

Biology and Seasonality of the Reemergent Pest *Rhynchaenus pallicornis* (Coleoptera: Curculionidae) and Methods for Monitoring Its Abundance

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Abstract

Rhynchaenus pallicornis (Say) is a pest of commercially grown apples in the upper Midwest. This historic pest has resurged and caused severe yield loss on farms using certified organic production practices. The life history and potential monitoring methods of *R. pallicornis* are presented. Seasonal abundance data were collected through beat and visual sampling. A phenological model was developed for *R. pallicornis*. The minimum developmental threshold of *R. pallicornis* was determined to be 3.5°C with a required degree-day accumulation of 125°D for first adult emergence. Larval damage was observed on >60% of leaves in unmanaged orchards and affected significantly fewer basal leaf clusters (near the trunk), than medially or apically located clusters. Of 2,900 *R. pallicornis* larval mines collected over two years at three different sites, 18.0% produced at least one adult parasitoid, but the targeted larval stage is unknown. Measurements of *R. pallicornis* larval head capsules and the simple frequency method were used to determine three larval instars of *R. pallicornis*. The number of larval instars could also be accurately determined by observing the presence or absence of two sets of thoracic sclerites. Pyramid traps, yellow sticky cards baited with olfactory cues (pear essence, benzaldehyde, and an aggregation of adult *R. pallicornis*) were evaluated as *R. pallicornis* monitoring tools. None of the traps or lures tested significantly affected the number of adult *R. pallicornis* per trap.

Key words: organic, instar, trapping, leaf miner, phenology

Rhynchaenus pallicornis (Say) (Coleoptera: Curculionidae), the apple flea weevil, is a reemerging pest of economic significance in Michigan organic apple orchards. *Rhynchaenus pallicornis* damage was first reported in organic orchards in 2008 but was misdiagnosed as frost damage. Since then, reported damage has continued to increase, with some growers experiencing up to 90% yield loss (Nielsen et al. 2012).

Adult *R. pallicornis* are small, matte black beetles 3 mm in length and 1.5 mm wide with enlarged saltatorial metathoracic femora and striated elytra. Adults superficially resemble flea beetles based on their size and jumping behavior. The larvae are generally concealed in leaf mines, but when removed are ivory in color and measure ~5 mm in length and ~2 mm in width (Houser 1923, Flint et al. 1924). *Rhynchaenus 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 consume leaf and bud tissue (Flint et al. 1924). Early reports of *R. pallicornis* infestation documented up to 1,400–1,700 adults emerging per tree in favorable weather conditions (Houser 1923, Flint et al. 1924). Mating reportedly begins almost immediately

after emergence, but 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 for pupation. The pupation chamber resembles a blister at the end of the leaf. Although multiple mines may be found within a single leaf, this is uncommon, and they are not contiguous. It is estimated that after 5–6 d, adult *R. pallicornis* emerge and feed for 6–12 d before returning to the soil duff to overwinter. *Rhynchaenus pallicornis* is believed to be a univoltine species, and is generally absent from the orchard canopy by mid-July (Houser 1923, Flint et al. 1924). There is currently no phenological model for *R. pallicornis*. The development of such a model is of particular importance because the timing of management applications is critical for keeping this pest below outbreak levels (Pote et al. 2015).

Very little is known about the role of natural enemies in the regulation of *R. pallicornis* populations. In the only published study, a variety of parasitic hymenopterans, including *Zatropis incertus* Ashmead, *Trichomalus inscitus* Walker (Hymenoptera: Pteromalidae), *Epius* sp., and *Chrysocharis pentheus* Walker

(Hymenoptera: Eulophidae), have been recovered from larval mines, with natural rates of parasitism exceeding 20% (Houser 1923), but it is not known which immature stage they attack, nor how many larval instars *R. pallicornis* has. Dyar's rule may be used to identify the number of larval instars. Dyar's rule states that sclerotized portions of the insect integument increase only between successive instars, the growth from which 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). Initially, it was believed that this method could not be applied to species that develop in an obscured or difficult to access manner (i.e., leaf-rolling or leaf-mining larvae). However, Peterson and Haussler (1928) determined the number of instars of *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), a leaf-rolling tortricid moth, from head capsule measurements alone through regular and repeated field collections of larvae within a single life cycle. The number of *R. pallicornis* larval instars is unknown.

Recent publications on *R. pallicornis* have provided management insights, but gaps in our knowledge still exist about the basic biology of this pest, especially in contemporary agricultural systems (Pote et al. 2015). The objectives of this study were to 1) determine the seasonality of *R. pallicornis* in Michigan organic orchards, 2) develop a phenological model for this pest, 3) determine the location of *R. pallicornis* larval damage, 4) determine an effective trapping system for monitoring *R. pallicornis* populations, 5) determine the number of *R. pallicornis* larval instars, and 6) determine the role of parasitoids in regulating *R. pallicornis* populations.

Materials and Methods

Research was conducted at three certified organic apple orchards in mid-Michigan that were experiencing very high levels of *R. pallicornis* populations. The first, a commercial mixed organic and conventional orchard located near Potterville, MI (42.635608, -84.788709); the second, a commercial certified organic orchard located near Flushing, MI (43.024371, -83.91168); and the third; an orchard at the Michigan State University Clarksville Research Center (42.875964, -85.248239). Research at the Potterville site was conducted in a 2.3-ha block of certified organic Cortland, Golden Delicious, Red Delicious, Jonamac, Jersey Mac, and Viking varieties. Research at the Flushing site was conducted in a 5-ha block of Golden Delicious, Gala, and Gold rush varieties. Research at the Clarksville site was confined to a 1-ha block of unmanaged Honey crisp, Gala, Gold rush, and Golden Delicious varieties. The Potterville and Flushing sites were managed in accordance with National Organic Program standards for insect, weed, and disease control. The Clarksville site was unmanaged.

Seasonality or Phenology

The seasonality of *R. pallicornis* was determined through beat sampling apple canopies and observation of larval infestation rates throughout the 2011 and 2012 growing seasons. Sampling was initiated as soon as temperatures began to rise in the early spring (24 April 2011 and 14 March 2012, respectively), which coincided with the beginning of silver tip phenological stage in apple, the first stage in breaking dormancy. Sampling was repeated every 1–3 d at all three locations until summer-generation adults were absent from the orchard canopy (1 August 2011–1 August 2012). 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 *R. pallicornis* adults that landed on a 1 m² mesh sheet held beneath the limbs (Bioquip Inc., Rancho Dominguez, CA). In 2011, 10 trees were sampled per site per sampling date. In 2012, the number of sampled trees was increased to 30 trees spread among three rows of varying bloom phenology. Rows sampled in 2011 were also sampled in 2012.

Minimum developmental threshold temperature and required accumulated degree days for spring adult *R. pallicornis* emergence were calculated from field data due to the difficulty of establishing an *R. pallicornis* laboratory colony. Temperature and emergence data from 2011 and 2012 were analyzed using a method of minimum developmental temperature calculation outlined in Snyder et al. (1999), namely the iteration method first described by Ring et al. (1983). Hourly temperature measurements were acquired from Michigan State University's EnviroWeather system of weather stations. Degree days were calculated from temperature readings using trapezoidal approximation of the region under the graph applied to hourly temperature measurements. Cumulative degree days were calculated with accumulation starting points (biofix points) of 1 January as well as 3 February, the first day with >10 h of daylight for all sites (NOAA Solar Calculator 2016). The iteration method minimizes differences between the number of predicted and observed days of development by calculating the root mean square error (RMSE) of that difference across a range of minimum developmental threshold temperatures (T_m) and cumulative degree days required ($^{\circ}D_c$). The combination with the lowest RMSE, when summed across all sites and years, is considered to be the best threshold. RMSE is calculated using the following equation:

$$\text{RMSE} = \sqrt{\left(\sum_{i=1}^n [dp_i - d_i]^2\right) \frac{1}{n}}$$

where dp_i is the expected number of days after biofix when degree-day accumulation exceeds $^{\circ}D_c$, d_i is the number of days after biofix when insect activity is first observed, and n is the number of instances (unique site-year combinations).

For this analysis, we iteratively calculated RMSE given values of T_m ranging from 1°C–10°C by 0.5°C and across values of $^{\circ}D_c$ from 35°D–260°D by 15°D. The combination of T_m and $^{\circ}D_c$ resulting in the lowest RMSE was selected as the ideal value for each. This method was used to predict the date of first *R. pallicornis* emergence as well as the date of peak *R. pallicornis* emergence (see Table 1 for dates).

In 2012, estimates of larval density were made in conjunction with adult sampling by counting the number of mines per 30 leaves, collected randomly from the canopies of three trees within one row. Leaf samples were selected from around the tree circumference and at varying heights and depths into the canopy. Leaves were considered infested if they housed at least one *R. pallicornis* larval mine.

Instar Determination

Leaf mines were collected throughout the 2011 and 2012 growing season to identify the number of *R. pallicornis* larval instars. Collections began at all three locations after the peak of the spring adult population and continued 1–2 times per week until intact mines could no longer be found. For each collection, 20–30 unopened larval mines were gathered from untreated trees and stored in 100% ethanol. A subsample of 100 mines were randomly selected

Table 1. Phenological parameters of *R. pallicornis* calculated using the RMSE method

		January 1 biofix					
		First emergence $T_m = 2.5; ^\circ D_c = 155$			Peak emergence $T_m = 3.0; ^\circ D_c = 260$		
		Date predicted (dp _i)	Date observed (d _i)	Diff. (d)	Date predicted (dp _i)	Date observed (d _i)	Diff. (d)
Clarksville	2011	28 April 2011	25 April 2011	3	12 May 2011	2 May 2011	10
Flushing	2011	23 April 2011	27 April 2011	4	7 May 2011	10 May 2011	3
Pottersville	2011	24 April 2011	25 April 2011	1	9 May 2011	25 April 2011	14
Clarksville	2012	20 Mar. 2012	21 Mar. 2012	1	6 April 2012	18 April 2012	12
Flushing	2012	18 Mar. 2012	19 Mar. 2012	1	25 Mar. 2012	24 Mar. 2012	1
Pottersville	2012	19 Mar. 2012	21 Mar. 2012	2	28 Mar. 2012	4 Mar. 2012	6
		RMSE = 2.007			RMSE = 9.034		
		10 h daylight biofix					
		First emergence $T_m = 3.5; ^\circ D_c = 125$			Peak emergence $T_m = 2.5; ^\circ D_c = 260$		
		Date predicted (dp _i)	Date observed (d _i)	Diff. (d)	Date predicted (dp _i)	Date observed (d _i)	Diff. (d)
Clarksville	2011	27 April 2011	25 April 2011	2	11 May 2011	2 May 2011	9
Flushing	2011	23 April 2011	27 April 2011	4	6 May 2011	10 May 2011	4
Pottersville	2011	25 April 2011	25 April 2011	0	7 May 2011	25 April 2011	12
Clarksville	2012	20 Mar. 2012	21 Mar. 2012	1	6 April 2012	18 April 2012	12
Flushing	2012	19 Mar. 2012	19 Mar. 2012	0	27 Mar. 2012	24 Mar. 2012	3
Pottersville	2012	19 Mar. 2012	21 Mar. 2012	2	29 Mar. 2012	3 April 2012	5
		RMSE = 1.862			RMSE = 8.488		

Accumulation of degree days was calculated from biofix points of January 1 and the first day of 10 h of daylight (3 February for all sites); T_m = minimum developmental threshold temperature, $^\circ D_c$ = cumulative growing degree-day requirement.

and dissected from mines collected across all sites. To ensure adequate sampling of small larvae present only in the early season, 10 additional mines were selected from the earliest sampling date and dissected. Any larvae within were extracted, and larval 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 (Buffalo Grove, IL). Prior to each measurement, the software measurement utility was calibrated using a stage micrometer.

Larval Damage Position

To determine if *R. pallicornis* larval infestation rate is correlated with relative distance to the trunk, larval damage was assessed on basal, medial, and apical leaf clusters in 2011. At the Clarksville and Flushing sites, the number of larval or pupal infestations and the number of total leaves were assessed on basal (located within 30 cm of the trunk), medial (located between 30 and 60 cm of the trunk), and apical (located within 30 cm of the limb apex) clusters per terminal. This was repeated on five randomly selected chest high terminals per tree. To account for potential differences by variety, a randomized complete block design was used at Clarksville: three randomly selected trees were assessed in each of five blocks, with each block consisting of one single-variety row. Trees at the Flushing site were unable to be blocked by variety, so clusters were assessed in a completely randomized design by sampling on 10 randomly selected trees. Trees selected for sampling were located in rows unsprayed by insecticides. At both locations, clusters were assessed at least once per week starting after the first observed larval mine at each site (Flushing: 27 May 2011 and Clarksville: 31 May 2011). Assessments ended when occupied larval mines could no

longer be readily found (Flushing: 10 June 2011 and Clarksville: 13 June 2011)

Monitoring Tools

During the spring of 2011, several trap designs and potential trap baits were tested as potential tools for monitoring adult *R. pallicornis* populations. Black pyramid traps (Whalon Modified Tedders Trap, #IPM-503 Great Lakes IPM Inc., Vestaburg, MI) and 7.6- by 12.7-cm yellow sticky traps (Great Lakes IPM Inc.) were deployed in a two-way randomized complete block design on untreated trees at the Flushing site on 22 April 2011 prior to observed activity of *R. pallicornis*. To control for spatial variation in *R. pallicornis* abundance, pyramid traps and yellow sticky traps were both deployed on 20 randomly selected trees arranged in five blocks of four trees. Pyramid traps topped with a plum curculio cone and sealed with a 3.78 liter (1 gal) paint strainer bag were staked securely near the trunk of each tree, and yellow sticky traps were fastened to terminals within the canopy of the same tree ~1.5 m from the ground. Traps located at the same tree were baited with the same lure treatment, but these were rotated within blocks once per week. Traps within blocks were at least 10 m apart from each other. Bait treatments consisted of 1) benzaldehyde, 2) pear essence, 3) an unbaited control, and, because no aggregation or sex pheromones have been isolated from *R. pallicornis*, 4) a small mesh bag containing 10 *R. pallicornis* adults (there is no known nondestructive method to differentiate between sexes in adult *R. pallicornis*). Baits were secured 10 cm above pyramid traps using sturdy wire and within 5 cm of yellow sticky cards on the same terminal. Traps were checked for adult *R. pallicornis* every 3–5 d between 29 April and 25 May 2011. Adults caught in pyramid traps were not released back into the orchard.

Parasitoids

To determine the incidence and rate of *R. pallicornis* parasitism, and the identity of parasitoids, leaves containing larval mines were collected throughout the 2011 and 2012 growing season. Samples of 100 unopened mines were randomly collected across five trees per sampling date from the Clarksville, Potterville, and Flushing sites. Sampling occurred approximately once per week from 24 May to 24 June 2011 and from 24 April to 27 June 2012. Additional mines were collected in 2012 as part of concurrent insecticide trials at the Clarksville, Potterville, and Flushing sites (Pote et al. 2015). These samples were collected using similar methods, but only those leaves collected from untreated control blocks were used to identify the rate and diversity parasitism in the present study. Infested leaves were stored for 3–7 d at 10°C before being stored individually in small 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 *R. pallicornis*. After emergence, parasitoids were transferred into 80% ethanol for storage and were then identified to family (Johnson and Triplehorn 2005).

Statistical Analysis

All data were analyzed using the R statistical language (R Development Core Team 2011). The lme4 package was used to perform a repeated-measures linear mixed effects analysis of the location of larval damage and monitoring methods (Bates et al. 2014). Where appropriate, data from different sites were analyzed separately because of differences in experimental design. Larval damage data were $\log(x+1)$ transformed prior to analysis so that they met assumptions of normality and heteroscedasticity. Models were fit using Maximum likelihood estimation, and the central effect (location of cluster along terminal; effects of trap and bait) was tested with a likelihood ratio test. Means separations were analyzed using Tukey's HSD.

The number of *R. pallicornis* larval instars was 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 in the frequency histogram. A one-way analysis of variance was used to evaluate significant differences in head capsule widths between instar groups (using site of collection as a blocking factor) and to test for differences in average instar collected from each site. Estimates of model parameters in Dyar's Law:

$$\text{Head capsule width at instar } n = A \times B^{(n-1)},$$

where A is the head capsule width of first-instar larvae, and B is the growth rate between instars, were calculated using nonlinear least-squares analysis (Dyar 1890).

The rates of parasitism were compared among sites using a repeated-measures linear mixed effects model with binomial distribution. Models were fit using restricted maximum likelihood, and effect of site was tested with a likelihood ratio test (Bates et al. 2014). Means separations were analyzed using Tukey's HSD modified for linear hypotheses.

Results

Seasonality or Phenology

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

The iterative method was able to calculate values for T_m and $^{\circ}D_c$ when predicting first and peak emergence at both of the tested biofix points. Using the iterative method to predict first emergence resulted in consistently lower RMSE values than when predicting peak emergence. Similarly, using 3 February as the biofix point resulted in higher RMSE values than 1 January (Table 1). The predicted dates

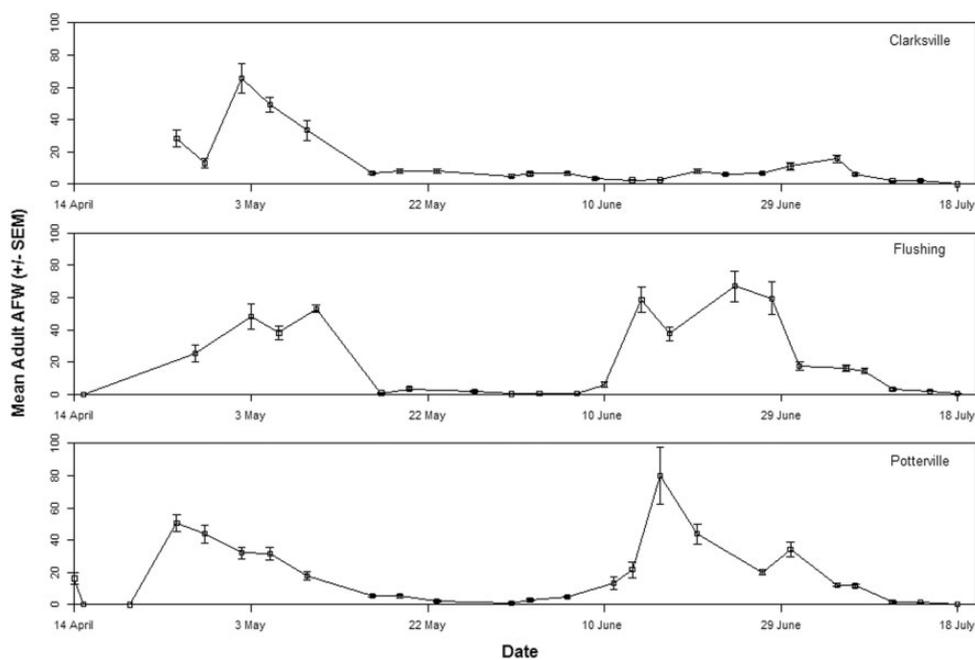


Fig. 1. Mean adult *R. pallicornis* adults (\pm SEM) collected per tree throughout 2011 in Michigan.

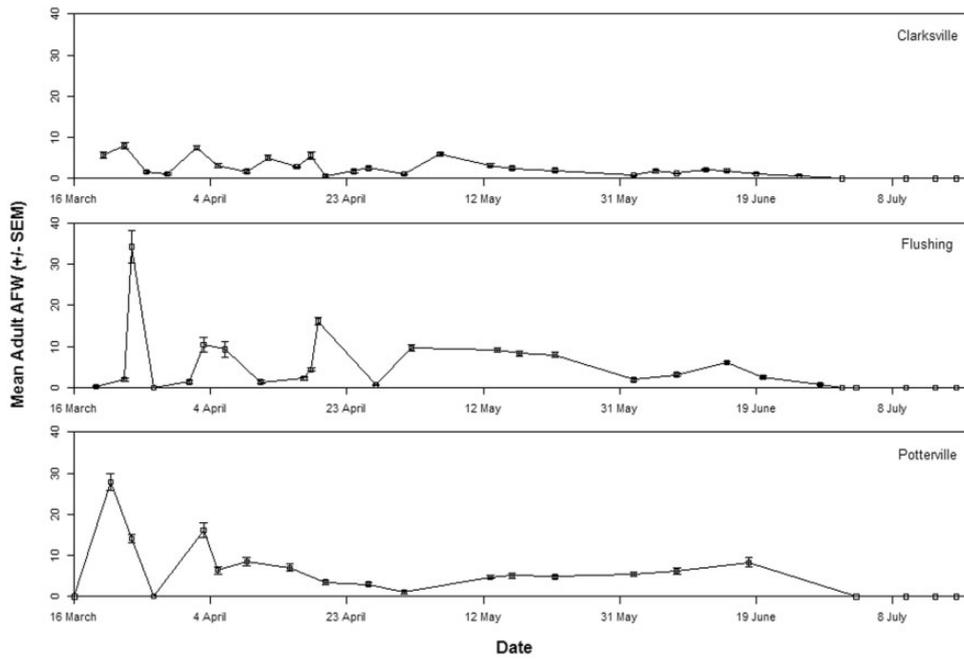


Fig. 2. Mean *R. pallicornis* adults (\pm SEM) per tree collected throughout 2012 in Michigan.

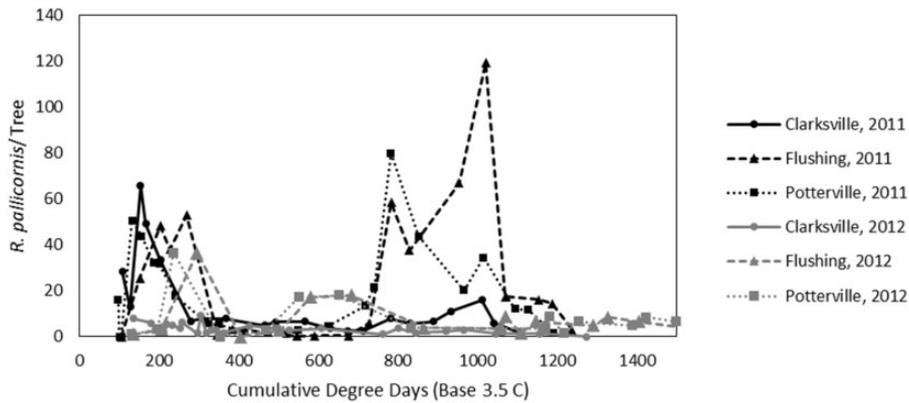


Fig. 3. Mean *R. pallicornis* adults per tree collected throughout 2011 and 2012, expressed as a function of cumulative growing degree days ($^{\circ}$ D, base 3.5° C) using 10 h of daylight as the biofix point (error bars omitted for clarity). Note: the iterative method predicted first *R. pallicornis* emergence at 125° D.

of first emergence using 1 January resulted in a T_m of 2.5° C with $^{\circ}$ D_c = 225° D (RMSE = 2.007). Predicting the date of first emergence using 10 h of daylight as a biofix predicted T_m of 3.5° C with $^{\circ}$ D_c = 125° D (RMSE = 1.862; Fig. 3). Predicting peak emergence given a biofix date of 1 January predicted T_m of 3.0° C with $^{\circ}$ D_c = 260° D (RMSE = 9.035) while using 10 h of daylight as the biofix date predicted a T_m of 2.5° C with $^{\circ}$ D_c = 260° D (RMSE = 8.488). *Rhynchaenus pallicornis* abundance as a function of accumulated growing degrees days (using the above method with the lowest RMSE) is displayed in Fig. 3. Table 1 summarizes the results of the iterative method.

In 2012, larval mines were first detected on 14 April (Fig. 4). 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. 4).

Instar Determination

Analysis of the frequency histogram of observed head capsule widths (simple frequency method) identified three significantly distinct ($F = 427.8$, $df_1 = 2$, $df_2 = 105$, $P < 0.0001$; Fig. 5) instar groups. Nonlinear regression of Dyar's geometric growth model generated parameter estimates of A (first-instar head capsule width): 0.278 ± 0.003 cm and B (per-instar growth rate): $123.9 \pm 1.01\%$. During larval measurements, dorsal thoracic shields and ventral thoracic sternites were observed that correlated strongly with larval size: the smallest group had neither structure, the middle group had only ventral sternites, and the largest group had both structures (Fig. 6). Separating larvae into groups based on these morphological differences also generated three significantly distinct instars ($F = 159.2$, $df_1 = 2$, $df_2 = 105$, $P < 0.0001$; Fig. 7). Regression analysis of Dyar's geometric growth model for morphologically determined instars calculated parameter estimates of A (first-instar head capsule width): 0.277 ± 0.005 cm and B (per-instar growth rate):

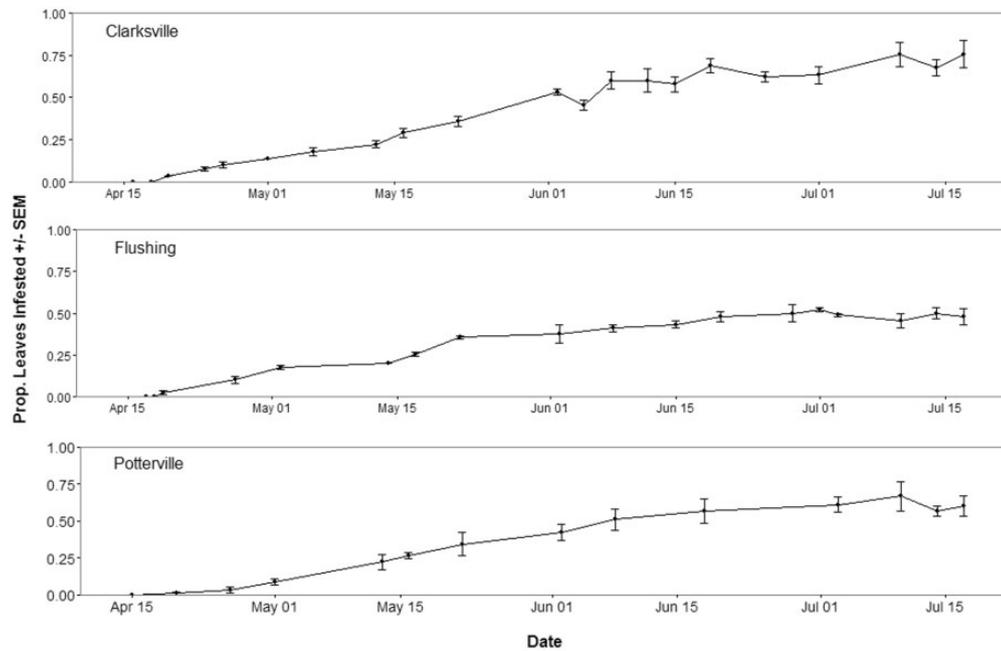


Fig. 4. Proportion of leaves infested with *R. pallicornis* larval mines collected in 2012 from organically grown apples in Michigan.

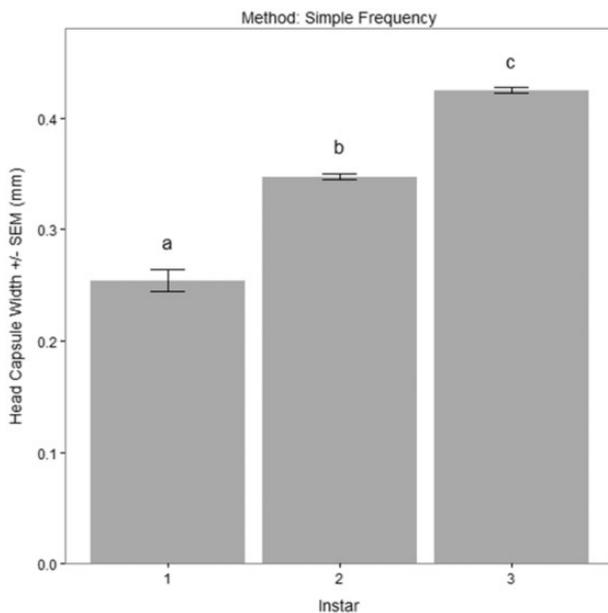


Fig. 5. Distribution of observed head capsule widths into graphically determined instar groups. Letters indicate significant differences among groups (Tukey's HSD, $\alpha = 0.05$).

$122.2 \pm 1.50\%$. Analysis of variance revealed significant differences in head capsule width between sites ($F = 5.858$, $df_1 = 2$, $df_2 = 105$, $P = 0.0039$) as well as a significant difference in the average instar collected between sites ($F = 16.56$, $df_1 = 2$, $df_2 = 107$, $P < 0.0001$). The average instar collected at Clarksville was 1.9 ± 0.08 compared with 2.6 ± 0.1 and 2.5 ± 0.08 from the Flushing and Pottersville sites, respectively.

Larval Damage Position

The proportion of leaves with larval damage was significantly affected by cluster location at the Flushing site ($\chi^2 = 29.961$, $df = 2$,

$P < 0.0001$) as well as the Clarksville site ($\chi^2 = 23.585$, $df = 2$, $P < 0.0001$). At both sites, basal clusters had a significantly lower proportion of leaves with larval damage than did medial or apical clusters. The proportion of larval damage did not differ significantly between medial and apical clusters at either site. At Flushing, the proportion of leaves with larval damage on basal clusters was $14.7 \pm 1.0\%$ compared with $22.1 \pm 1.3\%$ and $20.7 \pm 1.1\%$ on apical and medial clusters, respectively. Larval damage at Clarksville followed a similar trend with only $44.5 \pm 1.8\%$ damage on basal clusters, compared with $54.6 \pm 1.9\%$ and $53.1 \pm 2.0\%$ damage on apical and medial clusters, respectively.

Monitoring Tools

The number of *R. pallicornis* adults caught in traps was not significantly affected by trap type ($\chi^2 = 3.4618$, $df = 4$, $P = 0.484$). Pyramid traps caught an average of 25.2 ± 3.0 adults per sampling date, while sticky cards caught only 18.6 ± 1.9 adults per sampling date. Unbaited traps used as controls caught numerically more adult *R. pallicornis* than any of the lure treatments, although this effect was not statistically significant ($\chi^2 = 2.6506$, $df = 6$, $P = 0.851$). There was not a significant interaction between trap type and lure treatment ($\chi^2 = 1.447$, $df = 3$, $P = 0.6946$).

Parasitoids

Parasitoids collected from the three sites across 2011 and 2012 belonged to five families (Braconidae, Eulophidae, Eupelmidae, Ichneumonidae, and Pteromalidae; Fig. 8). The greatest number of individuals were from the family Pteromalidae in the superfamily Chalcidoidea. The Eupelmidae were represented by at least two genera, *Clostocerus* and *Eupelus*. The majority of Pteromalidae samples came from the genus *Trichomalus*. Of the 2,900 larval mines collected over both years, the total rate of parasitism was 18.0%. The majority of parasitoids emerged singly from mines, but in 10 instances (3.8% of total instances), more than one parasitoid emerged from a single mine. In 2012, the rate of parasitism was not

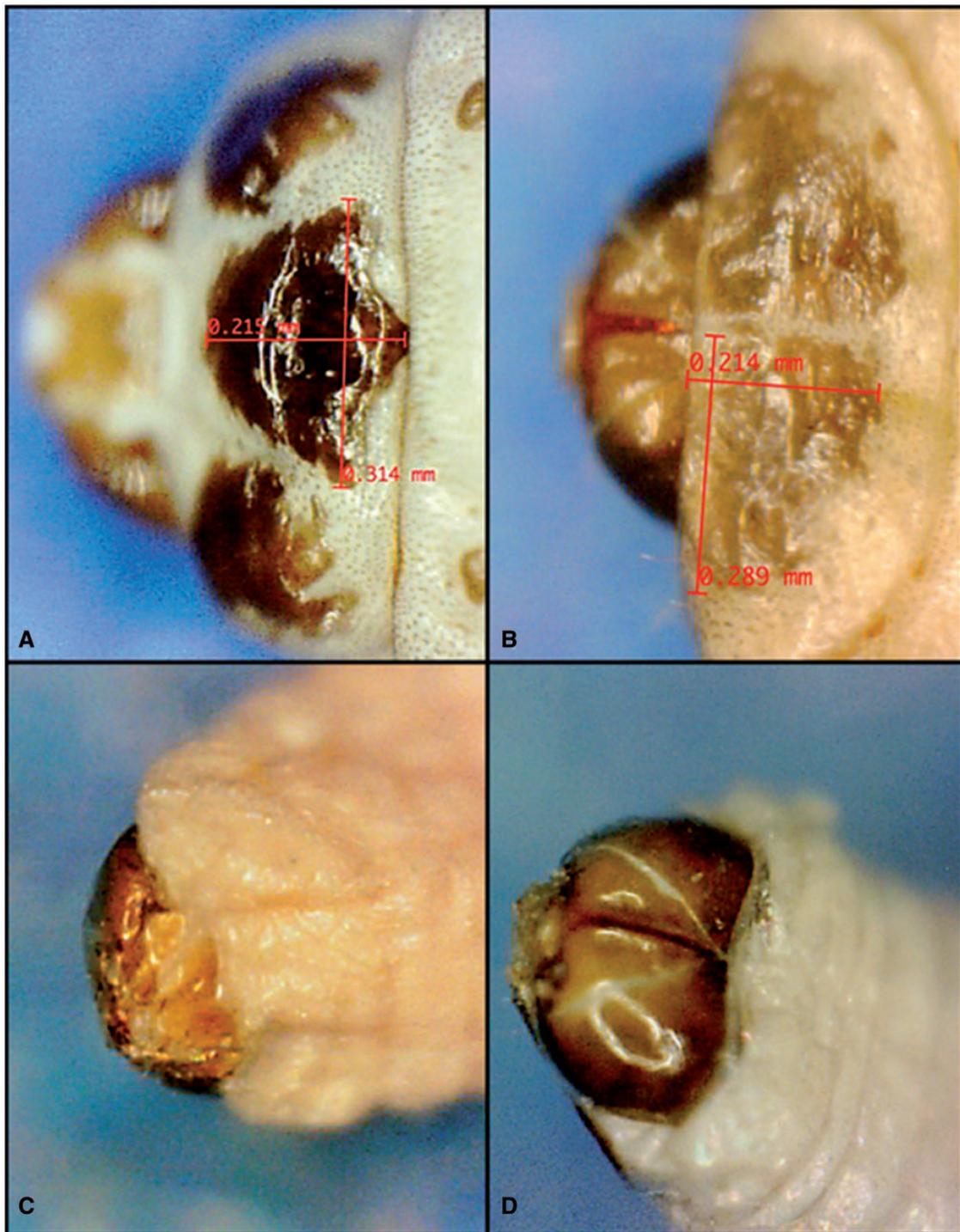


Fig. 6. Variation in larval *R. pallicornis* sclerotization used to create instar groups. (A) Ventral sclerite, (B) Dorsal sclerite, and (C and D) Larva without thoracic sclerotization.

significantly different among sites ($\chi^2 = 0.6568$, $df = 2$, $P = 0.7201$; Fig. 9).

Discussion

There was a substantial difference in the timing of spring emergence of *R. pallicornis* between 2011 and 2012, likely caused by record-setting warm temperatures during the spring of 2012 (Figs. 1 and 2). The great disparity in activity prevents the calculation of an

expected calendar date of peak activity. However, patterns of activity from the two years strongly indicate that *R. pallicornis* emergence is based on a combination of temperature and photoperiod (Danks 1987, Pote et al. 2015). Differences between the two years include the arrival of the spring peak almost 5 wk earlier in 2012 compared with 2011, and the lack of a true peak of summer adult activity in 2012 (Fig. 2). In March 2012, record-breaking high temperatures were followed by a period of typically cool spring weather. This may have generated two distinct periods of oviposition

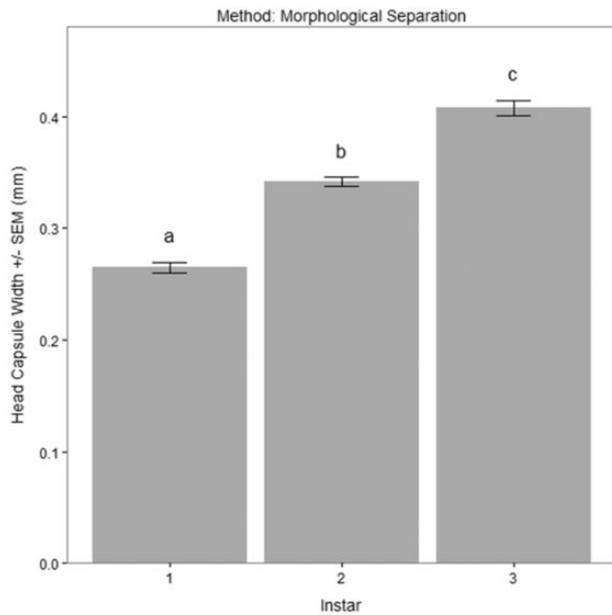


Fig. 7. Distribution of observed head capsule widths into morphologically determined instar groups. Letters indicate significant differences among groups (Tukey's HSD, $\alpha = 0.05$).

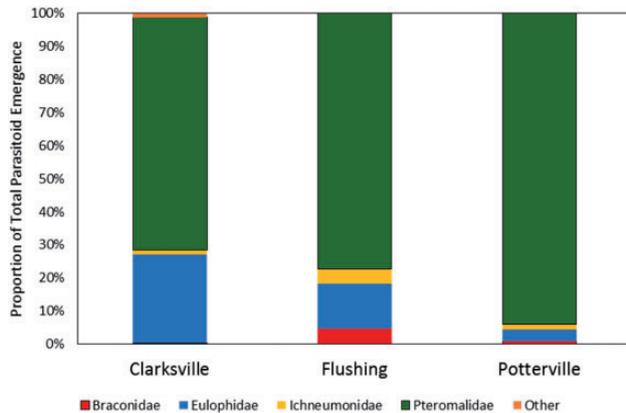


Fig. 8. Parasitoid diversity from three organically managed apple orchards in Michigan. Samples in the "Other" category included members of Eupelmidae as well as any parasitoids that could not be identified.

and thus divided the summer-generation activity into two graphically indistinguishable groups.

Predicting the first emergence of *R. pallicornis* using a biofix point of 3 February resulted in the lowest overall RMSE of any of the models tested. Using these conditions, the iterative method was able to accurately predict the exact date of first emergence for two of the six instances used in the present study. The average difference between the observed and predicted date of first emergence was only 1.5 calendar days, indicating a good fit between this phenological model and observed values (Table 1, Fig. 3). The iterative method was less accurate at predicting the date of peak emergence, with observed dates differing from predicted dates by an average of 7.5 d using a biofix point of 3 February and 7.6 d using 1 January as the biofix point. This is unsurprising, given the variability in date of peak *R. pallicornis* emergence. In 2011, the dates of peak emergence had a range of 15 d and in 2012, this range spanned 25 d. In comparison, the range of observed dates of first emergence spanned only two days in both years.

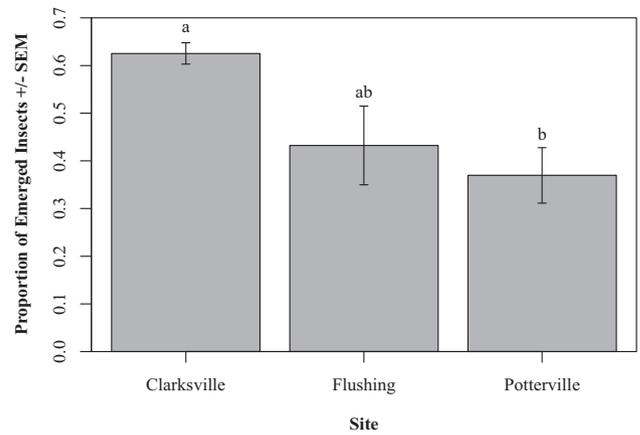


Fig. 9. Parasitoid emergence (+/- SEM) as a proportion of total insect emergence from field collected leaf mines. Letters indicate significant differences among sites (Dunnnett's modified Tukey-Kramer pair-wise comparison test, $\alpha = 0.05$; Ring et al. 1983, Snyder et al. 1999).

Although the iterative method created a phenological model that is reasonable and fits the biology of *R. pallicornis*, additional research is required to test the validity of this model. The accuracy of models created using the methods of Snyder et al. is likely lower than those created through rigorous developmental testing in the laboratory (Snyder et al. 1999). However, our results indicate that these methods are appropriate for the creation of a phenological model when laboratory developmental data are unavailable.

Analysis of larval head capsule width strongly indicates that *R. pallicornis* develops through three instars prior to pupation. Both the simple frequency method (from Gaines and Campbell 1935) and morphological method of instar analysis indicated three instars of *R. pallicornis* larvae. However, both methods have limitations. The simple frequency method relies on an assumed constant rate of growth and consistent numbers of instars, but both of these factors can be affected by field conditions in lepidopteran larvae (Schmidt et al. 1977, Fink 1984). 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 *R. pallicornis* second and third instars indicates that this is the likely number of instars. Between the two methods, the model created by the simple frequency method was more statistically robust. However, creation of instar groups based on this method, by design, prohibits any overlap in the range of observed head capsule widths, artificially increasing the likelihood of statistically significant groupings. The morphological method outlined here offers a simpler and more biologically relevant method for instar determination, where possible.

Significant discrepancies in head capsule sizes among the sites are most likely a function of the randomly uneven sampling of instars among sites. Sampling of larvae began when the mines were first observed in the field, but oviposition scars and first-instar mines are extremely minute and difficult to collect. Although sampling began at all sites on the same calendar day, the Clarksville site was phenologically delayed because of cooler weather (1,235°D as of 30 June 2012 compared with 2,165°D and 2,077°D at Flushing and Potterville, respectively). This delay may have increased the likelihood of sampling earlier instars at the Clarksville site and affected the mean head capsule width at that site.

Density of larval mines was significantly higher on apical and medial clusters than those near the base of terminals. Disparate levels of larval damage could be attributed to ovipositional preference for distal clusters, relatively higher mortality on proximal clusters, or other *R. pallicornis* behaviors that result in higher coincidental abundance near the apical ends of terminals. Additional research is needed to determine the cause of relatively higher distal larval damage.

Based on the findings of the present study, methods for monitoring *R. pallicornis* need further exploration. The use of monitoring tools is a key component of integrated pest management, and efforts to manage this pest will be improved by an effective trap or lure system. Pyramid traps may be an effective method for monitoring initial activity of *R. pallicornis* adults, which can be used as a proxy for management decisions (Pote et al. 2015).

After the early season peak of *R. pallicornis* activity in the spring of 2011, the population density at Clarksville was consistently lower than that at the other sites. Previous work has shown that *R. pallicornis* parasitism rates are significantly lower in rows treated with Entrust (spinosad) than in untreated rows (Pote et al. 2015). Throughout this study, all trees at Clarksville were not treated with insecticides, while the Potterville and Flushing sites were under a normal organic insect management regime including early season applications of Entrust (Dow AgroChemical, Indianapolis, IN), a spinosyn-based broad-spectrum insecticide widely used in organic agriculture. The absence of insecticide applications at Clarksville, specifically Entrust, may have fostered higher rates of parasitism compared with other sites (Pote et al. 2015). This higher top-down pressure may have contributed to the generally lower population of *R. pallicornis* at Clarksville compared with Flushing and Potterville. However, some of the less common parasitoid taxa (F: Eulophidae 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 *R. pallicornis* parasitoid survey (Houser 1923, Flint et al. 1924). Each site produced at least one specimen from each of the five identified families of *R. pallicornis* parasitoids.

Promoting parasitoids for control of *R. pallicornis* is especially important in organic settings, where natural pest control is recommended over the use of chemical control methods (Letourneau and Goldstein 2001, Wyss et al. 2005). Although chemical management tactics may be necessary as an emergency response, it is apparent that *R. pallicornis* is susceptible to numerous parasitoid species, which can regulate pest populations at low levels (Zehnder et al. 2007). During the present study, we showed that *R. pallicornis* in Michigan is susceptible to parasitoids of the same families as those previously described by Houser (1923) and Flint (1923). Additionally, we showed that parasitoids can account for up to 60% of emergence from *R. pallicornis* larval mines. However, previous research indicates that parasitism rates may be negatively impacted by applications of organic insecticides including Entrust (Pote et al. 2015). Thus, it is important to balance parasitoid conservation with the utilization of chemically based management tactics for control of *R. pallicornis*.

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References Cited

- (NOAA Solar Calculator) National Oceanic and Atmospheric Administration. 2016. NOAA Solar Calculator. (<http://www.esrl.noaa.gov/gmd/grad/solcalc/>) last accessed 27 April 2016.
- Bates, D., M. Maechler, B. Bolker, and S. Walker. 2014. lme4: Linear mixed-effects models using eigen and S4. *J. Stat. Softw.* 1–23.
- Danks, H. V. 1987. Insect dormancy: An ecological perspective, *Biol. Surv. Canada Monogr. Ser.*
- Dyar, H. G. 1890. The number of molts of lepidopterous larvae. *Psyche* 1: 420–422.
- Fink, T. J. 1984. Errors in instar determination of mayflies (Ephemeroptera) and stoneflies (Plecoptera) using the simple frequency, Janetschek, Cassie and Dyar's law methods. *Freshw. Biol.* 14: 347–365.
- Flint, W. P., S. C. Chandler, and P. A. Glenn. 1924. The apple flea-weevil, *Orchestes pallicornis* Say (Order Coleoptera; Family Curculionidae). *Illinois Nat. Hist. Surv. Bull.* 15.
- Gaines, J. C., and F. L. Campbell. 1935. Dyar's rule as related to the number of instars of the corn ear worm, *Heliothis obsoleta* (Fab.), collected in the field. *Ann. Entomol. Soc. Am.* 28: 445–461.
- Houser, J. S. 1923. The apple flea-weevil: *Orchestes pallicornis* Say. *Ohio Agric. Exp. Stn. Bull.* 372.
- Johnson, N. F., and C. A. Triplehorn. 2005. *Borror and DeLong's Introduction to the Study of Insects*. Thomson Brooks/Cole, Belmont, CA.
- Letourneau, D. K., and B. Goldstein. 2001. Pest damage and arthropod community structure in organic vs. conventional tomato production in California. *J. Appl. Ecol.* 38: 557–570.
- Nielsen, A. L., J. Pote, K. Buehrer, and M. J. Grieshop. 2012. The reemergence of an old pest, *Orchestes pallicornis* (Coleoptera: Curculionidae). *J. Integr. Pest Manag.* 3: 1–4.
- Peterson, A., and G. J. Haeussler. 1928. Some observations on the number of larval instars of the oriental peach moth, *Laspeyresia molesta* Busck. *J. Econ. Entomol.* 21: 843–852.
- Pote, J. M., A. L. Nielsen, L. Gut, and M. J. Grieshop. 2015. Organic management of apple flea weevil, a reemergent pest of commercial apples. *Org. Agric.* 1–11.
- Quaintance, A., and C. Brues. 1905. The Cotton Bollworm. *US Dep. Agric. Bur. Entomol. Bull.* 50: 1–112.
- R Development Core Team. 2011. R: A language and environment for statistical computing. *R Found. Stat. Comput.*, R Foundation for Statistical Computing.
- Ring, D. R., M. K. Harris, and J. A. Jackman. 1983. A Fortran computer program for determining start date and base temperature for degree-day models. *Bull. Texas Agric. Exp. Stn.* 1–10.
- Schmidt, F. H., R. K. Campbell, and S. J. Trotter. 1977. Errors in determining instar numbers through head capsule measurements of a lepidopteran—a laboratory study and critique. *Ann. Entomol. Soc. Am.* 70: 750–756.
- Snyder, R. L., D. Spano, C. Cesaraccio, and P. Duce. 1999. Determining degree-day thresholds from field observations. *Int. J. Biometeorol.* 42: 177–182.
- Wyss, E., H. Luka, L. Pfiffner, C. Schlatter, U. Gabriela, and C. Daniel. 2005. Approaches to pest management in organic agriculture: A case study in European apple orchards. *Cab Int. Org.* 33–36.
- Zehnder, G., G. M. Gurr, S. Kühne, M. R. Wade, S. D. Wratten, and E. Wyss. 2007. Arthropod pest management in organic crops. *Annu. Rev. Entomol.* 52: 57–80.