

# Influence of plant direction, layer, and spacing on the infestation levels of *Anthonomus eugenii* (Coleoptera: Curculionidae) in open jalapeño pepper fields in North Florida

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## Abstract

Pepper weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae), is a most serious pest of peppers in North Florida. To analyze *A. eugenii* infestation levels on jalapeño peppers as a reference for effectively controlling pepper weevil, we investigated infestation and population dynamics of *A. eugenii*, and influence of plant direction, layer, and spacing in open fields in North Florida. The results showed that adult pepper weevil infestation started in late Apr when plants started to produce buds and flowers. Three infestation peaks were recorded on 9 Jun, 24 Jul, and 11 Sep. The second peak showed the highest infestation level ( $14.6 \pm 2.7$  infested fruits per plant). The density curve of *A. eugenii* larvae within fruits lagged about 2 to 4 wk behind the curve of infestation level in the field. Three peaks occurred on 10 Jul, 21 Aug, and 25 Sep. Fruits on the eastern part and top third of pepper plants had the lowest infestation levels, as did plants spaced 40 cm apart. This study, focusing on the preferred locations of *A. eugenii*, will help improve sampling technique and pest management applications, and thus enhance the effectiveness of pesticide application, and ultimately reduce ecological damage.

Key Words: pepper weevil; preference; pest management; population dynamics

## Resumen

El picudo de chile, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae), es la plaga más grave de chile en el norte de la Florida. Para analizar el nivel de infestación de *A. eugenii* en chiles jalapeños como referencia para controlar eficazmente el picudo del chile, investigamos la infestación y la dinámica de la población de *A. eugenii*, y la influencia de la dirección, la capa y el espaciamiento de las plantas en campos abiertos en el norte de la Florida. Los resultados mostraron que la infestación de los adultos del picudo del chile comenzó a fines de abril, cuando las plantas comenzaron a producir brotes y flores. Se registraron tres picos de infestación, el 9 de junio, el 24 de julio y el 11 de septiembre. El segundo pico mostró el nivel más alto de infestación ( $14.6 \pm 2.7$  frutas infestadas por planta). La curva de densidad de las larvas de *A. eugenii* dentro de las frutas se retrasó aproximadamente 2 a 4 semanas por detrás de la curva del nivel de infestación en el campo. Tres picos ocurrieron en el 10 de julio, el 21 de agosto y el 25 de septiembre. Las frutas en la parte hacia el oriente y en el tercio parte superior de las plantas de chile tuvieron los niveles más bajos de infestación, al igual que las plantas separadas por 40 cm. Este estudio, que se enfoca en las ubicaciones preferidas de *A. eugenii*, ayudará a mejorar la técnica de muestreo y las aplicaciones de manejo de plagas y, por lo tanto, aumentará la efectividad de la aplicación de pesticidas y, en última instancia, reducirá el daño ecológico.

Palabras Clave: picudo de chile; gorgojo de chile; preferencia; manejo de plagas; dinámica poblacional

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Pepper weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae), is a serious pest of peppers in open fields in North Florida. Originating from Mexico, *A. eugenii* is widespread throughout Central America, southern USA, and Europe (Berdegue et al. 1994; Speranza et al. 2014). It is a potential risk to other pepper-growing areas due to

trade and frequent international shipments. The pest prefers young fruits in terms of feeding and oviposition. Pepper weevils emerge before pepper plant flowering, then infest budding and flowering plants (Seal & Bondari 1999). The infestation rate occasionally reaches 70 to 90% (Rolston 1977). During the post-harvest period when fruits are

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scarce, stamens and pollen are selected as alternative foods (Genung & Ozaki 1972). Larval feeding on internal tissues of fruits is the major damage of pepper weevil, causing the core to become brown and moldy (Brutton et al. 1989; Webb et al. 2014), and more susceptible to decay and disease (Burke & Woodruff 1980). Premature abscission is the most obvious sign of infestation (Riley 1990; Seal & Schuster 1995).

Three to 5 generations of pepper weevil occur each yr and the developmental period of a generation is 20 to 30 d (Elmore et al. 1934; Toba et al. 1969; Capinera 2014). The female deposits a single egg under the fruit surface by making a cavity, then covers it with an anal secretion that hardens and darkens (Toapanta et al. 2005). The female can identify its own pheromone to avoid oviposition in the same fruit (Addesso et al. 2007). More than 300 eggs can be produced by a female during her lifetime (Goff & Wilson 1937). The eggs begin to hatch in 3 to 4 d and the larvae subsequently develop within 12 d. After pupation, the emerging adult escapes from the pepper fruit via an exit hole (Capinera 2014). Once infestation by pepper weevil is detected, control with pesticides is difficult (Riley et al. 1992a, b). In addition, natural enemies suffer severe damage from increasing chemical sprays. This study was carried out to determine the infestation level of pepper weevil with respect to locations of pepper plants in open fields, and to access levels of infestation with respect to plant location, direction, layer, and spacing for proper monitoring and management in open fields where suitable pest management plans can be applied.

## Materials and Methods

### FIELD SITE

Open field experiments were conducted at the IPM demo plots located in the Center for Viticulture and Small Fruits Research, Florida Agricultural and Mechanical University (30.476888°N, 84.172611°W), Tallahassee, Florida, USA. Jalapeño peppers were planted in Mar 2017. Insect monitoring and data collection started twice per wk after planting. All experiments were carried out in pepper fields.

### INFESTATION LEVELS OF PEPPER FRUITS IN OPEN FIELDS

In the first survey of pepper weevil infestation and larval density in 2017, 55 samples were examined by collecting samples from Apr to Oct. During sampling, each plant was surveyed every 3 to 4 d. Plant observation times ranged from 10:00 AM to 2:00 PM Eastern Standard Time each d, and all fruits on the plant were examined for weevil infestation. The number of *A. eugenii* larvae within infested fruits was recorded after dissecting the fruits. The dissected fruits were left in the field to maintain the weevil population.

### INFLUENCE OF PLANT DIRECTION, LAYER, AND SPACING ON INFESTATION LEVELS OF PEPPER WEEVIL IN OPEN FIELDS

To determine the influence of plant direction, layering, and spacing on infestation by *A. eugenii*, pepper fruits were examined in 5 directions (north, south, east, west, and center part of plant), in 3 layers (upper, middle, and lower third of a plant), and at 5 spacing levels (20, 40, 60, 80, and 100 cm between plants). In addition, the wall thickness and single weight of fruits in each location were measured with digital calipers (model 500-474, Mitutoyo, Tokyo, Japan) and an analytical balance (model BS124S, Sartorius, Goettingen, Germany), respectively, to determine the relationship between wall thickness and fruit weight to infestation levels. The larvae were left in the field after data recording.

Each treatment examined 35 pepper plants from Apr to Oct, and treatments were replicated 5 times.

### STATISTICAL ANALYSIS

All data were subjected to 1-way ANOVA analysis and differences between means were calculated by post hoc Tukey's honest test of significance at 5% levels. All statistical analyses were performed with SPSS analysis software (IBM Corp. 2011). Regression analyses were performed with SigmaPlot 12.0 software (SigmaPlot 2007).

## Results

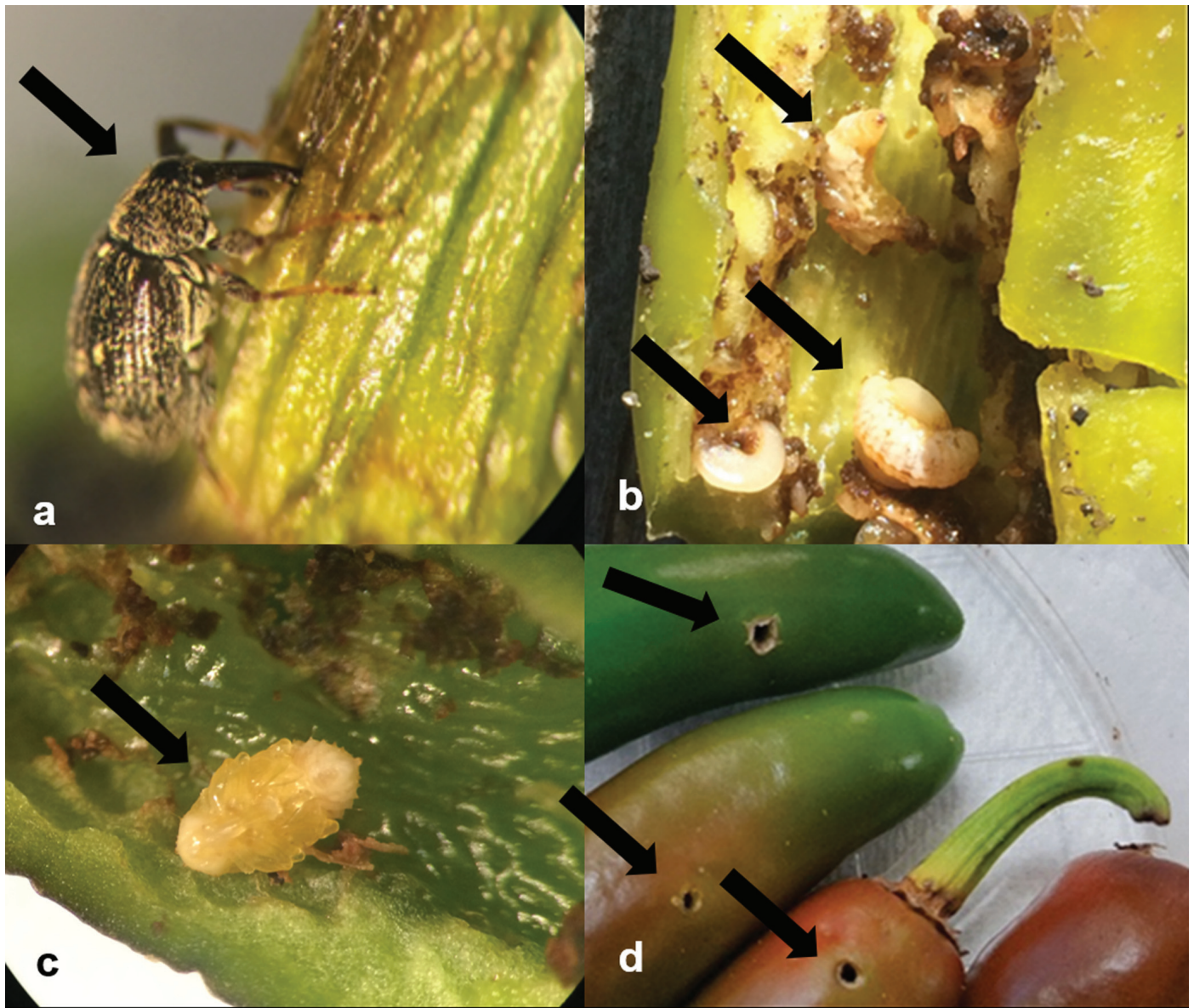
### INFESTATION LEVELS OF PEPPER FRUITS IN OPEN FIELDS

The different life stages of pepper weevils found on plants, or within fruit, and exit holes made as adults emerged from jalapeño fruits are shown in Fig. 1. Infestation of fruit per plant is shown in Figure 2A. Adult pepper weevils started mating and ovipositing in pepper field at the end of Apr. The infestation did not increase until 2 wk later. The number of infested fruits increased rapidly in the latter half of May and early Jun, reaching a peak ( $12.0 \pm 1.5$  fruits per plant) on 9 Jun. After this peak, the infestation level decreased to  $3.4 \pm 1.1$  fruits on 6 Jul, and then increased again during early Jul. The second peak of infested fruits occurred on 24 Jul ( $14.6 \pm 2.7$ ), and was the highest in the season. Fruit infestation decreased to  $3.4 \pm 0.9$  fruits on 14 Aug, followed by the third, and smallest, peak of infestation ( $9.2 \pm 1.4$ ) on 11 Sep. Infestation levels decreased although slight build-up of infested fruit was still detected in early Oct, but by the latter half of Oct the infestation gradually vanished after harvest.

Data on the number of pepper weevils in infested fruits per plant are shown in Fig. 2B. During this study, 3 generations of *A. eugenii* were observed in the open pepper fields in North Florida. The larval population started in early May and showed a peak on 10 Jul, reaching  $11.2 \pm 2.4$  larvae per pepper plant. The second ( $12.6 \pm 1.6$  larvae per plant) and third ( $8.4 \pm 1.9$ ) peaks of larvae per plant occurred on 21 Aug and 25 Sep, respectively. A density of  $2.8 \pm 1.5$  larvae per plant occurred on 31 Jul and again on 14 Sep ( $2.6 \pm 1.1$  larvae per plant). After the third peak, the population declined during early Oct and vanished at the end of Oct. The peaks in the number of *A. eugenii* larvae within fruits lagged 2 to 4 wk following an infestation peak (Fig. 2A), and an average of 1.12 larvae were found in an infested fruit.

### INFLUENCE OF PLANT DIRECTION, LAYER, AND SPACING ON THE INFESTATION LEVELS OF PEPPER WEEVIL IN THE OPEN FIELDS

The data are presented in Fig. 3. The number of infested fruits in the eastern side of pepper plant was markedly lower ( $2.0 \pm 0.3$  fruits per plant), while significant infestations occurred in the western ( $9.0 \pm 0.7$  fruits per plant) and central ( $9.5 \pm 0.9$  fruits per plant) parts ( $F_{4,170} = 21.827$ ;  $P < 0.001$ ; Fig. 3A). The number of weevil larvae in eastern fruits ( $3.0 \pm 0.7$  larvae per plant) was significantly less than in central fruits ( $10.3 \pm 1.1$  larvae per plant) ( $F_{4,170} = 12.85$ ;  $P < 0.001$ ; Fig. 3B). Pepper weevils infested more fruits in the middle third of a plant ( $7.1 \pm 0.8$  fruits per plant), and in the bottom third of a plant ( $6.4 \pm 0.8$  fruits per plant) than in the top third of plants ( $4.5 \pm 0.5$  fruits per plant) ( $F_{2,102} = 3.49$ ;  $P = 0.034$ ; Fig. 3C). There were more larvae in fruits in the bottom third of a plant ( $6.3 \pm 0.6$  weevils per plant) than in fruits in the middle third ( $4.9 \pm 0.6$  weevils per plant) or top of a plant ( $4.2 \pm 0.5$ ;  $F_{2,102} = 3.938$ ;  $P = 0.023$ ; Fig. 3D). Pepper fruits ( $4.3 \pm 0.6$  fruits per plant) on plants at the distance of 40 cm between plants showed the lowest



**Fig. 1.** (a) Adult pepper weevil feeding on the stalk of a pepper fruit; (b) young and full grown larvae inside a pepper fruit; (c) pupa inside the fruit; and (d) adult weevil exit holes in pepper fruits.

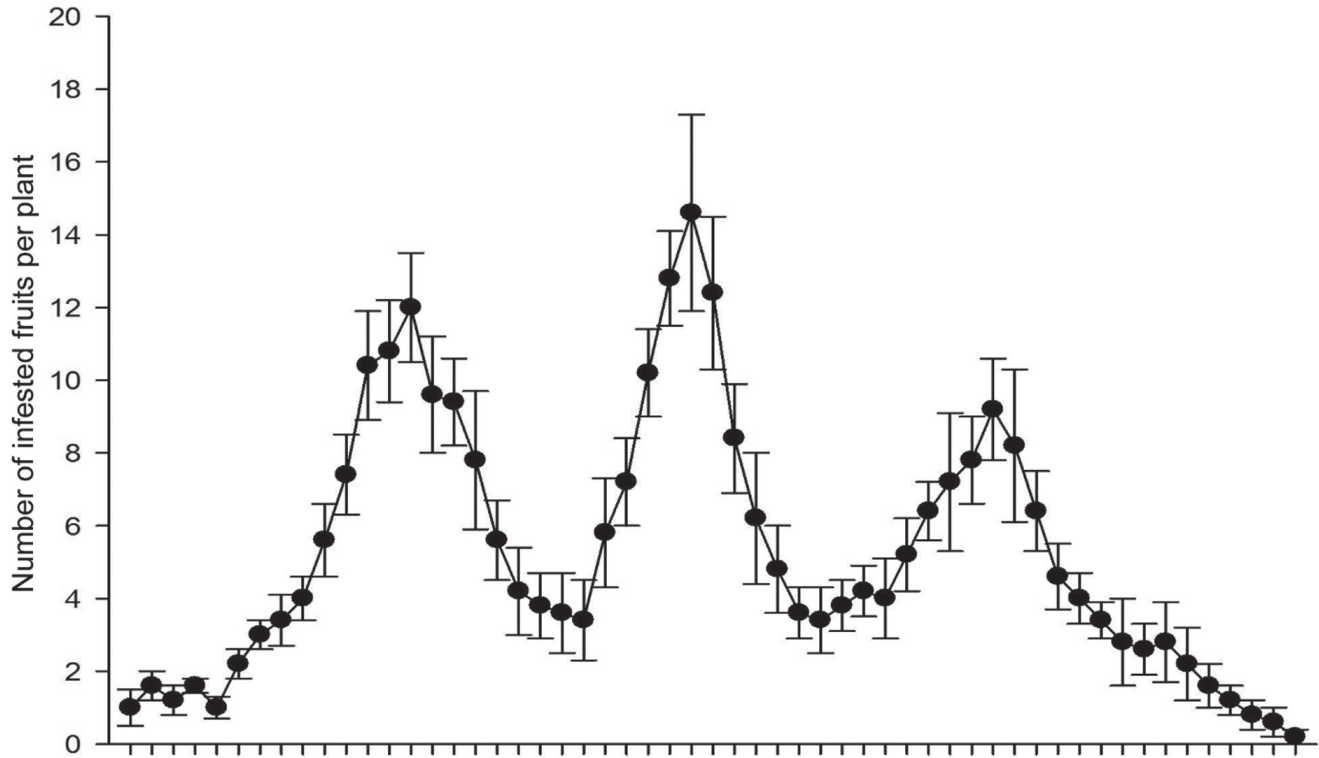
weevil infestation ( $F_{4,170} = 3.601$ ;  $P = 0.008$ ; Fig. 3E), leading to significantly fewer larvae in fruits ( $1.9 \pm 0.4$  larvae per plant) ( $F_{4,170} = 10.162$ ;  $P < 0.001$ ; Fig. 3F).

Pepper fruits on the eastern side of plants had thicker walls ( $2.84 \pm 0.11$  mm) and were heavier in weight ( $9.15 \pm 0.23$  g), whereas fruits on the western side and the central plant fruits had thinner walls (western,  $2.15 \pm 0.11$  mm; central,  $2.22 \pm 0.13$  mm), and were lighter in weight (western,  $7.11 \pm 0.34$  g; central,  $7.08 \pm 0.32$  g) (thickness,  $F_{4,45} = 5.349$ ;  $P = 0.001$ ; Fig. 4A; weight,  $F_{4,45} = 7.295$ ,  $P < 0.001$ ; Fig. 4B). However, no significant differences were detected among various layers in fruit wall thickness ( $F_{2,27} = 0.772$ ;  $P = 0.472$ ; Fig. 4C) and single fruit weight ( $F_{2,27} = 0.09$ ;  $P = 0.915$ ; Fig. 4D). More thick-walled fruit ( $2.72 \pm 0.11$  mm;  $F_{4,45} = 2.336$ ;  $P = 0.047$ ; Fig. 4E) and larger fruit ( $9.33 \pm 0.19$  g;  $F_{4,45} = 9.47$ ;  $P < 0.001$ ; Fig. 4F) were produced in plants spaced 40 cm apart compared to other spacing intervals (20, 60, 80, and 100 cm). Overall, based on these results, both the fruit wall thickness (Fig. 5A) and fruit weight (Fig. 5B) were negatively correlated with the infestation level of pepper weevil.

## Discussion

In North Florida, 3 generations of pepper weevil were recorded in our experiments. The second generation, occurring in Jul and Aug, triggered the highest infestation level and larval population, respectively, suggesting both times are most suitable for seasonal growth and development of the weevils. Wilson (1986) and Gordon and Armstrong (1990) also found that the pepper weevil population grew faster and higher during the summer months. In our study, the infestation level of *A. eugenii* and their larval population were close to zero at the end of Oct, indicating that pepper weevil failed to overwinter after harvest, although it has been suggested that in recent years weevils may have been overwintering in Georgia. Goff and Wilson (1937) indicated that pepper weevils cannot live through the winter where food and hiding places are not available. Based on the infestation and population dynamics, late Apr, late Jun, and early Aug are times associated with lower infestation, and those are potential periods to control *A. eugenii*.

A



B

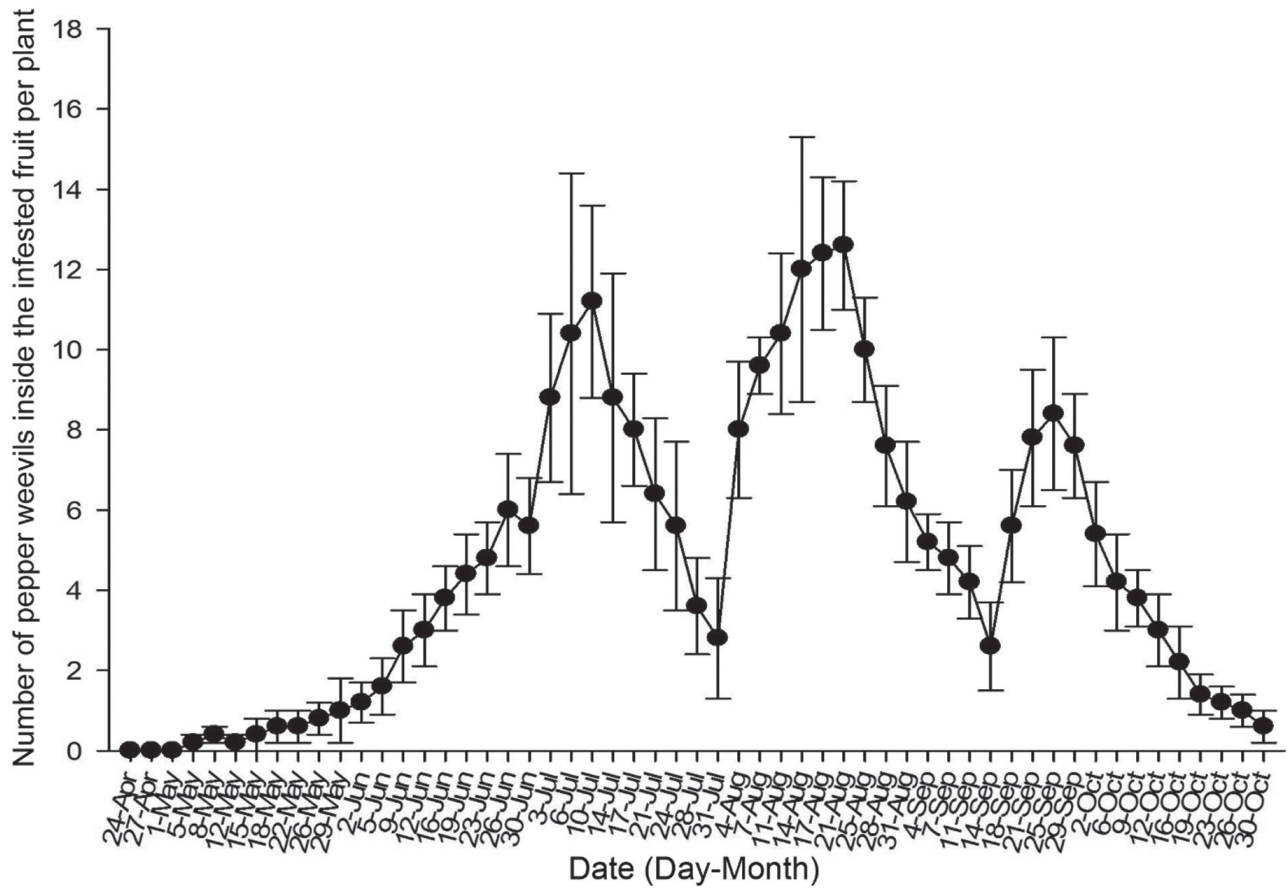
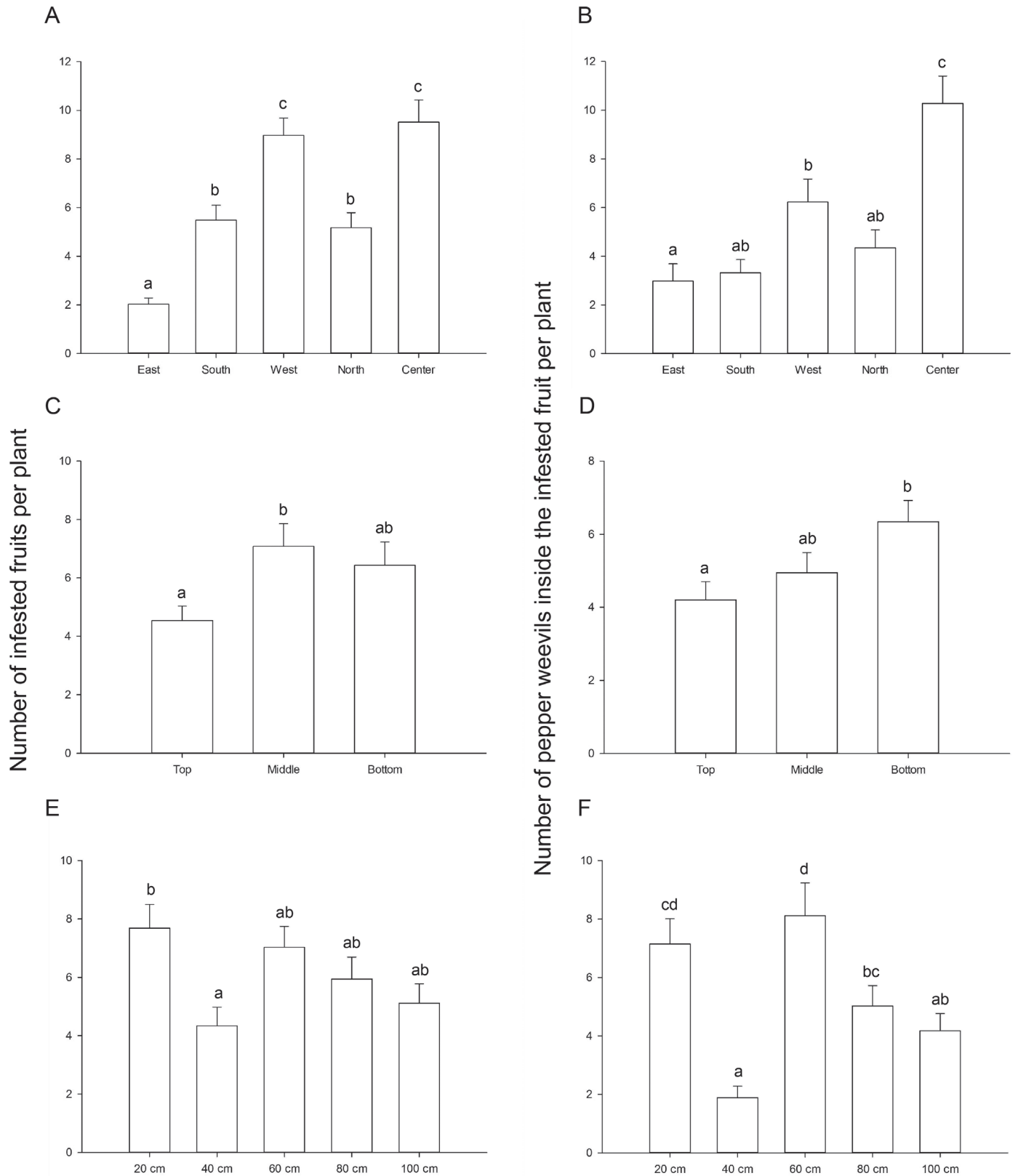


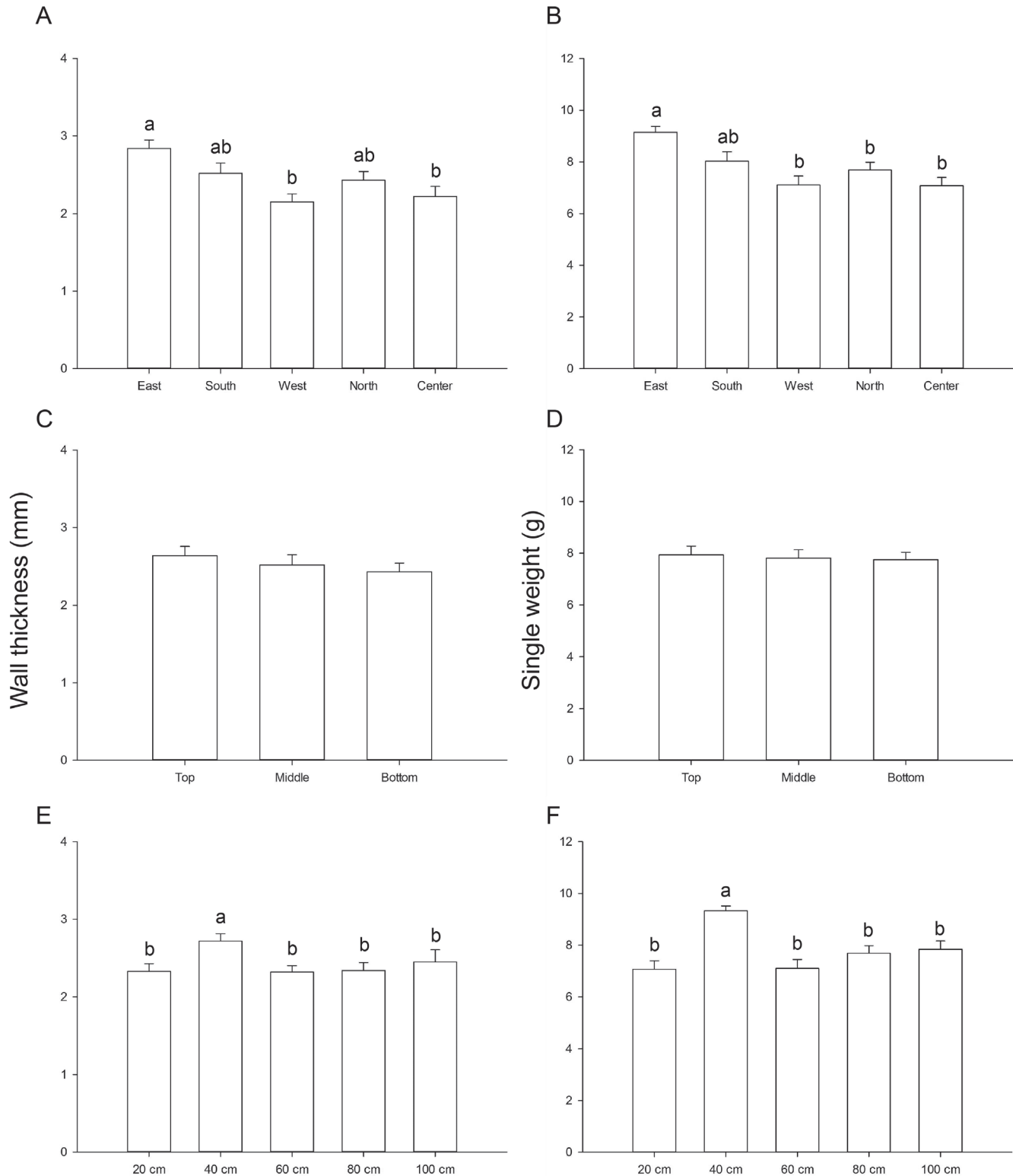
Fig. 2. Infestation and larval density of pepper weevil in 2017. Vertical bars are standard errors of the means.



**Fig. 3.** Number of infested fruits and presence of weevil larvae in different jalapeño plant parts (means ± SE). Number of infested fruits in 5 directions (A), in 3 layers (C), and at 5 spacings (E). Number of larval *A. eugenii* within infested fruits in 5 directions (B), in 3 layers (D), and at 5 spacings (F). Different letters indicate significant differences among the treatments (means separated by Tukey's HSD,  $P < 0.05$ ).

Overall, we found that jalapeño pepper plants with fruits on the eastern side of a plant with thicker walls and heavier fruits, and spacing of 40 cm between plants were attacked less often by pepper weevils.

Conventional varieties of pepper, especially those cultivars with thin-walled mesocarp, are susceptible to *A. eugenii* infestation (Elmore et al. 1934). Pepper weevils prefer smaller, thin-walled fruits over high-

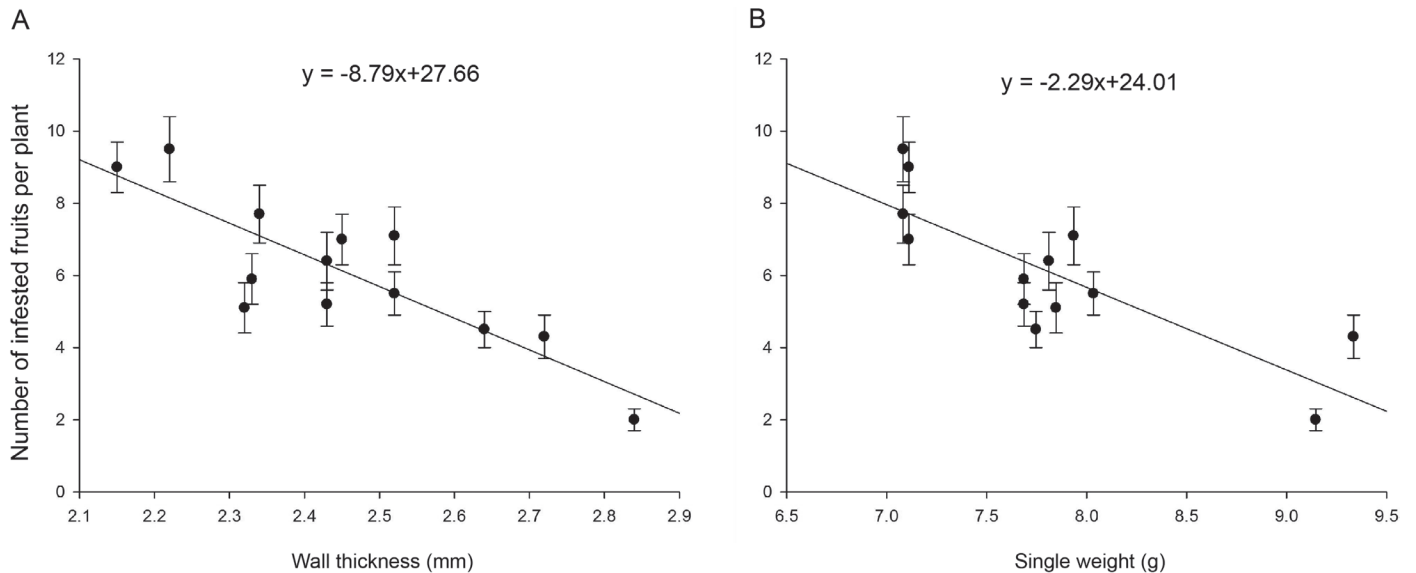


**Fig. 4.** Fruit wall thickness and single weight in different jalapeño plant parts (means  $\pm$  SE). Fruit wall thickness (A) and single weight (B) in 5 directions, fruit wall thickness (C) and single weight (D) in 3 layers, fruit wall thickness (E) and single weight (F) at 5 spacings. Different letters indicate significant differences among the treatments (means separated by Tukey's HSD,  $P < 0.05$ ).

quality fruits (Toapanta et al. 2005). Thick-walled fruits also are attacked by pepper weevil, but there is often a switch in preference to younger, thinner-walled fruits (Patrock & Schuster 1992). Choosing pepper cultivars for stronger resistance or lower susceptibility to *A. eugenii* (Quinones & Favela 2002), excellent cultivation conditions, suf-

ficient sunlight, and appropriate plant spacing can be effective strategies for controlling pepper weevil.

However, Seal and Martin (2016) suggested that larger fruits may result in more puncture marks and oviposition plugs than small fruits, due to the greater distances between sites of *A. eugenii* feeding and



**Fig. 5.** Relationship between the infestation level and the fruit wall thickness (A) and single weight (B). Each data point represents the number of infested fruits per plant in each fruit wall thickness or weight (means  $\pm$  SE). Line was fitted using linear regression analysis.

oviposition. Our data showed that the top third of plants triggered a significantly lower *A. eugenii* infestation level, further indicating that the top third of plants, which captures more sunlight, may produce better-growing fruits. Developing plant resistance is valuable from a pest management perspective (Kennedy 1978; Patrock & Schuster 1987). Pepper plants can produce high-quality fruits at a spacing of 40 cm (Li et al. 2011), and both the feeding and oviposition of pepper weevils were lowest at this spacing.

Although insecticide applications generally are effective, they are still challenging in controlling pepper weevil, because the eggs are deposited within flower buds and fruits (Riley et al. 1992a, b). The infestation rate of pepper weevils in the field occasionally reaches 66%, even when conventional insecticides are used (Addesso et al. 2014). More importantly, various natural enemies of *A. eugenii* (Pratt 1907; Wilson 1986; Cortez et al. 2005), such as the parasitoids *Catolaccus hunteri* Crawford (Hymenoptera: Pteromalidae) (Schuster 2007) and *Triaspis eugenii* Wharton and Lopez-Martinez (Hymenoptera: Braconidae) (Toapanta 2001), are not compatible with conventional insecticides. The center areas of plants were more susceptible to *A. eugenii* in 5 directions, and their middle and bottom third were less resistance from a layer perspective, indicating these locations were preferred by pepper weevils and should be the focus of insecticide applications. In further field investigations, weevil presence can be monitored with yellow sticky traps baited with aggregation pheromone to maintain the pest below the economic threshold (Andrews et al. 1986; Segarra-Carmona & Pantoja 1988; Cartwright et al. 1990; Riley & Schuster 1994).

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