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Evaluation of the Potential of Biocontrol Agent *Catolaccus hunteri,* a Hymenopteran Parasitoid in Reducing Pepper Weevil (*Anthonomus eugenii*) Population in Greenhouse Conditions

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Pepper *Capsicum annum* L. is an essential crop in the United States. The key pest of pepper is the pepper weevil (PW), *Anthonomus eugenii* Cano (Coleoptera: Curculionidae). It is distributed in tropical and sub-tropical parts of the United States. Adult females oviposit on flowers, buds, and fruits. Larvae cause significant damage by feeding inside the fruit, thus rendering the produce unmarketable. Farmers use insecticides to kill the adults, they are ineffective on the hidden immature stages that cause significant damage. There is a need to develop a sustainable approach that can reduce the damage inflicted by PW larvae. Developing a sustainable approach includes the use of biocontrol as a component for integrated management program. *Catolaccus hunteri*, has been found as a potential biocontrol agent of pepper weevil. However, the potential control offered by the parasitoid needs to be evaluated. In this study we evaluated the potential of *Catolaccus hunteri* **to control pepper weevil. An experiment was performed in cages in a glasshouse with three pots of jalapeño plants and a pair of pepper weevil adults in each cage. Treatment includes the release of three levels of** *C. hunteri* **adult densities 10, 20, 40 and a control without *C. hunteri*. The effectiveness of *C. hunteri* adults in suppressing pepper weevil was evaluated by counting the total number of infested flowers, fruits and buds on each plant at weekly intervals for twelve weeks. Infestation was confirmed by dissecting each plant part and observing them under a binocular microscope at 20×. The results indicated that the infestation rate was significantly lower in the treatment where 40 *C. hunteri* were released at weekly interval as compared to the control. Future studies are needed to incorporate *C. hunteri* as a component to develop sustainable management system for pepper weevil.**

Catolaccus hunteri Crawford is an ecto-parasitoid of coleopteran larva, particularly of Bruchidae and Curculionidae. It is the most common parasitoid of pepper weevil (PW), *Anthonomus eugenii* Cano, in the United States and Mexico (Seal et al., 2002). The first record of *C. hunteri* as a parasitoid of PW was in Baja California Sur, Mexico, in 1995 (Aguilar and Servin, 2000). Records indicate that *C. hunteri* was also found in Puerto Rico as a parasitoid of PW. Females of *C. hunteri* can lay 466.35 ± 28.39 eggs, out of which only 18.95% reach the adult stage (Rodríguez-Leyva et al., 2000). A couple of studies have demonstrated the potential of *C. hunteri* for management of PW. About 33.4% of *C. hunteri* was recovered from PW-infested nightshade berries. Schuster (2007) showed that the release of *C. hunteri* on bell pepper results in lower infestation than in pepper with no parasitoid. However, *C. hunteri* was not recovered from fruit larger than 2.5 cm (Riley & Schuster, 1992). Releasing adults in the off-season on nightshade and following with a second release in the main pepper season helps suppress larvae. Gómez-Domínguez et al. (2012) found that *C. hunteri* oviposits mainly on third instar larvae of PW, which makes some authors think it is ineffective. Monitoring this parasitoid in the field can be done using the same yellow sticky traps as are used for monitoring PW (Schuster, 2012). In Mexico, 13 species of parasitoid were reported to attack PW. The main species is the ecto-parasitoid, *C≠. hunteri*, on the third larval stage of PW. The larva develops on the host surface, and after two weeks, the adult emerges (Vásquez et al., 2005; Chabaane et al., 2021). *C. hunteri* feeds on all larval stages of PW but only parasitizes the third instar larva. The first and second larval instars of PW have been found to be more vulnerable to the attack of *C. hunteri* as they are nearer the pericarp. As the larva develops, it moves toward the placenta and seeds. The ovipositor length of *C. hunteri* is 1.91 ± 0.71 cm, and it does not reach the third instar if it is deeper. Mortality caused by the parasitoid is 39% (Riley et al., 1992 and Rodríguez-Levya et al., 2007). *C. hunteri* feeds on the first and second instar larvae of pepper weevil and parasitizes the third instar (Murillo-Hernández et al., 2019). In a survey of the abundance of this PW parasitoid in Mexico, *C. hunteri* was found at every site (Rodríguez-Levya et al., 2007). *Triaspis eugenii* Wharton and Lopez-Martinez (Hymenoptera-Braconidae) and *C. hunteri* Crawford (Hymenoptera-Pteromelidae) have been evaluated against PW infestations. *T. eugenii* attacks the egg stage of pepper weevil but fails to establish in the field. *C. hunteri* is native to Florida, where it is the species found most often attacking PW (Rodríguez-Levya, 2007; Addesso et al., 2014). Seal et al. (2002) found that the optimum temperature for rearing *C. hunteri* is 25 °C. The greatest number of progeny was found when the

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parasitoid was reared on *C. maculatus*. In the present study, we determined the potential of *C. hunteri* to suppress pepper weevil.

Materials and Methods

Study location. Studies were conducted in a greenhouse located at Tropical Research and Education Center (TREC) in Homestead, FL.

Greenhouse preparation. The roof of a 10.4 × 18.3 m^2 greenhouse was covered with a black, UV-resistant cloth netting which reduced the amount of sunlight coming into the greenhouse.

The plants used in the experiment were Jalapeño pepper (*C. annuum* var. PS 11435810). Styrofoam trays were used to plant the seeds of the pepper. Promix® potting medium, (Premier Tech, Quebec, Canada) was used to fill the trays. In each cell, two seeds were placed by making a shallow hole with the fingertip which was covered with t\soil. The trays were watered every day at 10 am to enhance germination and prevent desiccation. Three weeks after germination, the seedlings were fertilized with 20:20:20 (N: P: K) Peters water-soluble fertilizer (Scotts Co., Marysville, OH) at the rate of 1.198 kg/m^3 gallons of water. Each seedling was drenched with 30 mL of 20:20:20 solution. Six weeks after sowing, the seedlings were transplanted. The six-week-old seedlings were transplanted into 3.78-L plastic pots. The same potting was used. Plants were manually irrigated everyday with 0.059 L of tap water. All transplants were fertilized with 20:20:20 liquid fertilizer once each week. Granular fertilizer 6:12:12 (N: P: K) 896.6 kg/ha (Diamond-R Fertilizer, Fort Pierce, Fla.) was applied every two weeks. To protect the plants from fungal disease, chlorothalonil (1725 mL/ha, Bravo Weather Stik® (Syngenta crop protection LLC, Greensboro, NC) was applied at weekly intervals. The experiment was started forty five days after transplanting.

Plot design and data collection. Black plastic greenhouse benches (2.4m long × 0.91m wide × 60cm high) were used. The experimental design was a randomized complete-block design with four treatments and three replications per treatment. Each cage was of 45.72 cm × 45.72 cm × 91.44 cm size (Bioquip Products Inc., Compton, CA) and contained three jalapeño plants. The cages were placed on the bench in a row, spaced 1.83 m within the row and 0.91 m between rows on an adjacent bench.

The four treatments used in the present study were based on varying the number of *C. hunteri* released each time. The treatments included releasing of 10, 20, or 40 adults of *C. hunteri* (72 hrs old) in each cage plus a control without any adult release. In each cage, a pair (♂ × ♀) of pepper weevil (48-h-old) was released after placing the plants inside the cage. Adults of *C. hunteri* were released at weekly intervals in each cage according to the treatments listed above. Each cage was provided with a vial (30 drums) of 10% sugar solution as alternate nutrition source. The sugar solution was replaced in the cage every third day. Pepper plants were replaced by a new set (n = 3) of non-infested plants having flowers, buds, and young fruit in each cage three times at monthly intervals. Two pairs (♂ × ♀) of PW adults (48-h-old) were kept constant in each cage and released again if one died.

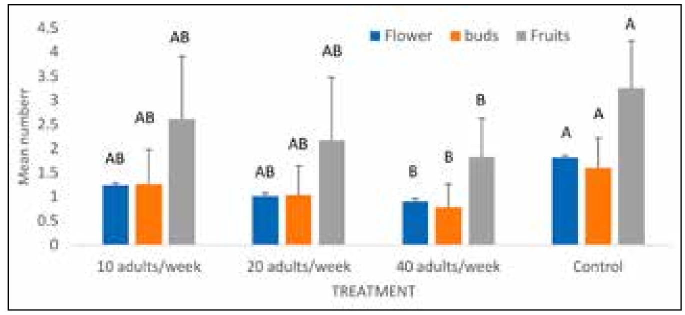

Fig. 1. Mean ±SE number of abscised flowers, fruits, and buds of pepper due to infestation of pepper weevil in different treatment plots in a greenhouse. Means within the same color followed by the different uppercase letter are significantly different at P≤ 0.05 according to Tukey's HSD test.

Treatments were evaluated by collecting all abscised flowers, fruit, and buds from each treatment at weekly intervals for 12 weeks. Samples of abscised flowers, fruits and buds from each treatment plot were placed in a Ziplock bag and were taken to the laboratory to check for the signs of parasitization by *C. hunteri*. Abscised flowers and buds were counted, cut open, and observed under a binocular microscope. The number of PW eggs and larvae, and parasitoid eggs and larvae were recorded. Any presence of PW and plus the parasitoid was considered as parasitization. When discarding old plants, all flowers, buds, and fruit were collected and checked for *C. hunteri* parasitisation.

Statistical analysis. Data were analyzed using SAS statistical software (SAS institute, Cary, NC, USA). Square-root transformation was use to normalize the data before analysis. Non-transformed means are in the tables. Transformed data were further analysed using mix model ANOVA using the mixed effects including treatment, sampling date and their interactions (PROC GLIMMIX model, SAS institute Inc. Cary, NC). Means were separated using Tukey's HSD (Honestly Significant Difference) test in SAS.

Results

A significantly lower number of abscised flowers (F = 3.57, df = 3,6, P = 0.0152), buds (F = 3.04, df = 3,6, P = 0.03) and fruit (F = 2.77, df = 3,6, P = 0.043) were observed in the treatment where 40 adults of *C. hunteri* were released weekly compared with the control (Fig.1). The highest parasitisation was recorded in the treatment with 40 adults released per week (Table 1).

Marketable fruit number and weight. There was no significant difference among treatments for the number of marketable fruit (Treatment; F = 2.36, df = 3,6, P = 0.17) or fruit weight

Table 1. The number of parasitoids (*Catolaccus hunteri*) in pepper fruit infested by pepper weevil collected in greenhouse on different sampling dates at TREC, Homestead, FL, in Jan. 2021.

	Sampling dates								
Treatment	15 Jan.	22 Jan.	19 Feb.	26 Feb.	12 Mar.	19 Mar.	26 Mar.	23 Apr.	30 Apr.
Control	0	0	0	0	0	0	0	0	0
10 adults/week	0	0	0	0	0	1	0	0	0
20 adults/week	0	0	0	1	1	1	0	0	0
40 adults/week	0	0	2	0	2	0	1	0	0

Table 2. The effect of the number adult *C. hunteri* parasitoid released weekly on marketable pepper fruit number and weight in a greenhouse at TREC, Homestead, FL, in Jan. 2021.

Treatment	Marketable fruit count (Mean ± SE)[z]	Marketable fruit wt. (Mean ± SE)
10 adults/week	16.3 ± 0.3 a	0.3 ± 0.0 a
20 adults/week	17.3 ± 1.3 a	0.4 ± 0.1a
40 adults/week	17.4 ± 0.3 a	0.4 ± 0.0 a
Control	13.0 ± 3.0 a	0.3 ± 0.2 a

[z]Means within the same column followed by the different letter are significantly different at $P \leq 0.05$ according to Tukey's HSD test.

(Treatment; $F = 0.81$, df = 3,6, $P = 0.53$). However, the number and weight of marketable fruit were the highest in the treatment with 40 adults released per week and the lowest in the control (Table 2).

Discussion

In the greenhouse study, there were significant differences in the number of abscised flowers, buds, and fruit among treatments. The treatment where 40 *C. hunteri*/week were released had the lowest number of abscised flowers, fruit, and buds. This result is supported by Schuster (2007), who also observed less PW infestation when parasitoids were released on bell pepper compared to the control with no parasitoid released. However, in the present study very few *C. hunteri* larvae were observed in the infested fruit. No adult emergence of *C. hunteri* was observed in the parasitized fruit due to the fruit rotting and the death of larvae after cutting infested fruit open in the laboratory. It was reported that *C. hunteri* fed on the first and second instar larvae of PW and parasitized the third instar (Murillo-Hernández et al., 2019). Feeding by the *C. hunteri* female on first and second instar larva of PW may be the reason for fewer infested fruit. In the present study, parasitisation by the female was observed when *C. hunteri* females were provided with third instar larvae in a Petri dish in the laboratory. Lower infestation of PW in pepper indicates that *C. hunteri* can be incorporated into a PW management program to decrease the damage by larvae and help reduce the PW population. There is good potential for using *C. hunteri* in a greenhouse, but more research is needed for large scale field applications. The appropriate time to start releasing the parasitoid is when the plant is in the flowering stage.

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