



## Effect of avocadofurans on larval survival, growth, and food preference of the generalist herbivore, *Spodoptera exigua*

Cesar R. Rodriguez-Saona & John T. Trumble

Department of Entomology, University of California Riverside, CA 92521, USA

Accepted: November 17, 1998

**Key words:** Lepidoptera, Noctuidae, avocadofurans, *Spodoptera exigua*, avocado, idioblast, oil cell, food preference

### Abstract

We examined the effect of two avocadofurans, 2-(pentadecyl)furan and 2-(heptadecyl)furan, from avocado idioblast oil cells on maturation and larval feeding behavior of a generalist insect herbivore, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). Experiments were conducted using two larval sizes: early-stadium larvae refer to those larvae from experiments initiated with neonates while late-stadium larvae refer to those larvae from experiments initiated with third instars. In order to use selected sublethal doses for developmental and behavioral studies on early- and late-stadium larvae, log-dose probit lines were determined using diet incorporation bioassays. Both avocadofurans had similar toxicities to early-stadium larvae [ $LC_{50} = 2.2$  and  $1.9 \mu\text{moles/g}$  of diet for 2-(pentadecyl)furan and 2-(heptadecyl)furan, respectively] and late-stadium larvae ( $LC_{50} = 3.0$  and  $3.4 \mu\text{moles/g}$  of diet, respectively). In diet bioassays extending from egg hatch to adult emergence, the avocadofurans significantly prolonged larval developmental times and reduced *S. exigua* pupal weights. In 7 d no-choice bioassays initiated with cohorts of newly-molted third instars, the avocadofurans significantly reduced larval weights at various sublethal concentrations (below  $LC_{50}$  values). To test larval feeding deterrence effects of these avocadofurans, choice tests were conducted using early and older instar larvae. A significantly higher proportion of early-stadium larvae preferred control diet over diet treated with either avocadofuran at several sublethal concentrations. Similarly, choice tests with late-stadium larvae showed greater proportions of larvae on control diet than treated diet even at concentrations below the  $LC_{50}$ . Moreover, late-stadium larvae consumed significantly more of the control diet than the treated diet. Thus, the avocadofurans may act as feeding deterrents as well as toxicants in plant protection against non-adapted insect herbivores.

### Introduction

Plants produce a diversity of biologically active substances that affect the growth and development of other organisms and can provide protection against herbivory. These plant products discourage or prevent attack from non-adapted organisms and play an important role in the ecology and physiology of phytophagous insects. Although some defensive compounds may affect insect growth or physiology, and many cause toxicity (Sukumar, 1993), their primary function may be to modify behavior (e.g., Jermy, 1984; Bernays & Graham, 1988).

Avocados, *Persea americana* Mill. (Lauraceae), contain idioblast oil cells that differentiate from other cells of the same tissue in form, structure, and content (Platt & Thomson, 1992). Avocado idioblast cells are randomly distributed in the leaves, seeds, roots (Armstrong, 1964), and fruit (Platt-Aloia et al., 1983). Few studies have examined the potential role of the oil in plant defense against pathogens or herbivores. Among them, Kobilier et al. (1993) showed that the oil from these cells inhibits the growth of the fungus, *Colletotrichum gloeosporioides* Penz., and Rodriguez-Saona & Trumble (1996) documented reduced feeding of the generalist insect herbivore, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). One compound

identified from the avocado idioblast oil and probably responsible for the detrimental effects of the oil cells to fungi and insects is (12Z, 15Z)-1-acetoxy-2-hydroxy-4-oxo-heneicosa-12, 15-diene (persin) (Prusky et al., 1982; Rodriguez-Saona et al., 1997).

More recently, Rodriguez-Saona et al. (1998) identified a group of alkylfurans from the avocado idioblast cells. These compounds, which are unique to the genus *Persea*, have received the common name of avocadofurans (Kashman et al., 1969a,b; Magalhaes et al., 1970). Avocadofurans have only been tested for activity against two insect herbivores. In whole leaf extractions, Murakoshi et al. (1976) found 2-(8Z, 11Z-heptadecadienyl)furan to have no activity against silkworm larvae, *Bombyx mori* L, at a concentration of 300  $\mu\text{g/g}$  in diet. Using diet-incorporation studies, Rodriguez-Saona et al. (1998) determined 2-(pentadecyl)furan and 2-(heptadecyl)furan to be the most toxic of several avocadofurans identified from the cell oil to *S. exigua* larvae. In 7 d no-choice bioassays initiated with neonate larvae, they demonstrated that these avocadofurans inhibit *S. exigua* larval growth at sublethal concentrations.

However, no data are available on the effect of avocadofurans on insect behavior. Because the larvae of *S. exigua* are highly mobile (Smits et al., 1987) and frequently make host plant choices (Berdegue & Trumble, 1996), the primary objective of our study was to test the hypothesis that the avocadofurans could deter feeding by this generalist herbivore.

## Materials and methods

**Insects.** *Spodoptera exigua* was chosen for this study because it is a generalist herbivore with an extensive host range (Mitchell, 1979; Steiner, 1936) which includes many horticulturally important crops (Van Steenwyk & Toscano, 1981; Trumble, 1990). *S. exigua* is a major pest in California (Carson et al., 1994), where it has developed resistance to several key synthetic pesticides (Brewer & Trumble, 1994). Therefore, new effective insecticides are needed to control this insect pest. The *S. exigua* colony was maintained on artificial diet (Patana, 1969). The colony was originally collected in 1982 from Orange Co., CA, and had new genetic material added from the same area six months prior to the study. To incorporate new genetic material into the lab colony, field collected material was maintained for two generations to eliminate any potential latent virus infections. Thus, all surviving in-

sects had demonstrated an ability to readily mate under lab conditions. Adults (1:1) from both colonies were then placed in adult cages for mating and oviposition. All neonates were used within 12 h of eclosion. Cohorts of recently molted third instars were selected by collecting large numbers of late second stadium larvae 1 d prior to the test. These larvae were reared on artificial diet from egg hatch to late second instars. All experiments were conducted in environmental chambers at  $28 \pm 2^\circ\text{C}$ , a relative humidity of 75%, and L14:D10 photoperiod with fluorescent lighting.

**Insect toxicity assays.** We tested two avocadofurans: 2-(pentadecyl)furan and 2-(heptadecyl)furan. Both were synthesized as described by Rodriguez-Saona et al. (1998). Purity was greater than 95%. Bioassays were conducted to determine the lethal concentration to kill 50% of a population ( $\text{LC}_{50}$ ) of early- and late-stadium *S. exigua* larvae, for 2-(pentadecyl)furan and 2-(heptadecyl)furan, and to determine the effect of these compounds on larval development and growth. Eight concentrations were assayed against early-stadium larvae: 0, 0.5, 1, 1.5, 2, 2.5, 3, and 3.5  $\mu\text{moles/g}$  of artificial diet. Six concentrations were assayed against late-stadium larvae: 0, 2, 3, 3.5, 4, 4.5  $\mu\text{moles/g}$  of artificial diet. Treatments were prepared by diluting the toxin with 10 ml of 0.1% Tween solution (Fisher, Pittsburgh, Pennsylvania). The mixture was vortexed and then homogenized for 30 s with an ultrasonic homogenizer (Cole-Palmer, Chicago, Illinois). Untreated artificial diet was added to the mixture to produce a total of 100 g. Control diets were prepared by adding  $\approx 90$  g of artificial diet to 10 ml of Tween solution to make a total of 100 g. Control and treated diets were poured into 30 ml plastic cups ( $\approx 7$  g of diet/cup). A standardized cohort of 3rd instars (starved for 12–18 h) was developed as previously described. One larva (neonate or 3rd instar) was placed in each cup, and cups were held in an environmental chamber. Fifteen larvae were tested at each concentration, and assays were repeated 3–4 times. In bioassays initiated with neonates, for each treatment pupal weights, days to pupation, and larval mortality were recorded. In bioassays initiated with third instars, larvae were weighed prior to the experiments; mortality and larval weight were recorded after 7 d.

Growth inhibitory effects of the avocadofurans were measured using the following standard parameters: the effective concentration (EC) measures the effect of the allelochemical on weight gain, i.e., % weight reduction of larvae fed on treated diet com-

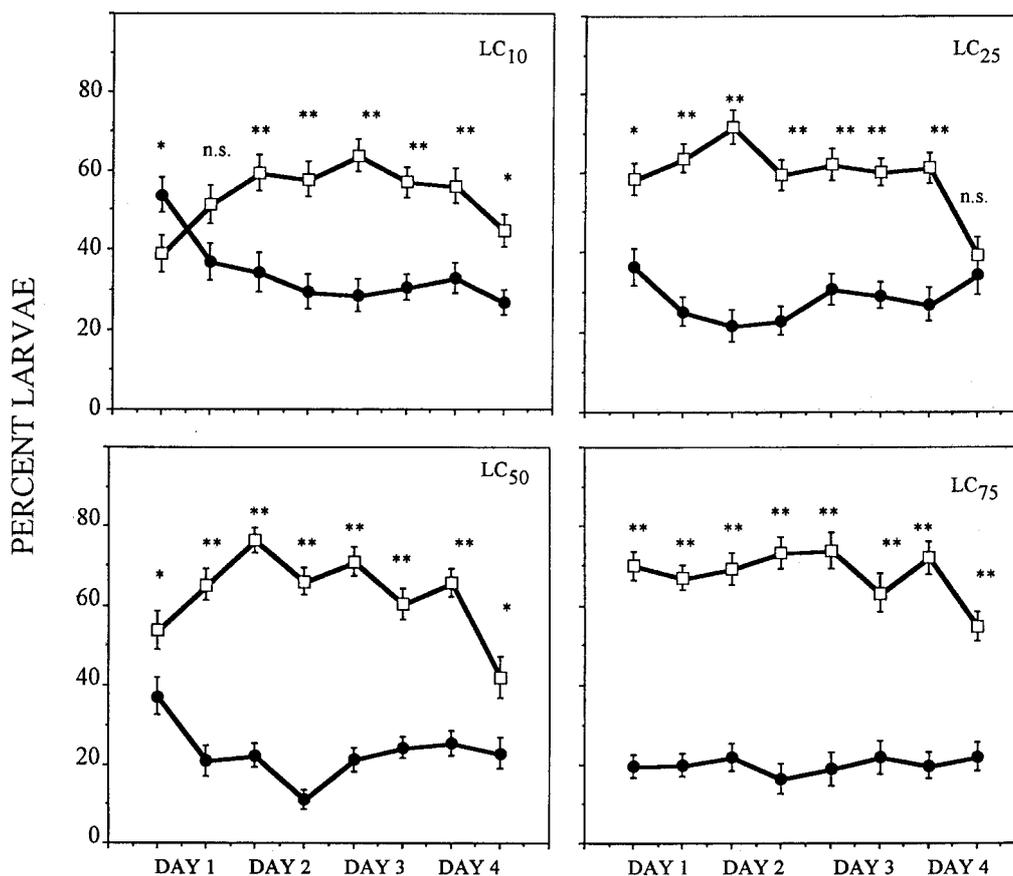


Figure 1. Percentage of early-stadia *S. exigua* on control (squares) and 2-(pentadecyl)furan treated (circles) diets over time (means  $\pm$ SE; sum does not need to be 100%). \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; n.s.,  $P > 0.05$ ; Wilcoxon signed-ranked test;  $n = 30$ .

pared to control larvae. The relative growth index (RGI) was proposed by Zhang et al. (1993). In this parameter, growth index (GI) is defined as the sum of growth stages attained by individuals under experimental conditions divided by the sum of the highest stages that would be reached in a control population. The RGI for each tested group is determined by dividing the GI of the test group by the GI of the control group. Control mortality for all bioassays was <10%.

*Choice tests with neonates.* Both avocadofurans, 2-(pentadecyl)furan and 2-(heptadecyl)furan, were tested for deterrence against early-stadia *S. exigua*. Five to seven *S. exigua* neonates were placed inside arenas (after Gould et al., 1991; as modified by Rodriguez-Saona et al., 1997). The arenas were constructed from 30 ml plastic cups with 4% agar (w/v) in the bottom, with 2 holes at opposite sides of the cup, where two 1.5 ml microcentrifuge tubes were placed. One of the tubes contained diet alone (control diet) and

the second contained the treated diet. To determine if low concentrations of avocadofurans elicit a larval response, two concentrations below the LC<sub>50</sub> were examined (at the LC<sub>10</sub> and LC<sub>25</sub>). In addition, one concentration at the LC<sub>50</sub> and one at the LC<sub>75</sub> were tested. The concentrations tested for 2-(pentadecyl)furan were 1.4, 1.8, 2.3, and 2.9  $\mu\text{moles/g}$ . Concentrations tested for 2-(heptadecyl)furan were 1.1, 1.4, 1.9, and 2.7  $\mu\text{moles/g}$ . The tests for all concentrations were run concurrently. The positions of the larvae were recorded twice/day (1 h after and 4 h before scotophase) for 4 days. Each arena was treated as a replicate and the experiment had a total of 30 replicates per concentration.

*Choice tests with third instars.* The two avocadofurans, 2-(pentadecyl)furan and 2-(heptadecyl)furan, were also tested for feeding deterrence to late-stadia *S. exigua*. Newly molted third instars (starved for 12–18 h), obtained as previously described, were placed

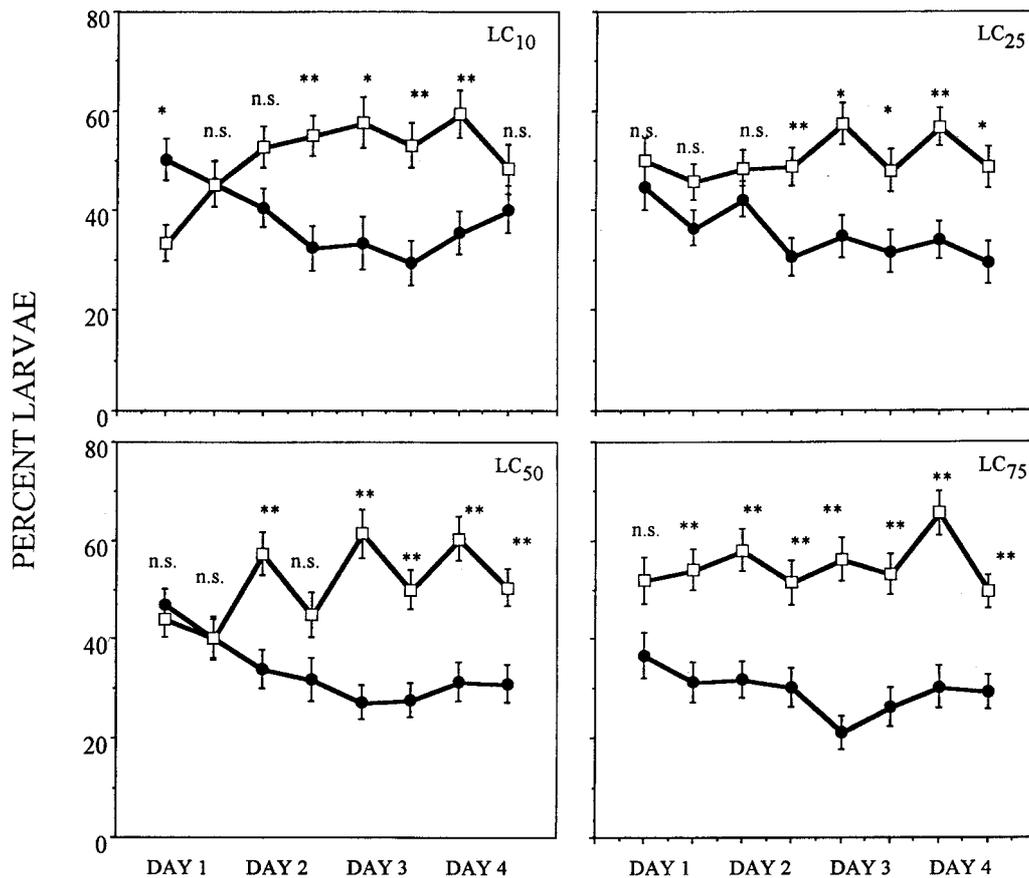


Figure 2. Percentage of early-stadia *S. exigua* on control (squares) and 2-(heptadecyl)furan treated (circles) diets over time (means  $\pm$ SE; sum does not need to be 100%). \*,  $P \leq 0.05$ ; \*\*,  $P = 0.01$ ; n.s.,  $P > 0.05$ ; Wilcoxon signed-ranked test;  $n = 30$ .

inside arenas constructed with 150 ml plastic cups with 4% agar in the bottom and with 4 holes in a cross arrangement in which four 1.5 ml microcentrifuge tubes were placed (Rodriguez-Saona & Trumble, 1996). Opposing tubes contained the same type of diet (control or treated diet). Four concentrations at the LC<sub>10</sub>, LC<sub>25</sub>, LC<sub>50</sub>, and LC<sub>75</sub> were examined. The concentrations tested for 2-(pentadecyl)furan were 2.1, 2.5, 3.0, and 3.7  $\mu$ moles/g; concentrations for 2-(heptadecyl)furan were 2.4, 2.8, 3.4, and 4.1  $\mu$ moles/g diet. The tests for all concentrations were run concurrently. The positions of the larvae were recorded twice/day (1 h after and 4 h before scotophase) for a total of 5 days. Consumption was determined by obtaining the difference between initial and final weights of the microcentrifuge tubes. Water loss for each treatment and concentration was corrected by determining weight differences of the microcentrifuge tubes in at least 2 arenas ( $n = 8$  tubes/concentration, 32 tubes per compound) held under the same conditions without

larvae. Each arena was treated as a replicate and each concentration tested had a total of 25–26 replicates.

*Data analysis.* Mortality data from toxicity bioassays were analyzed using probit analysis (Finney, 1971). Choice test data were analyzed using the number of larvae present in the control diet minus the number of larvae present in the treated diet divided by the total number of larvae at each observation. Percentage differences significantly different from zero indicated a preference either for controls or for treated diets, depending on the direction of the difference. The difference in larval preference and consumption between control and treated diets were compared to zero using Wilcoxon signed-ranked test (after Tallamy et al., 1997). Statistical analyses were performed using SuperAnova (1989). Duncan's new multiple range test was used to compare differences among treatment means (SuperAnova, 1989).

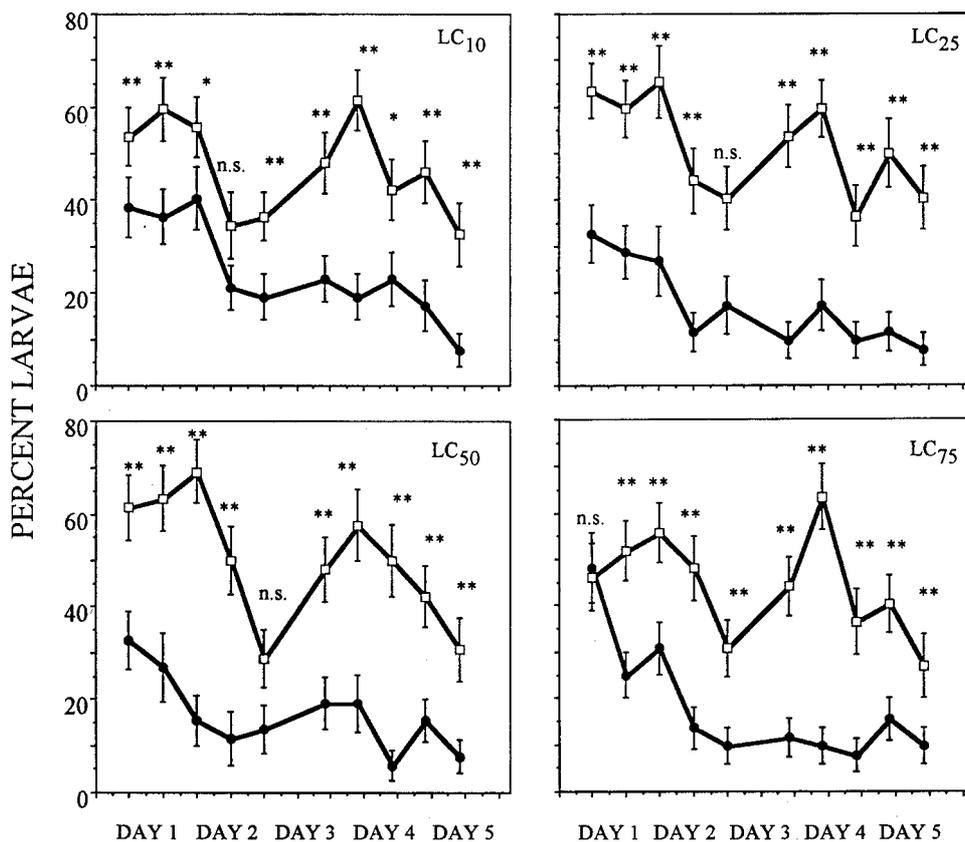


Figure 3. Percentage of late-stadia *S. exigua* on control (squares) and 2-(pentadecyl)furan treated (circles) diets over time (means  $\pm$ SE; sum does not need to be 100%). \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; n.s.,  $P > 0.05$ ; Wilcoxon signed-ranked test;  $n = 26$ .

## Results

**Insect toxicity assays.** Both avocadofurans had similar toxicities against early- and late-stadia *S. exigua*. In bioassays initiated with neonates, the  $LC_{50}$  [95% fiducial limit (FL)] for 2-(pentadecyl)furan was 2.24  $\mu\text{moles/g}$  of artificial diet (2.08–2.39  $\mu\text{moles/g}$ ) with a log dose-probit regression line slope of  $6.52 \pm 0.81$ . The  $LC_{50}$  (95% FL) for 2-(heptadecyl)furan was 1.94  $\mu\text{moles/g}$  in diet (1.53–2.47  $\mu\text{moles/g}$ ) with a log dose-probit regression line slope of  $4.84 \pm 1.21$ . In bioassays initiated with third instars, the  $LC_{50}$  for 2-(pentadecyl)furan was 3.03  $\mu\text{moles/g}$  of artificial diet (2.81–3.2  $\mu\text{moles/g}$ ) with a log dose-probit regression line slope of  $7.71 \pm 1.02$ . The  $LC_{50}$  (95% FL) for 2-(heptadecyl)furan was 3.41  $\mu\text{moles/g}$  in diet (3.19–3.60  $\mu\text{moles/g}$ ) with a log dose-probit regression line slope of  $8.63 \pm 1.32$ .

Pupal weights were significantly reduced as 2-(pentadecyl)furan ( $P < 0.001$ ,  $F = 5.75$ ,  $df = 7, 273$ ) or 2-(heptadecyl)furan ( $P < 0.001$ ,  $F =$

4.21,  $df = 6, 229$ ) concentrations increased in diet (Table 1). Compared to control larvae, 2-(pentadecyl)furan and 2-(heptadecyl)furan significantly reduced pupal weight at concentrations of 3  $\mu\text{moles/g}$  or higher in diet. In addition, larval developmental times from neonate to pupa were significantly prolonged as dietary concentrations of 2-(pentadecyl)furan ( $P < 0.001$ ,  $F = 29.54$ ,  $df = 7, 273$ ) and 2-(heptadecyl)furan ( $P < 0.001$ ,  $F = 40.57$ ,  $df = 6, 229$ ) increased (Table 1).

Late-stadium larval growth was significantly reduced even when the avocadofurans were applied at sublethal concentrations to artificial diets (Table 2). Larval growth of *S. exigua* fed avocadofuran-treated diets was reduced by more than 70% compared to the controls even at concentrations that killed only 0–20% of the larvae. Furthermore, high larval mortality (>75%) and significant growth inhibition (>90%) were obtained at concentrations of 4.0  $\mu\text{moles/g}$  or higher.

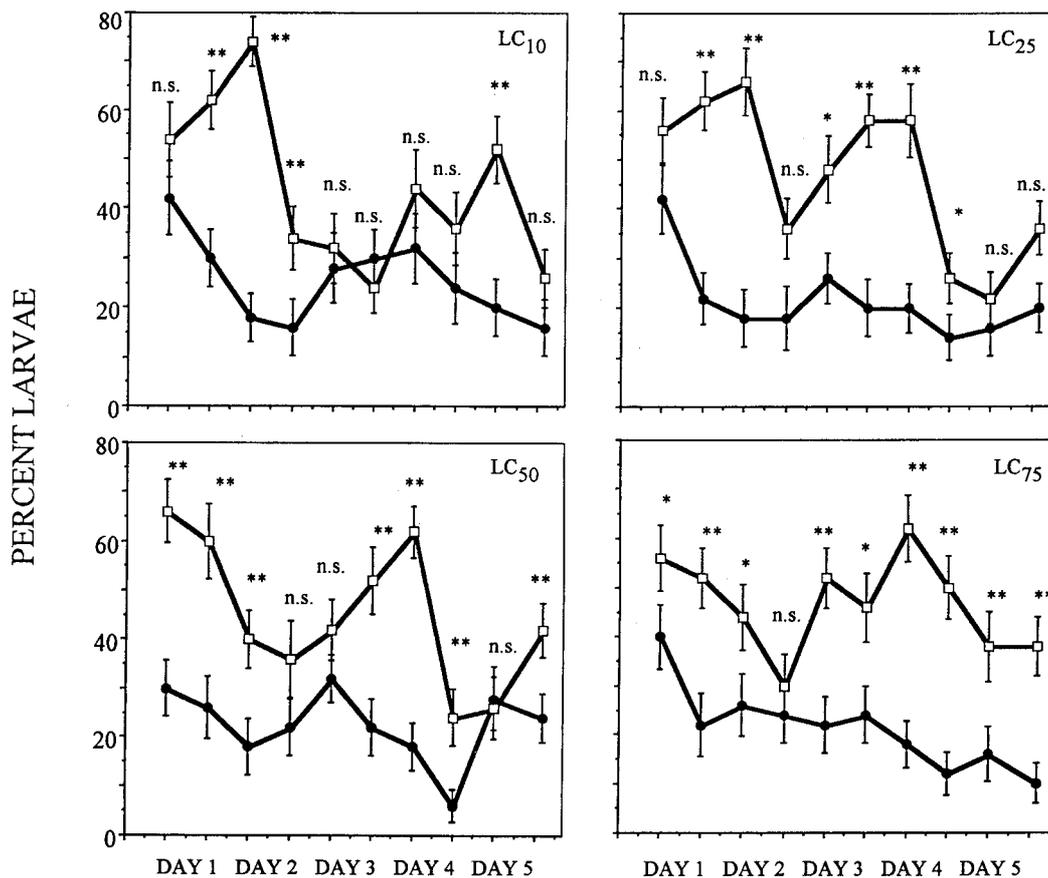


Figure 4. Percentage of late-stadia *S. exigua* on control (squares) and 2-(heptadecyl)furan treated (circles) diets over time (means  $\pm$ SE; sum does not need to be 100%). \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; n.s.,  $P > 0.05$ ; Wilcoxon signed-ranked test;  $n = 25$ .

RGI values decreased with increasing concentrations of the avocadofurans in diet (Tables 1 and 2). Based on these values, toxic and inhibitory effects of both avocadofurans [2-(pentadecyl)furan and 2-(heptadecyl)furan] were comparable. Late-stadium larvae fed concentrations of either avocadofuran at  $4.0 \mu\text{moles/g}$  or higher in diet attained  $<20\%$  of the total growth reached by control larvae.

*Choice tests with neonates.* Figures 1 and 2 show *S. exigua* larval preference in tests initiated with neonates for 2-(pentadecyl)furan and 2-(heptadecyl)furan, respectively. At the lowest concentration tested ( $\text{LC}_{10}$ ), 6 out of 8 observations showed significantly greater proportion of larvae on control diet than on diet treated with 2-(pentadecyl)furan (Figure 1). Increasing concentrations of 2-(pentadecyl)furan in diet caused higher larval avoidance, increasing the number of larvae present in control diet compared to treated diet. Sim-

ilarly, the lowest concentration tested ( $\text{LC}_{10}$ ) of 2-(heptadecyl)furan showed significantly greater proportions of larvae on control diet than on treated diet in only half of the observations. Furthermore, increasing concentrations of 2-(heptadecyl)furan in diet increased larval avoidance, causing more larvae to move to the control diet as compared to the treated diet (Figure 2).

*Choice tests with third instars.* A significantly greater proportion of late-stadium larvae preferred the control diet than diet treated with either avocadofuran at concentrations of about the  $\text{LC}_{25}$  and higher (Figures 3 and 4). At the  $\text{LC}_{10}$ , only half of the observations showed significantly higher proportions of larvae on control diet than on diet treated with either avocadofuran.

Additionally, larvae consumed more control diet than diet treated with 2-(pentadecyl)furan at all concentrations ( $\text{LC}_{10}$ : rank =  $-52$ ;  $\text{LC}_{25}$ : rank =  $0$ ;  $\text{LC}_{50}$ : rank =  $-43$ ;  $\text{LC}_{75}$ : rank =  $-52$ ;  $P < 0.01$ ;

Table 1. Larval mortality and developmental effects of 2-(pentadecyl)furan and 2-(heptadecyl)furan, in studies initiated with early-stadia *S. exigua*

Treatment ( $\mu\text{moles/g}$ )	$n^1$ (mg $\pm$ SE)	Pupal weight <sup>2</sup> (days $\pm$ SE)	Developmental times <sup>2</sup> (%)	Mortality	RGI <sup>3</sup>
2-(pentadecyl)furan					
0.5	60	118.2 $\pm$ 3.1b	15.5 $\pm$ 0.5a	5.0	1.02
1.0	60	119.2 $\pm$ 2.4b	16.1 $\pm$ 0.4a	11.7	0.94
1.5	60	105.8 $\pm$ 3.1b	16.2 $\pm$ 0.3a	26.7	0.88
2.0	60	101.9 $\pm$ 2.5ab	19.4 $\pm$ 0.5b	45.0	0.72
2.5	60	104.1 $\pm$ 2.9ab	21.2 $\pm$ 0.6b	53.3	0.61
3.0	60	93.7 $\pm$ 9.1ab	24.9 $\pm$ 1.8c	88.3	0.26
3.5	60	100.3 $\pm$ 11.0a	25.8 $\pm$ 1.9c	91.7	0.17
2-(heptadecyl)furan					
0.5	60	114.7 $\pm$ 2.9b	15.8 $\pm$ 0.4a	18.3	0.96
1.0	60	104.9 $\pm$ 2.8b	17.8 $\pm$ 0.5a	26.7	0.91
1.5	60	102.8 $\pm$ 2.8b	19.4 $\pm$ 0.6ab	48.3	0.78
2.0	60	106.7 $\pm$ 3.1b	23.1 $\pm$ 0.7bc	48.3	0.72
2.5	60	103.3 $\pm$ 4.2b	23.5 $\pm$ 1.0bc	63.3	0.49
3.0	60	86.6 $\pm$ 3.1a	24.9 $\pm$ 1.9c	88.3	0.22
3.5	60	0.0 $\pm$ 0.0c	0.0 $\pm$ 0.0d	100.0	0.00
Control					
0	60	116.3 $\pm$ 3.2b	14.6 $\pm$ 0.3a	8.4	1.00

<sup>1</sup>Number of larvae.

<sup>2</sup>Treatments with the same letter are not significantly different from each other (Duncan new multiple range test,  $P < 0.05$ ).

<sup>3</sup>After Zhang et al. (1993);  $i_{\text{max}} = 6$  (pupal stage), the highest attained stage by the insects in the controls.

Figure 5A). Similarly, larvae ate more control diet than diet treated with 2-(heptadecyl)furan at all concentrations (LC<sub>10</sub>: rank = -90; LC<sub>25</sub>: rank = -1; LC<sub>50</sub>: rank = -1; LC<sub>75</sub>: rank = -18;  $P = 0.05$ ; Figure 5B).

## Discussion

Although secretory idioblast cells containing oils are reported to occur in several plant families, their content is not usually specified (Baas & Gregory, 1985). However, Baas & Gregory (1985) suggest that, in general, the chemistry of their secretion is composed mainly of terpenes, fats, and flavonoids. For example, Mariani et al. (1989) reported insecticidal sesquiterpene lactones in the idioblast oil of *Liriodendron tulipifera* L. and Maron & Fahn (1979) indicated possible occurrence of monoterpenes in oil cells of *Laurus nobilis* L.

Avocado idioblast cells compose approximately 2% of the tissue volume and are characterized by a single large drop of oil filling the cell (Cum-

mings & Schroeder, 1942). Preliminary histochemical tests indicated the presence of alkaloids and sesquiterpene hydroperoxides, and, possibly, other terpenes in the oil cells (Platt & Thomson, 1992). In the present study, we report a new role for a previous described class of compounds from these cells. Although the avocadofurans were first reported by Kashman (1969a,b), little is known of their effects against insect herbivores and no previous studies have shown antifeedant effects. Murakoshi et al. (1976) tested 2-(8Z, 11Z-heptadecadienyl) furan against *B. mori*, however this compound had little insecticidal activity. More recently, other avocadofurans were tested for activity against *S. exigua* larvae (Rodriguez-Saona et al., 1998). Of them, 2-(pentadecyl)furan and 2-(heptadecyl)furan had the greatest toxicity. Rodriguez-Saona et al. (1998) in bioassays initiated with neonates, reported an LC<sub>50</sub> of 3.7 and 3.9  $\mu\text{moles/g}$  in diet for 2-(pentadecyl)furan and 2-(heptadecyl)furan, respectively. Their higher LC<sub>50</sub> values (>50%) may be due to a shorter (7 d) bioassay used, as they also reported that the surviving

Table 2. Larval mortality and developmental effects of 2-(pentadecyl)furan and 2-(heptadecyl)furan, in studies initiated with late-stadia *S. exigua*

Treatment ( $\mu$ moles/g)	$n^1$	7-d Larval weight <sup>2</sup> (mg $\pm$ SE)	7-d Mortality (%)	EC <sup>3</sup> (%)	RGI <sup>4</sup>
2-(pentadecyl)furan					
2.0	45	52.9 $\pm$ 7.9b	17.8	75.1	0.74
3.0	45	17.6 $\pm$ 3.2ab	35.5	91.7	0.50
3.5	45	0.7 $\pm$ 2.5a	71.1	94.9	0.23
4.0	45	5.9 $\pm$ 1.8a	86.7	97.2	0.07
4.5	45	13.7 $\pm$ 1.2a	93.3	93.5	0.05
2-(heptadecyl)furan					
2.0	45	54.1 $\pm$ 6.3b	4.4	74.6	0.85
3.0	45	18.3 $\pm$ 2.0ab	35.6	91.4	0.48
3.5	45	11.3 $\pm$ 1.3a	48.9	94.7	0.39
4.0	45	8.5 $\pm$ 1.9a	77.8	95.9	0.17
4.5	45	7.4 $\pm$ 1.7a	84.4	96.5	0.09
Control					
0	45	212.8 $\pm$ 14.6c	2.2	100.0	1.00

<sup>1</sup>Number of larvae.

<sup>2</sup>Treatments with the same letter are not significantly different from each other (Duncan new multiple range test,  $P < 0.05$ ).

<sup>3</sup>(control weight – treatment weight)/control weight  $\times 100$ .

<sup>4</sup>After Zhang et al. (1993);  $i_{\max} = 5$ , the highest attained instar by the insects in the controls.

larvae fed with avocadofuran-treated diets had  $>90\%$  weight loss compared to controls.

We showed in no-choice bioassays that 2-(pentadecyl)furan and 2-(heptadecyl)furan reduce late-stadium larval growth and extended larval developmental times, at sublethal concentrations (Tables 1 and 2). In bioassays initiated with neonates, the GI for *S. exigua* larvae fed either avocadofuran at 3  $\mu$ moles/g was reduced by 1/4 of the controls, while the GI for late-stadium larvae was reduced by 1/2 of the controls at the same concentration (Tables 1 and 2).

Choice bioassays with early- and late-stadium larvae indicated that *S. exigua* larvae have the capacity to detect and avoid diet treated with 2-(pentadecyl)furan and 2-(heptadecyl)furan at below lethal concentrations. However, discriminatory responses were not always immediate particularly at the lower concentrations, reflecting a period of larval probing and testing of diets (Figures 1 and 2). Although we observed larvae making contact with the treated diet, no actual sensory or behavioral mechanism of action of the compounds can be determined on the basis of the observation methods employed. Another pattern observed was a decline in deterrence with time, especially at the lower concentrations. We attribute this

response to the capability of larger larvae to tolerate the treated diet or to larval habituation.

In choice bioassays started with early third instars, we observed two decreases in the percent of larvae on control diet. The first occurred at the end of day 2 through early day 3, the second during late day 4 through early day 5. These movements away from control diets corresponded to molts to the 4th and 5th instars, respectively. Although this movement represents a wandering behavior just prior to the molt, *S. exigua* larvae apparently must re-learn the location of the preferred diet. Shortly after the molt, the proportion of larvae found on control diets increased to near premolt levels (Figures 3 and 4).

Our results indicate a concentration dependent effect on larval behavior by the two forms of avocadofurans tested that was consistent with the relative amount of diet consumed. The proportions of larvae on control diet compared to treated diet increased at concentrations above the  $LC_{10}$ . This increase was also evident in the differences in consumption between control and avocadofuran-treated diets; larvae ingested less treated diet and more of the control diets at concentrations higher than the  $LC_{10}$  (Figure 5).

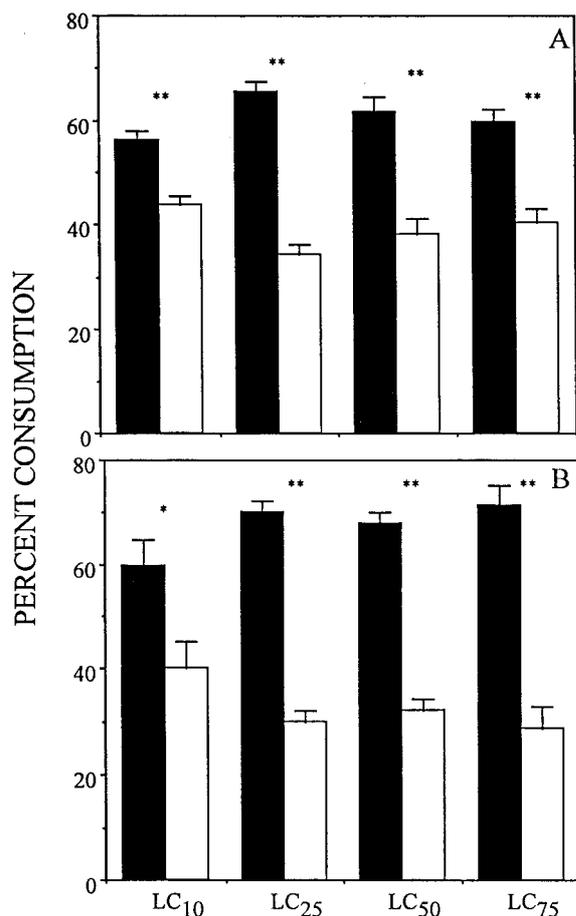


Figure 5. Percent consumption by 3rd- to 5th-instar *S. exigua* (means  $\pm$ SE), given a choice between control (black bars) and diets treated with 2-(pentadecyl)furan (A) and 2-(heptadecyl)furan (B) (white bars). \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ , Wilcoxon signed-ranked test.

To date, the level of specific avocadofurans present in avocados is unknown. However, based on data provided in Rodriguez-Saona et al. (1998), the estimated production for 1-(pentadecyl)furan was below  $500 \mu\text{g/g}$  (or  $<2.0 \mu\text{moles/g}$ ) of tissue. This concentration can deter early stadium feeding but might not have a behavioral effect on later instars. 2-(Heptadecyl)furan was found at a lower concentration,  $<50 \mu\text{g/g}$  of tissue. Nevertheless, because: (1) these concentrations may change in space and time; (2) other compounds present in the idioblast oil may have larval feeding deterrent effects (Rodriguez-Saona et al., 1997); (3) a previous study indicated that some oil components may act synergistically (C.R. Rodriguez-Saona, unpubl.); and (4) the mode of action and the mechanism through which avocadofurans

affect feeding behavior of the larvae remain unknown, additional studies will be necessary to document the collective deterrent effects of the oil.

Currently, the potential of the avocadofurans as new class of insecticides is under investigation. Recent *in vitro* studies showed that furan-containing compounds extracted from avocado seed oils may cause lysyl oxidase (protein-L-lysine: oxidoreductase) inhibition, and may serve as an antifibrotic drug in diseases involving excess collagen and elastin deposition (Rosenblat et al., 1995). Thus, more research on mammalian toxicity and phytotoxicity will be required before these compounds are considered for insect control.

Furthermore, *S. exigua* feeds on over 35 host plants around the world (Steiner, 1936), however avocados are not listed as a suitable host plant. The present results and those reported by Rodriguez-Saona & Trumble (1996) suggest that the avocado idioblast cell oil might be one possible reason why this polyphagous herbivore has not adapted to feed on avocados.

#### Acknowledgements

We are thankful to Kristina White, Jessica Young, William Carson, and Gregory Kund for their assistance in the laboratory. Also, we are grateful to Dr David F. Maynard and Joshua C. Hoerger, Jefferson M. Caparas, and Theresa A. Aguilar (Department of Chemistry, California State University, San Bernardino) for the synthesis of the avocadofurans. Drs Robert Beaver, Kathryn Platt, and William Thomson provided helpful advice. We appreciate the critical reviews of early drafts of this manuscript by Drs. David Morgan, Stuart Reitz, Daniel Hare, Jocelyn Millar, and Kirk Visscher. This research was supported in part by the California Celery Research Advisory Board, the California Tomato Commission, and the University of California, Riverside.

#### References

- Armstrong, W. W. Jr., 1964. Distribution of oil cells in *Persea*. Masters Thesis. University of California, Riverside, 40 pp.
- Baas, P. & M. Gregory, 1985. A survey of oil cells in the dicotyledons with comments on their replacement by and joint occurrence with mucilage cells. *Israel Journal of Botany* 34: 167-186.
- Berdegue, M. & J. T. Trumble, 1996. Effects of plant chemical extracts and physical characteristics of *Apium graveolens* and *Chenopodium murale* on host choice by *Spodoptera exigua* larvae. *Entomologia Experimentalis et Applicata* 78: 253-262.

- Bernays, E. A. & M. Graham, 1988. On the evolution of host specificity in phytophagous arthropods. *Ecology* 69: 886–892.
- Brewer, M. J. & J. T. Trumble, 1994. Beet armyworm resistance to fenvalerate and methomyl: resistance variation and insecticide synergism. *Journal of Agricultural Entomology* 11: 291–300.
- Carson, W. G., K. K. White & J. T. Trumble, 1994. Impact of insecticides on insects of tomatoes, 1993. *Arthropod Management Tests* 19: 148–149.
- Cummings, K. & C. A. Schroeder, 1942. Anatomy of the avocado fruit. *California Avocado Society*. 1942: 56–64.
- Finney, D. J., 1971. *Probit Analysis*. Cambridge University Press, Cambridge, U.K.
- Gould, F., A. Anderson, D. Landis & H. van Mellaert, 1991. Feeding behavior and growth of *Heliothis virescens* larvae on diets containing *Bacillus thuringiensis* formulations or endotoxins. *Entomologia Experimentalis et Applicata* 58: 199–210.
- Jerny, T., 1984. Evolution of insect/host plant relationships. *American Naturalist* 124: 609–630.
- Kashman, Y., I. Néeman & A. Lifshitz, 1969a. New compounds from avocado pear. *Tetrahedron* 25: 4617–4631.
- Kashman, Y., I. Néeman & A. Lifshitz, 1969b. Six new C17-olefinic and acetylenic oxygenated compounds from avocado pear. *Israel Journal of Chemistry* 7: 173–176.
- Kobiler, I., D. Prusky, S. Midland, J. J. Sims & N. T. Keen, 1993. Compartmentation of antifungal compounds in oil cells of avocado fruit mesocarp and its effect on susceptibility to *Colletotrichum gloeosporioides*. *Physiological and Molecular Plant Pathology* 43: 319–328.
- Magalhaes, A. H., D. T. Coxon, C. P. Falshaw, W. O. Godtfredsen & W. D. Ollis, 1970. The avocatin – a new class of natural products. *Anais da Academia Brasileira de Ciencias* 42(suppl.): 45–48.
- Mariani, P., E. M. Cappelletti, D. Campoccia & B. Baldan, 1989. Oil cell ultrastructure and development in *Liriodendron tulipifera* L. *Botanical Gazette* 150: 391–396.
- Maron, R. & A. Fahn, 1979. Ultrastructure and development of oil cells in *Laurus nobilis* L. leaves. *Botanical Journal of the Linnean Society* 78: 31–40.
- Mitchell, E. R., 1979. Migration by *Spodoptera exigua* and *Spodoptera frugiperda* North American style. In: R. L. Rabb & G. G. Kennedy (eds.), *Movement of Highly Mobile Insects: Concepts and Methodology*. University Graphics, North Carolina State Univ., Raleigh, N.C., pp 386–395.
- Murakoshi, S., E. Kanagawa, A. Isogai, C. F. Chang, T. Kamikado, A. Sakurai & S. Tamura, 1976. Effects of two components from the avocado leaves (*Persea americana* Mill.) and the related compounds on the growth of silkworm larvae, *Bombyx mori* L. *Japanese Journal of Applied Entomology and Zoology* 20: 87–91.
- Patana, R., 1969. Rearing cotton insects in the laboratory. US Department of Agriculture Product Research Report 108, 6 p.
- Platt, K. A. & W. W. Thomson, 1992. Idioblast oil cells of avocado: distribution, isolation, ultrastructure, histochemistry, and biochemistry. *International Journal of Plant Sciences* 153: 301–310.
- Platt-Aloia, K. A., J. W. Oross & W. W. Thomson, 1983. Ultrastructure study of the development of oil cells in the mesocarp of avocado fruit. *Botanical Gazette* 144: 49–55.
- Prusky, D., N. T. Keen, J. J. Sims & S. L. Midland, 1982. Possible involvement of an antifungal diene in the latency of *Colletotrichum gloeosporioides* on unripe avocado fruits. *Phytopathology* 72: 1578–1582.
- Rodriguez-Saona, C. & J. T. Trumble, 1996. Toxicity, growth, and behavioral effects of an oil extracted from idioblast cells of the avocado fruit on the generalist herbivore beet armyworm (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 89: 1571–1576.
- Rodriguez-Saona, C., J. G. Millar, D. F. Maynard & J. T. Trumble, 1998. Novel antifeedant and insecticidal compounds from avocado idioblast cell oil. *Journal of Chemical Ecology* 24: 867–890.
- Rodriguez-Saona, C., J. G. Millar & J. T. Trumble, 1997. Growth inhibitory, insecticidal, and feeding deterrent effects of (12Z, 15Z)-1-acetoxy-2-hydroxy-4-oxo-heneicosa-12, 15-diene, a compound from avocado fruit, to *Spodoptera exigua*. *Journal of Chemical Ecology* 23: 1819–1831.
- Rosenblat, G., H. M. Kagan, M. A. Shah, G. Spiteller & I. Neeman, 1995. Chemical characterization of lysyl oxidase inhibitor from avocado seed oil. *Journal of the American Oil Chemists' Society* 72: 225–229.
- Smits, P. H., M. C. van Velden, M. van de Vrie & J. M. Vlak, 1987. Feeding and dispersion of *Spodoptera exigua* larvae and its relevance for control with a nuclear polyhedrosis virus. *Entomologia Experimentalis et Applicata* 43: 67–72.
- Steiner, P., 1936. Beiträge zur Kenntnis der Schädlingfauna Kleinasiens III. *Laphygma exigua* Hb., ein Großschädling der Zuckerrübe in Anatolien. *Zeitschrift für Angewandte Entomologie* 23: 177–222.
- Sukumar, K., 1993. Role of allelochemicals in the control of phytophagous insects. In: T. N. Ananthkrishnan & A. Raman (eds.), *Chemical Ecology of Phytophagous Insects*. International Science Publisher: New York, USA, pp. 82–89.
- SuperAnova, 1989. Abacus Concepts Inc., Berkeley, CA.
- Tallamy, D. W., J. Stull, N. P. Ehresman, P. M. Gorski & C. E. Mason, 1997. Cucurbitacins as feeding and oviposition deterrents to insects. *Environmental Entomology* 26: 678–683.
- Trumble, J. T., 1990. Vegetable insect control with minimal use of insecticides. *HortScience* 25: 159–164.
- Van Steenwyk, R. A. & N. C. Toscano, 1981. Relationship between lepidopterous larval density and damage in celery and celery plant growth analysis. *Journal of Economic Entomology* 74: 287–290.
- Zhang, M., S. K. Chaudhuri & I. Kubo, 1993. Quantification of insect growth and its use in screening of naturally occurring insect control agents. *Journal of Chemical Ecology* 19: 1109–1118.