Artificial Substrates for Oviposition and Larval Development of the Pepper Weevil (Coleoptera: Curculionidae)

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ABSTRACT   The pepper weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae), is a major pest of cultivated peppers (*Capsicum* spp.) and other cultivated and wild species within the family Solanaceae. Laboratory study of this insect, as well as its biological control agents, will be greatly facilitated by an artificial rearing system that does not rely on pepper fruit. An egg collection method and amendments to a standard larval diet were investigated for use in the rearing of this weevil. Spherical sachets made of Parafilm or netting enclosing leaves of pepper, American black nightshade, eggplant, tomato, potato, and jasmine tobacco induced oviposition. Tomato, potato, and jasmine tobacco leaves were accepted despite the fact that these are not oviposition hosts for pepper weevils in the wild. A standard larval diet formula was modified in an attempt to improve egg hatch, larval survival, developmental time, and adult mass. The diet formula was modified with the addition of freeze-dried jalapeño pepper powder, an additional lipid source, alternate protein sources, and the removal of methyl paraben. None of the aforementioned treatments resulted in a significant improvement over the standard diet. Egg hatch was greater when eggs were incubated on moist paper towels rather than in diet; thus, placement of neonates rather than eggs into diet improved production of adults. Suggestions for more efficient rearing of weevils on the currently available diet and future directions for the development of an artificial rearing system for pepper weevil are discussed.

KEY WORDS   pepper weevil, artificial diet, oviposition, egg collection

The pepper weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae), is a significant pest of cultivated pepper (*Capsicum* spp.) throughout the southern United States, Central America, and the Caribbean region. Females oviposit preferentially in young fruit, but they also will use mature fruit or flower buds for oviposition, where larvae may feed on anthers, seeds, or placental material (Elmore et al. 1934). The resulting infestation renders fruit unmarketable and causes fruit drop with consequent yield loss. Although restricted to species within the Solanaceae, pepper weevils can reproduce on several wild and cultivated species, in addition to pepper (Tejada and Reyes 1986, Wilson 1986, Diaz et al. 2004), and adults will feed on an even wider range of species within the family (Patrock and Schuster 1992).

A mass rearing system for pepper weevil is desirable to facilitate study into chemical, behavioral, and biological control methods. Ideally, pepper weevils would be mass reared on an artificial diet suitable for both oviposition and larval development; however, such diets often take years to formulate, as was the case with the boll weevil, *Anthonomus grandis grandis* Boheman (reviewed by Lindig 1993). A semiartificial method of egg collection using pepper leaf "sachets" has been developed for pepper weevil (Calderon-Limon et al. 2002). Although pepper weevil females do not deposit eggs on host leaves in the wild, they did lay eggs when presented with pepper leaves shaped into spheres and covered with Parafilm.

The only published artificial diet formula for pepper weevil is a modification of a diet for cabbage looper, *Trichoplusia ni* (Hübner), which successfully reared the weevils from egg to adult (Toba et al. 1969). The effectiveness of this diet for larval rearing is uncertain, however, with one study of the commercial formulation (Bio-Serv, Frenchtown, NJ) reporting extremely high levels of egg and larval mortality (Toapanta 2001).

The purpose of the following experiments was to identify artificial oviposition and diet substrates for use in laboratory rearing of pepper weevil. We investigated several modifications of the leaf sachets developed by Calderon-Limon et al. (2002), including tests of alternate host plants and other sachet covering material for use in egg collection. We also tested modifications to the pepper weevil diet developed by Toba et al. (1969) for improved survival, developmental time, and adult mass. Our diet modifications included

the addition of host material to incorporate feeding stimulants into the formula, because this has been shown to improve the success of artificial rearing in phytophagous and entomophagous insects (Trudel et al. 1994, Blossey et al. 2000, Ferkovich and Shapiro 2004). We investigated alternative protein sources as a way of improving the diet's nutritional content by more closely mimicking the natural larval food source as well as a providing a source of additional feeding stimulants (Sterling et al. 1965, Sterling and Adkisson, 1966). We compared diets with and without methyl paraben, an antifungal agent that has been shown to negatively affect the fitness of a wide range of insect species including the mirid *Lygus hesperus* Knight (Alverson and Cohen 2002), the blow fly *Protophormia terraenovae* Robineau-Desvoidy (Voss 2000), cabbage looper (Kishaba et al. 1968), and pepper weevil (Toba et al. 1969). Finally, we investigated diets containing an additional source of essential polyunsaturated fatty acids (linoleic and linolenic acid) and phytosterols (campesterol and β-sitosterol) (Canavoso et al. 2001).

Materials and Methods

Insects and Plants. A laboratory colony of pepper weevils was established at the University of Florida, Gainesville, FL, from field collections made in south Florida near the city of Clewiston in spring 2004. Additional insects collected from Immokalee, Bradenton, and Wimauma in fall 2005 and 2006 were introduced into the colony to maintain genetic diversity. Insects were maintained in the laboratory (photoperiod of 14:10 [L:D] h), $\approx$ 27°C, and 30% RH) on excised greenhouse-grown jalapeño peppers (*Capsicum annuum* L.) with water and honey supplements. Females were removed from stock colony cages 10 d after emergence, giving them sufficient time for mating, and then they were transferred into oviposition containers made from 250-ml, 8.5-cm-diameter waxed cardboard cans with screened lids (The Fonda Group, Inc., Union, NJ). The number of eggs deposited by each female was monitored weekly to ensure only gravid females were maintained in cups.

Plant material in the following experiments came from greenhouse-grown jalapeño pepper, 'Ghostbuster' eggplant (*Solanum melongena* L.), 'Better Boy' tomato (*Solanum lycopersicum* L.), 'Fordhook 242′ bush lima bean (*Phaseolus lunatus* L.), DPL90 cotton (*Gossypium hirsutum* L.), tropical soda apple (*Solanum viarum* Dunal), and jasmine tobacco (*Nicotiana alata* Link & Otto) grown from seed, and American black nightshade (*Solanum americanum* Mill.) grown as wild transplants obtained in Gainesville. All plants were grown under greenhouse conditions ($\approx$34°C daytime temperature) and fertilized using Osmocote 14–14-14 slow release pellets (The Scotts Company, Marysville, OH).

Egg Collection Assays. Several physical factors may influence acceptability of leaf sachets to weevils for oviposition, including color, shape, and type of covering. In addition, we wanted to test different plant species, including known alternate hosts and non-

hosts, in an effort to offer researchers attempting to mass rear pepper weevil a wider range of plant material for use in egg collection protocols.

Leaf Sachet General Bioassay Conditions. For each of the following 12 oviposition assays, females (>10 d old), taken directly from the colony cage, were used. Weevils were held individually on an uninfested pepper fruit for 24 h before assay. Only females that were actively ovipositing during that 24-h period were used. All assays were performed in small plastic 10- by 10- by 8-cm boxes with screen lids. Sachets were made by rolling a young leaf into a 1-cm-diameter ball and wrapping the ball in Parafilm, unless otherwise noted. Individual females were offered a leaf sachet for feeding and oviposition in no-choice tests. Assays were conducted in a climate-controlled room (27°C and 30% RH) and assays ran for 6 h with 15 different females, with each female as a replicate. Treatments were randomized in blocks for all assays, and the numbers of feeding punctures and eggs deposited in the sachets were recorded after 6 h.

Effect of Physical Factors on Acceptance of Sachets. Three sets of assays were conducted to test physical factors influencing sachet acceptance. In the first assay, we tested whether Parafilm alone or a green-colored substrate wrapped in Parafilm, in the absence of plant odor, could induce pepper weevils to oviposit in sachets. Individual females were presented with one of three types of sachets in a no-choice assay. Parafilm enclosed either 1) a 1-cm-diameter clear glass marble, 2) a piece of green construction paper rolled up to mimic a 1-cm-diameter leaf ball, or 3) a young pepper leaf rolled into a 1-cm-diameter ball.

In a second assay, weevils were presented with pepper leaf-containing sachets wrapped in Parafilm that were either formed into spheres or flattened ($\approx$2 by 2 cm square and 1 cm thick) to investigate the influence of shape on feeding and oviposition under no-choice conditions. Both sachet treatments contained a similar mass of leaves, and leaves to be used in the flattened treatment were first rolled into spheres to ensure an equivalent level of tissue damage.

In a third set of assays, two sachet-covering options were compared with the standard Parafilm covering in separate no-choice assays. In the first assay, weevils were presented with pepper leaf-containing sachets wrapped in cheesecloth (grade 50, 28 by 24 weave, John L. Lyman Co., Chicopee, MA). The second assay examined pepper leaf sachets covered in bridal netting (1- by 1-mm mesh).

Effect of Plant Species on Acceptance of Sachets. In the first series of assays, Parafilm sachets containing leaves from eight plant species were examined for feeding and oviposition in short-term no-choice tests conducted for 6 h: two known solanaceous host plants (American black nightshade and eggplant), four non-host plants within the Solanaceae (potato, jasmine tobacco, tomato, and tropical soda apple), and two nonhost plants outside the Solanaceae (cotton and bean). For each of the eight assays, pepper leaf sachets were run as a control alongside the alternative plant species.

Table 1. Amounts and sources of chemicals used to produce 1 liter of standard pepper weevil artificial diet, modified from Toba et al. (1969)

Diet ingredient	Amount	Source
Dry mix		
Casein	33.2 g	BioServ
Sucrose	33.2 g	Bio-Serv
Wheat germ	28.5 g	Bio-Serv
Alfalfa meal	14.2 g	Bio-Serv
Wesson salt mix	9.5 g	Bio-Serv
Alphacel	4.8 g	MP Biomedicals, Irvine, CA
Water	825 ml	
Agar, USP	23.7 g	Bio-Serv
Vanderzant vitamin mix	19.2 g	Bio-Serv
Choline chloride (10% aq.)	9.5 ml	Bio-Serv
Formaldehyde, 37% (wt:wt)	1.08 ml	Fisher Scientific
KOH, 4 M	4.8 ml	Fisher Scientific
Methyl paraben	1.5 g	Bio-Serv
Sorbic acid	1.0 g	Bio-Serv
Ascorbic acid, 97%	4.0 g	Bio-Serv
Aureomycin, 14.1%	0.13 g	Bio-Serv

In the second series of assays, jalapeño pepper fruit (4–5 cm long) and Parafilm sachets of pepper, eggplant, tomato, or bean leaves were presented to females in no-choice tests for an extended oviposition period. Females were permitted to oviposit for 24 h to determine whether results from long-term exposure to the sachets would be consistent with the results from the 6-h studies. Twenty replicates of this long-term exposure assay were conducted. Only the number of eggs was recorded for this assay.

Artificial Diets for Larval Development. Eggs were collected using sachets made from rolled up pepper leaves wrapped in Parafilm (Calderon-Limon et al. 2002). Sachets were placed in a cage with 50–100 gravid females overnight (from 1700–0900 hours). Eggs were collected from the unrolled pepper leaves using a fine-tipped paintbrush (2/0) sterilized in a 1% chlorine bleach solution. Eggs were placed in a petri dish on Kleenex Brand Premiere paper towel (Kimberly-Clark Global Sales, Inc., Neenah, WI) moistened with the bleach solution. Eggs were incubated in a climate-controlled room until hatch 2–3 d later (27°C and 30% RH) and neonates were transferred to the diet.

Diet Preparation and Assay Design. A pepper weevil diet developed by Toba et al. (1969) was used as the standard diet in all assays (Table 1). Diets were dispensed immediately into 6.0- by 1.5-cm Fisherbrand petri dishes (Fisher Scientific, St. Louis, MO) for the hatching assays or 48-well disposable Falcon enzyme-linked immunosorbent assay (ELISA) plates (Thomas Scientific, Swedesboro, NJ) for the diet assays and were stored at 4°C until use (within 48 h). During the diet assays, one neonate pepper weevil <6 h old was placed in each well (or the 20 interior wells in some assays when it was noted that the wells along the edge of the ELISA plate dried out). Neonates were placed on a slit made in the diet using a bleach-sterilized probe and the diet was covered with the ELISA plate lid. Eight to 10 replicate plates were set up for each diet assay. Upon emergence, adult weevils were sexed,

killed by freezing (−20°C), and dried in an oven (45°C) to obtain adult dry mass.

Hatching success on the petri dishes was evaluated after incubating eggs in a climate-controlled room (27°C and 30% RH). For diet assays, neonates were transferred onto the diets using a fine-tipped brush and the ELISA plates were held in an incubator (27°C and 60% RH). Developmental time from hatch to eclosion, sex ratio, adult mass, and survival were recorded. Ten replicate petri dishes containing 10 eggs each or ELISA plates containing one egg per well were set up for each hatching and diet treatment, respectively, unless stated otherwise.

Pepper-Augmented Diets. Weevils were reared on seven diet treatments: two controls and five with varying amounts of freeze-dried jalapeño pepper powder incorporated. Treatments included 1) standard diet, 2) a jalapeño fruit, and standard diets with replacement of either 3) 5%, 4) 10%, 5) 20%, 6) 50% or 7) 100% of the dry mix with freeze-dried jalapeño pepper powder. For the jalapeño fruit diet, the oviposition plug was removed and larval development within the fruit was observed without further disturbance. Fruit were obtained from colony oviposition cups and contained four or five eggs, as is normal for colony rearing. Egg hatch was evaluated on the six artificial diets, jalapeño pepper and on Kleenex Brand Premiere paper towel moistened with 1% bleach solution for the "paper" treatment. The assay was replicated 10 times.

Methyl Paraben and Lipid-Modified Diets. Eight diet treatment combinations were evaluated: 1) standard diet, 2) diet with methyl paraben removed, 3) diet with Wesson corn oil added (10 ml/liter diet), 4) diet with methyl paraben removed and Wesson corn oil added, 5) diet with 20% of the dry mix (by weight) placed by ground jalapeño pepper powder (=20% pepper diet), 6) 20% pepper diet with methyl paraben removed, 7) 20% pepper diet with Wesson corn oil added, and 8) 20% pepper diet with methyl paraben removed and Wesson corn oil added. The assay was replicated eight times.

Protein Alternative Diets. Five different preparations of the standard diet were compared in an assay with the following treatments: 1) standard diet, which contains casein as the protein source; 2) cottonseed meal (Traders Protein, Memphis, TN) substituted for casein; 3) ground jalapeño pepper seeds (Dorsing Seeds, Inc., Parma, ID) substituted for casein; 4) 20% pepper diet; 5) 20% pepper diet with cottonseed meal substituted for casein; and 6) 20% pepper diet with ground pepper seeds substituted for casein. Pepper seed was dried in an oven (45°C) and ground in a Wiley Mill (size 20 mesh; Thomas Scientific). The assay was replicated 10 times.

Data Analysis. For egg collection assays the number of feeding punctures and eggs laid were recorded for all assays, unless otherwise noted. Data were analyzed by the Mann–Whitney *U* test and the Kruskal–Wallis test for multiple treatment comparisons (SAS Institute 2006). Egg hatch, adult dry mass, time to 50% emergence, sex ratio, and survival data were analyzed with one-way analysis of variance (ANOVA) for a com-

plete randomized block design with each ELISA plate considered one block. Non-normal data were log- or square-root transformed, as necessary, to reduce heterogeneity of variances. If transformation did not achieve normality, data were analyzed under a Poisson distribution (PROC GLIMMIX, SAS Institute 2006), and means were compared using least significant difference (LSD) for all ANOVA analyses. Correlation analysis (PROC CORR, SAS Institute 2006) of adult mass and larval developmental time was performed for each diet.

Results

Egg Collection Assays. *Effect of Physical Factors on Acceptance of Sachets.* When presented with sachets formed from Parafilm alone, Parafilm and green paper, or Parafilm containing pepper leaves, females did not feed or oviposit in sachets lacking plant material, but they did feed ($\chi^2 = 20.84$, df = 2, $P < 0.0001$) and oviposit ($\chi^2 = 16.11$, df = 2, $P < 0.0003$) on pepper leaf sachets. There was no difference between the number of feeding punctures in flat (0.9 ± 0.5) and spherical sachets (1.5 ± 0.4). However, weevils laid more eggs in the sphere-shaped sachets (1.1 ± 0.3) than in the flat sachets (0.2 ± 0.1) ($Z = 2.70$, $P = 0.0068$). Females made more feeding punctures (3.2 ± 0.1) ($Z = -2.98$, $P = 0.0029$) and laid more eggs (1.1 ± 0.2) ($Z = -3.92$, $P < 0.0001$) in the Parafilm sachets than in the cheesecloth-covered sachets (1.1 ± 0.5 and 0.1 ± 0.1, respectively). Females made statistically equivalent numbers of feeding punctures (3.7 ± 0.5 and 3.0 ± 0.4) ($Z = 0.89$, $P = 0.3743$) and laid equivalent numbers of eggs (1.2 ± 0.1 and 1.1 ± 0.2) ($Z = 0.41$, $P = 0.6797$) in the bridal netting and Parafilm treatments.

Effect of Plant Species on Acceptance of Sachets. Sachets made from leaves of the two nonsolanaceous plants, cotton and bean, stimulated less feeding and oviposition than did sachets made with pepper leaves (Fig. 1A and B) (pepper versus cotton: feeding $Z = 3.14$, $P = 0.0017$; oviposition $Z = 2.65$, $P = 0.0081$; and pepper versus bean: feeding $Z = 2.59$, $P = 0.0095$; oviposition $Z = 2.74$, $P = 0.0062$). Two known larval host plants, nightshade and eggplant, were as stimulatory to feeding and oviposition as was pepper (Fig. 1C and D). The four solanaceous nonhost plants, however, differed in acceptability. Sachets made from the leaves of potato and jasmine tobacco accumulated an equal number of feeding punctures and eggs compared with pepper sachets (Fig. 1E and F) but tomato and tropical soda apple leaves did not (Fig. 1G and H). Females made fewer feeding punctures in tomato ($Z = 3.40$, $P = 0.0007$) and tropical soda apple ($Z = 3.39$, $P = 0.0007$) sachets than in pepper sachets. They also laid fewer eggs in tomato ($Z = 2.01$, $P = 0.0440$) and tropical soda apple ($Z = 2.82$, $P = 0.0047$) compared with pepper sachet controls.

There was an overall difference in egg deposition when females were allowed a 24-h exposure period to pepper fruit or to sachets containing bean, tomato, eggplant, or pepper leaves ($\chi^2 = 30.97$, df = 4, $P < 0.0001$). Females laid the most eggs in pepper leaf
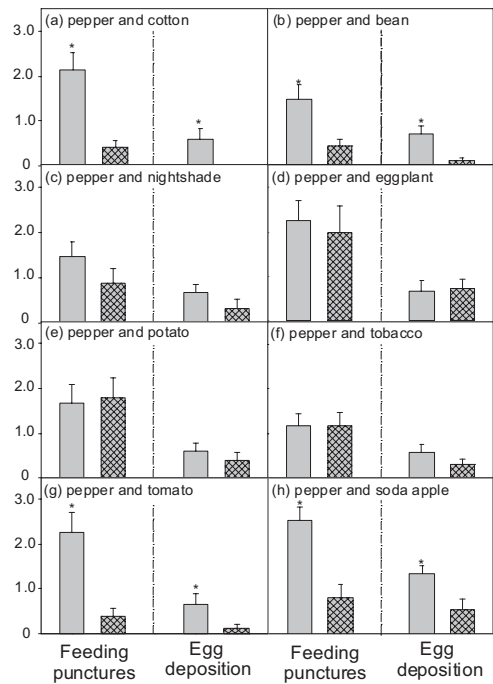

Fig. 1. Average number of feeding punctures and eggs laid by pepper weevil females in sachets containing leaves of host and nonhost plant species in no-choice tests run concurrently with jalapeño pepper leaf sachets during a 6-h exposure period (mean ± SE). Pepper (in solid bars) is presented first in each panel. Hatched bars indicate plant material other than pepper leaves. Bars marked with an asterisk (*) are significantly different using Mann–Whitney U test at $P = 0.05$.

sachets (3.8 ± 0.5), followed by pepper fruit (3.5 ± 0.6), eggplant (2.3 ± 0.5), and tomato (2.3 ± 0.6). No eggs were laid in bean. Females laid more eggs in pepper sachets than eggplant ($Z = 1.78$, $P = 0.0373$) or tomato sachets ($Z = 1.91$, $P = 0.028$). All other pairwise comparisons between pepper, eggplant, tomato, and pepper fruit were not significantly different.

Artificial Diets for Larval Development. *Pepper-Augmented Diets.* Total egg hatch differed significantly among treatments (Fig. 2A) ($F = 19.74$; df = 7, 72; $P < 0.0001$). Percentage of hatch was highest in pepper fruit and on moist paper towel. The best egg hatch in the artificial diet treatments was observed in the 20% pepper treatment.

When adult mass was analyzed there was no significant interaction between sex and diet but there were significant differences due to diet (Table 2) ($F = 2.77$; df = 5, 402; $P = 0.0180$) and sex ($F = 10.00$; df = 1, 402; $P = 0.0017$). Across treatments, males were heavier than females (male mass = 1.40 ± 0.02 mg, female mass = 1.31 ± 0.02 mg). Male and female mass were pooled for pairwise comparisons across diets because of the nonsignificant interaction between diet and sex. Weevils reared on standard and 5, 10, and 20% pepper-augmented artificial diet were heavier than weevils reared on pepper fruit in pairwise LSD tests. There
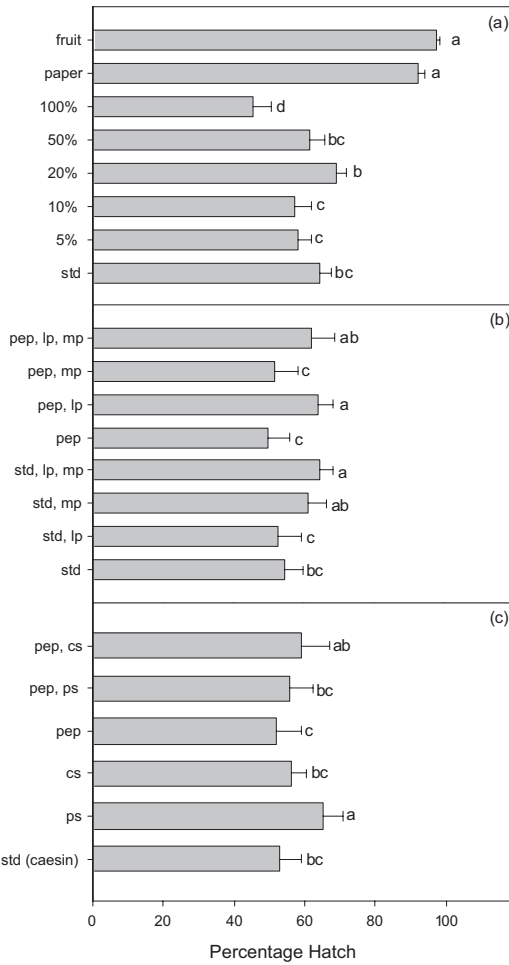
Fig. 2. Percentage of egg hatch (mean ± SE) after 72 h. In three diet assays, eggs were incubated in/on (a) pepper fruit, paper towel, and jalapeño pepper-augmented diets; (b) methyl paraben and lipid modified diets; and (c) protein alternative diets. Standard diet, std; 20% pepper-augmented diet, pep; lipid added, lp; methyl paraben removed, mp; pepperseed meal, ps; and cottonseed meal, cs. Means followed by the same lowercase letter are not different using LSD at $P = 0.05$.

was no difference in the number of days to 50% emergence, sex ratio, or survival among diet treatments (Table 2), and no correlation between adult mass and

time to 50% emergence for the standard, 10 and 50% pepper-augmented artificial diet, and fruit-reared weevils. Mass and emergence time were positively correlated in the 5% ($r = 0.42$, $P = 0.0006$) and 20% ($r = 0.28$, $P = 0.0077$) diets, accounting for 17 and 7% of the variation observed, respectively.

Methyl Paraben and Lipid-Modified Diets. The total number of eggs hatching on each diet treatment differed (Fig. 2B) ($F = 5.01$; df $= 7, 56$; $P = 0.0002$). Hatch was not improved by the addition of lipid or removal of methyl paraben alone, but the combination treatments did have higher hatch rate than the standard diet and the pepper-augmented diet. Adult dry mass was not different between diet treatments (Table 3) and there was no interaction between diet and sex. Across treatments, males were heavier than females (male mass $= 1.43 \pm 0.02$ mg; female mass $= 1.35 \pm 0.02$ mg; $F = 7.62$; df $= 1, 320$; $P = 0.0032$). There was no difference in days to 50% emergence or sex ratio across treatments (Table 3). Percentage of survival was different across treatments ($F = 6.24$; df $= 6, 49$; $P < 0.0001$) and low overall, ranging from 6.9% (20% pepper diet without methyl paraben) to a high of 36.9% (standard diet). The low survivorship in the methyl paraben removed and lipid added assay was due to fungal contamination of the diets. In this assay only the standard diet showed a significant correlation between mass and development time ($r = 0.30$, $P = 0.0247$) with changes in developmental time being responsible for ≈9% of the variation in mass.

Protein Alternative Diets. Treatment differences were detected in egg hatch (Fig. 2C) ($F = 4.13$; df $= 5, 54$; $P = 0.0030$) with the standard diet containing pepperseed as the protein source having significantly higher egg hatch than the standard diet containing casein. However, only the standard and pepper-augmented diets containing casein as a protein source had sufficient larval survival to adult emergence for data analysis (Table 4). Weevil mass was different between diet treatments ($F = 8.94$; df $= 1, 100$; $P < 0.0035$) and by sex (female mass $= 1.27 \pm 0.04$ mg, male mass $= 1.46 \pm 0.04$ mg; $F = 13.29$; df $= 1, 100$; $P = 0.0004$), but there was no interaction of sex and diet treatment. There was no difference in days to 50% emergence, survival or sex ratio between casein diets with and without freeze-dried pepper but mass was greater on the 20% pepper augmented diet (Table 4). Regression

Table 2. Developmental fitness parameters (mean ± SE) for pepper weevil neonates reared on standard diet substituted with varying amounts of jalapeño pepper powder

Diet treatment	Adult dry mass (mg)	Days to 50% emergence	Survival %	Sex ratio (f:m)
Pepper fruit	1.26 ± 0.04c	12.4 ± 0.2	58.4 ± 6.8	1.4 ± 0.6
Standard diet	1.38 ± 0.03ab	14.6 ± 0.4	50.6 ± 4.5	1.1 ± 0.2
5% pepper	1.39 ± 0.04ab	14.4 ± 0.4	47.3 ± 4.4	1.8 ± 0.5
10% pepper	1.40 ± 0.03a	15.5 ± 0.8	49.6 ± 4.4	1.9 ± 0.4
20% pepper	1.40 ± 0.03a	14.6 ± 0.2	53.9 ± 4.4	1.2 ± 0.2
50% pepper	1.30 ± 0.03bc	16.1 ± 0.4	54.1 ± 5.8	1.0 ± 0.2
100% pepper	n/a[a]	n/a	n/a	n/a

Means followed by the same lowercase letter are not significantly different ($P = 0.05$; LSD).
[a] n/a, no adults emerged.

Table 3. Developmental fitness parameters (mean ± SE) for pepper weevil neonates reared on standard or pepper-augmented diets with methyl paraben removed and lipid added

Diet treatment	Adult dry mass (mg)	Days to 50% emergence	Survival %	Sex ratio (f:m)
Standard	1.41 ± 0.04	17.6 ± 1.0	36.9 ± 3.9a	1.9 ± 0.8a
Standard + lipid added	1.39 ± 0.04	14.0 ± 0.4	24.4 ± 5.0bc	1.2 ± 0.2ab
Standard + no methyl paraben	1.34 ± 0.03	14.8 ± 0.5	35.0 ± 3.5ab	1.4 ± 0.3ab
Standard + lipid added + no methyl paraben	1.36 ± 0.03	14.6 ± 0.6	36.6 ± 4.4ab	0.9 ± 0.2ab
20% pepper	1.42 ± 0.05	17.4 ± 1.3	23.8 ± 3.9bc	1.8 ± 0.5a
20% pepper + lipid added	1.41 ± 0.04	16.1 ± 0.7	33.8 ± 5.3ab	0.7 ± 0.2bc
20% pepper + no methyl paraben	1.45 ± 0.05	17.0 ± 0.8	6.9 ± 3.5d	0.7 ± 0bc
20% pepper + lipid added + no methyl paraben	1.42 ± 0.04	15.4 ± 0.4	16.9 ± 4.4cd	1.7 ± 0.8ab

Means followed by the same lowercase letter are not significantly different ($P = 0.05$; LSD).

analysis performed on the standard and 20% pepper diets revealed no correlation between mass and development time in either treatment.

Discussion

The leaf sachet method (Calderon-Limon et al. 2002) was used to collect eggs for the diet assays reported here when extensive studies showed that weevils would not oviposit in agar or artificial diet presented in any of several different ways (Addesso 2007). Parafilm alone and green-colored sachets were not sufficient to stimulate feeding or oviposition. The shape of the substrate influenced the number of eggs laid but not feeding initiation with weevils preferring to oviposit in spherical sachets. In the field, pepper weevil adults will feed on terminal buds, leaves, and stem tissue of host plants, but females will only deposit eggs in the spherical flower buds and fruiting bodies (Elmore et al. 1934, Patrock and Schuster 1992). These field observations of the weevil explain why the weevils preferred spherical sachets to flat sachets.

Females preferred sachets made from Parafilm and bridal netting over cheesecloth. We believe the rejection of cheesecloth sachets was due to a lack of mechano- or chemoreceptor stimulation when females are in contact with the cheesecloth. When presented with cheesecloth sachets, females were sometimes observed tapping their tarsi against the surface while waving their antennae, suggesting that the weevils could smell pepper volatiles but were reluctant to initiate feeding due to the tactile quality of the cheesecloth. The netting treatment allowed the weevils to make physical contact with the leaf surface providing

sufficient stimulation and the Parafilm wax may contain compounds similar to those encountered on the waxy surface of pepper fruit. Extracts of pepper fruit surface waxes will stimulate feeding of pepper weevils on filter paper and may also help initiate oviposition (K.A., unpublished data). The Parafilm also may be able to absorb pepper leaf surface compounds, causing the females to come into contact with plant-derived oviposition stimulants (Justus et al. 2000).

Females were stimulated to feed and oviposit on leaf sachets made from known host plants: pepper, eggplant, and American black nightshade and two non-host plants within the Solanaceae, potato, and jasmine. Tomato and tropical soda apple, also members of the nightshade family, were not used as hosts in the 6 h assay, but females did lay eggs on tomato when held for 24 h. The differences we observed in sachet acceptability in the 6- and 24-h assays can be potentially attributed to several factors. First, a female's motivation to oviposit on a less suitable host may increase with time when alternatives are unavailable. Additionally, the chemistry of the excised plant material could have changed over the duration of the assay, resulting in an increase in stimulatory compounds, a decrease in repellent or deterrent compounds, or a combination of the two. These changes in the plant may thus have resulted in a change in a female's willingness to oviposit. It is clear from our results that the shape, type of covering, and species of plant used to make sachets is important for weevil acceptance. Eggs can be collected for further experimentation or for mass rearing by presenting cages of gravid female pepper weevils to round Parafilm or net sachets containing leaves of several plant species.

Table 4. Developmental fitness parameters (mean ± SE) for pepper weevil neonates reared on standard or pepper-augmented diets with casein, cottonseed, or pepperseed meal as protein sources

Diet treatment	Adult dry mass (mg)	Days to 50% emergence	Survival %	Sex ratio (f:m)
Standard (casein)	1.32 ± 0.04b	15.7 ± 0.4	30.5 ± 4.6	1.4 ± 0.4
Standard (cottonseed)	n/a	n/a	n/a	n/a
Standard (pepperseed)	n/a	n/a	n/a	n/a
20% pepper (casein)	1.47 ± 0.04a	15.6 ± 0.4	31.5 ± 2.6	1.2 ± 0.3
20% pepper (cottonseed)	n/a[a]	n/a	n/a	n/a
20% pepper (pepperseed)	n/a	n/a	n/a	n/a

Means followed by the same lowercase letter are not significantly different ($P = 0.05$; LSD).
[a] n/a, no adults emerged.

Modifications of the diet described by Toba et al. (1969) were not successful in improving survival, shortening developmental time, or increasing adult mass. Despite this fact, several important observations were made. First, egg hatch on the diets was substantially higher than observed by Toapanta (2001) (0–14%) but not as high at that observed by Toba et al. (1969) (98.6%). Our best hatch rates were between 65 and 70% and were observed in the standard and 20% pepper diets. We were able to obtain 93% hatch when eggs were incubated on moist paper towel. Removing methyl paraben from the standard diet did not have a significant impact on egg hatch, making the antifungal agent an unlikely cause of egg mortality.

The results of the pepper-augmented diet assay showed that male and female weevils reared on all artificial diets, including the standard diet, weighed more than weevils reared in naturally infested jalapeño fruit. There are two potential causes for this observation. If the artificial diets are nutritionally superior to the fruit, we would expect larger, healthier weevils to emerge from the diet (Reese and Field 1986). Alternatively, if there are inadequate levels of essential nutrients in the artificial diets the greater mass might be the result of compensatory feeding (Waldbauer and Friedman 1991, Yang and Joern 1994, Lee et al. 2004). The addition of freeze-dried pepper did not increase mass beyond that of the standard diet as would have been expected if the addition of plant feeding stimulants increased diet consumption. Males, however, were consistently heavier than females except on poor diets when male and female masses converged. There were no differences in developmental time or survival between the diet treatments with survival averaging 50% in all diets containing from 0 to 50% pepper powder. In contrast, incorporation of plant material improved these performance measures for the white pine weevil, *Pissodes strobe* (Peck) (Trudel et al. 1994), and the purple loosestrife biological control agent *Hylobius transversovittatus* Goeze (Blossey et al. 2000).

The major problems we encountered with artificial diets was their tendency to became moldy and/or to desiccate. Each well in the 48-well ELISA plates held more than enough diet to sustain a single larva and confinement in a well prevented the neonates from cannibalizing one another. Unfortunately, wells at the edge of the plates were subject to desiccation, whereas the central wells could become moldy if condensation built up in the plates resulting in larval mortality.

The original diet described by Toba et al. (1969) remains the most cost effective option for artificial rearing of pepper weevil. We recommend the use of leaf sachets contained in Parafilm or netting as oviposition substrates. Select solanaceous plants in at least three genera (*Capsicum, Solanum,* and *Nicotiana*) may be used for egg collection if pepper is not available. Testing of other species also may prove effective. Eggs should be incubated on moist paper towel for optimal hatching and neonates transferred to the diet. Future investigations into pepper weevil feeding and

oviposition stimulants may facilitate the development of a more efficient rearing system for the weevil.

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