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Secretory avocado idioblast oil cells: evidence of their defensive role against a non-adapted insect herbivore

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Abstract

We tested the hypothesis that avocado idioblast oil cells play a defensive role against herbivorous insects. Toxicities of the intact avocado idioblast oil cells and the extracted idioblast oil were compared for three insect herbivores. *Spodoptera exigua* (Hübner) larvae are generalists that do not feed on avocados. By contrast, *Sabulodes aegrotata* (Guenée) and *Pseudoplusia includens* (Walker) larvae are generalist herbivores that readily feed on avocados. All bioassays were performed at a naturally occurring concentration of idioblast oil cells (2% w/w). Choice experiments showed that *S. exigua* larvae avoided diet treated with avocado idioblast oil cells and consume more control than treated diet. In contrast, idioblast oil cells had no significant antifeedant effects on the adapted *S. aegrotata* and *P. includens* larvae. Subsequent experiments designed to assess resistance mechanisms separated pre-ingestive (behavioral) and post-ingestive (physiological) effects of the avocado idioblast oil cells, and the extracted idioblast oil, on the two adapted herbivores. Post-ingestive adaptation was the mechanism that allows feeding. Because the impact of the avocado idioblast oil cells was greatest on the performance of non-adapted *S. exigua*, additional experiments determined that larvae fed diet containing the oil cells had higher mortality and reduced larval growth compared to controls. Developmental times were significantly prolonged for the survivors. Thus, increased mortality, reduced developmental rates, and antifeedant activity in the non-adapted insect indicate that defense against non-adapted herbivores may be an important function of idioblast cells in avocados.

Introduction

Plants frequently produce substances that are stored inside simple (unicellular) or complex (multicellular) structures (Esau, 1967). Plant structures that contain these substances vary widely in their degree of specialization and location. Some of these secretory structures are external, such as trichomes, epidermal glands, and nectaries. Others are internal, such as secretory cells and secretory spaces or cavities. This latter group includes the specialized idioblast oil cells which markedly differ from other constituents of the same tissue in form, structure, or content (Esau, 1967). Idioblast oil cells are characterized by possessing oil drops (sometimes called 'oil sacs' [Fahn, 1979]) and by a three-layer cell wall consisting of an outer cellulotic layer, an intermediate suberin layer, and an inner cellulotic layer (Fahn, 1979; Baas & Gregory, 1985). Such oil cells occur in a variety of plant families including the Araceae, Aristolochiaceae, Calycanthaceae, Lauraceae, Magnoliaceae, Piperaceae, and Saururaceae (Fahn, 1979; Baas & Gregory, 1985).

Despite the fact that they have attracted the curiosity of botanists for more than a century mainly because of their taxonomic value (Baas & Gregory, 1985), the function of idioblast oil cells is not well-known (Platt & Thomson, 1992; Lersten & Curtis, 1993). Fahn (1988) speculated that the substances from plant secretory tissues might deter phytophagous animals. He also proposed that idioblast oil cells were precursors of other more complex plant secretory structures such as glandular trichomes that have long been known to af-

fect insect herbivores (Levin, 1973). Platt-Aloia et al. (1983) indicated that the specialized suberin layer of the idioblast cell may serve to isolate the contents from neighboring parenchyma cells, thus preventing autotoxicity. Recently, Lersten & Curtis (1998) suggested that plant idioblasts may primarily serve a defensive function, particularly because of their prevalence in young leaves where plant defenses are frequently greatest; however, the hypothesis was not evaluated.

Avocados, *Persea americana* Mill., contain idioblast oil cells in the leaves, seed, roots (Armstrong, 1964), and fruit (Platt-Aloia et al., 1983). In the fruit, these thick-walled cells are distributed uniformly through the mesocarp and make up 2% by volume of the total tissue (Cummings & Schroeder, 1942). A typical idioblast cell measures 80 μm in diameter compared to the parenchyma cells that compose the bulk of the mesocarp, which measure about 60 μm in diameter (Cummings & Schroeder, 1942). Idioblast oil differs from other oils in the avocado fruit (Platt & Thomson, 1992). Platt & Thomson (1992) indicated the presence in the avocado oil cells of alkaloids, sesquiterpene hydroperoxides, and possibly other terpenes, whereas parenchymal cell lipids are primarily triacylglycerides.

Oil extracted from the avocado idioblast cells was toxic to the fungus *Colletotricum gloeosporioides* (Penz.) Penz. & Sacc. (Kobiler et al., 1993). However, Kobiler et al. (1993) also indicated that intact idioblast cells in the fruit mesocarp were not toxic to *C. gloeosporioides*, and proposed that the cell thickness blocks contact with fungal hyphae. Thus, the oil in idioblast cells would have minimal effect on the susceptibility of the mesocarp to fungal attack. Similarly, oil extracted from idioblast cells was toxic to the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) (Rodriguez-Saona & Trumble, 1996). However, no studies have examined the toxicity of whole avocado idioblast cells, the form which would be encountered by insects.

Rodriguez-Saona et al. (1997) identified (12Z, 15Z)-1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene (persin) as a compound responsible for the antifeedant and toxic effects of the avocado idioblast oil against *S. exigua* larvae. More recently, a group of 2-alkylfurans were identified from a second active fraction obtained after fractionationation of the crude oil (Rodriguez-Saona et al., 1998). The 2-alkylfurans were reported to cause toxic (Rodriguez-Saona et al., 1998) and feeding deterrent effects (Rodriguez-Saona & Trumble, 1999) on the non-adapted *S. exigua* larvae.

The research reported here examined the hypothesis that idioblast oil cells protect plants against non-adapted insect herbivores. To test this hypothesis we chose idioblast oil cells from avocados because they are present in relatively large amounts within the avocado tissue and a rapid extraction method is available to isolate the oil cells from avocado mesocarp (Platt & Thomson, 1992). We conducted experiments to compare the effects of intact avocado oil cells on adapted and non-adapted insect herbivores. First, we investigated whether insects that feed on avocados have developed behavioral (pre-ingestive) or physiological (post-ingestive) adaptations to the idioblast oil cells and the extracted idioblast oil. Two avocado-feeding lepidopterans were chosen: the omnivorous looper, *Sabulodes aegrotata* (Guenée) (Lepidoptera: Geometridae) and the soybean looper, *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae). *S. aegrotata* is one of the most important leaf-feeding insects of avocados in California and accepts a large number of host plants ($>$ 35 species from over 20 plant families; McKenzie, 1935). Although the omnivorous looper usually feeds on foliage, it may attack the avocado fruit peel (early instars) or mesocarp (late instars) (Ebeling & Pence, 1953). Likewise, *P. includens* is a polyphagous herbivore with an extensive host-plant range ($>$ 20 species in over 12 plant families) that includes avocados (Eichlin & Cunningham, 1978). Both insects may encounter idioblast oil cells in their diet while feeding on avocado foliage, which contains the oil cells throughout the mesophyll layer (Armstrong, 1964).

Second, we determined if whole idioblast oil cells could account, at least in part, for the toxicity of avocados to the non-adapted generalist herbivore, *S. exigua*. This generalist herbivore feeds on over 35 host plants around the world (Steiner, 1936) from more than 18 families (Mitchell, 1979) with variable defensive chemistries, and occurs in many geographic areas where avocados are planted (e.g. southern California). However, *S. exigua* has not been reported to feed on avocados.

Our specific objectives were:

(1) Compare the effects of avocado idioblast oil cells on the non-adapted *S. exigua* and the adapted *S. aegrotata* and *P. includens*.

(2) Compare the effects of the extracted avocado idioblast oil on non-adapted and adapted insect herbivores.

(3) Test for toxicity, growth inhibition, and feeding deterrence of avocado idioblast oil cells on the non-adapted generalist herbivore, *S. exigua*.

Materials and methods

Study species

The *S. exigua* colony was initially collected in 1982 from Orange Co., CA, and maintained on artificial diet [modified from Patana (1969)] at 28 ± 2 °C and a photoperiod of L14:D10 h. New genetic material, collected from the same location, was added to the colony every 6–12 months.

S. aegrotata and *P. includens* colonies were initiated in September 1998 from larvae collected in avocado orchards in San Diego Co., CA. The *S. aegrotata* colony was maintained on pinto bean diet (Johnson & Federici, 1982). *P. includens* colony was reared on lima bean diet [modified from Patana (1969)]. Neither of the two rearing diets includes avocado material. Both colonies were kept at 22 ± 2 °C and a photoperiod of L10:D14 h.

S. exigua neonates were standardized by selecting those which had eclosed within 12 h of the initiation of the experiments. A standardized cohort of recently molted *S. exigua* third instars was created by collecting second instars close to molting approximately 12 h before initiation of the experiments and holding them on non-nutritional agar. Only those larvae which had molted to the third instar were used in the tests. Similarly, third instars of *S. aegrotata* and *P. includens* were selected from lab colonies and placed on agar 6–12 h prior to the bioassays. Unless stated, all experiments were conducted in environmental chambers set at 28 ± 2 °C and a photoperiod of L14:D10 h with fluorescent lighting.

Separation of the oil cells

Avocado idioblast cells were extracted from ripe avocado fruit as described by Platt & Thomson (1992) and Rodriguez-Saona & Trumble (1996). Armstrong (1964) and Platt & Thomson (1992) indicated a similar structure of the oil cells in the avocado leaf to those in the mature fruit. Avocados, *P. americana* variety Hass, were obtained from the University of California South Coast Research and Extension Center (Santa Ana, CA) and Index Fresh of California (Bloomington, CA). The avocados were held at room temperature until ripe (ca. 22 °C). When ripe, the mesocarp was removed and placed in a blender with water (5:1 water:tissue). The tissue was blended until liquid. The mixture was then sieved through a 149 μm mesh screen and the sieved material collected in a large Erlenmeyer flask. The collected material was sieved again through a 106 μm mesh screen. The filtrate was then poured through a 63 μm mesh screen. In this last stage, the residue remaining on the surface of the screen, which consisted mostly of oil cells and water, was collected and washed with water until pale in color. The water was removed using a filter paper (No. 1; Whatman, Maidstone, UK) under vacuum. Dried oil cells were collected from the surface of the filter paper and stored at 4 °C. All experiments were conducted at a concentration of 2% (w/w) of idioblast cells in diet. This concentration was chosen because Cummings and Schroeder (1942) reported that the oil cells composed about 2% of the avocado fruit tissue volume, a concentration that did not change significantly during fruit ripening.

Extraction of the avocado idioblast oil

The avocado idioblast oil was extracted using a methodology described by Platt & Thomson (1992) and Rodriguez-Saona & Trumble (1996). To measure the amount of oil per g of avocado idioblast cells, six g of oil cells were dissolved in 16 ml of chloroform and methanol (1:1). The mixture was vortexed for 5 min. The mixture was then filtered through filter paper with suction to remove solids. The filtrate was concentrated using a rotary evaporator. To remove traces of water, the resulting oil was dissolved in chloroform and dried by addition of anhydrous $MgSO_4$. The dried solution was filtered, concentrated, and weighed. The extraction yielded 0.16 g of oil per g of avocado idioblast cells.

Comparisons among species

Feeding deterrence studies. Experiments were conducted to compare the effects of avocado idioblast oil cells on the food preference of the non-adapted *S. exigua*, and the adapted *S. aegrotata* and *P. includens* larvae. The methodology used for these experiments was adapted from Gould et al. (1991) and successfully used for *S. exigua* by Rodriguez-Saona & Trumble (1996) and Berdegue et al. (1997). Choice tests, initiated with third instars, used arenas constructed from 150 ml plastic cups with approximately 1 cm of 4% agar (w/v) on the bottom. Four 1.5 ml microcentrifuge tubes were inserted in holes just above the agar in a

cross arrangement. The tube openings are located just above the agar, affording easy entry. Opposing tubes contained the same type of diet (control or treated diet). Treated diets were prepared by diluting 2 g of cells in 2 ml of distilled water and were vortexed until homogeneous. Diet was then added to obtain a final weight of 100 g. Control diet was prepared by adding artificial diet to 2 ml of distilled water to produce a total of 100 g, and vortexing until homogeneous. Two third instars were placed inside each arena.

The first observation was taken approximately 12 h after placing the larvae inside the arenas. The position of the larvae (e.g., larvae making physical contact with control or treated diet) was recorded twice each day (1 and 10 h after beginning of photophase) for 4 days. Because *S. aegrotata* larvae are nocturnal, the L:D period in the environmental chamber was altered to allow observations during the dark cycle (1 and 10 h after beginning of scotophase). For each species, each arena was treated as a replicate and the experiment had a total of 29–30 replicates. In addition, larval consumption was recorded by obtaining the difference between initial and final weights of the microcentrifuge tubes. Water loss was controlled by determining weight differences of diet in microcentrifuge tubes in arenas (without larvae) held under the same conditions ($n = 4$). Water loss was less than 5% in all microcentrifuge tubes.

To examine the possible effects of avocado idioblast oil cells on the food preference of early instar *S. exigua*, we conducted choice tests initiated with neonates using control diet and diet containing 2% idioblast oil cells. Arenas were constructed from 30 ml plastic cups with approximately 1 cm of 4% agar (w/v) on the bottom. Two 1.5 ml microcentrifuge tubes were placed in two holes at opposite sides of the cups (just above the agar). One of the tubes contained control diet while the other contained treated diet. Treated and control diets were prepared as previously described. Five to seven *S. exigua* neonates were placed inside each cup. The position of the larvae (e.g., larvae making physical contact with control or treated diet) was recorded twice a day (1 and 10 h after beginning of photophase) for 4 days, with the first observation recorded approximately 12 h after placing the larvae inside the arenas. Each arena was treated as a replicate and the experiment included a total of 50 replicates.

Separating pre-ingestive and post-ingestive effects. Bioassays were conducted to separate the physiological (post-ingestive) and behavioral (pre-ingestive) effects of the avocado oil cells on performance of adapted *S. aegrotata* and *P. includens*, and non-adapted *S. exigua*. Idioblast oil cells were extracted from avocado fruit and mixed with artificial diet at an ecologically relevant concentration (2%), as previously described. Treated and control diets were poured into plastic containers. After cooling at room temperature, the diet was cut into disks (1.5 cm diameter by 0.5 cm high) using a cork borer. Diet disks were then placed individually into 30 ml plastic cups lined with 1 cm of agar at the bottom. One 3rd instar was placed inside each cup. Trays holding the cups were placed inside plastic boxes (26.5 by 30 by 10 cm) (TriState Plastics, Dixon, KY), with two Petri dishes at the bottom containing a saturated solution of ammonium sulfate to maintain a continuous humidity of ca. 80% r.h. inside the boxes (The Merck Index 1989; Merck & Co., Inc., Rahway, N.J.). Boxes were then transferred to an environmental chamber. Larvae were allowed to feed on the diet *ad libitum* for a total of 5 days.

Wet weights of diet and larvae were recorded prior to starting the experiment. Fifteen additional samples of both larvae and diet disks were weighed, oven dried at 60 °C for 48 h, and weighed again to obtain values for wet weight to dry weight conversions. After 5 days, larvae and remaining diet were removed, oven-dried, and weighed. Thirty larvae were used per treatment, and each bioassay was replicated twice for each species-treatment combination.

To determine whether the insects are able to break the oil cells after ingestion we tested the effects of the extracted avocado idioblast oil at a concentration equivalent to 2% of oil cells in assays like those just described. The oil was extracted as previously described. To prepare a concentration equivalent to 2% of oil cells, 0.32 g of oil was diluted in 2 ml of 0.1% Tween-80 solution (Fisher, Pittsburg, PA). The mixture was vortexed for 5 min, and then homogenized using an ultrasonic homogenizer (Cole-Parmer, Chicago, IL). Subsequently, diet was added to obtain a final weight of 100 g. Control diet was prepared by adding artificial diet to 2 ml of Tween solution to produce a total of 100 g.

Effects on the non-adapted herbivore

Physiological studies. The effects of the avocado idioblast oil cells on mortality and growth of *S. exigua* were quantified using diet incorporation bioassays initiated with both first and third instars. Control and treated diets were prepared as previously described

and poured into 30 ml plastic cups (7 g of diet/cup). Larval (see below) and pupal weights and mortality were recorded. With early instars, 29–30 neonates were used per treatment, with a single larva per cup. Larval weights were recorded at 8 and 10 days. The bioassay was replicated 3 times. In experiments with 3rd intars, 20 larvae were used per treatment. Larvae were weighed at the beginning of the experiment and after 4 days. This bioassay was repeated twice.

Data analysis

In no-choice experiments, comparisons of growth and developmental parameters between control and treatment groups were analyzed using F-tests (SuperAnova, 1989). Where appropriate, the data were log-transformed and the results compared to those using non-transformed data. If the results were not different after transformation, the results from the non-transformed data are presented. In choice experiments, larval preference was analyzed using Wilcoxon signed-rank test (Statview, 1992; after Tallamy et al., 1997 and Rodriguez-Saona & Tumble, 1999). For each observation point, the percent of total larvae on control and treated diets was calculated. If the difference in percentages (control minus treatment) was significantly different from zero, then we concluded that a significantly higher proportion of larvae preferred one diet over the other. Differences in absolute amount of diet consumed between control and treated diets were analyzed using paired *t*-tests (SuperAnova, 1989; after Horton, 1995). Comparisons were made among species by contrasting the proportion of larvae feeding on the treated diet after appropriate transformation (ANOVA with repeated measures; SuperAnova, 1989).

Analyses to separate the pre- and post-ingestive effects of avocado idioblast cells, and idioblast oil, on performance (weight gain) of non-adapted and adapted insect herbivores were conducted using analysis of covariance (ANCOVA; SuperAnova, 1989; after Horton & Redak, 1993). These results are typically presented in a graphical format: if correlation lines of biomass gained versus food consumption for treated and untreated diets show that, at a common consumption rate, diets differed in their effects on performance (weight gain), then post-ingestive effects are important. However, if the consumption rates differed between diets, and treatment effects become less pronounced or disappear, then pre-ingestive effects are important (Horton & Redak, 1993).

In the ANCOVA analysis, the variate was biomass gained, while amount of diet consumed was used as covariate. The differences in mean biomass gained between treatment diets were compared in ANCOVA, after they were adjusted for any variation due to consumption. If the treatment effect was significant ($P \leq 0.05$) after the adjustment, then it suggests that post-ingestive effects altered gains in biomass. But, if there is no significant ($P > 0.05$) treatment effect when variation in consumption is removed by the ANCOVA, then the variation in weight gain is probably due to pre-ingestive effects.

Results

Comparisons among species

Feeding deterrence studies. Choice tests initiated with older instar *S. exigua* larvae showed a significantly greater proportion of larvae on control diets in 5 of 8 observations (Figure 1A). Thus, avocado idioblast oil cells are acting as deterrents to feeding by the non-adapted *S. exigua*.

Two of the observations for tests initiated with older instar *S. exigua*, that did not show significant differences between larval preference of control and treated diets corresponded to molting events (Figure 1A). The first event happened at the end of day 1, which corresponded to a molt to fourth instar, while the second happened at the end of day 3 through early day 4, corresponding to a molt to the fifth instar. This same pattern also was observed when *S. exigua* larvae were given a choice between control diets and diets incorporating oil extracted from avocado idioblast cells (Rodriguez-Saona & Trumble, 1996, 1999). This observation suggests that increased *S. exigua* larval locomotion is associated with molting, followed by random choice, which then sorts out by a kinesis.

Choice tests initiated with older instar *P. includens* (Figure 1B) and *S. aegrotata* (Figure 1C) showed no significant larval preference between control diet and diet incorporating avocado idioblast oil cells. Thus, unlike *S. exigua*, neither of these adapted species was significantly deterred by the presence of the oil cells in diet. Choice tests found differences in feeding deterrence by avocado oil cells among the three species evaluated ($F = 22.6$; $df = 2, 14$; $P = 0.0001$; Figure 1A–C), with most of the variance explained by the difference between non-adapted and adapted herbivores (Contrast, $F = 28.2$; $df = 1$; $P = 0.0001$).

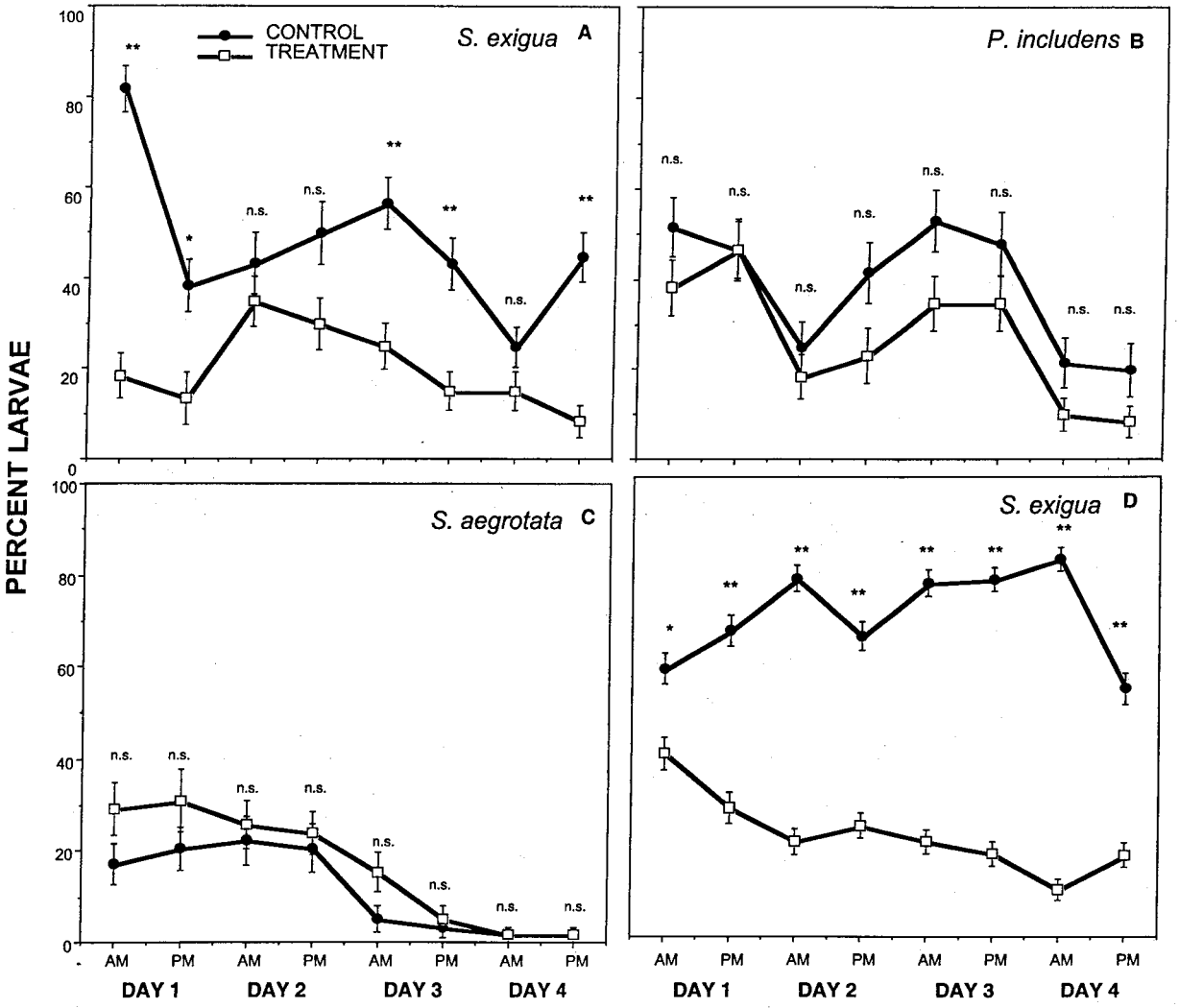

Figure 1. Percentage of larvae on control diets and diets treated with avocado idioblast oil cells at a concentration of 2%. Choice tests were initiated with third instar *S. exigua* (A), *P. includens* (B), and *S. aegrotata* (C); and neonate *S. exigua* (D). Not significant = n.s., $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; Wilcoxon signed-rank test. Bars at each observation point indicate standard errors.

In choice tests initiated with *S. exigua* neonates, a significantly higher proportion of larvae preferred control diet than diet with avocado idioblast oil cells for all observations (Figure 1D).

Consumption measurements support the assumption that the idioblast oil cells acted as antifeedants to the non-adapted *S. exigua* larvae. Larvae consumed significantly more of the control diet than diet with idioblast cells (*t*-test = 13.4; df = 29; $P < 0.001$). Overall, the control diet accounted for more than 70% of the total food consumed by *S. exigua* larvae (Figure 2A).

Although larvae of the adapted *P. includens* (Figure 2B) and *S. aegrotata* (Figure 2C) consumed slightly less of the oil-cell-treated diet, we found no significant differences in total amount of diet consumed between treatment diets for either *S. aegrotata* (*t*-test = 1.77; df = 28; P = 0.09), or *P. includens* (*t*-test = 1.62; df = 29; P = 0.12).

Separating pre-ingestive and post-ingestive effects. Intact avocado idioblast oil cells significantly affected *S. exigua* pre-ingestion (consumption) (ANOVA; $F_{1,107} = 35.1$; $P < 0.001$) and post-ingestion (biomass gain) (ANCOVA; $F_{1,106} = 12.3$; $P < 0.001$) (Figure 3A). Larvae gained less weight and consumed

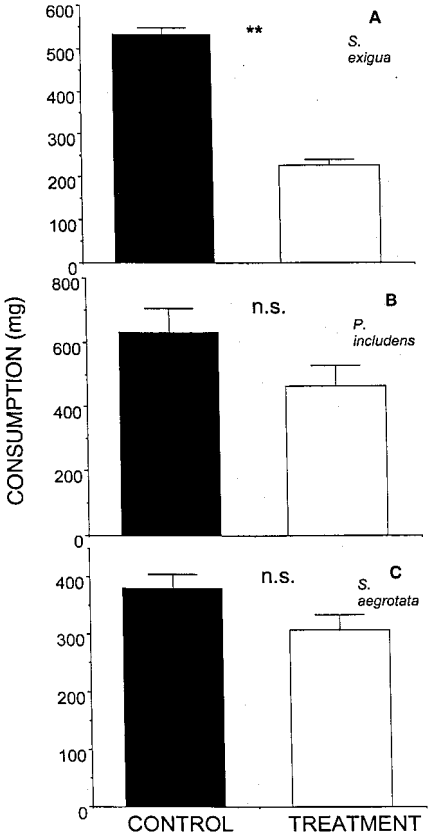

Figure 2. Mean percent consumption per arena by *S. exigua* (A), *P. includens* (B), and *S. aegrotata* (C) larvae given a choice between control diets and diets incorporating avocado idioblast oil cells at a concentration of 2%. Tests were initiated with third instars. Not significant = n.s., $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; paired *t*-test. Bars indicate standard errors.

less food when fed diet incorporating oil cells compared to controls. In contrast, biomass gain for *P. includens* (ANCOVA; $F_{1,116} = 0.76$; $P = 0.38$) and *S. aegrotata* (ANCOVA; $F_{1,113} = 2.25$; $P = 0.14$) was not affected significantly (Figure 3B–C). However, the oil cells reduced consumption by *P. includens* (ANOVA; $F_{1,117} = 5.44$; $P = 0.02$), but not *S. aegrotata* (ANOVA; $F_{1,114} = 3.57$; $P = 0.06$).

The extracted oil had a negative effect on *S. exigua* larvae both pre-ingestion (ANOVA; $F_{1,75} = 53.1$; $P < 0.001$) and post-ingestion (ANCOVA; $F_{1,74} = 7.67$; $P = 0.007$) (Figure 4A). The detrimental effect of the oil on *S. exigua* larval growth and consumption was greater than that caused by intact oil cells. However, the extracted oil had no effect on weight gain of either *P. includens* (ANCOVA; $F_{1,116} = 0.30$; $P = 0.58$) or *S. aegrotata* (ANCOVA; $F_{1,115} = 0.20$; $P = 0.66$) (Figure 4B–C). Of the two adapted herbivores, only

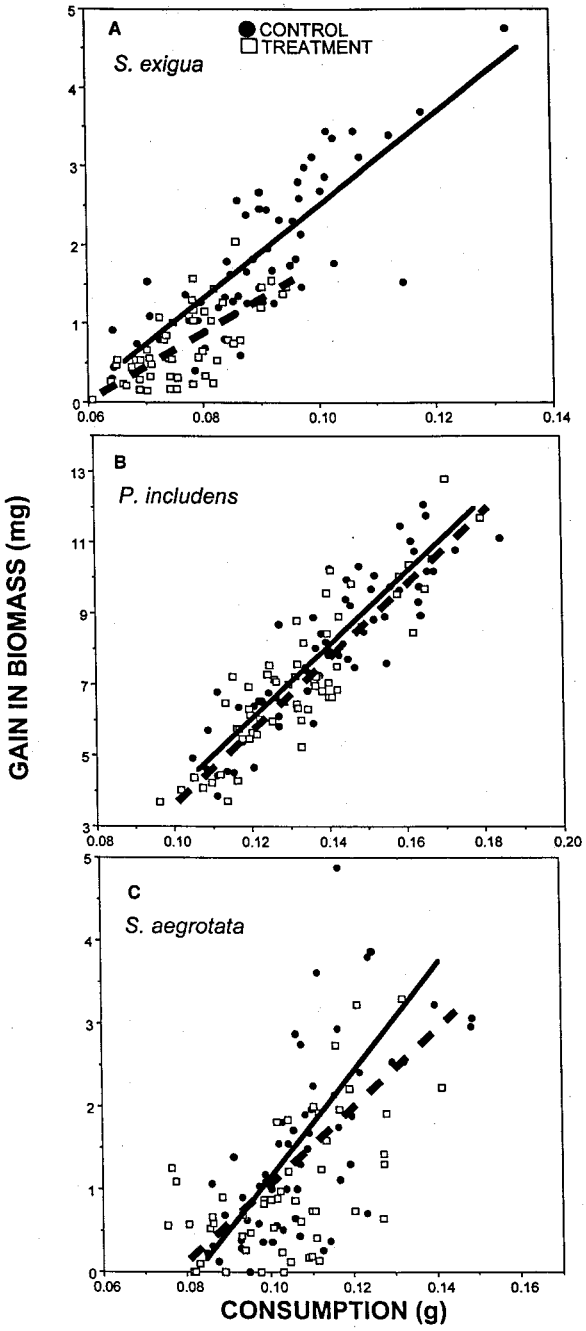

Figure 3. Effects of avocado idioblast oil cells (treated diet) and food consumption on gain in biomass by *S. exigua* (A), *P. includens* (B), and *S. aegrotata* (C) larvae. *S. exigua*: control $Y = -3.55 + 60.12X$ $r^2 = 0.69$; treatment $Y = -2.42 + 41.27X$ $r^2 = 0.48$. *P. includens*: control $Y = -5.68 + 97.79X$ $r^2 = 0.79$; treatment $Y = -6.15 + 100.14X$ $r^2 = 0.78$. *S. aegrotata*: control $Y = -5.54 + 66.02X$ $r^2 = 0.52$; treatment $Y = -3.53 + 44.47X$ $r^2 = 0.30$.

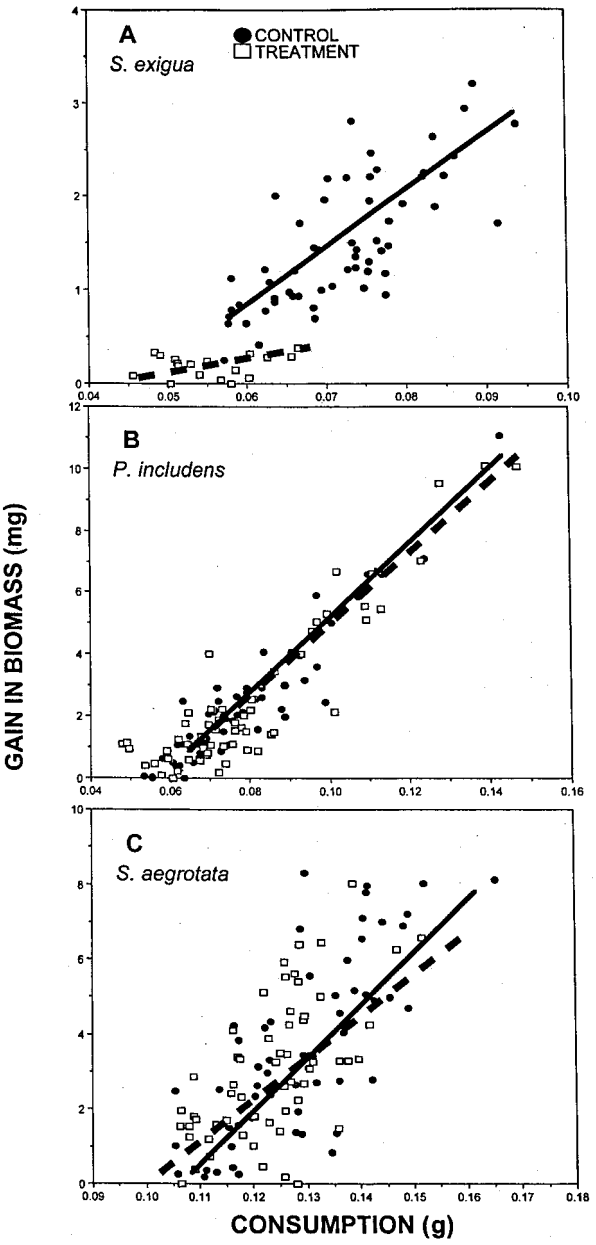

Figure 4. Effects of avocado idioblast oil (treated diet) and food consumption on gain in biomass by *S. exigua* (A), *P. includens* (B), and *S. aegrotata* (C) larvae. *S. exigua*: control $Y = -2.72 + 58.41X$ $r^2 = 0.58$; treatment $Y = -0.06 + 4.63X$ $r^2 = 0.05$. *P. includens*: control $Y = -6.27 + 110.52X$ $r^2 = 0.89$; treatment $Y = -5.88 + 105.45X$ $r^2 = 0.84$. *S. aegrotata*: control $Y = -13.89 + 135.88X$ $r^2 = 0.56$; treatment $Y = -10.63 + 110.64X$ $r^2 = 0.38$.

S. aegrotata food consumption was reduced when the avocado oil was incorporated in diet (ANOVA; $F_{1,116} = 4.33$; $P = 0.04$). No effect of the idioblast oil on *P. includens* food consumption was demonstrated (ANCOVA; $F_{1,117} = 0.18$; $P = 0.67$).

Effects on the non-adapted herbivore

Physiological studies. In no-choice tests initiated with first instar *S. exigua*, avocado idioblast oil cells increased larval mortality, inhibited larval growth, and prolonged larval developmental times when incorporated into artificial diet (Table 1). Eight-day weights ($F = 177.1$; df = 1, 153; $P < 0.001$) and 10-day weights ($F = 177.2$; df = 1, 153; $P < 0.001$) were decreased by more than 75% compared to the controls. Pupal weights also were reduced among the larvae fed diet containing oil cells ($F = 7.6$; df = 1, 123; $P = 0.007$).

Larval ($F = 82.6$; df = 1, 123; $P < 0.001$) and larval-pupal ($F = 80.5$; df = 1, 116; $P < 0.001$) developmental times were prolonged by approximately 5 days for larvae fed diet containing oil cells. In addition, larval ($F = 19.3$; df = 1, 4; $P = 0.01$) and larval-pupal ($F = 17.3$; df = 1, 4; $P = 0.01$) mortality was more than 3 fold greater for larvae fed diet with oil cells (Table 1).

Growth rates also were reduced in tests initiated with third instar *S. exigua*. Initial weights (mean $\pm$ SE) of third instars were not significantly different for larvae from the control (2.34 ± 0.08 mg) and treatment (2.33 ± 0.11 mg) groups ($F = 0.004$; df = 1,78; $P = 0.94$). However, after 4 days, larvae fed control diet were significantly heavier (72.6 ± 4.3 mg) than larvae fed diet containing idioblast cells (50.6 ± 4.2 mg) ($F = 13.2$; df = 1,78; $P < 0.001$).

Discussion

It is common for adapted insect herbivores to be minimally or even unaffected by the defensive chemistry of their hosts (at ecologically relevant concentrations), while the same compounds are toxic to non-adapted insects (e.g., Blau et al., 1978; Berenbaum, 1981, 1983). In the present study, we documented that avocado idioblast oil cells increase mortality, reduce growth, and prolong development of the non-adapted herbivore *S. exigua* (Table 1). Thus, our results support the hypothesis that one important function of these oil

Table 1. Mortality (mean ± SE) and growth inhibition of avocado idioblast oil in tests initiated with neonate *S. exigua*

Treatment[a]	Control diet	Treated diet	Significance[b]
n[c]	89	89	
8-d larval weight[d]	49.2 ± 2.5	9.3 ± 1.4	***
10-d larval weight[d]	194.2 ± 9.4	42.6 ± 5.4	***
Pupal weight[d]	116.1 ± 2.5	104.5 ± 3.4	**
Larval developmental time[e]	13.9 ± 0.2	19.1 ± 0.7	***
Developmental time[e] from neonate to adult	21.3 ± 0.2	26.3 ± 0.7	***
% Larval mortality	14.6 ± 2.3	47.1 ± 7.0	**
% Mortality (neonate-adult)	15.7 ± 2.9	54.0 ± 8.7	**

[a]Control larvae fed artificial diet (modified from Patana, 1969); treated diet contained 2% avocado idioblast oil cells.
[b]Significance level between control and treated groups; ** $P \leq 0.01$; *** $P \leq 0.001$.
[c]Total number of larvae used per treatment.
[d]In mg, based on surviving larvae.
[e]In days, based on surviving larvae.

cells is to protect plants against non-adapted insects. In contrast, adapted insect herbivores such as *P. includens* and *S. aegrotata* were not as affected by idioblast oil cells as compared to the non-adapted *S. exigua* (Table 2). If in fact other host plants are available in the field when such foods are encountered, pre-ingestive effects could result in a host shift.

The susceptibility of *S. exigua* to the avocado oil cells is not surprising, given the relatively short time period for this insect species to adapt to the defensive chemistry of the avocado idioblast oil. *S. exigua* originated from southern Asia and was first collected in California in 1882 (Wilson, 1932). Avocados, a New World species, were first introduced to California from tropical America in 1856 (Hodgson, 1930). Because of the availability of many alternative host plants, the insect has not been under a strong selective pressure to adapt to the unusual chemistry of the oil (see Rodriguez-Saona et al., 1997, 1998) during the last 150 years. Unlike the other species tested, we could find no reports in the literature of oviposition and feeding on avocados by *S. exigua*. Thus, the presence of an oviposition deterrent(s), or the lack of stimulant(s), could partially account for the lack of adaptation. However, this species is highly mobile in the larval stage, often making host plant decisions (Berdegue et al., 1998). Because avocados are grown in close association with many acceptable hosts for *S. exigua* (J.T. Trumble, personal observation), it is likely that larval feeding would have been observed if the plant was not sufficiently defended.

The mechanism by which the idioblast cells elicit avoidance behavior in *S. exigua* is unknown. However, because the active compounds are isolated by a thick three-layered cell wall (Platt-Aloia, 1980), we hypothesized three possible ways (after Bernays, 1998) in which phytophagous insects may break the cells and come into contact with the contents. First, pre-ingestive salivary enzymes may expose the toxic compounds to peripheral taste receptors. This would allow a rapid avoidance response. Second, the simple mastication of the oil cells may cause the breakage of the cells and release of the oil content. Third, post-ingestive digestive enzymes or the grinding action of the proventricular valve may break the oil cells. The resulting intestinal release would require a physiological feedback enabling the insect to 'learn' to reject the oil cell-treated diet.

Even though the three hypotheses are not mutually exclusive, the fact that *S. exigua* consumes about 30% of its total food intake from the idioblast-treated diet (Figure 2A) supports the latter hypothesis. This finding also suggests that idioblast cells do not represent a pre-feeding deterrent and is consistent with the lack of such cells in the epidermal layers (Armstrong, 1964).

P. includens is a generalist herbivore that has been only occasionally found on avocados. We found only one record (Eichlin & Cunningham, 1978) that reported this insect as a pest on avocados. This may be explained, in part, because the oil cells provide some feeding deterrence. However, *P. includens* does not show post-ingestive effects of oil cells on growth and

Table 2. Percent mortality, interpretation of results shown in Figures 3 and 4, and associated outcomes by ANCOVA[a]

	Mortality (%)[b]	Consumption (biomass gain)	Performance[c]	Outcome of effect
A. Avocado idioblast oil cells				
S. exigua	15.0	Significant	Significant	Pre-and post-ingestive
P. includens	1.7	Significant	Non-significant	Pre-ingestive
S. aegrotata	3.3	Non-significant	Non-significant	No effects
B. Idioblast oil				
S. exigua	70.0	Significant	Significant	Pre-and post-ingestive
P. includens	0.0	Non-significant	Non-significant	No effects
S. aegrotata	0.0	Significant	Non-significant	Pre-ingestive

[a]After Horton & Redak (1993).
[b]Total number of larvae used per treatment = 60. Control mortality for all bioassays was < 5%.
[c]Adjusted for consumption.

development of larvae, so the herbivore is capable of survival and development on avocados. LaFontaine & Poole (1991) indicate that many members of the family Lauraceae, which includes avocados, serve as hosts for *P. includens*. Some of these plant species have similar idioblast oil cells (Armstrong, 1964), although their contents have not been analyzed. Because *P. includens* is distributed over much of North, South, and Central America, and is native to the Western Hemisphere (Sullivan & Boethel, 1994), presumably has had a long period of coexistence with *P. americana* and its relatives.

S. aegrotata appears to be well adapted to avocado idioblast oil cells. Larval feeding, growth, and development were not affected by the presence of oil cells. Although we found a feeding deterrent effect of the extracted oil on *S. aegrotata*, there was no reduction in growth or development. Because this species has been recorded from California (Zhang, 1994) and from Brazil (as *S. caberata* Guenée; Fletcher, 1979), we suspect that *S. aegrotata* is a widely distributed in the Western Hemisphere. Thus, a long period of coexistence between avocados and *S. aegrotata* appears likely.

To date, with the limited information available on the content (Baas & Gregory, 1985; Platt & Thomson, 1992) and anti-herbivore effects of oil cells, it would be premature to infer a ubiquitous defensive role of idioblast oil cells for other plant-herbivore systems. However, idioblast cells in at least one other plant species (*Laurus nobilis* L.) have been shown to contain sesquiterpene lactones (Maron & Fahn, 1979). Many of the sesquiterpenes (Eigenbrode et al., 1994), and the sesquiterpene lactones (Castillo et al., 1998) in particular, have documented insecticidal activity. In addition, the occurrence of fully developed oil cells in young plant tissues (Armstrong, 1964; Lersten & Curtis, 1998) and the thick cell wall suitable for preventing autotoxicity (Baas & Gregory, 1985), suggest that idioblast oil cells may serve a protective function in other plant-herbivore systems.

Our previous studies identified various compounds in the avocado idioblast oil, including persin and several alkylfurans, which are responsible for the toxicity and antifeedant effects observed for *S. exigua* (Rodriguez-Saona et al., 1997, 1998; Rodriguez-Saona & Trumble, 1999). In this study, we provide the first experimental evidence that idioblast oil cells can serve a defensive function in deterring non-adapted herbivores.

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