LIFE HISTORY OF *Triaspis eugenii* Wharton and Lopez-Martinez (HYMENOPTERA: BRACONIDAE) AND EVALUATION OF ITS POTENTIAL FOR BIOLOGICAL CONTROL OF PEPPER WEEVIL *Anthonomus eugenii* Cano (COLEOPTERA: CURCULIONIDAE)

By

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The pepper weevil, *Anthonomus eugenii* Cano, is considered the most important pest of peppers (*Capsicum* spp.) in Tropical and Subtropical America. Juvenile stages develop inside buds and immature fruits, so that only adults are susceptible to insecticidal control. No viable biological tactic has been developed to combat this pest but *Triaspis eugenii*, a braconid recently collected in Mexico that attacks pepper weevil eggs, could offer an important addition to its management. The parasitoid was collected from Nayarit, Mexico, during 2003, and a rearing methodology was developed using pepper weevil reared on immature 'Jalapeño' peppers. Low levels of parasitism and high levels of superparasitism hampered the rearing and reliable estimation of parasitoid demography until the weevil rearing system was improved. Confining individual mated females to a single immature 'Jalapeño' fruit maximized weevil fecundity, and also the number of oviposition punctures that were plugged by female. Weevils fed with pepper floral buds,

a high nitrogen source, and immature 'Jalapeños' laid 5.5 eggs per female per day over a life span of 64.5 d at 27 ± 2ºC. The net reproductive rate (Ro=158.1) and intrinsic rate of increase (r_m = 0.14) obtained in a life table study were higher than any previous report.

T. eugenii is a solitary egg-prepupal parasitoid of the pepper weevil. At 27 ± 1ºC, *T. eugenii* eggs hatched at 23 ± 1 h. Females developed in 16.6 ± 0.9 d and males in 16.4 ± 0.9 d. Females (n=10) laid 402 ± 199 eggs of which less than 50% reached the adult stage because of high levels of superparasitism in the laboratory. Ovipositing females lived 16.5 ± 3.02 d, and parasitoids died in fewer than 48 h without carbohydrates (honey). Net reproductive rate (Ro) was estimated at 106 and 167, and intrinsic rate of increase (r_m) at 0.24 and 0.26 with and without superparasitism respectively.

The oviposition plug deposited by the pepper weevil played a decisive role in the ability of *T. eugenii* to find and to parasitize weevil eggs. Crowded rearing conditions inhibited deposition of oviposition plugs and consequently efficiency of parasitism. Wasps were able to find hosts in field cages, and semiochemicals from weevil-damaged plants appeared to play a role in host patch location. However, *T. eugenii* did not stay more than 120 min in the patch regardless of the presence of weevil damaged plants. Superparasitism was less frequent in the field cages (28%) compared to the laboratory (55-64%). The relatively short duration of foraging on large patch size compared to the rearing environment might explain why.

T. eugenii was recovered in low numbers from field releases in spring 2005 at Immokalee, FL, and at least one generation was completed in the field. No wasps have been recovered after that although sampling has been limited. Even if *T. eugenii* does not establish in Florida, its host specificity, its ability to parasitize weevil eggs, and its almost

double intrinsic rate of increase, compared to the pest, make this parasitoid an excellent prospect for biological control by augmentation against the pepper weevil.

CHAPTER 1
INTRODUCTION

The pepper weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae), is considered one of the most important insect pests of all cultivated varieties of chile = pepper (*Capsicum* spp.) in the New World. Pepper weevil is considered a key pest of this crop in the southern United States, Hawaii, Mexico, Central America, and Puerto Rico (Elmore et al. 1934, Burke and Woodruff 1980, Abreu and Cruz 1985, Riley and King 1994, Coto 1996, Arcos et al. 1998).

Pepper weevil biology, control tactics, distribution, and sampling methods have been studied (Elmore et al. 1934, Wilson 1986, Patrock and Schuster 1992, Riley et al. 1992a,b, and Toapanta 2001). Nevertheless, insecticidal control is necessary once the pest is present in pepper crops (Riley and King 1994, Riley and Sparks 1995, Arcos et al. 1998, Mariscal et al. 1998). Unfortunately, the use of insecticides as the principal method of control can provoke many adverse effects such as marketing restrictions, exposure of non-target organisms, environmental contamination, pesticide resistance, and secondary pest outbreaks within the same crop (Doutt and Smith 1971, Van Driesche and Bellows 1996).

Biological control could be an alternative strategy to incorporate into the integrated pest management (IPM) of pepper weevil (Riley and King 1994, Mariscal et al.1998, Schuster et al.1999). However, little is known of the natural enemies of this pest. Pepper weevil arrived or was incidentally introduced from Mexico to the United States at the beginning of the last century (Walker 1905, Pratt 1907). Early reports indicated two

species of ants, three pteromalids, one braconid, and one mite that attack this pest in the United States (Pratt 1907, Pierce et al. 1912, Elmore et al. 1934, Cross and Chesnut 1971). However, the effect of those enemies on pepper weevil populations was not considered important (Pratt 1907, Elmore et al. 1934, Elmore and Campbell 1954).

A few decades ago, Wilson (1986) reported on the ectoparasitoid *Catolaccus hunteri* Crawford (Hymenoptera: Pteromalidae) attacking pepper weevil in Florida. *C. hunteri* is the dominant parasitoid attacking this pest in Florida and Puerto Rico (Wilson 1986, Schuster et al. 1999), and one of the most abundant in some states of Mexico (Mariscal et al. 1998, Schuster et al. 1999, Rodriguez et al. 2000).

C. hunteri has been collected from a closely related pest of cotton, the cotton boll weevil *Anthonomus grandis* Boheman (Coleoptera: Curculionidae), in several countries in Central and South America, Mexico, and the United States (Pierce et al. 1912, Towsend 1912, Cross and Mitchell 1969). Biological studies showed that C. *hunteri* has a higher fecundity than that of the pepper weevil (Rodriguez et al. 2000, Seal et al. 2002). Those findings stimulated and supported the evaluation of *C. hunteri* for controlling this pest (Schuster et al. 1999, Rodriguez et al. 2000).

Releasing *C. hunteri* in field plots in Mexico and Florida produced equivocal results; in Mexico this parasitoid was not effective for combating pepper weevil on bell pepper (Corrales 2002). However, some augmentative releases of this parasitoid prior to and during the pepper season suggested that this parasitoid might have potential to reduce weevil damage (D. Schuster, UFL, personal communication). These contradictory results may be due, in part, to the fact that C. *hunteri* attacks the 3rd instar host that is usually inaccessible deep within the pepper fruit. Therefore, augmentative releases of *C. hunteri*

prior to the crop cycle could reduce pepper weevil populations in alternative host plants like nightshade, while releases in early infestations of pepper weevil, or in small fruited varieties, might be effective because floral buds and small fruits allow *C. hunteri* to reach its host. Once the pepper fruits increase in size, there is no way to expect parasitism. In fact, Riley and Schuster (1992) indicated that *C. hunteri* was not detected in fallen fruits larger than 2.5 cm in diameter. According to this information, an effective biological control agent for pepper weevil might be one that attacks earlier and more accessible life stages.

The search for biological control agents in the native region of the target pest is one of the first steps in a successful biological control program (DeBach 1964, Hagen and Franz 1973, Huffaker and Messenger 1976). Mexico and Central America have been indicated as a center of origin and domestication of *Capsicum annuum* L., the most important cultivated species of chile = pepper (Vavilov 1951, MacNeish 1964, Pickersgill 1969, 1971, Pickersgill and Heiser 1971, Eshbaugh 1976, 1980, Loaiza-Figueroa et al. 1989). Furthermore, it was in Mexico that the pepper weevil was first reported as a pepper pest (Cano and Alcacio1894).

Mariscal et al. (1998) collected nine different genera of hymenopteran parasitoids from pepper weevil in Nayarit, Mexico, including a subsequently described species *Triaspis eugenii* Wharton & Lopez-Martinez 2000 (Hymenoptera: Braconidae). Only Mexico appears to have this diversity of parasitoids of pepper weevil, and additional search would likely yield more potential species.

T. eugenii was the most abundant parasitoid emerging on pepper weevil in Nayarit. Incidence of parasitism ranged from 18 to 40%, making it the most important parasitoid

of this pest in the field (Mariscal et al. 1998, Schuster et al. 1999, Toapanta 2001). This level of parasitism in unsprayed fields suggests that *T. eugenii* could be a good candidate for inclusion in an IPM program against pepper weevil in the United States, Mexico, and Central America. Nevertheless, information on the biology and ecology of this braconid is needed to evaluate its potential as a biological control agent.

Literature Review

Origin of *Capsicum* spp. and Distribution of Pepper Weevil

Central and southern Mexico and part of Central America, a region called Mesoamerica according to anthropologists, has been indicated as a center of origin and domestication of the most important cultivated species of chile = pepper, *Capsicum annuum* L. (Vavilov 1951, MacNeish 1964, Pickersgill 1969, 1971, Eshbaugh 1976, Loaiza-Figueroa et al. 1989, Conciella et al. 1990). The other four important species of cultivated chiles (or peppers) are *Capsicum frutescens* "Tabasco"; *C. chinense* Jacquin "Habanero or Scotch Bonnet"*; C. pubescens* Ruiz and Pavon "Manzano or Pera"; and *C. baccatum* L. All are endemic to South America (Heiser and Smith 1951, Smith and Heiser 1957, Pickersgill 1969, 1971, Pickersgill and Heiser 1971, Eshbaugh 1976, McLeod et al. 1982).

A Mesoamerican origin of the pepper weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae), is suggested by the following facts: a) the origin of the most important species of cultivated pepper (*C. annuum*) is probably Mesoamerica; b) the pepper weevil has not been reported from South America (Clark and Burke 1996); and c) this insect was found for the first time damaging peppers in Central Mexico in Guanajuato (Cano and Alcacio 1894).

A few years after the report from Guanajuato, Mexico, the pepper weevil was reported from Texas (Walker 1905). According to Pratt (1907) this insect was introduced from Mexico on infested fruit shipments. After affecting pepper crops in Texas, the insect was reported from California in 1923, New Mexico and Arizona in 1927, and Hawaii in 1933 (Elmore et al. 1934). It was reported from the west coast of Florida in 1935 (Goff and Wilson 1937), and the east coast in 1972 (Genung and Ozaki 1972). During 1935-1972, when high levels of organochlorine insecticides were used on the east coast, there were no records of pepper weevil in the zone, but in 1972 it was a widespread pest (Burke and Woodruff 1980).

Presently, the pepper weevil is a pest in all pepper growing areas of the United States including North Carolina and New Jersey (Burke and Woodruff 1980). It was once reported in greenhouses in Canada, probably introduced on seedling plants from California (Riley and King 1994). Mexico, Guatemala, El Salvador, Honduras, Nicaragua, and more recently Puerto Rico, Costa Rica, Jamaica, and Panama have been added to the list of countries where this insect is considered a serious problem on peppers (Cano and Alcacio 1894, Elmore et al. 1934, Burke and Woodruff 1980, Abreu and Cruz 1985, Andrews et al. 1986, Riley and King 1994, Clark and Burke 1996, Coto 1996).

Economic Importance

The pepper weevil lays eggs, feeds, and develops completely inside the fruits, which contribute to the difficulty of controlling the insect. The damage to flowers and/or young pods causes abscission and diminishes yield up to 30 to 90% if treatment is not implemented (Campbell 1924, Elmore et al. 1934, Goff and Wilson 1937, Velasco 1969, Genung and Ozaki 1972, Riley and Sparks 1995). In 1990, losses in the United States due to the pepper weevil were estimated at 23 million dollars on the 31,000 ha grown in

California, New Mexico, Florida and Texas (Riley and King 1994). By 2003, the growing area of the crop in the United States had increased to close to 32,000 ha and the value of production reached 550 million dollars (National Agriculture Statistics Service 2004). Using the same proportion of damage (10%) estimated by Riley and King (1994), the economic damage in 2003 could have reached more than 50 million dollars. In Mexico, Central America, and the Caribbean islands, serious economic impact is indicated but not quantified (Riley and King 1994). Additional costs include the many adverse affects of pesticides such as exposure of non-target organisms, environmental contamination, pesticide resistance, and secondary pest outbreaks within the same crop (Doutt and Smith 1971, Van Driesche and Bellows 1996).

The pepper weevil shares many similarities with its congener the cotton boll weevil: Mesoamerica as the likely origin, the damage to their hosts by feeding and developing inside the fruits, and the history of dispersal in the United States (Burke et al. 1986). However, the difference in acreage and, consequently, economic importance of cotton and pepper in the United States, explains why pepper weevil has never received the same attention as the boll weevil, and why even a hundred years after the original detection, there is relatively little information about biogeography, evolution, and biological control of the pepper weevil (Riley and King 1994).

Description

The pepper weevil was described from specimens collected from Guanajuato, Central Mexico, by Cano and Alcacio (1894). The author mentioned as a motive the importance of the insect as a pest of pepper crops in that region. This weevil belongs to the subfamily Anthonominae and shares many characteristics with the other 330 described species of the genus *Anthonomus* (Burke 1976, Anderson 1992, Clark and

Burke 1996): basically robust and convex body, elongation of anterior part of the head to form a rostrum longer than broad, reduced mandibles on the tip of this rostrum, and flat scales like hair covering elytra, scutellum, and to some extent other parts of the body (Burke 1976).

Detailed descriptions of immature lifestages and adults have been written by Elmore et al. (1934), Burke (1968), and Clark and Burke (1996). Pepper weevil adults are usually not longer that 3 mm, and males possess larger metatibial mucrones than females, a characteristic useful to determine sex (Eller 1995). As with boll weevils, pepper weevil males have a notch visible on the 8th tergum of the abdomen, which females do not have (Agee 1964, Sappington and Spurgeon 2000). However, the small size of the pepper weevil makes the last characteristic difficult to see (Eller 1995).

Biology and Life History

The biology of the pepper weevil was first described by Cano and Alcacio (1894), Walker (1905), and Pratt (1907); however, Elmore et al. (1934) made the first complete biological study. The pepper weevil feeds and develops on several species of Solanaceae, but it is only a pest on peppers, *Capsicum* spp. (Elmore et al. 1934, Wilson 1986, Patrock and Schuster 1992). Adults feed on buds, flowers, fruits, and even leaves. Larvae feed and develop completely inside floral buds and immature fruits. Premature abscission is a consequence of feeding and developing inside buds and fruits resulting in loss of production (Cano and Alcacio 1894, Walker 1905, Pratt 1907, Elmore et al. 1934).

Even if there were no abscission of fruits, there could be loss, as the fruits have larvae inside and are often spoiled. Actually, abscission of fruits could be considered an advantage because fallen fruits can be gathered to avoid reinfestation of the crop (Berdegue et al. 1994) and because fewer damaged fruits make it to the market.

There are three instars and the number of generations per year depends only of temperature and availability of food resources (Elmore et al. 1934). Pepper weevil develops within a wide range of temperature (10-30ºC), but the ideal range is from 27 to 30ºC (Toapanta et al. 2005).

Other reports that included information on generation time, oviposition, fecundity, fertility, and host plant associations have advanced our understanding of the biology of this species (Genung and Ozaki 1972, Burke and Woodruff 1980, Wilson 1986, Gordon and Armstrong 1990, Patrock and Schuster 1992, Arcos et al. 1998). In addition, a life table study by Toapanta et al. (2005) provided additional demographic parameters such as net reproductive rate, intrinsic rate of increase, and finite rate of increase at different temperatures. With this information the biology of the pepper weevil is reasonably well known (Table 1), but is not complete. Various factors that were not included in fecundity experiments could affect fitness of this species, including type and quality of food. It is well known that the boll weevil needs to feed on floral buds, because of their high nitrogen content, for ovarian development and oviposition (Hunter and Hinds 1905, Isley 1928). Other studies demonstrated that fecundity was optimal in boll weevil fed with a diet containing high nitrogen content (10% cottonseed flour as the amino acid nitrogen source) but drastic nitrogen reduction (2.5% cottonseed flour) reduced egg production (Hilliard 1983). Oogenesis and oviposition in pepper weevil proceeds only on peppers (almost any reference in Table 1). However, adults prefer to feed on floral buds instead of fruits (Patrock and Schuster 1992), so the importance of this type of food and its effect on fecundity should be considered for future studies.

Table 1. Biological data of pepper weevil, selected references until 2005

Parameter (average in days)	Conditions	Reference
Generation time		
25		Walker 1905
20.9	Summer	Elmore et al. 1934
32.1	Fall	
17-18	25.7-27.7ºC; 70% RH; on an artificial diet	Toba et al. 1969
17.5	23-27ºC	Genung & Ozaki 1972
14.2	25.7-27.7ºC; 40-100% RH	Wilson 1986
22.7, 13.9, 12.9	21.0, 27.0, and 30ºC, 60% RH; 14:10 (L:D), respectively	Toapanta et al. 2005
Longevity of adults		
78.7	Laboratory reared	Elmore et al. 1934
90	Insectary	Goff & Wilson 1937
31.7	22-27ºC; 60-70% RH	Gordon & Armstrong 1990
Oviposition period		
72	Laboratory reared	Elmore et al. 1934
30	Insectary	Goff & Wilson 1937
76, 50, 52	21.0, 27.0, and 30ºC, 60% RH; 14:10 (L:D), respectively	Toapanta et al. 2005
Oviposition rate (eggs/ female/ day)		
4.7	Laboratory reared	Elmore et al. 1934
6.6	Insectary	Goff & Wilson 1937
6.0	23-27 ºC	Genung & Ozaki 1972
7.1	25.7-27.7ºC; 40-100% RH	Wilson 1986
8	22-27 ºC; 60-70% RH	Gordon & Armstrong 1990
1.9, 1.7, 3.1	21.0, 27.0, and 30ºC, 60% RH; 14:10 (L:D), respectively	Toapanta et al. 2005
Fecundity (eggs/female)		
341		Elmore et al. 1934
198	Insectary	Goff & Wilson 1937
253	22-27ºC; 60-70% RH	Gordon & Armstrong 1990
144, 85, 161	21.0, 27.0, and 30ºC, 60% RH; 14:10 (L:D), respectively	Toapanta et al. 2005
Population values		
[a] R_o = 25.15, 11.76, 33.57	21.0, 27.0, and 30ºC, 60% RH; 14:10 (L:D), respectively	Toapanta et al. 2005
[b] r_m = 0.06, 0.07, 0.11		
[c] T = 52.51, 35.79, 32.39		

[a] Net reproductive rate (R_o); [b] Intrinsic rate of increase (r_m); [c] Generation time (T)

Host Plant Association

Capsicum spp.

Host plants utilized by pepper weevil for reproduction are confined to the genera *Capsicum* and *Solanum*, both in the family Solanaceae. All of the five species of pepper

grown as crops (*C. annuum*, *C. frutescens, C. chinense*, *C. pubescens or C. baccatum)* are suitable for oviposition and development of the pepper weevil (Elmore et al. 1934, Wilson 1986, Patrock and Schuster 1992). The majority of studies describing feeding, oviposition, survival and reproduction have been developed in these hosts.

Pepper weevil adults feed on floral buds, flowers, fruits, and sometimes leaves of pepper plants; they lay eggs inside floral buds and young pepper pods. For feeding, females and males bore a small hole with the mandibles, which are located at the end of the rostrum, to reach internal tissue (Walker 1905, Elmore et al. 1934).

For oviposition, females spend a short time in selecting a site on the fruits, after which they bore a small hole with the mandibles, turn completely around and deposit an individual egg into the hole. Once the egg is in the cavity, the female deposits a yellowish or brownish substance that turns black on drying and seals the hole (=oviposition plug) (Elmore et al. 1934, Goff and Wilson 1937, Genung and Ozaki 1972). After hatching, the larva bores into the interior of the fruit until reaching the placenta and seeds. Once development is completed, the insect pupates inside the fruit (Pratt 1907, Elmore et al. 1934, Goff and Wilson 1937, Burke and Woodruff 1980, Riley and Sparks 1995). The particular feeding and reproduction behavior of this insect make it inaccessible to many natural enemies, and also to most insecticides (Pratt 1907, Elmore et al. 1934, Elmore and Campbell 1954).

The size, quality, and availability of buds, flowers, and fruits could be factors that affect the selection of oviposition sites for pepper weevil (Walker 1905, Elmore et al. 1934, Riley and King 1994). More eggs were laid in fruits than in flowers (Patrock and Schuster 1992). For feeding and oviposition, adults prefer small fresh fruits rather than

mature fruits (Walker 1905, Wilson 1986). According to Bruton et al. (1989), the pepper weevil preferred developing bell pepper fruits from 1.3 to 5.0 cm in diameter rather than smaller (less than 1.3 cm in diameter) or larger mature fruits (bigger than 5 cm in diameter) for oviposition.

***Solanum* spp.**

The suitability for feeding, oviposition and development of the pepper weevil has been confirmed for at least 10 species of the genus *Solanum* (Table 2). Six additional species of the family Solanaceae served as food, but were not suitable for oviposition in non-choice tests (Wilson 1986, Patrock and Schuster 1992). Nevertheless, laboratory results do not necessary apply to the field. For example, the pepper weevil was able to develop in eggplant (*S. melongena*) in the laboratory, but rarely is observed to attack this crop in the field.

The developmental time of pepper weevil reared on bell peppers was not different from those reared in American black nightshade (*S. americanum*) or eastern black nightshade (*S. ptycanthum*), but the dry weight of adults from pepper was greater than those from the other hosts (Patrock and Schuster 1992). A hypothesis of restriction of resources, where bigger fruits could offer more food for the developing larva, could help to explain why pepper weevil females prefer fruits instead of flowers for oviposition. The same hypothesis could explain the greater dry weight of pepper weevil adults reared from bell pepper fruit than those from nightshade fruit (Patrock and Schuster 1992). The qualitative differences in suitability among hosts of pepper weevil have not been evaluated.

Table 2. Non *Capsicum* Solanaceae used by pepper weevil*

Plant species	Feeding	Oviposition	Development
Solanum americanum Mill.	L, F, Fr[1]	Fr	S[4]
S. carolinense L.	L, F, Fr	F, Fr	S
S. dimidiatum Fav.	L, F, Fr	F, Fr	S
S. eleganifolium Cav.	L, F, Fr	F, Fr	S
S. melongena L.	L, F, Fr	F, Fr	S
S. pseudocapsicum L.	L, F, Fr	Fr	S
S. pseudogracile Heiser	L, F, Fr	Fr	S
S. ptycanthum Dun	L, F, Fr	Fr	S
S. rostratum Dunal	L, F	F	S
S. triquetrum Cav.	L, F[2]	F[2]	S
S. tuberosum L.	L, F[2]	N[3]	
Datura stramonium L.	L, F	N	
Lycopersicon esculentum Mill.	L, F, Fr	N	
Nicotiana alata Link and Otto	L, F	N	
Petunia parviflora Vilm	F	N	
Physalis pubescens L.	L, F, Fr	N	

*Source: Wilson (1986), Patrock and Schuster (1992)
[1]L= Leaves, F = Flowers, Fr = Fruits. [2]Fruit unavailable. [3]No oviposition observed on flowers or fruits.
[4]Successful development.

Management Strategies

Host plant resistance

Host plant resistance is an important component of IPM (Painter 1951, NAS 1969). Berdegue et al. (1994) indicated that some types of pepper exhibited less damage from pepper weevil because of an escape mechanism –concentrated production before the presence of the pest – instead of antibiosis. Quiñones and Lujan (2002) indicated that some 'Jalapeño' lines might have some tolerance mechanism against damage from this pest. Seal and Bondari (1999) indicated that only two commercial varieties of peppers, one of them Habanero, had lower rates of infestation by pepper weevil than the remaining

nine varieties tested in field evaluations. Unfortunately, there is not a single cultivated variety known to provide any important resistance characteristic against this pest.

Cultural and chemical control

Current management practices against pepper weevil consist of a combination of cultural and chemical control. According to Riley and King (1994), control practices against pepper weevil focused on cultural control during almost the first 40 years following introduction into the United States. The lack of other tactics was explained by the difficulty of reaching the insect inside the fruit (Walker 1905). The inorganic insecticides, available during those years, especially calcium arsenate, could not be used because of human toxicity (Elmore et al. 1934, Elmore and Campbell 1954) and were observed to provoke secondary pest problems in the crop (Folsom 1927, Elmore et al. 1934). Insecticides have improved in selectivity for other pests, but they have not improved in efficacy against pre-imaginal stages of the pepper weevil.

Recommendations for avoiding damage included establishing a pepper free period —some months if possible— to reduce populations by food deprivation. Other recommended practices included 1) using weevil-free seedlings to establish new crops, 2) removing alternative hosts inside and around the fields, 3) collecting and destroying fallen fruits to reduce reinfestation, and 4) destroying crop residues immediately after harvest (Cano and Alcacio 1894, Walker 1905, Pratt 1907, Elmore at al. 1934, Goff and Wilson 1937, Watson and Lobdell 1939).

From the mid 1940's to 1980's, chemical control gained favor due to the availability of synthetic insecticides (Elmore and Campbell 1954, Riley and King 1994). Increasing knowledge of dispersal and sampling techniques (Andrews et al. 1986, Riley et al. 1992a b, Riley and Schuster 1994) aided in managing this pest without calendar

spraying of pesticides (Riley et al. 1992a b, Riley and King 1994). When 5% of the terminals were damaged (Cartwright at al. 1990) or when one adult pepper weevil was present per 400 terminal buds (Riley and Schuster 1992 b), significant economic loss in highly productive crops resulted. These criteria could serve as action thresholds. Azadirachtin, bifenthrin, cyfluthrin, cryolite, esfenvalerate, oxamyl, permethrin, acetamiprid, and thiamethoxam are some of the insecticides labeled for combating the pepper weevil in Florida (Olson et al. 2005, P. Stansly, personal communication).

Biological control

Known natural enemies of the pepper weevil are summarized in Table 3. Although biological control is usually considered an integral part of the IPM of any pest, natural enemies of pepper weevil in Florida have not been shown to play an important role in suppression of this pest (Elmore and Campbell 1954, Wilson 1986, Schuster et al. 1999). For this reason, although efforts to introduce natural enemies against pepper weevil have been considered, no specific parasitoid had been introduced and released for this pest in the United States until the present study (Elmore et al. 1934, Elmore and Campbell 1954, Clausen 1978, Schuster et al. 1999).

Entomopathogenic nematodes (*Heterorhabditis* sp. and *Steinernema* sp.) have proved effective against certain insect pests that develop some part of their life cycle in the soil (Glaser 1931, Bell at al. 2000). Studies in Texas have shown that *S. riobravis* can kill boll weevil larvae inside abscised squares and bolls of cotton if applied to the soil under ideal conditions of moisture (Cabanillas 2003). In a similar way, entomophagous nematodes might attack the pepper weevil inside buds or pods that are on the ground, and could thus diminish pest populations; however, there is little information in regard to entomopathogenic fungi, as well as viruses or bacteria that might attack this pest. In a

recent work in Florida, a commercial formulation of *Beauveria bassiana* (Balsamo) was used against pepper weevil adults, but proved to be ineffective in the field (Schuster et al. 1999).

Three pteromalids, one braconid, two species of ants, and one mite were identified attacking the pepper weevil in the United States (Pratt 1907, Pierce et al. 1912, Elmore et al. 1934, Cross and Chesnut 1971). Nevertheless, their impact on the pest was not considered important (Elmore and Campbell 1954, Genung and Ozaki 1972). The cited natural enemies that are endemic to the United States would be unlikely to act effectively against an exotic pest.

Before 1950, two attempts of classical biological control of this pest were made in the United States. *Eupelmus cushmani* Crawford and *Catolaccus (Heterolaccus) hunteri* Crawford were collected from Guatemala and released in Hawaii during 1934-37, where they established, but did not provide encouraging results (Clausen 1978). The other attempt during 1942-1943 occurred in California with the release of *Triaspis vestiticida* Viereck, a parasitoid of *Anthonomus vestitus* Boheman, the Peruvian cotton boll weevil in Peru (Berry 1947, Clausen 1978). However, the parasitoid was never recovered from peppers (Clausen 1978). These three natural enemies were originally obtained for biological control programs against the boll weevil (*Anthonomus grandis* Boheman) and evaluated against pepper weevil in the hope that similarities between these two pests, might make possible the use of the same natural enemies (Clausen 1978). Nevertheless, there are no reports of *E. cushmani* or *T. vestiticida* ever parasitizing pepper weevil.

C. hunteri has the widest distribution of any parasitoid of boll weevil or pepper weevil in the United States, Mexico, and Central America (Pierce et al. 1912, Cross and

Mitchell 1969, Cross & Chesnut 1971, Mariscal et al. 1998, Schuster et al. 1999, Rodriguez et al. 2000). It is the most important parasitoid attacking pepper weevil in Florida (Wilson 1986, Schuster et al. 1999), and one of the most widely distributed in Mexico (Mariscal et al. 1998, Schuster et al. 1999, Aguilar and Servín 2000, Rodriguez et al. 2000). It is also present in Honduras (Riley and Schuster 1992) Costa Rica (Schuster et al. 1999), and it is the only one known from Puerto Rico (Schuster et al. 1999).

C. hunteri is a generalist that is known to attack at least 17 species of Curculionidae and 2 of Bruchidae (Cross and Mitchell 1969, Cross and Chesnut 1971); it usually develops on the last instar of its host (Wilson 1986, Cate et al. 1990, Rodriguez et al. 2000). The particular biology of this species was useful for mass rearing at a moderate scale on the cowpea weevil, *Callosobruchus maculatus* F. (Rodriguez et al. 2002, Vasquez et al. 2005). Those findings, and some biology studies that showed that *C. hunteri* has greater fecundity and intrinsic rate of increase than pepper weevil (Rodriguez et al. 2000, Seal et al. 2002), stimulated and supported the plan of evaluating *C. hunteri* as a biological control agent. Releases of 1050 *C. hunteri* per hectare in Sinaloa, Mexico, were not effective in combating pepper weevil on bell pepper (Corrales 2002). Nevertheless, in Florida results of weekly releases of the equivalent of 7900 *C. hunteri* per hectare suggested that this parasitoid has potential to reduce damage of pepper weevil on peppers (D. Schuster, personal communication). One of the most important disadvantages of using *C. hunteri* to combat pepper weevil is the fact that it attacks the 3rd instar host, which is usually inaccessible deep within the fruit of commercial pepper varieties.

Table 3. Natural enemies of the pepper weevil, references until 2005

Natural enemies	Reference
Predators	
Hymenoptera: Formicidae	
Solenopsis geminata Fab.	Pratt 1907, Hinds 1907
Tetramonium guinense Fab.	Wilson 1986
Passeriformes: Icteridae	
Sturnella magna (Easter meadowlark bird)	Genung & Ozaki 1972
Parasitoids	
Pteromalidae	
Zatropis incertus Ashmead (=*Catolaccus incertus* Ashmead)	Pierce 1907, Cross & Chesnut 1971
Catolaccus hunteri Crawford	Pierce et al. 1912, Cross & Chesnut 1971, Mariscal et al. 1998
Habrocytus piercei Crawford	Cross & Chesnut 1971
Braconidae	Pratt 1907, Pierce et al. 1912,
Bracon mellitor Say	Cross & Chesnut 1971
Triaspis eugenii Wharton & Lopez-Martinez	Mariscal et al. 1998, Wharton & Lopez-Martinez 2000, Toapanta 2001
Urosigalphus sp.	Mariscal et al. 1998, Toapanta
Bracon sp.	2001
Aliolus sp.	Mariscal et al. 1998
Eulophidae	
Euderus sp.	
Syempiesis sp.	
Eupelmidae	
Eupelmus sp.	Mariscal et al. 1998, Toapanta 2001
Eurytomidae	
Eurytoma sp.	Mariscal et al. 1998
Mites	
Acarina: Pyemotidae	
Pyemotes ventricosus Newport	Pierce et al. 1912, Cross & Chesnut 1971

Even though Mexico and Central America have been indicated as the center of origin and domestication of the most important cultivated species of peppers (Vavilov 1951, Pickersgill 1969, 1971, Pickersgill and Heiser 1971, Eshbaugh 1976, Loaiza-

Figueroa et al. 1989, Conciella et al. 1990), and the pepper weevil was first described as a pest of that crop in Mexico (Cano and Alcacio 1894), no significant surveys for natural enemies were undertaken until 1997 (Mariscal et al. 1998). A survey by these authors in the west central coastal state of Nayarit, Mexico, detected four species of Braconidae, one of Pteromalidae, two of Eulophidae, and one each from Eupelmidae and Eurytomidae (Table 3). Of these, the most abundant was a new species, later described as *Triaspis eugenii* Wharton and Lopez-Martinez (Hymenoptera: Braconidae) (Wharton and Lopez-Martinez 2000), which was reported to reach incidences of 18-40% parasitism under field conditions (Mariscal et al. 1998, Schuster et al. 1999, Toapanta 2001). This is a solitary parasitoid which was reported to parasitize the egg, and to complete its life cycle in less than three weeks with a life span of less than four (Mariscal et al. 1998, Toapanta 2001, Rodriguez et al. 2004).

The genus *Triaspis* Haliday (Hymenoptera: Braconidae) is placed in the tribe Brachistini of the subfamily Helconinae (Martin 1956, Sharkey 1997). This genus could be represented by 100 species in the New World, but just a few of them have been described from this region (Sharkey 1997). In general, biology and life history of the genus *Triaspis* is poorly known. Some species of Brachistini are egg-larval parasitoids of weevils, bruchids, and anthribids, and the last larval instar has an ectoparasitic phase (Clausen 1940, Berry 1947, Saw and Huddleston 1991, Sharkey 1997). In spite of the fact that the biology of the genus *Triaspis* is not completely known, some species of this genus have been used in biological control programs in North America.

T. thoracicus was imported to Canada and the United States to combat species of Bruchidae, especially the pea weevil, *Bruchus pisorum* L. Apparently this species failed

to establish because it has a preference for laying eggs in plant tissue instead of dry seeds (Martin 1956, Clausen 1978). Efforts to combat the cotton boll weevil in USA with *Triaspis vestiticida* Viereck, were previously mentioned. *T. vestiticida* is a solitary parasitoid which attacks the egg of the host (peruvian cotton boll weevil) and develops as an endoparasitoid in early instars. At the end of the last instar, the larva emerges from the host to feed as an ectoparasitoid, leaving only the host cephalic capsule (Berry 1947). Although these habits may prevail throughout the tribe, more detailed studies of *T. eugenii* are necessary to understand its habits and potential use.

Objectives

The high incidences of parasitism by *T. eugenii* under natural conditions in Nayarit, Mexico and the recent information that confirms that this species attacks the weevil egg make it a good candidate for biological control of pepper weevil. Attempts to use this species for biological control will require more specific information on host/parasitoid relationships. Therefore, the objectives of the present study were the following:

1. Clarify key aspects of the biology of the pepper weevil, including demographic parameters, to improve rearing methodologies
2. Evaluate oviposition behavior and population dynamics of *T. eugenii* to optimize rearing procedures and to assess control potential
3. Test the ability of *T. eugenii* to control pepper weevil

CHAPTER 2
REARING METHODOLOGY AND INFLUENCE OF FOOD QUALITY ON BIOLOGY OF PEPPER WEEVIL

Introduction

Adult pepper weevils, *Anthonomus eugenii* Cano, feed on floral buds, flowers, fruits, and even pepper leaves, but the larvae feed and develop completely inside floral buds and immature fruits (Cano and Alcacio 1894, Walker 1905, Pratt 1907, Elmore et al. 1934). When feeding in floral buds, weevil larvae damaged especially the anthers (Walker 1905, Elmore et al. 1934, Patrock and Schuster 1992). During the oviposition process on fruit, females spend a short time in selecting a site on the pod, usually close to the calyx. They then bore into the pod, turn completely, and deposit an individual egg in the hole. Once the egg is in the cavity, the female deposits a brownish substance that turns black on drying and seals the hole (oviposition plug) (Walker 1905, Elmore et al. 1934, Goff and Wilson 1937). The pepper weevil laid more eggs in pepper fruits than in flowers (Patrock and Schuster 1992), and adults preferred small and immature fruits to mature fruits for feeding and oviposition (Elmore et al. 1934, Wilson 1986, Bruton et al. 1989).

Although the pepper weevil pepper weevil has been considered the major pest of peppers for more than eight decades in Mexico and the United States (Cano and Alcacio 1894, Walker 1905, Pratt 1907, Elmore et al. 1934, Riley and King 1994), no artificial rearing system has been developed to facilitate biological studies. Consequently, most studies have depended on weevils emerging of infested peppers collected from the field

(Elmore et al. 1934, Wilson 1986, Patrock and Schuster 1992). An attempt to rear pepper weevil on artificial diet can be excluded from this generalization (Toba et al. 1969). These authors were able to rear 6 generations of weevils using diet. However, females would not lay eggs in the diet, so the processes of collection and manipulation of eggs are laborious and ineffective (Toapanta 2001).

Toapanta et al. (2005) described a methodology to produce weevils in peppers, but these authors did not define the quality of fruits offered. A similar methodology with a few variations is practiced at the Golf Coast Research and Education Center (GCREC), Wimauma, FL. Both methodologies consist basically of exposing peppers for 48-72 h to weevil adults in cages 50x40x40 cm. Fruits are subsequently removed and held in ventilated plastic containers until the emergence of weevils. Toapanta (2001) used mainly 'Serrano' peppers, whereas 'Jalapeño' peppers from the supermarket were used at GCREC. Toapanta (2001) also indicated that the largest number of weevils, 1.7 adults per fruit, was obtained using 5 weevils per fruit. At GCREC a ratio of 15 adults per fruit is used, although an evaluation of efficiency has not been conducted (D. Schuster, personal communication). These methodologies have been useful to maintain dozens of generations in the laboratory, but efficiency could be improved by considering factors such as competition within fruits, fruit decay, and effect of food quality. Therefore, modifying fruit quality and decreasing competition could improve the laboratory rearing of the weevil.

Even though there is a basic understanding of the biology of pepper weevil, there are no studies that refer to food quality and its effect on fitness of this species. For example, there are no reports that explain why weevils prefer feeding on floral buds

instead of fruits. This might be related to food quality (nitrogen concentration or protein content). That is suggested because the ovarian development and oviposition of the boll weevil, *Anthonomus grandis* Boheman, are biological processes that depend upon the presence of cotton squares, a high nitrogen content food (Hunter and Hinds 1905, Hunter and Pierce 1912, Isley 1928). The pepper weevil does not need floral buds for ovarian development as has been proved by authors who reared the weevil in the laboratory with only peppers available for feeding and oviposition (Toapanta et al. 2005, D. Schuster, personal communication). However, studies that include feeding on floral buds are necessary to understand nutritional factors in this species.

The biology of the pepper weevil has been studied by several authors (as summarized in Riley and King 1994), but most, including Toapanta et al. (2005), reported a fecundity of no more than 150 or 160 eggs per female. Only Elmore et al. (1934) reported a fecundity as high as 340 eggs per female. Possibly a better understanding of the effects of food quality and the availability of immature fruits and floral buds could allow a further realization of the reproductive potential of this species and thereby improve the rearing system. The objectives of this chapter were to determine the effects of crowding on oviposition and the effects of fruit quality on rearing of the pepper weevil, and to provide demographic parameters when immature fruits and floral buds are offered to adults.

Materials and Methods

Laboratory Weevil Colony

A colony of the pepper weevil was established in a laboratory at the Entomology and Nematology Department in Gainesville, UFL, with 200-250 insects that emerged

from infested fruits collected during the Spring of 2003 at the Southwest Florida Research and Educational Center (SWFREC), Immokalee, FL. Genetic enrichment was provided by addition of insects collected periodically from the same site, particularly during Spring 2004 and 2005. The insects were reared continuously on fresh peppers, 'Jalapeño M' (Harris Seeds Rochester, NY), and 'Jalapeño Mitla' (Otis S. Twilley Seed Co. Hodges SC), but all the experiments were conducted with 'Mitla' fruits.

The colony was moved in April 2004 to a rearing room at the SWFREC maintained at 27 $\pm$ 2°C , 60-70% RH, and 14:10 (L:D) h photoperiod, and the experiments with the pepper weevil were conducted at those conditions, unless otherwise indicated. Pepper fruits were exposed to unsexed adult weevils using a ratio of 10:1 insects per fruit. Plastic jars 3.8 L (14x14x25 cm Rubbermaid Home Products Wooster, OH), with four lateral holes 3 cm in diameter covered with polyethylene screen, were used as oviposition cages. The cages had a knit sleeve (Kimberly-Clark Co. Roswell, GA) 50 cm long taped to the opening of the jar to prevent escape of insects during manipulations. Each cage held ca. 100 unsexed weevil adults. Water was provided in a cotton wick placed in 28-mL plastic cups, and lines of honey were dispensed every day on the upper side of the cage. Every 24 h, 10 small fresh 'Jalapeños' (ca. 5 cm long) were placed in the cages with the weevils, and 10 fruits placed 24 h previously were removed. The fruits were then held in plastic containers, 60-70 fruits each, until the emergence of adults. These containers (33x21x11 cm Rubbermaid Home Products Wooster, OH) had six lateral holes 3 cm in diameter covered with polyethylene screen to ensure ventilation. Weevils that emerged within the same week were collected and confined in a new oviposition cage. Each cage

was used for four or five weeks, because fecundity was assumed to decline thereafter and because many weevils died during daily manipulations.

Crowding Effects on Oviposition

Production of eggs and presence or absence of oviposition plugs was evaluated for different numbers of mated females alone and for different numbers of males and females together. Weevils were held in clear plastic cups 250 mL, each provided with a single fresh 'Jalapeño' pepper (4.95 $\pm$ 0.61 cm long, 1.86 $\pm$ 0.24 cm wide, and 8.57 $\pm$ 2.02 g weight, n=49). Individual peppers were vertically oriented using adhesive poster putty (Henkel Consumer Adhesives, Inc. OH) on the tip of the fruits. Water was provided by daily saturating a cotton wick placed in the bottom of each cup (Fig. 1).

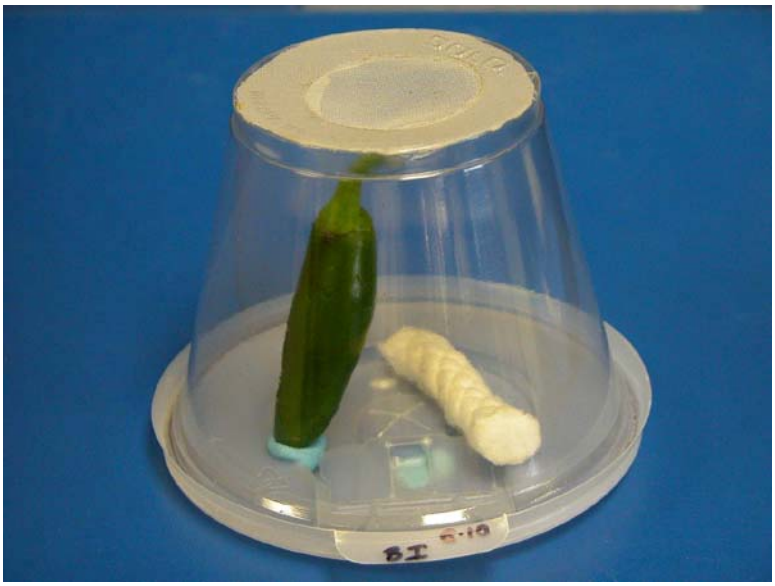

Figure 1. Device for exposing different densities of weevil adults to 'Jalapeño' peppers.

The treatments were 1 female, 1 female/ 1 male, 2 females, 2 females/ 2 males, 5 females, 5 females/ 5 males, and 10 females. The large metatibial mucrones of males were used to sex weevils (Eller 1995). To facilitate sexing, groups of 10-12 weevil adults were placed in Petri dishes over a white background and were observed through a dissection microscope at 16X magnification. An average of 22 females and 16 males

could be sexed in 10 min using this method (n=4). These seven treatments were arranged in a randomized complete block design with three replications. Because it is assumed that fecundity of pepper weevil is higher in the first weeks of its life (Patrock and Schuster 1992) and because of the availability of insects, each block was set up at different time using insects 7-10 days old. 'Jalapeño' fruits were replaced daily for 7 d and the number of eggs per day, and number of eggs with or without an oviposition plug, was evaluated.

To compare the number of eggs per female per day, data were analyzed using an analysis of variance (PROC ANOVA, SAS Institute 2000) and means were compared by Least Significant Difference (LSD) ($P \leq 0.05$). Data were normalized prior to the analysis using the square root transformation (PROC CAPABILITY, SAS Institute 2000), but data are presented in the original scale. The presence or absence of oviposition plugs by treatment was analyzed using a chi-square test ($df = 1$, $P \leq 0.05$) to determine differences between observed and expected (1:1) values (PROC FREQ, SAS Institute 2000). When one of the cells had expected counts less than 5, the Fisher's exact test to contrast the null hypothesis was used (PROC FREQ, SAS Institute 2000).

Fruit Quality

The number of adult weevil progeny obtainable using the ratio of 10 unsexed weevils per fruit was compared for two fruit qualities: immature green and green mature (marketable fruits). Prior to evaluation oviposition cages (3.8 L) containing 100 ± 10 unsexed weevils 1-5 d old were provided with 10 immature fruits and 5 or 10, depending on availability, floral buds. The fruits and buds were replaced daily for 3 days. After that, one cage was assigned to one of two treatments: 10 immature green fruits (4.75 ± 0.61 cm long, 1.77 ± 0.25 cm wide, and 7.66 ± 2.01 g weight, n=37), harvested from potted plants grown outdoors, or 10 mature green 'Jalapeño' fruits (6.67 ± 0.77 cm long, $2.06 \pm$

0.41 cm wide, and 14.96 ± 3.58 g weight, n=37), bought at the supermarket. Both qualities of fruit were washed with liquid dish detergent and rinsed up with tap water before offering to the weevils. Fruits were replaced every 24 h (5:00 PM to 5:00 PM) and exposed fruits were held until adult emergence in the ventilated plastic containers used in colony maintenance. Each cage of weevils was considered an experimental unit and was evaluated for 22 days. Because of the availability of peppers three replicates were conduced at different times during July and September of 2003. After checking the data for normality (PROC CAPABILITY, SAS Institute 2000), statistical differences were determined using the student's t-distribution (PROC TTEST, SAS Institute 2000).

Adult Feeding and Oviposition

One pepper weevil female and one male both 18 h old or younger were placed in individual 250 mL plastic cups. A lid closed each cup and a 3 cm diameter organdy-sealed hole provided ventilation (Fig 1). Each weevil pair was considered an experimental unit and assigned to one of two treatments: a) peppers only or b) peppers plus floral buds. Every day during the first 7 days of life, each weevil pair was provided a cotton wick saturated in water, as well as either a single young pepper (length 3.23 ± 0.40, width 1.07 ± 0.15, n = 15) or a single young pepper plus three floral buds before anthesis. The peppers were vertically oriented in the cups, as indicated above, and floral buds were placed in the bottom of the cups. Each treatment had 8 replicates. Because there were not enough floral buds for doing all replicates at the same time, the replicates were performed in groups at different times (3, 3, and 2). After 24 h of exposure, food items were replaced and observed under a stereoscopic microscope to evaluate number of feeding and/or oviposition punctures. Punctures were dissected to determine the presence or absence of eggs. Before the analysis, the data were normalized by the arcsine square

root transformation (PROC CAPABILITY, SAS Institute 2000), but means are presented in the original scale. Statistical differences were determined using the student's *t*-test (PROC TTEST, SAS Institute 2000). Results from weevils that received both floral buds and fruits in the same cup were further analyzed to compare the preference of feeding and ovipositing in floral buds or fruits using student's t-test (PROC TTEST, SAS Institute 2000).

Life Table

Fourteen weevil pairs 18 h old or younger were caged individually in plastic cups as previously described. Males were removed after 7 d to avoid possible interference with oviposition behavior (Elmore et al. 1934). A male was subsequently introduced into each cage for 2 d every 4 wk for mating. The females, or pairs when the males were present, received one fresh 'Jalapeño' pepper daily (length 3.23 $\pm$ 0.40, width 1.07 $\pm$ 0.15, n = 25). The pepper was removed and dissected to count the number of eggs. Two floral buds were offered every day in addition to the fruits for the lifetime of the female, except during week 2-4 due to short supply. The procedures were followed until death of the females.

The sex ratio of the species was estimated from field and laboratory samples, and survivorship was evaluated from a sample of 102 peppers with only one weevil egg per fruit from the laboratory colony. In addition, fertility was estimated from a sample of 171 eggs laid in immature peppers from the same colony. Previous observations indicated that an oviposition plug ensured the presence of an egg in 91% of all cases (SE = 0.018, n = 216). Therefore, removing oviposition plugs to check for the presence of eggs was not necessary. A permanent marker was used to mark the oviposition plugs and the fruits were dissected 4 d later to look for larvae.

Using LIFETABLE.SAS (Maia et al. 2000), the whole data set was used to estimate net reproductive rate (Ro), defined as the number of females produced by one adult female during its mean life span; generation time (T), the period between the birth of parents and the birth of the offspring; doubling time (Dt), the time necessary to double the initial population; intrinsic rate of increase (r_m), the potential growth of a population under given conditions; and finite rate of increase (λ), the daily rate of increase of each cohort (Birch 1948, Maia et al. 2000, Southwood and Henderson 2000, Toapanta et al. 2005). Data reported by Elmore et al. (1934) and Toapanta et al. (2005) were subjected to the same analysis and compared to results obtained in the present study.

Nitrogen Content of Fruits and Buds

The nitrogen content of anthers, pericarp and a mix of placenta and seeds of three maturation stages of 'Jalapeños Mitla' was evaluated. These maturation stages were classified according to size and appearance as: a) immature green, b) mature green, c) mature red (Table 4). Four samples were collected from plants 2-4 months old which were grown in a greenhouse. During each sampling, 70-90 floral buds just prior dehiscence and 6-7 peppers of each maturation stage were collected. Anthers were removed with forceps, and fruits were dissected to separate pericarp from placenta and seed. Because of possible variation among harvests (June and July 2005), each sampling was considered as a block.

Table 4. Characteristics of 'Jalapeño Mitla' used for nitrogen content determinations

Pepper quality (n=10)	Length, cm	Width, cm	Wall thickness
Immature Green	4.24 $\pm$ 0.24	1.12 $\pm$ 0.14	0.18 $\pm$ 0.02
Mature Green[1]	6.76 $\pm$ 0.60	2.42 $\pm$ 0.24	0.39 $\pm$ 0.03
Mature Red	7.72 $\pm$ 0.67	2.57 $\pm$ 0.20	0.41 $\pm$ 0.03

[1] Similar to fresh market quality

The samples of each botanical structure were placed in individual paper bags, dried at 50º C for three days, and ground to a powder. Nitrogen content was determined by the soil laboratory at the SWFREC using the alternative dry Micro-Dumas combustion analysis for total nitrogen in solid phase samples. This method is based on transformation to the gas phase by extremely rapid and complete flash combustion of the sample material (Matejovic 1993, 1995, Anonymous 1997). There were four replications (samples) in a randomized block design. The data were normalized by square root transformation (Sokal and Rohlf 1969, Ott and Longnecker 2001) prior to ANOVA (PROC ANOVA, SAS Institute 2000); however, the means and standard deviations are presented in the original scale. Means were compared by the LSD test (LSD) ($P \leq 0.05$) when the F value was significant.

Results

Crowding Effects and Oviposition

The number of weevils per fruit had a significant influence on the numbers of eggs per female per day ($F = 12.47$; $df = 8,138$; $P \leq 0.0001$). The largest number of eggs per female per day was obtained with a single female per fruit followed by one female and one male (Table 5). There were no differences among the remaining treatments (LSD test $P \leq 0.05$). Increasing the number of weevils from 1 female to 2 females/2 males or even 5 females had no effect on the number of plugged eggs (Fisher's exact test $P = 0.266$ and 0.139, respectively). However, the percentage of unplugged eggs increased 5 or 6 fold at densities of either 5 females/5 males or 10 females (Table 5). There was no differences between these treatments ($X^2 = 2.61$, $P = 0.1064$).

Table 5. Numbers of eggs per female per day for different numbers of females and males per single 'Jalapeño' pepper fruit

Weevil density	Eggs/female/day (Mean ± SD)[1]	Unplugged eggs (%)
1 ♀	5.0 ± 2.43 a	5 (4.76)
1 ♀ 1 ♂	3.38 ± 1.99 b	2 (2.82)
2 ♀	2.05 ± 1.37 c	1 (1.16)
2 ♀ 2 ♂	1.97 ± 0.8 c	3 (3.61)
5 ♀	1.68 ± 0.54 c	4 (2.26)
5 ♀ 5 ♂	1.74 ± 0.82 c	25 (13.66)
10 ♀	1.57 ± 0.48 c	30 (9.10)

[1]Means with the same letter are not significantly different (LSD $P \leq 0.05$)

Fruit Quality

Immature green and green marketable fruits were both able to support the rearing of pepper weevil, although there were significant differences. Using the 10 weevils per fruit density 2.65 ± 1.16 weevil adults emerged per immature fruit, compared to only 1.11 ± 0.81 weevils per marketable fruit ($t = 8.81$, $df = 65$, $P \leq 0.0001$).

Feeding and Oviposition in Peppers versus Floral Buds (7d)

When floral buds and peppers were offered at the same time, females fed and laid eggs in both (Table 6). Nevertheless, weevils preferred feeding on floral buds instead of fruits ($t = 13.39$, $df = 55$, $P \leq 0.0001$). In contrast, females preferred to lay eggs in pepper fruits rather than floral buds ($t = 9.59$, $df = 55$, $P \leq 0.0001$).

Table 6. Feeding and oviposition of pepper weevil adults when given a choice of 'Jalapeño' pepper fruit or floral buds (7d)

Parameter	Pepper fruits	Floral buds	*t-test* ($P \leq 0.05$)
Feeding punctures per day	3.27 ± 4.99	18.75 ± 8.64	*
Eggs per day	12.73 ± 10.46	0.16 ± 0.49	*

Oviposition with or without Floral Buds (7d)

Differences in oviposition were observed between females that were provided with pepper fruits only, compared to those that were provided with both floral buds and fruits (Table 7). Females provided with fruits and buds laid many more eggs the first day of oviposition ($t = 2.81$, $df = 14$, $P = 0.0261$), and over the entire 7 d test period ($t = 5.08$, $df = 14$, $P = 0.0002$) than females provided only with fruits. Pepper weevil females laid only 1.25% of eggs in the floral buds when both floral buds and fruits were provided at the same time.

Table 7. Oviposition of pepper weevil females fed with 'Jalapeño' pepper fruits only or with pepper fruits plus floral buds

Parameter	Peppers	Pepper plus floral buds	*t-test* ($P \leq 0.05$)
Days to begin oviposition	2.87 ± 0.99	2.62 ± 0.52	ns
Eggs at first day of oviposition	3.50 ± 1.41	11.87 ± 8.30	*
Total number of eggs (7 d)	23.75 ± 11.16	89.12 ± 34.65	*

Life Table

The 14 females survived 28 to 103 d, with an average of 64.5 ± 25.8 d. During this time 8 of 14 females (57%) began oviposition on the second day, and 13 of 14 females were depositing eggs by the third day (Fig. 2). The remaining female took seven days to begin oviposition. Fecundity ranged from 0 to 32 eggs per female per day with an average of 5.51 ± 5.31 eggs per female per day, and declined to near zero by 86 d. The fertility of eggs deposited in immature fruits was 97.66% ($n = 171$, $SD = 0.15$) and the survivorship of all preimaginal stages was 89% ($n = 102$, $SD = 0.31$). These data and the sex ratio of the species, that was not significantly different from 1:1 either in the field or

in the laboratory (X^2 =0.95, P ≤ 0.05, X^2 =0.35, P ≤ 0.05, respectively), were used to estimate the demographic parameters of pepper weevil (Table 8).

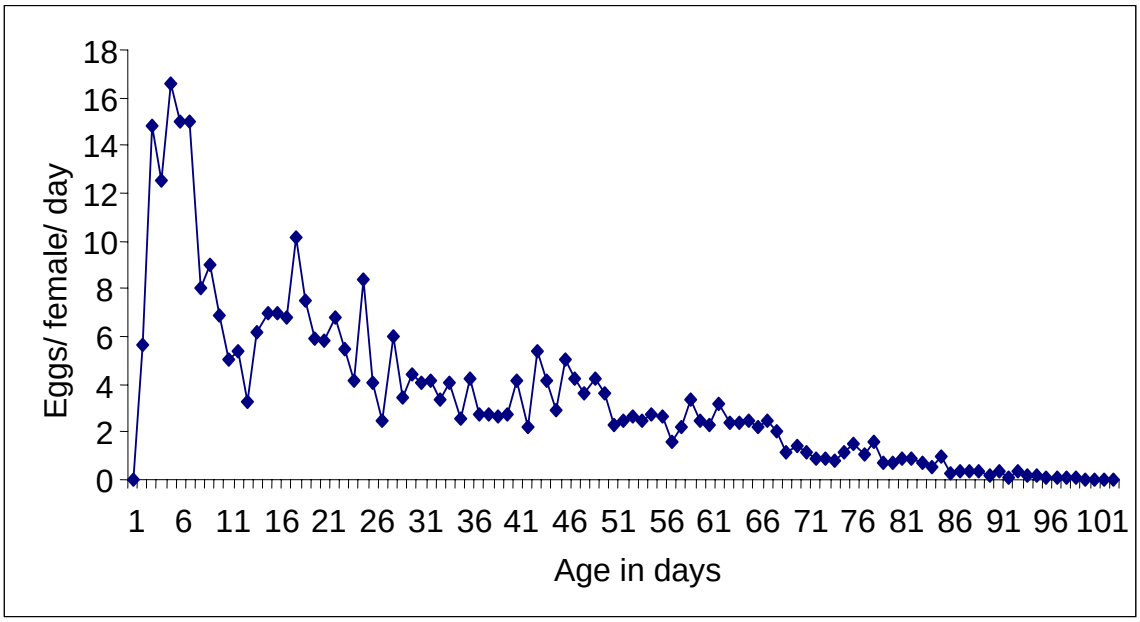

Figure 2. Mean fecundity of adult pepper weevil females (n = 14) reared on 'Jalapeño' peppers at 27 ± 2 ºC and 14:10 (L:D) photoperiod.

Table 8. Life table parameters for *A. eugenii* females (n =14) reared on 'Jalapeño' peppers at 27 ± 2°C, and 14:10 (L:D) photoperiod

Parameter	Value (± 95% confidence limits)
Net reproductive rate (Ro)[a]	158.1 (115.1 - 201.1)
Intrinsic rate of increase (r_m)[b]	0.14 (0.12 - 0.15)
Generation time (T)[c]	36.04 (31.26 - 41.22)
Doubling time (Dt)[c]	4.93 (4.42 - 5.49)
Finite rate of increase (λ)[d]	1.15 (1.13 - 1.17)

[a] Female/female, [b] $\log_e$ (Ro)/T, [c] Day, [d] Female/female/day.

Nitrogen Content of Fruits and Buds

Nitrogen content was different among botanical structures and maturation stages of peppers (F = 41.61; df = 6, 27; P ≤ 0.0001). Highest nitrogen content occurred in the anthers of floral buds. An intermediate concentration occurred in placentas and seeds at

any maturation stage, and in pericarp of the immature green fruits. The lowest nitrogen concentration was detected in pericarp of mature green and mature red fruits (Table 9).

Table 9. Nitrogen content (Mean ± SD) of anthers in floral buds and 'Jalapeño' fruits at three maturation stages

Maturation stage	Botanical structure	N content (%) dry weight
Floral buds	Anthers	5.23 ± 0.09 a[1]
Immature green	Placenta and seeds	3.39 ± 0.04 b
Mature red	Placenta and seeds	3.21 ± 0.25 bc
Immature green	Pericarp	3.19 ± 0.38 bc
Mature green	Placenta and seeds	2.96 ± 0.09 c
Mature green	Pericarp	2.25 ± 0.42 d
Mature red	Pericarp	2.01 ± 0.35 d

[1]Means followed by the same letter are not significantly different (LSD, $P \leq 0.05$)

Discussion

Crowding Effects and Oviposition

Increasing density of female pepper weevils per fruit reduced oviposition and increased the number of eggs lacking their normal oviposition plugs. An individual female produced more eggs than a female and a male together, and both treatments had higher number of eggs per female than two females together. The first comparison confirmed a previous study by Elmore et al. (1934) that suggested that the presence of males could inhibit oviposition behavior of females. However, the second comparison indicated that inhibition of oviposition also could occur with the presence of more females. Similar results with unsexed weevils were reported by Toapanta (2001), who indicated that a ratio of five unsexed weevils per fruit were more efficient for producing adults than larger ratios (10:1, 15:1, 20:1).

Crowding by either males or females interfered with the oviposition process. Males may interfere by seeking to mate with females, and females may interfere by contending with each other for oviposition resources. The final phase of oviposition is deposition of the plug, so the same factors might have influenced the presence of eggs lacking oviposition plugs.

Feeding could also contribute to the reduction of oviposition plugs by crowding. Pepper weevil prefers both to oviposit and to feed close to the calyx (Walker 1905, Elmore et al. 1934, Wilson 1986, Toapanta et al. 2005). More weevils per fruit would mean more feeding punctures and, consequently, more mechanical damage to tissue close to the calyx, including the plugs.

These results demonstrated why rearing the pepper weevil by using large numbers of weevils per fruit is inefficient. A small number of insects per fresh 'Jalapeño', even a single mated female, may be the most efficient combination. Additionally, this system maximizes the number of oviposition plugs which may prevent dehydration of eggs as well as exposure to opportunistic predators, such as mites or ants.

Fruit Quality

Quality of the host can exert an important influence on fecundity and survival of insects (Awmack and Leather 2002). Exposing pepper weevil females to immature green peppers resulted in more offspring than exposing females to marketable fruits (= mature green). The nitrogen content in the pericarp of immature fruits was higher than in mature green fruits (Table 9). Partitioning of nitrogen by fruit age and tissue could affect the capacity of both larvae and adults to acquire nitrogen.

Eggs are laid generally in the pericarp of peppers (Walker 1905, Elmore et al. 1934, Goff and Wilson 1937) due to the small size of the pepper weevil proboscis (Clark and

Burke 1996). Eggs laid in older fruits will produce larvae that have to bore through a thicker pericarp, which is lower in nitrogen compared to an immature fruit (Tables 4, 9). Additionally, the maturation process in peppers includes substituting soft tissues with fiber, the clearest example being the seed coat on mature fruits (Bosland and Votava 1999). The high nitrogen content in mature seeds may not be accessible to the larva. Thus, thickening of the pericarp and redistribution of nitrogen to seeds during maturation of pepper fruits could explain why the pepper weevil prefers to feed and to oviposit on immature fruits, and why the rearing process of weevils could be improved if immature fruits are used.

Adult Feeding and Oviposition

Early observations of pepper weevil adults in the field indicated a feeding preference for floral buds compared to fruits (Walker 1905, Elmore et al. 1934). These observations were later corroborated with laboratory tests (Wilson 1986, Patrock and Schuster 1992). The data presented here corroborate this behavior in 'Jalapeño' floral buds and fruits, and demonstrate the likely cause. Many more feeding punctures were made per floral bud compared to 'Jalapeño' fruit. Patrock and Schuster (1992) reported a mean of 0.74 and 0.75 feeding punctures for floral bud and fruit, respectively, produced by a single female. This contrasted with 3.27 feeding punctures in fruits against 18.75 feeding punctures on floral buds using a pair of weevils in this study. Patrock and Schuster (1992) used 10-20 d old females in bell pepper fruits ('Early Calwonder') at 22 $\pm$ 4ºC, while 1-7 d old weevils in 'Jalapeño' fruits at 27 $\pm$ 2ºC were used in the present study. More information related to the quality of the varieties of peppers and the vigor of the insects used could help to better understand those differences.

Patrock and Schuster (1992) indicated that a female laid more eggs on immature fruits than floral buds (0.96 and 0.36 eggs, respectively), because fruits offered more resources to support larvae. Data collected here (0.16 eggs per floral bud and 12.73 eggs per fruit) followed the same trend, although the number of eggs laid per fruit was 10 times greater.

The seven day oviposition experiment provided information on the ability of floral buds to increase fecundity. The preoviposition period was not affected by access to floral buds, and a similar value (2 or 3 days) was reported by Elmore et al. (1934), Wilson (1986), Gordon and Armstrong (1990), and Toapanta et al. (2005). Nevertheless, the number of eggs at the first day of oviposition (3.5 vs 11.9) and the total number of eggs during the first seven days after emergence (23.7 vs 89.1) was always higher for females that received anthers. The total number of eggs deposited in the first seven days of life of females provided with immature 'Jalapeño' fruits and floral buds was greater than any number reported before for this species in previous works (Elmore et al. 1934, Wilson 1986, Gordon and Armstrong 1990, Patrock and Schuster 1992, Toapanta et al. 2005), probably because of feeding on anthers. An effect from feeding on floral buds (= cotton squares) has been reported for the boll weevil, which needs to feed on floral buds for the ovaries to develop (Hunter and Hinds, 1905, Hunter and Pierce 1912, Isley 1928, 1932).

The pepper weevil is able to obtain sufficient nutrients for egg production from pepper fruits alone, as the majority of references in Table 1 indicate, and it is common practice to rear the weevils on a strict diet of fruit. The present comparison was conducted for only 7 days, so it is not possible to say that total fecundity would be lower on a strict fruit diet. Possibly, the resulting nitrogen deficiency could be compensated by

increasing consumption, as has been observed for other species including *Celerio euphorbiae* L. (Lepidoptera: Sphingidae), and *Pieris rapae* L. (Lepidoptera: Pieridae) (House 1965, Slansky and Feeny 1977, Awmack and Leather 2002).

In addition to nitrogen content, a proper balance of amino acids is critical for growth and reproduction in insects (Linding 1984, Weis and Berenbaum 1989, Nation 2002). Therefore, the increased fecundity observed for the pepper weevil could also be due to the balance of amino acids that might be present in floral buds. Suitable diets for the boll weevil were developed only after developing a basic knowledge of the proteins and free amino acids in anthers of young cotton flowers (Earle et al. 1966, Hilliard 1983). Further studies that identify the proteins and amino acids of anthers of peppers could be useful in the development of a better artificial diet for pepper weevil adults.

Life Table

Pepper weevils laid a mean of 341 eggs per female with an average of 4.7 eggs per day over an average life span of 78.7 d (Elmore et al. 1934). No temperature was recorded in that study. Goff and Wilson (1937) reported a fecundity of 198 eggs with an average of 6 eggs per day. Gordon and Armstrong (1990) reported 253.6 eggs over a life span of 31.7 d, with an average of 8 eggs per day at 22 to 27ºC. Recently, Toapanta et al. (2005) reported a wide range of fecundity for this species according to the number of fruits offered to the weevils and temperature. Using eight females per group, these authors reported 3.1 eggs per day at 30°C and 158 eggs per female in single peppers over 51 days. Offered 3 peppers per day at 27°C, females were able to lay 3.9 eggs per day or 281 eggs over 72 d. The fecundity of pepper weevil females fed with floral buds observed in this study was 355 eggs per female, with an average of 5.5 eggs per day and a life span of 64.5 d.

During the adult stage of insects, the reproductive potential is fully realized only if the females have an adequate supply of energy for surviving and of nutrients for egg production (Hilliard 1983, Awmack and Leather 2002). The biology of the pepper weevil has been studied for more than a hundred years, but just three studies (Elmore et al. 1934, Gordon and Armstrong 1990, and the present) provided floral buds along with fruits to females when estimating fecundity. Not surprisingly, these studies reported the highest fecundity for this species. Fecundity is the major component determining population dynamics of a species and, along with survivorship, influences all demographic parameters (Birch 1948, Southwood and Henderson 2000). Consequently, the demographic parameters based on fecundity were higher for data reported by Elmore et al. (1934) and the present study (Table 10).

Table 10. Life table parameters for the pepper weevil

Parameter	Elmore et al. (1934)[1]	Toapanta et al. (2005) 30°C	This study 27 ± 2°C
Net reproductive rate (Ro)	153.45	33.57	158.10
Intrinsic rate of increase (r_m)	0.09	0.11	0.14
Generation time (T)	53.22	32.39	36.04
Doubling time (Dt)	7.33	6.35	4.93
Finite rate of increase (λ)	1.10	1.11	1.15

[1]The sex ratio and survivorship used to do the statistical analysis were 0.5 and 0.9, respectively. No temperature data provided.

The Ro value for pepper weevil females fed with immature 'Jalapeño Mitla' and floral buds was 4.6 times greater than the highest value reported by Toapanta et al. (2005). These authors fed the insects only with 'Serrano' or 'Jalapeño' peppers, but no floral buds. Furthermore, the condition or quality of fruits was not indicated. The

Generation time (T), and Doubling time (Dt) reported by Elmore et al. (1934) were larger than those observed in this study. This could be a consequence of distributing the eggs evenly over the entire oviposition period. It was not possible to know the true distribution of eggs over time to make a better estimation. Therefore, both parameters might be overestimated. If the generation time of this study is used with the approximation indicated by Birch (1948) to calculate the intrinsic rate of increase ($r_m = \log_e Ro / T$), the r_m for Elmore et al. (1934) data set is similar to this study ($r_m = 153.45/36.04 = 0.14$).

The difference in results in this study versus Elmore's data are due to generation time, with fecundity (as indicated by Ro) being the same. These values represented the potential of this species when feeding on floral buds and immature fruits, a situation that would occur in the field. It might explain in part the success of this species in infesting a young pepper crop, when floral buds and immature fruits are abundant. These demographic parameters could be useful for: a) comparisons of different rearing conditions, b) comparisons of population dynamics with natural enemies, and c) predictions of the potential of biological control agents to control this pest.

CHAPTER 3
LIFE HISTORY OF *Triaspis eugenii* Wharton and Lopez-Martinez (HYMENOPTERA: BRACONIDAE)

Introduction

The pepper weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae), is considered one of the key pests of peppers in the United States, Mexico, Central America, and some Caribbean Islands (Elmore et al. 1934, Riley and King 1994, Mariscal et al.1998, Schuster et al.1999). Adults lay eggs in floral buds and immature peppers, and larvae develop inside beyond the reach of insecticides. Consequently, chemical control targets only adults. An action threshold of a single adult weevil per 400 pepper terminals has been proposed (Riley et al. 1992 a,b). This low action threshold, and the use of insecticides as the principal method of control, can result in adverse effects including marketing restrictions, exposure of non-target organisms, environmental contamination, pesticide resistance, and secondary pest outbreaks (NAS 1969, Luckmann and Metcalf 1982, Doutt and Smith 1971). Unfortunately, no other viable control strategy for the pepper weevil has been developed during the one hundred years that have elapsed since the pest was first reported in Mexico (Cano and Alcacio 1894) and the United States (Walker 1905, Pratt 1907).

Biological control could be an alternative tactic to incorporate into integrated pest management (IPM) of the pepper weevil; however, sufficiently effective natural enemies have yet to be discovered. One of the natural enemies of pepper weevil which has received attention as a potential biological control agent is the generalist and

cosmopolitan parasitoid *Catolaccus hunteri* Crawford (Hymenoptera: Pteromalidae). This ectoparasitoid of the last instar of the pepper weevil has a greater fecundity than its host (Rodriguez et al. 2000, Seal et al. 2002). It has been recorded as one of the most common parasitoids of pepper weevil in some states of Mexico (Mariscal et al. 1998, Rodriguez et al. 2000), Central America, and Florida (Cross and Mitchell 1969, Wilson 1986, Riley and Schuster 1992, Schuster et al. 1999). However, releasing the equivalent of 1150 *C. hunteri* adults per hectare per week in field plots at Sinaloa, Mexico, was not effective against this pest (Corrales 2002). On the other hand, results of releasing 7900 *C. hunteri* per hectare, prior to and during the early pepper season, indicated that this parasitoid could reduce damage by the pepper weevil in Florida (D. Schuster, personal communication). Divergent results may be due, in part, to the fact that *C. hunteri* attacks the 3rd instar host that is inaccessible deep within the pepper fruit. Augmentative releases of this parasitoid prior to the crop cycle on the alternative host plant nightshade, which has small fruits, could reduce pepper weevil populations. Releases early in the crop cycle when fruits are small, or in small fruited varieties, might be effective because the host is more accessible to *C. hunteri*. Unfortunately, once the fruits increase in size the effect of the parasitoid is limited. In fact, Riley and Schuster (1992) indicated that *C. hunteri* was not detected in fallen fruits larger than 2.5 cm in diameter. Therefore, it is desirable to find species that may be effective as biological control agents of this pest, preferably those that attack earlier and more accessible life stages.

A candidate parasitoid was recently reported in Mexico, where pepper, pepper weevil, and parasitoids likely evolved. This species is considered the most important parasitoid of the pepper weevil in Nayarit, Mexico, where it causes the highest incidence

of parasitism on pepper weevil (from 18 to 40%) in field samples (Mariscal et al. 1998, Schuster et al. 1999, Toapanta 2001). This solitary endoparasitic wasp was described in 2000 as *Triaspis eugenii* Wharton & Lopez-Martinez (Hymenoptera: Braconidae). However, there is still little information available on its biology and behavior (Sharkey 1997, Mariscal et al. 1998, Toapanta 2001).

This Chapter focuses on the biology and life history of *T. eugenii*. Initial efforts to maintain the parasitoid colony were hampered by low levels of parasitism and high levels of superparasitism. These factors limited reproductive potential and, thus, reliable estimates of demographic parameters. In addition, inefficient rearing demanded excessive resources (peppers and hosts). Preliminary observations in the laboratory suggested two possible causes for low levels of parasitism: 1) a limited acceptable range of host-age for parasitizing, and 2) the inability of females to find or recognize the host. One hypothesis to explain this latter difficulty was that exposing pepper fruit to too many weevil adults in the colony resulted in fewer eggs sealed with an oviposition plug (Chapter 2). The lack of acceptable hosts could then result in either retention of eggs or in excessive superparasitism. These questions required a solution before demographic parameters could be reliably estimated and before the potential of the parasitoid as a biological control agent could be evaluated. The objectives of this study then were to determine the host lifestage suitable for parasitization by *T. eugenii*, to determine the parasitoid's ability to recognize and to parasitize plugged and unplugged host eggs, to describe the life cycle of the parasitoid, and to estimate demographic parameters for this species.

Materials and Methods

Surveys

Based on results of Mariscal et al. (1988) and Toapanta (2001), two surveys for *T. eugenii* were conducted in pepper growing regions at Nayarit, Mexico. In April and May of 2003, sampling was conducted in Puerta de Mangos, Cañada del Tabaco, Villa Juárez, Los Corchos, Los Medina, and Palma Grande, all located in Santiago Ixcuintla county, Nayarit, between 21º 36' and 22º 17' north latitude, and between 104º 53' and 105º 40' west longitude (INEGI 1999).

Hurricane Kenna made land fall in San Blas on October 25, 2002, just 30 km from Santiago Ixcuintla and many pepper fields were destroyed. Late replanting resulted in delayed harvest, with the result that fields included in the first survey in April 2003 were still being managed for weevils with insecticides. During the second survey in May 2003, the majority of peppers were collected from abandoned pepper crops. Pepper fruits with signs of damage of the weevil (including presence of feeding and/or oviposition punctures) were collected from the plants and/or from the ground. A random sample (n=57) of these fruits was dissected to confirm weevil presence. Around 25 kg of fruit or about 3,300 peppers were collected in each survey. All were *Capsicum annuum* L. The dominant variety was ‘Serrano’ but local cultivars such as ‘Serranillo’, ‘Caloro’, and a few ‘Jalapeño’ were also collected. Fruits were held in plastic nets of 2 kg in the field and later at room temperature to avoid water condensation. Within 3 d the plastic nets containing the peppers were placed in individual Ziploc® plastic bags (S.C. Johnson & Son, Inc. WI) provided with a paper towel to absorb moisture, and transported by air to the United States in two insulated coolers provided with ice substitute (Rubbermaid Inc.,

Wooster, OH). The material was allowed to enter in the United States under the permit 46799 of the Animal and Plant Health Inspection Service (APHIS), USDA.

Upon arrival in Miami, the material was brought immediately to the quarantine facilities at the Florida Department of Agriculture and Consumer Services (FDACS), Division of Plant Industry (DPI), in Gainesville, Florida. In the presence of the quarantine officer, the peppers were distributed into lidded plastic containers (30x23x10 cm). Each container had 4 lateral holes, 5 cm in diameter, covered with fine mesh screen for ventilation. A paper towel was placed at the bottom of each container to absorb excess moisture. These emergence containers were held in the maximum security room in quarantine at 27 $\pm$ 3°C , 50 to 70% RH, and 12:12 (L:D) h photoperiod. All the parasitoids except *T. eugenii* were preserved in 70% ethanol and labeled and identified with the aid of available literature. Identifications of braconids and chalcids were verified by R. Wharton and R. Lomelí, respectively, both of Texas A&M University, College Station. Voucher specimens were deposited at the Florida State Collection of Arthropods at DPI.

Parasitoid Rearing

Wasps were held in 3.8 L plastic cages (Chapter 2) with the lateral holes sealed with organdy. Parasitoids had free access to water in a cotton wick placed in 28-mL plastic cups, and to lines of honey dispensed every day on the upper side of the cage. In addition, a 7-8 cm long pepper flush (of leaves and floral buds) was inserted in a 28 mL plastic cup filled with water and placed in each cage to simulate natural host finding conditions. The flush had been exposed for 48 h to three pepper weevil adults.

Pepper weevil hosts were obtained from a colony held in Florida Reach-In Incubation chambers (Walker et al. 1993) maintained at 27 $\pm$ 1°C and 70-80% RH, and

14:10 (L:D) h photoperiod. Immature 'Jalapeño' fruits (4.7 ± 0.77 cm length, 1.56 ± 0.27 cm width, and 5.57 ± 1.07 g weight, n = 25) were exposed to weevils for 24 h at a ratio of 10 weevils per fruit. Under these conditions, pepper weevil eggs take 2.9 ± 0.1 d to hatch, and an additional 1.7 ± 0.1 d to reach the second instar (Toapanta et al. 2005). Accordingly, two age ranges of eggs (2-24 h and 26-48 h after weevil exposure) and first or early second instars (4 to 5 d after infestation), were offered to the wasps. Each cage contained one or two *T. eugenii* females. At least two pepper fruits of each age category were offered daily to the wasps beginning the second day after emergence. Following exposure, fruits were held in ventilated containers until emergence of insects.

When it became apparent that parasitoids were only recovered from peppers that had weevil eggs, the exposure period of fruits to weevils in the colony was limited to 24 h. Fruits were replaced between 4:00 to 6:00 PM, held at 22 ± 2°C until 10:00 AM the next day, exposed to parasitoids for 24 h, and then held as above.

After the second generation of parasitoids, 10-15 females and 10-12 males were included per cage to maintain a ratio of one parasitoid female per infested fruit per day. Each cage was used 10-12 d. Females emerging from one cage were used to mate with males emerging from a different cage to prevent inbreeding within the colony. Using this rearing system, 80-100 females and 100 males were held each generation until the colony could be moved from quarantine during October and November, 2003. The colony was then held at the Entomology and Nematology Department, University of Florida, Gainesville, at 27 ± 1°C and 70-80% RH, and 14:10 (L:D) h photoperiod for five months. The colony was moved in April 2004 to the South West Florida Research and Education Center (SWFREC) at Immokalee FL, and was maintained in a rearing room at 27 ± 2°C,

60-70% RH, and 14:10 (L:D) h photoperiod. Experiments were conducted under these environmental conditions, unless otherwise indicated. 'Jalapeño Mitla' was the variety used most in rearing the colony and in conducting experiments, but when this cultivar was not available, 'Jalapeño Ixtapa' was used.

Host-Age Range

Choice tests were conducted during December 2003 to compare the acceptance of eggs either 24-40 h or 48-64 h old. 'Jalapeño' fruits 5.25 $\pm$ 0.53 cm length, 1.68 $\pm$ 0.15 cm width, and 7.41 $\pm$ 0.70 g weight, (n = 15), were exposed the required time to 10 weevil females per fruit. Fruits with eggs were offered to the parasitoids for 8 h.

A no-choice test was conducted during June 2005 and included eggs either 3.5, 27.5, 51.5 or 75.5 $\pm$ 3.5 h old. 'Jalapeño' fruits (4.73 $\pm$ 0.68 cm length, 1.48 $\pm$ 0.26 cm width, and 5.26 $\pm$ 0.67 g weight, n = 10) were exposed seven hours to female weevils at a density of one weevil per fruit. Fruits with eggs of the first age class were used without storage while fruits in the other age classes were held at 22 $\pm$ 2°C and 50-60% RH for 24-72 h before being offered to *T. eugenii*. Infested fruits were exposed to parasitoids for 2 h. Three randomly selected weevil eggs were removed under a dissecting microscope from peppers in each age class to observe development. Heat units for each age, including the time exposed to the wasp, were proportionally estimated using the average temperature for each period. Heat units = (maximum temperature + minimum temperature/2) – threshold temperature. The threshold temperature was considered to be 11°C.

For the choice test, an experimental unit was considered a group of 10 infested peppers, five for each egg age class, offered at the same time to 20 *T. eugenii* females.

Five replicates were run. For the no-choice test, the experimental unit was five infested peppers of each egg age class, which were offered simultaneously to 10 *T. eugenii* females, there were three replicates. Because parasitoid females were used only once in each replicate in both assays and many parasitoids were needed, replicates were carried out in different days. Parasitoids were 5-10 d old and they were randomly taken from the colony in any replicate. Fruits were removed after exposure, and held in ventilated containers until the emergence of insects. Chi-square analysis (PROC FREQ, SAS Institute 2000) was used to test if parasitism was independent of age.

Role of the Oviposition Plug in Host Location

Choice and no-choice tests were conducted during May and June 2004 to determine the importance of the oviposition plug to *T. eugenii* for finding and parasitizing host eggs. Previous observations showed that the oviposition plug indicated the presence of an egg in 91% of all cases (Chapter 2). Host eggs for these tests were obtained from groups of three weevil females, 7-10 d old, placed in 250 mL plastic cups. Each group was provided with a 'Jalapeño' pepper (4.65 $\pm$ 0.48 cm length, 1.93 $\pm$ 0.29 cm width, and 7.40 $\pm$ 1.47 g weight, n=50) for 24 h. Subsequently, fruits were held at room temperature (22 $\pm$ 2°C and 60-70% RH), before exposure to the parasitoid the next day. Only pepper fruits with 10 eggs were used. Excess plugs and eggs (Fig. 3) were removed from the fruits using fine-tipped forceps.

Each experimental unit was considered as one or two pepper fruits, depending on the test, offered to an individual *T. eugenii* female 4-6 d old. Subject parasitoids were randomly selected from the colony and used only once. Each female was placed individually in 1.9 L plastic containers (12x11x15 cm) with two males to ensure mating.

Water and honey were provided as previously described. Infested fruits were exposed to the parasitoids for 24 h.

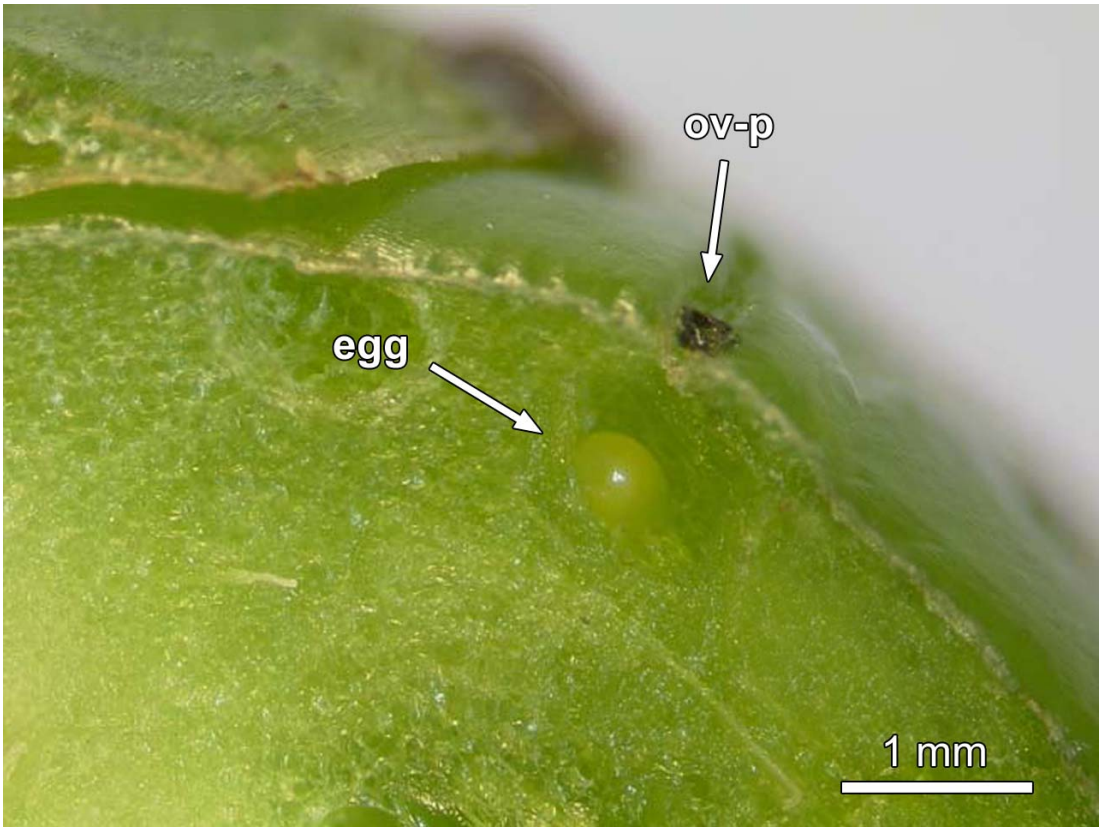

Figure 3. Longitudinal section of an infested 'Jalapeño' fruit showing a pepper weevil egg and oviposition plug (ov-p).

Choice tests were conducted by offering two pepper fruits (10 weevil eggs each) to individual *T. eugenii* females. The oviposition plugs were removed from half of the eggs of each pepper 24 h before the test. Plugs were removed under a stereoscopic microscope using forceps, and a cotton swab was used to clean any visible residue. No-choice tests were conducted by offering a single pepper fruit with ten weevil eggs, either with or without oviposition plugs. Weevil eggs were later removed, placed in a drop of tap water on a microscope slide (4 or 5 at once), and crushed under a glass cover slide. A binocular composed microscope was then used to observe parasitoid eggs (100X magnification). Fourteen replicates were run for each treatment, and data were analyzed using chi-square

tests to determine the differences between observed and expected values (PROC FREQ, SAS Institute 2000).

Effect of Weevil: Fruit Ratio on Parasitoid Production

Cages (3.8 L) containing 20-30 *T. eugenii* females and 20-25 males, 1-3 d old, were used as the experimental unit. The percentage of parasitism was compared between 'Jalapeño' fruits exposed 24 h to unsexed weevils (ratio 10:1 fruit), or exposed 24 h to mated females (ratio 1:1 fruit). Daily during 10 consecutive days 13 infested fruits (4.44 $\pm$ 0.72 cm length, 1.47 $\pm$ 0.23 cm width, and 4.83 $\pm$ 1.10 g weight, n = 30) of each pepper weevil ratio, exposed to weevil adults as above, were placed in the corresponding parasitoid cage. Fruits were exposed for 24 h to the parasitoids the first and the last day, but only 2.5 h the remaining 8 d. Fruits were removed and held in plastic containers until emergence of insects. Three replications were run for each treatment during September and October, 2004. Data were evaluated for normality (PROC CAPABILITY, SAS Institute 2000) before the analysis, and statistical differences were determined using student's t-distribution (PROC TTEST, SAS Institute 2000).

Life Cycle

'Jalapeño' pepper fruits containing pepper weevil eggs 18-40 h old were obtained by exposing the fruits 24 h to a density of 10 weevils per fruit (5.29 $\pm$ 0.80 cm length, 1.67 $\pm$ 0.24 cm width, and 5.90 $\pm$ 1.40 g weight, n = 10). An experimental unit consisted of eight infested fruits exposed 2 h to *T. eugenii* in cages each containing 15 females and 20 males 5-7 d old. Fruits were then removed and incubated in ventilated containers at 27 $\pm$ 1°C, 50-60% RH, and 14:10 (L:D) photoperiod. Eight experimental units were run

concurrently. In addition, 10 infested pepper fruits from the same batch, but not exposed to the parasitoids, served as control for the whole experiment.

To determine incubation time of *T. eugenii* 40 to 60 weevil eggs, usually one egg per fruit of each experimental unit, were removed and dissected from fruits at 18, 20, 22, and 24 h after parasitoid exposure. In addition, ten to 15 control eggs were dissected each time to confirm the absence of parasitoids. Length and greatest width of parasitoid eggs 18-20 h old (n=33) were measured using a binocular microscope fitted with an ocular micrometer calibrated with a stage micrometer (parallel lines at 10 μ).

The remaining pepper fruits (64 exposed and 10 unexposed) were held under conditions described above for 9 days after parasitoid exposure. Peppers were then dissected and all prepupae were removed, together with the completed or partially completed pupal cell, and held in individual polystyrene cells (Multiwell®, 24 well plates with lid, Becton Dickinson and Co.). Cells were filled with paper towel packed into the cells with no water to support the pupal cells, which were observed every 4 h during the first 3 d, and then twice a day (9:00 AM and 6:00 PM) afterwards until the emergence of adults. The Multiwell® cells with the host and parasitoids were maintained at 27 $\pm$ 1°C, 50-60% RH, and 14:10 (L:D) photoperiod, but the observations were made at room temperature (22°C).

Twenty unexposed and 160 exposed pepper weevil larvae were isolated as above, but only data from those parasitoids that were observed emerging from the host or spinning their cocoons and successfully reaching the adult stage are reported. Development times were calculated for the egg, endoparasitic and ectoparasitic larval stages, prepupa (spinning cocoon), and pupa. Stages were considered completed when

50% plus 1 individuals reached the next stage. The prepupal stage was considered to occur from the cessation of larval feeding through the completion of the cocoon. Means and standard deviations were calculated for each stage.

Fecundity

During September to December 2004, 10 adult *T. eugenii* females 18 h old or younger were placed individually in 3.8 L plastic containers. Three males were included in each cage to ensure mating. Each cage was provided daily with 'Jalapeño' fruits previously exposed for 24 h to pepper weevil females at the density of one pepper weevil per fruit (4.60 $\pm$ 0.45 cm length, 1.94 $\pm$ 0.30 cm width, and 7.37 $\pm$ 1.52 g weight, n = 46). Weevil adults were provided water and honey as previously described. Five of the *T. eugenii* females were each provided every day from 9:00 AM to 6:00 PM with 12 fresh peppers, each containing 4-8 pepper weevil eggs. The other five females were each provided with two peppers fruits replaced every 1.5 h during the same period, so that the total number exposed was also 12 fruits per female. Every day, half of the pepper weevil eggs of each fruit were removed and dissected to count the number of *T. eugenii* eggs per host using the methodology described above. The remaining eggs and fruits were held until the emergence of *T. eugenii* to estimate survival and sex ratio. The program LIFETABLE.SAS (Maia et al. 2000) was used to estimate net reproductive rate (Ro), generation time (T), doubling time (Dt), intrinsic rate of increase (r_m), and finite rate of increase (λ) (Chapter 3) (Birch 1948, Maia et al. 2000, Southwood and Henderson 2000). Frequency of superparasitism between treatments was compared using chi-square analysis (PROC FREQ, SAS Institute 2000).

Adult Longevity

T. eugenii adults 12 h old or less were caged in two 1.8 L containers as follows: 1) 10 females and 10 males provided with water only, and 2) 10 females and 10 males provided with honey and water. The wasps were observed twice a day (9:00 AM and 7:00 PM) and mortality was recorded until all died. The two treatments were replicated three times. A comparison of longevity was also made between these females and ovipositing females from the fecundity experiment. The Student's t test (PROC TTEST, SAS Institute 2000) was used to make all comparisons.

Results

Surveys

About 50 kg of pepper fruits infested by pepper weevil larvae were collected under natural conditions from Nayarit, Mexico. Weevil and parasitoid recovery varied with location (Table 11), although fruits from all sites were severely infested. Although a mean of 1.40 $\pm$ 0.97 weevil per fruit was observed in the field from a random sample of 57 fruits, only an average of 0.73 weevils per fruit emerged in quarantine. Two genera of Braconidae (including *Triaspis eugenii*) and one each of Pteromalidae, Eurytomidae and Eupelmidae were recovered, accounting for only 2.85% of total parasitism (Table 11). The dominant species recovered from both collecting trips was *C. hunteri*, which represented about 83% of emerged parasitoids.

Table 11. *A. eugenii* and parasitoids emerged from approximately 50 kg of infested hot peppers collected in the state of Nayarit, Mexico, April and May, 2003.

Location (sites visited)	*Anthonomus eugenii*	*Catolaccus hunteri*		*Triaspis eugenii*		*Eurytoma sp.*		*Eupelmus sp.*		*Bracon sp.*	
				April 2003							
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Cañada del Tabaco (3)	1260		1	1							
Los Corchos (2)	427	7	4	1	1						
Puerta de Mangos (1)	1484		1								
				May 2003							
Cañada del Tabaco (1)	151	11	1	1		1		2		1	
Los Corchos (5)	698	32	12	2	3			2			
Los Medina(3)	251	15	4				1	1			
Palma grande (1)	237	4	1			4	1	1	1		
Villa Juárez (1)	226	19	3								
Total	4734	88	27	5	4	5	2	6	1	1	0

Host-Age Range

Heat units were calculated for each age class to precisely determine which ones could be parasitized (Table12, 13). *T. eugenii* preferred younger eggs to older eggs in choice tests ($X^2 = 5.88$, df = 1, P= 0.015) (Table 12). Nevertheless, *T. eugenii* was equally able to parasitize eggs over four age classes when given no choice ($X^2 = 1.53$, df = 1, P = 0.674) (Table 13). The eyes and mandibles of pepper weevil larvae were visible through the chorion in the most mature eggs before wasp exposure.

Table 12. Parasitism of pepper weevil eggs by *T. eugenii* when given the choice of two ages of eggs

Host maturity		Parasitism		
Heat units[1]	Age (h)	Not parasitized	Parasitized	
			Number	Percentage[2]
12.66 - 28.66	24 – 48	51	64	55.6
23.66 – 39.66	48 – 72	98	68	40.9

[1]Accumulated heat units based on 11ºC threshold temperature
[2]$X^2 = 5.88$, df = 1, P = 0.015

Table 13. Parasitism of pepper weevil eggs by *T. eugenii* when given no choice of egg age

Host maturity		Parasitism		
Heat units[1]	Age (h)	Not parasitized	Parasitized	
			Number	Percentage[2]
0.10-4.66	0 - 7	6	13	68.4
12.45-15.66	24 – 31	10	29	74.3
23.45-26.66	48 – 55	16	26	61.9
34.45-37.66	72 – 79	16	30	65.2

[1]Accumulated heat units based on 11ºC threshold temperature
[2]$X^2 = 1.53$, df = 1, P = 0.674

Role of the Oviposition Plug in Host Location

Parasitism of pepper weevil eggs by *T. eugenii* was clearly affected by the presence or absence of the oviposition plugs (Table 14). Over two and a half more eggs were

parasitized when covered normally by an oviposition plug, compared to eggs over which the plugs were removed in choice tests.

The predominance of parasitism in plugged eggs was just as evident under no choice conditions (Table 15). Given no choice, *T. eugenii* parasitized three times more eggs covered normally by an oviposition plug, compared to unplugged eggs.

Table 14. Parasitism of pepper weevil eggs by *T. eugenii* when given the choice of eggs covered with an oviposition plug and eggs with no plug

Oviposition plug	Parasitism		
	Not parasitize	Parasitized	Percentage[1]
Normal	69	71	50.7
Removed	114	26	18.6

[1]$X^2 = 31.94$, df = 1, $P \leq 0.0001$

Table 15. Parasitism of pepper weevil eggs by *T. eugenii* when given no choice of eggs covered with an oviposition plug and eggs with no plug

Oviposition plug	Parasitism		
	Not parasitize	Parasitized	Percentage[1]
Normal	51	89	63.6
Removed	111	29	20.7

[1]$X^2 = 52.73$, df = 1, $P \leq 0.0001$

Effect of Weevil: Fruit Ratio on Parasitoid Production

When *T. eugenii* females were exposed to 'Jalapeño' pepper fruits infested with pepper weevil eggs by mated weevil females at a ratio of one weevil per fruit more parasitoids ($t= 5.26$, df = 58, $P \leq 0.001$) and fewer weevil adults ($t= 5.26$, df = 58, $P \leq 0.001$) emerged per fruit, compared to when *T. eugenii* were exposed to fruits infested by unsexed weevils at a ratio of 10 weevils per fruit (Table 16). Percentage of arasitism was similarly affected ($t= 4.40$, df = 58, $P \leq 0.0001$). Therefore, infesting pepper fruits with

one weevil female per fruit made more efficient use of weevils, weevil eggs, and fruits than infesting pepper fruits with 10 weevils per fruit.

Table 16. *T. eugenii* produced with two pepper weevil densities

Weevil adult density	Insects per fruit		Parasitism (%)
	T. eugenii	Weevils	
Unsexed weevils (10/ fruit)	0.81 ± 0.26	0.94 ± 0.35	45.5 ± 7.1
Mated weevil females (1/ fruit)	1.25 ± 0.48	0.52 ± 0.30	71.2 ± 12.3

Life Cycle

The eggs of *T. eugenii* were translucent and elongated with no evident surface sculpturing. They were 246 ± 33.6 μm long by 73.4 ± 16 μm wide. The length included a small pedicel of 31.2 ± 5.7 μm long (Fig 4A). The majority of the eggs hatched 23.0 ± 1 h after the 2 h exposure to *T. eugenii* (Table 17). Emergence was completed (41 larvae of 42 eggs) after 25 ± 1 h. After emergence and for a few more hours, first instar *T. eugenii* were translucent, except for the large sclerotized head and large mandibles. The segments of the body were nearly of equal size, except for the last one which was rounded and slightly larger than the rest (Fig 4B). Some of the larvae observed on the microscope slides appeared to be attacking unhatched parasitoid eggs within the host-egg.

The endoparasitic phase lasted almost 10 d and comprised more than half of the total parasitoid developmental time (Table 17). Parasitized pepper weevil larvae, even late 3rd instars, appeared to develop and to molt normally and had no evidence of decreased activity, compared to the observed control. The parasitoid larva emerged from the pepper weevil prepupa, usually after the completion of the pupal cell. The parasitoid larva emerged through a hole made in the distal end of the abdomen of the host. Bending the base of the head forward, it began to feed immediately on the weevil prepupa (Figure

5A). The last few abdominal segments of the parasitoid remained in the host at the beginning of the ectoparasitic phase (Figure 5B). In about 8 h the parasitoid completely consumed the host, except for the cephalic capsule (Table 17). The parasitoid then spun its cocoon and pupated inside the pupal cell of the host, where it remained for 6-7 d. Some parasitoid larvae removed from the pupal cell of the host and placed directly into the polystyrene cells made partial cocoons; however, they failed to complete the cocoon, and eventually died. Possibly, the lack of the pupal cell of the host caused dehydration. The developmental times of males and females did not differ (Table 17), but it is common to see in the laboratory that males emerge before females.

Table 17. Developmental times of *T. eugenii* in days (Mean ± SD) at 27 ± 1ºC

Sex	Egg	Parasitic development		Prepupa[1]	Pupa	Total
		Endo	Ecto	♀ or ♂	♀ n= 25	♀ n= 25
	n= 197	♀ n= 8	♀ n= 9	n=14	♂ n= 35	♂ n= 35
Female	0.96 ± 0.2	9.87 ± 0.30	0.27 ± 0.22	0.40 ± 0.17	6.63 ± 0.82	16.63 ± 0.88
Male				0.42 ± 0.18	6.40 ± 0.52	16.36 ± 0.88

[1]Spinning cocoon

Adults emerged by chewing through the cocoon then through the fruit wall. The emergence from infested peppers could be noticed as a small rounded hole. Adults were no longer than 2.4 mm and completely black. The only evident difference between sexes observed was the presence of the ovipositor in the female (Figure 6 C, D).

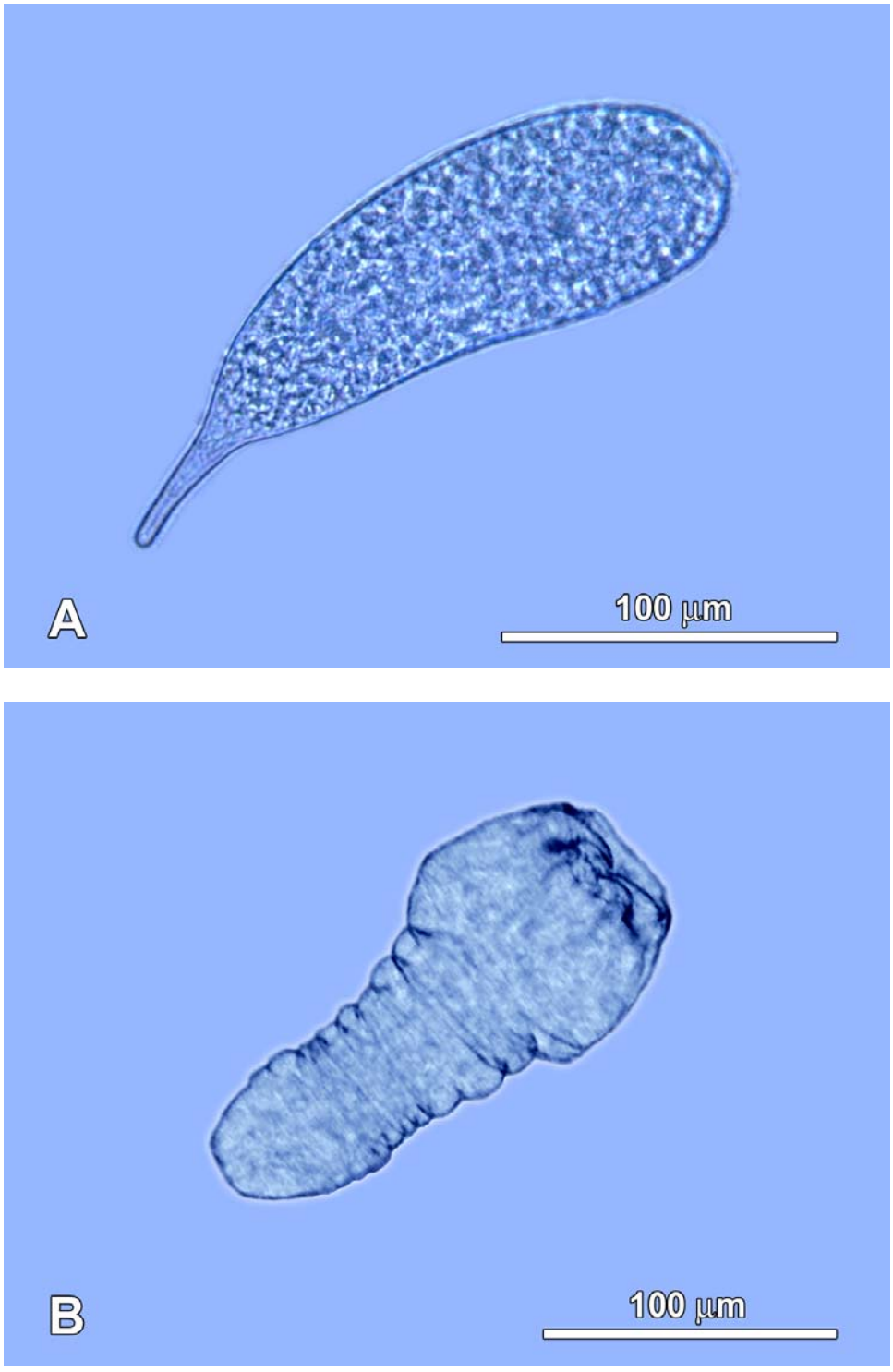

Figure 4. Immature stages of *T. eugenii*: (A) Egg 22 h after oviposition, (B) First instar, 30-33 h after oviposition, with well developed mandibles.

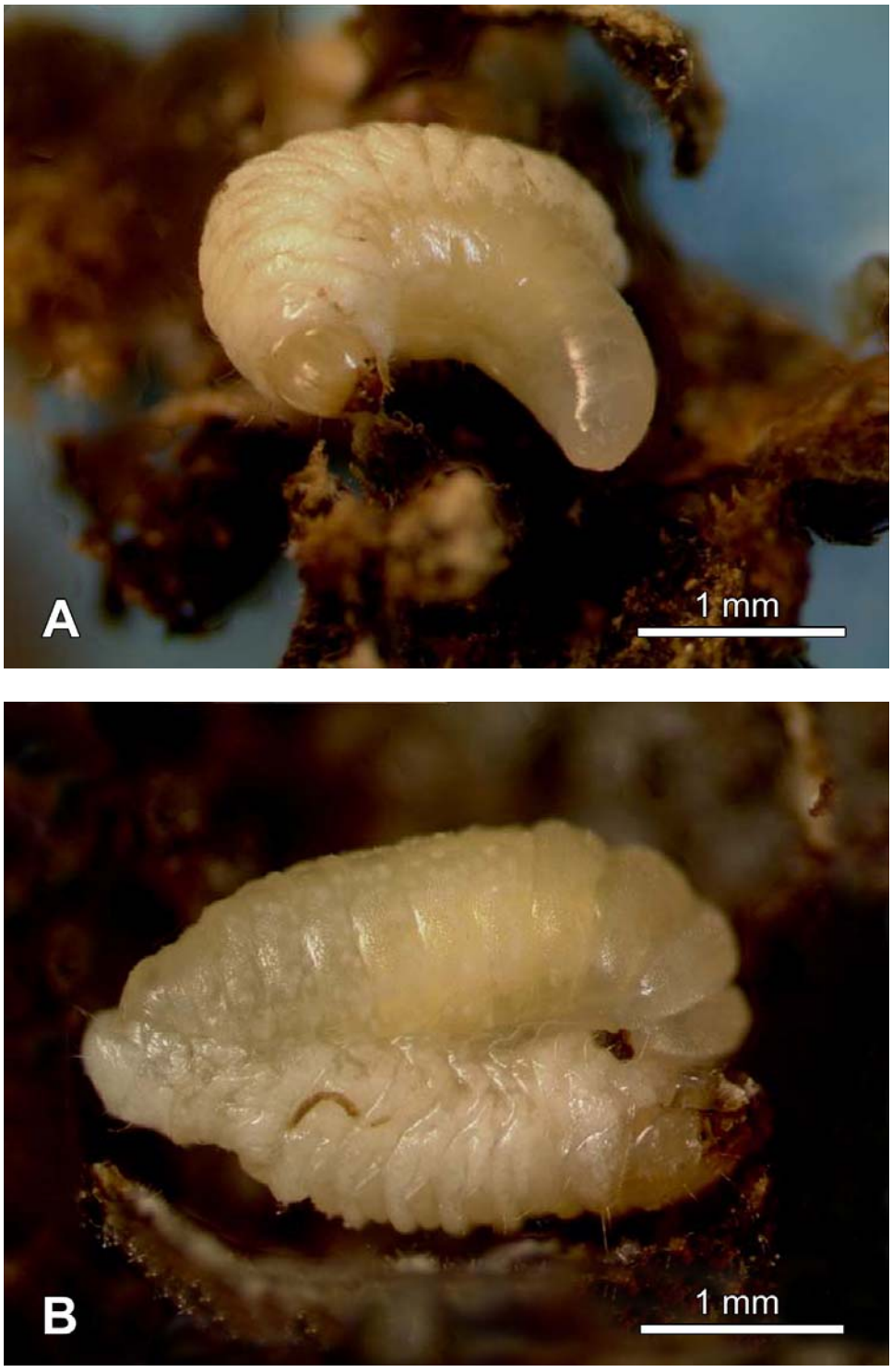

Figure 5. Larva of *T. eugenii*. (A) Emerging from the posterior end of the host, (B) Beginning to feed on the host even before emerging completely.

Figure 6. T. *eugenii* adults. (A) Female, (B) Male.

Fecundity

The preoviposition period was shorter than 24 h for six of ten females, between 24-48 h for three females, and between 48-72 h for one female. Little difference in the oviposition pattern was observed between host exposure treatments (Fig. 7). Maximum fecundity was reached on the 4^{th} day after emergence and then gradually declined, reaching zero by day 18. Females deposited 95% of their eggs by day 12 when hosts were changed every 1.5 h, and 92% when hosts were left for 9 h. During that period, females laid 29.63 $\pm$ 9.8 and 33.13 $\pm$ 12.2 eggs per day, respectively, for the 1.5 h and 9 h host exposure periods. All females died by day 21 (Figure 7).

When hosts were changed every 1.5 h, females produced an average of 372.40 ± 223.14 eggs, compared to 433.20 ± 193.80 when hosts were left for 9 h; however, the difference was not significant (t= 0.5, df = 164, P = 0.901).

Superparasitism was common with both treatments, although the proportion of superparasitized hosts was significantly greater when *T. eugenii* females were exposed to infested fruits for 9 h, compared to when females were exposed to infested fruits every 1.5 h (64.4% vs 55.3%, X^2= 7.95, df = 1, P = 0.0048). Superparasitism ranged from two to nine eggs, and averaged 2.24 $\pm$ 1.34 for the 9 h exposure period compared to 2.10 $\pm$ 1.40 for the 1.5 h exposure treatment. These values were not significantly different (t= 1.76, df = 924, P = 0.0779).

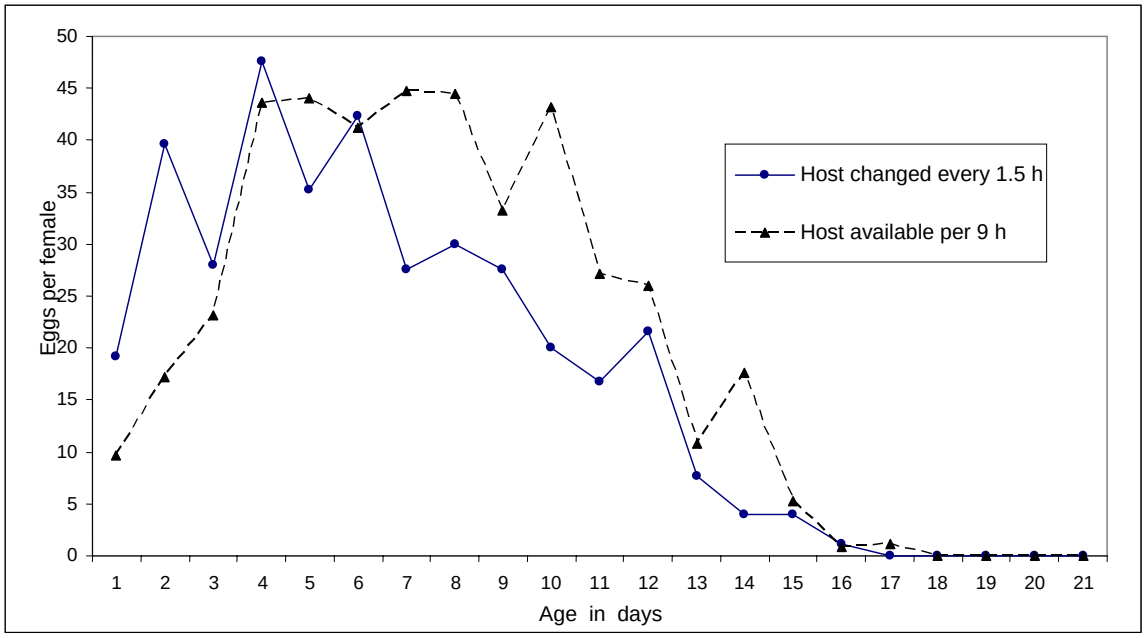

Figure 7. Fecundity of *T. eugenii* females at 27 ± 2ºC either exposed to hosts changed every 1.5 h (n=5) or exposed to hosts per 9 h (n=5).

The proportion of superparasitism and the numbers of emerged *T. eugenii* and pepper weevils suggested strongly that more than half of the host eggs were not dissected. Consequently, the proportion of superparasitism from the dissected host eggs, and the survivorship of pepper weevil (90%) estimated in Chapter 2, were used to estimate survivorship of the parasitoid. The estimate was made using the proportion of superparasitism observed from the dissected hosts each day. Demographic parameters for *T. eugenii* were estimated for the 9 h and 1.5 h treatments (Table 18). Values were not significantly different between the two host exposure intervals according to Student's t-test comparison, $P < 0.05$ (Maia et al. 2000).

Adult Longevity

Males and mated females with access only to water lived 2.3 ± 0.7 d and 2.3 ± 0.6 d, respectively, with no significant difference between the sexes (t=0.15, df = 58, P=0.881). Mated females provided with honey lived significantly longer (24.7 ± 10.6 d)

that did males (15.8 ± 9.2 d) (t=3.47, df = 58, P ≤ 0.001). Finally, females laying eggs and provided with honey lived 16.5 ± 3 d, which was significantly less than the 24.7 ± 10.6 d for females not laying eggs (t=3.26, df = 38, P ≤ 0.002).

Table 18. Demographic parameters and confidence limits (± 95%) for *T. eugenii* reared on pepper weevil eggs in 'Jalapeño' fruits when parasitoid females were offered hosts for different exposure intervals at 27 ± 2ºC

Parameter[1]	Host changed every 1.5 h	Host available for 9 h
Net reproductive rate (Ro)	102. 83	106.15
	(8.47 - 197.18)	(26.89 - 185.40)
Generation time in days	19.22	19.95
(G)	(18.13 - 20.31)	(18.90 - 21.00)
Intrinsic rate of increase	0.24	0.24
(r_m)	(0.20 – 0.29)	(0.20 – 0.28)
Doubling time (Dt)	2.82	2.92
	(2.24 – 3.40)	(2.37 - 3.47)
Finite rate of increase (λ)	1.28	1.27
	(1.22 - 1.34)	(1.21 – 1.32)
Sex ratio	1:1	1:1

[1] Difference between treatments was not significantly (Student's t-test for pairwise comparison, P ≤ 0.05, Maia et al. 2000).

Discussion

Surveys

The total number of parasitoids recovered from both trips was 139, most of which emerged from weevil infested fruits collected in May. *C. hunteri* and *T. eugenii* represented 83% and 6.5%, respectively, but parasitism reached only 2.85%. These

numbers were lower than those previously reported from that region: 865 and 1 210 parasitoids (recovered of 45 and 80 kg of infested fruit by pepper weevil, respectively) according to Mariscal et al. (1988) and Toapanta (2001). *T. eugenii* was the dominant species (84% and 88%, respectively), with levels of parasitism ranging from 29 to 40%, respectively.

The destruction by hurricane Kenna and subsequent reduced acreage of the growing pepper area in Nayarit during 2003, the possible damage to alternative hosts by the hurricane, and the intensified use of insecticides during late months in the season, are possible explanations for low levels of parasitism of the pepper weevil observed during the surveys in 2003 (2.85%) compared to previous reports (29-40%) (Mariscal et al. 1988, Toapanta 2001). Nevertheless, the 115 *C. hunteri* observed in the present study was not very different from 87 and 102 individuals observed in the previous studies. *C. hunteri* is a generalist ectoparasitoid of more than 17 species of Curculionidae and Bruchidae (Cross and Mitchell 1969, Cross and Chesnut 1971). It can survive as an adult for more than 40 days and, in addition to carbohydrates, can feed on host larvae or, sometimes, pupae to survive (Cate et al. 1990, Rodriguez et al. 2000). Thus, *C. hunteri* is well equipped to survive unstable environmental conditions. In contrast, the pepper weevil is the only known host of *T. eugenii*. It has not been observed to host feed and cannot survive as long as *C. hunteri*, especially without a carbohydrate source. Thus, *T. eugenii* would have fewer resources to draw upon under rapidly changing conditions.

Host-Age Range

During choice tests, *T. eugenii* preferred younger eggs to older eggs. Age may be related to host suitability (Godfray 1994). Because eggs may lack a cellular defensive response to foreign intrusion (Askew 1971) or because, with the exception of eggs in

diapause, eggs rapidly change from storage nutrients to chemically more complex embryonic tissue (Sander et al. 1985), parasitoids may prefer young eggs. These explanations have been supported by authors who found that parasitism by some egg parasitoids declines as the host ages (Strand and Vinson 1983a, b, Ruberson et al. 1987, Reznik and Umarova 1990, Castillo et al. 2005). Nevertheless, this information has been supported mainly in observations with parasitoids from Trichrogrammatidae, Scelionidae, Mymaridae, and Eulophidae. They are idiobionts that complete their development in the eggs, and they may have differences with parasitoids which develop as koinobionts, such as egg-larval or egg-pupal parasitoids (Strand 1989, Vinson 1998, Godfray 1994, Quicke 1997).

In contrast to many idiobiont egg parasitoids, egg-larval or egg-pupal parasitoids may lay eggs in a more advanced stage of host development (Clausen cited by Quicke 1997, Abe 2001, Quimio and Walter 2001, Kaeslin et al. 2005). Strand and Pech (1995) suggested that egg parasitoids may evade the immune response of the host by preferentially ovipositing in pregastrula embryos in which the immune system has not yet become functional. However, Kaeslin et al. (2005) observed that all stages of *Spodoptera littoralis* (Boisduval) eggs can be parasitized by *Chelonus inanitus* (L.), because the behavior of the parasitoid larva or adult assure that the larva will be located in the haemocoel of the host. When freshly deposited host eggs are parasitized, the parasitoid hatches in the yolk and enters the host embryo either after waiting or immediately through the dorsal opening. When host eggs are parasitized at 1 or 2 d old, the host embryo is covered by an embryonic cuticle through which the hatching parasitoid larva

bores with its abdominal tip. When old eggs (2.5-3.5 d) are parasitized, the female parasitoid oviposits directly into the haemocoel of the host embryo.

T. eugenii was able to parasitize young and old eggs, an ability which is shared with other koinobiont species that attack eggs of their hosts but later emerge from larvae or pupae (Clausen cited by Quicke 1997, Abe 2001, Quimio and Walter 2001, Kaeslin et al. 2005). The physiological interactions between host and parasitoid are not completely understood, but the ability to use either young or mature hosts represents a desirable characteristic that increases probability of locating an appropriate host.

Role of the Oviposition Plug in Host Location

Host habitat location by parasitoids often involves chemical cues, as well as physical and electromagnetic signals (Quicke 1997, Vinson 1976, 1998, Turlings et al. 1998, Havill and Raffa 2000). These signals include odors emanating from host frass, symbiotic fungi, or host-damaged plants (Quicke 1997, Vinson 1976, 1998, Turlings et al. 1998). Parasitoids attacking concealed larvae, such as fruit flies or wood borers, can locate their hosts by detection of movement transmitted as vibrations in the substrate (Godfray 1994, Quicke 1997). Such stimuli are not available to parasitoids of concealed eggs. Such parasitoids could rely on olfactory signals from chemicals such as kairomones of the host (e.g. accessory gland secretions), or from tactile and chemical signals emanating from mechanical damage such as oviposition wounds (Strand 1989, Godfray 1994). Once a probable site of a host has been located, parasitoids respond to less volatile substances, or to tactile cues where closer antennation and the ovipositor might play more important roles (Godfray 1994, Quicke 1997).

This study demonstrated that the host oviposition plug is important for parasitoid host location. A high proportion, around 80%, of pepper weevil eggs were not parasitized by *T. eugenii* when the oviposition plug was removed. The oviposition plug, then, provided means for the parasitoid to detect and, subsequently, to parasitize its host. It is possible that the antennae were used to detect host kairomones found in the plugs, because drumming was observed to increase as soon as the female was close to the plugs. The response of some parasitoids to these substances has been demonstrated in more than one species (Quicke 1997, Turlings et al. 1990, 1998, Vinson 1998). However, the design of the present study did not permit the evaluation of other sources of stimuli, such as physical or visual characteristics of the plugs, which may be important in the host detection and oviposition by *T. eugenii*. More studies need to be done in this area.

When the pepper weevils were reared under crowded conditions, the proportion of eggs lacking ovipositions plugs increased (Chapter 2). For this reason, it was more efficient to rear the pepper weevil by exposing pepper fruit to one female weevil per fruit rather than 10 unsexed weevils per fruit. Additionally, less feeding damage on pepper fruits by weevils helped to diminish the number of rotten fruits in the colony (E. Rodriguez, unpublished data).

Life Cycle

T. eugenii is a member of the tribe Brachistini in the subfamily Helconinae and the family Braconidae. The members of Brachistini are cosmopolitan and are known as egg-larval endoparasitoids of different families of Curculionidae and Bruchidae (Beirne 1946, Martin 1956, Shaw and Huddleston 1991, Sharkey 1997). Additionally, there is at least one species, *Nealiolus curculionis* (Fitch), which has been reported parasitizing early larvae of the sunflower stem weevil, *Cylindrocopturus adspersus* (LeConte) (Charlet and

Seiler 1994, Charlet et al. 2002). Early reports confirmed and described the endo- and ectoparasitic phases of the immature stages of some *Triaspis* species, such as *T. vestiticida* and *T. caudatus* (Berry 1948, Obrtel 1960); however, few species of this genus have been studied in detail (Sharkey 1997).

Females of *T. eugenii* were able to lay eggs from the first day of life. This behavior is characteristic of many koinobiont parasitic wasps which do not need to consume protein-rich host hemolymph to initiate oogenesis (Quicke 1997). The parasitoid egg was deposited in the host egg, where it hatched in less than 24 h. No parasitoids were recovered from larvae (4-5 d after infestation). The endoparasitic phase thus initiated in the egg and continued until the prepupal stage of the host. Only one parasitoid emerged from each host egg. The first larval instar possesses large mandibles. The presence of such mandibles in the early instars of solitary parasitoids has been suggested as useful for physical defense against either the same or different species in case of superparasitism or multiple parasitism, respectively (Clausen 1940, Godfray 1994, Quicke 1997, Ming et al. 2003). In contrast, physiological suppression by asphyxiation may be a common mechanism by which established larvae eliminate younger competitors (Fisher 1971, Godfray 1994, Quicke 1997).

Egg-larval parasitoids occur in several subfamilies of Braconidae (Alysiinae, Helconinae, Ichneutinae), but members of the subfamily Cheloninae more often use this strategy (Clausen 1940, Shaw 1997). The potential as biological control agents of some members of this subfamily could be one reason for the availability of information on species such as *Chelonus* sp. nr. *curvimaculatus* Cameron, an egg-larval parasitoid of the pink bollworm, *Pectinophora gossypiella* (Saunders) (Hentz et al. 1997), and *Chelonus*

inanitus, an egg-larval parasitoid of *Spodoptera littoralis* (Kaeslin et al. 2005). The information reported in the present study confirms similarities in the life cycle of *T. eugenii* with that of these species of Cheloninae, and with that of *Triaspis vestiticida* (Berry 1947). In addition, the present study describes life table parameters of *T. eugenii*, information which is needed to establish the potential of this species.

Potential of *T. eugenii* as a Biological Agent of the Pepper Weevil

Despite the fact that five species and at least five other genera of parasitoids have been collected from the pepper weevil (Pratt 1907, Pierce et al. 1912, Elmore et al. 1934, Cross and Chesnut 1971, Mariscal et al. 1998, Toapanta 2001), *T. eugenii* and *Urosigalphus* sp. are the only known species that parasitize eggs of this pest. *Urosigalphus* sp. (14 females and 9 males) was collected by P. Stansly during July 2003 in Oaxaca, Mexico, and was held in quarantine facilities and reared in the same conditions that *T. eugenii*. However, the parasitoids produced only males in its offspring, making it impossible to establish a colony (E. Rodriguez, unpublished data).

The egg of the pepper weevil could be considered the stage more ecologically susceptible to be parasitized, because it is defenseless and is deposited close to the surface in the pepper fruits. Therefore, the ability to parasitize pepper weevil eggs could be one of the most important biological advantages of *T. eugenii*. Because *T. eugenii* can reach the host before the larva migrates deep inside into the fruit, it could attack the pepper weevil in any cultivar, regardless of fruit size.

The reproductive capacity of *T. eugenii* is another important biological characteristic of the parasitoid. Females were able to produce an average of 402.8 $\pm$ 199.5 eggs. Females laid more than 90% of their eggs during the first 12 days of life, depositing 31.4 $\pm$ 22.6 eggs per female per day.

Superparasitism was common, although the proportion of superparasitized hosts observed was greater where all infested fruits were left for 9 h rather than being changed at 1.5 h intervals (64% versus 55%). *T. eugenii* lays eggs individually in only 28 sec (E. Rodriguez, unpublished data). The short time required for oviposition coupled with the longer exposure period may explain the higher rate of superparasitism. However, it does not explain the apparent inability of the parasitoid to distinguish parasitized hosts, even in the presence of nonparasitized hosts.

Decreasing exposure time from 9 h to 1.5 h reduced superparasitism. Further reduction of exposure time would be impractical within the context in the rearing system developed here. Additional manipulations may be necessary such as more hosts per female, fewer hosts per fruit, and larger cage size. On the other hand, superparasitism is relatively common in laboratory conditions in other rearing systems of parasitoids, such as *Chelonus* sp. nr. *curvimaculatus* (Hentz et al. 1997), or *Catolaccus grandis* Burks (Morales-Ramos and Cate 1993); however, superparasitism does not occur often in the field according to the same authors. Given these considerations, the reproductive potentials of the pest and parasitoid were compared using fecundity and demographic parameters of the pepper weevil (Chapter 2) and of *T. eugenii* using the data set of the combined 10 *T. eugenii* females. It was assumed that superparasitism does not occur under field conditions (Table 19).

The fecundity of the pepper weevil (355.28 $\pm$ 167.51) and that of *T. eugenii* (402.8 $\pm$ 199.55) with the same sex ratio (0.5) and survivorship (0.9) indicated no differences in the net reproductive rate (Ro) ($P = 0.82$). Nevertheless, the remaining parameters favored *T. eugenii* ($P \leq 0.00001$). The intrinsic rate of increase (r_m), which might be one of the

most important parameters to compare populations because it combines Ro and generation time (T) ($r_m = \log_e Ro / T$, Birch 1948, Southwood and Henderson 2000), was almost twice as great for *T. eugenii* as for the pepper weevil. Therefore, the important pest/parasitoid difference was generation time.

Table 19. Life table parameters (± 95% confidence limits) estimated for 10 T. *eugenii* and 14 pepper weevil adults at 27 ± 2°C

Parameter	Pepper weevil[1]	*T. eugenii*[1]
Net reproductive rate (Ro)	158.10	167.1
	(115.1 – 201.1)	(92.7 – 241.3)
Intrinsic rate of increase (r_m)	0.14	0.26
	(0.13 – 0.16)	(0.24 – 0.28)
Generation time (T)	35.4	19.6
	(30.4 – 40.4)	(19.0 – 20.2)
Doubling time (Dt)	4.8	2.6
	(4.3 – 5.4)	(2.4 – 2.8)
Finite rate of increase (λ)	1.2	1.3
	(1.1 – 1.2)	(1.2 – 1.3)

[1]The sex ratio and survivorship used to do the statistical analysis were 0.5 and 0.9, respectively

T. eugenii has been cited as the most abundant parasitoid of the pepper weevil in field samples from Nayarit, Mexico, a region where the pepper weevil occurs naturally (Mariscal et al 1988, Schuster et al. 1999, Toapanta 2001). It parasitizes the egg of its host and has almost twice the potential for increase than the pepper weevil. These attributes make *T. eugenii* an excellent candidate for biological control of the pepper weevil.

CHAPTER 4
RELEASE AND RECOVERY OF *Triaspis eugenii* Wharton and Lopez-Martinez (HYMENOPTERA: BRACONIDAE) IN FIELD CAGES AND IN THE FIELD

Introduction

The pepper weevil is considered one of the key pests in peppers (*Capsicum* spp.) in many growing regions of the United States, Mexico, and Central America, and only cultural and chemical tactics are presently available for its control (Elmore et al. 1934, Riley and King 1994, Mariscal et al. 1998, Schuster et al. 1999). Since the pepper weevil was reported in Mexico (Cano and Alcacio 1894) and in the United States (Walker 1905, Pratt 1907, Elmore et al. 1934), no viable biological control strategy has been developed to manage the pest.

Some reasons for the lack of successful biological control of the pepper weevil may include: (1) a paucity of natural enemies recorded from this pest (Pratt 1907, Pierce et al. 1912, Elmore et al. 1934, Cross and Chesnut 1971, Clausen 1978), (2) limited impact of those parasitoids (Elmore and Campbell 1954, Genung and Ozaki 1972), (3) incompatibility of natural enemies with insecticides used to control pepper weevil, and (4) limited biological control research because of the relatively small economic importance of pepper compared with other crops (Riley and King 1994).

Recently Mariscal et al. (1998) identified nine different species of parasitoids attacking pepper weevil larvae in the state of Nayarit, Mexico, which is located in the central pacific coast and which is a likely center of origin of the pest. The most abundant of these parasitoids was the braconid *Triaspis eugenii* Wharton and Lopez-Martinez,

which attained 18-40% parasitism in the field (Mariscal et al. 1998, Schuster et al.1999, Toapanta 2001). These relatively high levels of parasitism, complemented with the continual presence of *T. eugenii* during surveys between 1997 and 2003, and with its ability to attack pepper weevil eggs, renewed interest in biological control to combat the pepper weevil (Mariscal et al. 1998, Schuster et al. 1999, Toapanta 2001).

T. eugenii is a solitary egg-prepupal parasitoid of the pepper weevil. Eggs are deposited into pepper weevil eggs, and hatching larvae feed as endoparasitoids; however, larvae later emerge from the host prepupa and complete development as ectoparasitoids (Chapter 3). The habit of attacking the egg, located close to the fruit surface, rather than the larva that burrows deep into the fruit, in addition to its reproductive superiority to pepper weevil, make *T. eugenii* a good candidate for biological control of the pest. Nevertheless, the reproductive potential in the field could be influenced by many factors, including the ability of the parasitoid to locate host and food, and the influence of environmental conditions. Therefore, the evaluation of field releases is an indispensable step in assessing the effectiveness of the parasitoid as a biological control agent.

T. eugenii cannot survive for more than two or three days at 27 $\pm$ 1ºC without a carbohydrate supplement such as honey. Under field conditions, many parasitoids use nectar or honeydew for this purpose (House 1977, Powell 1989, Baggen and Gurr 1998). It cannot be assumed that these sources would always be adequate in or near every pepper crop. Many parasitoids and predators also use plant volatiles to locate the host plants of their hosts or preys (Vinson 1976, Alphen and Vet 1989, Godfray 1994, Quicke 1997). The volatiles often are released from damaged plants (Turlings et al. 1990, 1995, 1998, Powell et al. 1998, Takabayashi et al. 1998, Havill and Raffa 2000, James and

Grasswitz 2005). There is no information about the importance of weevil damage in host location or foraging behavior of *T. eugenii* within a patch. This information could be used to improve the effectiveness of establishing the parasitoid in the field. The objectives of these studies were to assess the ability of *T. eugenii* to survive and parasitize the pepper weevil in large field cages and in the open field, and to evaluate the effect of honey supplements and weevil damage on this ability.

Materials and Methods

Insects

Pepper weevil and *T. eugenii* adults were obtained from colonies maintained at the SWFREC in Immokalee, FL, as described in Chapters 2 and 3. Mated weevil females, 8 to 10 d old, and parasitoids 3 d old or younger were randomly chosen from the colonies for release into field cages. Parasitoids that were released in the open field were 4 d old or younger.

Field Cages

Experiments were conducted in two screenhouses 7.3 x 3.6 x 3.65 m, with sides of 50 x 24 mesh screen (266 x 818 μm openings) and roofs of polyethylene (Figure 8A). Each screenhouse was divided into 2 large cages 3.65 m in length by an organdy cloth partition. A zipper was sewn into the partition to serve as a door. Each cage represented an experimental unit (Figure 8B). Temperature and humidity were recorded during assays using HOBO® data loggers (Onset Computer Corporation, Bourne, MA).

Figure 8. Field cages used at the SWFREC. (A) General view of a unit containing two cages. (B) Organdy partition and zipper between two experimental units.

Effect of Honey Supplement

Five pots 7.8 L, each containing two ‘Jalapeño’ pepper plants, were placed in each cage. Plants were 20.6 $\pm$ 5.2 cm tall (n=10) and were spaced 35 cm apart. At the time of the evaluations, plants had 4.0 $\pm$ 2.4 fruits smaller than 4 cm in length. To provide natural host finding conditions for *T. eugenii*, two pepper weevil adults were confined in organdy sleeves on floral buds on each of two plants (in the same pot) per cage two days before experiments commenced.

Honey was offered in lines and replaced every other day on two plastic yellow cards (5 x 8 cm) which were protected from sunlight by a cork roof (15 x 22 cm) supported by wire mesh (Figure 9A). A pepper flush damaged by pepper weevil adults was placed in a 28 mL plastic cup filled with water (Chapter 3) and attached to the wire under the cork roof to attract parasitoids to the source of carbohydrates (Figure 9B). One of these devices (wasp refuge) was suspended 30 cm above the plants in each cage.

The experiment had two treatments, a refuge with honey and a refuge without honey, which were replicated four times. Because replications were developed at different times, each screenhouse containing both treatments was considered a block. The two weevil damaged plants per cage of each block were replaced with newly damaged plants prior to the release of *T. eugenii*. Using a fine brush, 10 *T. eugenii* females 3 d old or younger were placed onto the pepper flush of the refuge of each cage (Figure 9B), which was located 1.6 m from the infested fruits. Refuges and pepper flushes were observed for 5 sec at 1, 2, 3, 8, 24, and 48 h after release to check for the presence of parasitoids. Parasitism was evaluated daily in each cage for 3 d by hanging two infested fruits per each of the damaged plants. The fruits contained a total of 20 weevil eggs 24-40 h old.

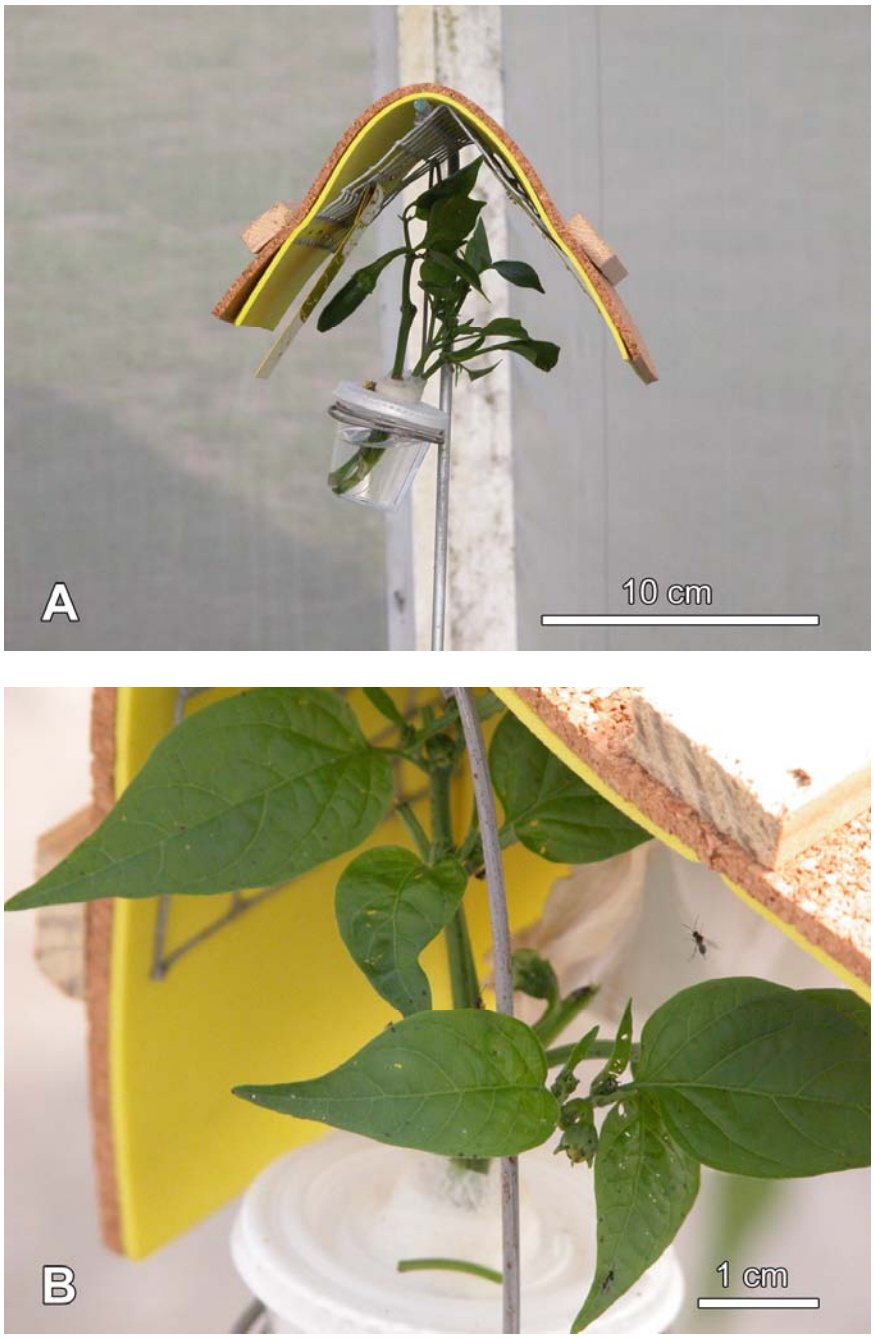

Figure 9. Honey-wasp-refuge. (A) General view of the set up. (B) Using a unit as *T. eugenii* release point.

After 8 hours (9:00 AM to 5:00 PM), the exposed fruits were removed and dissected in the laboratory to assess the percentage of parasitism and superparasitism (Chapter 3). A new group of infested fruits was hung in the same location at the same time the next morning. Each block was replicated four times and each cage received the

alternative treatment for the following replication to nullify an influence of cage orientation. Chi-square analysis (PROC FREQ, SAS Institute 2000) was used to test whether parasitism was independent of the presence of food (honey).

Survival

Survival of *T. eugenii* was evaluated using females confined in 250 mL plastic cups that had 3 cm diameter organdy sealed holes in the tops for ventilation (Chapter 2, Fig. 1). Cups were placed inside the canopy of potted plants to avoid direct sunlight. Five *T. eugenii* females 3 d old or younger per cup represented an experimental unit. Treatments were cups without honey or cups with honey streaked every 2 or 3 d on the internal side of the cups. Survival was checked every 1, 3, 9, 24, and 32 h, and then was checked each 24 h thereafter until all parasitoids were dead. Five replicates were performed in a single block. Student's t-test (PROC TTEST, SAS Institute 2000) was used to evaluate differences between treatments.

Effect of Host-Damaged Plants

Twenty 7.8 L pots, each containing two 'Jalapeño' pepper plants were placed in each cage. The pots were spaced 30 cm apart and plants were 40 $\pm$ 14.3 cm tall with 15 $\pm$ 7.8 fruits less than 4 cm long at the beginning of evaluations. Treatments in this assay were non-damaged plants and weevil damaged plants replicated five times. Because replications were performed at different times, each screenhouse containing both treatments was considered a block. Plants were noticeably larger and more mature during the last two blocks, and the presence of open flowers which could be a source of nectar was estimated from five plants per cage. At the same time, plants infested with aphids and leafminers in these two blocks were redistributed among cages to maintain a homogenous environment. At the end of each replication, plants used to support fruits

with hosts were replaced, and cages received the alternative treatment. A "Jalapeño' plant was damaged by using an organdy sleeve to confine three mated females (8-10 d old) on one terminal with floral buds 48 h prior to releasing *T. eugenii*. The damaged plant was used to hang infested fruits with pepper weevil eggs daily as above. The same procedure without weevils was followed for the control. Five *T. eugenii* females 3 d old or younger were then introduced into each cage, using a fine brush to place the parasitoids onto the foliage of the potted plants furthest (3.6 m) from infested fruits. Previous observations indicated that this number of wasps was sufficient to detect a response.

Parasitism was evaluated daily in each cage for two days by hanging five 'Jalapeño' pepper fruits containing a total of 25 pepper weevil eggs, 24-40 h old, in a random pattern on the previously infested plant or in the previously designated plant, which was placed in an equivalent position among the 20 plants, in undamaged treatments. Infested fruits were held for 9 h as in the previous experiment and dissected to check parasitism. In addition, these fruits were observed for 20 sec at 10, 20, 40, 60, 120, and 180 min after releasing the parasitoids. Cage walls were also observed for parasitoids for 30 sec at 1, 2, 3, 8, 24, 32, and 48 h after release. A chi-square test (PROC FREQ, SAS Institute 2000) was used to compare parasitism in cages with and without weevil damaged plants. The numbers of parasitoids observed in infested fruits were compared in cages with and without weevil damaged plants using student's t test (PROC TTEST, SAS Institute 2000). Before analysis, data were transformed using the square root of $y \pm 0.5$ to satisfy assumptions of normality (Sokal and Rohf 1969). Student's t test was applied also to data on wasps observed on cage walls after verifying normality of the data (PROC CAPABILITY, SAS Institute 2000). All means are reported in the original scale.

Field Release and Establishment

On June 17, 2004, 100 *T. eugenii* females and 70 males 4 d old or younger were released at the SWFREC in Immokalee, FL. Parasitoids were released from 7:30 to 8:30 AM using a fine brush to place them on the foliage of pepper plants that were at the end of the fruiting cycle. Parasitoids were released on six 100 m rows of 'Jalapeño' pepper. Three hundred randomly selected terminals were observed to assess the adult pepper weevil population. No flowers or buds were seen and fruits were large (7.68 $\pm$ 1.46 cm length, 2.77 $\pm$ 0.73 cm width, n=10) and mature. Fruits (n=10) were dissected to estimate the infestation of weevil larvae. The crop was destroyed a week after the release. Before eliminating plants, 6 kg of fruits were collected and held in an organdy fabric cage (60 x 60 x 60 cm) at 27 $\pm$ 2ºC to allow weevil or parasitoid adults to emerge. The number of collected fruits was estimated by weighting 20 fruits.

During January to April of 2005, *T. eugenii* was released five times on 90 m row of approximately 400 'Jalapeño' plants of different ages maintained at the SWFREC (Table 20). Parasitoids 4 d old or younger were placed with a fine brush on floral buds, or foliage, in groups of 5-10 females at 10 m intervals along the row.

Table 20. Releases of *T. eugenii* during 2005 at the SWFREC in Immokalee, FL.

Date	*T. eugenii*	
	♀	♂
January 10	100	80
February 8	80	40
February 18	35	
March 25	60	50
April 20	80	60
Total	355	230

Before each release, 10 immature pepper fruits with weevil feeding punctures were collected and dissected to confirm the presence of weevil eggs. The pepper weevil

infestation was also estimated by checking 50 terminals of pepper plants just prior to each parasitoid release. Fruits were harvested every 1- 2 wk, and peppers with weevil feeding punctures or chlorotic calyxes were held in the laboratory until emergence of weevil or parasitoid adults. On March 25, three honey-wasp-refuges were established in the field and 60 parasitoid females and 50 males, 4 d old or younger, were released at those points at 5:00 PM. Five second observations at those honey-wasp-refuges were made at 1, 15, and 24 h after releasing in each refuge to check for the presence of parasitoids.

During the summer of 2005 at the SWFREC, 10 potted pepper plants 5-6 months old and with mature fruits present were transplanted 10-20 m from a 'Jalapeño' plot severely infested by the pepper weevil during the previous spring. These plants were watered and fertilized during that season. In August 8, 2005, 70 fruits from those plants, and 15 more from a greenhouse nearby, were collected by P. Stansly. Fruits were held at 27 $\pm$ 2ºC until the emergence of weevil or parasitoid adults. During the fall of 2005, experimental 'Jalapeño' peppers were destroyed at the SWFREC by hurricane Wilma. No peppers were grown in the field during spring of 2006 at the SWFREC, but 25-30 potted (7.8 L) 'Jalapeño' plants were kept outdoors during spring 2006 and any pepper fruit with feeding punctures or chlorotic calyxes were collected to attempt further recovery of *T. eugenii.*

Results

Effect of Honey Supplement

T. eugenii found and parasitized pepper weevil eggs in the presence or absence of honey in the wasp-refuge during the first day of release. Eggs from the second and third days were not parasitized. No difference in parasitism was found between refuges with

honey (94.3%) or without honey (82.3%) ($X^2 = 2.29$, df = 1, P = 0.1299). After releases, some parasitoids remained a few minutes in the wasp-refuge searching the pepper flushes, whether honey was present or not, but soon left. No parasitoids were subsequently observed at any of the refuges.

Survival

Parasitoid females in cups with honey survived longer (3.65 ± 2.06 d) than parasitoids without honey (0.62 ± 0.56 d) (t = 7.11, df = 24, P ≤ 0.0001). In the presence of honey, more than 50% of the females were alive at day four, and the last females died at day seven. In contrast, females without honey died in an average of 1.3 days (Fig. 10).

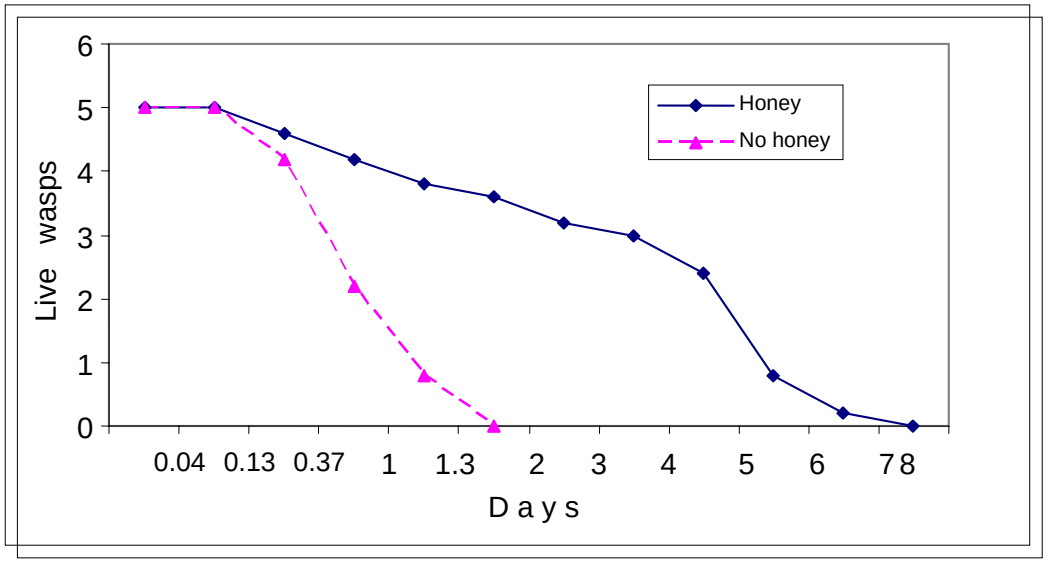

Figure 10. Survival of *T. eugenii* adults in plastic cups with and without honey, when the cups were placed inside the canopy of pepper plants, 25.4 ± 4.9ºC, 67 ± 19% RH.

Effect of Host-Damaged Plants

T. eugenii females were able to find and to parasitize pepper weevil eggs in pepper fruits hung in damaged and undamaged 'Jalapeño' plants; however, the level of parasitism was higher in fruits hung in damaged plants (57.6%) than that in fruits hung in

undamaged plants (26.4%) ($X^2 = 24.97$, df = 1, P = 0.001). No parasitism was observed on eggs in fruits hung in cages a day after the release of parasitoids.

Overall, the numbers of *T. eugenii* adults observed on fruits hung on plants damaged by the pepper weevil decreased with time (Table 21). More *T. eugenii* females were observed on fruits hung in plants damaged by the pepper weevil 10 and 20 min after release, compared to the number observed on fruits hung on undamaged plants (t= 2.45, df = 8, P = 0.040). Fewer parasitoids were observed at 40 and 60 min after release, with no differences between treatments. No *T. eugenii* adults were observed on fruits hung on undamaged plants after 60 min, or hung on any plant after 120 min. However, parasitoids were seen on the upper wall of cages during the entire 48 h observation period. The number decreased with time; 3.4 $\pm$ 0.9 versus 3.6 $\pm$ 0.5, respectively for damaged and undamaged plants at 1 h; 2.2 $\pm$ 0.8 versus 2.0 $\pm$ 1.2, respectively at 24 h; and 0.4 $\pm$ 0.5 versus 0.6 $\pm$ 0.9 respectively at 48 h. There were not differences between treatments at any time (t-test, P $\leq$ 0.05).

Table 21. Number of *T. eugenii* adults released in field cages and subsequently observed on pepper fruits (n=5) hung on pepper plants either damaged or undamaged by the pepper weevil, 28.2 $\pm$ 4.7ºC, 55 $\pm$ 9% RH

Time after releases (min)	Adults observed on fruits of pepper plants damaged by weevil	undamaged	t-test (P $\leq$ 0.05)
10	2.0 $\pm$ 1.22	0.4 $\pm$ 0.55	*
20	2.0 $\pm$ 1.22	0.4 $\pm$ 0.55	*
40	1.4 $\pm$ 0.89	0.8 $\pm$ 0.84	ns
60	0.6 $\pm$ 0.89	0.6 $\pm$ 0.89	ns
120	0.2 $\pm$ 0.45	0	
180	0	0	

Field Release and Establishment

At the time of the first release of parasitoids on 17 June 2004, 3.3 weevil adults were observed per 100 terminals, and 1.1 ± 0.57 weevil larvae were seen per fruit (n=10), although no weevil eggs were detected. An estimated of 731 pepper fruits were collected before the crop was destroyed, from which 380 adult weevils but no wasps emerged.

Environmental conditions during *T. eugenii* releases at SWFREC during 2005 are shown in Figure 11. Pepper weevil adults were found in the crop in excess of one adult per 100 hundred terminals at the time of four of the five releases of *T. eugenii*, and more larvae than eggs were detected in the fruits (Table 22). Overall, the number of fruits damaged by pepper weevil larvae and the number of emerged weevil adults increased through time (Table 22). No *T. eugenii* adults were observed in honey-wasp-refuges following releases of the parasitoid during March 25.

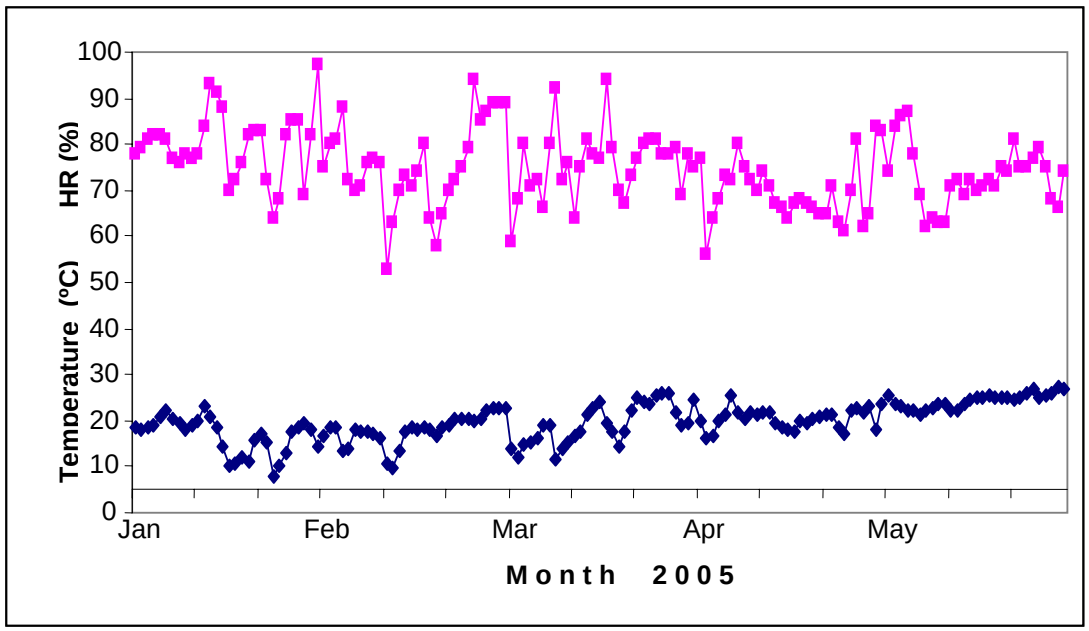

Figure 11. Daily temperatures and humidity during *T. eugenii* field releases on 'Jalapeño' pepper at the SWFREC in Immokalee, FL, 2005.

T. eugenii emerged for the first time on April 14 from infested fruits collected on April 6. Infested fruits were then collected almost every week, and parasitoids were recovered in low numbers through May 2 (Table 23). A few *Catolaccus hunteri* adults also emerged. *T. eugenii* and *C. hunteri* together caused 6.7% parasitism. The pepper row was eliminated during the last week of May, 2005.

Table 22. Adults per 50 terminals, and the number of weevil lifestages per 10 fruits, 1 or 2 d before field releases of *T. eugenii* on 'Jalapeño' pepper plots at SWFREC, Immokalee, FL, 2005

T. eugenii release (date)	No. of pepper weevils			
	Adults	Eggs	Larvae	Emerged adults
January 10	0	0	0	0
February 8	2	0	12	12
February 18	1	0	2	148
March 25	3	2	5	300
April 20	1	2	7	

Table 23. Parasitoid and pepper weevil adults recovered from 'Jalapeño' pepper following field releases of *T. eugenii* at the SWFREC, Immokalee, FL, 2005

Emergence	*Triaspis eugenii*		*Catolaccus hunteri*		Pepper weevil
	♀	♂	♀	♂	
April 1					180
April 14	6	10	4	1	147
April 19	1		1		
April 25	5	8	2		535
April 30	20	5			
May 2	9	5			206
Total	41	28	7	1	1068

Pepper weevil adults emerged from the field sample and greenhouse samples collected by P. Stansly during the summer 2005 at the rate 0.9 and 0.53 weevils per fruit, respectively. However, no *T. eugenii* emerged. No pepper weevils were detected in the

potted ‘Jalapeño’ plants established at the SWFREC by the time sampling was terminated on April 14, 2006.

Discussion

Effect of Honey Supplement

T. eugenii searched the pepper flushes offered in the wasp refuges for a few minutes but left, apparently not to return. Parasitism did not differ between honey and no honey treatments, and was observed only during the first day of exposure.

As expected, survival of *T. eugenii* adults was greater when adults were caged in plastic cups with honey. Nevertheless, longevity of adults was drastically reduced to 3.7 d in the plastic cups, compared to 16.5 d for ovipositing females in the laboratory at 27 $\pm$ 1ºC (Chapter 3). The difference on survivorship may be due to heat stress that might have occurred in the cups or the field cages.

It is known that floral and extrafloral nectars are indispensable sources of carbohydrates for most adults of Hymenoptera (House 1977, Powell 1989, Baggen and Gurr 1998, Kogan et al. 1999). Availability of these foods will affect longevity, fecundity, and parasitism (Powell 1989, Idris and Grafius 1995, Baggen and Gurr 1998). It is expected that efficiency, biological potential and survival of parasitoids that are dependent upon these sources of energy to survive could be limited in monocultures that provide those resources only for a limited time (Altieri 1992, Kogan et al. 1999). In the field cages, *T. eugenii* in the plastic cups died in less than 48 h without a carbohydrate source. Offering honey in the wasp-refuges did not appear to increase foraging time. However, it is possible that food was not a limiting factor in the field cages. Parasitoids might have used honey or left the patch before any carbohydrate was required.

Effect of Host-Damaged Plants

More *T. eugenii* adults and higher parasitism were observed on infested fruits on damaged plants compared to fruits on undamaged plants. Thus, the difference in parasitism could be explained by the greater portion of parasitoids that found hosts in damaged plants. *T. eugenii* found infested fruits in less than 10 min in both treatments; however, less than 50% of the released parasitoids were observed on fruits hung in damaged plants, and less than 10% were observed on fruits in undamaged plants (Table 22). On the other hand, the majority of parasitoids left the foraging patch within 120 min or less, regardless of treatment. No parasitism was observed 24 h after the first exposure, but *T. eugenii* adults were observed on the cage walls where they apparently spent the rest of their lives.

Parasitoids use chemical, visual, and/or electromagnetic cues from the host habitat to locate their hosts (Vinson 1976, 1998, Alphen and Vet 1989, Godfray 1994, Quicke 1997). The oviposition plug is important for *T. eugenii* to identify and to parasitize hosts (Chapter 3), but might also serve as a cue for short distance host location. In this experiment both treatments offered similar conditions and plants damaged by the pepper weevil attracted more parasitoids. *T. eugenii* then was able to detect and to recognize a host patch (infested fruits in damaged plants). It is possible that the more likely cues used by *T. eugenii* to find their hosts from a distance are volatiles produced by damaged plants or left by the pest. Volatiles produced by pest-damaged plants have been suggested as important cues for other parasitoids to find their hosts. In wind tunnels plant volatiles, particularly those produced by aphid feeding, were important in long-range cues in the initial stage of host location by the braconid *Aphidius ervi* Haliday (Powell et al. 1998). Poplar tree leaves damaged by the gypsy moth (*Lymantria dispar* L.) were three times

more attractive to its braconid parasitoid, *Glyptapanteles flavicoxis* (Marsh), than undamaged leaves (Havill and Raffa 2000). The studies on pest-damaged emissions clearly suggest that these volatiles are important for parasitoids to locate hosts. These volatiles, many times related to terpenoids, may be produced in defense against pests, but may also serve in attracting the natural enemies of these pests (Turlings et al. 1995, 1998).

It is possible that *T. eugenii* was able to recognize pepper weevil damaged plants from a distance, and that volatile chemicals then might play an important role in a host patch selection. Even with the presence of 25 host eggs and a pepper plant damaged by pepper weevils, *T. eugenii* did not remain more than 120 min on the host patch. The hypothesis of host marking by an ovipositing parasitoid can be proposed to explain the observed behavior. Some parasitoids mark their hosts once the hosts are parasitized, thus reducing the acceptability of the patch to themselves or to other females. After ovipositing and marking a host, parasitoids may disperse from the immediate vicinity of the host (Vinson 1984, Van Dijken et al. 1992, Potting et al. 1997). In support of this hypothesis, the rate of superparasitism observed in the field cages (28%) was lower than that reported in the laboratory (55%) (Chapter 3). These observations are consistent with the presence of chemical marking of parasitized hosts, inducing females to reject and to leave a patch already explored. If that is true, it could explain why *T. eugenii* females left the patch in less than 120 min, and why superparasitism was less often detected in the field cages. On the other hand, chemical markings may be less effective in small cages in the laboratory because females cannot leave the patch, and because females may become habituated to the chemical in the confined space.

Field Release and Establishment

T. eugenii completes its life cycle in 16.6 d at 27 ± 1ºC, requiring about 265 heat units based on a development threshold of 11ºC (Chapter 3). The first successful recovery of parasitoids from the field occurred on April 14, 2005. Considering field temperatures and the time that fruits were held in the laboratory, these parasitoids could have originated from releases made on February 18 (267 heat units). Parasitoids emerging April 19 could have originated from the release on March 25 (272 heat units). However, *T. eugenii* that emerged on April 25 do not correspond to a precise date of release, and parasitoids that emerged on April 30 and May 2 should have originated around the second week of April, possibly on days 13-14 (266 heat units) when there were no releases. It appears likely, then, that *T. eugenii* was able to complete at least one generation in the field. No parasitoids were recovered subsequently from the field, but the sampling was very limited because the lack of pepper crops during the summer of 2005, and because of the destruction of crops in the fall of 2005 by hurricane Wilma.

Pepper plants do not set fruit at temperatures above 32ºC (Bosland and Votava 1999). Therefore, the high temperatures during late spring and summer in southwest Florida preclude commercial production of peppers. Nevertheless, adults of the pepper weevil survive the temperatures of summers in Florida (Toapanta 2001) or the winters of California (Elmore et al. 1934), apparently feeding and ovipositing on alternative hosts such as nightshade (Elmore et al. 1934, Wilson 1986, Genung and Ozaki 1972, Patrock and Schuster 1992). Pepper weevil adults feed on buds and immature fruits but, when these are not available, adults can feed on foliage (Elmore et al. 1934, Goff and Wilson 1937). As result, adults can live at least two or three months during the hot season of the year (Elmore et al. 1934, Toapanta 2001), or maybe longer in less extreme conditions.

Elmore et al. (1934) reported that overwintering weevils may live as long as 10 months. The amount of time between peaks of reproductive structures, which offer the best quality of fruits for ovipositing, does not seem to represent a problem for the survivorship of this species. However, the lack of eggs of the pepper weevil in some seasons in the year, or even between peaks of commercial production within the same crop, may represent one of the most important barriers for establishment of *T. eugenii* in Florida. Considering these facts, at least two factors may explain the unsuccessful parasitism by *T. eugenii* during the first release in 2004, and the inconsistent parasitism in some releases in 2005. First, there may have been few or no pepper weevil eggs present at the time of the releases. Only pepper weevil larvae were observed in fruits in the field during the first release in 2004. The presence of pepper weevil eggs was confirmed by sampling on only two of five release dates in the spring of 2005. Second, *T. eugenii* may be inefficient in locating host eggs. Females were able to find hosts in the field cages at release rates equivalent to 3,753 and 7,507 females per hectare. In the open field parasitoid females were released at rates equivalent to 3,500 (Feb 18) and 6,000 (March 25) per hectare. In two other releases, the equivalent release rates of females per hectare was 10,000 (January 10) and 8,000 (February 8, April 8) respectively. Thus, it would seem that the lack of host eggs during wasp releases was more important in explaining no or inconsistent parasitism in the field releases.

Considering (1) that pepper weevil adults can survive without laying eggs during unfavorable seasons or between peaks of production in the pepper cycle, (2) that *T. eugenii* leaves an explored patch in a short time and survives less than two weeks as an adult, and (3) that pepper crops do not consistently provide hosts and nectars, different

methods of ensuring the presence of pepper weevil eggs at the moment of parasitoid releases must be developed in order to establish this parasitoid in the field in Florida.

CHAPTER 5
CONCLUSIONS

Rearing Methodology and Influence of Food Quality on Biology of Pepper Weevil

The pepper weevil preferred feeding on the anthers of floral buds rather than on fruits. This behavior may be related to the higher nitrogen content of anthers compared to fruits. The pepper weevil is able to obtain nutrients for egg production by feeding only on peppers. However, because of the nitrogen content and, maybe, the balance of amino acids, weevils fed on floral buds and immature 'Jalapeño' peppers had higher fecundity than weevils fed only on immature 'Jalapeño' fruits.

Pepper weevil females preferred to oviposit in immature pepper fruits compared to floral buds and mature fruits. Females may prefer immature fruits over floral buds for oviposition because fruits provide a greater resource to support larval development. Actually, weevils developing in floral buds are usually smaller than weevils developing in immature fruits (Patrock and Schuster 1992). On the other hand, immature fruits could be preferred to mature fruits not only because the nitrogen content of the pericarp is higher, but also because the thinner pericarp offers a shorter distance for the offspring to reach the immature placenta and seeds where nitrogen content is higher yet.

Overcrowding of pepper weevil females by males or other females resulted in reduced fecundity of females and increased incidence of eggs lacking the normal oviposition plug. A single mated female per immature fruit appeared to be the most efficient combination to maximize fecundity, and to maximize the number of eggs with oviposition plugs.

Life History of *Triaspis eugenii*

T. eugenii is a solitary egg-prepupal parasitoid of the pepper weevil. Eggs are deposited in young or mature eggs of the pepper weevil. Hatching larvae feed as endoparasitoids, but emerge from the prepupa of the host to complete development as ectoparasitoids. At 27 $\pm$ 1ºC, females developed in 16.6 $\pm$ 0.9 d, just a few hours less than males. Adults of *T. eugenii* were not observed to feed on the host and needed a carbohydrate source to survive. Mated females provided with honey and water lived 24.7 $\pm$ 10.6 d and females without honey lived 2.3 $\pm$ 0.7 d. Females ovipositing constantly lived 16.5 $\pm$ 3 d and were able to produce 402.8 $\pm$ 199.5 eggs. About 90% of the eggs were laid during the first 12 d of life at the rate of 31.4 $\pm$ 22.7 eggs per female per day.

The oviposition plug of the pepper weevil played an important role in the ability of *T. eugenii* to find and to parasitize pepper weevil eggs. Further research is necessary to determine if the cues utilized by the wasp to recognize and/or locate the plugs are chemical, physical or both.

When pepper fruits with weevil eggs were exposed for 2 h or more to *T. eugenii* in the laboratory, less than 50% of the parasitoid eggs reached the adult stage because of a high level of superparasitism. Exposing fruits for 1.5 h to single ovipositing females slightly reduced superparasitism.

Demographic parameters, such as the intrinsic rate of increase (r_m), estimated without considering superparasitism, indicated the greater reproductive potential of *T. eugenii* ($r_m = 0.26$) compared to the pepper weevil ($r_m = 0.14$). So *T. eugenii* could build upon pepper weevil populations, and superparasitism must be reduced to improve efficiency in the rearing system.

Field Cage and Field Release

T. eugenii was able to find pepper weevil eggs in fruits hung on weevil-damaged and undamaged plants in the field cages; however, more parasitoids were observed in weevil-damaged plants. Thus, volatile chemicals (plant or weevil plus plant) might play a role during the location of host patches by the parasitoid. Wasps did not remain more than 120 min in the host patch and did not come back to a new group of hosts provided 24 h later.

Superparasitism was observed at a lower frequency in the field cages (28%), compared to the laboratory (55 to 64%).This may indicate that *T. eugenii* marks parasitized hosts, inducing the parasitoid to leave the host patch in 2 h or less. If that is true, it is possible that in confined conditions (laboratory cages 3.8 L) host marking would have less of an effect for at least two reasons. First, females cannot leave the patch, thus increasing the number of times that they locate the same host. Second, the reduced space and abundant stimuli might cause habituation to the substance that helps to prevent superparasitism by *T. eugenii*.

Parasitoids died in less than 48 h without honey as a carbohydrate source, although a device offering honey in the field cages was not efficient in attracting them. This observation strongly suggests the importance of further research to develop a better method of providing an attractive source of carbohydrates to *T. eugenii* in the field.

T. eugenii was recovered in low numbers from the field after releases in the spring of 2005 at the SWFREC in Immokalee, FL. Although at least one generation was completed in the field, no parasitoids were recovered subsequently. However, subsequent sampling also was limited.

The pepper weevil has the ability to survive as an adult for more than two or three months by feeding on foliage or flowers of different hosts. Thus, the pepper weevil can survive even without the presence of pepper fruit or floral buds for oviposition. Conversely, the longevity of *T. eugenii* adults is short. Thus, in order for the parasitoid population to survive longer periods, weevil eggs are needed for reproduction. Therefore, it might be difficult for this parasitoid to survive during summers or winters when pepper is not present, or to survive during the pepper season when plants may not have fruits acceptable for pepper weevil oviposition. Even if *T. eugenii* fails to become established in the pepper agroecosystem as it currently exists in Florida, this parasitoid is still an excellent prospect for biological control of the pepper weevil by augmentation. It is highly host specific, it parasitizes weevil eggs, and it has an intrinsic rate of increase that is almost twice that of the pepper weevil.

Further Research

T. eugenii may be the parasitoid with more potential for biological control of the pepper weevil, but there is no information to support that it may colonize and self perpetuate under annual pepper crop production systems or environmental conditions different from Nayarit, Mexico. The possibility of establishment of *T. eugenii* in different locales will be greater if the ecology of the parasitoid is studied in the natural pepper weevil-host plant-parasitoid system in indigenous sites in Mexico. How does *T. eugenii* survive in Nayarit when there are no peppers in the field? Are there any alternative host plants for the pepper weevil that can offer flowers or fruits for oviposition? How does *T. eugenii* survive when there is a lacking of pepper weevil eggs? Are there any other insect hosts for *T. eugenii*?

Mexico is the center of origin and domestication of *Capsicum annuum* L., the most important cultivated species of chile-peppers (Vavilov 1951, MacMeish 1964, Pickersgill 1969, Eshbaugh 1976, Loaiza-Figueroa 1989). Wild and semi-domesticated peppers grow everywhere in the country. With this base we may propose at least four different ways for *T. eugenii* to survival when no commercial peppers are grown in Nayarit. First, the alternative plant hosts that offer floral buds and/or acceptable fruits for constant pepper weevil oviposition are wild or semi-domesticated peppers. Second, the alternative host plant that plays the same role is a weed (*Solanum* spp.?). Third, wild or semi-domesticated peppers and weeds may serve as alternative host plants. Four, there is an alternative insect host of *T. eugenii* that develops in the same or different plants. All of these options need to be studied before the survival of *T. eugenii* can be understood.

Even if *T. eugenii* does not become established in Florida, its host specificity and reproductive potential make it an excellent prospect for augmentative biological control of the pepper weevil. However, as with any exotic or indigenous natural enemy with potential to combat a pest, a large number of parasitoids would be needed to determine the parasitoid/host ratio that would provide effective control in the open field. Therefore, improved and economical rearing systems need to be developed for both the pepper weevil and *T. eugenii.* Research is needed to develop and artificial diet for pepper weevil larvae in order to eliminate the need of immature peppers. An artificial means of collecting and then offering pepper weevil eggs to *T. eugenii* and different ways of diminishing superparasitism are also needed.

The system developed for mass rearing *Catolaccus grandis* (Hymenoptera: Pteromalidae) with cotton boll weevil larvae reared in an artificial diet (Cate 1987, Cate

et al. 1990) might aid in the development of a rearing system for *T. eugenii*. In the former system third instars of the boll weevil are encapsulated in Parafilm bubbles for presentation to *C. grandis.* The Parafilm bubble method was satisfactory for rearing *Bracon mellitor* Say, which also attacks boll weevil larvae developing concealed inside fruits and floral buds (Cate 1987). Hopefully, the rearing system for the pepper weevil and *T. eugenii* could be enhanced using information gained in the present study, including the use of nitrogen to increase the fecundity of the pepper weevil, and the use of signals (chemical or physical) from the oviposition plug to increase parasitization of pepper weevil eggs by *T. eugenii.* On the other hand, if an alternative host for *T. eugenii* could be identified, that host might be reared more easily in the laboratory thus leading to an improved rearing system for the parasitoid.

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BIOGRAPHICAL SKETCH

Esteban Rodríguez-Leyva was born on November 28, 1966, in Mexico City, to Benito Rodríquez and Pilar Leyva. He earned his Bachelor of Science in plant science in 1992, from Universidad Autónoma Chapingo, Chapingo, México. Upon graduation he worked as a research assistant in the Fruit Crops Center at Colegio de Postgraduados en Ciencias Agrícolas (Graduate College of Agriculture Sciences) at Chapingo, México. In the fall of 1993 he started his Master of Science in entomology. Before completion of his M.S. degree, in spring of 1996, he was offered an opportunity to work as research assistant at the Entomology and Acarology Department, Colegio de Postgraduados at Montecillo, México. Under the supervision of Drs. Jorge L. Leyva and Nina M. Bárcenas, he began to work with parasitoids of the pepper weevil and boll weevil in 1997. He started his Ph.D. research under the supervision of Drs. P. A. Stansly and D. J Schuster in August 2001. His research focused on *Triaspis eugenii,* a parasitoid described from Mexico, and its potential for biological control of the pepper weevil. He will receive his degree in spring 2006, and he will be joining the Entomology program in the Colegio de Postgraduados at Montecillo-Texcoco, México, as a research associate. E-mail address is esteban669@hotmail.com.