Biochemical crypsis in the avoidance of natural enemies by an insect herbivore

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Plant–herbivore interactions provide well studied examples of coevolution, but little is known about how such interactions are influenced by the third trophic level. Here we show that larvae of the specialized lepidopteran herbivore *Heliothis subflexa* reduce their vulnerability to natural enemies through adaptation to a remarkable and previously unknown feature of their host plant, *Physalis angulata*: The fruits of this plant lack linolenic acid (LA), which is required for the development of most insects. By overcoming this nutritional deficiency, *H. subflexa* larvae achieve numerous advantages. First, they gain near-exclusive access to a food resource: we demonstrate that closely related *Heliothis virescens* larvae cannot develop on *P. angulata* fruit unless the fruit are treated with LA. Second, they reduce their vulnerability to enemies: LA is a key component of volicitin, an elicitor of plant-volatile-signaling defenses. We demonstrate that volicitin is absent in the oral secretions of fruit-feeding caterpillars, that the volatile profiles of plants induced by fruit feeding differ from those induced by leaf feeding or by feeding on LA-treated fruit, and that the former are far less attractive to female *Cardiochiles nigriceps* parasitoids. Finally, they render themselves nutritionally unsuitable as hosts for enemies that require LA for their own development: we show that *C. nigriceps* larvae fail to develop within the bodies of fruit-feeding caterpillars but do develop in caterpillars feeding on LA-treated fruit. Thus, *H. subflexa* larvae not only overcome a serious dietary deficiency but also reduce their vulnerability to natural enemies through a form of "biochemical crypsis."

The vulnerability of insect herbivores to attack by predators and parasitoids is often mediated by interactions with the host plant on which the herbivore feeds (1–3). Specialist herbivores that have overcome certain plant defenses may subsequently co-opt those defenses, for example, by sequestering chemical toxins produced by the plant within their bodies as a defense against their own enemies (2, 4–6). Plants, in turn, often respond to herbivory by releasing volatile chemical compounds that are attractive to predators and parasitoids that are natural enemies of the herbivores (7, 8). Thus, the particular physical and physiological characteristics of the host plant are thought to be major features influencing the vulnerability of insect herbivores to attack by predators and parasitoids, and adaptation to those specific characteristics may be expected to play an important role in avoiding attack by natural enemies (9–11). Lill *et al*. (3) demonstrated a strong effect of host-plant identity on parasitism rates and suggested that these differences might be influenced by features of the host plant including plant-volatile-related differences in parasitoid attraction and retention but did not address the specific mechanisms that might underlie such differences.

In this article we describe behavioral and physiological adaptations of the specialist lepidopteran herbivore *Heliothis subflexa* to features of its solanaceous host plant, *Physalis angulata*, that confer a number of advantages on the caterpillar, including reduced production of herbivore-induced volatile signals that are attractive to natural enemies. *H. subflexa* larvae feed exclusively on the fruits of *Physalis spp*., which are physically encased within an inflated calyx of the flower and defended by the presence of secondary compounds (e.g., withanolides and flavonol glycosides) (12, 13). We have discovered an additional feature of these fruits that might be expected to deter insect herbivory: *P. angulata* fruits lack an important fatty acid, linolenic acid (LA), which is required for the normal development and metamorphosis of most insect larvae (14–16).

The series of experiments reported below explored the implications of this remarkable feature of the plant's biology on tritrophic (plant–herbivore–natural enemy) interactions. We show that *H. subflexa* larvae, by overcoming the nutritional barrier posed by the lack of LA in *P. angulata* fruit, gain access to a food resource that is unavailable to most of their competitors. Moreover, by feeding exclusively on the fruits of *P. angulata* (after boring through the protective calyx in which they are enclosed), the larvae are able to co-opt the physical and chemical (17) defenses of the plant and also render themselves nutritionally unattractive to parasitic wasps and other potential natural enemies that require LA for their own development. Most remarkably, *H. subflexa* larvae avoid triggering certain induced plant-signaling responses, because LA is an essential component of the chemical compound volicitin, which has been demonstrated (18) to be a critical elicitor of these responses.

Materials and Methods

Absence of LA in *P. angulata* Fruit and of Volicitin in Fruit-Feeding Caterpillars. ***Experimental design and objectives.*** This series of experiments explored the chemical composition of the regurgitant of *H. subflexa* and *Heliothis virescens* caterpillars feeding on various *P. angulata* tissues and of the plant tissues themselves. Second-instar larvae of both species were placed in diet cups and fed either *P. angulata* fruits, fruits sprayed with LA, or leaves. The larvae were allowed to feed until the molt to fourth instar when oral secretions were collected. Regurgitant was analyzed for the presence of fatty acids and fatty acid conjugates. Plant tissues from undamaged fruit, calyx, and leaves then were analyzed for the presence of fatty acids.

Insects and plants. *H. subflexa* larvae were obtained from the F. L. Gould laboratory (North Carolina State University, Raleigh) and reared on *P. angulata* fruit or an artificial corn/soy meal diet. *H. virescens* larvae were obtained from W. J. Lewis (U.S. Department of Agriculture) and reared on *P. angulata* fruit or an artificial pinto-bean diet. All larvae were maintained under a 14-h/10-h light/dark cycle at 60% relative humidity and 25°C. *H. subflexa* utilizes several species of *Physalis*, but larvae are most commonly found on *P. angulata* (19, 20). *P. angulata* plants were grown from seed obtained from the F. L. Gould laboratory.

Oral secretions. Caterpillar oral secretions were collected following the procedure described by Turlings *et al*. (21). Each sample of pooled regurgitant (≈30 larvae) was immediately stored at −80°C. Samples were centrifuged at 16,000 × *g* for 10 min, and the supernatant was filtered consecutively through 0.45-μm

Abbreviation: LA, linolenic acid.

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(Millex-HV, Millipore) and 0.22-μm (Millex-GX, Millipore) sterile Millipore filters to remove bacteria.

Plant extractions. Plant materials (undamaged fruits, calyxes, or leaves) were weighed (2.5 g), ground in liquid nitrogen in a precooled mortar and pestle, diluted in a 50-ml solution of chloroform/methanol (2:1), and left in the dark for 24 h at 20°C. After the sample was centrifuged at 3,000 $\times$ *g*, the supernatant was saved, and the tissue pellet was reextracted with a solution of chloroform/methanol/water (1:10:10). Lipids were recovered in the chloroform phase, dried under N_2, and redissolved in methanol/acetic anhydride (MeOH/Ac_2O) (method described below). After methanolysis, the fatty acid methyl esters were quantified with GC/MS as described below.

HPLC analysis. Caterpillar oral secretions and plant extracts were analyzed by HPLC with UV detection at 200 nm (constaMetric 4100 pump, SpectroMonitor 3200 detector, Spectra System AS 3500 autosampler, Thermo Separation Products, Riviera Beach, FL). A reverse-phase column (YMC-Pack ODS-AMQ, 250 $\times$ 4.6-mm i.d.; YMC, Kyoto) was eluted (1 ml/min) with a solvent (High Purity Solvent, gradient 20–95% CH_3CN, Burdick and Jackson) containing 0.8% acetic acid, in water (Milli-Q UV Plus system, Millipore) containing 0.5% acetic acid, over 40 min and then returned to initial conditions at 45 min. The column was maintained at 60°C. For quantitative analyses, 5 μl of *N*-palmitoleoyl-L-glutamine solution (1 μg/μl) in CH_3CN/H_2O (8:2, μvol/vol) was added to each sample (50 μl) as an internal standard, and 10 μl of each sample was injected for HPLC analysis.

Acid methanolysis and chemical analysis. HPLC-purified compounds were vacuum-dried and treated with MeOH/Ac_2O following the procedure described by Mori *et al*. (22). The samples were analyzed by GC/MS using both chemical ionization and electron ionization (6890 gas chromatograph with a 30-m $\times$ 0.25-mm i.d., 0.25-μm-film-thickness HP-5 capillary column, interfaced to a Hewlett–Packard 5973 mass selective detector). The column was held at 40°C for 1 min after injection and then heated 10°C/min to 180°C. The carrier gas was helium at a velocity of 30 cm/sec. Isobutane was used as the reagent gas for chemical ionization, and the ion source temperature was set to 250°C. Compound identifications were confirmed by comparing chromatographic retention times and mass spectra for authentic standards.

Composition of fatty acid conjugates. Caterpillar oral secretions and plant extracts were heated to 95°C for 20 min and centrifuged at 16,000 $\times$ *g* for 10 min. Internal standard (*N*-palmitoleoyl-L-glutamine) was added, and each sample was analyzed by HPLC. The results were calculated as nanograms of each component per microliter.

Adaptation of *H. subflexa* Larvae to LA-Deficient *P. angulata* Fruit.

Experimental design and objectives. These experiments explored the ability of other herbivore species to survive and develop on *P. angulata* fruit, which lack LA. Larvae of the closely related species *H. virescens* (three repetitions of 30 larvae each per treatment) were subjected to three diet treatments: LA-sprayed fruits, fruits sprayed with a control solution, or an artificial pinto-bean diet. Survival of the larvae was assessed daily, and pupae were classified as healthy or defective.

Insects and plants. *H. virescens* larvae and *P. angulata* plants were maintained according to the protocols described for the previous experiment.

LA-treated fruit. Mature fruits (marble-sized) were collected from *P. angulata* plants and sprayed with either a solution of LA and ethyl alcohol (1:9) or a control solution (ethyl alcohol) by using a microspray device that creates a fine, uniform spray (23). Each fruit's calyx was cut and temporarily opened by pulling it apart at the distal end to allow the fruit to be sprayed and then reclosed. (Despite the opening created by this process, caterpillars almost invariably persisted in boring through the reclosed calyx to reach the fruit.) Once dry, the fruits were placed in diet cups and changed daily. A *G* test (log-likelihood ratio) was used to analyze differences between treatments (SAS Institute, Cary, NC).

Vulnerability to Natural Enemies. ***Experimental design and objectives.*** These experiments explored the effects of adaptation by *H. subflexa* to a diet of LA-deficient *P. angulata* fruit on the vulnerability of the larvae to natural enemies. We examined volatile profiles from plants induced by feeding on different *P. angulata* tissues (leaves, fruits, or fruits treated with LA) and the relative attractiveness of each of these profiles to female *Cardiochiles nigriceps* parasitoids. We then measured the survival and viability of *C. nigriceps* larva developing on *H. virescens* and *H. subflexa* caterpillars that were fed different diets (*P. angulata* fruits, fruit sprayed with LA, or artificial diet).

Insects and plants. *H. virescens* larvae and *P. angulata* plants were maintained according to the protocols described above. *C. nigriceps* were reared on *H. virescens* larvae according to the procedure of Lewis and Burton (24). Parasitoids were held at 25°C, 14-h/10-h light/dark, and 70% relative humidity until used for experiments.

Volatile collection and analysis. Five third-instar *H. subflexa* larvae were placed on 9-week-old greenhouse-grown *P. angulata* plants, which then were enclosed in a volatile collection chamber. Caterpillars were either allowed to move freely within the chamber (in which case they fed exclusively on fruits) or confined to feeding exclusively on the leaves. Volatile collections (at 6-h intervals) started when the caterpillars were placed in the chambers and continued for 7 days. Volatile collections and analyses followed the method described by De Moraes *et al*. (8) but with larger chambers (48 $\times$ 22 cm in diameter). Samples were analyzed by GC (GC/flame ionization detector) and mass spectrometry (GC/MS electron ionization and chemical ionization). The structure of the volatile components was confirmed by comparing GC retention times and mass spectral data with those of commercially available standards.

Behavioral assays. To assess parasitoid preference, dual-choice tests were performed in a wind tunnel by using (*i*) plants induced by leaf feeding versus plants induced by fruit feeding and (*ii*) plants induced by fruit feeding versus plants induced by larvae feeding on LA-treated fruits; undamaged plants with LA-treated fruits were used as a control. Twelve third-instar caterpillars were caged on the leaves (two per leaf) or fruit (one per fruit) of greenhouse-grown *P. angulata* plants for 48 h. The plant–caterpillar complex then was placed at the upwind end of a 60 $\times$ 60 $\times$ 180-cm wind tunnel. A wind speed of 60 $\pm$ 2 cm/sec was used at 25 $\pm$ 2°C and 40 $\pm$ 10% relative humidity and a light level of 500 W/m^2. A female *C. nigriceps* was released at the downwind end of the tunnel. The first plant terminal on which the female landed was recorded. Fifty complete flights were conducted for each treatment. Plant positions were switched after every fifth flight. Parasitoid preferences in the dual-choice tests were analyzed by a χ^2 goodness-of-fit test (SAS Institute).

Effect of LA on survival and development of parasitoids. To determine whether the absence of LA affects parasitoid (*C. nigriceps*) emergence and survival, we allowed female parasitoids to parasitize both *H. virescens* and *H. subflexa* larvae that were kept on the three diet treatments: LA-sprayed fruits, fruits sprayed with the control solution, or artificial diet (pinto bean for *H. virescens* or corn/soy meal for *H. subflexa*). For each treatment, three replicates with 30 caterpillars each were performed. Differences between treatments were analyzed by using ANOVA (SAS Institute).

Results and Discussion

Absence of LA in *P. angulata* Fruit and of Volicitin in Fruit-Feeding Caterpillars. Under natural conditions, *H. subflexa* larvae feed exclusively on the fruits of *Physalis spp*. Chemical analyses (HPLC-UV) of the regurgitant of *H. subflexa* caterpillars fed on

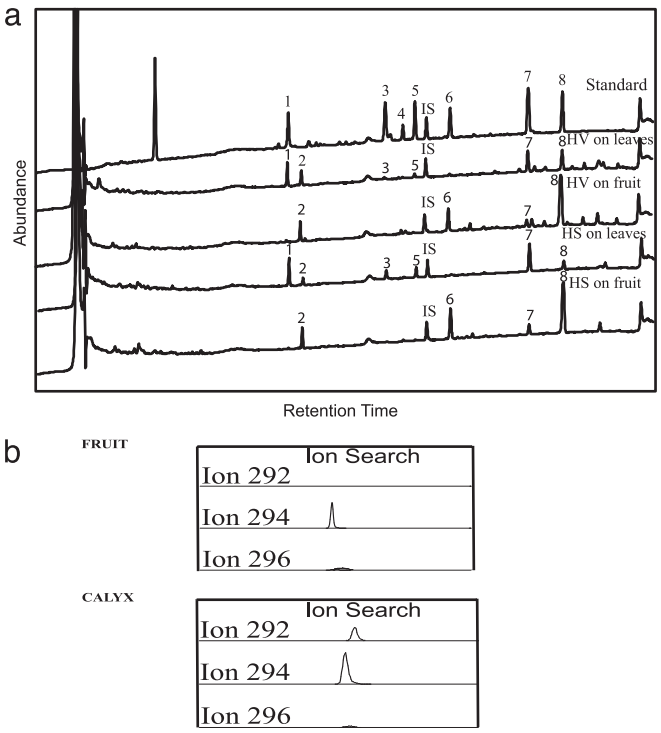

Fig. 1. (*a*) Typical chromatograms of oral secretions from *H. virescens* (HV) and *H. subflexa* (HS) fed on *P. angulata*. Chromatograms (HPLC-UV analysis at 200 nm) show the compounds detected in the supernatant of oral secretions from third-instar *H. virescens* and *H. subflexa* fed on *P. angulata* leaves or fruit for 48 h. Represented are: 1, *N*-(17-hydroxylinolenoyl)-L-glutamine (volicitin); 2, *N*-(17-hydroxylinoleoyl) glutamine; 3, 17-hydroxylinolenic acid; 4, 17-hydroxylinoleic acid; 5, *N*-linolenoyl-L-glutamine; 6, *N*-linoleoyl glutamine; 7, LA; 8, linoleic acid; internal standard (IS), *N*-palmitoleoyl-L-glutamine. It is significant that compound 1, volicitin, is not detectable in the oral secretions when either species fed on fruit. The very small amount of LA detected is likely caused by the presence of LA in the protective calyx, through which the caterpillar must bore to reach the fruit. (*b*) Analyses of fatty acid compounds (GC/MS electron ionization) in the fruit and calyx of *P. angulata*. Represented are: 1, linoleic acid (ion 294); 2, LA (ion 292); 3, oleic acid (ion 296). An ion search was performed to make sure that LA was not present in the fruits.

P. angulata fruit revealed the absence of volicitin [*N*-(17-hydroxylinolenoyl)-L-glutamine], an important elicitor of induced plant-volatile responses (Fig. 1*a*). This result was surprising because volicitin has been found in the regurgitant of the closely related species *H. virescens* and *Helicoverpa zea* (22) as well as in seven additional noctuid species (25).

We repeated our chemical analyses by using regurgitant from larvae that had been fed *P. angulata* leaves rather than fruits and from larvae that had been fed on tobacco and found that volicitin was present in both cases. These findings suggested that the absence of volicitin in fruit-fed caterpillars was caused by some property of the fruits. To confirm this, we repeated our analyses with a second caterpillar species, *H. virescens*, and obtained similar results (Fig. 1*a*): volicitin was absent in the regurgitant of *H. virescens* larvae fed on *P. angulata* fruit but present in the regurgitant of caterpillars fed on *P. angulata* leaves or on tobacco.

Because volicitin is synthesized within the caterpillar by addition of a hydroxyl group and glutamine to LA obtained from the diet (26), we hypothesized that the absence of volicitin in fruit-feeding caterpillars might be explained by an absence of LA in *P. angulata* fruit. Chemical analyses (HPLC/GC/MS) of fruit extracts confirmed the absence of LA (Fig. 1*b*). A search of mass spectra for ions representing fatty acid compounds in the fruit and calyx of *P. angulata* revealed that LA (ion 292) was present only in the latter.

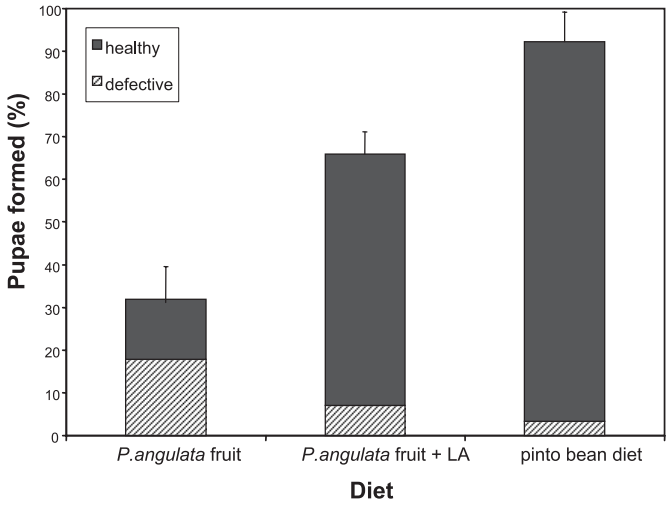

Fig. 2. Suitability of *P. angulata* fruit for *H. virescens*. Survival and pupation rates (for healthy and defective pupae) on *P. angulata* were assessed. Second-instar larvae were individually placed on fresh fruit in small plastic cups. Fruits were replaced daily. Three treatments were tested: (*i*) *P. angulata* fruit sprayed with ethyl alcohol; (*ii*) fruit sprayed with a solution of ethyl alcohol and LA (9:1); and (*iii*) artificial pinto-bean diet. Each replicate differed significantly from expectation ($G > 31.0$, df = 2, $P < 0.0001$).

We then sprayed *P. angulata* fruits with a synthetic solution containing LA to manipulate the presence of LA without affecting other factors such as the presence of secondary compounds. We found that volicitin was synthesized by *H. subflexa* caterpillars feeding on sprayed fruit, which confirmed that the absence of volicitin in the regurgitant of *P. angulata* fruit-fed caterpillars is attributable to the absence of LA in these fruits.

We cannot entirely rule out the possibility that small amounts of LA are present in the fruits but undetectable by our methods: LA is present in plant tissues in both free and membrane-bound forms (e.g., glycolipids, phospholipids, etc.); although our methods should extract both forms, it remains possible that some fatty acids, particularly those bound to membranes, were not completely extracted. Nevertheless, the undetectability of LA in the fruits compared with the high levels detected in other plant tissues, together with the strong effects obtained by adding supplemental LA, strongly indicate that a deficiency of LA in the fruits of *P. angulata* explains the absence of volicitin in fruit-fed caterpillars and the other phenotypic effects described below.

Adaptation of *H. subflexa* Larvae to LA-Free *P. angulata* Fruit. LA is essential for the normal development and metamorphosis of most insect larvae (15), especially Lepidoptera and Hymenoptera (16), which suggests that the ability of *H. subflexa* larvae to develop on the LA-deficient fruit of *P. angulata* may provide them with access to a food resource that is unavailable to lepidopteran herbivores lacking this adaptation. To explore this possibility, we examined the development of larvae of the closely related species *H. virescens*. We found that *H. virescens* larvae rarely survived on a diet of *P. angulata* fruit, and those larvae that did survive developed slowly and frequently displayed morphological defects (Fig. 2). To confirm that the inability of *H. virescens* larvae to develop on *P. angulata* fruit was attributable to the absence of LA, we reared larvae on *P. angulata* fruits that had been sprayed with a solution containing LA: survival, development, and pupal eclosion were all greatly enhanced (each of these replicates differed significantly from expectation: $G > 31.0$, df = 2, $P < 0.0001$) (Fig. 2).

The ability of *H. subflexa* larvae to develop in the absence of LA seems to provide them with almost exclusive access to *P. angulata* fruit. Over 2 years of fieldwork in Georgia and Florida,

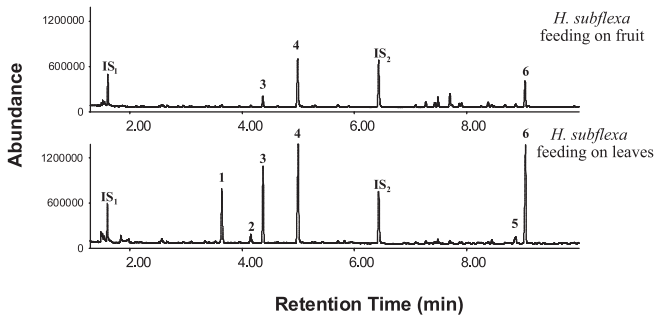

Fig. 3. Chromatographic profiles of volatiles from *P. angulata* during a 6-h interval after 48 h of feeding by *H. subflexa* on leaves or fruit. Represented are: 1, (*E*)-β-ocimene; 2, linalool; 3, (*E*)-4,8-dimethyl-1,3,7-nonatriene; 4, methyl salicylate; 5, an unidentified sesquiterpene; 6, (*E*,*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene; internal standards, *n*-octane (IS_1) and nonyl acetate (IS_2).

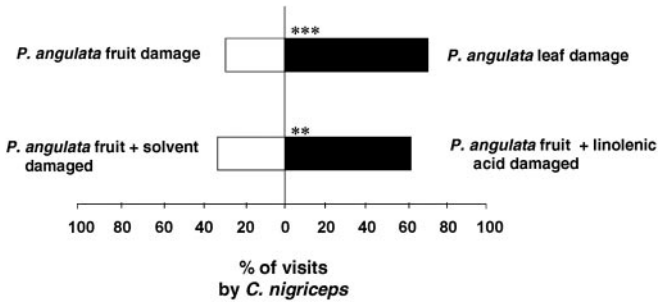

Fig. 4. Flight response of *C. nigriceps* to *P. angulata* plants subjected to damage by *H. subflexa*. Fruits were untreated or treated with LA in ethyl alcohol. Fruits treated with solvent alone were used as a control. Bars indicate the percentage of complete flights ($n = 50$). Asterisks indicate a significant difference within the choice test (χ^2 test: **, $P < 0.05$; ***, $P < 0.005$). Additional controls testing parasitoid response to filter paper treated with solvent and LA and to solvent alone resulted in no complete flights.

during which we collected hundreds of fruits from dozens of *P. angulata* plants, the only other insect observed was a single pyralid moth pupae. Their ability to develop on *P. angulata* fruits also makes it possible for *H. subflexa* larvae to exploit the protection from enemies provided by the fruit's protective calyx (27). In their natural habitat, *H. subflexa* larvae experience much lower parasitism rates than do closely related species such as *H. virescens*. Reported rates of parasitism for *H. virescens* on tobacco vary from 25% (28) to 72% (29), and rates as high as 96% have been observed on cotton (30). In contrast, Roach (31) found no parasitism of *H. subflexa* on *P. angulata*, whereas Lewis *et al.* (32) reported 2% parasitism and Sisterson and Gould (27) found 7% parasitism. It has been suggested that these low rates of parasitism are below expectations based on the protection afforded by the calyx alone (33), indicating that other factors must be involved.

Vulnerability to Natural Enemies. One factor that might account for the low parasitism rates observed for *H. subflexa* relative to closely related species is a reduction in plant-signaling defenses resulting from the absence of volicitin, an important elicitor of such defenses, in the regurgitant of *H. subflexa* larvae, which feed exclusively on the LA-deficient fruits of *P. angulata*. Gas chromatographic analyses revealed significant differences between the volatile profiles of *P. angulata* plants on which *H. subflexa* fed exclusively on fruits (as they do in nature) and those on which they were forced to feed exclusively on leaves (Fig. 3), with several major compounds present only in the profiles of leaf-damaged plants. Although some parasitoid-attracting volatiles were released by fruit-damaged plants, those typically elicited by volicitin were absent.

We directly tested the attractiveness of plants subjected to these two treatments, fruit feeding versus leaf feeding, to the parasitoid wasp *C. nigriceps* through behavioral assays (Fig. 4). *C. nigriceps* females were significantly more attracted to volatiles from *P. angulata* plants induced by *H. subflexa* feeding on leaves than to those from plants induced by fruit feeding ($\chi^2 = 8.00$, df = 1, $P < 0.005$). Furthermore, *C. nigriceps* females were significantly more attracted to volatiles from plants on which *H. subflexa* larvae fed on LA-treated fruits than to those from plants on which caterpillars fed on untreated fruits ($\chi^2 = 4.52$, df = 1, $P < 0.03$), strongly supporting the hypothesis that the relatively low attractiveness of volatiles induced by fruit feeding results from the absence of compounds elicited by volicitin, which is absent in the regurgitant of *H. subflexa* caterpillars feeding exclusively on the LA-deficient fruits of *P. angulata*.

Discrimination of these alternative volatile profiles may be adaptive for the parasitoids if LA-free caterpillars are unsuitable hosts. To determine the importance of LA for parasitoid survival, we allowed *C. nigriceps* females to parasitize *H. subflexa* and *H. virescens* larvae that were fed on fruit, fruit treated with LA, or an artificial diet. *C. nigriceps* larvae formed cocoons on only 23% of parasitized *H. virescens* and 33% of parasitized *H. subflexa* caterpillars fed on untreated fruit (Fig. 5). Of the parasitoids that did emerge from fruit-fed caterpillars, many (65% on *H. virescens* and 57% on *H. subflexa*) exhibited defects in their cuticles and wings. In contrast, 83% of wasp larvae from *H. virescens* and 67% from *H. subflexa* caterpillars fed on artificial diet formed cocoons (Fig. 5). When caterpillars were allowed to feed on fruits that had been sprayed with LA, 61% of wasp larvae from *H. virescens* and 47% from *H. subflexa* larvae produced cocoons (Fig. 5). ANOVA showed no caterpillar species effect but a highly significant diet effect [ANOVA: caterpillar species, $F(1,17) = 2.34$, $P = 0.149$; diet, $F(2,17) = 44.0$, $P < 0.00001$]. Morphological defects were rarely observed in parasitoids that emerged from *H. virescens* or *H. subflexa* larvae fed on fruit sprayed with LA and were not observed at all in parasitoids emerging from larvae fed on an artificial diet.

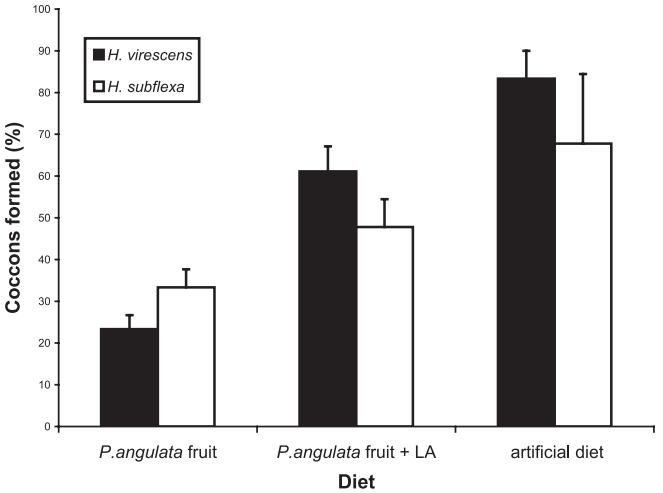

Fig. 5. Effect of diet on the suitability of *H. virescens* and *H. subflexa* larvae as hosts for *C. nigriceps*. Survival and pupation rates for the parasitoid were assessed. Female wasps parasitized third-instar larvae that then were individually placed on fresh fruit in small plastic cups. Fruits were replaced daily. Three treatments were tested: (*i*) *P. angulata* fruit sprayed with ethyl alcohol; (*ii*) fruit sprayed with a solution of ethyl alcohol and LA (9:1); and (*iii*) artificial diet. ANOVA: *H. virescens*, $F(2,8) = 101.6$, $P < 0.0001$; *H. subflexa*, $F(2,8) = 17.7$, $P < 0.005$; caterpillar species, $F(1,17) = 2.34$, $P = 0.1485$; diet, $F(2,17) = 44.02$, $P < 0.00001$.

The very low survival rate for *C. nigriceps* larvae developing in caterpillars fed on untreated fruits indicates that LA is needed for successful development in the caterpillar host. Thus, the ability of *H. subflexa* larvae to develop on LA-deficient *P. angulata* fruit seems to make them unsuitable hosts for natural enemies that require LA.

We also observed that transferring caterpillars from fruit to artificial diet after parasitism increased the rate of parasitoid emergence, which suggests that previously published parasitism rates for *H. subflexa*, although already quite low, may be inflated relative to what would have occurred in the field because it is a common practice in studies that measure parasitism rates to collect caterpillars in the field and then rear them on an artificial diet containing LA.

Conclusions

Through their close adaptation to particular features of the morphology, physiology, and biochemistry of *P. angulata*, *H. subflexa* larvae seem to achieve a level of protection from natural enemies exceeding that available to less-specialized herbivores. Our results indicate that *H. subflexa* larvae achieve multiple benefits from such narrow specialization: they gain access to a food resource that is unavailable to most of their competitors, co-opt the plants' physical and chemical deterrents for their own defense, render themselves nutritionally unattractive to parasitoids, and circumvent induced plant defenses elicited by volicitin, including the production of some volatiles attractive to natural enemies.

Investigation of plant defense mechanisms and herbivore countermeasures continues to reveal unexpected layers of complexity (8, 34, 35). The findings presented here illustrate that the vulnerability of insect herbivores to attack by natural enemies is mediated by complex interactions with the host plant and provide a documented example of a case in which adaptive specialization results in reduced vulnerability to natural enemies. This system highlights the complexity of tritrophic systems and the potential for intricate coevolutionary relationships to emerge within them.

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