BIOLOGY OF THE REEMERGENT PEST APPLE FLEA WEEVIL (ORCHESTES PALLICORNIS, SAY) AND METHODS FOR ITS ORGANIC CONTROL

By

John Pote

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ABSTRACT

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The goal of this research was to explore the basic life-history and seasonality of *O. pallicornis*, and provide affected growers with OMRI-approved tactics to control it. I conducted beat sampling and leaf collections throughout the growing seasons of 2011 and 2012 at three Michigan orchards to identify the seasonality of *O. pallicornis* adults and larvae. *O. pallicornis* adults are active in the early Spring, timed with the Green Tip or Tight Cluster stages of apple bloom phenology. I determined that there were three instars by measuring the head capsules of field-collected larvae. Parasitoids of *O. pallicornis* were studied through observation of field-collected larval mines stored in petri-dishes. At least five families of parasitic hymenopterans were found to parasitize *O. pallicornis*. I tested conventional and organic insecticides against *O. pallicornis* adults in a lab bioassay. Most conventional products caused high *O. pallicornis* mortality. Entrust (Spinosad) was the only tested OMRI-approved product to cause high mortality. I then conducted field insecticide trials emphasizing Entrust to determine appropriate management practices. Entrust applied at Tight Cluster is suggested for control of the economically damaging Spring adults, due to reduced potential for parasitoid toxicity and high pest mortality. Half-rate applications of Entrust may be sufficient to control adult populations, especially those of the Summer generation. This information was synthesized into an extension bulletin for distribution to afflicted growers.

For Leah,
who I cannot wait to meet.

ACKNOWLEDGEMENTS

I'd like to thank Dr. Matthew "Big G" Grieshop first because he's my boss and he probably wouldn't like being in the middle of the list. But also for the priceless help, wisdom and instruction he's given me over the last 4 years as a professor and then as my graduate advisor. Thank you for the nicknames, the yet-unbroken lab equipment, and most importantly for seeing my intellectual potential. I'd also like to thank Dr. Anne Nielsen for authoring the grant that allowed me to pursue my graduate studies and being on my advisory committee throughout said studies. She also deserves a commendation for tolerating my shenanigans for over a year, and for some reason, agreeing to tolerate them for four more years. Dr. Larry Gut and Dr. Ronald Perry also have my deepest thanks for guiding my research as members of my advisory committee. I would like to thank my lab-mates for their assistance and wisdom, but mostly beg their forgiveness for having to tolerate the chaos that inevitably consumes everywhere I go. I'd like to thank my parents for everything, including their supportive yet challenging brand of nurturing that made me the awesome, lame, brilliant idiot I am today. I begrudgingly thank my sister Alex who helped me accept my inner nerd by unceasingly making fun of me for the last two decades. My deepest affection goes out to F.R.D. You have always been a source of adventure and a desperately needed haven from reality. I owe much of my success to my close group of friends: thank you for letting me take advantage of your kindness for so long (especially KK and PQ). Finally, I'd like to thank the 2009-2010 residents of 212 Milford St.: Annie, Christine, Elizabeth, Phillip and Lanny. Two-One-Two means family and family means no one gets left behind. You have no idea how much I appreciate all five of you for making my time as a Spartan so entertaining, so hazy, so personally and financially destructive and so damn hard to leave behind.

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CHAPTER 1

Introduction

Orhestes pallicornis was first discovered in Indiana in 1831. Since then it has been reported in Ohio, Illinois, New York, New Jersey, Michigan, Montana, Arkansas, Connecticut, Georgia, Louisiana, Maine, New Hampshire, North Carolina, Ohio, Texas, Virginia and Washington as well as Alberta, Ontario and Quebec, Canada (Houser 1923, Flint et al. 1924, Anderson 1989). Because of its ubiquitous presence, it is believed to be native to the New World (although this is unconfirmed in modern literature) (Flint et al. 1924).

Orchestes pallicornis adults are small, matte black beetles with long snouts. They are approximately 3mm in length and 1.5mm wide with enlarged saltatorial metathoracic femorae and striated elytra. The larvae develop while concealed in leaf mines, but when removed are ivory in color and measure ~5mm in length and ~2mm in width. Like most leaf-mining larvae, they are legless and dorso-ventrally flattened (Houser 1923, Flint et al. 1924).

Orchestes pallicornis overwinters in the adult stage by burrowing into partially decayed plant-matter directly above the top-soil. In early Spring, adults emerge and crawl or fly to the developing apple canopy where they begin consuming leaf and bud tissue (Flint et al. 1924). Early reports of *O. pallicornis* infestation documented an average of 1400-1700 adults emerging per tree in favorable weather conditions (Houser 1923, Flint et al. 1924). Mating begins almost immediately after emergence. Gravid females oviposit only after leaf flush and lay eggs singly in the mid-vein of low to mid canopy leaves (Houser 1923, Flint et al. 1924). Eggs hatch in roughly 7 d. The larvae are leaf-miners, developing between the leaf epidermal layers and consume mesophyll tissue in a winding path to the leaf margin. Larval development takes ~17 d to complete. Pupation causes the dead outer layers of leaf tissue to separate and create a

characteristic “blister”. After 5-6 d adult *O. pallicornis* emerge by chewing a hole in the dried tissue of the blister. The number of instars is unknown and there is no published phenological data on *O. pallicornis*. Second generation adults feed for 6-12 d before returning to the soil duff to overwinter. *Orchestes pallicornis* is a univoltine species, and is generally absent from the orchard canopy by mid-July (Houser 1923, Flint et al. 1924).

Although the primary economic host of *O. pallicornis* is domesticated apple (*Malus domestica* Bork.), it has been collected off several other species of Rosaceae. Larval *O. pallicornis* have been documented on winged elm (*Ulmus alata*), Amercian elm (*Ulmus americana*), quince (*Prunus virginiana*), hazlenut (*Corylus americana*), hawthorn (*Crataegus mollis*) and elder (Alnus sp.). Adult feeding has also been reported on wild crab apple (*Pyrus coronaria*), willow and saskatoon flowers (Amelanchier sp.) (Houser 1923, Flint et al. 1924). Despite its wide host range, *O. pallicornis* has never been observed severely infesting any host other than domesticated apple, even in cases where alternate hosts were located adjacent to heavily infested orchards (Houser 1923, Flint et al. 1924).

Economic damage from *O. pallicornis* occurs when spring generation adults consume developing bud tissue. This damage frequently causes the termination of flower development leading to decreased fruit yield. Larvae and summer generation adults both feed on leaf tissue which can lead to a decrease in tree health, reduced winter hardiness and (after repeated years with high damage) tree mortality (Houser 1923, Flint et al. 1924).

Orchestes pallicornis (Say) is a re-emerging pest of economic significance in Michigan organic apple orchards. *Orchestes pallicornis* damage was first reported in organic orchards in 2008 but was initially misdiagnosed as frost damage. Since then, reported damage has increased dramatically, with some growers experiencing up to 90% yield loss (Nielsen et al. 2012).

Historical research on this pest is extremely limited, the most recent of which was conducted in 1950 on the effect of DDT on *O. pallicornis* (Cutright 1950). Nielsen et al. (2012) hypothesized that the recent emergence of this pest is due to the limited efficacy of narrow-spectrum insecticides used in organic agriculture, as *O. pallicornis* is not a pest in conventional orchards. Changes in chemical management regimen have been shown to markedly impact pest complexes (Lu et al. 2010). As conventional growers shift to increasingly narrow-spectrum insecticides as part of integrated pest management (IPM), *O. pallicornis* may emerge as a pest of significance in those systems as well. A better understanding of the biology of this pest and investigation of management techniques to control are thus timely.

Parasitoids and pathogens contribute to the natural mortality of *O. pallicornis*. *Orhcestes pallicornis* populations can be regulated by a variety of parasitic hymenopterans. *Zatropis incertus* Ashm., *Trichomalus inscitus* Walker (Hymenoptera: Pteromalidae), *Epiusus* sp., and *Chrysocharis pentheus* Walker (Hymenoptera: Eulophidae) have been collected from larval mines (Houser 1923). In addition, overwintering *O. pallicornis* adults are susceptible to white muscardine disease (*Beauveria bassiana* Vuill.), a globally distributed entomopathogenic soil fungus.

Chemical management of *O. pallicornis* has been largely unresearched since the 1950's. Early chemicals tested for *O. pallicornis* control included: arsenate of lead and calcium, nicotine sulfate and kerosene emulsion (Houser 1923). After World War II, synthetic organic compounds like DDT, parathion and methoxychlor were all successfully used for control of *O. pallicornis* (Cutright 1950, Mundinger 1951).

Successful integrated pest management requires knowledge of pest phenology for adequate timing of control tactics (Ascerno, 1991). Insect phenology is a function of the insect's

specific minimum developmental threshold temperature and the required growing degree day (GDD) accumulation for a given life-stage (Arnold 1959, Snyder et al. 1999). The minimum temperature threshold is typically calculated through laboratory rearing of insects at a number of constant temperatures (Campbell et al. 1974). For insects that cannot be lab-reared, estimations of insect development must be made from field data. Several methods exist to determine phenology from field-collected data. Snyder et al. (1999) evaluated the accuracy of several such methods including the Standard Deviation of Days Method, the Standard Deviation of Degree Days Method and the Linear Regression Method, all of which involve the statistical manipulation of temperature data to determine the most appropriate developmental minimum and degree day accumulation required for a given phenological period.

Presently, little is known about *O. pallicornis* phenology, larval development, biological control, or effective chemical control strategies. Thus the objectives of this research are:

1. Determine *O. pallicornis* basic phenology and biology including the identity and prevalence of parasitoids.
2. Develop an effective pest management program based on National Organic Program (NOP) compatible insecticides.
3. Develop extension materials to disseminate relevant information to the grower community

CHAPTER 2

Biology, Seasonality and Parasitoids of the Reemergent Pest Apple Flea Weevil (Orchestes pallicornis, Say)

1. Introduction

Orchestes pallicornis (Say) is a re-emerging pest of economic significance in Michigan organic apple orchards. *Orchestes pallicornis* damage was first reported in organic orchards in 2008 but was initially misdiagnosed as frost damage. Since then, reported damage has increased dramatically, with some growers experiencing up to 90% yield loss (Nielsen et al. 2012). Past research on this pest is extremely limited. The most recent work conducted in 1950 on the efficacy of DDT on *O. pallicornis* (Cutright 1950).

Adult *O. pallicornis* are small, matte black beetles with long snouts. They are approximately 3mm in length and 1.5mm wide with enlarged saltatorial metathoracic femorae and striated elytra. The larvae are generally concealed in leaf mines, but when removed are ivory in color and measure ca. 5mm in length and ca. 2mm in width. Like most leaf-mining larvae, they are legless and dorso-ventrally flattened (Houser 1923, Flint et al. 1924).

Orchestes pallicornis overwinter in the adult stage by burrowing into partially decayed plant-matter directly above the topsoil. In early spring, adults emerge and crawl or fly to the developing apple canopy where they begin consuming leaf and bud tissue (Flint et al. 1924). Early reports of *O. pallicornis* infestation documented an average of 1400-1700 adults emerging per tree in favorable weather conditions (Houser 1923, Flint et al. 1924). Mating begins almost immediately after emergence. Gravid females oviposit only after leaf flush and lay eggs singly in the mid-vein of low to mid canopy leaves (Houser 1923, Flint et al. 1924). Eggs hatch in roughly 7 d, after which larvae consume mesophyll tissue in a winding path to the leaf margin. Larval

development takes ~17 d to complete. The number of larval instars has not been determined. Pupation causes the dead outer layers of leaf tissue to separate and create a characteristic "blister". After 5-6 d adult *O. pallicornis* emerge by chewing a hole in the dried tissue of the blister. Second generation adults feed for 6-12 d before returning to the soil duff to overwinter. *Orchestes pallicornis* is a univoltine species, and is generally absent from the orchard canopy by mid-July (Houser 1923, Flint et al. 1924).

Leaf-miners, when considered as a non-taxonomic feeding guild, have more parasitoids per host than any other feeding guild (Askew and Shaw 1979, Hawkins 1988, 1990; Hawkins et al. 1992, Askew 1994). Data directly comparing differences in predatory mortality between mining and external feeders is severely lacking, but the few taxa where these comparisons have been made show profoundly higher predation and parasitism rates in miners than external feeders (Cornell 1990, Hawkins and Sheehan 1994). Very little is known about the role of natural enemies in regulation *O. pallicornis* populations. In the only published study, a variety of parasitic hymenopterans, including *Zatropis incertus* Ashm., *Trichomalus inscitus* Walker (Hymenoptera: Pteromalidae), *Epiusus* sp., and *Chrysocharis pentheus* Walker (Hymenoptera: Eulophidae), were recovered from larval mines with natural rates of parasitism exceeding 20% (Houser 1923).

Understanding larval development is a prerequisite for implementation of selective bio-insecticides that may affect instars with differing efficacy (Liang et al. 2002), thus determining the number of instars should be a prime concern for management of a new or understudied pest. Parasitoid species and rates may also depend on suitability of host life stage. The number of larval instars for *O. pallicornis* is unknown, however Dyar's law may be used to identify instars. Dyar's Law states that sclerotized portions of the insect integument increase only between

successive instars and this increase can be expressed in a step-wise geometric pattern (Dyar 1890). Dyar and other early researchers showed that it was possible to describe the pattern of geometric growth *post-hoc* in lab-reared species by observing the number of molts and applying a geometric series to the observed pattern of head-capsule widths (Dyar 1890, Quaintance and Brues 1905). However, through repeated field collections of *Grapholita molesta* (Busck) at regular intervals within a single life-cycle, Peterson and Haeussler (1928) showed that it is possible to determine the number of instars as well as the existence of missing instars from the head capsule measurements of obscured or inaccessible species.

The objectives of this study were to determine: 1) the phenology of *O. pallicornis* in Michigan organic orchards, including the number of larval instars, and 2) the potential role of parasitoids in regulating *O. pallicornis* populations.

2. Materials and Methods

Research was conducted at three certified organic apple orchards in mid-Michigan, the first located near Potterville, MI (42.635608,-84.788709), the second located near Flushing MI (43.024371,-83.91168), and the third at the Michigan State University Clarksville Research Station (42.875964,-85.248239). Research at the Potterville site was principally conducted in an *O. pallicornis*-infested 2.3 ha block of Cortland, Golden Delicious, Red Delicious, Jonamac, Jersey Mac and Viking varieties planted in 1987 on MM.111 rootstock. Trees were planted North to South with a 3 m x 5.5 m (10 ft. x 18 ft.) tree-row spacing, and trained in a non-supported central leader system. Research at the Flushing site was primarily conducted in an *O. pallicornis* -infested 5 ha block of Golden Delicious, Gala and GoldRush on MM.7 rootstock. Trees were planted East to West on a 3 m x 5.5 m (10 ft. x 18 ft.) tree-row spacing and trained in a non-supported central leader system. Research at the Clarksville site was confined to an

unmanaged organic block of mixed apple varieties on a dwarfing rootstock and planted at high density with rows running North to South, trained in a supported slender spindle system. Portions of orchards selected for sampling at each site were either historically not treated with insecticides, or were the untreated control for ongoing insecticide efficacy trials, and thus were not treated with insecticides for the duration of the study (unless specifically stated below).

2.1. Phenology

The seasonality of *O. pallicornis* was determined through beat-sampling apple canopies and observation of larval infestation rates throughout the 2011 and 2012 growing seasons. Beat-sampling consisted of firmly tapping three terminal-bearing limbs (>3 cm in diameter) at three different heights within the canopy architecture and collecting any dislodged *O. pallicornis* adults that landed on a 1 m^2 mesh sheet held beneath the limbs (Bioquip Inc., Rancho Dominguez, CA, product # 2840 R). In 2011, ten trees of intermediate bloom phenology were sampled per site per sampling date. In 2012, the number of sampled trees was increased to 10 trees in three rows of varying bloom phenology. Rows sampled in 2011 were also sampled in 2012. Sampling was initiated as soon as temperatures began to rise in the early spring (24 April, 2011 and 14 March, 2012, respectively) and was repeated every 1-3 d until summer-generation adults were absent from the orchard canopy (1 August for 2011 and 2012). Temperature data was collected over the course of the study from weather stations in the MSU Enviroweather network (http://www.enviroweather.msu.edu/) and of the National Ocean and Atmospheric Administration (http://cdo.ncdc.noaa.gov/).

Levels of larval infestation were measured at the same time points as beat samples. Infestation rate was determined by counting the number of mines per 30 leaves, collected randomly from the canopies of three randomly selected trees within one row. Leaf samples were

selected from around the tree circumference and at varying heights and depths into the canopy (Note: Data was recorded as total number of mines per 30 leaves, thus a value higher than 30 was theoretically possible).

2.2. Instar Determination

Specimens were collected to identify larval growth stages at the three research sites throughout the 2011 and 2012 growing season in untreated trees. Collections began after the peak of the spring adult population and continued 1-2 times per wk until unopened mines could no longer be found. For each collection, 20-30 leaves per site that contained unopened larval mines were gathered and stored in 100% ethanol. Larvae were extracted from the mines and their head capsule width was measured using the DinoXcope v. 1.9.1 software (New Taipei City, Taiwan) paired with a USB Dino-Eye digital eyepiece camera mounted on a Leica S8APO stereo-microscope. Prior to each measurement, the accuracy of the software measurement utility was calibrated using a stage micrometer.

2.3. Parasitoids

To determine the rate of parasitism in under organic management and determine the identity of associated parasitoids, *O. pallicornis*-infested leaves were collected throughout the 2011 and 2012 growing seasons. Random samples of 100 unopened mines were collected among five trees per sampling date. Sampling occurred on seven dates from 24 April to 27 June 2012. In addition, mines were collected in 2012 as part of concurrent insecticide trials at the Clarksville, Potterville and Flushing sites. These samples consisted of 60 randomly mines collected per day (20 mines from at least five randomly selected trees per treatment) but only the 20 collected from untreated blocks were used for this study.

Infested leaves were stored for 3-7 d at 10° C before being stored individually in 60mm x 15mm petri dishes sealed with acetate tape. Petri dishes were then stored at room temperature and checked weekly for emergence of either a parasitoid wasp or an adult *O. pallicornis*. Parasitoids were transferred into 1.5 ml microcentrifuge vials containing 80% ethanol. Parasitoid specimens were identified to family and reference photographs collected using a Dino-Eye digital eyepiece camera and Dino-Lite software (New Taipei City, Taiwan, prod. # AM423XC) mounted on a Leica S8APO (Buffalo Grove, IL) stereo- microscope. Specimens have been sent to experts for species identification.

2.4 Statistical Analysis

Data were analyzed in the R statistical language (R core development team 2013).

2.4.1. Seasonality Analysis

Minimum developmental threshold temperature for spring *O. pallicornis* emergence were calculated from field data due to the difficulty of establishing an *O. pallicornis* colony and conducting laboratory studies. Temperature and emergence data from 2011 and 2012 was analyzed using the methods of minimum developmental temperature determination from field data outlined in Snyder et al. (1999) including the SD_{day} method first outlined by Arnold (1959). This was completed using both the mathematical average for mean daily temperature as well as the rectangle method (mean = (Tmax-Tmin)*0.5). Due to variability of temperature, field population data collected in 2011 and 2012 can be considered a measure of insect activity rather than insect development. The methods for regression analysis of insect activity as a function of temperature proposed by Taylor (1963) were modified and applied to observed adult activity. For each year, peak spring generation populations were averaged across sites and bloom phenologies. Using weather station data, growing degree day (GDD) accumulation from 1 Jan to the date of

peak *O. pallicornis* spring generation development was calculated using both the simple and sine methods of GDD calculation (from equations provided in Allen 1976) and averaged across sites. Heat accumulation models were calculated using projected minimum threshold temperatures ranging from 0-10 C° (32-50 F°). Heat accumulation models were compared to *O. pallicornis* population accumulation using multiple linear regression. Each individual model was compared to the population accumulation of the appropriate year using singular linear regression to determine the R^2 and Akaike Information Criterion (AIC) values for each temperature minimum model. The minimum temperature threshold that best fit the criteria and population data was identified.

2.4.2. Instar Determination Analysis

The number of *O. pallicornis* larval instars was indirectly determined using the simple frequency method (Peterson and Haeussler 1928, Gaines and Campbell 1935): a frequency histogram was generated for head capsule width measurements. The measurements were split into groups based on the loci of discontinuities and peaks on the frequency histogram. Widths between groups determined graphically were analyzed using a two-way ANOVA with site of collection as a blocking factor. Widths between groups determined morphologically were analyzed using a two-way ANOVA with site of collection as a blocking factor. Means separation was determined using Tukey's HSD.

2.4.3. Parasitoid Analysis

Parasitoids were collected as part of a concurrent *O. pallicornis* insecticide study and the preliminary parasitoid sampling from 2011 were included in the by-site assessment of *O. pallicornis* parasitoid diversity (Pote 2013). To guard against treatment effects, only mines

collected during 2012 from untreated blocks were included in the by-site analysis of *O. pallicornis* parasitism.

The rates of parasitism (# parasitoids/ #mines) were compared between sites. Site means were compared by date using Chi-square analysis. Due to unequal sample size and variance between sites, means separation was determined using Dunnett's Modified Tukey-Kramer pair-wise comparison test.

3. Results

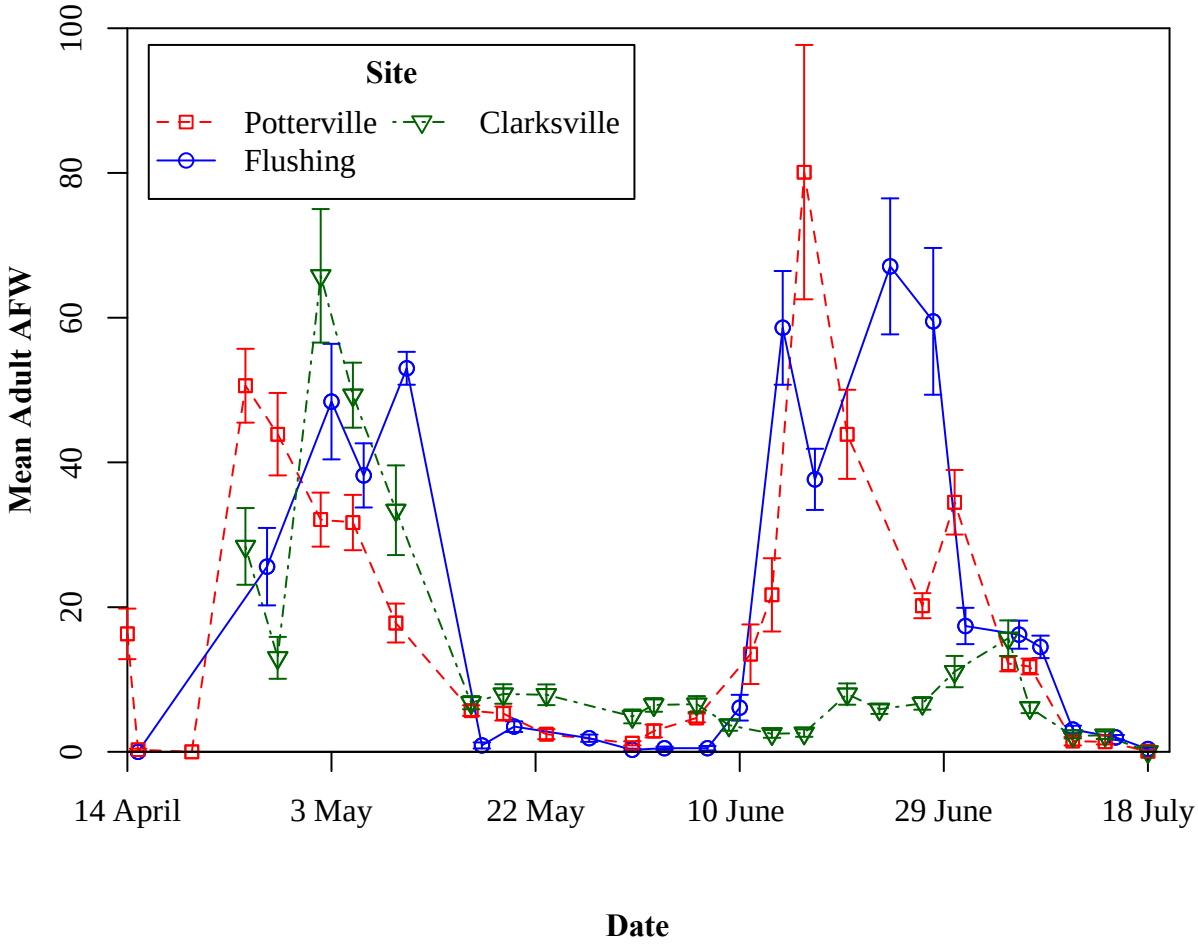

Figure 2.1. Mean adult *O. palicornis* adults (± SEM) collected per tree throughout 2011. Spring and summer population peaks occurred on 2 May and 16 June, 2011, respectively. (For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.)

3.1 Seasonality

In 2011, spring emergence of *O. pallicornis* populations peaked on 2 May, 2011 (Fig 2.1). The second (summer) generation peaked on 16 June, 2011 and by 18 July, 2011 no *O. pallicornis* adults could be observed in the orchard canopy (Fig. 2.1). Spring emergence peaked on 24 March in 2012 (Fig. 2.2). There was no clearly defined peak of the summer generation in 2012, but its numerical maximum was near 1 May (Fig. 2.2).

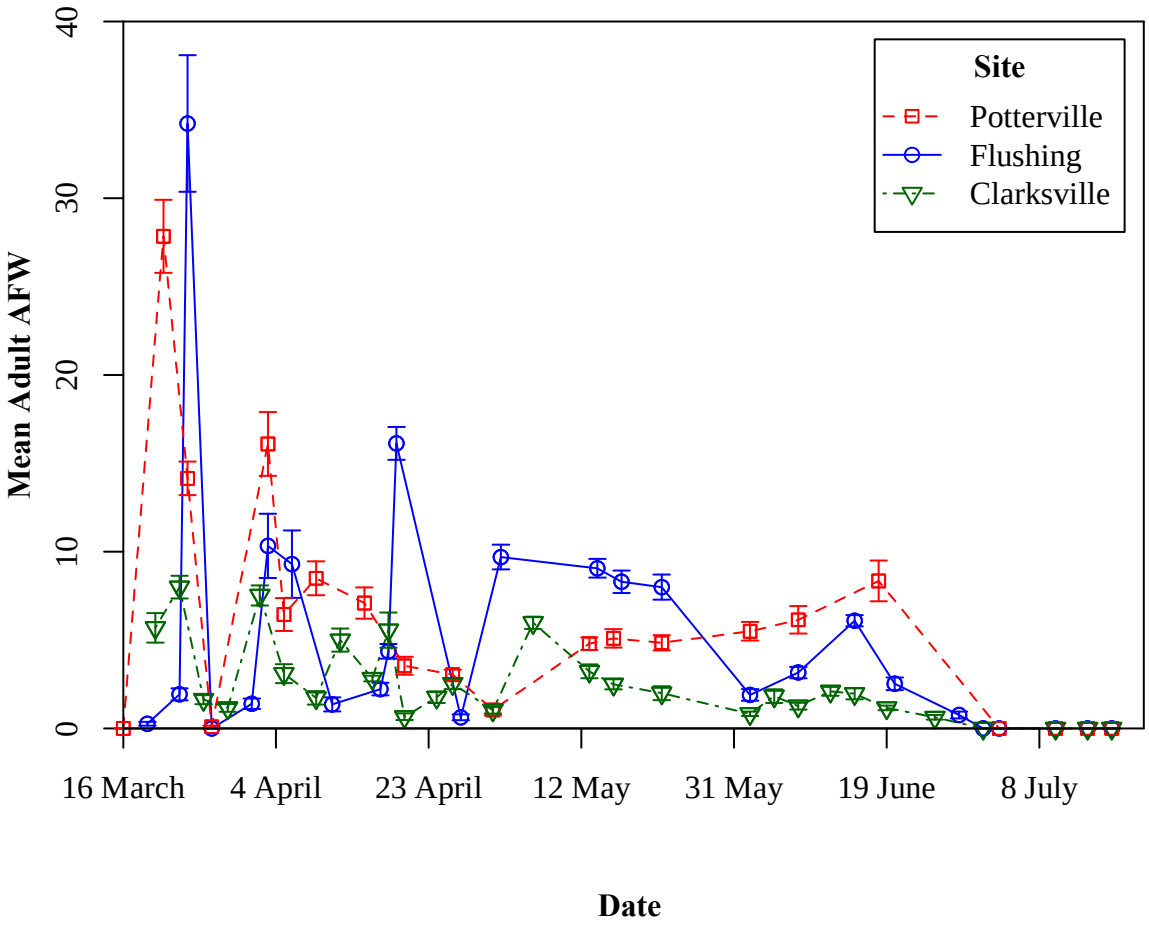

Figure 2.2. Mean *O. palicornis* adults (± SEM) collected per tree throughout 2011. Spring population peak occurred on 24 March, 2012. The summer *O. palicornis* population did not have a clear peak but, numerically, the most adults were recorded on 1 May, 2012.

In 2012, larval mines were first detected on 14 April (Fig. 2.3). The proportion of leaves with mines increased in a similar fashion at all three sites from mid-April to mid-May. By June, Clarksville had a numerically higher proportion of leaves with mines than the other two sites. The Flushing site had the lowest proportion of leaves with mines (Fig. 2.3).

The SD_{day} method for determination of minimum developmental thresholds (Snyder et al. 1999) calculated the most plausible minima for *O. pallicornis* development (Table 2.1). Inputting simple daily means for individual years into the SD_{day} calculations did not provide valid minima (44° C and 108° C for 2011 and 2012, respectively). Inputting rectangle-method daily means for individual years into the SD_{day} calculations also determined illogical minima (33° C and 62° C for 2011 and 2012, respectively). Inputting simple daily means into the SD_{day} calculations yielded a minimum of 10.7° C (51.4° F) when data from both years were analyzed together (Table 2.1). In contrast, using the rectangle method of daily means calculations (Snyder et al.

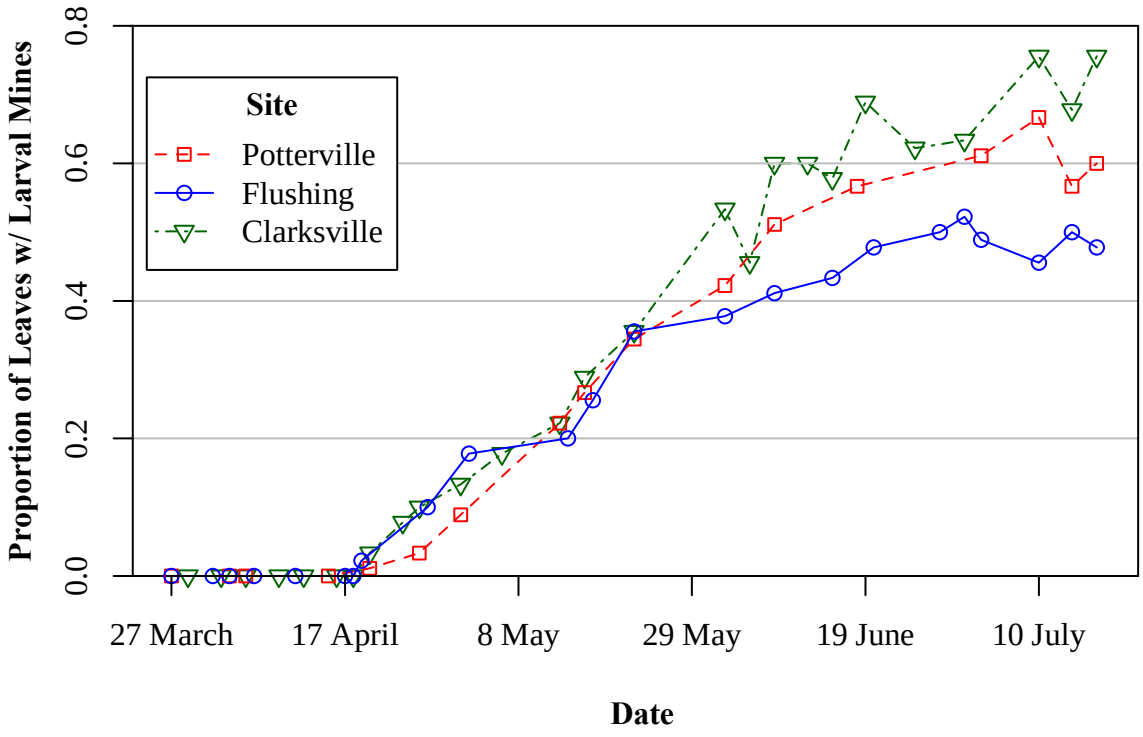

Figure 2.3. Mean proportion of leaves housing at least one *O. palicornis* larval mine collected in 2012.

1999) yielded a minimum of 5.4° C (41.7° F) when both years were analyzed together (Table 2.1).

Table 2.1. Proposed *O. palicornis* minimum developmental threshold temperatures calculated using the SDday method from Snyder et al. (1999). Minima were derived from daily means calculated using both the simple method and rectangle method.

Daily Mean Calculation Method	**Calculated Minima (° C)**		
	2011	**2012**	**Both Years**
Simple:	44.6°	108.7°	10.8°
Rectangle:	33.2°	62.1°	5.4°

Table 2.2. A summary of *O. palicornis* minimum developmental threshold temperatures calculated using a multitude of statistical methods. Of the Snyder et al. methods, only SDday for both years yielded realistic minima.

	Year	
Method	**2011**	**2012**
Calculation	Temp C *(F)*	Temp C *(F)*
SD*day*		
Simple	10.7 *(51.4)*	
Rectangle	5.4 *(41.7)*	
Regression		
Sine	8.3 *(47)*	10 *(50)*
Simple	5.0 *(41)*	-
AIC		
Sine	9.4 *(49)*	10 *(50)*
Simple	8.8 *(48)*	10 *(50)*

Developmental thresholds were also generated based on the sine wave calculated mean temperatures. The sine-calculated heat accumulation model with the highest regression

coefficient (R^2) was generated using a base developmental threshold of 8.3° C (47° F) for 2011 and 10° C (50° F) for 2012.

Comparison of AIC values indicated that models corresponding to a minimum threshold of 9.4° C (49° F) and 10° C (50° F) (for 2011 and 2012, respectively) were the most parsimonious within the tested range. The GDD model calculated using simple mean temperatures with the highest regression coefficient (R^2) was 5° C (41° F) for 2011. No model based on simple means was significantly correlated with spring *O. pallicornis* population accumulation in 2012. Comparison of AIC values indicated that simple-method models corresponding to minima of 8.8° C (48° F) and 10° C (50° F) (for 2011 and 2012, respectively) were the most parsimonious within the tested range. These results are summarized in Table 2.2. Models corresponding to minima greater than 10 C° were included in the regression and AIC analysis for 2012. The R^2 and AIC values had not yet reached a point of inflection when temperatures up to 18.3 C° (65 F°) were included. The maximum temperature for this period in 2012 was 22.5 C° (72.5 F°).

3.2 Instar Determination

Graphical separation of the range of observed head capsule widths identified three significantly distinct ($F = 323$, $p = <0.001$) (Fig. 2.4) instar groups. During larval measurements

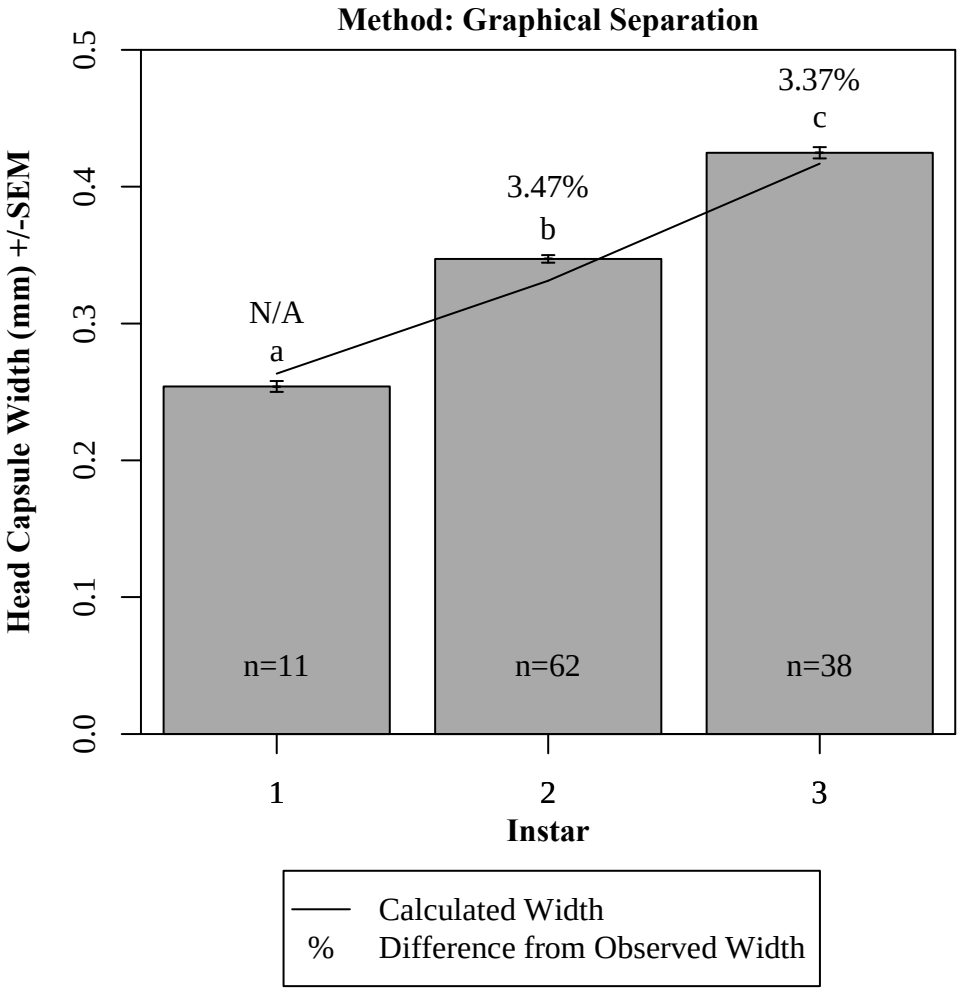

Figure 2.4. Distribution of observed head capsule widths into graphically determined instar groups. The trend line indicates the head capsule widths predicted by Dyar's Law based on the graphical method of instar grouping. Letters indicate significant differences among sites (means separation determined by Tukey's HSD, $\alpha = 0.05$).

it became evident that larvae could be classified into three groups based on the presence or absence of dorsal thoracic shields and ventral thoracic sternites: the smallest group had neither structure, the middle group had only ventral sternites and the largest group had both structures. Separating the collected larvae into groups based on sclerite presence/absence on the thorax also generated three significantly distinct ($F = 304$, $p = <0.001$)(Fig. 2.5) instar groups. For instar groups created graphically, mean group head capsule width was significantly different from that of the other instar groups ($p < 0.001$)(Fig. 2.4). For instar groups created morphologically, mean

group head capsule width was significantly different from that of the other instar groups ($p <$ 0.001)(Fig. 2.5). For both methods of instar differentiation, head capsules widths of instars varied significantly between the Potterville and Clarksville sites ($p <$0.001).

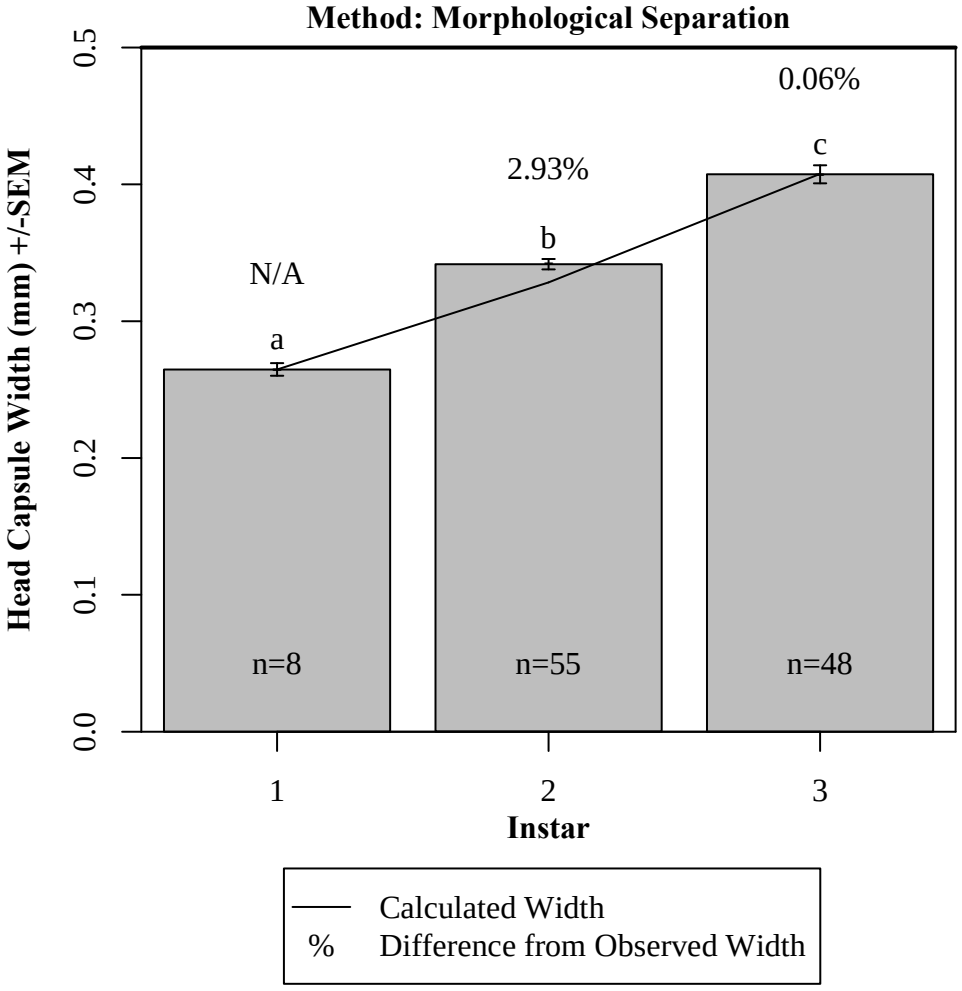

Figure 2.5. Distribution of observed head capsule widths into morphologically determined instar groups. The trend line indicates the head capsule widths predicted by Dyar's Law based on the morphological method of instar grouping. Letters indicate significant differences among groups (means separation determined by Tukey's HSD, $\alpha = 0.05$).

The geometric series to describe the increase in head capsule by instar is as follows:

Head Capsule Width of Instar $n = ax^{(n-1)}$,

where *a* is the head capsule width of the first instar and *x* is the rate of increase . Because the series relies on an observed value of *a*, it is impossible to generate a calculated value for *a.* However, calculating the average value of *x* for each instar separation method allowed for the calculation of the percent difference between the observed and calculated head capsule widths of instars 2 and 3 (Figs. 2.4-2.5, Table 2.3). The average difference between observed and calculated head capsule widths for the graphical and morphological methods of instar determination were 3.42% and 1.49%, respectively (Table 2.3).

Table 2.3. A summary of the methods of instar determination used. The morphological method generated a geometric series that differed from observed head capsule widths less than the simple frequency (graphical) method.

	Simple Frequency Method			**Morphological Separation Method**		
	Observed	Calculated	% Diff	Observed	Calculated	% Diff
Instar 1 (a)	0.2633	–	–	0.2647	–	–
Instar 2 (ax)	0.3429	0.3313	3.47%	0.3381	0.3284	2.93%
Instar 3 (ax^2)	0.4310	0.4169	3.37%	0.4074	0.4076	0.06%
		Ave. % Diff :	3.42%		Ave. % Diff :	1.49%

3.3 Parasitoids

Parasitoids collected from the three sites across 2011 and 2012 belonged to at least five families (Braconidae, Eulophidae, Eupelmidae, Ichneumonidae, and Pteromalidae). The greatest number of individuals were from the family Pteromalidae in the superfamily Chalcidoidea. In 2012, the rate of parasitism was not significantly different between sites (F=0.078, p = 0.925)(Fig. 2.6).

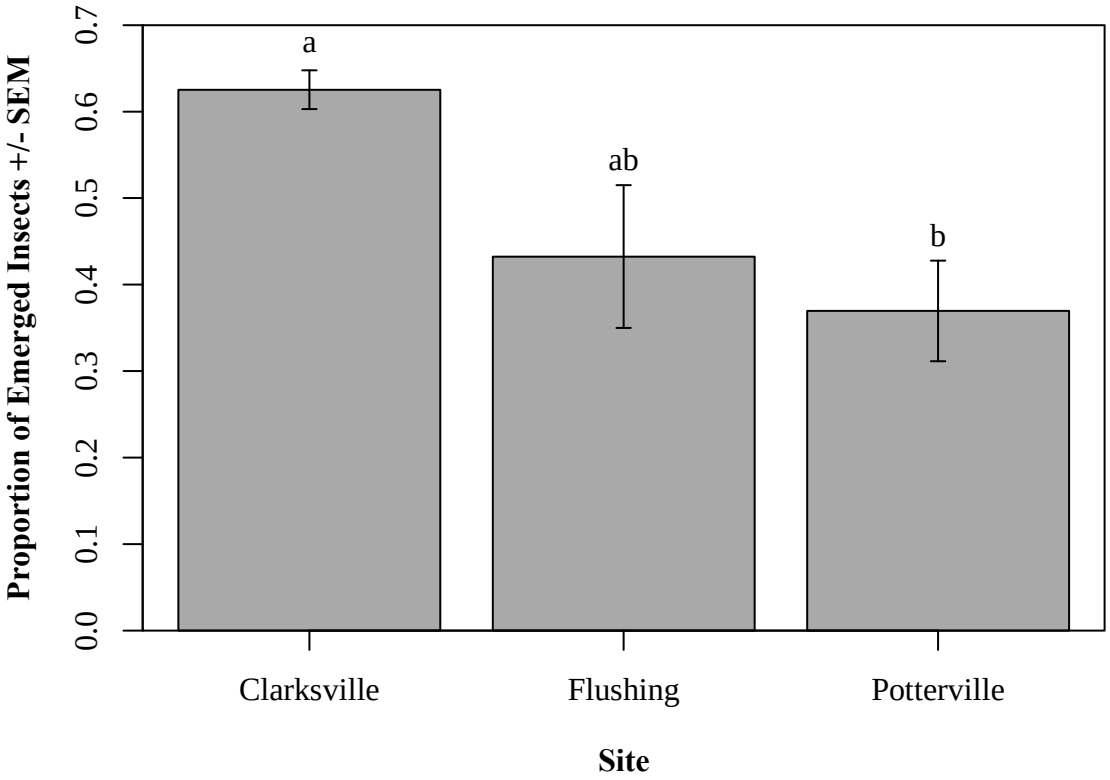

Figure 2.6. Parasitoid emergence as a proportion of total insect emergence from field collected leaf mines. Letters indicate significant differences among sites (means separation determined by Dunnett's Modified Tukey-Kramer pair-wise comparison test, $\alpha = 0.05$).

4. Discussion

There was a substantial difference in the timing of spring emergence of *O. pallicornis* between 2011 and 2012, likely caused by the record-setting warm temperatures during the spring of 2012 (Figs. 2.1 and 2.2). The great disparity in flights prevents the calculation of an expected calendar date of peak activity. However, patterns of activity from the two years strongly indicate that *O. pallicornis* emergence is based on temperature, not on photo-period (Danks 1987). Differences between the two years include the arrival of the spring peak almost 5 weeks earlier in 2012 compared to 2011, and the lack of a true peak of summer adult activity in 2012 (Fig. 2.2). After record breaking high March temperatures in 2012, there were a few days of "normal"

temperatures. This may have generated two distinct periods of ovipostion and thus divided the summer generation activity into two graphically indistinguishable cohorts.

The methods for calculating minimum temperature threshold from Snyder et al. (1999) yielded mixed results (Table 2.1). The minima generated by all methods yielded biologically unrealistic temperatures when applied to individual years. While this may imply an incongruity between the collected *O. pallicornis* phenology data and the Snyder et al. methods, when applied to both years, the SD_{day} and SD_{dd} methods generated temperatures that are consistent with typical insect physiology and temperatures generated by the regression methods (Table 2.3). While plant and insect development are both obviously tied to ambient atmospheric temperature, the Snyder et al. (1999) methods were designed for determining the minimum developmental threshold between stages of the plant life cycle within a single growing season. Because these equations rely heavily on mean temperatures, the long period of very low mean daily temperatures preceding peak *O. pallicornis* activity may have limited the accuracy of these methods.

Analysis of heat accumulation models with multiple linear regression prevents the incidental correlation between early season inactivity of *O. pallicornis* and early season low temperatures from effecting the significance of any individual heat accumulation model. This method was most appropriate for 2011, when *O. pallicornis* populations developed steadily for several weeks. However, regression analysis is unable to project a viable minimum for 2012 due to the rapid growth of *O. pallicornis* populations in that year (Table 2.2). As previously mentioned, models corresponding to minimum thresholds as high as 18.3° C (65° F) were included in the linear regression and AIC analysis for 2012. The estimated best-fit temperatures from regression and AIC analysis in 2012 were significant (where stated) but the R^2 value of

models with minima up to 18.3° C (65° F) continued to increase (as the associated AIC values decreased). Thus it is unlikely that these temperatures are representative of the true minimum developmental or activity thresholds for *O. pallicornis*. The best estimate for minimum developmental threshold of *O. pallicornis* is 8-10 C (47-50 F) based on the minima temperatures estimated by the SD_{day} method analysis of both years, and those minima determined by regression and AIC analysis of 2011. Continued monitoring of *O. pallicornis* populations over multiple years is required to verify this mimum.

Analysis of larval head capsule width strongly indicates that *O. pallicornis* experience three instars prior to pupation. Both the graphical (simple frequency from Gaines and Cambell 1935) and morphological method of instar analysis indicated three instars of *O. pallicornis* larvae. However, both methods have limitations. The simple frequency method relies on an assumed constant rate of growth and consistent numbers of instars (Fink 1984). Schmidt et al. (1977) found that field conditions could affect both factors in lepidopteran larvae. The morphological method hinges on the assumption that larval sclerites are consistent across all populations and can therefore be used as a diagnostic indicator of instar. The high level of accuracy with which both methods predict the head capsule widths of *O. pallicornis* second and third instars indicates that this is the likely number of instars. Between the two methods, the morphological separation of instars appears to be the most robust (Figs 2.6-2.7, Table 2.3). Not only is it based on biological delineation of instars, but the geometric series generated from the morphological method also had a lower overall percent difference from observed head capsule widths than the graphical method.

The cause of discrepancies in head capsule size between the Clarksville and Potterville sites is unknown and deserves further investigation. Based on the work of Schmidt et al. (1977) it

is likely that the observed differences are related to variation in field conditions and their impact on *O. pallicornis* development. Notable variations between the two sites that may affect development include: average tree age (considerably higher at the Potterville site), nutrient and pest management regimes (Clarksville is unmanaged), variety and training system (Central Leader at Potterville, Slender Spindle at Clarksville).

Differences in chemical management at the Clarksville and Potterville sites may explain the discrepancy in proportion of parasitoids recovered at the two sites. Clarksville has not been under any chemical management for the past five years. The apple plots we sampled at the Potterville site were only recently (~ 3-5 years) transitioned to organic management, with a significant portion of the acreage on the Potterville farm remaining under conventional management. Additionally, varieties between sites varied. The highest rate of parasitism on a single sample date was observed at the Clarksville site (>55%). Figures 2.1 and 2.2 show that after the spring peak of *O. pallicornis* activity in the spring of 2011, the population density at Clarksville was consistently lower than that at the other two sites. The absence of insecticide applications may have fostered high rates of parasitism which in turn may have contributed to the generally lower population of *O. pallicornis* at Clarksville compared to Flushing and Potterville since the spring of 2011. However, some of the less common parasitoid taxa (F: Eulophidae G: Closterocerus, and F: Braconidae) were observed in higher frequency at the two sites with a history of insecticide intervention. All sites displayed similar levels of parasitoid diversity and collected parasitoids were of the same families as those collected in an earlier *O. pallicornis* parasitoid survey (Houser 1923, Flint 1924). Each site produced at least one specimen from each of the five identified families of *O. pallicornis* parasitoids.

Promoting parasitoids for control of *O. pallicornis* is especially important in organic settings, where natural pest control is recommended over the use of chemical control methods (Wyss et al. 2005). Although chemical management tactics may be necessary as an emergency response, it is apparent that *O. pallicornis* is host to numerous parasitoid species, which can regulate the population at low densities and may be enhanced through conservation biological control (Zehnder et al. 2007). Conservation biological control has been shown to provide adequate natural control of leaf-mining pests. For example, the mango flea weevil (*Rhyncaneus mangiferae,* Marshal), a pest of mangoes that is closely related to *O. pallicornis,* has been successfully controlled through conservation of its parasitoids alone (Mani and Krishamoorthy 2001, Peter and Balasubramanian 1984). Rates of parasitism that provided adequate suppression of the mango flea weevil were ~50% lower than those recorded for *O. pallicornis* in the present study. Further understanding of the parasitoid complex of *O. pallicornis* is required before a successful conservation biological control program can be attempted. The development of a chemical management program for control of *O. pallicornis* must include a study on the effects of potential insecticides on the parasitoid community of *O. pallicornis*. Further research into *O. pallicornis* phenology would benefit from an exploration of parasitoid phenology. An integrated pest management approach to controlling *O. pallicornis* would incorporate the phenology of both pest and parasite to provide growers with a phonological model for optimal timing of insecticidal controls.

CHAPTER 3

Organic Management of the Apple Flea Weevil (Orchestes pallicornis, Say), a Reemergent Pest of Commercial Apples

1. Intro

Orchestes pallicornis (Say) is a re-emerging pest of economic significance in Michigan organic apple orchards. *Orchestes pallicornis* damage was first reported in organic orchards in 2008 but was initially misdiagnosed as frost damage. Since then, reported damage has increased dramatically, with some growers experiencing up to 90% yield loss (Nielsen et al. 2012). Historical research on this pest is extremely limited, the most recent of which was conducted in 1950 on the effect of DDT on *O. pallicornis* (Cutright 1950).

Orchestes pallicornis adults are small, matte black beetles with long snouts (typical of Curculionids). They are approximately 3mm in length and 1.5mm wide with enlarged saltatorial metathoracic femorae and striated elytra. The larvae are generally concealed in leaf mines, but when removed are ivory in color and measure ~5mm in length and ~2mm in width. Like most leaf mining larvae, they are legless and dorso-ventrally flattened (Houser 1923, Flint et al. 1924).

Orchestes pallicornis overwinter in the adult stage by burrowing into partially decayed plant-matter directly above the topsoil. Adults emerge in spring after accumulating 55-70 GDD (base 10° C) and crawl or fly to the apple canopy where they begin consuming leaf and bud tissue (Flint et al. 1924, Pote 2013). Early reports of *O. pallicornis* infestation documented an average of 1400-1700 adults emerging per tree in favorable weather conditions (Houser 1923, Flint et al. 1924). Mating begins almost immediately after emergence. Gravid females oviposit only after leaf flush and lay eggs singly in the mid-vein of low to mid canopy leaves (Houser 1923, Flint et al. 1924). Eggs hatch in roughly 7 d, after which larvae consume mesophyll tissue

in a winding path to the leaf margin. Larval development takes ~17 d to complete through 3 instars (Pote 2013). Pupation causes the dead outer layers of leaf tissue to separate and create a characteristic "blister". After 5-6 d adult *O. pallicornis* emerge by chewing a hole in the dried tissue of the blister. Second generation adults feed for 6-12 d before returning to the soil duff to overwinter. *Orchestes pallicornis* is a univoltine species, and is generally absent from the orchard canopy by mid-July (Houser 1923, Flint et al. 1924, Pote 2013)

Research pertaining to insecticidal control of *O. pallicornis* has been absent in the literature for over 50 years. Early insecticide efficacy trials for *O. pallicornis* control included arsenate of lead and calcium, nicotine sulfate and kerosene emulsion (Houser 1923). After World War II, synthetic organic compounds like DDT, parathion and methoxychlor were all successfully used for control of *O. pallicornis* (Cutright 1950, Mundinger 1951). No research has been conducted testing the efficacy of contemporary insecticides against *O. pallicornis*.

Leaf miners, when considered as a non-taxonomic feeding guild, have more parasitoids per host than any other feeding guild (Askew and Shaw 1979, Hawkins 1988, 1990; Hawkins et al. 1992, Askew 1994). *Orchestes pallicornis* populations can be maintained at non-damaging levels by an array of parasitic hymenopterans. Houser (1923) recovered *Zatropis incertus* Ashm., *Trichomalus inscitus* Walker (Hymenoptera: Pteromalidae), *Epiusus* sp., and *Chrysocharis pentheus* Walker (Hymenoptera: Eulophidae) from larval mines. Our previous work found up to 60% parasitism of *O. pallicornis* in a chemically unmanaged orchard (Pote 2013). Furthermore, leaf mines collected from an unmanaged orchard produced significantly more parasitoids than those collected from organically managed orchards (Pote 2013). Reduced parasitism in an organically managed orchard is consistent with the finding that many parasitoids are susceptible

to the lethal and sub-lethal effects of organic compounds, including the common organic apple control option, spinosad (Entrust) (Mason et al. 2002).

The goal of this research was to identify potential OMRI-approved insecticides for *O. pallicornis* management, identify the appropriate application timing for insecticides targeting spring *O. pallicornis* populations and determine the seasonal requirements for insecticidal control of Summer *O. pallicornis* populations. Additionally, I investigated the potential impact of these insecticides on *O. pallicornis* parasitoids and natural control.

2. Materials and Methods

2.1.1 Lab Insecticide Bioassays, 2011

Insect Culture: Adult *O. pallicornis* used in insecticide bioassays were obtained from leaf litter and straw mulch collected from the organic apple blocks at the MSU Clarksville Horticultural Experiment Station in Clarksville, MI (42.875964,-85.248239) from October 2010 – March 2011. Litter was stored in black plastic contractor bags at 10C° until extraction. Litter was placed in a Berlese funnel (Bioquip #2832, Rancho Dominguez, CA), modified to collect and retain live insects. Adults were extracted no more than 48 h prior to use in the bioassays. The wet and dry residue of Assail® , Avaunt®, Danitol®, Delegate®, Entrust®, Guthion®, Rex Lime, M-pede®, MycotrolO®, Neem Blend 45, PermaGuard™, Pyganic®, were tested for efficacy against adult *O. pallicornis*. Table 3.1 contains a list of the products and rates tested in this bioassay.

Insecticide lab bioassays were conducted between March and May 2011. Contact efficacy was evaluated using formulated product in deionized water at the maximum label rate for weevil pests at 935 L/ha (100 gal/acre). Material was applied to Petri dish lids and bottoms (100mm x

20mm; BD Falcon, Franklin Lakes, NJ) with a Potter Spray tower (Burkard Scientific, Uxbridge, UK). Efficacy of the material was tested while wet and dry (dried for 3 h in fume hood) against 10 individuals per Petri dish with five replications and repeated at least twice. Mortality was assessed at 24 h, 48 h, and 72 h after exposure for dry trials, and at 24 h and 48 h for wet trials. Moribund insects that did not recover were considered dead.

Table 3.1. Treatments used in the *O. palicornis* insecticide lab bio-assay. Rates were calculated based on an application volume of 935 L/ha (100 gal/acre). Trade names followed by * are approved by the Organic Materials Review Institute for use in organic production.

Trade Name	Active Ingredient	Rate
Assail 70WP	Acetamiprid	80 g/ha
Avaunt 30WG	Indoxacarb	400 g/ha
Danitol EC	Fenpropathrin	1.5 L/ha
Delegate WG	Spinetoram	512 ml/ha
Entrust SC ∗	Spinosad	210 g/ha
Guthion Solupak	Azinophosmethyl	3.36 kg/ha
Rex Lime ∗	Calcium Polysulphide	143 kg/ha
M-Pede ∗	Potassium Salts	37.5 L/ha
Mycotrol-O ∗	*Beauvaria bassiana*	2.34 L/ha
Neem Blend 45 ∗	Neem Oil	239 g/ha
Permaguard	Diatomaceous Earth	5.6 kg/ha
Pyganic EC ∗	Pyrethrins	4.67 L/ha

2.1.2-2.1.7 Field Insecticide Bioassays

On-farm efficacy trials were conducted during the 2011 and 2012 growing season at two participating certified organic orchards. The first site was in Potterville, MI (42.635608,-

84.788709). Research at this site was principally conducted in an *O. pallicornis* -infested 2.3 ha block of Cortland, Golden Delicious, Red Delicious, Jonamac, Jersey Mac and Viking varieties planted in 1987 on MM.111 rootstock. Trees were planted North to South with a 3 m x 5.5 m (10 ft. x 18 ft.) tree-row spacing, and trained in a non-supported central leader system. The second farm was in Flushing, MI (43.024371,-83.91168). Research was conducted in an *O. pallicornis*-infested 5 ha block of Golden Delicious, Gala and GoldRush on MM.7 rootstock. Trees were planted East to West with a 3 m x 5.5 m (10 ft. x 18 ft.) tree-row spacing and trained in a non-supported central leader system. Neither orchard was under irrigation but both were under mating disruption for codling moth and Oriental fruit moth and received organic fungicide sprays during the study.

At both farms, the same trees were used for population assessment and caged adult mortality. Treatments were applied to blocks of a minimum of 3 orchard rows using a farmer-cooperator supplied airblast sprayer in the early morning. Within each treatment block, ten trees (replicates) spaced two to four trees apart within the middle row were selected for data collection.

To test the efficacy of treatment residues, *O. pallicornis* adults were caged on treated terminals. Adults were collected from either of the research sites no more than 48 h prior to introduction into the cages by beat-sampling untreated trees. Adults were stored at 10 C° with access to fresh apple cuttings in floral foam (Smithers-Oasis Co., Kent, OH) prior to deployment in cages. On the day treatments were applied, *O. pallicornis* adults were removed from cold-storage, and transferred to 9 dram styrene aspirator tubes (Bioquip Inc., Rancho Dominguez, CA, product # 8909), ten adults per tube. Tubes were transported to field sites on ice. After re-entry interval expiration, weevils were exposed to treated foliage by transferring them from the chilled tubes to mesh paint strainer bags ((18.9 L (5 gal) paint strainer bags, The Cary Co., Addison, IL)

and attaching the bags to treated terminals. Bags containing weevils were applied to apple terminals such that they enclosed three to five fruiting buds and were sealed with twist ties on both ends to prevent weevils from escaping. For each treatment block, this was replicated once per tree on ten trees within the same row. After 48 h of exposure, each terminal was firmly jarred to dislodge weevils and the bag containing weevils was removed from the tree and resealed. Sealed bags were transported back to the lab on ice where adults were removed from bags, transferred into Petri dishes and assessed for mortality at ~48 h and 72 h post-treatment

The effect of treatments on natural *O. pallicornis* population densities was measured by the limb-jarring technique. One day before treatment application, ten trees per treatment block (the same trees which were later affixed with caged adults) were sampled by firmly tapping three terminal-bearing limbs (>3 cm in diameter) each at a different height within the tree canopy and collecting dislodged *O. pallicornis* s that landed on a 1 m^2 mesh sheet held beneath the limbs (Bioquip Inc., Rancho Dominguez, CA, product # 2840 R). This procedure was repeated on the same trees three days after treatment application (or as close as possible, weather permitting).

2.1.2. Entrust Timing Trial, Spring 2011: Potterville

In a mixed-variety apple block at the Potterville site, two application timings of Entrust were compared for efficacy against adult *O. pallicornis*. Treatments consisted of 219 ml per ha (3 oz. per acre) of Entrust applied at Green Tip (29 April), 219 ml per ha (3 oz. per acre) of Entrust applied at Green Tip and Pink (11 May), and an untreated control. Treatments were applied in a complete randomized block design with three blocks per treatment. . On both the green tip and pink treatment dates, caged adult mortality was tested using the aforementioned methodology. Larval mines (10 leaves/tree x 10 trees) were counted on the same trees within each treatment block.

2.1.3. Field Insecticide Trial, Spring 2011: Flushing

In a mature 4.7 ha mixed-variety apple block, four insecticide treatments were evaluated for control of the spring generation of *O. pallicornis*. Treatments consisted of 1) Entrust (219 ml/ha; 3 oz./acre) applied at Tight Cluster (##date?), 2) Entrust applied at 219 ml/ha (3 oz./acre) at Tight Cluster and Pink (13 May), 3) three applications (dates) of Surround WP (Englehard Corp., Iselin, NJ) at 60 g/ L, 50lb/100 gal, 4) three applications (dates?) of Diatomaceous Earth (Alar Engineering Corp., Mokena, IL) at 12 g/L, 10 lb/100 gal. Each treatment was applied to at least four rows in a completely randomized design. On both the tight cluster and pink treatment dates, caged adult mortality was tested using the aforementioned methodology. Larval mines (10 leaves/tree x 10 trees) were counted on the same trees within each treatment block. Adult density change was assessed by limb-jarring 10 trees per block one day before application and 3 days post-treatment (or as close as possible, weather permitting).

2.1.4. Entrust Rate Trials, Summer 2011

At the Potterville and Flushing sites, we evaluated the efficacy of Entrust applied at full-rate (219 ml/ha; 3 oz./acre) and half-rate (110 ml/ha, 1.5 oz./acre) for control of the summer *O. pallicornis* generation. In the organic apple orchard at Potterville, Entrust treatments and an untreated control were assigned in a complete randomized block design, using the same control blocks as the spring Entrust rate trial, and replicated three times. In the Flushing orchard, a complete block design was used and within each block, Entrust and control treatments were replicated three times, again using the same control trees as spring insecticide trial. Adult *O. pallicornis* were exposed to treated foliage as previously described with ten cages placed within each block. Adult density change was assessed by limb-jarring 10 trees per block one day before application and 3 days post-treatment (or as close as possible, weather permitting).

2.1.5. Entrust Timing Trials, Spring 2012

During the spring of 2012, the efficacy of Entrust sprayed at various apple bloom phenologies was evalutated. The treatments were 1) Entrust (219 ml/ha; 3 oz./acre) applied at Tight Cluster, 2) Entrust (219 ml/ha; 3 oz./acre) applied at Pink and 3) an untreated control. Treatments were applied to three 3-row blocks in a completely randomized block design at both sites. At both treatment dates (Tight Cluster on 3/22/12 at Potterville and 3/25/12 at Flushing and Pink on 4/4/12 at both sites) adults were caged on treated foliage as previously described. Larval mines (10 leaves/tree x 10 trees) were counted on the same trees within each treatment block. Adult density change was assessed by limb-jarring 10 trees per block one day before application and 3 days post-treatment (or as close as possible, weather permitting).

2.1.6. Entrust Parasitoid Trials, Spring 2012

To identify the effects of Entrust application timing on the parasitoids of *O. pallicornis*, large numbers of larval mines were collected from the Potterville and Flushing orchards. Once *O. pallicornis* larvae were first detected in the field (~24 April 2012), larval mines were collected every 2-4 d from each of the treatment blocks of the Entrust timing trials at both sites. Twenty leaves infested with unopened larval mines were collected per treatment for ~six weeks. This period was determined to be the peak parasitoid activity period from preliminary data collected in 2011. Mines were stored for three to five days in a 10° C refrigerator before being stored individually in 60mm x 15mm petri dishes sealed with acetate tape. Petri dishes were stored at room temperature and checked approximately once per week for emergence of either a parasitoid wasp or an adult *O. pallicornis*. After emergence ceased, parasitoids were transferred into microcentrifuge vials containing 80% ethanol for later identification.

2.1.7. Entrust Rate Trials, Summer 2012

The efficacy of Entrust at full-rate (219 ml/ha; 3 oz./acre) and half-rate (110 ml/ha; 1.5 oz./acre) for summer *O. pallicornis* control was evaluated again in 2012. At the Potterville, MI orchard, the two treatments and an untreated control were applied in a complete randomized block design replicated three times. In Flushing, MI, a complete block design was used and within each block, each of the three treatments were replicated three times, again using the same control trees as the spring insecticide trial. At both sites, adult *O. pallicornis* caged mortality was tested as described above. On the same trees, limb-jarring samples were taken to assess population density 1d prior to application and ~3d after application.

2.2. Statistical Analysis

2.2.1. Lab Insecticide Bioassay, Spring 2011

Wet and dry residue efficacy of each compound was determined by comparing the Abbott's corrected mortality (Abbott 1925) by block between treatments using a Kruskal-Wallis rank sum test.

2.2.2. Entrust Timing Trial, Spring 2011: Potterville

Mortality of caged adults from the Entrust timing trial at Potterville was corrected using Abbott's Corrected Mortality and compared to the mortality of the appropriate control using Welch two-sample t-tests. This was repeated for each mortality evaluation interval (48 h and 72 h). Proportion of leaves with mines were arcsine transformed and compared across Entrust application timings using analysis of variance with tree variety as an interactive factor. Tukey's HSD ($p < 0.05$) was used to determine significant differences in larval infestation rates between treatments.

2.2.3. Field Insecticide Trial, Spring 2011: Flushing

Mortality of caged adults from the Spring insecticide trial at Flushing was corrected using Abbott's Corrected Mortality and compared across treatments using a Kruskal Wallis rank sum test. Larval infestation rates were arcsine square-root transformed and compared across treatments using analysis of variance. Tukey's HSD ($p < 0.05$) was used to determine significant separation of mean larval infestation rates across treatments. The change in *O. pallicornis* population before and after application was compared across treatments using analysis of variance. Tukey's HSD ($p < 0.05$) was used to determine significant differences in mean population change between individual treatments.

2.2.4. Entrust Rate Trials, Summer 2011

Mortality of caged adults from the Entrust rate trial at both sites was corrected using Abbott's Corrected Mortality and compared across treatments using Kruskal Wallis rank sum test. The change in *O. pallicornis* population before and after application at both sites was compared across treatments using analysis of variance. Tukey's HSD ($p < 0.05$) was used to determine significant differences in mean population change between individual treatments.

2.2.5. Entrust Timing Trials, Spring 2012

Mortality of caged adults from the Entrust timing trial at both sites was corrected using Abbott's Corrected Mortality and compared across treatments using a Wilcoxon rank-sum test. Proportions of leaves with mines were arcsine square-root transformed and compared across treatments using analysis of variance. Tukey's HSD ($p < 0.05$) was used to determine means separation across treatments. The change in density after application at both sites was compared across treatments using analysis of variance. Tukey's HSD ($p < 0.05$) was used to determine significant differences in mean population change between individual treatments. The proportion

of parasitoids emerged from leaf mine samples was analyzed using repeated-measures multivariate analysis of variance.

3. Results

3.1. Lab Insecticide Bioassay, 2011

3.1.1. Wet Residuals

The lab bioassay of wet insecticides residues revealed significant differences in *O. pallicornis* mortality between treatments at both 24 h and 48 h post-treatment (24 h: Chi^2 = 81.18, *df* =*30, p* = < 0.0001; 48 h: Chi^2 = 77.98, *df* = 28, *p* = < 0.0001)(Table 3.2a). The results are summarized in Table 3.2a. Many of the synthetic insecticides caused very high *O. pallicornis* mortality. Guthion and Danitol caused 100% mortality at both 24 h and 48 h post-treatment. Of the organic-approved treatments tested, only Entrust and Pyganic caused >85% mortality after 24 h, and after 48 h, only Entrust and M-Pede caused >95% mortality.

3.1.2. Dry Residuals

There were significant differences in *O. pallicornis* mortality between dry residue treatments at 24 h, 48 h and 72 h mortality assessment intervals (24 h: Chi^2 = 176.6, *df* =55, *p* < 0.0001; 48 h: Chi^2 = 180.5, *df* = 45, *p* < 0.0001; 72 h: Chi^2 = 138.59, *df* = 28, *p* < 0.0001) The results of the dry residue efficacy trial are summarized in Table 3.2b. As in the wet residue trials, dry residues of synthetic insecticides caused high *O. pallicornis* mortality. At 24 h post-application, exposure to Guthion and Assail resulted in 100% mortality. Exposure to Assail,

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Table 3.2a. Lab insecticide bio-assay mortality caused by wet residuals. Letters indicate significant differences among treatments (Kruskal-Wallis Rank Sum Test, $\alpha = 0.05$). Treatments followed by * are approved by the Organic Materials Review Institute.

	24h Mortality		48h Mortality	
Treatment	**(%) ± SEM**		**(%) ± SEM**	
Assail	79.9 ± 7.8	b	94.0 ± 4.8	a
Avaunt	10.0 ± 4.2	cd	17.2 ± 5.9	b
Danitol	100.0 ± 0.0	a	100.0 ± 0.0	a
Delegate	91.3 ± 4.6	ab	100.0 ± 0.0	a
Entrust*	87.8 ± 4.5	b	95.8 ± 2.2	a
Guthion	100.0 ± 0.0	a	100.0 ± 0.0	a
Lime Sulfur*	13.3 ± 4.6	c	21.6 ± 9.9	b
M-pede*	82.2 ± 6.5	b	96.5 ± 3.5	a
MycotrolO*	5.7 ± 1.9	cd	9.8 ± 4.8	bc
Neem*	4.1 ± 2.9	d	6.7 ± 3.3	bc
PermaGuard	7.7 ± 3.2	cd	14.2 ± 8.2	bc
Pyganic*	88.1 ± 3.8	b	94.2 ± 4.0	bc
Control	1.7 ± 0.6	d	7.2 ± 3.2	c

Danitol, Delegate, Guthion and Entrust (an OMRI-approved treatment) resulted in 100% mortality 72 h post-application. Entrust was the only organic treatment that provided significant mortality, with > 30% mortality after 24 h, > 50% mortality after 48 h, and > 70% mortality after 72 h. At 48 h and 72 h post-application intervals, the mortality caused by exposure to Entrust residues was not significantly different from that of Guthion. After 72 h, Entrust, M-Pede and Pyganic were the only organic treatments that provided mortality significantly different than that in the control group (Kruskal Wallis, $p < 0.05$).

Table 3.2b. Lab insecticide bio-assay mortality caused by wet residuals. Letters indicate significant differences among treatments (Kruskal-Wallis Rank Sum Test, $\alpha = 0.05$). Treatments followed by * are approved by the Organic Materials Review Institute.

	24h Mortality		48h Mortality		72h Mortality	
Treatment	(%) ± SEM		(%) ± SEM		(%) ± SEM	
Assail	100.0 ± 0.0	ab	100.0 ± 0.0	a	100.0 ± 0.0	a
Avaunt	20.9 ± 6.5	ef	58.5 ± 6.8	c	76.3 ± 4.9	bc
Danitol	99.2 ± 0.8	a	100.0 ± 0.0	a	100.0 ± 0.0	a
Delegate	84.2 ± 5.0	bc	98.3 ± 1.2	a	100.0 ± 0.0	a
Entrust*	78.3 ± 7.0	c	91.8 ± 6.7	a	100.0 ± 0.0	a
Guthion	100.0 ± 0.0	a	100.0 ± 0.0	a	100.0 ± 0.0	a
Lime Sulfur*	6.2 ± 3.3	efg	15.9 ± 6.5	de	25.7 ± 7.8	d
M-pede*	11.6 ± 3.6	d	16.9 ± 6.0	b	63.6 ± 6.6	b
MycotrolO*	2.2 ± 0.8	fg	5.9 ± 2.3	ef	16.0 ± 6.4	d
Neem*	2.9 ± 1.7	g	4.7 ± 2.0	f	9.3 ± 3.7	d
PermaGuard	15.4 ± 4.9	e	29.0 ± 7.8	d	30.5 ± 8.5	d
Pyganic*	27.0 ± 7.7	d	45.5 ± 9.5	b	50.9 ± 14.5	c
Control	8.2 ± 3.2	g	14.7 ± 5.4	ef	9.4 ± 5.2	d

3.2. Entrust Timing Trial, Spring 2011: Potterville

3.2.1. Field Bioassay of Caged Mortality

Entrust applied at Green Tip provided significant mortality to caged *O. pallicornis* at 48 h and 72 h after treatment (48 h: t = -13.5, df = 84, $p < 0.0001$, 72 h: t = -14.5, df = 76, p = < 0.0001) (Table 3.3a). Applications of Entrust at Green Tip and Pink also resulted in significantly higher mortality compared to the untreated control at 48 h and 72 h (48 h: t = -19.8, df = 39, p = < 0.0001; 72 h: t = -16.5, df = 31, p = < 0.0001) (Table 3.3a). Applications of Entrust at both Green Tip and Pink caused numerically higher mortality than a single treatment at Green Tip (Table 3.3a).

Table 3.3. Potterville, 2011: Effects of Entrust application timing on caged adult *O. palicornis* mortality and prevalence of larval mines.. P-value for caged adult mortality study determined by Welch Two-Sample T-Test. Means separation for proportion of leaves with mines determined by Tukey's HSD ($\alpha = 0.05$)

a. Caged Adult Mortality

	48 Hour Mortality		**72 Hour Mortality**	
Timing	**% ± SEM**	**p-val**	**% ± SEM**	**p-val**
Green Tip + Pink	98.3 ± 0.8	**< 0.001**	99.6 ± 0.4	**< 0.001**
Control	16.8 ± 3.5		26.2 ± 4.5	
Green Tip	32.2 ± 2.3	**< 0.001**	79.9 ± 2.9	**< 0.001**
Control	2.1 ± 0.8		12.7 ± 2.3	

b. Proportion of Leaves with Mines

Timing	**Prop Leaves w/ Larvae**	
Green Tip + Pink	20.0 ± 2.7	a
Green Tip	53.0 ± 5.2	b
Control	78.3 ± 4.8	c

3.2.2. Mine Proportion

There were significant differences between treatments in proportion of leaves with mines ($F = 58.1$, $df = 2, 80$, $p = < 0.0001$) (Table 3.3b). Average proportion of leaves with mines was significantly higher in blocks treated with Entrust at Green Tip than in blocks treated with Entrust at Green Tip and Pink (GT: 20.2%, GT+P: 12.4%) (Table 3.3b). Untreated blocks had a higher proportion of mines than insecticide-treated blocks (Control: 28.0%) (Table 3.3b).

3.3. Field Insecticide Trial, Spring 2011: Flushing

3.3.1. Field Bioassay of Caged Mortality

There were significant differences in caged adult mortality between the spring insecticide treatments at 48 h and 72 h after treatment (48 h: $Chi^2 = 39.0$, $df = 20$, $p = 0.0066$; 72 h: $Chi^2 = 22.07$, $df = 10$, $p = 0.0147$) (Table 3.4a). Entrust applied at Pink or at Pink and Tight Cluster

caused significantly higher mortality than other treatments after 48 h, The levels of mortality provided by the two Entrust programs were not significantly different from each other (P: 78%, P+TC: 99%). After 72 h, higher mortality was recorded in the Entrust treatments compared to Diatomaceous Earth (P: 94%, P+TC: 100%) or the untreated control. However, significantly higher mortality after 72 h was recorded in the Entrust at Pink compared to the Surround treatment (Surround: 73%)(Table 3.4a).

3.3.2. Change in Adult Density as Measured by Beat-Sampling

There were significant treatment effects on endemic *O. pallicornis* populations as measured by changes in adult densities before and after application of insecticides (F = 5.4856, df= 4, p = 0.0011) (Table 3.4b). Adult densities declined significantly following application of Entrust applied at Pink and Tight Cluster compared to the untreated control (P+TC: -33.6, Control: -0.7)(Table 3.4b).

3.3.3. Mine Proportion

The proportion of leaves with mines differed significantly between treatments (F = 10.32, df = 4, p = < 0.0001)(Table 3.4b). None of the treatments provided a significant reduction in the proportion of leaves with mines compared to the controls, although Entrust applied at Pink and Tight cluster had the lowest proportion, numerically (P: 39%, P+TC: 7%, DE: 49%, Control: 29%)(Table 3.4b).

Table 3.4. Flushing, 2011: Effects of Entrust application timing on caged adult *O. palicornis* mortality, change in natural population density and prevalence of larval mines. Letters indicate significant differences among treatments (For caged adult mortality, means separation determined by Kruskal-Wallis Rank Sum Test, α = 0.05; For change in adult density and proportion of leaves with mines, means separation determined by Tukey's HSD, α = 0.05)

a. Caged Adult Mortality

	48 Hour Mortality		**72 Hour Mortality**	
Treatment	**% ± Std Err**		**% ± Std Err**	
Tight Cluster + Pink	98.5 ± 1.5	a	100.0 ± 0.0	a
Pink	78.5 ± 7.2	a	93.9 ± 4.2	ab
Surround	25.7 ± 9.3	b	72.8 ± 11.9	b
DE	11.5 ± 8.0	b	8.0 ± 6.4	c
Control	7.4 ± 3.4	b	23.7 ± 10.7	c

b. Change in Adult Density and Proportion of Leaves with Mines

Treatment	**△ Adult Density**		**Proportion of Leaves w/ Mines**	
Tight Cluster + Pink	−21.6 ± 4.6	a	0.07 ± 0.03	a
Pink	−33.6 ± 5.5	ab	0.39 ± 0.07	bc
Surround	−12.6 ± 11.6	ab	0.61 ± 0.12	c
DE	− 8.8 ± 3.1	b	0.49 ± 0.07	bc
Control	− 0.7 ± 4.0	b	0.29 ± 0.05	ab

3.4. Entrust Rate Trial, Summer 2011: Potterville

3.4.1. *Field Bioassay of Caged Mortality*

There were significant differences in the 48 h and 72 h mortality of caged *O. pallicornis* between treatments in the summer Entrust rate trials at Potterville in 2011 (48 h: Chi^2= 39.09, *df* = 2, *p* = < 0.0001; 72 h: Chi^2= 35.99, *df* = 2, p = < 0.0001)(Table 3.4). Mortality was significantly higher after 48 h and 72 h in blocks treated with the full label rate of Entrust than blocks treated at half rate (48 h: Full= 68%, Half= 33%, Control= 17%; 72: Full= 96%, Half= 81%, Control= 43%)(Table 3.5).

3.4.2. Change in Adult Density as Measured by Beat-Sampling

There also were significant treatment effects on endemic *O. pallicornis* populations as measured by changes in adult densities before and after application of insecticides (F = 5.38, df = 2, p = 0.0068)(Table 3.4). The two Entrust treatments and the control all had significantly different impacts on adults. While *O. pallicornis* adult density increased in the untreated control (9.8), density decreased on average by 17.4 in the full rate Entrust blocks and increased by only 0.53 in the half rate Entrust blocks (Table 3.5).

Table 3.5. Potterville, 2011: Effect of Entrust application rate on average mortality of caged adults and change in density of wild adults. Full-rate treatments of Entrust were applied at 219 ml/ha (3 oz./acre) and half-rate treatments were applied at 110 ml/ha (1.5 oz./acre).

	Caged Adult Mortality				*Change In Density*	
	48 Hour Mortality		**72 Hour Mortality**		**Δ Adult Density**	
Treatment	**(%) ± SEM**		**(%) ± SEM**			
Full	68.1 ± 4.7	a	96.5 ± 2.3	a	-17.4 ± 5.5	a
Half	32.9 ± 3.9	b	80.8 ± 4.0	b	0.5 ± 1.6	b
Control	17.3 ± 4.9	c	42.8 ± 6.9	c	9.8 ± 10.6	c

3.5. Entrust Rate Trial, Summer 2011: Flushing

3.5.1. Field Bioassay of Caged Mortality

There were significant differences in the 48 h and 72 h mortality of caged *O. pallicornis* between treatments (48 h: Chi^2 = 40.3, df = 2, p = < 0.0001; 72 h: Chi^2 = 44.8, df = 2, p = < 0.0001)(Table 3.6). Mortality after 48 h was significantly higher in Entrust treated blocks than the control, but there was no significant difference between Entrust application rates (Full: 53%, Half: 51%, Control: 6%). Mortality after 72 h was also significantly higher in Entrust treated

blocks than the control, but again there was no significant difference between Entrust application rates (Full: 86%, Half: 84%, Control: 7%)(Table 3.6).

Table 3.6. Flushing, 2011. Effect of Entrust application rate on average mortality of caged adults and change in density of wild adults. Full-rate treatments of Entrust were applied at 219 ml/ha (3 oz./acre) and half-rate treatments were applied at 110 ml/ha (1.5 oz./acre).

	Caged Adult Mortality				*Change In Density*	
	48 Hour Mortality		**72 Hour Mortality**		**$\triangle$ Adult Density**	
Treatment	**(%) $\pm$ SEM**		**(%) $\pm$ SEM**			
Full	50.6 $\pm$ 4.7	a	84.0 $\pm$ 2.3	a	-32.6 $\pm$ 5.5	a
Half	53.3 $\pm$ 3.9	a	86.1 $\pm$ 4.0	a	-19.3 $\pm$ 1.6	b
Control	5.7 $\pm$ 4.9	b	6.7 $\pm$ 6.9	b	-18.2 $\pm$ 10.6	b

3.5.2. Change in Adult Density as Measured by Beat-Sampling

There were significant treatment effects on *O. pallicornis* populations as measured by changes in adult densities before and after application of insecticides (F = 5.91, df = 2, p = 0.0042)(Table 3.6). Change in density was significantly different in blocks treated with Entrust at full label rate than in half-rate and control blocks. Adult density decreased on average by 32.5 in the full rate Entrust blocks, while similar and lower declines were recorded in the half-rate Entrust (-19.3) and control (-18.2).

3.6. Entrust Timing Trial, Spring 2012: Potterville

3.6.1. Field Bioassay of Caged Mortality

Each Entrust application timing was significantly different from the respective controls at both 48 h and 72 h (Pink: 48 h: W = 804, p < 0.0001; 72 h: W = 898, p < 0.0001; Tight Cluster: 48 h: W = 852, p < 0.0001; 72 h: W = 878, p < 0.0001)(Table 3.7a). At 48 h, mortality in bags treated with Entrust at Pink was numerically higher than bags treated with Entrust at Tight Cluster (48 h: Pink= 36%, Tight Cluster= 56%). However, at 72 h mortality in bags treated with

Entrust at Pink was numerically lower than bags treated with Entrust at Tight Cluster (72 h: Pink= 77%, Tight Cluster= 70%).

3.6.2. Change in Adult Density as Measured by Beat-Sampling

There were no significant treatment effects on endemic *O. pallicornis* populations as measured by changes in adult densities before and after application of insecticides (F = 0.028, df = 2, p = 0.972)(Table 3.7b).

3.6.3. Mine Proportion

There were significant treatment effects on the proportions of leaves with mines (F = 256.6, df = 2, p = < 0.0001)(Table 3.7b). All treatments differed significantly from each other, but the fewest proportion of mines were found in the blocks treated with Entrust at Tight Cluster (P: 9.3%, TC: .06%, Control: 56%)(Table 3.7b).

Table 3.7. Potterville, 2012: Effects of Entrust application timing on caged adult *O. palicornis* mortality, change in natural population density and prevalence of larval mines. P-value for caged adult mortality study determined by Wilcoxon Rank-Sum Test. Means separation for change in adult density and proportion of leaves with mines determined by Tukey's HSD (α = 0.05).

a. Caged Adult Mortality

	48 Hour Mortality		**72 Hour Mortality**	
Timing	**% ± Std Err**	**p-val**	**% ± Std Err**	**p-val**
Tight Cluster	56.4 ± 3.6	< **0.001**	69.1 ± 3.1	< **0.001**
Control	8.4 ± 3.2		10.4 ± 3.4	
Pink	33.6 ± 4.1	< **0.001**	76.8 ± 3.2	< **0.001**
Control	2.2 ± 0.8		8.7 ± 2.0	

b. Change in Adult Density and Proportion of Leaves with Mines

Treatment	**△ Adult Density**		**Proportion Leaves w/ Mines**	
Tight Cluster	-7.0 ± 1.0	a	0.01 ± 0.004	a
Pink	-6.7 ± 1.3	a	0.09 ± 0.009	b
Control	-7.1 ± 1.3	a	0.56 ± 0.031	c

Table 3.8. Flushing, 2012: Effects of Entrust application timing on caged adult *O. palicornis* mortality, change in natural population density and prevalence of larval mines. P-value for caged adult mortality study determined by Wilcoxon Rank-Sum Test. Means separation for change in adult density and proportion of leaves with mines determined by Tukey's HSD ($\alpha = 0.05$).

a. Caged Adult Mortality

	48 Hour Mortality		**72 Hour Mortality**	
Timing	**% ± Std Err**	**p-val**	**% ± Std Err**	**p-val**
Tight Cluster	24.4 ± 2.9	< **0.001**	54.1 ± 3.4	< **0.001**
Control	5.0 ± 1.3		9.5 ± 1.6	
Pink	45.1 ± 3.8	< **0.001**	80.1 ± 3.4	< **0.001**
Control	24.4 ± 4.6		32.9 ± 4.6	

b. Change in Adult Density and Proportion of Leaves with Mines

Treatment	**Δ Adult Density**		**Proportion Leaves w/ Mines**	
Tight Cluster	-25.5 ± 2.7	a	1.23 ± 0.24	a
Pink	-1.7 ± 0.3	b	1.00 ± 0.23	b
Control	-12.5 ± 4.7	c	6.67 ± 0.23	c

3.7. Entrust Timing Trial, Spring 2012: Flushing

3.7.1. Field Bioassay of Caged Mortality

There was a significant difference in caged *O. pallicornis* mortality between blocks treated with Entrust at Pink and the control at 72 h post-application, not 48 h (Pink: 48 h= 45%, 72 h= 80%; Control: 48 h= 24%, 72 h= 33%)(48 h: W = 518, $p = 0.3168$; 72 h: W = 769, $p < 0.0001$)(Table 3.8a). There was also a significant difference in caged *O. pallicornis* mortality between blocks treated with Entrust at Tight Cluster and the control at 48 h and 72 h post-application (Tight Cluster: 48 h= 24%, 72 h= 54%; Control: 48 h= 5%, 72 h= 10%)(48 h: W = 583, $p = 0.0458$; 72 h: W = 856, $p < 0.0001$)(Table 3.8a).

3.7.2. Change in Adult Density as Measured by Beat-Sampling

There were significant treatment effects on native *O. pallicornis* populations as measured by changes in adult densities before and after application of insecticides (F = 12.37, df = 2, p < 0.0001)(Table 3.8b). All treatments were significantly different from each other, but the greatest decrease in density was recorded following application of Entrust at Tight Cluster (-25.5 adults/tree)(Table 3.8b).

3.7.3. Mine Proportion

Proportions of leaves with mines differed significantly between treatments (F = 320.8, df = 2, p = < 0.0001)(Table 3.8b). All treatments differed significantly from each other, but blocks treated with Entrust at Tight Cluster had the lowest proportion overall (0.003)(Table 3.8b).

3.8. Entrust Parasitoid Trial, Spring 2012: Potterville

There were significant differences in the number of parasitoids that emerged from leaf samples between treatments (F = 4.84, df = 2, p = 0.0202). Leaves treated at Tight Cluster yielded significantly fewer parasitoids than those treated at Pink or not treated with insecticide (Tight Cluster= 0.05, Pink= 0.14, Control= 0.18)(Table 3.9).

3.9. Entrust Parasitoid Trial, Spring 2012: Flushing

There were no significant treatment effects on the number of parasitoids that emerged from leaf samples (Tight Cluster= 0.19, Pink= 0.16, Control= 0.20)(F = 0.27, df = 2, p = 0.755)(Table 3.9).

3.10. Entrust Rate Trial, Summer 2012: Potterville

3.10.1. Field Bioassay of Caged Mortality

There was a significant difference in caged mortality between treatment blocks at both 48 h and 72 h post-application (48 h: $F = 74$, $df = 2$, $p < 0.0001$, 72 h: $F = 226.8$, $df = 2$, $p < 0.0001$)(Table 3.10). Mortality of caged adults did not differ significantly between half and full rate applications of Entrust at 48 h or 72 h (Full: 48 h= 80%, 72 h= 95%; Half: 48 h= 75%, 72 h= 90%, Control: 48 h= 30%, 72 h= 37%)(Table 3.10).

Table 3.9. Effect of Entrust application timing on the proportion of insects emerged from *O. palicornis* larval mines made up by parasitoids. Means separation was determined by Tukey's HSD for repeated-measures multivariate analysis ($\alpha = 0.05$).

	Potterville		**Flushing**	
Treatment	**Proportion ± Std Err**		**Proportion ± Std Err**	
Tight Cluster	0.05 ± 0.01	a	0.19 ± 0.04	a
Pink	0.14 ± 0.03	b	0.16 ± 0.04	a
Control	0.18 ± 0.03	b	0.20 ± 0.05	a

Table 3.10. Potterville, 2012: Effect of Entrust application rate on average mortality of caged adults and change in density of wild adults. Full-rate treatments of Entrust were applied at 219 ml/ha (3 oz./acre) and half-rate treatments were applied at 110 ml/ha (1.5 oz./acre).

	Caged Adult Mortality				*Change In Density*	
	48 Hour Mortality		**72 Hour Mortality**		**Δ Adult Density**	
Treatment	**(%) ± SEM**		**(%) ± SEM**			
Full	79.5 ± 3.7	a	95.0 ± 1.4	a	-5.95 ± 1.6	a
Half	74.5 ± 4.5	a	89.5 ± 2.5	a	-2.25 ± 0.5	ab
Control	29.5 ± 3.0	b	37.0 ± 2.9	b	-1.75 ± 0.7	b

Table 3.11. Flushing, 2012: Effect of Entrust application rate on average mortality of caged adults and change in density of wild adults. Full-rate treatments of Entrust were applied at 219 ml/ha (3 oz./acre) and half-rate treatments were applied at 110 ml/ha (1.5 oz./acre).

	Caged Adult Mortality				*Change In Density*	
	48 Hour Mortality		**72 Hour Mortality**		**Δ Adult Density**	
Treatment	**(%) $\pm$ SEM**		**(%) $\pm$ SEM**			
Full	64.0% $\pm$ 3.6%	a	68.7% $\pm$ 3.3%	a	-1.13 $\pm$ 0.3	a
Half	51.3% $\pm$ 3.9%	a	60.7% $\pm$ 3.9%	a	-0.90 $\pm$ 0.3	a
Control	23.0% $\pm$ 2.8%	b	32.0% $\pm$ 3.2%	b	-1.27 $\pm$ 0.4	a

3.10.2. Change in Adult Density as Measured by Beat-Sampling

There were significant treatment effects on native *O. pallicornis* populations as measured by changes in adult densities before and after application of insecticides (F = 3.63, df = 2, p = 0.0328) (Table 3.10). The decline in adult density in blocks treated with full-rate Entrust was significantly greater than that recorded in the other treatments. (Full: -5.95, Half: -2.25, Control: -1.75)(Table 3.10).

3.11. Entrust Rate Trial, Summer 2012: Flushing

3.11.1. Field Bioassay of Caged Mortality

There was a significant difference in caged mortality between treatments at both 48 h and 72 h (48 h: F = 47.4, df = 2, p < 0.0001, 72 h: F = 37.3, df = 2, p < 0.0001)(Table 3.11). Mortality did not differ significantly between half and full rate applications at 48 h or 72 (Full: 48 h= 64%, 72 h= 69%; Half: 48 h= 51%, 72 h= 61%, Control: 48 h= 23%, 72 h= 32%)(Table 3.11).

3.11.2. Change in Adult Density as Measured by Beat-Sampling

There were no significant treatment effects on endemic *O. pallicornis* populations as measured by changes in adult densities before and after application of insecticides (Full= -1.1, Half= -0.9, Control= -1.3)($F = 1.093$, $df = 2$, $p = 0.34$)(Table 3.11).

4. Discussion

Laboratory bioassays demonstrated that *O. pallicornis* were susceptible to an array of conventional insecticides (Table 3.2). In contrast, *O. pallicornis* were relatively unaffected by all but a few of the organic insecticides (Table 3.2). These findings support the general hypothesis that *O. pallicornis* is more common in organically managed orchards compared to conventional orchards because of differences in insecticide regimes. Wet residues of the OMRI approved compounds, Entrust, M-Pede and Pyganic, did cause high levels of mortality. However, Entrust was the only OMRI-approved product providing > 65% mortality following exposure to dry residues (Table 3.1b). Dry residues of Entrust at 48 h and 72 h caused mortality that was not significantly different from Guthion, a broad-spectrum conventional compound recently phased-out for use in apple and other crops in the US (Table 3.1b)(EPA News Brief).

The field trials conducted over the spring of 2011 highlight the importance of Entrust as a tool for the successful organic control of *O. pallicornis*. Two applications of Entrust was overall the most effective treatment for killing caged adults and reducing larval infestation rates (Tables 3.3-3.4). However, at the Flushing site the mortality caused by two applications of Entrust was not significantly different from that of the single application of Entrust (Table 3.4). Although Surround was only tested at one site, our findings indicated this material had potential for controlling *O. pallicornis*. Caged adult mortality (72 h), change in population density and proportion of leaves with mines were similar following applications of Surround or a single

application of Entrust (Table 3.4). Due to abnormal weather conditions in 2012, O. pallicornis populations throughout that year were unpredictable. This did not affect the results of the caged mortality experiments, where flea weevil populations were controlled. It should be noted however, the extreme unpredictability of natural O. pallicornis densities affected direct study of adult population densities during the spring and summer 2012 (Pote 2013).

Tight Cluster and Pink applications of Entrust were effective for *O. pallicornis* control. Significant mortality of caged *O. pallicornis* adults occurred following applications of Entrust at Tight Cluster or at Pink. The Tight Cluster treatment significant decreased natural populations at Flushing, and significantly lowered the proportion of leaves with mines at Potterville (Tables 3.3-3.4, 3.7-3.8).

Comparison of a full label rate of Entrust or a half rate of Entrust provided confounding results. In 2011, a full rate application of Entrust resulted in significantly higher caged adult mortality and significantly decreased populations at Potterville (Table 3.5). At this same site in 2012, however, the two rates of Entrust provided similar impacts on *O. pallicornis* (Table 3.10). At Flushing in 2012, significantly higher mortality in caged adults at 48 h but not at 72 h was recorded for the full compared to the half rate of Entrust (Table 3.11). Delayed mortality of half-rate applications was also observed at Potterville in 2011, although it was not significant (Table 3.5). This effect was not observed at Flushing in 2011 (Table 3.6). Whether Entrust should be applied at the full label rate or a reduced rate remains unclear.

Overall, these findings provided strong evidence that Entrust is currently the most effective organically-approved option for controlling *O. pallicornis*. Unfortunately, Entrust has been shown to negatively affect natural enemies including hymenopterous parasitoids (Cisneros et al. 2002, Mason et al. 2002) and in this study decreased emergence of *O. pallicornis*

parasitoids at one of the sites. In an effort to mitigate potential impacts on parasitoids, reduced-risk Entrust application practices were explored in the present study. Applying Entrust at Pink or at Tight Cluster provided significant control of *O. pallicornis*. However, application at Tight Cluster, being phenologically earlier than Pink, may provide more time for residues to decline and therefore protect pollinators and parasitoids from the deleterious effects of Entrust (Mayes et al. 2003, Mason et al. 2002).

Considering both efficacy and conservation of beneficials, I suggest applying Entrust at Tight Cluster. Half-rate applications of Entrust also may be a viable strategy for the control of *O. pallicornis* adults. However, this strategy is most useful against the summer generation where immediate mortality is less of a concern. Considering the relatively high cost of a full label rate application of Entrust (~ $320 / ha; ~$130/acre) and the maximum annual application cap (0.65 L/ ha; 9 oz. / acre), future research should explore Surround as a tool for control of *O. pallicornis* (Table 3). In all organic agricultural systems, priority should be given to preventative measures, like cultural manipulation of the agro-ecosystem (Zehnder et al. 2006). Houser (1923) found that in a completely cultivated orchard, *O. pallicornis* damage was well below the economic threshold. Although Entrust is currently the only tool available to control *O. pallicornis* at outbreak levels, cultivation in concert with conservation biocontrol and optimization of timings and rates of organic insecticides may be able to suppress *O. pallicornis* populations to non-damaging levels in the future.

CHAPTER 4

Conclusions and Future Research

The goal of this research was to expand our understand of *O. pallicornis* basic biology and seasonality and provide affected growers with satisfactory control measures that are congruent with current National Organic Standards. *Orchestes pallicornis* has recently resurfaced as a pest of economic significance in Michigan's organic apple orchards, and has caused significant yield losses across the state (Nielsen et al 2012). *Orchestes pallicornis* has not been a prominent pest in several decades, thus modern research pertaining to its biology and control with contemporary insecticides is completely lacking from historical literature. The objectives of this research were to:

1. Collect phenological and basic biological information regarding *O. pallicornis* as well as that of its parasitoids.
2. Establish an effective control program for *O. pallicornis* using materials and methods compatible with current National Organic Program regulations.
3. Communicate relevant information pertaining to the biology and control of *O. pallicornis* to the grower community.

As a result of this research, our understanding of *O. pallicornis* biology has been updated for the 21st century. I am now able to suggest an organic-approved insecticide to affected growers seeking management options. My exploration of *O. pallicornis* parasitoids has shown that natural biological control may contribute to economic suppression of *O. pallicornis* especially under systems that minimize the use of chemical management tactics. By developing a

preliminary development minimum, I have taken the first step toward developing a phonological model. Such a model could be used to predict Spring *O. pallicornis* emergence, which growers may use to integrate knowledge of this pest's biology with measures for its control by appropriately timing applications of Entrust. I also showed that Entrust may negatively affect parasitoids of the *O. pallicornis*. Growers may protect parasitoids by including the phenology of these natural enemies in the determination of appropriate Entrust application timing.

The methods used to determine the appropriate GDD model for *O. pallicornis* Spring emergence could be applied to the case of almost all emergent or re-emergent pests. For pests such as these, an imperfect phenological model is acceptable, so long as growers can calculate rough estimates of pest activity for treatment timing. These imperfect models can then be revised after several years of population and temperature data have been collected, or lab rearing methods are developed and can be used to determine phenology (as in Campbell et al. 1974). This regression method of GDD model determination could also be used to predict the activity of new invasive species. Between increased international trade as a result of globalization and global climate change, invasive pests will continue to be a primary concern for pest managers in all realms of entomology (Dukes and Mooney 1999, Perrings et al. 2005) thus methods for preliminary descriptions of pest phenology will be paramount.

The use of reduced-risk or narrow-spectrum insecticides is an increasingly important aspect of IPM (Kogan 1998). Many modern narrow-spectrum insecticides mimic or block the behavior of juvenile hormones (Dhadialla et al. 1998), and others are variably effective against different larval instars of the same species (Liang 2002). Describing the instars of *O.palicornis* larvae by conspicuous morphological characters may also allow scouts, growers and researchers to determine the age of an unknown larva in the field. Additionally, larval instar determination

through analysis of absent sclerites may be a novel approach to determining this basic biological information. When applicable, this method may assist in the study of insects without distinct head capsule width distributions, as is the case for insects with variable rates of inter-instar growth (Fink, 1984).

The phenological data provided in Chapter 2 is of great value to the grower community, however it is admittedly preliminary. Definitive phenological models should ideally be determined from many years of temperature and activity data. The atypical weather in the Spring of 2012 liklely decreased the accuracy of our proposed lower developmental threshold and any subsequent degree day model. Future attempts to describe the phenology of *O. pallicornis* should be paired with attempts to develop a method of lab-rearing. In this way, lab and field phenological data could be synthesized into an extremely accurate and comprehensive model of *O. pallicornis* development.

Numerous species of agricultural pests have developed resistance to spinosad (the active ingredient of Entrust) (Zhao et al. 2002; Shono and Scott 2003 among others). Because Entrust is the only chemical control tactic yet explored, *O. pallicornis* may be at high risk for resistance development (Wise et al. 2008). Entrust was shown to be an extremely effective method for controlling *O. pallicornis* but was also shown to negatively affect parasitoids of *O. pallicornis*. Ideally, *O. pallicornis* would be controlled by non-chemical strategies. Early research indicated that wide-scale cultivation may be instrumental in controlling *O. pallicornis* (Flint 1923, Houser 1924). Cultivation is also commonly used for organic control of weeds thus future research could integrate these two approaches for optimum control of both weeds and *O. pallicornis* (Bond and Grundy 2001). Even after further research, chemical methods like Entrust may still be the only available option for control of outbreak populations of *O. pallicornis*. If chemical control is

required, continued research should further refine the appropriate timings of these applications to protect natural enemies of *O. pallicornis*.

Even our preliminary understanding of *O. pallicornis* control would benefit greatly from a better understanding of *O. pallicornis* parasitoid phenology. At one field site, applying Entrust at Tight Cluster caused a decrease in parasitoid emergence compared to the same application at Pink. However, logic would dictate that applying earlier would allow time for Entrust residuals to degrade before parasitoids become prevalent in the orchard ecosystem. Although Entrust is an OMRI-approved product, its broad-spectrum of activity and detrimental effects on pollinators and parasitoids seem incongruent with the spirit of the National Organic Program. The relationship between Entrust applications and parasitoid health is worthy of further study in this and other systems.

Although our insecticidal research did not focus on Surround, caged adult mortality (72 h), change in population density and proportion of leaves with mines in trees treated with Surround were statistically similar to that caused by a single full rate application of Entrust. Surround is already commonly utilized in organic apple production for the control of plum curculio, apple maggot and some diseases (Friedrich et al. 2003). However, Surround applications for other orchard pests are not synchronized temporally with periods of peak *O. pallicornis* activity. Surround may provide adequate control of *O. pallicornis* populations at typical level without the negative ecological effects and high monetary cost of Entrust applications.

Leaf mining larvae are particularly susceptible to attack by parasitoid wasps (Askew, 1980; Hawkins, 1988, 1990, 1994; Hawkins et al., 1992). By manipulating the agro-ecosytem, parasitoid populations can be increased to a level capable of suppressing citrus leaf-miner

populations (Jacas and Urbaneja 2010). Through habitat management alone, parasitoids of the mango flea weevil (Rhyncaneus mangiferae, Marshal) can successfully control this pest without the need for chemical applications (Mani and Krishamoorthy 2001, Peter and Balasubramanian 1984). An adequate understanding of the resources required by parasitoids of *O. pallicornis* is a prerequisite for successful implementation of a conservation biological control program (Landis et al. 2000).

Throughout the course of this study we explored the phenology of *O. pallicornis*, determined the number of *O. pallicornis* larval instars, and identified the parasitoids of *O. pallicornis*. We also identified Entrust as an organic insecticide capable of providing significant control of *O. pallicornis* adults. We determined Tight Cluster as the most appropriate stage of apple bud development at which Entrust can be applied for control of Spring *O. pallicornis* and showed that half rate applications of Entrust may be capable of controlling Summer *O. pallicornis* adults. As broad-spectrum insecticides become less available due to EPA restrictions, tree fruit growers will undoubted adopt integrated pest management programs. These programs, which emphasize behavioral and cultural control over chemicals, may allow the resurgence of *O. pallicornis*, as was seen in organic farms over the last half decade (Wyss et al. 2005, Nielsen et al. 2012). This research not only provides organic growers with an effective method for *O. pallicornis* control, but also provides fundamental biological data which could be invaluable should *O. pallicornis* emerge as a pest in conventional orchards.

APPENDIX

APPENDIX

Record of Deposition of Voucher Specimens

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: _____

Author and Title of thesis:
John McNamara Pote
Biology of the Reemergent Pest Apple Flea Weevil (*Orchestes palicornis*, Say) and Methods for its Organic Control

Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Curculionidae	*Orchestes palicornis*	adult	20	pinned

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