

Toxicity, Growth, and Behavioral Effects of an Oil Extracted from Idioblast Cells of the Avocado Fruit on the Generalist Herbivore Beet Armyworm (Lepidoptera: Noctuidae)

CESAR RODRIGUEZ-SAONA AND JOHN T. TRUMBLE

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

J. Econ. Entomol. 89(6): 1571-1576 (1996)

ABSTRACT An oil extracted from idioblast cells in avocado fruit was examined for toxicity, growth inhibition, and behavioral effects on the generalist herbivore *Spodoptera exigua* (Hübner). In diet incorporation studies, the oil was toxic to both early and late instars. At concentrations of 0.80% or higher, 100% mortality was observed within 7 d. Larval weights of 3rd instars were significantly reduced after feeding for 5 d on a diet containing 0.30% oil as compared with a control diet. At lower concentrations of 0.05, 0.10, and 0.20%, no significant differences in developmental times from neonate to adult emergence were observed. The oil also acted as a feeding deterrent. Choice tests initiated with neonates showed a significantly greater proportion of larvae on control diet rather than oil-containing diet for concentrations of 0.10% or greater. Similar results were obtained for choice tests initiated with 3rd instars for oil concentrations of 0.20% or greater. In these tests the larvae consumed significantly more control diet than treated diet. The potential of the oil for development as a pest management tool is discussed.

KEY WORDS *Spodoptera exigua*, avocado, oil, idioblast cells, biorational pesticide

NATURAL PLANT COMPOUNDS that have proven to be effective in insect control include precocenes from *Ageratum houstonianum* Mill. leaves, ryania from *Ryania speciosa* Vahl., caffeine, and nicotine, among others (Coats 1994). A subset of these compounds, antifeedants, offers a potential approach for pest management by rendering plants unattractive or unacceptable to pest insects (Saxena and Khan 1987). Azadirachtin, a complex tetranortriterpenoid from Neem tree, *Azadirachta indica* A. Juss, with well-documented antifeedant properties (Butterworth and Morgan 1971, Ruscoe 1972, Rembold et al. 1980, Redfern et al. 1981, Simmonds et al. 1995), may be the best known compound in this category. Crude extracts of leaves, seeds, and other elements of this plant are used in many developing countries as protectants against plant pests (i.e., Heyde et al. 1984). Extracts of the seeds have been registered or tested for use on a variety of crops (Meister 1995).

Unfortunately, these neem-based products have not proven efficacious for controlling *Spodoptera exigua* (Hübner), a generalist feeder and the major pest of many vegetables in California (Carson et al. 1994, Schuster 1995). *S. exigua* has a long history as a key herbivore on lettuce (Oatman and Platner 1972), celery (Van Steenwyk and Toscano 1981), and tomato (Lange and Bronson 1981). As a result of extensive pesticide application, this species has developed resistance in some locations to several key synthetic pesticides (Brewer and Trum-

ble 1991, 1994). Therefore, in an attempt to find alternatives for *S. exigua* control, the insecticidal activity of an oil extracted from idioblast cells of the avocado fruit, *Persea americana* Mill., was evaluated. Although *S. exigua* attacks >35 crops around the world (Steiner 1936), it has not been reported to feed on avocados.

Idioblast oil cells are secretory structures that differentiate in the parenchyma of many taxonomically diverse angiosperm species (West 1969). They occur in the leaves and other organs of dicotyledonous plant species in numerous families (Postek and Tucker 1983, Baas and Gregory 1985). Idioblast cells, isolated from ripe avocado fruit, contain an oil that differs from other oils present in the fruit; histochemical tests indicated the presence in the cells of alkaloids and sesquiterpene hydroperoxides and, possibly, other terpenes (Platt and Thomson 1992). Subsequent studies by Kobilier et al. (1993) indicated that the oil contains at least 2 constituents that have antifungal activity. Preliminary tests (J.T.T., unpublished data) suggested that oil from idioblast cells could affect the feeding activity of *S. exigua*.

Therefore, we examined the hypothesis that the oil could act to provide protection for plants from generalist herbivores. To test this hypothesis, bioassays were conducted to determine the effect of the oil against *S. exigua*. Specific objectives were to assess the toxicity of the idioblast oil against both early and late instars, to document any effects

of the oil on insect growth, and determine if the oil affected larval behavior.

Materials and Methods

Extraction of the Oil. Avocados, *P. americana* variety Hass, were harvested from trees grown at the University of California's South Coast Research and Extension Center in Santa Ana. The oil was isolated from idioblast cells in soft, ripe fruit because the extraction of the cells is easier with ripe avocados. During ripening of the fruit, the activity of the wall hydrolytic enzymes (cellulase and polygalacturonase) increases substantially (Awad and Young 1979), but the suberized walls of the idioblast cells are immune to the activity of these enzymes and remain intact (Platt and Thomson 1992). Idioblast cells were extracted, using a modification of the technique described by Platt and Thomson (1992). The mesocarp from avocados was excised and placed in a blender with water. The tissue was homogenized until liquid. The blended mixture was then sieved through a 149- μ mesh screen followed by a 106- μ mesh screen. The substrate on the upper surface of the 106- μ mesh was washed with tap water until clean, and the filtrate was collected in large Erlenmeyer flasks. The filtrate, which was green because of the presence of large amounts of chlorophyll, was then poured through a 63- μ mesh screen. The idioblast cells remained on the upper surface of this screen. The cells were washed with tap water, until they were straw colored (pale brown), and then transferred to a flask.

To obtain the oil from the idioblast cells, a chloroform and methanol mixture was added to the flask containing the cells at a ratio of 1:2:1.8 (chloroform:methanol:cells). The mixture was stirred for 15–30 min before more chloroform was added to obtain a final ratio of 2:2:1.8. The mixture was stirred for at least 5 min more. The resulting material was poured into 30-ml centrifuge tubes and spun at $1.12\text{--}4.47 \times g$ for 35 min. Centrifugation resulted in 3 solvent layers and the pellet. The more polar components that correspond to the 2 top layers were pipetted off. The bottom layer, containing the lipids dissolved in chloroform, was poured through filter paper No. 1 (Whatman, Maidstone, U.K.) to remove any pelleted material that might remain. The solvent then was evaporated using a rotary evaporator. The oil was refrigerated at 4°C until needed. An average of 7.3 ± 1.5 g of oil were extracted per kilogram of ripe avocados.

Insects. *S. exigua* larvae used in all experiments were maintained on artificial diet (modified from Patana [1969]) at $28 \pm 2^\circ\text{C}$ and a photoperiod of 14:10 (L:D) h. The colony was originally collected from Orange County, California, in 1982 and had new genetic material added within 12 mo before the study. All neonates were used within 12 h of eclosion. Cohorts of recently molted 3rd instars

were created by collecting large numbers of late 2nd instars ≈ 12 h before the test, and randomly assigning those that molted to 3rd instars to the various treatments. Unless otherwise stated, incubator conditions were $28 \pm 2^\circ\text{C}$ and a photoperiod of 14:10 (L:D) h for all experiments.

Toxicity Study. The oil extracted from idioblast cells of ripe avocado fruit was examined for effect on mortality of *S. exigua* using a diet bioassay. For tests initiated with neonates, a 10% stock solution was prepared by mixing 1 ml of the oil with 9 ml of 0.1% Tween-80 solution (Fisher, Pittsburgh, PA) using an ultrasonic homogenizer (Cole-Parmer, Chicago, IL). Seven different concentrations (0, 0.05, 0.10, 0.15, 0.20, 0.25, and 0.30% vol:vol) were prepared by diluting the 10% stock solution with 0.1% Tween solution to make a final volume of 2 ml. Untreated diet was then added to the mixture to produce a total of 10 g. The mixture was vortexed for 3 min. Control and treated diets were poured into 24-well bioassay trays (C-D International, Pitman, NJ). Each bioassay tray is divided into 8 groups of 16 wells. One neonate was added per well, and the trays were placed in an incubator. Thirty-two neonates, occupying 2 of the 16 well groups, were used for each concentration. A tray could hold a total of 4 treatments; 2 trays per replicate were filled to test all concentrations. This complete assay was repeated 4 times. Mortality was recorded after 7 d. Control mortality was $<10\%$ in all bioassays.

For tests initiated with 3rd instars, dietary concentrations were prepared as previously described; enough diet was made to fill 30-ml plastic cups with ≈ 10 ml of diet. A standardized cohort of 3rd instars was developed as previously described. One 3rd instar was placed in each cup and cups were held in an incubator. Twenty larvae were tested at each concentration, and assays were repeated 4 times. Mortality was recorded after 7 d. Previous tests with 3rd instars indicated that the LC_{50} occurs within 0.3 and 0.8% of oil in diet (J.T.T., unpublished data). Therefore, a control diet and 3 concentrations between that range (0.30, 0.45, and 0.75%) were examined. Control mortality was $<10\%$.

Growth and Development. The effect of the oil on larval weight was tested using a standardized cohort of 3rd instars. Treated and control diets were prepared as previously described, but the treated diets were prepared with a sublethal concentration of 0.30% of oil. Diet with a 0.30% concentration of oil was selected because survivorship was high ($>97.0\%$) in both the treatment and control. Forty larvae per treatment were placed individually in 30-ml diet cups filled with ≈ 10 ml of diet, and cups were held in an incubator. Larval weights were recorded before initiation of the bioassays and after 5 d.

We also tested the effect of the oil on larval development for 40 larvae per treatment at 4 dietary concentrations: 0, 0.05, 0.10, and 0.20% of oil in

diet. All diets were prepared as previously described. Developmental time was recorded from egg hatch to adult emergence. Mortality was recorded daily and larval weight was recorded 7 d after the initiation of the experiment. Diets were replaced every 2 or 3 d, and all larvae were held in an incubator.

Larval Behavior Studies. Larval choice between treated and control diets was examined using a methodology modified from Gould et al. (1991). Five neonates were placed inside an arena constructed of 30-ml plastic cups lined with 4% agar (wt:vol) and with 2 holes at opposite sides of the cup where two 1.5-ml polypropylene microcentrifuge tubes were placed. One of the tubes contained the control diet alone and the other contained the treated diet. The tubes were completely filled with the diet. To determine if low concentrations of oil could elicit a larval response, 2 concentrations below the LC_{50} were tested (0.05 and 0.10%). In addition, 1 concentration (0.20%) above the LC_{50} was examined. The arenas were held in an incubator. The position of the larvae was recorded twice a day for a total of 4 d. Each arena was treated as a replicate, and the experiment had a total of 27, 40, and 38 replicates for the 0.05, 0.10, and 0.20% concentrations, respectively.

Choice tests were also performed with 3rd instars. Two 3rd instars were placed inside arenas constructed with 150-ml plastic cups lined with 4% agar and 4 holes in opposing arrangement where four 1.5-ml microcentrifuge tubes were placed. Opposing tubes contained the same type of diet (control or treated diet). The concentrations tested were 0.20, 0.40, and 0.60% of oil in diet. As in the neonate tests, 2 concentrations below and 1 concentration above the LC_{50} were examined. A standardized cohort of 3rd instars was produced using the technique described previously. Replicates for all concentrations were run simultaneously in an incubator. The positions of the larvae were recorded twice a day for a total of 4 d. Consumption data were obtained by calculating the difference between initial and final weights of the microcentrifuge tubes. Water loss for each concentration was controlled for by measuring weight differences in arenas without larvae ($n = 12$ microcentrifuge tubes) and subjected to the same conditions as the others. Each arena was treated as a replicate and a total of 25 replicates were used per concentration.

Data Analysis. Mortality data from toxicity bioassays were analyzed using probit analysis (Finney 1971). Data from choice tests (proportion larvae on each diet) were arcsine square-root transformed. Transformed preference data for neonates and 3rd instars were analyzed with the paired-sample t -test (Horton 1995) using the PROC UNIVARIATE procedure (SAS Institute 1990).

Results

Toxicity Study. The idioblast oil proved toxic for both size classes of larvae. The LC_{50} (95% FL)

for early instars was 0.16% (0.15–0.17%) of oil in the diet, with a log dose-probit regression line slope of 4.05 ± 0.32 . The LC_{50} (95% FL) for late instars was 0.51% (0.44–0.61%) of oil in diet, with a log dose-probit regression line slope of 5.84 ± 1.28 .

Growth and Development. Initial weights among 3rd instars were not different at the beginning of the test (control = 15.93 ± 0.5 mg, 0.30% oil diet = 15.78 ± 0.4 mg; $t = -0.24$, $P = 0.81$). However, after 5 d on the diets, weights of larvae on the 0.30% oil diet averaged significantly less than for larvae on control diet (control = 231.91 ± 7.8 mg, 0.30% oil diet = 28.95 ± 1.6 mg; $t = -25.3$, $P < 0.001$).

Developmental time from 1st instar to adult emergence was not significantly different among survivors feeding on diets with 0.05, 0.10, or 0.20% oil as compared with controls ($F = 1.93$; $df = 3, 87$; $P = 0.13$). Larvae on control diet developed in 29.5 ± 0.5 d, whereas larvae on the highest concentration tested developed in 30.5 ± 0.6 d.

Larval Behavior Studies. Choice tests initiated with neonates at the lowest concentration tested (0.05%) showed a significantly greater proportion of larvae on control diets in 3 of the 8 observations (Fig. 1). At concentrations of 0.10% or above, larvae significantly preferred control diet over diets incorporating the avocado oil in at least 6 of 8 observations (Fig. 1).

A significantly greater proportion of late instars preferred the control diet than treated diet at all the concentrations tested (Fig. 2). A decrease in the percentage of larvae on control diets observed at the 4th observation in all treatments corresponded to a molt to the 4th instar (Fig. 2). Following this molt, the proportion of larvae found on control diets increased to near premolt levels. In addition, larvae consumed significantly more control diet than treated diet at all concentrations tested (0.20% diet, $t = -12.9$; 0.40% diet, $t = -14.3$; 0.60% diet, $t = -2.7$; $df = 24$; $P < 0.001$) (Fig. 3).

Discussion

The oil extracted from idioblast cells in the avocado fruit appears to have potential as a natural insecticide against the generalist herbivore, *S. exigua*. Not only was the oil toxic at relatively low levels, but it had substantial effects on weight gain and behavior of the larvae at sublethal levels. Choice tests indicated that both early and late instars avoided the treated diet. This suggests that the idioblast oil is acting as a feeding deterrent in our laboratory studies. Another possible explanation is that the larvae become intoxicated after ingestion of small amounts of the oil-containing diet, stop feeding, and start wandering. After they recover and encounter the control diet, they stop wandering and feed and remain on the control diet. Kobiler et al. (1993) reported that this oil had antifungal activity, but it was primarily located in

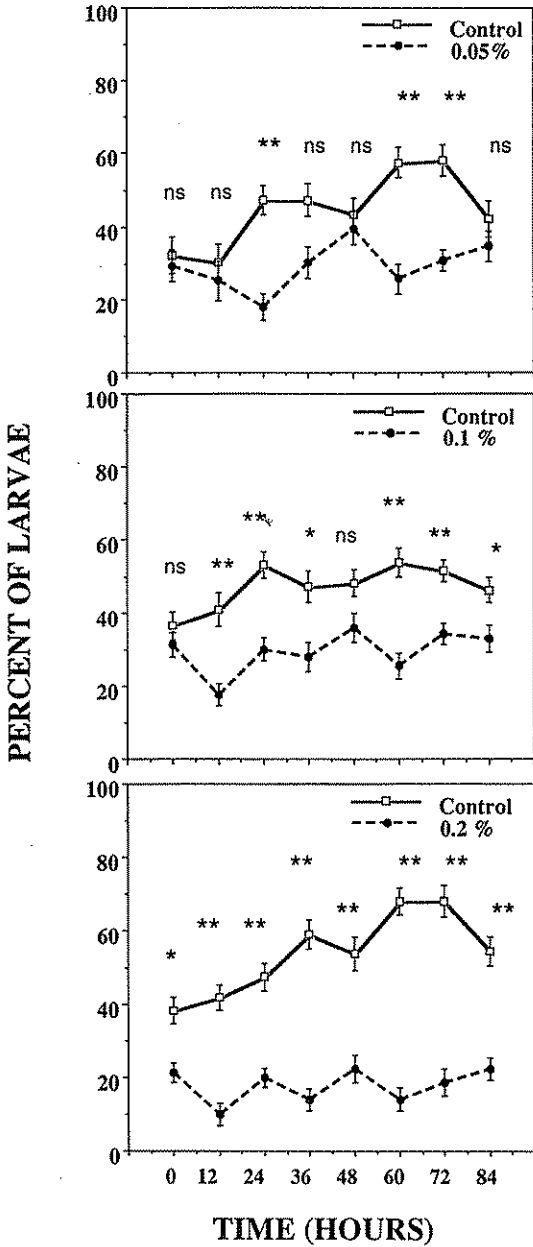


Fig. 1. Percentage of early-instar *S. exigua* on control and treated diets over time. *, $P < 0.05$; **, $P < 0.01$; paired *t*-test; 0.05% oil, $n = 27$; 0.1% oil, $n = 40$; 0.2% oil, $n = 37$.

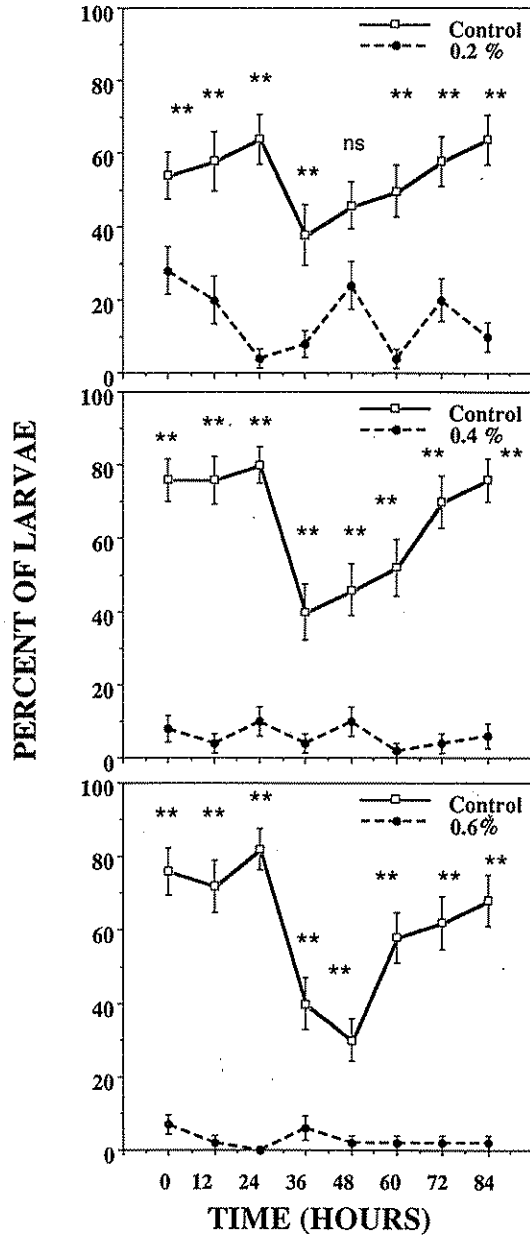


Fig. 2. Percentage of late-instar *S. exigua* on control and treated diets over time. *, $P < 0.05$; **, $P < 0.01$; paired *t*-test; $n = 25$.

the mesocarp in the specialized idioblast cells where it was unavailable to serve as an antifungal agent. However, no conclusive evidence is available on the potential role of the oil as a plant defense against pathogens or herbivores that commonly occur on avocado fruit.

Because feeding deterency was observed for larvae at a concentration of 0.20% oil in diet an increase in larval developmental time was expect-

ed, but no significant increase was observed. However, because $>50\%$ mortality occurred in the developmental study, these data were based on a small cohort of surviving larvae. Nonetheless, larvae that can survive the dose are capable of feeding and completing development in a normal amount of time. However, at concentrations of 0.30% oil in diet or higher, significant developmental effects such as weight reduction occur in no-choice situations even for large larvae.

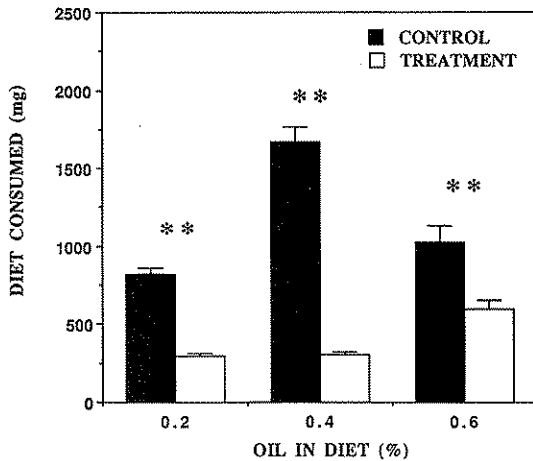


Fig. 3. Mean consumption per arena by two 3rd- to 4th-instar *S. exigua* given a choice between controls and treated diet at various concentrations of oil in diet (0.2, 0.4, and 0.6%). **, $P < 0.01$, paired *t*-test.

Although substantial research is needed before considering commercialization, the potential for this material appears high. Even if the specific constituents prove too expensive to synthesize, extraction of the oil from avocados may still be commercially feasible. For the purposes of this study the oil was extracted from avocados that were at an advanced stage of ripening and were no longer suitable for consumer use. In addition, many avocados become unmarketable each year because of premature abscission during windstorms. Thus, there may be a practical application for these avocados that otherwise would be an economic liability. However, the synthesis of key insecticidal components might offer a more economical solution to manufacturing. The potential for mammalian toxicity appears low because avocado oils are used in cosmetics and the fruit has been consumed for centuries without reports of significant effects on the general populace (Anonymous 1980).

Acknowledgments

We thank Kristina White, Mitchel White, and William Carson for their assistance on the project. We appreciate the collaborations of Kathryn Platt and William Thomson. We also thank Mariano Berdegue, Stuart Reitz, Jocelyn Millar, Kirk Visscher, and Robert Beaver for their critical reviews of the manuscript. The support of the California Celery Research Advisory Board and the California Tomato Commission is acknowledged.

References Cited

- Anonymous. 1980. Final report of the safety assessment for avocado oil. *J. Environ. Pathol. Toxicol.* 4: 93-103.
- Awad, M., and R. E. Young. 1979. Postharvest variation in cellulase, polygalacturonase, and pectinmethylesterase in avocado (*Persea americana* Mill. cv. Fuerte) fruits in relation to respiration and ethylene production. *Plant Physiol.* 64: 306-308.
- Baas, P., and M. Gregory. 1985. A survey of oil cells in the dicotyledons with comments on their replacement by and joint occurrence with mucilage cells. *Israel J. Bot.* 34: 167-186.
- Brewer, M. J., and J. T. Trumble. 1991. Classifying resistance severity in field populations: sampling inspection plans for an insecticide resistance monitoring program. *J. Econ. Entomol.* 84: 379-389.
1994. Beet armyworm resistance to fenvalerate and methomyl: resistance variation and insecticide synergism. *J. Agric. Entomol.* 11: 291-300.
- Butterworth, J. H., and E. D. Morgan. 1971. Investigation of the locust feeding inhibition of the seeds of the neem tree (*Azadirachta indica*). *J. Insect. Physiol.* 17: 969-977.
- Carson, W. G., K. K. White, and J. T. Trumble. 1994. Impact of insecticides on insects of tomatoes, 1993. *Arthropod Manage. Tests* 19: 148-149.
- Coats, J. R. 1994. Risks from natural versus synthetic insecticides. *Annu. Rev. Entomol.* 39: 489-515.
- Finney, D. J. 1971. Probit analysis. Cambridge University Press, Cambridge, U.K.
- Gould, F., A. Anderson, D. Landis, and H. Van Mel-laert. 1991. Feeding behavior and growth of *Heliothis virescens* larvae on diet containing *Bacillus thuringiensis* formulations or endotoxins. *Entomol. Exp. Appl.* 58: 199-210.
- Heyde, J. V. D., R. C. Saxena, and H. Schmutterer. 1984. Neem oil and neem extracts as potential insecticides for control of hemipterous rice pests, pp. 377-390. In H. Schmutterer and Ascher [eds.], *Natural pesticides from the neem tree and other tropical plants*. Deutsche Gesellschaft für Technische Zusammenarbeit, Eschborn, Germany.
- Horton, D. R. 1995. Statistical consideration in the design and analysis of paired-choice assays. *Environ. Entomol.* 24: 179-192.
- Kobiler, I., D. Prusky, S. Midland, J. J. Sims, and N. T. Keen. 1993. Compartmentation of antifungal compounds in oil cells of avocado fruit mesocarp and its effect on susceptibility to *Colletotrichum gloeosporioides*. *Physiol. Mol. Plant Pathol.* 43: 319-328.
- Lange, W. H., and L. Bronson. 1981. Insect pests of tomatoes. *Annu. Rev. Entomol.* 26: 345-371.
- Meister, R. T. [ed.]. 1995. *Farm chemicals handbook '95*. Meister, Willoughby, OH.
- Oatman, E. R., and G. R. Platner. 1972. An ecological study of lepidopterous pests affecting lettuce in coastal southern California. *Environ. Entomol.* 1: 202-204.
- Patana, R. 1969. Rearing cotton insects in the laboratory. U.S. Dep. Agric., Agric. Res. Serv. Prod. Res. Rep. 108: 1-6.
- Platt, K. A., and W. W. Thomson. 1992. Idioblast oil cells of avocado: distribution, isolation, ultrastructure, histochemistry, and biochemistry. *Int. J. Plant Sci.* 153: 301-310.
- Platt-Aloia, K. A., J. W. Oross, and W. W. Thomson. 1983. Ultrastructural study of the development of oil cells in the mesocarp of avocado fruit. *Bot. Gaz.* 144: 49-55.
- Postek, M. T. and S. C. Tucker. 1983. Ontogeny and ultrastructure of secretory oil cells in *Magnolia grandiflora* L. *Bot. Gaz.* 144: 501-512.
- Redfern, R. E., J. D. Warthen Jr., E. C. Uebel, and G. D. Mills, Jr. 1981. The antifeedant and growth-

- disrupting effects of azadirachtin on *Spodoptera frugiperda* and *Oncopeltus fasciatus*, pp. 129-136. In H. Schmutterer, K.R.S. Ascher, and H. Rembold [eds.], Natural pesticides from the neem tree (*Azadirachta indica* A. Juss). Deutsche Gesellschaft für Technische Zusammenarbeit, Eschborn, Germany.
- Rembold, H., G. K. Sharma, C. Czoppelt, and H. Schmutterer. 1980.** Evidence of growth disrupting in insects without feeding inhibition by neem tree fractions. *Z. Pflanzenkr. Pflanzenschutz* 87: 290-297.
- Ruscoe, C.N.E. 1972.** Growth disrupting effects of an insect antifeedant. *Nature (Lond.)* 236: 159-160.
- SAS Institute. 1990.** User's guide: statistics. SAS Institute, Cary, NC.
- Saxena, R. C., and Z. R. Khan. 1987.** New bioactive products: growth regulators, antifeedants, pheromones, and other attractants. *Mem. Sci. Fisiche Nat. Ser. 5. 11:* 303-317.
- Schuster, D. J. 1995.** Insect control on fresh market tomatoes in west-central Florida, fall 1993. *Arthropod Manage. Tests* 20: 136-137.
- Simmonds, M. S., W. M. Blaney, S. V. Ley, J. C. Anderson, R. Banteli, A. A. Denholm, P. C. Green, R. B. Grossman, C. Gutteridge, L. Jennens, S. C. Smith, P. L. Toogood, and A. Wood. 1995.** Behavioral and neurophysiological response of *Spodoptera littoralis* to azadirachtin and a range of synthetic analogues. *Entomol. Exp. Appl.* 77: 69-80.
- Steiner, P. 1936.** Beiträge zur Kenntnis der Schädlingsfauna Kleinasiens. III. *Laphygma exigua* Hb., ein Grossschädling der Zuckerrübe in Anatolien. *Z. Angew. Entomol.* 23: 177-222.
- Van Steenwyk, R. A., and N. C. Toscano. 1981.** Relationship between lepidopterous larval density and damage in celery and celery plant growth analysis. *J. Econ. Entomol.* 74: 287-290.
- West, W. C. 1969.** Ontogeny of oil cells in the woody Ranales. *Bull. Torrey Bot. Club* 96: 329-344.

Received for publication 22 January 1996; accepted 26 July 1996.