were performed with varying levels of predation by adult *Hippodamia convergens* as well as varying levels of habitat complexity. Across predation pressure the early growth of *A. gossypii* appears beneficial to the net change in abundance of *A. pisum* over the course of the experiment, however the early growth of *A. pisum* provides no explanatory power for the net change in abundance of *A. gossypii*. Both increasing predation pressure and decreasing habitat complexity appear to change the sign and strength of indirect interactions between *A. gossypii* and *A. pisum* as predicted by ecological theory.

The negative impacts of agricultural intensification are wide-reaching and insect communities respond to the changes in their environment at multiple levels. Sustainable pest control strategies must consider long-term data sets, whenever possible, take into account environmental, local and landscape variables and evaluate patterns in the context of community ecology.

Dedication

For Mimi, the Uruguayan field cat

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Chapter 1: Sources of variation in the adult flight of walnut husk fly (Diptera: Tephritidae): a phenology model for California walnut orchards

Abstract

A phenology model of the walnut husk fly, *Rhagoletis completa*, was developed to more accurately predict the timing of the flight period and optimize management decisions. A data set of 153 orchard-years in which adults were trapped throughout the season was used for the development and validation of this model. Data from CIMIS weather stations were used to match orchard-year data sets with historical climatic data on degree-day accumulation, winter chill and winter rainfall. A cumulative Weibull distribution was used to model the relationship between cumulative trap catch and degree-day accumulation for *R. completa* in California. The model was used to predict thermal requirements for the start (5% cumulative trap catch) and mid-point (50% cumulative trap catch) of the flight period, which were 1670 and 2179 degree days, respectively. The prediction for 50% cumulative trap-catch of *R. completa* in California was much higher than the thermal requirement estimated in Oregon previously (1751 degree days). Linear mixed effects models were used to evaluate other environmental and orchard-specific factors which could explain the large variation between predicted and observed thermal requirements for both the start and mid-point of the flight period. Latitude, walnut cultivar leaf-out time, orchard age and year, as a continuous variable, all contributed significantly to explain deviations from the predictions of the degree-day model for individual orchard-years. Such factors can be used both to adjust predicted thermal requirements for these two specific and informative stages of the flight period, and to provide a basis for ecological and evolutionary hypotheses.

Introduction

The Tephritidae (fruit flies) are one of the largest families of Diptera with more than 4,000 species worldwide, many of which are of economic importance (White and Elson-Harris 1992). Fruit flies in the genus *Rhagoletis*, with approximately 65 species, include some of the main pests of fruit crops in North America (Boller and Prokopy 1976, Yee et al. 2014). Unlike tephritids in other genera, most *Rhagoletis* species are univoltine and oligophagous (Boller and Prokopy 1976). Several species within this genus have become invasive, perhaps because they are less host specific and vulnerable to human-facilitated transportation within fruit, which has resulted in unintentional introductions (Bush 1966, Qin et al. 2015). There have also been multiple host shifts by individual species (*R. completa* Cresson, *R. pomonella* (Walsh), *R. cingulata* (Loew), *R. fausta* (Osten Sacken), *R. indifferens* Curran and *R. lycopersella* Smyth) from native host plants to closely related non-native crop plants during the last century resulting in genetically divergent races (Boller and Prokopy 1976).

R. completa, walnut husk fly, is one of six species in the *suavis* species group, all of which feed in the husk of nuts from the genus *Juglans* (walnut). Unlike the sympatric speciation observed for the *pomonella* species group (Rull et al. 2013), speciation in the *suavis* group has

been allopatric. Each species in the *suavis* species group is primarily associated with a distinct native *Juglans* species with disjunct geographic distributions in North America (Rull et al. 2013).

R. completa is an invasive pest in California that came from the southern United States, where it is found on *J. microcarpa* Berlandier and *J. hirsuta* Manning as native host plants; it later expanded its range to *J. nigra* L. in the Midwestern United States, where it overlaps with *R. suavis* (Rull et al. 2013). It has further expanded its host plant range to include *J. californica* S. Wats., *J. hindsii* (Jeps.) R.E. Smith and the cultivated walnut *J. regia* L. (Bush 1966, White and Elson-Harris 1992). *R. completa* was first found in a California walnut orchard in 1926 (Boyce 1934). It has since spread throughout California and the western region of the United States. Previously, *R. completa* was not a major concern to walnut growers as its abundance was kept under control through applications of organophosphate insecticides that were used for codling moth (*Cydia pomonella* L.) management (Riedl and Barnes 1979). *R. completa* adults feed on honeydew produced by the walnut aphid (*Chromaphis juglandicola* Kalt.) in the canopy of walnut trees, which makes them highly susceptible to insecticide residues. In the past twenty years codling moth management has shifted away from the use of organophosphates with the passage of the Food Quality Protection Act in 1996 (Jones et al. 2009). As alternatives to organophosphates have been adopted, growers have reported increasing levels of nut damage by *R. completa* requiring multiple applications of neonicotinoids and pyrethroids to achieve control.

R. completa is a mid to late-season pest with adult trap-catch from June to September and peak trap-catch occurring in late July to early August. Mating occurs about one week after emergence, and females lay 300-400 eggs in batches of approximately 20 in the husk of a single nut (Duso and Lago 2006, Verheggen et al. 2017). Females preferentially lay eggs in large fruit (Guillén et al. 2011), and consequently cultivars may differ in susceptibility with percent of damaged fruit ranging from 0.3 to 75% (Coates 2005). *R. completa* larvae develop in the husk of the walnut, and the decaying husk tissue stains the shell of the nut. Early infestations damage the kernel of the nut, which then becomes darkened, shriveled and susceptible to mold. According to a multi-year study, walnuts attacked by *R. completa* had a 14% increased chance of mold, 47% fewer light kernels, and total edible yield was reduced by 10% (Coates 2008). After 2-5 weeks larvae drop to the ground and pupate a few centimeters below the soil surface to overwinter. In one study, approximately 84% of adults emerged the following season, with 9% emerging in a subsequent season while the remaining 7% did not emerge (Duso and Lago 2006).

R. completa has no effective natural enemies in California, and management is based on insecticide treatments. The use of acetamiprid, even in low doses, can be effective for control of *R. completa*, but timing is critical according to studies conducted in the Central Valley and along the Central Coast (Coates 2008). Since the timing of adult emergence varies between locations and years, flights are typically monitored in individual orchards through the use of yellow sticky traps charged with ammonium carbonate and hung high on the north side of the tree canopy. Insecticide treatments are initiated by a sudden increase in trap catch of adults and repeated at intervals throughout the flight period. In contrast, spray timing for other tephritid fruit fly pests in tree crops in the western region of the United States is predicted by use of degree-day phenology models, which have been developed for the closely related apple maggot (*R. pomonella*) and western cherry fruit fly (*R. indifferens*) (Jones et al. 1989, 1991).

Phenology models for insect pests are important in agricultural systems to provide managers with effective decision-making tools that are based on accumulated degree days rather than calendar dates to optimize the timing of insecticide applications. Properly timed spray applications are not only more effective as IPM tactics, but can also reduce the need for subsequent treatments and reduce negative impacts on the environment (Pedigo and Rice 2009, Evans et al. 2014, D’Auria et al. 2016). Temperature drives the development and timing of life cycle events of all ectothermal organisms, including insect pests (Jarosik et al. 2004, 2011). Degree-day models provide a way to predict the timing of life cycle events from the accumulation of heat units between a lower and upper temperature threshold and the thermal requirement for completion of specific stages in the life cycle (Pruess 1983). Kasana and AliNiazee (1994) estimated the lower and upper developmental thresholds for overwintered *R. completa* puparia in Oregon to be 5 and 30 °C, respectively. A phenology model based on three years of trap-catch data in Oregon predicted a thermal requirement for *R. completa* of 1751 degree days centigrade (DD) for 50% cumulative trap catch (Kasana and AliNiazee 1997). Thermal requirements for insects can vary with latitude, however, and adult trap catch of *R. pomonella* differed by 600 DD between Illinois and Texas (Dambroski and Feder 2007) and by 150 DD between Washington and California for overwintered codling moth (Jones et al. 2013).

The timing of life cycle events and thus the thermal requirement for adult emergence of tephritid fruit flies can also be influenced by environmental factors other than temperature. Extended winter chill can lead to a reduction in the DD requirement for adult emergence of *R. pomonella* (Smith and Jones 1991) and *R. indifferens* (Brown and AliNiazee 1977). Similarly, rainfall and soil moisture can reduce the DD requirement for *R. pomonella* (Smith and Jones 1991), but effects on *R. indifferens* emergence appear variable (Song et al. 2003, Yee 2013a).

The primary objective of this research was to develop a degree-day phenology model that most closely matches the patterns of early season *R. completa* trap catch using an unusually extensive historical data set. The secondary objective was to determine whether predictions from this overall model could be adjusted and improved using site and season-specific environmental factors, such as latitude, walnut cultivar, orchard age, precipitation and winter chill.

Materials and Methods

Monitoring Flights of R. completa

Our analysis included data collected from trap catches of *R. completa* in walnut orchards from northern California over an 18-year period. *R. completa* populations were monitored using 1-3 yellow sticky traps (Pherocon AM, Trece Inc., Adair, OK) per walnut orchard super-charged with ammonium carbonate and were checked either weekly or bi-weekly for adult flies from June through September. Some orchards were only monitored in one year, and others were monitored for as many as 12 years in succession. Two criteria were used to ensure that the trap-catch data for a particular location and year were of sufficient quality to be included in our data set. Firstly, at least 50 adults needed to be caught in an orchard over the duration of the season to provide a sufficient cumulative distribution of the timing of trap catch. Secondly, the records for an orchard could have no more than 2% of the total adults trapped from the entire season caught

on the first and last observation date to ensure that the beginning and end of the flight period were sufficiently well represented in the data. After these criteria for inclusion had been applied, our data set consisted of 153 orchard-years over an 18-year period from 49 walnut orchards (1810 trap-catch records) in California from Shasta county in the north to San Joaquin county in the south. Since the majority of *R. completa* emerge the year following pupation (Duso and Lago 2006), the trap-catch data used in this study were assumed not to be unduly influenced by flies emerging from two years prior.

Climate Data and Cumulative Degree Days

Each orchard-year in the data set for trap catch of *R. completa* was matched with historical temperature data (max and min daily temperature) from the nearest CIMIS (California Irrigation Management Information System) station located within 5-20 km of each orchard. Degree-day temperature accumulation for each orchard-year was calculated with the University of California Integrated Pest Management Degree Day calculator (UC IPM 2016) using the single sine method (Baskerville and Emin 1969) with lower and upper temperature thresholds of 5 and 30 °C, respectively, as estimated by (Kasana and AliNiazee 1994) which are assumed not to vary among orchards or between Oregon and California. Temperature accumulation from March 1 of each year was used, a date selected for the phenology models developed for apple maggot, western cheery fruit fly and blueberry maggot (Jones et al. 1989, Smith and Jones 1991, Teixeira and Polavarapu 2001, Song et al. 2003) as well as *R. completa* in Oregon (Kasana and AliNiazee 1997). Temperature accumulation was also evaluated for starting dates of January 1 and May 1, but March 1 provided a thermal requirement for adult emergence that better matched our laboratory estimates based on California populations of *R. completa* (Emery and Mills, unpublished observation).

Development of Degree-Day Phenology Models

To develop a phenology model for the flight of *R. completa* in California walnut orchards, the relationship between cumulative percent trap catch $f(x)$ and degree-day accumulation x was fitted to a three-parameter cumulative Weibull distribution as:

$$f(x) = 100 * [1 - e \left(-\frac{x-\gamma}{\alpha} \right)^\beta] \quad \text{Eqn 1.}$$

where α is a scale parameter that alters the spread of the curve, β is a shape parameter that influences the steepness of the curve and γ is a threshold parameter that determines the location of the curve along the x-axis. A three parameter cumulative Weibull distribution was selected for model development as it is commonly used to develop phenology models for other insect pests (Wagner et al. 1984, D'Auria et al. 2016).

To maximize the range of variation in flight phenology represented in the data set for model development, orchards were grouped by year and nearest CIMIS weather station. When there were only one or two orchards paired with the same CIMIS weather station for any given year, the trap-catch data from those orchard-years were used for model development. However, when there were three or more orchards paired with the same CIMIS weather station for a given

year, trap-catch data for two-thirds of these orchard-years were randomly selected for model development so that more orchard-years were allocated to model development than to model validation (Nowatzki et al. 2002). This gave a subset of 113 orchard-years used for model development. The other third was set aside for model validation (see below). Maximum likelihood was used to estimate the scale, shape and threshold parameters of a cumulative Weibull distribution for each orchard-year using the `pweibull3` function from the `FAdist` package (Aucoin 2015) and the `mle2` function from the `bbmle` package (Bolker 2017) in R version 3.4.2 (R Core Team 2017).

As phenology models are primarily a tool for pest managers to use in predicting specific life cycle events for insect pests, here we focus on the start (5%), mid-point (50%) and end (95%) of the adult flight period of *R. completa*. We use 5% cumulative trap catch to represent the start of the flight period as this excluded the first few adults trapped each season, which tend to be outliers often occurring several weeks before any others are caught, perhaps representing individuals that had overwintered for more than one year. Consequently, we considered 5% cumulative trap catch to be a better measure of the start of the main flight than the first adult trapped.

Cumulative Weibull distributions were first fitted separately for all 113 data sets to calculate the observed degree-day thermal requirement (x) for 5%, 50% and 95% cumulative trap catch ($f(x)$) in each orchard-year from rearranging Equation 1:

$$x = \alpha * -\ln\left(1 - \left[\frac{f(x)}{100}\right]^{\frac{1}{\beta}}\right) + \gamma \quad \text{Eqn. 2}$$

When using trap-catch data from multiple locations and years, there are three different ways in which a phenology model can be developed. The mean and median phenology models were cumulative Weibull distributions in which the scale, shape and threshold parameters were represented by either the mean or the median, respectively, of the values from the 113 fitted distributions for each orchard-year. The maximum likelihood phenology model was a cumulative Weibull distribution with parameters estimated directly by maximum likelihood for the combined data from all 113 orchard years used for model development. A comparison of the overall fit of these three models was based on Akaike's Information Criterion (AIC) (Burnham and Anderson 2002, Arnold 2010).

Validation of the Degree-Day Phenology Models

We used all three phenology models and compared them for best overall fit to the combined validation set of 40 orchard-years using AIC. We also evaluated the fit of each model to each orchard-year separately. The accuracy of the model predictions was evaluated at specific stages of the flight period (5%, 50% and 95% cumulative trap catch) by analyzing the residuals (observed minus predicted thermal requirements in degree days). Firstly, the spread of the residuals around 0, the predicted thermal requirement, was estimated from the RMSE using the function `rmse` in the `Metrics` package (Hamner and Frasco 2017). Subsequently, the distribution of residuals around 0 was examined for evidence of bias in either under- or over-prediction and

tested, based on the proportion of orchard-years with positive residuals and the function `binom.test` (exact binomial test) in base R.

Adjustment of Degree-Day Predictions Based on Other Environmental Factors

Linear mixed effects models using the `lmer` function in the `lme4` package (Bates et al. 2015) were used to explore the observed variation for individual orchard-years around the degree-day thermal requirement predicted by the mean phenology model (see the Results section below for justification) for 5% and 50% cumulative trap catch of *R. completa* in California. We focused here on just the start and the mid-point of the flight period as these are likely to be of greatest significance for pest management decisions. We selected environmental factors to include in the models based on previous research from the closely related apple maggot and western cherry fruit fly (Smith and Jones 1991, Feder et al. 2010, Yee 2013b). Chill hours (either cumulative hours between 0 – 7.2 °C or cumulative hours below 0 °C from November 1 to March 1), chill portions (a dynamic model in which warm days set back chill-hour accumulation (Luedeling et al. 2009a)), and rainfall (cumulative November to June or cumulative March to June) were obtained from the CIMIS weather stations for the years represented in the model development data set. Latitude, cultivar leaf-out time (vars. Chico, Payne, Serr, Vina were classified as early and vars. Chandler, Hartley, Howard as late from (Tulecke and McGranahan 1994)) and orchard age (in years) were also considered as potential explanatory variables. As there was minimal variation in elevation among walnut orchards we did not include this factor in model development. Year was evaluated both as a fixed effect, based on anecdotal evidence that *R. completa* is now emerging earlier in the season, and as a random intercept to account for the lack of independence from data sets collected within the same year. We also included orchard as a random effect. As orchard age and walnut cultivar (for leaf-out time) were available for only 77 of the initial 113 orchard-years used for model development, this reduced data set was used for the development of each mixed effects model. Standard model reduction based on corrected AIC and least significant explanatory variable was used (Bozdogan 1987, Arnold 2010). Visual inspection of residual plots for the fitted models did not reveal any obvious deviations from homoscedasticity or normality. A posterior predictive *P*-value (PPP) based on a set of 1000 simulated interquartile ranges was used to ensure that the final linear mixed effects models represented the data adequately (Bates et al. 2015). PPP can be used as a goodness of fit test with extreme values indicating a poor fit of a model to the data (e.g., $0.05 < P < 0.95$) (Gelman et al. 1996, Gelman 2013).

Validation of Adjusted Degree-Day Predictions Based on Other Environmental Factors

The final linear mixed effects models for 5% and 50% cumulative trap catch were then applied to a reduced validation data set (20 of the initial 40 orchard-years) for which both orchard age and cultivar were known. PPP was again used to check for the adequacy of the fit of the linear mixed effects models to the data. The improvement provided by the linear model predictions for the reduced validation data set was evaluated by plotting observed values on predicted values (Piñeiro et al. 2008). Observed values were the degree-day thermal requirement estimates for each orchard-year, and predicted values were obtained from the `predict` function in R for each orchard-year from the corresponding linear mixed effects model. Perfect adjustment would be represented in these plots by all observed thermal requirements lying along the 45

degree line, and evidence of bias in either under- or over-prediction was tested using the `binom.test` function on the proportion of observed thermal requirements above this line. Similarly, RMSE estimates were used to compare the residuals of the observed thermal requirements from both the constant prediction (horizontal line) of the mean degree-day phenology model and the improved prediction (45 degree line) of the linear mixed effects model.

Results

Development of Degree-Day Phenology Models

The estimates for the three parameters of the cumulative Weibull distribution models (shape, scale and threshold) varied considerably between each of the 113 orchard-years of the model development data set. The shape parameter varied from 0.5 to 62.9 (mean = 14.3, SD = 16.8), scale parameter from 60.9 to 11835.8 (mean = 2952.0, SD = 3401.7) and threshold parameter from -9448.5 to 2039.7 (mean = -692.4, SD = 3284.5). The accumulated degree days (thermal requirements) for 5% cumulative trap catch ranged from 1028 to 2379 (mean = 1745, SD = 225), for 50% cumulative trap catch from 1445 to 2724 (mean = 2174, SD = 247) and for 95% cumulative trap catch from 1954 to 3183 (mean = 2605, SD = 214) (Figure 1.1A). Cumulative trap catch increased from 5% to 50% in as little as 29 to as much as 919 (mean = 427, SD = 158) degree days. Likewise, the range from 5% to 95% occurred over a period of between 130 and 1477 degree days (mean = 858, SD = 240).

All three phenology models provided a reasonable fit to the model development data set (Figure 1.1B, Table 1.1). The mean phenology model had a steeper curve than the other two models (larger shape parameter β) and consequently predicted a later 5% cumulative trap catch and earlier 95% cumulative trap catch. In contrast, the maximum likelihood model predicted the earliest 5% and latest 95% cumulative trap catch, but as it had the lowest AIC value it provided the best overall fit to the model development data set.

As our main objective in developing a phenology model for *R. completa* in California is to inform growers of the timing of adult flight events to assist them in making management decisions, we focused on predicted thermal requirements for 5%, 50% and 95% cumulative trap catch. The predicted thermal requirement for 50% cumulative trap catch was very similar for all three models (Table 1.1), but differed to a greater extent for both 5% and 95% cumulative trap catch. The predicted requirement for 5% cumulative trap catch was very similar for the mean and median models, but much lower for the maximum likelihood model. For 95% cumulative trap catch all three models gave different predictions with that of the maximum likelihood model being greatest and that of the mean model being lowest.

Validation of the Degree-Day Models

The fit of the three degree-day phenology models to the combined data set for the 40 orchard-years used for model validation gave AIC values of 54353 for the mean model, 53968 for the maximum likelihood model and 53937 for the median model (Figure 1.2). In contrast to the model development data set, the median model had the lowest AIC value for the validation

data set indicating that it provides the best overall fit to the pooled data. However, the combined validation data set gives greater weight to orchard-years with more frequent sampling dates since they contribute more data points to the data set. Therefore, we also evaluated AIC values for the fit of the three phenology models to each orchard-year separately. For the mean phenology model AIC values ranged from 366 to 1848 (mean = 608, SD = 266), for the median model from 376 to 1081 (mean = 533, SD = 149) and for the maximum likelihood model from 399 to 890 (mean = 512, SD = 109). In this context, the maximum likelihood model showed less variation in AIC values among orchard-years and the lowest overall mean.

Although the median and maximum likelihood phenology models generated lower AIC values for overall fit to the combined validation data set, they did not necessarily provide the most accurate predictions of the thermal requirements for specific stages of the flight period when residuals from the fitted models were examined (Table 1.2). All three phenology models underestimated the thermal requirement for 5% cumulative trap catch (Figure 1.3A), but the mean phenology model was most accurate as the residuals showed both lower spread (RMSE) and the least bias in being centered around 0 (proportion of positive residuals). For 50% cumulative trap catch all three models were similarly accurate (Figure 1.3B) with equivalent spread of residuals (RMSE) and no significant bias in underestimation. However, of the three models, the mean model again had the lowest RMSE value and least bias. In contrast for 95% cumulative trap catch the mean model performed poorly (not shown) with both greater spread of residuals (RMSE) and a significant bias toward underestimation.

Accuracy in predicting the timing of specific stages in adult flight, namely 5% and 50% trap catch, is often more important for pest management decision making than a description of the full flight curve. As such, all further model development was restricted to the mean degree-day phenology model.

Adjustment of Degree-Day Predictions Based on Other Environmental Factors

Linear mixed effects models were used to explore whether other environmental and orchard-specific factors could explain the large amount of variation around the degree-day thermal requirement predictions of the mean phenology model for 5% and 50% cumulative trap catch of *R. completa* in California. After model reduction, latitude ($P = 0.005$), cultivar leaf-out time ($P = 0.03$) and year ($P = 0.03$) were significant explanatory factors for 5% cumulative trap catch (Table 3), and a one-tailed PPP of 0.57 indicated that the model provided a good representation of the data set. Twenty-nine percent of the variance in observed thermal requirements was explained by the fixed effects alone and 66% was explained when random effects (year and orchard) were also included. A significant interaction between latitude and cultivar leaf-out time ($P = 0.03$) indicated that, although flights of *R. completa* in orchards with late leaf-out cultivars were not affected by latitude, those in orchards with early leaf-out cultivars were likely to begin earlier than predicted by the mean phenology model in more southern latitudes (Figure 1.4A). There was also a significant decline in observed thermal requirement between 1998 and 2015 (Figure 1.4B).

To further explore the year effect on the timing of 5% cumulative trap catch we found that chill hours below freezing increased over the same period (Figure 1.5A), while chill portions

showed a non-significant decline (Figure 1.5B). This suggests that while winter chilling has been less consistent through this period with a greater occurrence of warmer days that set back the accumulation of chill portions, there have also been a greater number of days with freezing temperatures that may be more likely to contribute to chilling of *R. completa* puparia in the soil and reduce their subsequent thermal requirement for adult emergence.

For 50% cumulative trap catch the fixed effects with significant explanatory power for the variation around the predicted thermal requirement were latitude ($P = 0.04$), cultivar leaf-out time ($P = 0.03$), and orchard age ($P = 0.02$) (Table 1.3). This mixed effects model also gave an adequate representation of data set with a one-tailed PPP of 0.18. Twenty-six percent of the variance in observed thermal requirements was explained by the fixed effects alone, and 63% was explained when the random effects (year and orchard) were included. As for the 5% cumulative trap catch data there was a significant increase in the thermal requirement for 50% cumulative trap catch at more northerly latitudes, but in this case for all cultivars rather than just early leaf-out cultivars (Figure 1.6A). In addition, a significant interaction ($P = 0.004$) between cultivar leaf-out time and orchard age indicated a marginal increase in the thermal requirement with orchard age for late leaf-out cultivars and a contrasting decline for early leaf-out cultivars in older orchards (Figure 1.6B).

Validation of Adjusted Degree-Day Predictions Based on Other Environmental Factors

The linear mixed effects models for adjustment of the predicted degree-day thermal requirements for 5% and 50% cumulative trap catch were subsequently used to predict values for the orchard-years that were available from the reduced model validation data set ($n = 20$). The one-tailed PPP for 5% cumulative trap catch was 0.55 and for 50% cumulative trap catch was 0.49, indicating that both of the linear mixed effects models gave a good representation of the reduced validation data set. A comparison of observed versus predicted (linear mixed effects model adjustment) values show a good match for both stages in the flight period of *R. completa* (Figure 1.7). There was no bias toward over- or underestimation in this data set for either the mean degree-day phenology models or the linear mixed effects models (Table 1.4). However, consideration of other environmental factors through the linear mixed effects models substantially improved upon the predictions of the mean phenology models as evidenced by a 33% reduction in RMSE for 5% cumulative trap catch and a 26% reduction for 50% cumulative trap catch (Table 1.4).

Discussion

Development of a Degree-Day Phenology Model and Model Validation

The primary objective of this study was to develop a degree-day phenology model that most closely matched the pattern of early-season trap catch of adult *R. completa* observed from an extensive historical data set. Cumulative trap catch through the season for individual orchard-years was well described by a cumulative Weibull distribution, and three different approaches were compared for the development of a single Weibull model that best describes the dependence of cumulative trap catch on degree-day accumulation among orchard-years. As

expected, the maximum likelihood model provided the best overall fit to the full flight period of *R. completa* for both the larger model development data set (lowest AIC) and the smaller model validation data set (lowest mean AIC). In contrast, however, the mean model provided a more accurate estimate of the thermal requirements for 5% and 50% cumulative trap catch (lowest RMSE and bias in the distribution of residuals). This finding underscores the importance of considering the practical application of a model when using model selection approaches like AIC to indicate overall fit. If a phenology model is needed to predict thermal requirements for any or all stages of the flight period of *R. completa*, the best model is the maximum likelihood model with the lowest overall AIC. However, if a model is needed to predict the thermal requirement for a specific stage in the flight period, such as the start (5%) or mid-point (50%), then the mean model might be more accurate. Our goal was to develop a model that provides robust predictions for the start of the flight period to reduce the potential for overuse of insecticides, but it could also be argued that it is more effective for pest managers to get the alert out earlier than needed. In this regard it is also worth noting that the predicted thermal requirement for 5% and 50% trap catch are likely within four days of each other across models (Table 2) based on the accumulation of 25 degree days per day during that period. Although the frequency of trap monitoring through the season varied between orchards and years, as at least four different data collectors were involved, this distinction between models was facilitated by the larger size and longer time period of the data set (153 orchard-years and 18 years), which allowed us to analyze the distribution of residuals around model predictions for specific stages of the flight period.

The mean phenology model predicted a thermal requirement of 2179 DD for 50% cumulative trap catch of *R. completa* in California, much higher than the 1751 DD requirement for 50% cumulative trap catch predicted from a study in Oregon (Kasana & AliNiazee 1997). It is worth noting that the sample size used in the latter study was quite small, but nonetheless, the observed difference is likely due to the fact that *R. completa* populations in California experience fewer chill hours during the winter than those in Oregon. Alternatively, or as a complementary effect, populations in California may have become locally adapted to warmer post-diapause temperatures to influence the difference in thermal requirements between California and Oregon (Dambroski and Feder 2007).

For other *Rhagoletis* species, winter diapause is completed after a relatively long period of winter chilling, and consequently phenology models for apple maggot (Jones et al. 1989), western cherry fruit fly (Smith and Jones 1991, Song et al. 2003) and blueberry maggot (Teixeira and Polavarapu 2001) have been based on temperature accumulation after March 1. *R. completa*, on the other hand, seems not to need chilling at all to be able to complete diapause and emerge as adults (AliNiazee et al. 1988). Thus, while chilling does synchronize adult emergence of the closely related *R. indifferens* (Brown and AliNiazee 1977), and synchronized development has been suggested as a definition of successful completion of diapause (Hodek 2002), it is less clear that March 1 is consistently the best date to begin post-diapause degree day accumulation for the flight phenology of *R. completa*. From an initial exploration of start dates, we found that both January 1 and May 1 showed even greater variation in predicted thermal requirements between orchard-years than March 1, but it is nonetheless an arbitrary date selected for practical necessity rather than based on known biology. It is certainly an oversimplification of how degree-day accumulation is experienced by insects as they must first become physiologically susceptible to temperature accumulation after a process of temperature mediated diapause termination before

post-diapause development can be initiated (Williams et al. 2015). It is more likely that actual dates when puparia in the soil became physiologically responsive to temperature accumulation varied between orchards and years and that this is a significant source of the observed variation in the phenology of the trap catch data for *R. completa*. Another limitation of this study is that air temperatures were used to predict the flight phenology of an insect that pupates and overwinters in the soil. Soil characteristics, radiation and moisture are known to influence disparities between soil and air temperatures (Langholz 1989, Chen et al. 1996, Ashcroft and Gollan 2013) and likely varied between orchards and years, further contributing to the variability in degree-day prediction of the phenology of adult flight.

Adjustment of Degree-Day Predictions Based on Other Environmental Factors

A secondary objective of this study was to determine whether the thermal requirement predictions of a degree-day phenology model could be adjusted and improved using other environmental and orchard-specific factors. Although winter chill has been shown to affect the timing and synchrony of adult emergence for insects with winter diapause (van Asch and Visser 2007), it was not a significant predictor of variation for either the start (5% cumulative trap catch) or mid-point (50% cumulative trap catch) of the flight period for *R. completa*. This was particularly surprising given that the duration of chilling is known to affect the thermal requirement for adult emergence of other *Rhagoletis* species under laboratory conditions (Brown and AliNiazee 1977, Smith and Jones 1991, Moraiti et al. 2014). However, a significant decline in the thermal requirement for 5% cumulative trap catch with year did correlate with an increase in chill hours of below-freezing temperatures, although not with chill portions. While other studies have shown that the number of chill portions has been in decline in the Central Valley of California (Baldocchi and Wong 2008, Luedeling et al. 2009b), this did not appear to be the case for the CIMIS weather stations used in this study over the 18 year period of trap catch data for *R. completa*. Winter chilling of *R. completa* puparia in the soil may be more responsive to air temperatures below freezing than to those above freezing and more likely to be affected by extreme temperature events than by trends in mean winter temperatures. Alternatively, the decline in thermal requirement for 5% cumulative trap catch with year could have resulted from warmer winter temperatures that impose a higher metabolic cost on overwintering puparia and advanced the timing at which diapause ends and degree-day accumulation begins (Williams et al. 2015).

Some research has suggested that adult emergence of *Rhagoletis* species is responsive to patterns of precipitation and soil moisture (Boyce 1934, Boller and Prokopy 1976, Yee 2013a, 2013b), however, this was not directly supported by our study. Precipitation was analyzed as total winter precipitation (Nov-June) and spring precipitation (March-June), and neither provided any explanatory power for the variation in either 5% or 50% cumulative trap catch for *R. completa*. Other studies have found that rainfall (or increased soil moisture) can accelerate the timing of adult emergence in the months immediately prior to initial emergence (Smith and Jones 1991, Song et al. 2003). In California there is no, or very little, precipitation in the months immediately prior to *R. completa* emergence, but walnut orchards are irrigated during this period. Irrigation practices vary widely from flood irrigation to sprinkler and drip systems, and this variation could help explain why precipitation has little explanatory power for the timing of adult flights in this system.

Research has also shown that the canopy cover provided by trees has a significant regulating effect on both soil temperature and moisture (Breshears et al. 1998, Ashcroft and Gollan 2012). Canopy cover seems to be particularly important at regulating soil temperature when temperatures are high and humidity is low (Ashcroft and Gollan 2013). The role of microclimate in walnut orchards on adult trap-catch data for *R. completa* was apparent from the effects of both orchard age and cultivar leaf-out time. For 5% cumulative trap catch the microclimate effect of leaf-out time interacted with latitude, such that orchards at southern latitudes only appeared to have a lower thermal requirement for early-leaf out, but not for late leaf-out cultivars. However, this result could be partially conflated with a lack of late leaf-out cultivars monitored in the more southerly latitudes and so should be interpreted with caution. Additionally, most of our orchards were located between the 39th and 40th parallel so the broader effect of latitude on the phenology of trap catch was less clear, though there is a consistent pattern that the linear effect of latitude delays accumulated degree days at 5% cumulative trap catch by 89–134 degree days, or 4–6 days, in June and July when 20–25 degree days are accumulated per day. For 50% cumulative trap catch an interaction of orchard age with leaf-out time resulted in a lower thermal requirement for early leaf-out cultivars than for late leaf-out cultivars in older orchards. An earlier leaf-out time combined with a greater canopy cover in older orchards may combine to reduce soil temperature and raise soil moisture. As greater soil moisture levels can reduce the thermal requirement for adult emergence of other *Rhagoletis* species in the western United States (Smith and Jones 1991, Song et al. 2003), it could also be an explanation for the lower thermal requirement for 50% cumulative trap-catch of *R. completa* in older walnut orchards with early leaf-out cultivars.

Conclusions

In this study we have shown that when multiple data sets are available for the development of a degree-day phenology model, while a maximum likelihood model provides the best overall fit to the combined data, an alternative model based on the mean parameter values for each individual data set can improve the accuracy of predicted thermal requirements for specific stages of the phenology. Furthermore, the value of considering other environmental and orchard-specific variables to better explain the variation in thermal requirements of *R. completa* was clearly demonstrated. The degree-day phenology models developed for *R. completa* in this study provide a new tool that can be used by managers for improved decision making in walnut pest management and to adjust predictions for 5% and 50% cumulative trap catch based on orchard age, cultivar and latitude. Lastly, the clear trend in declining thermal requirement for 5% emergence over time should be taken into consideration as growers consider the varied effects of climate change on emergence patterns of crop pests.

Table 1.1 - Parameter estimates, AIC values and predicted accumulated degree days (DD) for 5%, 50% and 95% cumulative trap catch of *Rhagoletis completa* for each of the three degree-day phenology models.

Phenology model	Model parameters of the cumulative Weibull distribution				Predicted DD for cumulative trap catch		
	Scale α	Shape β	Threshold γ	AIC	5%	50%	95%
Mean	2952	14	-692	169893	1670	2179	2493
Median	1276	5	988	168869	1694	2174	2576
Max Likelihood	1536	5.1	724	168495	1580	2153	2630

Table 1.2 - Analysis of the residuals from the degree-day phenology models for *Rhagoletis completa* to estimate the spread of the distribution from the RMSE and potential bias toward under- or overestimation from the proportion of positive residuals (Prop +) and associated probability from the exact binomial test.

Phenology model	5%		50%		95%	
	RMSE	Prop +	RMSE	Prop +	RMSE	Prop +
Mean model	264.2	0.68	282.4	0.55	239.4	0.67
		($P = 0.04$)		($P = 0.63$)		($P = 0.04$)
Median model	266.5	0.68	283.1	0.63	217.4	0.50
		($P = 0.04$)		($P = 0.15$)		($P = 1.00$)
Maximum likelihood model	330.0	0.83	285.9	0.65	217.8	0.50
		($P < 0.01$)		($P = 0.08$)		($P = 1.00$)

Table 1.3 - Parameter estimates from the linear mixed effects models for significant environmental factors that help to explain variation from the predicted degree-day thermal requirements of the mean phenology model for 5% and 50% cumulative trap catch of *Rhagoletis completa*. (NA is used for factors that were not significant in the model).

Model	R^2	Intercept	Year	Latitude	Orchard age
5% early leaf-out		24109.2	-13.8	135.8	NA
5% late leaf-out	0.29	31107.1	-13.8	-41.2	NA
50% early leaf-out		-1090.2	NA	89.1	-13.4
50% late leaf-out	0.26	-1476	NA	89.1	3.7

Table 1.4 - An analysis of the residuals of the observed degree-day thermal requirements for cumulative trap catch of *Rhagoletis completa* from the predictions of the mean phenology model and the linear mixed effects models for the reduced validation data set ($n = 20$). RMSE is used to evaluate the spread of the distribution of residuals, and the proportion of positive residuals and associated probability from the exact binomial test estimate the potential bias toward under- or overestimation.

Model		5%		50%	
	RMSE	Prop +		RMSE	Prop +
Mean model (reduced data set)	204.6	0.45		300.6	0.60
		($P = 0.82$)			($P = 0.50$)
Linear mixed model	136.0	0.60		223.7	0.50
		($P = 0.50$)			($P = 1.00$)

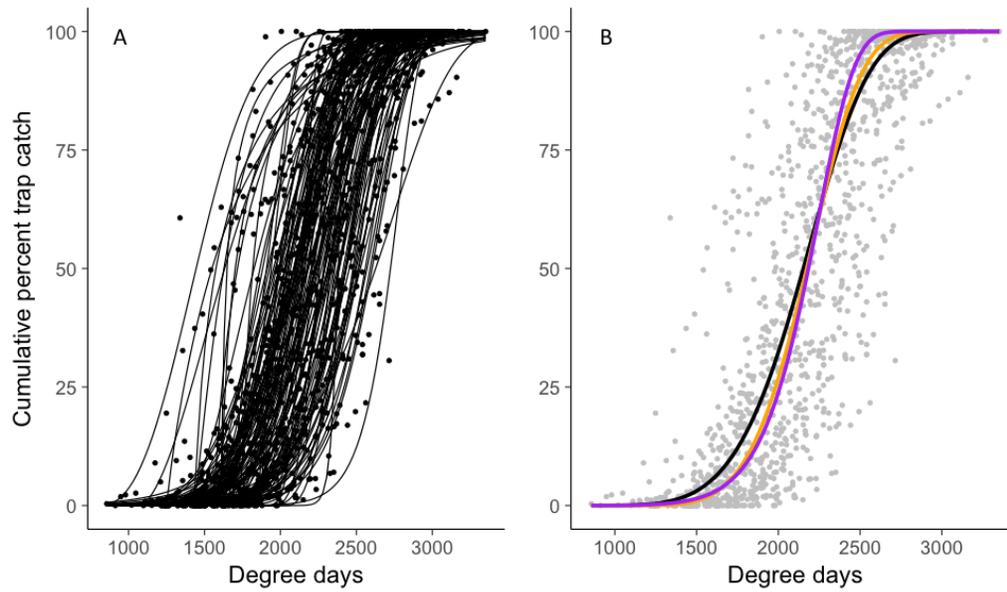


Figure 1.1 - Cumulative Weibull distributions fitted to cumulative trap catch data for *Rhagoletis completa* in relation to accumulated degree days from the model development data set (A) for each of the 113 orchard-years separately and (B) for combined data from the 113 orchard-years fitted to three phenology models described in the text, mean phenology model (purple), median phenology model (orange) and maximum likelihood phenology model (black).

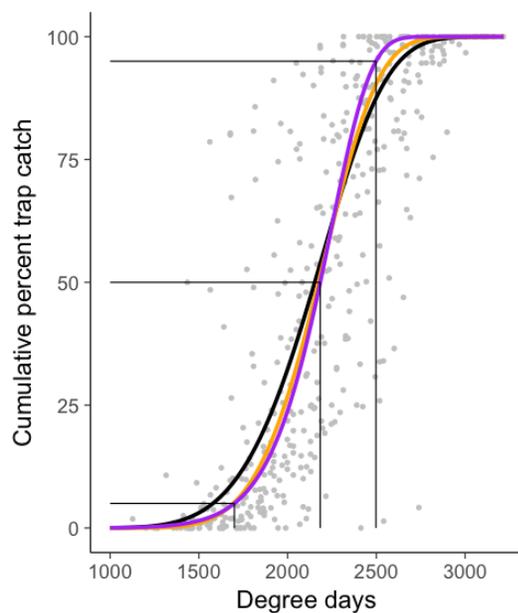


Figure 1.2 - The fit of the degree-day phenology models to the combined data on cumulative trap catch for *Rhagoletis completa* from the 40 orchard-year data set used for model validation. Mean phenology model (purple), median phenology model (orange) and maximum likelihood phenology model (black).

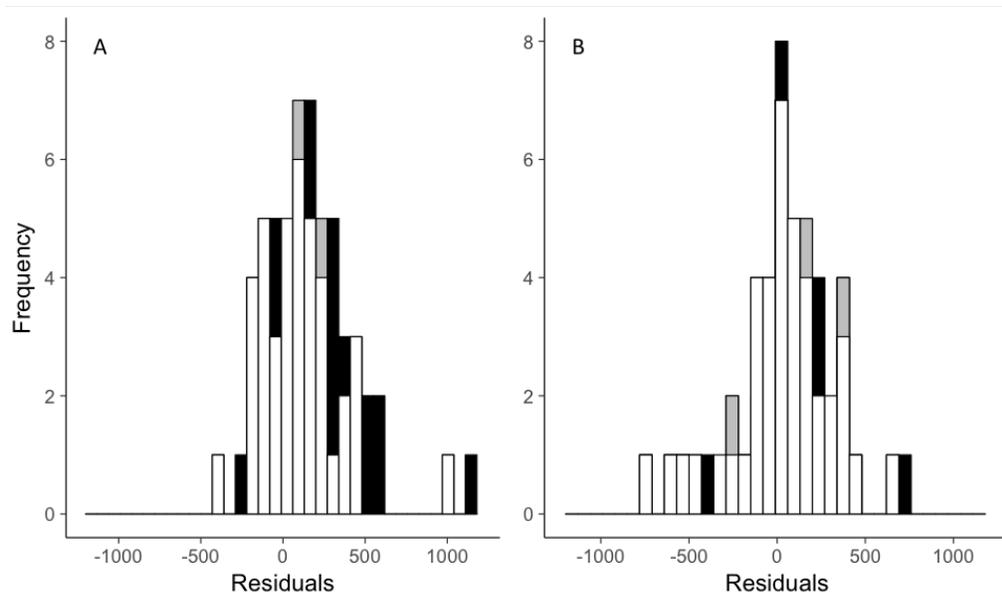


Figure 1.3 - Frequency distributions of the residuals (observed minus predicted thermal requirements) produced from degree-day phenology models for *Rhagoletis completa* using the combined validation data set consisting of 40 orchard-years for (A) 5% and (B) 50% cumulative trap catch. The frequency distributions are shown layered in sequence from the mean (white), to median (grey), and maximum likelihood (black) phenology models.

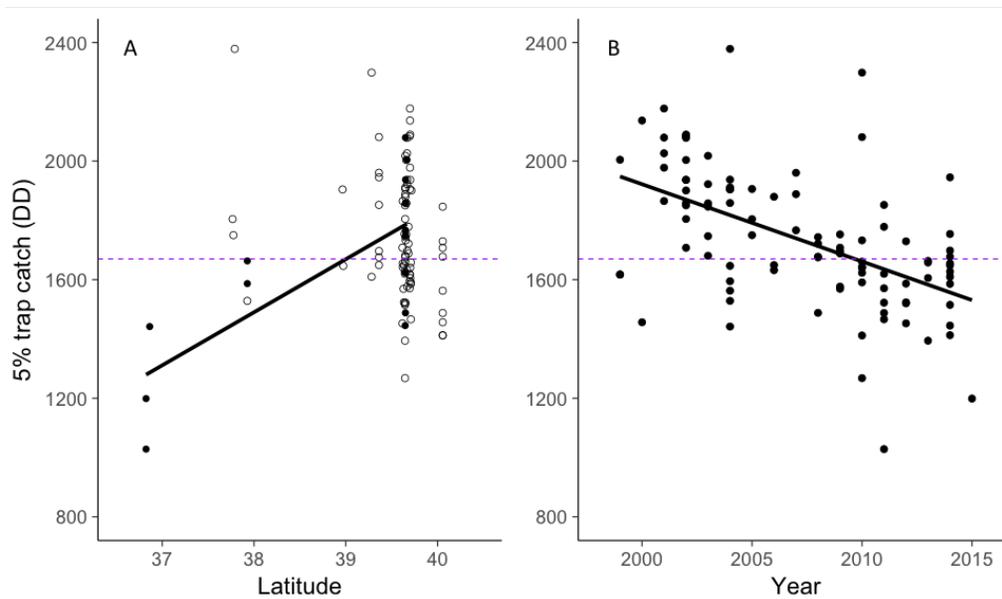


Figure 1.4 - Variation in observed thermal requirements for 5% cumulative trap catch of *Rhagoletis completa* for each orchard-year in the reduced model development data set in relation to (A) latitude alone for early leaf-out cultivars ($y = 168.3x - 4912.6$, $R^2 = 0.53$, $P = 0.005$, solid circles and solid line) and late leaf-out cultivars ($y = -93.8x + 5445.5$, $R^2 = 0.04$, $P = 0.1$, open circles), and (B) year alone ($y = -22.7x + 47142$, $R^2 = 0.25$, $P < 0.001$). The degree-day thermal requirement predicted by the mean phenology model is shown as a dashed horizontal line.

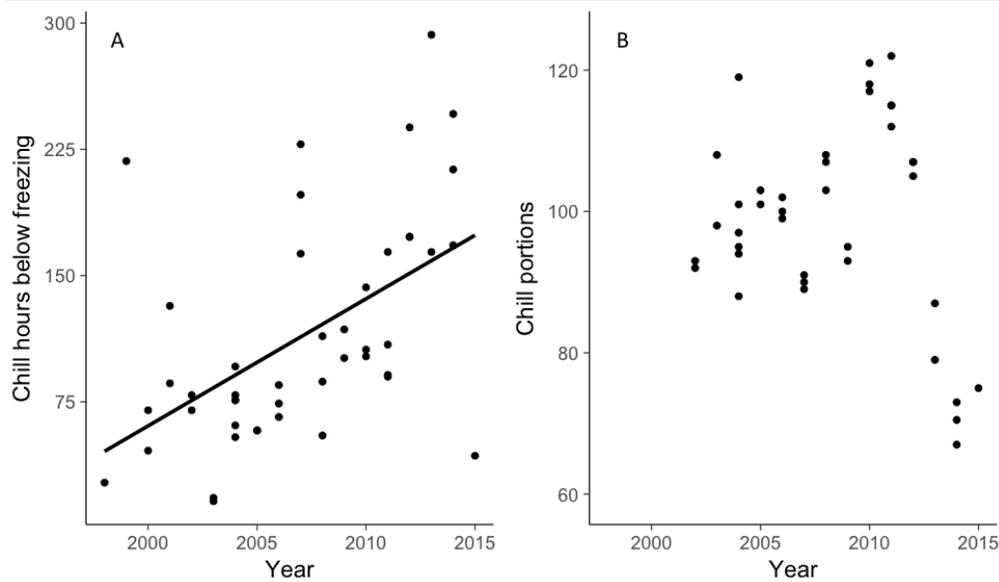


Figure 1.5 - Extent of winter chill from November 1 to March 1 for the period 1998-2015 from the CIMIS weather stations used in this study expressed as (A) chill hours below 0 °C ($y = 7.5x - 15036.6$, $R^2 = 0.27$, $P < 0.001$), and (B) chill portions ($y = -0.7x + 1572.4$, $R^2 = 0.04$, $P = 0.22$).

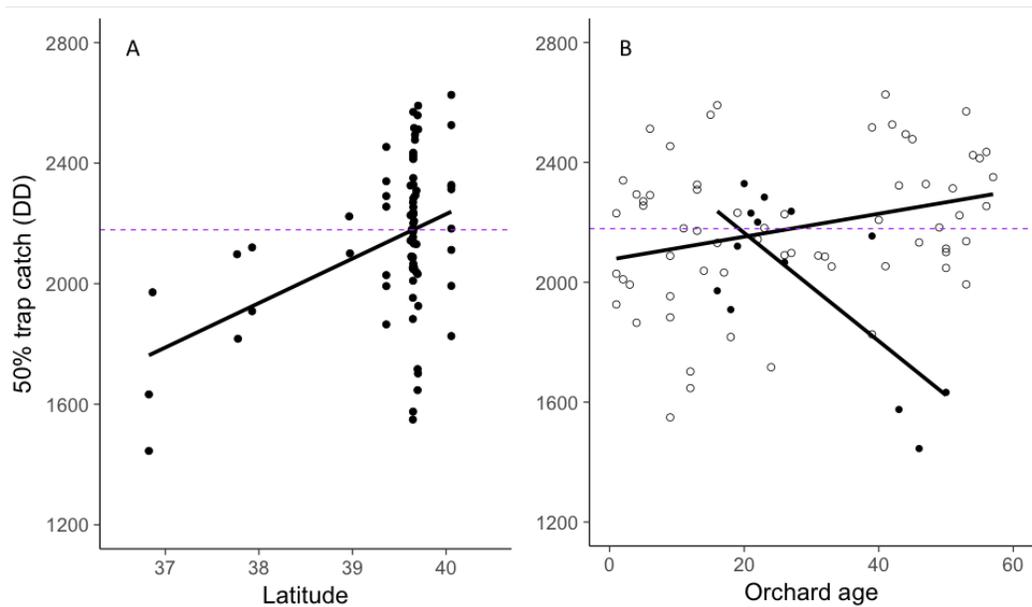


Figure 1.6 - Variation in observed thermal requirements for 50% cumulative trap catch of *Rhagoletis completa* for each orchard-year in the reduced model development data set in relation to (A) latitude alone ($y = 147.5x - 3668$, $R^2 = 0.16$, $P < 0.001$), and (B) orchard age alone for early leaf-out cultivars ($y = -18.1x + 2525.8$, $R^2 = 0.53$, $P = 0.005$, solid circles) and late leaf-out cultivars ($y = 3.8x + 2075.3$, $R^2 = 0.09$, $P = 0.01$, open circles). In each graph the degree-day thermal requirement predicted by the mean phenology model is shown as a dashed horizontal line.

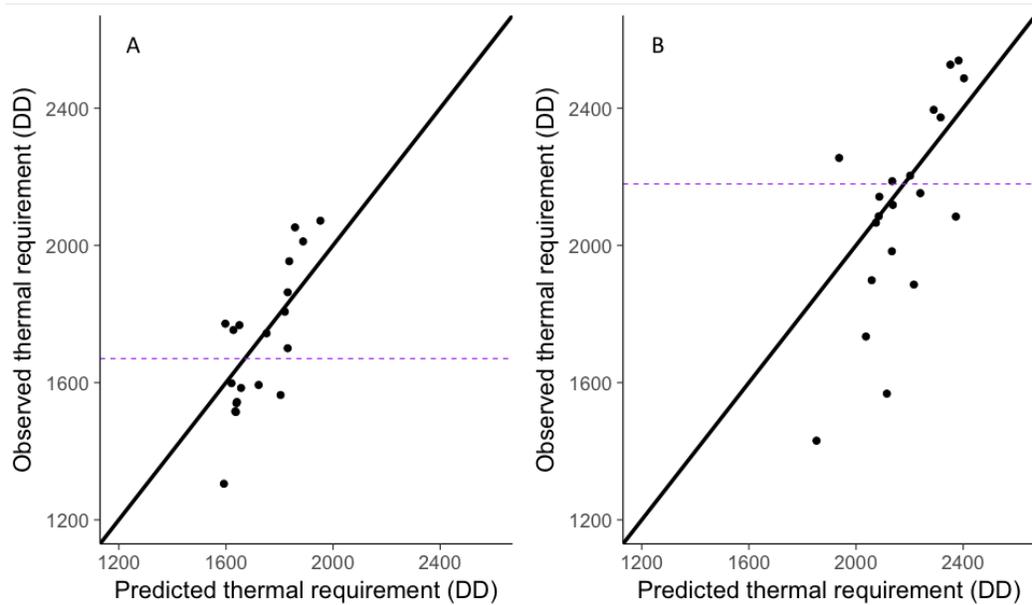


Figure 1.7 - A comparison of the observed degree-day thermal requirements (data points) and the predictions of the mean phenology (horizontal dashed line) and linear mixed effects models (45 degree solid line) for *Rhagoletis completa* trap catch data from the reduced validation data set ($n = 20$) for (A) 5% cumulative trap catch and (B) 50% cumulative trap catch.

Chapter 2: Effects of temperature and other environmental factors on the post-diapause development of walnut husk fly, *Rhagoletis completa* (Diptera: Tephritidae)

Abstract

To more effectively manage walnut husk fly, *Rhagoletis completa* (Diptera: Tephritidae), in California walnut orchards, it is important to understand the factors that affect the timing of adult emergence. In this study we examined the effects of incubation temperature, pre-chill and chill durations, latitude, cultivar and size on the post-diapause development of *R. completa* puparia. The lower developmental threshold, upper developmental threshold and optimal temperature for puparial development were estimated to be 4 °C, 34 °C and 26.6 °C, respectively. The thermal requirement for adult emergence after 120 days of chilling was estimated to be 2024 degree days. Percent adult emergence declined at both higher and lower incubation temperatures. Chill duration at 5 °C for diapausing puparia had a nonlinear negative effect on the thermal requirement, but no effect on percent emergence. Insufficient chilling lead to poor synchronization of adult emergence. Greater pre-chill duration at room temperature increased the thermal requirement and slightly decreased percent emergence. Latitude had a negative effect on the thermal requirement. Puparia from northern California black walnut (*Juglans hindsii*) had a slightly greater thermal requirement than puparia from cultivated walnut (*J. regia*). There was no significant difference in puparial fresh weight or mean thermal requirement between males and females, but the positive correlation between thermal requirement and puparial fresh weight was stronger for females than males. The effects of temperature and other environmental factors on the post-diapause development of *R. completa* are discussed in relation to observations from other *Rhagoletis* species.

Introduction

The Tephritidae (fruit flies) is one of the largest families of Diptera with more than 4,000 species worldwide, many of which are of economic importance (White and Elson-Harris, 1992). Fruit flies in the genus *Rhagoletis*, comprising approximately 65 species, include some of the main pests of fruit crops in North America (Boller and Prokopy, 1976; Yee et al., 2014). The walnut husk fly, *Rhagoletis completa* Cresson, is an invasive pest in California and native to the southern and midwestern United States where it is found on *Juglans microcarpa* and *J. hirsuta* as ancestral host plants; it later expanded its range to *J. nigra* in the midwest (Rull et al. 2013). It has further expanded its host plant range to include *J. californica*, *J. hindsii* and the cultivated walnut *J. regia* (Bush, 1966; White and Elson-Harris, 1992) and was first found in a California walnut orchard in 1926 (Boyce 1934). Over the past 90 years it has spread throughout the western region of the United States.

R. completa is a mid to late-season pest in walnut orchards in California with a flight period extending from June to September. The percent damaged fruit ranges widely from 0.3 to

75% (Coates 2005). Attacked walnuts can have 14% increased chance of mold, 47% fewer light kernels, and 10% reduced edible yield (Coates 2008). As control of *R. completa* is based on insecticide treatment of adults to reduce oviposition and nut damage, the timing of adult emergence from overwintered puparia is critical for pest management (Riedl and Barnes, 1979). A degree-day phenology model recently developed for *R. completa* in California predicted a thermal requirement of 1670 degree days for 5% cumulative trap catch and 2179 degree days for 50% cumulative trap catch (Chapter 1). However, there was considerable variation in the start of the flight period between orchards and years, and this suggested that in addition to post-diapause heat accumulation, other factors like soil temperature and moisture, ground cover or shading may also influence overwintering diapause, post-diapause development and the timing of adult emergence.

Species in the genus *Rhagoletis* are univoltine and typically undergo obligatory pupal diapause expressed regardless of environmental conditions (Košťál, 2006; Ragland et al., 2011; Papanastasiou and Papadopoulos, 2014), but see Feder et al. (1997). Diapause is characterized by developmental arrest, lowered metabolic rate and an increased resistance to environmental stress (Tauber et al., 1986). Ragland et al. (2009) found the metabolic rate of diapausing *R. pomonella* puparia is only 7.5% of that for puparia in the post-diapause stage. Winter chill for dormancy of fruit and nut trees is defined as hours between 0 and 7.2 °C (Luedeling and Brown, 2011) and this also seems to apply to diapause for species in the genus *Rhagoletis* (Moraiti et al., 2014). Many insects have been shown to need chilling to complete diapause, and for *R. cerasi* there is no adult emergence from puparia with insufficient chilling (Moraiti et al., 2014). However, others, including *R. completa* and *R. pomonella*, appear not to require cold temperatures to complete diapause (AliNiazee et al. 1988, Feder et al. 1997, Hodek 2002). Hodek (2002) suggested that in addition to adult emergence another measure of successful completion of diapause is the synchronization of emergence. Indeed, research has shown that extended chilling and lower chill temperatures lead to greater synchronization of post-diapause development in *R. indifferens* (Brown and AliNiazee, 1977) as well as in butterflies (Stålhandske et al. 2015, Lehmann et al. 2017). Nonetheless, diapause of insects in temperate regions is thought to usually terminate in late fall or early winter after which a period of quiescence persists until conditions are favorable for post-diapause development (Tauber et al., 1986, Feder et al., 1997; Hodek, 2002; Košťál, 2006).

As well as synchronizing adult emergence, the extent of chilling in winter can also influence the thermal requirement for adult emergence of tephritid fruit flies. Extended winter chill can lead to a reduction in the degree-day requirement for adult emergence of *R. cerasi* (Moraiti et al., 2014), *R. indifferens* (Brown and AliNiazee, 1977) and *R. pomonella* (Smith and Jones, 1991), but warmer temperatures appear not to be an immediate cue for diapause termination. After transition from 4 °C to 24 °C the metabolic rate of *R. pomonella* puparia did not increase for 10 days (Ragland et al. 2009). Papanastasiou & Papadopoulos (2014) identified morphological markers for diapause termination in *R. cerasi* that align with the metabolic timeline for *R. pomonella* found by Ragland et al. (2009).

The extent of winter chilling and thermal requirement for adult emergence can also vary with latitude. Laboratory research on other *Rhagoletis* species has found a lower thermal requirement at more northerly latitudes for both *R. mendax* and *R. pomonella* (Dambroski and

Feder, 2007). Lower thermal requirements are also typical of more northerly populations of Lepidoptera, but this pattern is inconsistent (Jones et al. 2013, Posledovich et al. 2014, Stålhandske et al. 2015).

The main aim of this study was to investigate, under controlled laboratory conditions, the influence of chill duration and subsequent incubation temperature on the post-diapause development of California populations of *R. completa*. We use post-diapause development to refer to the period post-chilling, though we do not directly measure the timing of diapause termination using morphological or metabolic characteristics for verification. We expected developmental thresholds for post-diapause development to be similar to those found in an earlier study from Oregon (Kasana and AliNiazee, 1994) and for extended chill durations to lead to a reduction in the thermal requirement needed for adult emergence as found for other *Rhagoletis* species (Brown and AliNiazee, 1977; Smith and Jones, 1991; Moraiti et al., 2014). In addition, we sought to determine whether pre-diapause conditions such as date of collection of larval-infested fruit from the field and the duration of warm temperatures prior to chilling would influence post-diapause development. Since prolonged pre-diapause metabolic activity can deplete resources and cause earlier post-diapause emergence in other insects (Williams et al., 2015) we expected greater pre-chill durations to reduce the thermal requirement for adult emergence of *R. completa*. Other environmental factors such as latitude (Dambroski and Feder, 2007) and cultivar (Guillén et al. 2011) have also been shown to affect diapause in *Rhagoletis* species and we included these two factors in our study of potential influences on the thermal requirement of *R. completa*. Finally, the relationships between sex, puparial weight, and thermal requirement were evaluated since research on *R. cerasi* reported the common occurrence of protogyny (Moraiti et al., 2017), earlier emergence of females than males, and anecdotal evidence of this has been reported for *R. completa* (Boyce 1934).

Materials and methods

To examine the effects of a variety of climatic and environmental factors on the thermal requirement for adult emergence and percent adult emergence of *R. completa* a series of laboratory experiments were performed between 2013 and 2015. These are shown schematically to illustrate the sequence of factors examined in relation to the lifecycle and overwintering puparial diapause of *R. completa*, with levels indicated for environmental factors that were directly manipulated in the laboratory (Figure 2.1). Larval-infested fruit were collected from commercial walnut (*Juglans regia*) orchards planted to different cultivars and from northern California black walnut (*J. hindsii*) trees in walnut growing regions of California from Hollister to Red Bluff. Unfortunately, no information was available on the management practices used in these orchards. Infested fruit were placed on sand in well aerated plastic bins (0.6 x 0.4 x 0.1m) to allow pupation at room temperature (15 - 20 °C). After a pre-chill period of one month, puparia in the bins were sorted from walnut husks and sand, dipped in a 0.5% sodium hypochlorite (common bleach) solution to reduce the incidence of fungal contamination (Etzell and Legner 1999), dried and placed in clear plastic sandwich boxes (18 x 13 x 8 cm) with peat moss in a 5 °C ($\pm 2^\circ$) cold room to chill for 90 days unless otherwise stated. After chilling, replicates with ten puparia each were placed in Petri dishes (52mm diam.) without any accompanying substrate in an incubator at 21 °C as replicates for each experiment. The Petri

dishes were monitored for adult emergence every two days until no new adults emerged. For each Petri dish the median emergence date of adult flies was recorded and converted to accumulated degree days above a lower temperature threshold of 5 °C (Kasana and AliNiazee, 1994). For all experiments, replicates in which less than three flies emerged were excluded from analysis unless otherwise noted. Pre-chill and chill durations, incubation temperature and measures of adult emergence were altered for specific experiments and are noted in the relevant sections below. All data were analyzed in R version 3.4.2 (R Core Team 2017) and nonlinearity in observed responses to experimental treatments was tested using model comparison (ANOVA) with either generalized additive models (thermal requirements) or polynomial models (percent adult emergence).

Effect of temperature on development rate and adult emergence

To evaluate the effect of incubation temperature on development rate of chilled puparia and percent adult emergence we collected infested *J. regia* var. Cisco fruit from Davis, CA on 4 September, 2013. After a chill duration of 120 days (following AliNiazee et al., 1988), 10 replicate Petri dishes of puparia were placed at a series of seven constant temperatures (12, 15, 18, 21, 24, 27 and 30 °C) to monitor adult emergence. Median emergence time from each replicate was used to evaluate the effect of temperature on development rate. As percent adult emergence at 30 °C was low, replicates in which only two adults emerged ($n = 4$) were included in the analysis.

The relationship between the development rate (the reciprocal of the time to adult emergence) of chilled puparia and temperature was fitted to both a linear model (Campbell et al. 1974) and the nonlinear Brière 1 model (Brière et al., 1999). The linear model was used to estimate t , the lower developmental threshold temperature (for a development rate of 0) and k , the thermal requirement for adult emergence (based on the reciprocal of the slope). The linear model was applied to development rate data for intermediate temperatures from 12 – 21 °C only as linearity no longer applies at higher temperatures (Campbell et al. 1974, Deutsch et al. 2015). The nonlinear Brière 1 model for development rate (d):

$$d = aT(T - T_0)(T_L - T)^{1/2}$$

has T as temperature in degrees Celsius, a as a scale parameter, and T_0 and T_L as lower and upper developmental threshold temperatures, respectively, and was fitted to data from all temperatures. The Brière 1 model was used to estimate T_L and T_{opt} , the temperature with the most rapid rate of development, and to describe the nonlinearity of development rate at higher temperatures using the devRate package in R (Rebaudo and Struelens, 2018). The potential influence of incubation temperature on the probability of adult emergence was then examined using the package MuMIn in R (Bartoń 2017) to fit a third order polynomial to the percentage of puparia that emerged as adults.

To evaluate the occurrence of protogyny in *R. completa* development rate the first adult to emerge in each replicate Petri dish was analyzed by sex using a linear mixed effects model with temperature as a random effect and ANOVA to test for the significance of sex as an explanatory variable. Replicates in which at least two adults emerged were included for analysis.

Effect of chill duration on the thermal requirement and adult emergence

To evaluate the effect of chill duration on the thermal requirement and percent adult emergence of *R. completa* infested *J. regia* var. Tehama fruit were collected on 21 August, 2015 from an orchard in Durham, CA. One hundred puparia were chilled at each of a series of 12 chill-hour durations (0, 180, 360, 720, 1080, 1440, 1800, 2160, 2520, 2880, 3240 and 3600). This range of hours was selected since *R. completa* has been shown to emerge successfully without any chilling (AliNiazee et al. 1988) and 3600 hours represents more than double the maximum number of chill hours typically experienced in California walnut orchards (data from 1998-2005 for seven weather stations from the California Irrigation Management Information System (CIMIS)). After chilling, ten replicate Petri dishes of puparia from each treatment were placed in an incubator to monitor median adult emergence. A generalized additive model (GAM) was fitted to the relationship between the thermal requirement for adult emergence and chilling hours using the *mgcv* package in R (Wood 2011). A linear model was used to evaluate the effect of chill time on percent adult emergence.

To determine the effect of maximum chill duration on the thermal requirement for adult emergence, puparia were obtained from infested fruit of both *J. hindsii* and *J. regia* var. Chandler collected in Davis, CA in early September 2013 and chilled for two full years. Forty-one replicates of 10 pupae each from both species were incubated and accumulated degree days for median emergence and percent adult emergence were compared between walnut species using ANOVA.

Effect of pre-chill duration, collection date, latitude and cultivar on the thermal requirement and adult emergence

To investigate the effect of pre-diapause conditions and other environmental factors on the thermal requirement for adult emergence of *R. completa*, separate sets of infested fruit were collected from different locations in 2015. All puparia were sorted from walnut husks in late September and chilled on 20 October, unless otherwise stated. After chilling, ten replicate Petri dishes of puparia from each treatment were placed in an incubator to monitor adult emergence. To evaluate a possible effect of collection date from the same orchard and cultivar, infested *J. regia* var. Tehama fruit were collected weekly over a four-week period (21 August, 28 August, 4 September and 11 September) from an orchard in Chico, CA. To determine the effect of pre-chill duration, the time spent at room temperature between pupation and the start of chilling, infested fruit were collected from Hollister, CA (*J. regia* var. Hartley) on 22 August and from Chico, CA (*J. regia* var. Tehama) on 11 September. For both locations chilling was initiated on each of the following dates, 15 October, 30 October, 15 November and 30 November. To investigate the effect of latitude infested *J. hindsii* fruit were collected on the same day (28 August) from three different latitudes 37.93, 38.69 and 39.73 °N in Brentwood, Esparto and Chico, CA respectively. To test for an effect of walnut cultivar infested fruit were collected from *J. hindsii* and from *J. regia* vars. Payne and Hartley in Hollister, CA on 22 August. In addition, to further test for a difference between *J. hindsii* and *J. regia* as resources for *R. completa*, infested fruit from *J. hindsii* and *J. regia* vars. Hartley, Payne, Tehama and Vina were collected between 22 August and 11 September in Hollister, Brentwood, Esparto and Chico, CA.

Linear models were used to test the effects of pre-chill duration, collection date and latitude, and ANOVA was used to test the effect of cultivar on the thermal requirement and percent adult emergence.

Effect of adult size and sex on the thermal requirement for adult emergence

Infested *J. regia* var. Cisco were collected from an orchard in Davis, CA on 4 September, 2013 and puparia were chilled for 120 days. After chilling, puparia were weighed and placed individually into gelatin capsules (size 0) in an incubator at 24 °C to monitor adult emergence. Separate ANOVAs were used to test for a difference in both puparial fresh weight and the thermal requirement for adult emergence by sex of the emerging adults. A linear model was used to examine the relationship between puparial fresh weight and the thermal requirement for both sexes.

Adults that emerged from other experiments were dried and weighed to assess the influence of sex on adult dry weight using ANOVA. For a small subset of these individuals it was possible to link puparial fresh weight with adult dry weight for both sexes which was evaluated using a linear model.

Results

Effect of temperature on development rate and adult emergence

The effect of temperature on the development rate of chilled puparia of *R. completa* at intermediate temperatures between 12 and 21 °C (Figure 2.2A) was described using a linear model. The lower developmental threshold (t) was estimated to be 4 °C, with a thermal requirement for adult emergence (k) of 2023.9 degree days. The Brière 1 model, fitted to the full range of temperatures, provided estimates of -10.1 °C for the lower temperature threshold (T_0), 34.4 °C for the upper temperature threshold (T_L), and 26.6 °C for the optimal temperature (T_{opt}). Percent adult emergence declined at both the upper and lower temperatures used in this experiment (Figure 2.2B), with a peak of 97% at 21 °C and a low of 11% at 30 °C. A linear mixed effects model found no evidence of protogyny in *R. completa* as sex did not explain the variation in development rate for emergence of the first adult in each replicate ($\chi^2 = 0.1$, $p = 0.75$, $n = 64$).

Effect of chill duration on the thermal requirement and adult emergence

A generalized additive model indicated a significant nonlinear decline in the thermal requirement for adult emergence of *R. completa* (ANOVA, $F = 30.8$, $df = 6.5, 110.5$, $p < 0.001$) in response to increasing chill hours from 0 to 3600 (Figure 2.3). At a low level of chill hours, below 1080, the thermal requirement was highly variable indicating poor synchronization of emergence (SD from 626 to 1112). A value of 1080 chill hours provided the lowest thermal requirement and showed greatly reduced variation among replicates (mean = 963, SD = 210). The estimated thermal requirement increased from 1080 to 2160 chill hours but then remained more or less constant from 2160 to 3600 chill hours. There was no significant effect of chill hours on percent adult emergence

(Figure 2.3). When puparia experienced maximal chilling for two full years the mean percent emergence was 81% (SE = 17%) and the thermal requirement was estimated to be 958 degree days (SE = 7.4). There was also no significant difference in the thermal requirement for adult emergence (ANOVA, $F = 1.7$, $df = 1,82$, $p = 0.2$) between puparia collected from *J. hindsii* and *J. regia* var. Chandler after maximal chilling.

Effect of pre-chill duration, collection date, latitude, and cultivar on the thermal requirement and on adult emergence

The thermal requirement for adult emergence of *R. completa* increased with pre-chill duration for puparia reared from infested fruit from two cultivars in different locations (Figure 2.4A). The pre-chill duration effect was similar but significantly different for the two locations (ANOVA, $F = 39.3$, $df = 1,75$, $p < 0.001$). This suggests that either cultivar (Tehama versus Hartley) or latitude (Chico versus Hollister) also had an influence on the thermal requirement at the two locations. In contrast, percent adult emergence decreased significantly with increasing pre-chill duration for both collections (Figure 2.4B). The date of collection of infested fruit from the same orchard over a four-week period did not influence the thermal requirement ($y = -8.75x + 3766.51$, $R^2 = 0.04$, $p = 0.12$). Adult emergence from chilled puparia collected from *J. hindsii* at three different latitudes on the same date showed a negative effect of latitude on the thermal requirement (Figure 2.5). For fruit collected at the same location and date there was no effect of cultivar on the thermal requirement (Figure 2.6A). However, when data were pooled over collection date, which provided no explanatory power, a linear mixed model with location as a random effect provided weak support for a lower thermal requirement for *R. completa* collected from *J. regia* (vars. Hartley, Payne, Tehama and Vina) than from *J. hindsii* (Figure 2.6B).

Effect of adult size and sex on the thermal requirement for adult emergence

There was no significant difference (ANOVA, $F = 0.03$, $df = 1,135$, $p = 0.86$) between mean puparial fresh weight of females (6.91 mg, SE = 0.22) and males (6.97 mg, SE = 0.30). Similarly, there was no significant difference (ANOVA, $F = 0.62$, $df = 1, 427$, $p = 0.43$) between the mean dry weight of adult females (1.64 mg, SE = 0.04) and adult males (1.60 mg, SE = 0.04). Though the sample size was small ($n = 14$), there was a positive linear relationship between puparial fresh weight and adult dry weight for both males and females (Figure 2.7A). There was also a linear relationship between puparial fresh weight and the thermal requirement for adult emergence, though this relationship was stronger for females than for males (Figure 2.7B). Small females emerged sooner than small males of the same size, but large females emerged later than large males of the same size. Overall, however, there was no significant difference ($F = 1.56$, $df = 1,135$, $p = 0.8$) between the mean thermal requirements for female (2531 degree days, SE = 23) and male (2573 degree days, SE = 24) emergence.

Discussion

Our observations of optimum and threshold temperatures for development of chilled puparia of *R. completa* in California are very similar to those made earlier in Oregon by Kasana and AliNiasee (1994). The Brière 1 model, fitted to the full range of temperatures, provided a

much lower estimate of the lower temperature threshold (T_0) at $-10.1\text{ }^\circ\text{C}$, but this is unlikely to be accurate since this model is less effective at estimating development rates for temperatures outside of the range observed (Brière et al., 1999). The lower developmental threshold estimates for puparia from California and Oregon were almost identical (4 versus $5\text{ }^\circ\text{C}$), but the optimal temperature ($26.6\text{ }^\circ\text{C}$) and upper developmental threshold ($34.4\text{ }^\circ\text{C}$) were approximately $5\text{ }^\circ\text{C}$ higher for puparia from California compared to those from Oregon. This may in part be due to the use of different models to describe the data (Brière 1 model for California versus quadratic polynomial for Oregon). It could also be due to an effect of latitude and adaptation of California populations of *R. completa* to slightly higher soil temperatures.

The thermal requirement (k) of 2024 degree days estimated from the linear model provides a good match to an estimated 2179 degree days for 50% cumulative trap catch of *R. completa* in California (Chapter 1). Research on *R. completa* as an invasive species in Europe has found that under field conditions peak trap catch coincides with peak oviposition and that peak oviposition is somewhat resilient to environmental fluctuations (Duso and Lago, 2006). Trap catch data over a 15 year period in California confirmed this observation for 50% cumulative trap catch of *R. completa*, but the timing of the start of the flight period at the beginning of the season was found to be far more variable (Chapter 1). In addition, our data showed no support for protogyny in *R. completa*. While we have no explanation for this, it suggests that no distinction needs to be made between males and females in examining the effect of other factors on the thermal requirement for adult emergence.

The most striking difference between this study and Kasana and AliNiazee (1994) was the estimated thermal requirement for median adult emergence from chilled puparia of 2024 DD for California compared to 976 DD for Oregon. Rather than an effect of latitude, this difference is likely due to the more limited 120 day chill duration (2880 chill hours) used in our study versus 215 days (5160 chill hours) used in the Oregon study. When given maximum chilling for two years we found that the thermal requirement for adult emergence was reduced to 958 degree days. This suggests that the effect of maximum chilling on the post-diapause development of *R. completa* may saturate at around 5000 chill hours as found for *R. indifferens* and *R. pomonella* (Brown and AliNiazee 1977, Feder et al. 1997). Research on other *Rhagoletis* species has reported a lower post-diapause thermal requirement for populations at more northerly latitudes (Dambroski and Feder, 2007) and our results from three different latitudes in California suggest a similar pattern for *R. completa*. Although this might appear to be evidence for adaptation to local differences in prevailing soil temperatures it may also suggest phenotypic plasticity and caution is needed in extrapolating from our data for *R. completa* which spanned only 2 degrees of latitude.

Theory predicts that as specialist and univoltine insects have narrower and more critical physiological requirements for resource use they are likely to be more vulnerable to climate change and at greater risk from variability in seasonal temperature patterns (Miller-Rushing et al., 2010; Williams et al., 2015). In this context *R. completa* is univoltine, but is oligophagous and has a phenotypically plastic response to variable overwintering conditions, with no minimum duration of chilling required for diapause termination and no effect of variable chill duration on percent emergence even after cold storage for two years. In contrast, *R. cerasi* (Vallo et al., 1976; Moraiti et al., 2014) expresses rather less phenotypic plasticity showing poor emergence with

both insufficient and extended periods of chilling. The plasticity of *R. indifferens* and *R. pomonella* appears intermediate as although adults can emerge in the absence of chilling, a much higher proportion emerge after chilling (AliNiazee et al. 1988, Feder et al. 1997, Neven and Yee 2017). Consequently, the greater phenotypic plasticity of *R. completa* seems likely to make it less susceptible to changing winter climatic conditions than has been suggested for other univoltine insects (Williams et al., 2015).

The effect of greater chill duration on the thermal requirement for adult emergence of *R. completa* was greater synchronization of emergence, as found previously for *R. indifferens* (Brown and AliNiazee, 1977; Neven and Yee, 2017), and a nonlinear decline to a minimum at 1080 chill hours over the lower range of chill hours. This decline in thermal requirement was opposite to the pattern of increase to a peak observed for *R. pomonella* over a lower range of chill hours (Feder et al. 1997). As pointed out by Hodek (2002) the effects of chill duration can be misinterpreted in laboratory studies when the chill temperature is greater than the lower threshold temperature for development. However, since the threshold for *R. completa* development was found to be 4 °C and the chilling temperature was set at 5 °C ($\pm 2^\circ\text{C}$) in our study, the difference is minimal and seems unlikely to have affected the observed pattern of decline in thermal requirement with increased chill duration. The minimum thermal requirement for *R. completa* was observed at 1080 chill hours, a value that is quite close to the mean chill hours recorded from 1998-2015 from a set of seven CIMIS weather stations in California located near to the walnut orchards from which *R. completa* were collected for this study (mean = 993, SE = 189 chill hours). This suggests that *R. completa* may be locally adapted to the amount of winter chill experienced in walnut orchards in California which likely helps mated females to physiologically align with nut development on their host plants, *J. hindsii* and *J. regia*. However, as soil temperatures are buffered to a greater degree than air temperatures in winter (Ashcroft and Gollan, 2013), puparia of *R. completa* overwintering in the soil in California probably experience less than the optimum chill hours and consequently adult emergence may continue to become less synchronized as winters become warmer.

We expected that prolonged pre-chill durations at room temperature would reduce the thermal requirement for adult emergence of *R. completa*, as observed for the yellowheaded spruce sawfly (Eller et al. 1989), but found the opposite effect of an increased thermal requirement. It is possible that *R. completa* uses warmer temperatures during the pre-chill phase of diapause as a cue for a winter with less winter chilling, which has been shown to increase the thermal requirement for overwintering pupae of pierid butterflies (Stålhandske et al. 2015). In addition, it seems likely that *R. completa* enters diapause during larval-pupal apolysis, as is the case for *R. cerasi* (Papanastasiou and Papadopoulos, 2014), and an early onset of diapause in late summer likely minimizes subsequent metabolic costs of prolonged exposure to warm temperatures in the fall (Ragland et al. 2009). However, we did find a decline in percent adult emergence with increasing pre-chill duration, which, though not as pronounced as that found by Feder et al. (1997) for *R. pomonella*, may be a consequence of resource depletion, one component of the pupal energy reserve hypothesis of Feder et al. (2010).

Previous research on *R. completa* from Switzerland suggests diapause length is influenced by walnut cultivar and negatively correlates with fruit weight (Guillén et al. 2011). In addition, research from other *Rhagoletis* species has shown higher thermal requirements for adult

emergence from populations feeding on wild versus cultivated host plant species as an adaptation to later fruiting times (Dambroski and Feder, 2007; Teixeira et al., 2009; Smith et al., 2014). We did not observe any effect of walnut cultivar on the thermal requirement of *R. completa*, but there was a slight effect of wild (*J. hindsii*) versus cultivated walnuts (*J. regia*). As *J. hindsii* and *J. regia* grow in the same regions of California, it seems less likely that populations of *R. completa* are adapted to differences in the timing of fruit maturation, and more likely that the greater thermal requirement is due to the smaller fruit size of *J. hindsii* (Tulecke and McGranahan, 1994; Forde and McGranahan, 1996).

Although there was no significant difference in mean size between male and female *R. completa*, we observed a stronger effect of size on the thermal requirement for adult emergence of females which is likely tied to energetics, since smaller puparia have lower nutrient reserves and lower post-diapause fitness (e.g. fecundity) (Honěk, 1993; Hahn and Denlinger, 2007). It was surprising to find that the thermal requirements for adult emergence in this experiment were approximately 500 degree days higher (see Figure 7B) than for all other experiments. One difference was that puparia for this experiment were kept individually in gelatin capsules rather than grouped in Petri dishes. This likely lowered the relative humidity, a factor which has been shown to increase the thermal requirement of *R. indifferens* (Yee 2013a).

From our study of post-diapause development of *R. completa* we have been able to demonstrate that pre-chill duration, chill duration and puparial weight are among the most important factors that influence the propensity for adult emergence and associated thermal requirement. Additional factors that we did not include in our study are soil temperature, moisture and the importance of ground cover and irrigation (Kasana and AliNiazee 1997, Yee 2013a, Yee and Chapman 2018). Since both ground cover and irrigation practices vary widely in walnut orchards in California these factors could also contribute to variation in thermal requirements and the timing of emergence of *R. completa* in commercial orchards.

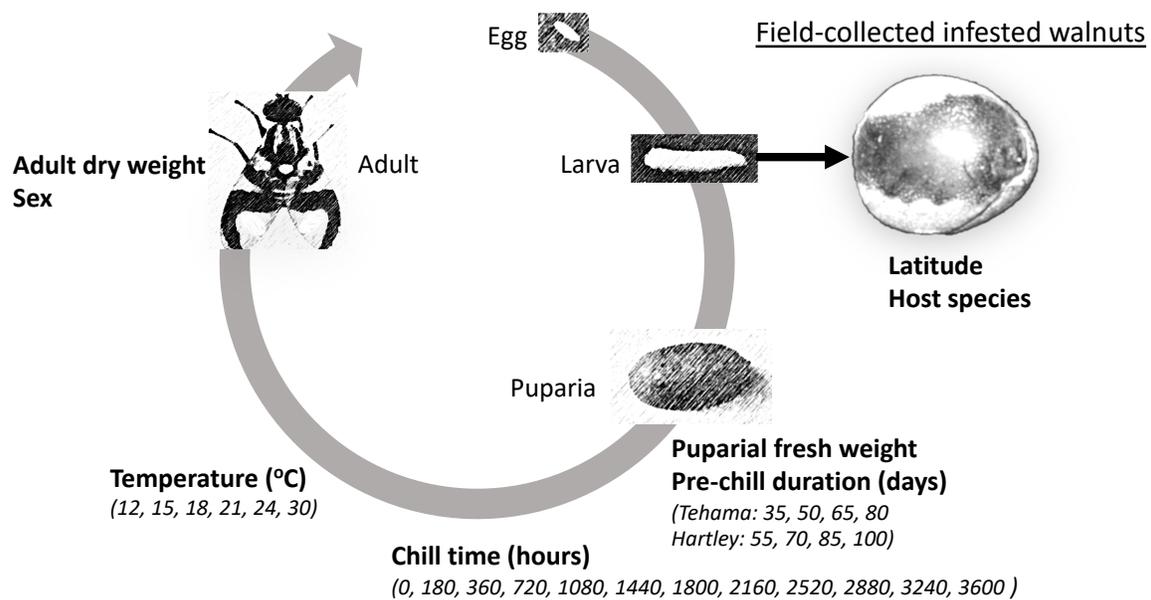


Figure 2.1 - A schematic illustration of the sequence of environmental factors examined in relation to the lifecycle and overwintering puparial diapause of *Rhagoletis completa*. Environmental factors are shown in bold with accompanying italicized text to indicate the levels used for those that were directly manipulated in the laboratory. Puparia for all experiments were collected from larval-infested walnuts in the field.

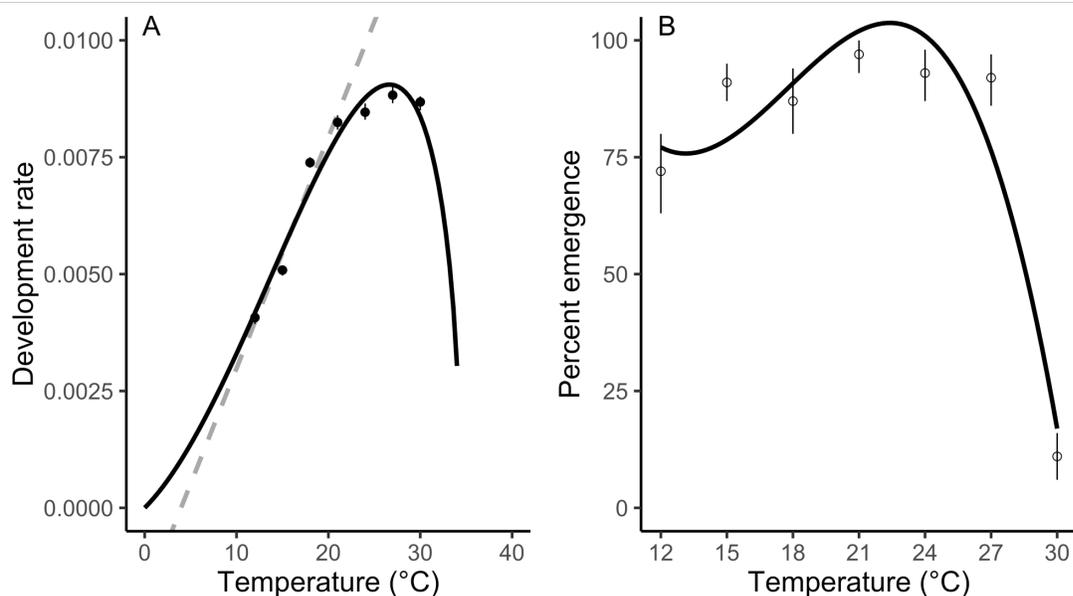


Figure 2.2 - The influence of incubation temperature on the post-diapause development of chilled puparia of *R. completa*, showing (A) mean development rate (\pm SE) fitted to a linear model for temperatures from 12 - 21°C (---), $y = 0.00049x - 0.00196$, $R^2 = 0.95$, $p < 0.001$, $n = 40$, and to a curvilinear Briere 1 model for all temperatures (—) with $a = 0.0000033$, $T_0 = -10.1$ °C, $T_L = 34.4$ °C, adjusted $R^2 = 0.94$, $p < 0.001$, $n = 64$, and (B) mean percent adult emergence (\pm SE) fitted to a third order polynomial curve, $y = 401.83 - 61.73x + 3.73x^2 - 0.07x^3$, adjusted $R^2 = 0.8$, $p < 0.001$, $n = 70$.

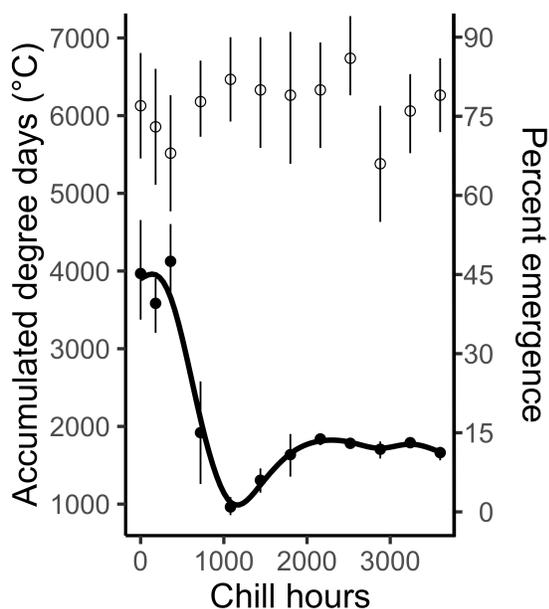


Figure 2.3 - The influence of chill duration on the post-diapause development of *Rhagoletis completa* showing mean thermal requirements (accumulated degree days) ($\bullet \pm$ SE) fitted to a GAM curve (adjusted $R^2 = 0.75$, $p < 0.001$) and mean percent adult emergence ($\circ \pm$ SE) ($y = 0.77 + 0.0002x$, $R^2 = -0.006$, $p = 0.58$, $n = 120$).

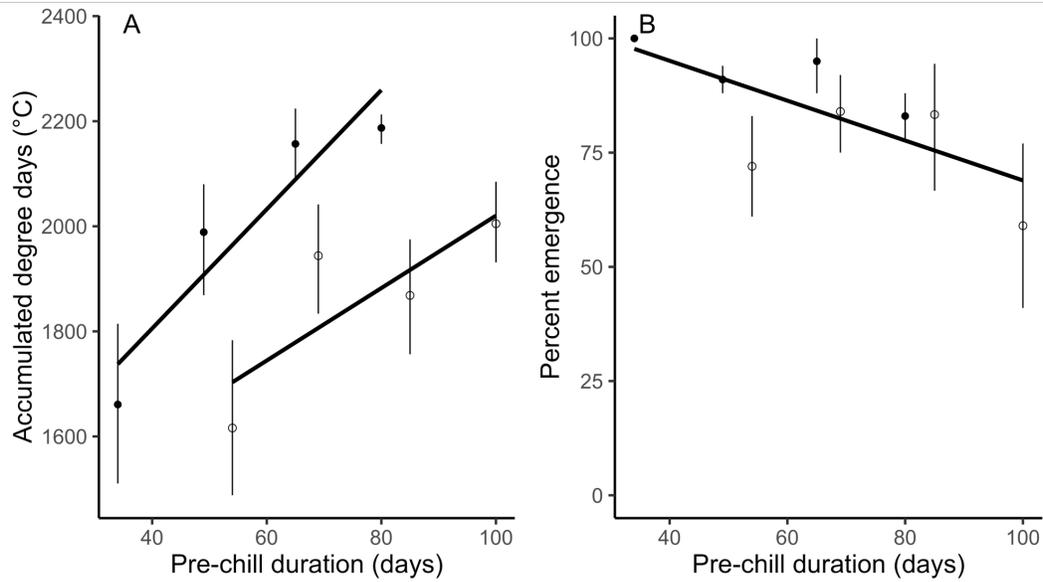


Figure 2.4 - The influence of pre-chill duration on the post-diapause development of *Rhagoletis completa* showing (A) mean thermal requirements (accumulated degree days) (\pm SE) fitted to separate linear models for two walnut cultivars, Tehama (\bullet) $y = 11.1x + 1352.1$, $R^2 = 0.52$, $p < 0.001$, $n = 40$ and Hartley (\circ) $y = 7.4x + 1283.5$, $R^2 = 0.31$, $p < 0.001$, $n = 39$, and (B) mean percent adult emergence (\pm SE) fitted to a pooled linear model $y = -0.4x + 112.6$, $R^2 = 0.19$, $p < 0.001$, $n = 79$.

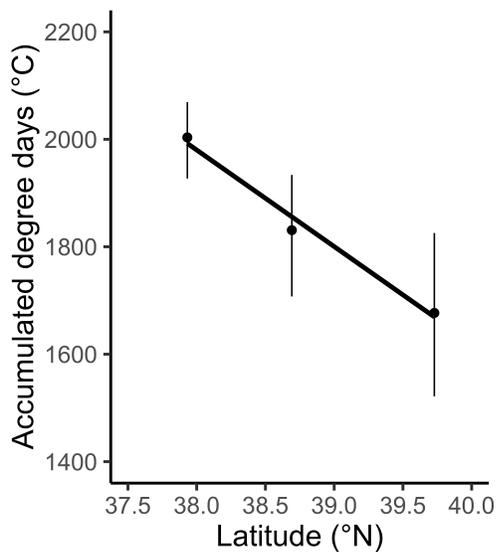


Figure 2.5 - The influence of latitude on the mean thermal requirement (accumulated degree days) (\pm SE) for post-diapause development of *Rhagoletis completa* fitted to a linear model $y = -179.8x + 8811.1$, $R^2 = 0.32$, $p = 0.001$, $n = 30$.

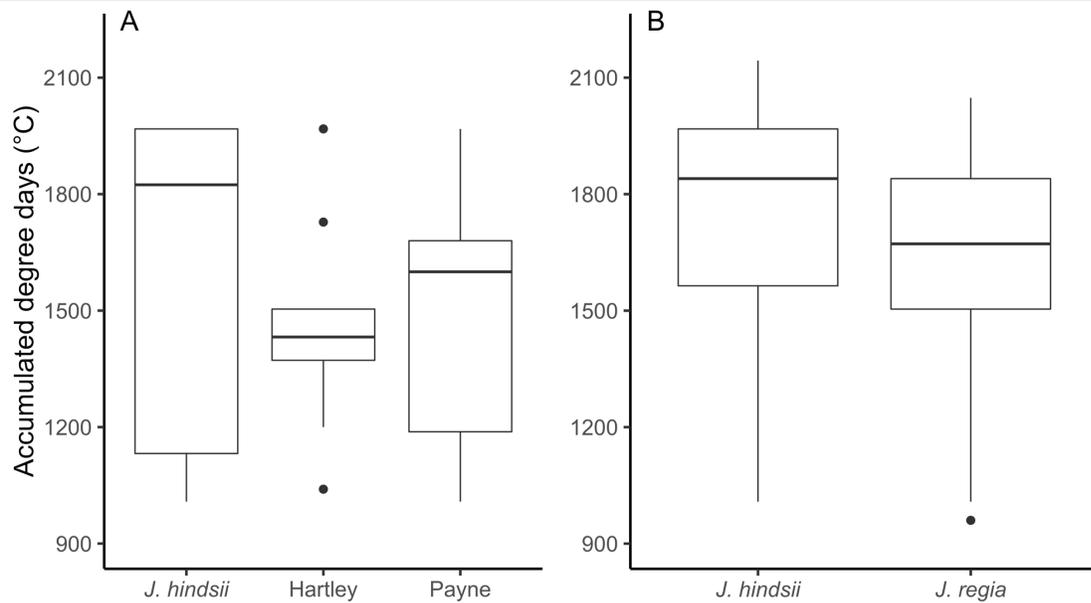


Figure 2.6 - The influence of walnut cultivar and species on the thermal requirement (accumulated degree days) for post-diapause development of *Rhagoletis completa* for (A) puparia from infested fruit collected from the same location on the same date (ANOVA, $F = 0.5$, $df = 2,27$, $p = 0.6$) and (B) puparia from *Juglans hindsii* versus *J. regia* pooled across dates with location as a random effect ($y = -134.4x + 1706.9$, $R^2 = 0.04$, $p = 0.04$ where $x = 0$ for *J. hindsii* and $x = 1$ for *J. regia*).

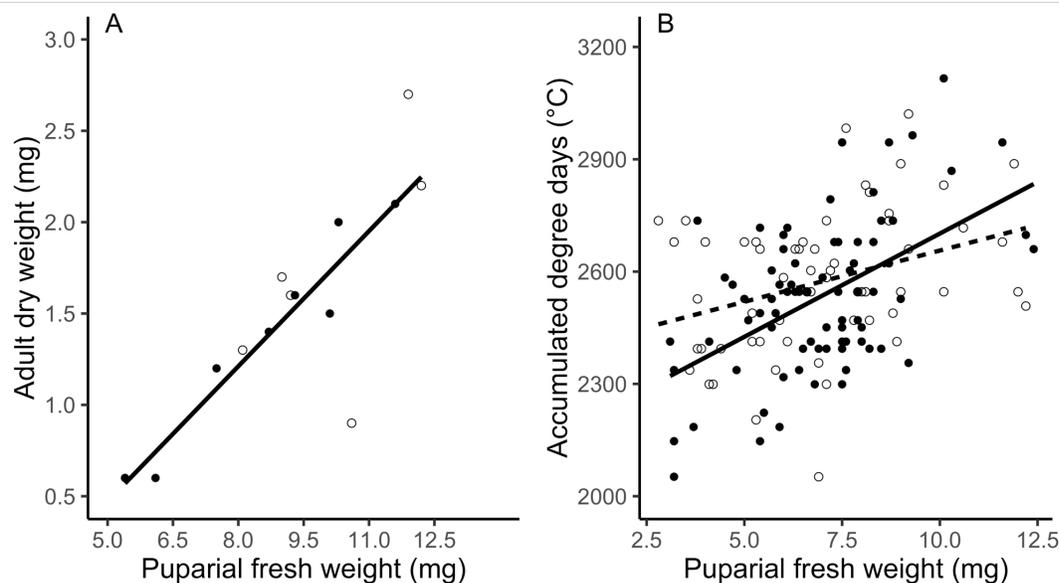


Figure 2.7 - Relationships for *Rhagoletis completa* between (A) puparial fresh weight and adult dry weight for pooled females (•) and males (◦) ($y = 0.25x - 0.76$, $R^2 = 0.68$, $p < 0.001$), and (B) puparial fresh weight and the thermal requirement for adult emergence (accumulated degree days) for females (• —) ($y = 55.2x + 2150.1$, $R^2 = 0.26$, $p < 0.001$) and males (◦ - -) ($y = 27.4x + 2382.5$, $R^2 = 0.1$, $p = 0.009$).

Chapter 3: Land-use intensity benefits agricultural pests at multiple scales, generalist predators respond locally

Abstract

As agricultural production has intensified on the local scale over the last century, landscapes have also become increasingly homogenized. Insect pests and natural enemies respond to land-use intensity at varying scales. This study analyzes the effects of land-use intensity at both landscape and local scales on the main insect pests, *Spodoptera frugiperda* and *Rhopalosiphum maidis*, the most abundant predators, Coccinellidae, and yield in grain sorghum as a summer crop in the southern hemisphere. Landscape intensity was evaluated using the proportion of annual crops within 1km of field sites. Local intensity included a multi-year component, a less intense crop-grazing rotation versus a more intense continuous crop rotation, and a within-year component based on insecticide use and winter crop cultivation prior to summer sorghum. Piecewise structural equation models were used to compare the direct and indirect effects of management at these different scales over a two-year period in 59 sorghum fields in western Uruguay. Greater landscape intensity increased densities of both pest species and provided evidence that they may interact indirectly through apparent commensalism leading to decreased coccinellid abundance. At a local scale, crop-grazing rotational systems had lower coccinellid species richness, winter crop cultivation caused a higher proportion of non-native coccinellid individuals, and insecticide use increased *R. maidis* density. In contrast to other studies, coccinellid species evenness was reduced with greater land-use intensity. The use of structural equation models provided a robust analysis of the effects of land-use intensification on insect communities in a lesser known multi-year management system and an under-studied region.

Introduction

Agricultural crops and livestock pastures cover approximately 38% of the available land surface globally (Smith et al. 2014b), and agricultural production has continued to increase in intensity over the past 300 years (World Resources Institute 2005). An important consequence of the intensification of management practices in agricultural ecosystems is the negative effect on biodiversity and ecosystem services (Tscharntke et al. 2005, Flynn et al. 2009, Molina and Perrachon 2010, Landis 2017). The species richness and abundance of vertebrates, invertebrates and plants are 40% lower in intensively managed agricultural landscapes than in landscapes less associated with human use (Newbold et al. 2015).

Agricultural intensification can occur at different spatial scales including a landscape scale and a local scale. Greater land-use intensity at a local scale, with shorter crop rotation cycles, decreased crop diversity and increased inputs is often correlated with greater landscape intensity, characterized by fragmented natural habitat and increasingly homogeneous land-use types (Tscharntke et al. 2005, Margosian et al. 2009, Ramankutty et al. 2018). Consequently, it is important to distinguish between local and landscape intensity factors when evaluating

downstream effects on biodiversity and ecosystem services. The goal of this study was to evaluate the effects of agricultural intensification at local and landscape scales on pest densities, predator evenness and origin (native/non-native), and crop yield in South America, specifically the Paysandú region in western Uruguay.

Land-use intensity at a local scale encompasses within-year management practices like pesticide-use, fertilizer inputs and crop rotation frequency as well as multi-year management practices (e.g. organic vs. conventional). Insecticide-use can significantly depress generalist predator abundance and alter the composition of predator communities (Lee et al. 2001, Roubos et al. 2014). Increased fertilizer use can also have negative effects on predator to prey ratios and species diversity (Zhao et al. 2015). In many cases lower intensity alternative management strategies can increase the level of biodiversity supported by agricultural systems (Tscharntke et al. 2005, Larsen and Noack 2017, Lichtenberg et al. 2017, Ponisio et al. 2017), but see Kleijn et al. (2011). It has been posited that increased biodiversity will only lead to greater biological control when the relative abundance of predator species is sufficiently even to provide functional complementary in hunting strategies (Crowder and Jabbour 2014). Such an effect has been demonstrated through a trophic cascade in potato fields where greater predator evenness increased pest mortality and resulted in higher yield (Crowder et al. 2010). The effects of organic versus conventional farming on natural enemy biodiversity seem to be context dependent, however, as one meta-analysis has demonstrated a consistent effect of greater evenness for organic farms (Crowder et al. 2012), while another has shown the opposite (Lichtenberg et al. 2017). Another multi-year management system, more commonly found in Uruguay than organic management, is the rotation between agricultural crops and pasture for livestock grazing. It involves winter and summer crops planted in sequence for 2-3 years followed by 2-4 years of grazing. The land-use intensity of this crop-grazing rotation is lower than for continuous crop rotation alone, and has been shown to reduce soil erosion and support a higher diversity of macrofauna (Molina and Perrachon 2010). Though the effect on arthropods at a local scale is unknown, other studies have shown that sheep grazing in dryland grain production systems can reduce pest abundance and support higher parasitoid populations (Goosey et al. 2005, 2013). Thus the crop-grazing rotation in Uruguay presents a valuable opportunity to examine the direct and indirect effects of rotational systems (Goosey et al. 2013, Crowder and Jabbour 2014) on the balance between pests and natural enemies in an agricultural crop in the southern hemisphere.

Intensification at a landscape scale is also known to have effects on insect communities such as distorting arthropod communities in favor of a few dominant species (Matson et al. 1997; Hillebrand, Bennett, and Cadotte 2008), and altering food web structure (Tylianakis, Tscharntke, and Lewis 2007). The percentage of landscape under cultivation is often used as a measure of landscape land-use intensity (Rand and Tscharntke 2007, Caballero-López et al. 2012, Tuck et al. 2014, Gagic et al. 2017). Homogenization of the landscape can increase habitat connectivity and population density for pest species (Meehan et al. 2011, Rand et al. 2014, Balzan et al. 2016). It can also reduce the biological control services provided by natural enemies (Bianchi et al. 2006, Gardiner et al. 2009a, Perović et al. 2010, Rusch et al. 2014). The crop-grazing rotation system in Uruguay is similar to the 6-year ley perennial grass/crop rotation in Sweden, a study of which showed that the biological control of pests increased with the proportion of ley in the landscape (Rusch et al. 2013). Other research has found that the species evenness of spiders can be negatively related to landscape scale factors, such as percent grazing area (Rusch et al. 2014).

Non-native coccinellids are often disturbance specialists whose establishment is facilitated by human-modified landscapes (Greze et al. 2013). Studies in both North and South America have shown that non-native coccinellid species negatively affect native coccinellid diversity and abundance (Harmon et al. 2007, Greze et al. 2016). Gardiner et al. (2009b) found that native coccinellids were most abundant in agricultural fields in landscapes with an abundance of grasslands surrounding the agricultural habitats. Most landscape studies have been carried out in the northern hemisphere (Chaplin-Kramer et al. 2011, Winqvist et al. 2011, Papanikolaou et al. 2017, Karp et al. 2018), however, and so this study from Uruguay adds an important contribution from an underrepresented region (New and Samways 2014).

There is a link, especially for mobile arthropods, between the landscape matrix and biodiversity conservation efforts at a landscape scale, and ecosystem services and agroecological management practices at a local scale (Perfecto and Vandermeer 2010, Power 2010, Tscharrntke et al. 2012, Gagic et al. 2012, Liere et al. 2017). The Paysandú region of western Uruguay presents a range of landscape homogeneities and intensities of local land use from which to study these interdependent relationships (Berretta 2006). Across South America, soybean production has been steadily increasing for the past four decades (Schnepf et al. 2001) homogenizing the landscape in step with global trends (Margosian et al. 2009, Ramankutty et al. 2018). In Uruguay production grew from just 7,600 ha of soybean harvested in 1996 to 1,140,000 ha harvested in 2016, despite the acreage of land in production having decreased over the same time period (Food and Agriculture Organization of the United Nations 2018). In addition, between 2002 and 2013 insecticide and fertilizer use for agriculture in Uruguay both jumped eight-fold (Food and Agriculture Organization of the United Nations 2018) indicating an intensification of agricultural land management. There have been efforts to support soil conservation and sustainable agriculture, however, with over 1 million ha under no-till management in 2013 (Kassam et al. 2015) and 2.9 million ha managed as a multi-year crop-grazing rotation that includes soybean (Ferrari et al. 2013).

The focus of this study is on sorghum, a less intensively managed alternative to soybean as a summer crop in both the multi-year crop-grazing rotation and continuous agriculture systems of the Paysandú region of Uruguay in which *Rhopalosiphum maidis* and *Spodoptera frugiperda* are the two main pest species and Coccinellidae is an abundant family of generalist aphid predators. Observations from a series of sorghum fields collected over two years were used to examine the effects of three different aspects of agricultural intensification at a landscape and local scale using structural equation modeling. Landscape intensity was quantified as the proportion of the landscape under annual crops within a 1km radius of sorghum fields. Greater landscape intensification was expected to positively correlate with local pest density (Rand et al. 2014) and to reduce the abundance of coccinellids and favor non-native species (Chaplin-Kramer et al. 2011, Greze et al. 2013). At a local scale, intensification was represented in this study by both a multi-year effect of production system (crop-grazing rotation versus continuous crop rotation) and a within-year effect of management decisions (fallow/grazed versus winter crop prior to sorghum planting, and whether insecticides were used). We expected the greater intensity of a multi-year continuous crop rotation to result in both higher pest densities and a greater proportion of non-native Coccinellidae. The greater intensity of within-year management decisions (winter crop grown before sorghum and use of insecticides) were expected to lead to increased pest densities and decreased coccinellid species evenness. This research informs our

understanding of land-use intensity at both local and landscape scales on sorghum pest densities and aphid natural enemy communities in a more unique agricultural rotation (Crowder and Jabbour 2014).

Materials and methods

Field sites and land-use intensity

To evaluate both local and landscape level effects of land-use intensity on pest and natural enemy abundance in sorghum as a summer crop in the Paysandú region of Uruguay a total of 59 sites were sampled over a period of two summer field seasons in 2016 and 2017. None of the sorghum fields were irrigated and the majority were under no-till systems. To address the effect of landscape level land-use intensity the extent of the landscape planted to annual summer crops was used. For local level land-use intensity two measures were used, the multi-year effect of the type of agricultural rotation system, and the within-year effects of insecticide use and planting a winter crop prior to sorghum. Thirty-three sites were under continuous crop rotation and 26 sites were under a crop-grazing rotation.

For landscape level land-use intensity the proportion of land under annual agriculture was estimated using the Plans of Use provided to the authors as GIS shape files from the Ministry of Agriculture (Ministerio de Ganadería, Agricultura y Pesca or MGAP) for the corresponding planting season. Plans of Use data were available for only 41 of the 59 sites, however, and so for the other 18 sites the extent of annual agriculture was estimated from Google Earth satellite images for 2015 and 2016 followed up by ground-truthing for each site (Midega et al. 2014, Plečáček et al. 2014). The software program QGIS (QGIS Development Team 2018) was used to delimit a 1 km radius around each site, a distance found to be most relevant in other landscape studies for both aphids and general predators (Thies et al. 2005, Rand and Tscharntke 2007, Winqvist et al. 2011, Caballero-López et al. 2012, Rusch et al. 2014). The proportion of land under annual crops was analyzed as a continuous variable (Zhao et al. 2014) ranging from less than 0.01 to 0.76 over the 59 sites.

Multi-year local level land-use intensity was categorized based on the whether a site was managed under a continuous crop rotation or under a crop-grazing rotation. Sites were classified as using a continuous crop rotation if they had been under annual cultivation without periods of grazing for five or more years. Seasonal fallow periods did not alter this categorization. Sites were classified as using a crop-grazing rotation if they had been used for grazing for a minimum period of three years and subsequently transitioned to annual crops a maximum of one year before sampling. In addition, these sites were planted to an annual crop a maximum of once post-grazing and prior to sorghum planting (either a summer crop followed by winter fallow or cover crop, or a winter crop only).

Within-year local level land-use intensity for each site was evaluated from both the use of insecticides on the sorghum crop that was sampled and from planting of a winter cereal crop immediately prior to the summer sorghum planting (as discussed in Tscharntke et al. 2005). Insecticides applied to the sorghum crop were either organophosphates or benzoylureas targeting

S. frugiperda larval populations. Peak abundance of *S. frugiperda* occurred early in the growing season and all growers who applied insecticides did so only once prior to the sampling period used in this study. Winter crops planted prior to the summer sorghum included oat, wheat and barley. If the field was grazed, fallow or planted to cover crops during the preceding winter it was categorized as not having a winter crop.

Sampling of field sites

The summer sorghum crops at each site were sampled within a 10 x 10 m sampling universe located at least 8 m from the edge of the field (following Jonsson et al. 2012) every three weeks from December to April each year. To estimate densities of the two key sorghum pests present at each site every 10th plant along two 10 m transects within the sampling universe was visually inspected on each sampling date and counts were recorded of *R. maidis* (all instars) and *S. frugiperda* larvae per plant. The abundance and diversity of aphidophagous coccinellid predators at each site were monitored using three half-sized yellow sticky traps (Alpha Scents, Inc., West Linn, OR) placed on 2 m high posts attached just above plant height and arranged along the diagonal of the sampling universe. Sticky traps were used to sample the predators as they have been shown to both collect more species and more mobile life stages than destructive or field-count sampling methods (Schmidt et al. 2008). Counts of adult coccinellids on yellow sticky traps from the same site and date were pooled and predator abundance was estimated as mean number of Coccinellidae per trap for the sorghum yield SEM. For the coccinellid evenness SEM only sites in which all traps were present were included and total abundance was used. Adult coccinellids were identified and the abundance data was used to calculate rarefied species richness, evenness and the proportion of non-native individuals at each site as described below.

In addition to sampling the insect pests and predators at each field site, sorghum yield (kg/ha) for each site was provided by pest control advisors from a variety of organizations (Copagran, Unicampo, Facultad de Agronomía UdelaR, Terra Nova, Alcoholes de Uruguay (ALUR), and Molino Americano). Planting date, measurements of plant vigor, rainfall and temperature were also included as potential explanatory variables in the analysis. Planting date was standardized over the two years using a discrete scale from 1 to 51 to correspond to the range of planting dates from 25 October to 15 December. For plant vigor a mean chlorophyll content index (CCI) was measured on young leaves from three representative sorghum plants along the two transects at each site using a chlorophyll meter (Apogee CCM-200, Apogee Instruments, Logan, Utah). Measurements were taken from when the fifth leaf was visible at all sites to the soft dough stage (Vanderlip and Reeves 1972), after which the chlorophyll content of leaves declines significantly as the plant transfers resources to the head of grain (Oyier et al. 2017). The CCI (a scale from 0-100 representing the transmission of light at 931/635 nm) has been shown to correlate well with plant nitrogen status (Richardson et al. 2002), although water stress and aphid infestation can negatively affect the CCI (Schepers et al. 1996, Golawska et al. 2010). Daily rainfall (mm) and maximum and minimum temperatures were obtained from three weather stations; all field sites were within 50 km of one of these weather stations. Data from the Glencoe Experimental Station and the Salto weather station was provided by the National Institute of Agro-fishery Research in Uruguay (INIA). Data from the Paysandú weather station was provided by the Mario A. Cassinoni Research Station (EEMAC) associated with the University of the Republic (UdelaR). The standard deviation of total rainfall between sampling

dates was used as a measure of seasonal rainfall variation. Rainfall in January was also evaluated as it varied greatly between years and sites. The number of days with a maximum temperature higher than 34 °C was used as a measure of extreme temperatures above which the juvenile development rates and survivorship of *R. maidis* and *S. frugiperda* decline (Kuo et al. 2006, Early et al. 2018).

Other variables that were measured at each site, but ultimately proved to be nonsignificant as explanatory factors in the analysis included carabid beetle abundance, field size, latitude and a soil productivity index using information from the National Commission for the Agronomic Study of Land (CONEAT) (Ministerio de Ganadería Agricultura y Pesca, 2016).

Data analysis

The statistical environment of R was used for all data analyses (R Core Team 2017) and the direct and indirect effects of landscape and local level land-use intensity on sorghum crop yield and coccinellid evenness were investigated using structural equation models (SEMs) (Jonsson et al. 2012, 2015). The R packages nlme (Pinheiro et al. 2018) and piecewiseSEM (Lefcheck 2016a) were used for SEM development and analysis. Piecewise SEMs (pSEMs) were used as they have relaxed sample size requirements and can manage nested and non-normally distributed data (Lefcheck 2016b). The pSEMs were generated from a set of component linear models (LM) or linear mixed effects models (LME) that were developed through standard model reduction using AIC and step-wise removal of non-significant variables to evaluate path directionality and strength of direct effects (Shipley 2000). The assumption of normality was visually checked with Q-Q plots of component model residuals and Shapiro-Wilks tests (Thode 2002). Any models for which rainfall SD or days above 34 °C significantly improved the fit, an LME was used in place of an LM to include weather station as a random effect accounting for the lack of independence among sampling sites. Both the marginal and conditional R^2 values are reported for any component model that included random effects. Cascading LMs and LMEs were pieced together using confirmatory path analysis to estimate a full causal model with indirect as well as direct effects. To determine standardized path coefficients raw coefficients were scaled using the standard deviation of x divided by the standard deviation of y (Lefcheck 2016b). Fischer's C statistic, based on p-values calculated from a test of directed separation, was used to evaluate full model fit (Shipley 2009). A test of directed separation evaluates whether missing paths, that is paths not defined in the component linear models, are significant ($p < 0.05$), but it controls for indirect effects already in the full model. A chi-squared test of Fischer's C with $p > 0.05$ indicates that the full model represents the data well and no missing paths contribute explanatory power. Alternately, for $p < 0.05$ the full model does not represent the data sufficiently and missing paths improve fit. If significant missing paths were identified the variables were considered for ecological significance. If a case for ecological significance could be made, the alternate model was compared using AIC. If the alternate model had an AIC value more than two below the original model, the alternate model with its additional specified path was accepted. Two separate pSEMs were developed and analyzed, one for crop yield and a second for coccinellid evenness.

Development of sorghum yield SEM

Three sample periods were used for this analysis, covering the period of peak abundance of the two key sorghum pests. Of the 59 sites sampled over the two-year period, only 49 had sufficient data available for all factors included in the sorghum yield pSEM. A set of five hierarchical linear models were used to capture the hypothesized causal relationships for sorghum yield (see Fig. 1a, Table 1). Linear models were developed for the density of the two key pests and for coccinellid abundance, CCI and sorghum yield. Coccinellid abundance and pest densities were log transformed to reduce the influence of outliers and to normalize the data (Beduschi et al. 2015). To account for observed zeros in the pest data, prior to transformation a constant equal to the square of the first quartile divided by the third quartile was added to the ranked observations (following Burton et al. 2016). The following factors were found to have significant effects in one or more of the hierarchical linear models used to develop the sorghum yield pSEM: proportion of land under annual agriculture within a 1 km radius (landscape intensity), insecticide use and the presence of a winter crop prior to sorghum (local within-year intensity), standardized sorghum planting date, standard deviation of accumulated rainfall between sampling periods and number of days above 34 °C. To give greater power to the analysis of some of the more important causal relationships revealed by the pSEM, a larger data set, that included some of the sites which were initially rejected due to absence of sufficient data for all factors, was used to further analyze the effects of specific factors alone.

Development of coccinellid evenness SEM

Only sites in which no traps were missing during the three sample dates that covered the peak period of coccinellid trap capture were used for this analysis and the total number of adults for each aphidophagous species from the nine traps was used for each site. Of the 59 original sample sites, 54 had sufficient information available for all factors included in the coccinellid evenness pSEM. As both species richness and evenness of predators can be differently affected by land-use intensity (Crowder and Jabbour 2014) a set of three hierarchical linear models were developed to capture the hypothesized causal relationships for coccinellid evenness (see Fig. 5a, Table 2). These included models for the proportion of non-native coccinellids, coccinellid species richness and coccinellid species evenness. Hill numbers were used to estimate rarefied/extrapolated species richness ($q = 0$) and evenness ($q = 1$) in the R package iNEXT (Hsieh et al. 2018). Species richness can accurately be extrapolated to twice the minimum number of individuals, while species evenness can reliably be extrapolated to the asymptote (Chao et al. 2014). Consequently, the sample size for interpolation/extrapolation was set at 62 for each site representing twice the minimum number of individuals captured at a single site. The following factors had a significant effect in one or more of the linear models used to build the pSEM: continuous crop rotation (local multi-year intensity), winter crop present (local within-year intensity), rainfall in January and coccinellid abundance.

Results

Sorghum yield SEM

Landscape and/or local land-use intensity factors had significant effects on four of the five component linear models used for the sorghum yield SEM, the exception being the model for CCI (Table 1). For the final pSEM (Figure 3.1b, AIC = 100.6) a Fischer's C statistic of 42.6 (df = 50, $p = 0.76$) provided evidence of a good fit to the data and directed separation tests indicated no missing paths (factors with $p < 0.05$). The proportion of land under annual agriculture within a 1km radius around field sites, a metric for landscape intensity, had a significant positive effect on *S. frugiperda* density as well as sorghum yield (Figures 3.1b, 3.2a,c). It also appeared to have a similar effect on *R. maidis* density in a univariate LM (Figure 3.2b), although the significance of this was lost in the context of the mixed effects model (Table 3.1). The relationship was further supported by an LME based on the larger sample size of the full ($n = 59$) rather than reduced ($n = 49$) data set ($y = 3.37x + 0.64$, $R^2 = 0.07$, $p = 0.04$).

Multi-year local land-use intensity (continuous crop versus crop-grazing rotations) appeared not to have any significant effects on the sorghum yield SEM based on the reduced data set used in the development of the pSEM ($n = 49$). The larger sample size of the full data set ($n = 59$) revealed however, that *R. maidis* density was positively related to *S. frugiperda* density in continuous crop rotations, but not in crop-grazing rotations (Figure 3.3a). Conversely, coccinellid abundance was negatively related to *S. frugiperda* density for both types of rotations (Figure 3.3b), such that *R. maidis* density was negatively related to the ratio of coccinellid abundance to *S. frugiperda* density in continuous crop rotations, but not in crop-grazing rotations (Figure 3.3c).

As a local within-year component of land-use intensity, insecticide use has no effect on *S. frugiperda* density, but positive effects on both *R. maidis* density and coccinellid abundance (Table 3.1, Figure 3.1b). In addition, further analysis of a larger data set suggested that for sites with insecticide use there was no relationship between landscape level land-use intensity and *S. frugiperda* density ($y = 0.38x - 1.74$, $R^2 = 0.002$, $p = 0.84$, $n = 30$), but a significant positive relationship for sites with no insecticide use ($y = 3.04x - 3.11$, $R^2 = 0.17$, $p = 0.04$, $n = 25$). Winter crop presence resulted in greater *S. frugiperda* density (Figure 3.4a) and later planting date also resulted in higher *S. frugiperda* and *R. maidis* densities (Figures 3.4b,c). Planting date was significantly later for sites with winter crop present (mean = 34 ± 2.4), than for sites with no winter crop (mean = 24 ± 2.3 , $F = 8.57$, $df = 1,47$, $p = 0.005$), with no significant interaction in the component LME model.

Seasonal variation in rainfall had a strong negative effect on both the CCI and sorghum yield. In contrast, the number of extreme heat days (above 34 °C) had a positive effect on CCI, but a negative effect on both *S. frugiperda* and *R. maidis* densities (Figure 3.1b).

Coccinellid evenness SEM

Landscape land-use intensity was not a significant effect in any of the component models of the coccinellid evenness SEM, but local effects were significant in two of the three models

(Table 3.2). The final pSEM (Figure 3.5b, AIC = 43.75) had a Fischer's C statistic of 9.75 (df = 14, $p = 0.78$) indicating a well specified model and directed separation tests showed no missing paths (factors with $p < 0.05$). The multi-year land-use intensity effect of continuous crop rotation had a direct positive effect on coccinellid species richness (Figure 3.6a) that also led to an indirect increase in evenness (Figure 3.5b). The presence of a winter crop prior to sorghum, the only local within-year metric of land-use intensity that had any effect on the component linear models (Table 3.2), caused a greater proportion of non-native coccinellids (Figure 3.6b). Again, a cascading effect of local land-use intensity on coccinellid evenness is mediated by a direct effect on the proportion of non-native individuals (Figure 3.5b). Other factors that had an influence on the coccinellid community were coccinellid abundance and January rainfall. Greater coccinellid abundance had a positive effect on coccinellid evenness and January rainfall had a negative effect on coccinellid species richness, evenness and the proportion of non-native individuals (Figure 3.5b).

Discussion

The primary objective of this study was to evaluate the effects of landscape and local scale land-use intensity on pest densities and their cascading effects on yield in sorghum as a summer crop in the Paysandú region of Uruguay and the structure of the associated coccinellid community over a two-year period. More annual crop acreage within a 1 km radius, representing greater landscape land-use intensity, had a positive influence on sorghum yield. This effect was likely due to a latent variable like soil quality. Though we included a general soil productivity index in our initial analysis, it proved not to be significant in any of the component linear models and may not have been sufficient to capture the complexity of soil quality and suitability for sorghum production. Greater landscape land-use intensity also resulted in higher *S. frugiperda* density as found in other studies of pests with high dispersal ability in increasingly homogeneous landscapes (Andow 1983, Tschardt et al. 2005). This was not the case for *R. maidis* density, however. It is also possible that 1km was not the ideal spatial scale to examine landscape effects on *R. maidis* densities, as aphids have been shown to respond to landscape factors up to 6 km away (Thies et al. 2005). Alternately, since aphid populations were generally low and highly variable, the sample size may have been inadequate to capture the nuance of a landscape intensity effect on *R. maidis* density. The greater multi-year local land-use intensity in continuous crop rotations also resulted in higher coccinellid species richness which may be due to an increased number of non-native species and individuals that thrive in more highly disturbed systems. It has often been suggested that non-native species benefit from habitat disturbance (Lozon and Macisaac 1997, Hierro et al. 2006), and Grez et al (2013) found that more disturbed agricultural crops had both a greater number of non-native coccinellid species and a greater proportion of non-native individuals.

The most surprising finding from this research, however, was a direct negative effect of *S. frugiperda* density on the abundance of aphidophagous coccinellids and the absence of a direct link between *R. maidis* density and coccinellid abundance. As these predators are not known to feed on lepidopteran larvae, no causal effects between *S. frugiperda* and coccinellids were considered. One hypothesis, which we did not test directly, is that the herbivore-induced plant volatiles (HIPVs) induced by *S. frugiperda* disrupted the HIPVs induced by *R. maidis*. One of

the main volatiles induced in plants by aphid feeding is also a component of the aphid alarm pheromone, (E)- β -farnesene (EBF). Several coccinellid species have been shown to respond, both behaviorally and physiologically, to EBF (Nakamuta 1991, Zhu et al. 1999, Abassi et al. 2000). The aphid alarm pheromone can be effective as a small-scale local attractant for coccinellid larvae, but any longer-range attraction of coccinellid adults would likely be in response to EBF as an HIPV since aphids don't emit enough EBF for long-range detection (Vosteen et al. 2016). A comprehensive metanalysis showed that chewers, like *S. frugiperda*, consistently induce greater quantities of HIPVs than sap feeders, like *R. maidis* (Rowen and Kaplan 2016). Furthermore, research in a comparable system, with both a lepidopteran and aphid pest, revealed a reduction in aphid parasitism when lepidopteran densities exceeded 0.5 larvae/plant (Blubaugh et al. 2018). Though Blubaugh et al. (2018) did not find the same effect for generalist aphid predators in their study, HIPV disruption of aphid predators has been observed in laboratory studies of indirect interactions in lepidopteran-aphid systems (Ponzio et al. 2016, 2017). Our finding that *S. frugiperda* and *R. maidis* densities were positively related in continuous crop rotations adds further support to the dual-herbivore facilitation hypothesis of Blubaugh et al. (2018) that is based on predator-mediated apparent commensalism. A further consequence of this relationship is that for sites with continuous crop rotations and lower *S. frugiperda* densities, coccinellid abundance was higher and *R. maidis* populations appeared to be better controlled.

It was interesting that the positive relationship between *S. frugiperda* density and landscape land-use intensity disappeared for sites with insecticide use. It is unclear however, whether insecticide use disrupted the link between *S. frugiperda* populations and the landscape or whether at higher densities *S. frugiperda* populations no longer had a relationship with the landscape and insecticide use was required. As a within-year measure of local land-use intensity insecticide use early in the season aimed at control of *S. frugiperda* did result in increased *R. maidis* densities. Secondary pest outbreaks, facilitated by early-season use of insecticides, are a well-known phenomenon (van den Bosch et al. 1973, Andow 1983, Crowder and Jabbour 2014), but in our study coccinellid abundance was also positively associated with insecticide use. Consequently, this finding seems to stand in contrast to other landscape research in which insecticide use has been shown to disrupt effective pest control by natural enemies (Hill et al. 2017). Evidence for secondary pest increase due to insecticide use aimed at *S. frugiperda* does seem evident in this system, however, possibly due to disruption of other aphid predators, as *R. maidis* populations reached greater abundance in sites that used insecticides.

The last within-year metric for intensity that we evaluated was the presence or absence of a winter crop grown in the season prior to sorghum planting. The finding that the greater intensity of winter crop presence resulted in higher *S. frugiperda* densities and higher proportions of non-native aphidophagous coccinellid individuals was not wholly unexpected. *Spodoptera frugiperda* has no diapausing stage and is unable to reproduce at mean temperatures below 10 °C (Luginbill 1928). Consequently, populations do not survive year-round in Uruguay and the furthest south that year-round populations have been found is 27 °S (Murúa and Virla 2004), almost 10 ° north of the study region. *Spodoptera frugiperda* migrate south each spring to regions in which they have seasonal populations (Mitchell et al. 1991) that have been found to be higher in density and more damaging on late-planted summer crops (Ayala et al. 2014). Since the presence of winter crop cultivation was significantly related to later spring planting date for

sorghum in this study and both had significant positive effects on *S. frugiperda* densities it is likely that later planting date provided younger, higher quality, plants for the return migration of *S. frugiperda* populations. In addition, the higher proportion of non-native coccinellid individuals associated with the presence of a winter crop seems likely to be linked to the propensity of non-native species to benefit from more highly disturbed environments as found in another study of coccinellid populations in South America (Grez et al. 2013).

Two aspects of local land-use intensity (agricultural rotation and presence of winter crop) seemed to affect the coccinellid communities in sorghum more than the landscape effects of land-use intensity (proportion of acreage under annual crops within a 1 km radius). This finding is consistent with other research that has found local effects to be more important than broader landscape effects in shaping generalist predator communities (Sarthou et al. 2014, Rusch et al. 2014). In our study greater species richness and proportion of non-native coccinellid species both contributed to greater species evenness. This finding contrasts with other research on land-use intensity effects on natural enemy communities which has found greater evenness associated with lower land-use intensity (Crowder et al. 2010, Mace and Mills 2017) and may have resulted from non-native species, which benefit from higher local intensity, reducing the dominance of native species and creating greater evenness. Greater species evenness within a single predator family, however, may not contribute to complementarity as much as evenness across different natural enemy groups in enhancing biological control (Crowder and Jabbour 2014). It is likely that the hunting mode of aphidophagous coccinellid species is very similar and consequently, that just a few better adapted native species might have become dominant in less intensively managed sorghum fields. Other research has found that simpler food webs, with lower species richness, can also result in higher predation rates (Gagic et al. 2011). This lends support to the perspective that greater evenness across a predator group with high functional redundancy, such as aphidophagous coccinellids, might actually impede the provisioning of biological control services (Martin et al. 2013).

The negative effect of *R. maidis* density on CCI is consistent with previous research demonstrating a direct negative effect of aphid feeding on chlorophyll a, chlorophyll b and total chlorophyll (Burd and Elliott 1996). It is also worth noting the variation in rainfall both among sites and between years is likely indicative of the effects of El Niño - Southern Oscillation (ENSO) coupled weather patterns. During the first sampling season (December 2015 - March 2016) the ENSO event was considered quite extreme (Hu and Fedorov 2017) and the conditions in Paysandú, Uruguay were unusually dry and warm during the sampling period, though the spring and fall were extremely wet, consistent with past ENSO effects found in the region (Bidegain and Krecl 1998).

The results from this study provide further evidence that greater land-use intensity benefits agricultural pests at both local and landscapes scales and that generalist predator communities are most susceptible to intensification at the local scale. In addition, it adds to discussions of under what conditions predator evenness is of importance for effective biological control and how intensification can have unexpected effects on community interactions like the predator facilitated apparent commensalism between *S. frugiperda* and *R. maidis*. In a broader context it also adds information from an under-studied multi-year management system and an under-studied geographic region.

This study presents a framework for parsing the effects of local and landscape level intensification on insect abundance and diversity in agricultural crops. The use of structural equation models provided a robust analysis of the effects of land-use intensification on insect communities in sorghum as a summer crop, but the low sample size still imposed some restrictions on the analysis. Efforts like the PREDICTS database (Hudson et al. 2016), a central repository of data from studies across managed and unmanaged landscapes that address questions of land-use intensity, might provide a more comprehensive way to increase predictive power and disentangle the mechanisms driving the complex direct and indirect relationships observed in agroecosystems. Landscape studies will almost always suffer from sample size constraints and using aggregate data would help to more accurately quantify the effects of environmental change and the cascading effects of land-use intensity.

Table 3.1 - Direct effects of environmental and land-use intensity factors on the dependent variables of the five component models used for the piecewise SEM for sorghum yield (NA = not applicable, NS = non significant, CCI = Chlorophyll content index).

Component model	R^2_m	R^2_c	Intercept	S_f density	R_m density	CCI	Proportion annual crop	Insecticide use	Winter crop	Planting date	SD seasonal rainfall	Days > 34 °C
<i>S. frugiperda</i> density (ln)	0.45	0.71	-3.20	NA	NS	NS	2.11	NS	0.81	0.04	NS	-0.11
<i>R. maidis</i> density (ln)	0.60	0.60	-0.77	NS	NA	NA	NS	1.11	NS	0.10	NS	-0.17
Coccinellid abundance (ln)	0.23	NA	0.84	-0.21	NS	NA	NS	0.41	NS	NS	NS	NS
CCI	0.36	0.67	31.38	NS	-0.69	NA	NS	NS	NS	NS	-0.17	1.20
Yield (kg/ha)	0.36	0.57	3214.00	NS	NS	58.3	2007.90	NS	NS	NS	-9.60	NS

Table 3.2 - Direct effects of environmental and land-use intensity factors on the dependent variables of the three component models used for the piecewise SEM for coccinellid evenness (NA = not applicable, NS = non significant).

Component model	R^2_m	R^2_c	Intercept	Coccinellid abundance	Coccinellid richness	Proportion non-native	Continuous agriculture	Winter crop	January rainfall
Coccinellid richness	0.18	0.18	5.71	NS	NA	NS	1.05	NS	-0.01
Proportion non-natives	0.23	0.27	0.04	NS	NS	NA	NS	0.05	-0.0005
Coccinellid evenness	0.71	0.71	1.15	-0.004	0.23	4.80	NS	NS	-0.003

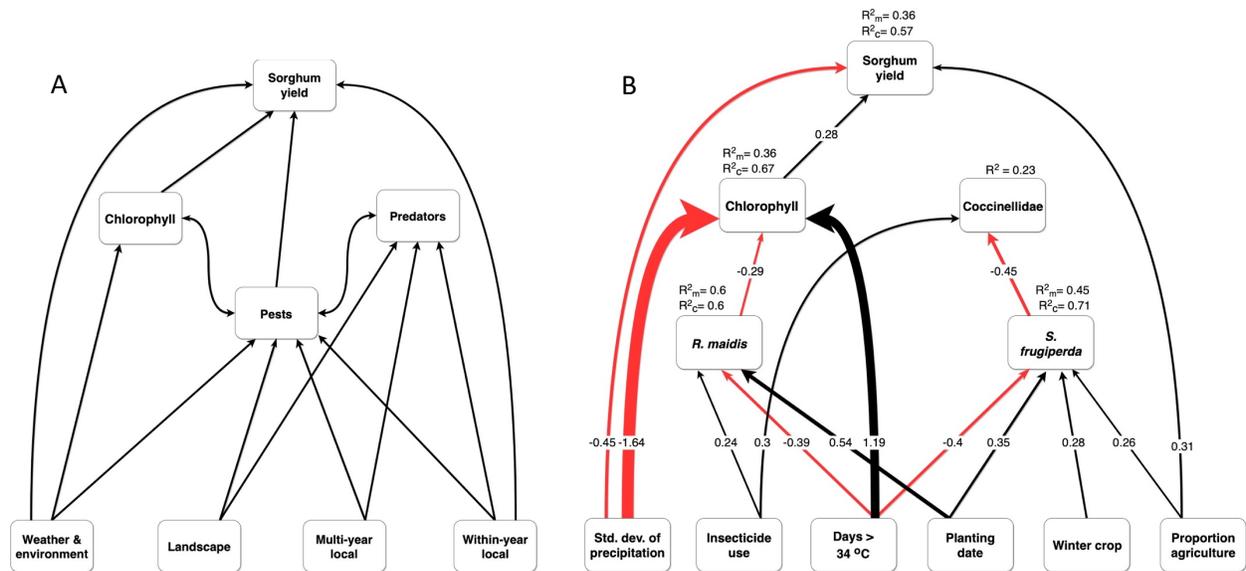


Figure 3.1 - Environmental and land-use intensity (landscape and local) effects for the sorghum yield structural equation model (SEM). The diagrams show (a) initial hypothesized relationships that were tested in the preliminary analyses and (b) the direct and indirect effects of explanatory factors on yield in the final pSEM. Red arrows indicate a negative effect, black arrows a positive effect and the width of the arrow corresponds to the standardized path coefficient for each significant effect ($p \leq 0.05$). Marginal and conditional R^2 values are given for all component LMEs in the final pSEM.

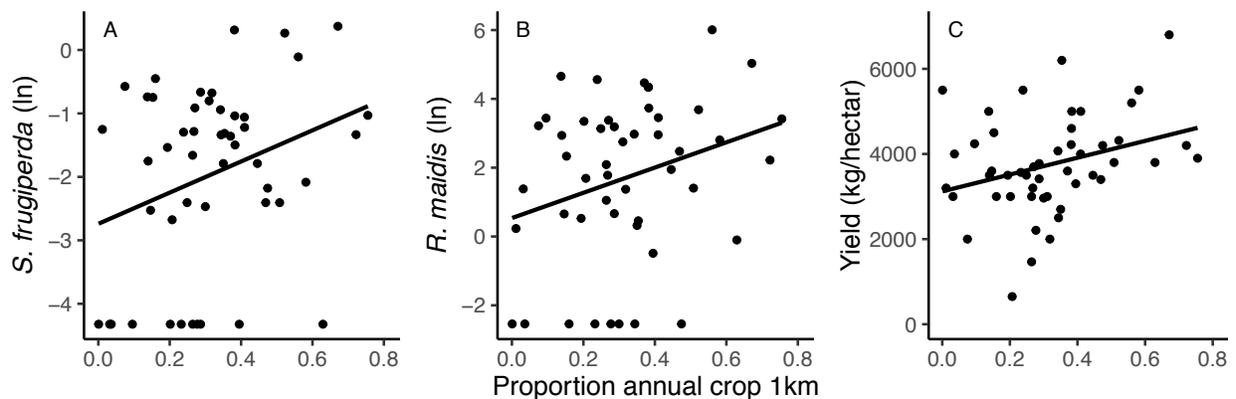


Figure 3.2 - The influence of landscape land-use intensity (proportion of land under annual agriculture within in 1km of each site, $n = 49$) on a) *Spodoptera frugiperda* density ($y = 2.46x - 2.74$, $R^2 = 0.09$, $p = 0.03$), b) *Rhopalosiphum maidis* density ($y = 3.67x + 0.54$, $R^2 = 0.08$, $p = 0.05$), and c) sorghum yield ($y = 1978x + 3120.5$, $R^2 = 0.09$, $p = 0.03$).

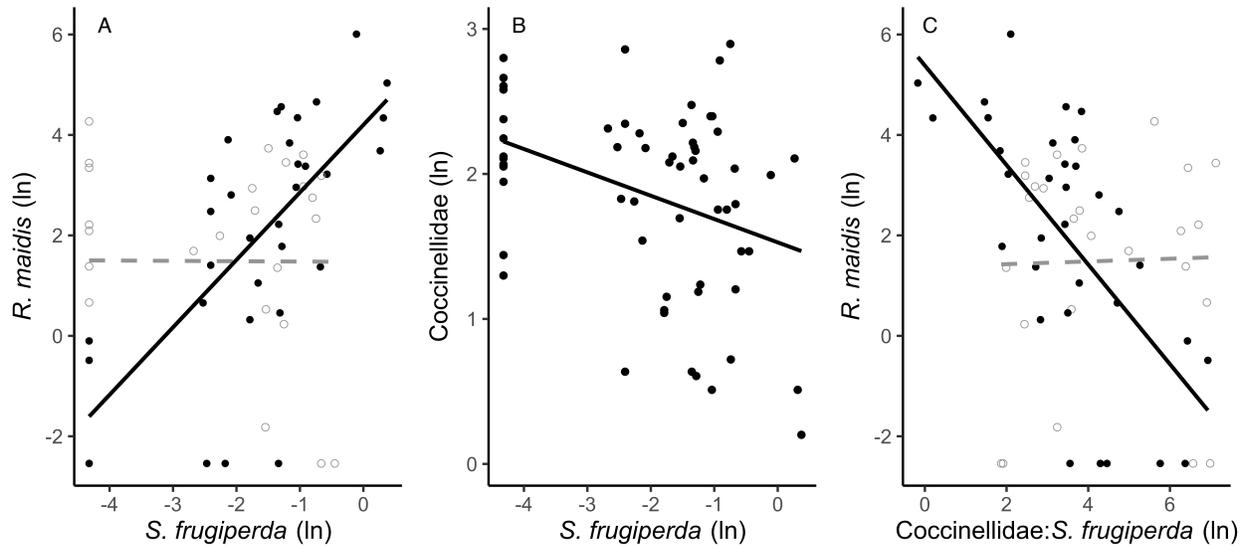


Figure 3.3 - The influence of *S. frugiperda* density on a) *R. maidis* density in continuous crop rotations (filled circles and solid line, $y = 1.34x + 4.2$, $R^2 = 0.48$, $p < 0.001$, $n = 33$) and in crop-grazing rotations (open circles and dashed line, $y = -0.007x + 1.47$, $R^2 < 0.001$, $p = 0.98$, $n = 26$), and b) coccinellid abundance for both types of rotation ($y = -0.18x + 1.53$, $R^2 = 0.14$, $p = 0.008$, $n = 49$). In c) the relationship between *R. maidis* and the ratio of coccinellid abundance to *S. frugiperda* density is shown for continuous crop rotations (filled circles and solid line, $y = -0.99x + 5.38$, $R^2 = 0.43$, $p < 0.001$, $n = 33$) and for crop-grazing rotations (open circles and dashed line, $y = 0.03x + 1.37$, $R^2 < 0.001$, $p = 0.91$, $n = 26$).

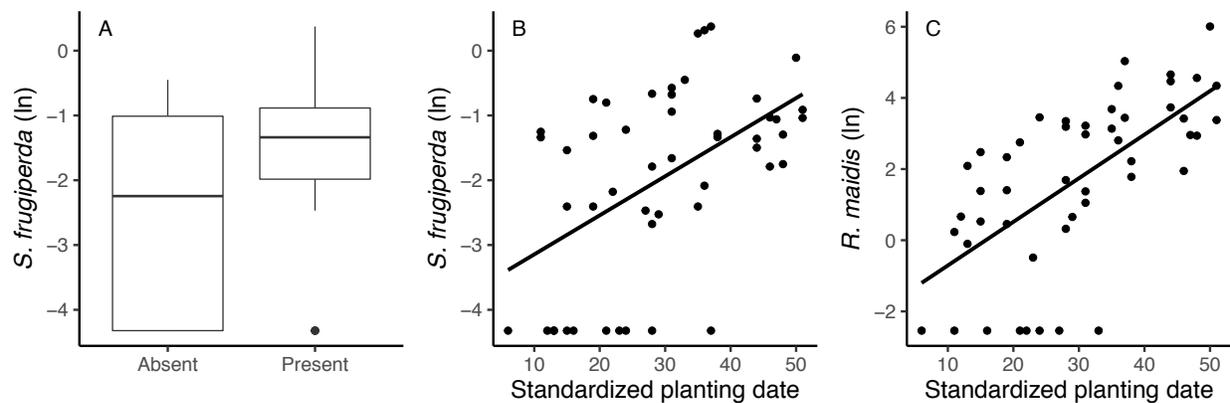


Figure 3.4 - The influence of a) the presence of a winter crop on *S. frugiperda* density ($F = 5.92$, $df = 1,47$, $p = 0.03$, mean winter crop absent = -2.46 ± 0.33 $n = 22$, mean winter crop present = -1.54 ± 0.24 $n = 27$) and of standardized planting date ($n = 49$) on b) *S. frugiperda* density ($y = 0.06x - 3.97$, $R^2 = 0.24$, $p < 0.001$), and c) *R. maidis* density ($y = 0.12x - 1.94$, $R^2 = 0.42$, $p < 0.001$).

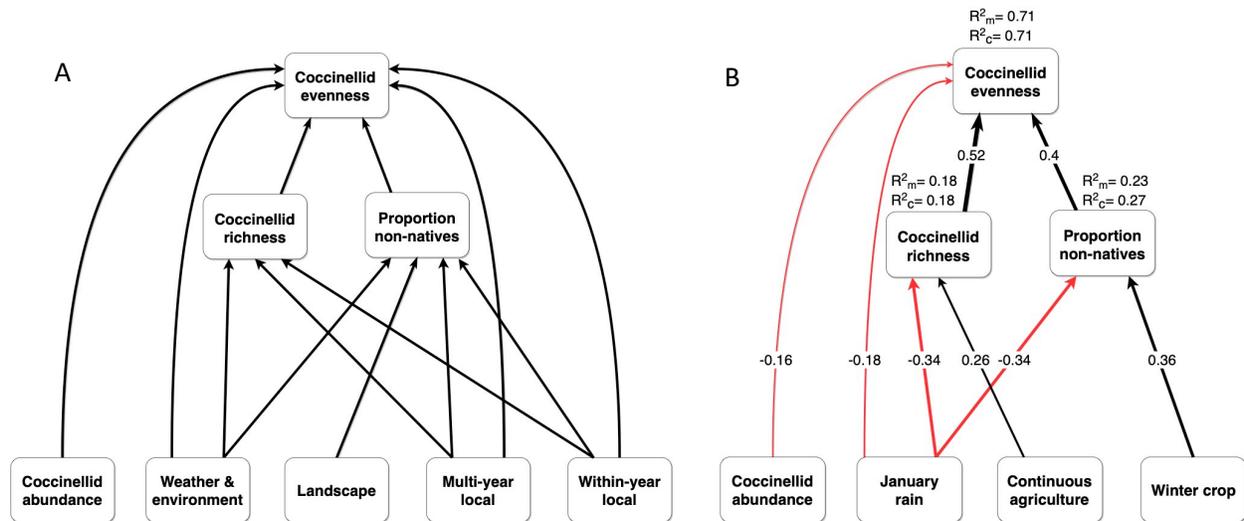


Figure 3.5 - Environmental and land-use intensity (landscape and local) effects for the coccinellid evenness structural equation model (SEM). The diagrams show a) initial hypothesized relationships that were analyzed in the preliminary analyses and b) the direct and indirect effects of explanatory factors on coccinellid evenness in the final pSEM. Red arrows indicate a negative effect, black arrows a positive effect and the width of the arrow corresponds to the standardized path coefficient for each significant effect ($p \leq 0.05$). Marginal and conditional R^2 values are given for all component LMEs in the final pSEM.

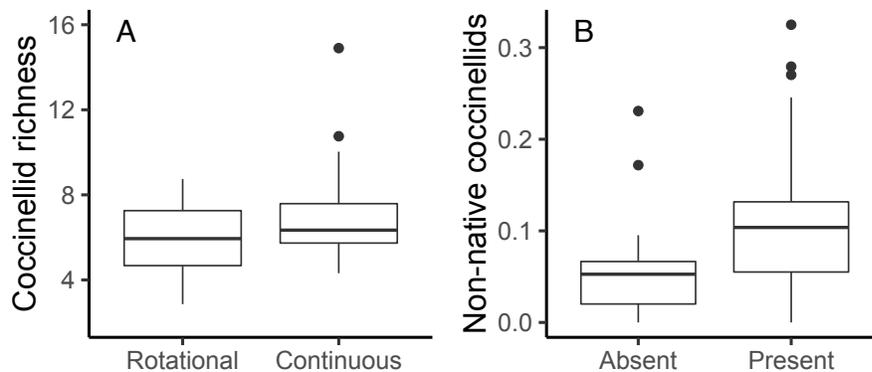


Figure 3.6 - The influence of local land-use intensity on coccinellid communities showing a) the multi-year influence of crop-grazing rotation ($n = 23$) versus continuous crop rotation ($n = 31$) on coccinellid species richness ($F = 3.62$, $df = 1,52$, $p = 0.06$), and b) the within-year effect of the absence ($n = 27$) or presence ($n = 27$) of a winter crop prior to sorghum on the proportion of non-native coccinellids ($F = 9.28$, $df = 1,52$, $p = 0.004$).

Chapter 4: Predation pressure and habitat complexity alter indirect interactions between two aphid species

Abstract

Generalist predators are increasingly recognized as important contributors to the reliability of conservation biological control. In addition, indirect interactions between prey species sharing a common predator are increasingly recognized as drivers of community dynamics. Though the impact of generalist predators has been studied frequently, it is often in the context of a single prey species with either single or multiple predator species. With their broad diet, however, consideration of how indirect interactions between prey species can affect biological control impacts is critical. Cage experiments with *Acyrtosiphon pisum* and *Aphis gossypii* were performed with varying levels of predation by adult *Hippodamia convergens* as well as varying levels of habitat complexity (numbers of plants per cage). Across predation pressure the early growth of *A. gossypii* appears beneficial to the net change in abundance of *A. pisum* over a period of 8 days, however the early growth of *A. pisum* provides no explanatory power for the net change in abundance of *A. gossypii*. A linear mixed effects model including early growth of both aphid species and predation pressure explained 96% of the variation in the net change in abundance of *A. pisum* and 92% of the variation in net change in abundance of *A. gossypii*. Both increasing predation pressure and decreasing habitat complexity appear to change the sign and strength of indirect interactions between *A. gossypii* and *A. pisum*.

Introduction

Numerous studies have addressed the importance of native predator and parasitoid assemblages in providing conservation biological control of herbivorous pests (Straub et al. 2008, Jonsson et al. 2015, Mace and Mills 2017, Rusch et al. 2017), and the question of whether interactions among multiple generalist predators can limit control through intraguild predation or add to it through complementarity (Ferguson and Stiling 1996, Brodeur and Rosenheim 2000, Snyder and Ives 2003, Costamagna et al. 2008, Martin et al. 2013, Gontijo et al. 2015, Tschardt et al. 2016, Riggi et al. 2017, Roubinet et al. 2017, 2018). However, most studies that have evaluated the biological control potential of generalist predators have focused on the impact of either single predators or predator assemblages on the population growth of a single focal prey species (Grevstad and Klepetka 1992, Obrycki et al. 1998, Donaldson et al. 2007, Gardiner and Landis 2007, Madadi et al. 2011). Another aspect of the biological control potential of generalist predators, less frequently considered, is their impact on a focal prey species in the inevitable context of multiple prey species (but see Koss et al., 2004). Multiple prey species may either coexist in the same crop or occur in perimeter vegetation as well as in a field crop. Whenever multiple prey species exist, however, both the direct effects of predation on multiple prey populations and the indirect effects of predators on community dynamics must be considered (Denno et al. 1995, Alhmedi et al. 2011).

Populations of two prey species that do not compete directly for resources can nonetheless interact indirectly via a shared predator. These indirect effects are common, can range from positive to negative and can be either symmetrical or asymmetrical between the two prey species. Apparent competition, the negative effect of one prey species on the population of another through a shared natural enemy (Holt 1977), is an important indirect interaction that can structure insect communities through both time and space (Blitzer and Welter 2011). Apparent amensalism (Chaneton and Bonsall 2000) in which one species is negatively affected but not the other, and apparent commensalism (Dethier and Duggins 1984) whereby one species benefits but not the other have also been found in systems with multiple prey species. Finally, apparent mutualism is a positive effect between prey species when the impact of a shared predator is limited and the two prey species effectively buoy each other to mitigate the negative effects of predation (Abrams and Matsuda 1996). Though often neglected, some research suggests that the effects of apparent mutualism in shaping community dynamics have been underestimated (Tack et al. 2011, Ims et al. 2011). Theory suggests that mutually positive and mutually negative indirect effects are the extremes of a continuum and that transition between them in a single community can be mediated by frequent shifts in the transient dynamics of consumer and resource populations (Abrams and Matsuda 1996). Food or space limitation and predator density are thought to be the factors responsible for changes in the strength and directionality of indirect effects. Most of the research on indirect interactions represent snapshots in time (Lawler 1993, Muller and Godfray 1997, Blitzer and Welter 2011, Blubaugh et al. 2018) and have not evaluated the possibility that such interactions within a community can range from positive to negative over time and under different environmental circumstances (but see Long et al., 2012).

The main goal of this study is to evaluate the effect of variable predation pressure on the population growth of two prey species and to examine whether the early growth rate of one prey species can add explanatory power to the change in abundance of the other. It is also an initial attempt to evaluate some of the factors predicted to affect the sign and strength of indirect interactions between two prey species that share a common predator. *Acyrtosiphon pisum* Harris and *Aphis gossypii* Glover (Hemiptera: Aphididae) are aphid pests that often cause damage in agricultural crops both directly and as vectors of disease (Blackman 2000, Capinera 2001). *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) is an important predator of both aphid species (Eigenbrode et al. 1998, Flint and Dreistadt 1998). Both predation pressure and habitat complexity (plants/cage) were manipulated in laboratory cage experiments to determine how the combined effects of predation by *H. convergens* and indirect interactions between the aphid species influenced their population growth. Increasing predator abundance and habitat complexity are hypothesized to determine the sign and strength of the indirect interaction between *A. pisum* and *A. gossypii* (Figure 4.1). By increasing the abundance of *H. convergens* and altering habitat complexity we seek to determine whether it is possible to shift the indirect interactions between the two aphid species from negative to positive.

Materials and methods

A series of laboratory cage experiments were performed to examine the direct and indirect effects of habitat complexity and predation pressure on the population growth and dynamics of *A. pisum* and *A. gossypii*. The two aphid species were collected from urban gardens in Berkeley, CA during the spring of 2015. Colonies of both species were kept separately in a greenhouse at 18 °C and reared on potted *Vicia faba* plants. *Hippodamia convergens* were obtained from Rincon-Vitova Insectaries, Inc. (Ventura, CA) as overwintering batches of 500 adults that were stored in a cold room at 5 °C. Before each experiment the adult beetles were transferred to an incubator at 18 °C in individual Petri dishes lined with moist filter paper and, after 48 h, vigorously active individuals were selected for experiments.

An initial experiment was conducted to evaluate whether *H. convergens* shows any prey preference between *A. gossypii* and *A. pisum*. A single adult *H. convergens* was placed in a 22 ml translucent polystyrene cup (SOLO, Highland Park, IL) with wet filter paper and 20 adults of each aphid species. A set of 36 replicate cups were kept at 18 °C over a 24 h period to compare the number of *A. gossypii* and *A. pisum* that remained alive, using a t-test.

Predation pressure and habitat complexity were varied in two series of experiments. The experiments were conducted in organdy-sided sleeve cages (1 x 1 x 1m) in an insectary room that was held at 20 °C and a 16 h light: 8 h dark photoperiod. Inside the cages 30-40 cm tall broad bean (*Vicia faba*) in individual 10 cm plastic pots provided a food source for both aphid species and were watered every second day. For each 8-day experiment every cage was started on day 0 with 40 4th instar and adult (mixed) *A. pisum* and an equal number of the same instars of *A. gossypii* with the aphid species on separate potted bean plants. Aphids were subsequently counted on day 4 and day 8 of each experiment and recorded as total number of each species per cage. Each predation pressure and habitat complexity treatment consisted of 15 replicates carried out in blocks of five cages initiated on each of three separate weeks. Order of blocks was randomized between experiments. To reduce the likelihood of direct interaction between the aphid species, plants were placed equidistant from each other in two rows on either side of the cage with one aphid species assigned randomly to plants to either side. At the beginning of each experiment plants touched neither each other nor the sides of the cages.

Predation pressure

To evaluate the effect of predation pressure four treatments with 0, 1, 5 or 10 adult *H. convergens* per cage and an initial prey population of 40 aphids of each species were used. Each cage had eight potted bean plants and ten *A. pisum* or *A. gossypii* were placed on the apical meristem of each plant on day 0.

Habitat complexity

In a separate set of experiments predation pressure was held constant at five adult *H. convergens*, while habitat complexity was simplified with fewer plants. To evaluate the effect of habitat complexity three treatments were used with eight, four, or two potted bean plants per cage such that the initial aphid density per plant was 10, 20 or 40, respectively.

Statistical analysis

All statistical analyses were conducted using version 3.4.2 of R (R Core Team 2017). Linear mixed effects models were developed using the function `lmer` of the `lme4` package (Bates et al. 2015) to evaluate the direct effect of predation pressure and early aphid growth (from day 0 to day 4) as well as any indirect effects of early growth (day 0 - 4) by the other aphid species on net change in abundance of each aphid species (from days 0 to day 8) over the course of the experiment. As some effects appeared nonlinear, models including second order polynomials were compared to models with linear effects only using AIC. A second order polynomial was used to evaluate the effect of number of *H. convergens* per cage on net change in abundance for each aphid species. Block was included in all models as a random effect.

To evaluate the sign and strength of indirect effects both the early growth (day 0 – 4) and the net change (day 0 – 8) in abundance of *A. pisum* per cage was compared to that of *A. gossypii* and linear regression was used to evaluate the relationship for each level of both predator pressure and habitat complexity. The ratio of *A. gossypii* to *A. pisum* in each treatment level of predation pressure was also compared to the same ratio in the absence of predation using a t-test. Similarly, the ratio of *A. gossypii* to *A. pisum* in the two lower habitat complexity treatments were compared to the ratio in the high complexity treatment with a t-test.

Results

There was no significant difference in the number of *A. pisum* adults (mean = 9.2 ± 3.8) and *A. gossypii* adults (mean = 9.8 ± 3.3) that remained alive after 24 h, $t = -0.7$, $p = 0.5$ indicating that *H. convergens* had no preference for either species of aphid prey. In the absence of predation *A. gossypii* exhibited exponential growth, but the population growth of *A. pisum* appeared linear (Figure 4.2). On day 4 of the experiment, the abundance of *A. pisum* was consistently higher than that of *A. gossypii* except for the highest level of predation pressure (ten adult *H. convergens* per cage). However, by day 8, the abundance of *A. gossypii* was always higher. Every direct comparison between the abundance of *A. gossypii* and *A. pisum* on day 4 and day 8 was significantly different as determined by t-tests, except day 8 with one *H. convergens* (*A. pisum* mean = 558.2 ± 239.2 , *A. gossypii* mean = 727.3 ± 353.8 , $t = -0.7$, $p = 0.1$) and day 8 with five *H. convergens* (*A. pisum* mean = 130.4 ± 138.8 , *A. gossypii* mean = 230 ± 152.8 , $t = -1.8$, $p = 0.07$) in which the abundance of the two aphid species was statistically equivalent.

Predation pressure

Both early growth of *A. pisum* and early growth of *A. gossypii* had significant positive effects on the net change in abundance of *A. pisum* over the course of the experiment (Figure 4.3a). In addition, the effect of predation pressure from *H. convergens* on the net change in abundance of *A. pisum* was described by a quadratic polynomial (Figure 4.3b, Table 4.1). The effect of predation pressure was better described by a second order polynomial (AIC = 704) than by a linear relationship (AIC = 720) ($X^2(1, 60) = 18.7$, $p < 0.001$).

For the net change in abundance of *A. gossypii*, both early growth of *A. pisum* and early growth of *A. gossypii* had significant positive effects (Figure 4.3c), but in the linear mixed effects model only early growth of *A. gossypii* provided significant explanatory power (Table 4.1). The effect of *H. convergens* pressure on *A. gossypii* net change in abundance was best described by a quadratic polynomial (Figure 4.3d). Again, the effect of predation pressure was better described by a second order polynomial (AIC = 816) than by a linear relationship (AIC = 821) ($X^2(1, 60) = 7.4, p = 0.007$).

At low predation pressure the early growth of *A. gossypii* appeared to be negatively related to the early growth of *A. pisum* (Figure 4.4a). Though not significant, this is the only early growth or net change in abundance relationship between aphid species that indicated a change in sign from negative to positive with increasing predation pressure. For the five and ten adult *H. convergens* treatments the relationships between the early growth of each aphid species were increasingly positive, though the 10 *H. convergens* treatment lacks significance (Figures 4.4b,c). As predation pressure increased, the net change in abundance of *A. pisum* had a stronger positive relationship with the net change in abundance of *A. gossypii* (Figures 4.4 a-c), although the relationship at low predation pressure (one adult *H. convergens*) was not significant (Figure 4.4a). The ratio of the early growth of *A. gossypii* to that of *A. pisum* in the absence of predation was 0.8 ± 0.2 , indicating that the *A. pisum* population grew more rapidly than that of *A. gossypii* during this phase of the experiment. The ratios for one adult *H. convergens* ($t = 1.1, p = 0.3$) and five adult *H. convergens* ($t = 1.4, p = 0.2$) were statistically equivalent, but a significantly higher ratio of *A. gossypii* to *A. pisum*, 24.0 ± 25.1 occurred with 10 adult *H. convergens* ($t = -3.6, p = 0.003$). The ratio of net change in abundance of *A. gossypii* to *A. pisum* in the absence of predation was 1.9 ± 0.7 , indicating that the *A. gossypii* population was generally more abundant than that of *A. pisum* by the end of the experiment. With one adult *H. convergens* this ratio was significantly lower at 1.3 ± 0.7 ($t = 2.4, p = 0.03$), but for five adult *H. convergens* the ratio was equivalent to that in the absence of predation ($t = -1.8, p = 0.1$). The ratio of the net growth in abundance at ten adult *H. convergens* was significantly greater at 19.4 ± 14.4 than in the absence of predation ($t = -4.7, p < 0.001$).

Habitat complexity

When predation pressure was held constant at five *H. convergens* per cage and habitat complexity (number of potted plants) was varied a nonlinear relationship was apparent between complexity and aphid abundance (Figures 4.5a,b). A second order polynomial provided a marginally better fit overall than a linear relationship for both *A. pisum* (AIC = 532 vs 540, $X^2(1, 45) = 3.2, p = 0.08$) and *A. gossypii* (AIC = 540 vs 543, $X^2(1, 45) = 4.9, p = 0.02$).

As habitat complexity decreased the net change in abundance of *A. pisum* had a weaker relationship with the net change in abundance of *A. gossypii* (Figures 4.6 a-c). When habitat complexity was at its highest with eight plants per cage there was a significant positive relationship between *A. pisum* and *A. gossypii* both during the early growth period and in the net change in abundance (Figure 4.6a). When there were only four plants these relationships became

less clear as they lacked significance for both early growth and net change in abundance, despite a positive trend (Figure 4.6b). With two plants per cage any marginal increase in the net change in *A. pisum* abundance disproportionately benefits *A. gossypii* (Figure 4.6c). The ratios of *A. gossypii* to *A. pisum* during the early growth period were significantly greater with four plants ($t = -2.9, p = 0.01$) and two plants per cage ($t = -3.3, p = 0.005$) than with eight plants per cage (0.7 ± 0.3). However, the ratios of the net change in abundance of *A. gossypii* to *A. pisum* were statistically equivalent to the eight-plant control (3.2 ± 2.8) for the four-plant ($t = -1.8, p = 0.1$) and two-plant ($t = -1.0, p = 0.3$) treatments, respectively, though the variance was quite large in both reduced complexity treatments.

Discussion

Establishing predator preference or lack thereof is a critical first step to making predictions about indirect interactions. Since there was no evidence for *H. convergens* preference between the two aphid species the likelihood for apparent mutualism was higher in this system (Bêty et al. 2002). This initial study, however, failed to consider foraging success in environments in which aphids were living on plants as a component of prey preference, although it is known to affect the predation efficacy of *H. convergens* (Grevstad and Klepetka 1992, Eigenbrode et al. 1998). This study also did not differentiate between a lack of preference by *H. convergens* for either prey species, in which we might expect *H. convergens* to consume all individuals on the plant that it finds first, and prey switching by *H. convergens* based on their relative abundance (Long et al. 2012). Since the abundance of *A. pisum* increased more quickly than that of *A. gossypii* early in the experiment, this distinction could actually be quite important in interpreting the results.

One of the goals of this study was to determine whether predation pressure and early population growth of both aphid species could reasonably predict the net change in aphid abundance from day 0 to day 8 of the experiment. Using a second order polynomial to explain the nonlinear effect of predation pressure the linear mixed effects models performed extremely well. It was surprising that the early growth of *A. pisum* did not contribute any explanatory power to the net change in abundance of *A. gossypii* considering that the *A. pisum* population grew significantly more quickly in the first half of the experiment than *A. gossypii* in all but the highest predation pressure treatment. Early growth of *A. gossypii*, however did contribute positively to the net change in abundance of *A. pisum*. This indicates that an apparent commensalism may be at play where *A. pisum* benefits from higher early growth of *A. gossypii* while *A. gossypii* is unaffected by the early growth of *A. pisum*. Additionally, habitat complexity seemed to be an important component since any decrease in complexity resulted in much higher consumption rates by the predator which certainly impacted the indirect interactions between aphid species.

The second goal of this study was to evaluate whether reduced levels of predator satiation and habitat complexity could change the sign and strength of the indirect interactions between *A. gossypii* and *A. pisum* and influence their net change in abundance. Across almost all predation pressure treatments the relationship between *A. gossypii* and *A. pisum* abundance was positive. In

general, greater predator pressure (decreased predator satiation, particularly for the ten adult *H. convergens* treatment) and fewer plants per cage (less complex habitat, particularly for the two plants per cage treatment) seemed to disproportionately benefit *A. gossypii* possibly indicating an asymmetrical apparent mutualism. These results should be treated with caution, however, as in almost all cages the abundance of *A. pisum* was reduced to 0 by day 8 in the less complex treatments (13 of 15 for the four plants per cage treatment and 14 of 15 for the two plants per cage treatment) and in the highest predation pressure treatment (11 of 15). If *A. pisum* is always driven to local extinction in less complex habitats and at higher predation pressure, this dynamic may, in fact, be apparent competition. At low predation pressure (one adult *H. convergens*) the relationship between *A. gossypii* and *A. pisum* was slightly negative. These data seemed to show a neutral relationship for this treatment level. However, as it was the only treatment that showed a negative relationship it deserves additional study. The fact that the inverse relationship between *A. pisum* and *A. gossypii* was more strongly negative may indicate asymmetrical apparent competition in which early growth of *A. gossypii* is more negative for *A. pisum* than the reverse. Since these relationships were not significant, however, additional experiments are needed to further assess whether the sign of the indirect effects between the aphid species can change under high predation pressure. In the lower predation pressure treatments (one and five adult *H. convergens* per cage) with high complexity (8 plants per cage) both aphid species were able to persist throughout the experiment and the final abundances were not significantly different between the species suggesting that they perhaps subsidized each other (apparent mutualism).

Though the ratios of the net change in abundance of *A. gossypii* to *A. pisum* stayed the same in less complex habitats, *A. pisum* was driven to local extinction in almost every replicate. This may indicate the hypothesized slide from apparent mutualism towards apparent amensalism whereby *A. pisum* was negatively affected by habitat simplification, but *A. gossypii* remained unaffected. Path analyses could also be helpful in disentangling interactions along this continuum (Wootton 1994).

This research would benefit from greater replication at low and intermediate levels of predation. Furthermore, in order to quantify the relative effect of *A. pisum* on *A. gossypii*, and vice versa, controls in which each aphid species is reared alone both with and without predation pressure are necessary to indicate more clearly whether the indirect interactions are positive or negative. Treatments in which predation pressure is low, but where habitat complexity varies would also give a more complete picture of conditions under which indirect interactions between aphid species can change. In addition, these experiments could be run for longer periods of time and be sampled more frequently to better capture when and how changes in the strength and sign of indirect interactions between aphid species occur (Holt and Lawton 1994). Negative indirect effects in particular are hypothesized to occur over longer time periods since many predator species show density-mediated responses (Muller and Godfray 1997, Chailleux et al. 2014).

The value of mesocosm experiments conducted under controlled environmental conditions is that the dynamics of direct and indirect interactions can be monitored over time which can add further detail to the snapshot dynamics that have been observed in larger field studies. It is especially important to experimentally determine how and when the sign and strength of these

interactions can be shifted and to be able to relate such changes to the context of how and when conservation biological control programs that depend on generalist predators succeed or fail (van Veen et al. 2006, Chailleux et al. 2014).

Table 4.1 - Effects of predation pressure from *Hippodamia convergens* and early aphid population growth on the net change in aphid abundance between day 0 and day 8 of the experiment for *Acyrtosiphon pisum* and *Aphis gossypii* from the fitted linear mixed effects models (NS = non significant).

Component model	R^2_m	R^2_c	Intercept	# <i>H. convergens</i>	# <i>H. convergens</i> ²	Early growth <i>A. pisum</i>	Early growth <i>A. gossypii</i>
<i>A. pisum</i> net change (d 0-8)	0.95	0.96	160.0	-90.9	7.3	1.2	0.7
<i>A. gossypii</i> net change (d 0-8)	0.89	0.92	466.0	-164.2	11.3	NS	3.3

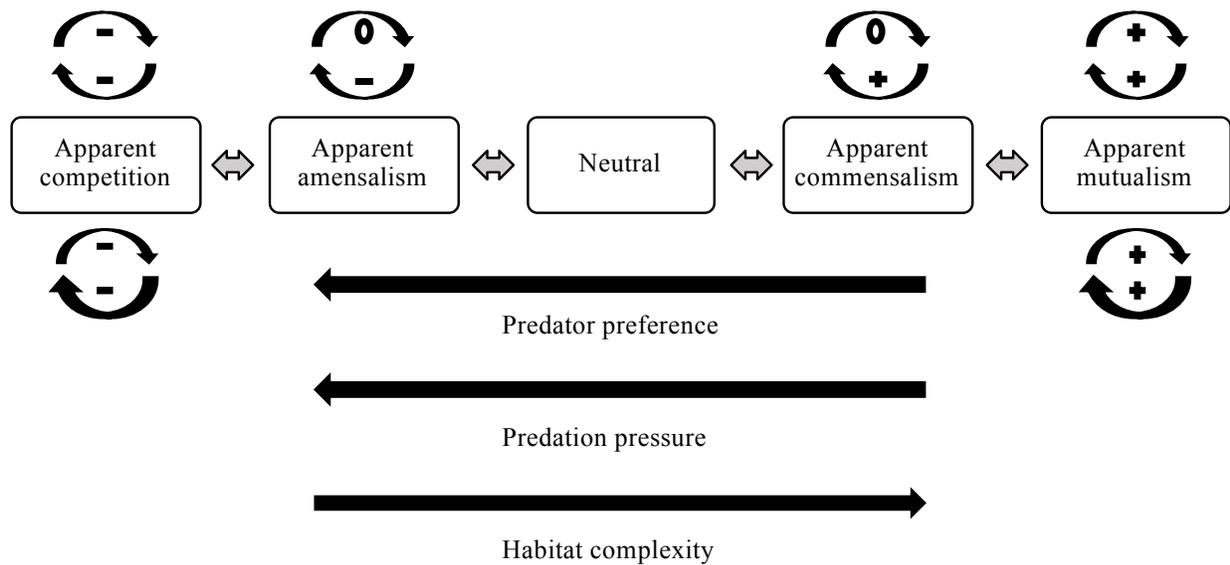


Figure 4.1 - A schematic illustration of the continuum of possible indirect interactions along with hypothesized factors that are expected to affect the sign and potentially the strength of the indirect interactions.

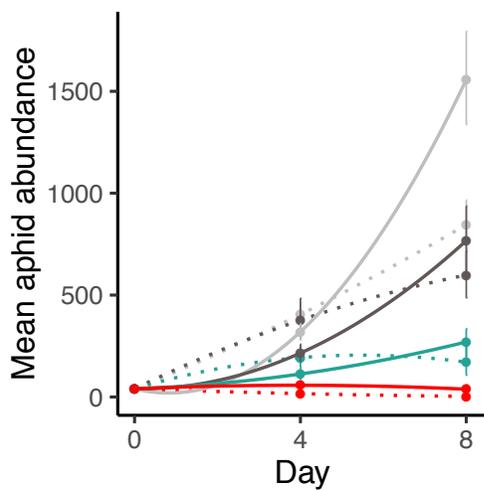


Figure 4.2 - Aphid population growth over the course of the experiment for *Acyrtosiphon pisum* (dotted lines) and of *Aphis gossypii* (solid lines) in relation to predation pressure. The points are means per cage \pm SE and the line colors are in the absence of predators (light grey) and in the presence of one (dark grey), five (teal) or 10 (red) adult *Hippodamia convergens*.

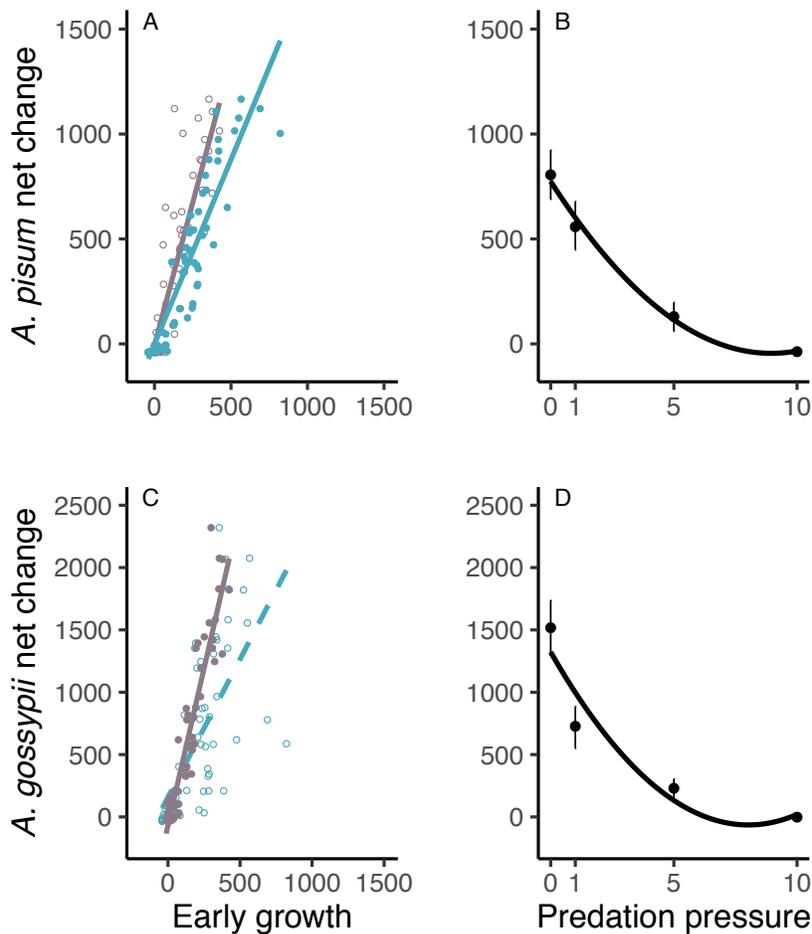


Figure 4.3 - The influence of early aphid population growth and predation pressure on the net change in abundance between day 0 and day 8 for *Acyrthosiphon pisum* (A, B) and *Aphis gossypii* (C, D). For the effects of early population growth (days 0 – 4) open circles represent the effect of the other aphid species, solid circles the effect of the same aphid species and solid versus broken lines indicate significance for that factor in the final linear mixed effects model. Fitted models are: A) same aphid species $y = -1.5 + 1.8x$, $R^2 = 0.84$, $p < 0.001$, other aphid species $y = -6.4 + 2.7x$, $R^2 = 0.74$, $p < 0.001$; B) predation pressure $y = 774.4 - 183.1x + 10.2x^2$, $R^2 = 0.77$, $p < 0.001$; C) same aphid species $y = -68.8 + 5.0x$, $R^2 = 0.88$, $p < 0.001$, other aphid species $y = 157.8 + 2.2x$, $R^2 = 0.46$, $p < 0.001$; D) predation pressure $y = 1322.3 - 345.7x + 21.5x^2$, $R^2 = 0.73$, $p < 0.001$.

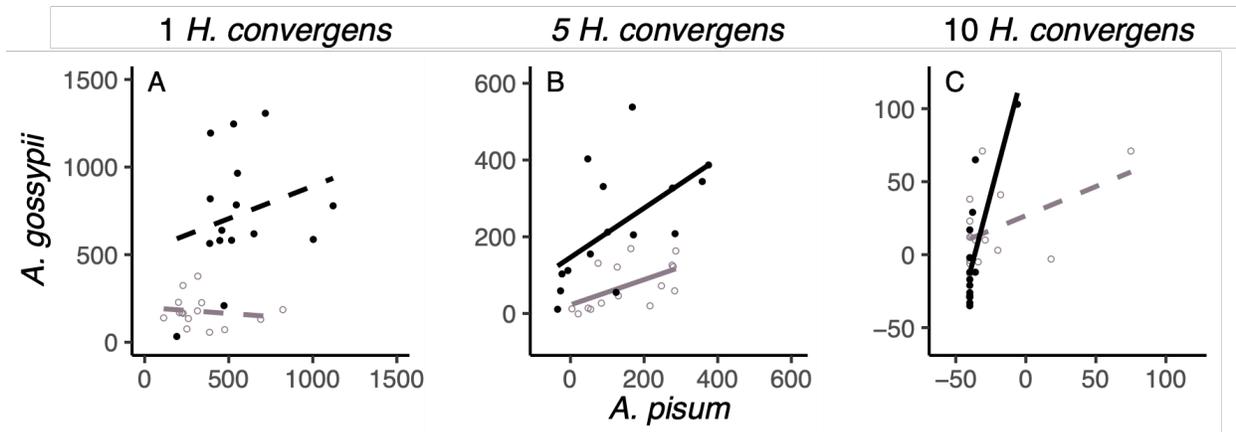


Figure 4.4 - The relationships between early population growth of *Acyrthosiphon pisum* and *Aphis gossypii* (days 0 – 4, grey open circles and lines) and net change in abundance (days 0 – 8, black solid circles and lines) for different levels of predation pressure. Solid versus broken lines indicate significance in the final linear mixed effects model. Fitted models are: A) one adult *Hippodamia convergens*, early growth $y = 198.6 - 0.1x$, $R^2 = 0.02$, $p = 0.59$, net change in abundance $y = 519.8 + 0.4x$, $R^2 = 0.06$, $p = 0.37$, B) five *H. convergens*, early growth $y = 22.5 + 0.3x$, $R^2 = 0.32$, $p = 0.03$, net change in abundance $y = 146.2 + 0.6x$, $R^2 = 0.34$, $p = 0.02$, and C) 10 *H. convergens*, early growth $y = 26.7 + 0.4x$, $R^2 = 0.22$, $p = 0.08$, net change in abundance $y = 132.6 + 3.6x$, $R^2 = 0.62$, $p < 0.001$.

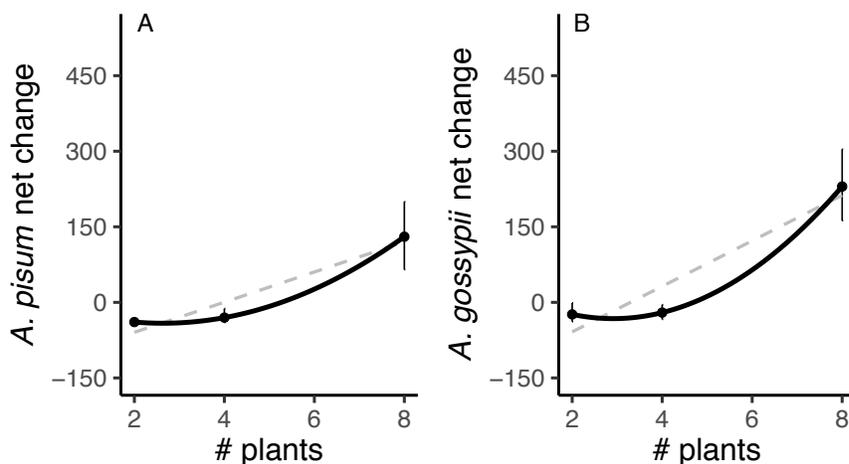


Figure 4.5 - The effect of habitat complexity (number of potted plants) on the net change in aphid abundance from day 0 to day 8 in the presence of five adult *Hippodamia convergens* for A) *A. pisum* in which $y = 395.2 - 31.7x + 0.5x^2$, $R^2 = 0.49$, $p < 0.001$ (black solid) and $y = -119.4 + 19.9x$, $R^2 = 0.45$, $p < 0.001$ (dashed grey) and B) *A. gossypii* in which $y = 645.9 - 49.9x + 0.8x^2$, $R^2 = 0.64$, $p < 0.001$ (solid black) and $y = -149.0 + 42.2x$, $R^2 = 0.57$, $p < 0.001$ (dashed grey).

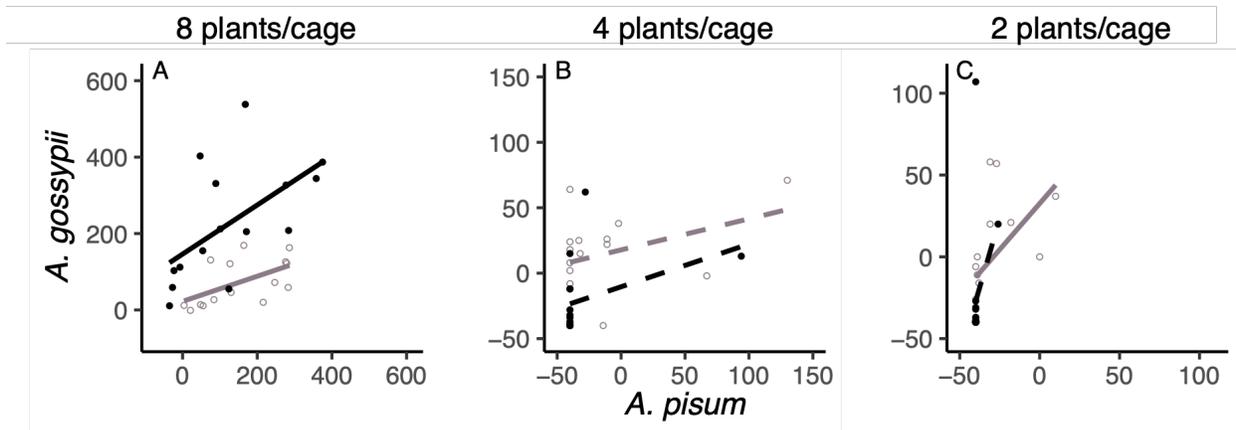


Figure 4.6 - The relationships between early population growth of *Acyrthosiphon pisum* and *Aphis gossypii* (day 0 – 4, grey open circles and lines) and the net change in abundance (day 0 – 8, black solid circles and lines) for different levels of habitat complexity. Solid versus broken lines indicate significance in the final linear mixed effects model. Fitted models are: A) eight plants/cage, early growth $y = 22.5 + 0.3x$, $R^2 = 0.32$, $p = 0.03$, net change in abundance $y = 146.2 + 0.6x$, $R^2 = 0.34$, $p = 0.02$; B) four plants/cage, early growth $y = 17.8 + 0.2x$, $R^2 = 0.14$, $p = 0.17$, net change in abundance $y = -10.4 + 0.3x$, $R^2 = 0.15$, $p = 0.16$; and C) two plants/cage, $y = 32.6 + 1.1x$, $R^2 = 0.28$, $p = 0.04$, $y = 107.2 + 3.4x$, $R^2 = 0.1$, $p = 0.26$.

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