

REPORT

An ecological cost of plant defence: attractiveness of bitter cucumber plants to natural enemies of herbivores

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Abstract

Plants produce defences that act directly on herbivores and indirectly via the attraction of natural enemies of herbivores. We examined the pleiotropic effects of direct chemical defence production on indirect defence employing near-isogenic varieties of cucumber plants (*Cucumis sativus*) that differ qualitatively in the production of terpenoid cucurbitacins, the most bitter compounds known. In release–recapture experiments conducted in greenhouse common gardens, blind predatory mites were attracted to plants infested by herbivorous mites. Infested sweet plants (lacking cucurbitacins), however, attracted 37% more predatory mites than infested bitter plants (that produce constitutive and inducible cucurbitacins). Analysis of the headspace of plants revealed that production of cucurbitacins was genetically correlated with large increases in the qualitative and quantitative spectrum of volatile compounds produced by plants, including induced production of (*E*)- β -ocimene (3*E*)-4,8-dimethyl-1,3,7-nonatriene, (*E,E*)- α -farnesene, and methyl salicylate, all known to be attractants of predators. Nevertheless, plants that produced cucurbitacins were less attractive to predatory mites than plants that lacked cucurbitacins and predators were also half as fecund on these bitter plants. Thus, we provide novel evidence for an ecological trade-off between direct and indirect plant defence. This cost of defence is mediated by the effects of cucurbitacins on predator fecundity and potentially by the production of volatile compounds that may be repellent to predators.

Keywords

Acari, cucurbitacins, indirect defence, plant–insect interactions, induced plant volatiles, Phytoseiidae, sequestration, tritrophic interactions.

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INTRODUCTION

Plants, herbivores, and natural enemies that consume herbivores rarely behave like linear food chains. Indirect effects typically influence such tritrophic interactions (Price *et al.* 1980; Sabelis *et al.* 1999; Agrawal 2000). For example, some attributes of the plant may attract natural enemies of herbivores or facilitate their efficacy (e.g. nectar: Koptur 1992; volatiles: Dicke & Sabelis 1988; leaf domatia: O'Dowd & Willson 1989) whereas other attributes may repel or interfere with the action of natural enemies (e.g. leaf hairs: Krips *et al.* 1999; plant toxins: Campbell & Duffey 1979). Because a single aspect of the plant may have positive and/or negative effects on herbivores and/or natural enemies of herbivores, studies of multitrophic interactions are essential for understanding

the net consequences of putative defensive traits for organisms. For example, a plant trait that has a negative effect on herbivores and a positive effect on predators is the most desirable from the perspective of plant fitness. It is unknown, however, how common such synergies may be, and plants generally have to balance the positive and negative effects of individual defensive traits.

Both the genotype and the environment of plants can influence tritrophic interactions. We have been studying the influence of bitter cucurbitacins (tetracyclic triterpenoids) in cucumber plants (*Cucumis sativus*) on herbivory by spider mites (*Tetranychus urticae*). Cucumber varieties differ qualitatively in production of cucurbitacins (hereafter, high cucurbitacins = bitter plants, and no cucurbitacins = sweet plants) (Da Costa & Jones 1971; Gould 1978). Bitter plants

are typically hostile hosts for spider mites (Da Costa & Jones 1971; Gould 1978; Agrawal 2000) as well as a wide range of herbivores including: several beetles, lepidopteran larvae, cockroaches, mice, and vertebrate grazers including humans (Metcalf *et al.* 1980; Metcalf & Lampman 1989; Tallamy & Krischik 1989; Tallamy *et al.* 1997). Humans can detect cucurbitacins at dilutions of 1 part per billion (Metcalf & Lampman 1989)! Other herbivores, such as specialist diabroticite beetles, compulsively feed on and sequester cucurbitacins, which may protect them against attack by natural enemies (Ferguson & Metcalf 1985; Barbercheck *et al.* 1995). In addition, spider mites can evolutionarily adapt to cucurbitacins in 5–10 generations and can have equal, sometimes even higher, fecundity on bitter plants compared to sweet plants (Gould 1979; Agrawal 2000). Several studies have taken advantage of the simple genetics of qualitative cucurbitacin production to demonstrate the repellent and toxic effects of cucurbitacins on herbivores and predators of herbivores (Da Costa & Jones 1971; Gould 1978; Tallamy & Krischik 1989; Barbercheck *et al.* 1996; Agrawal *et al.* 1999a).

Spider mite herbivory on cucumber plants results in increased cucurbitacin production (Agrawal *et al.* 1999a) and in the emission of volatiles (Takabayashi *et al.* 1994; Bouwmeester *et al.* 1999). The induced volatiles attract predatory mites (*Phytoseiulus persimilis*) and bugs (*Orius laevigatus*) (Takabayashi *et al.* 1994; Janssen 1999; Venzon *et al.* 1999) to plants infested with mites. In this study we examine the potential for pleiotropic effects of genetically determined cucurbitacins on the plant–predator interaction. We hypothesized that because both cucurbitacins and volatiles have a common origin in terpenoid biosynthesis, there may be a link between bitterness and attraction of natural enemies of herbivores. We use a population of herbivorous spider mites that is not affected by cucurbitacins to induce volatile production and to serve as food for predators to avoid the confounding issue of direct effects of cucurbitacins on herbivores.

Specifically, we asked the following questions. (1) How does the production of cucurbitacins affect the relative attractiveness of herbivore-infested and uninfested plants to predatory mites? (2) How does the production of cucurbitacins affect the volatile profiles of herbivore-infested and uninfested plants? (3) Do predatory mites have reduced fecundity when consuming spider mites reared on bitter plants compared to mites reared on sweet plants?

MATERIALS AND METHODS

Study system and general procedures

We used cucumber varieties Marketmore 76 (bitter) and Marketmore 80 (sweet) that are genetically very similar

except for those genes tightly linked with the gene responsible for cucurbitacin production (H. M. Munger, personal communication). Sweet plants were backcrossed at least four times with the bitter plants, and should thus differ by a maximum of 1/32 of their genes (T. Wehner, personal communication). Cucurbitacin production is controlled by a two-allele, single-locus gene, where cucurbitacin production is dominant (Andeweg & de Bruyn 1959). Cucumber plants were grown from seed in 0.8-L pots in growth chambers or greenhouses. Spider mites were maintained on intact sweet cucumber plants (var. Ventura RZ) in a growth chamber. Predatory mites were reared on detached cucumber leaves (var. Ventura RZ) infested with spider mites.

Effect of cucurbitacins on spider mite performance

To determine if our population of herbivorous spider mites was affected by cucurbitacins, we conducted an assay in which we inoculated 1.5 cm diameter leaf discs of bitter or sweet cucumbers each with 20 adult female spider mites ($n = 32$ for each treatment). A single leaf disc was taken from each plant. We counted the number of eggs laid after 48 h and tested for effects of plant variety on mite fecundity with a *t*-test.

Indirect effects of cucurbitacins: attraction of predators to plants

To test if *P. persimilis* was attracted to herbivore-infested plants and to assess the role of cucurbitacins in this attraction, we conducted a greenhouse release–recapture experiment. We buried eight potted cucumber plants in a large plastic tray (1.74 m × 1 m × 0.19 m) filled with soil (Fig. 1). The plants were placed in an octagon, an array with two replicate plants of each treatment: (1) an uninfested sweet plant, (2) a sweet plant infested with ≈100 spider mites

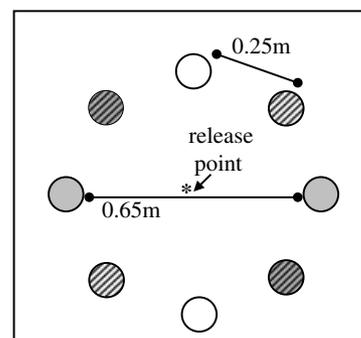


Figure 1 Experimental design of the release–recapture experiments to detect the effects of treatments on attraction of predatory mites. The design was a 2 × 2 factorial with cucumber variety (bitter or sweet) and infestation by spider mites (with and without) as the main effects.

3 days earlier, (3) an uninfested bitter plant, and (4) a bitter plant infested with ≈ 100 spider mites 3 days earlier. The two replicates of each treatment were at opposite sides of the octagon (Fig. 1). The plants had three expanded true leaves and none of the leaves touched the soil. Plants infested with mites showed visual signs of damage, although this was not severe. Spider mites did not move between plants (i.e. we never found mites on uninfested plants). After the plants were allowed to acclimate to the experimental arena for 24 h, we began our experiment by releasing 150 predatory mites in the centre of the octagon. *P. persimilis* does not have eyes and is blind. After 1 h, and approximately every 45 min for eight censuses, we counted the number of predatory mites on the plants. Predatory mites were also censused once on the following day, at which time the experiment was terminated. Mites had to walk a linear distance of 32.5 cm over soil to reach a plant, an equivalent of ≈ 600 body lengths. At each census we removed any predatory mites found on plants. By frequently sampling the plants and removing predators, we reduce the chance that predators on uninfested plants were missed because they left the plant in the absence of prey. Similar experiments revealed no difference when plants were sampled every 20 min vs. every hour (Janssen 1999). Prey quality is also not likely to influence the rate that predators leave plants (personal observations).

Each of our experimental trials with eight plants can be thought of as a small common garden experiment with two replicates of each treatment. We replicated this experiment six times over the course of 3 months (i.e. a total of 12 replicates for each treatment). Each time the experiment was replicated, treatment positions were shifted so that all treatments experienced all positions and had different neighbours each time. For each experiment we calculated the percentage of predatory mites recovered on each plant relative to the total number of mites recovered. To assess how our plant treatments affected recovery of predatory mites we used a factorial ANCOVA with plant variety (fixed effect), mite infestation (fixed effect), trial (random effect), plant position (random effect), and fresh plant weight (covariate) as the five main effects. Plant position was an arbitrary and unbiased covariate that was removed from the final analysis because it did not explain variance in predator attraction. Since all experiments were conducted in the greenhouse, trial was treated as an arbitrary blocking factor included in the analyses to correct for variance associated with the different trials, and thus trial-by-treatment interactions were not included in the model (model two described by Newman *et al.* 1997). Percentage data were arcsine square-root transformed before analyses because this improved the normality of the residuals. The above-ground mass of the plants was used to assess the influence of potential differences in plant size among treatments.

Indirect effects of cucurbitacins: volatile collection and analysis

Each volatile collection replicate consisted of a pooled sampling of two plants. Volatile samples were collected on three separate days, and all treatment combinations were represented on each day. Just prior to headspace sampling, stems of the two plants were cut right above the soil surface. The plants were put in glass vials with tap water and a cotton plug, and transferred into a 5-L glass desiccator. Incoming air was purified by drawing it through silica gel, activated charcoal (both 400 mL) and Tenax adsorbant. Volatiles were trapped on Tenax adsorbant (90 mg) packed in a 160×4 mm i.d. glass tube (Chrompack, the Netherlands). Airflow rate was ≈ 100 mL/min. and we sampled each pair of plants for 60 min. The plants were weighed immediately after headspace sampling. Tenax tubes were closed and stored in the dark at room temperature until analysis.

Adsorbents were first released from the Tenax by thermodesorption at 250 °C for 10 min with a helium flow of 10 mL/min. Desorbed compounds were cryofocused in a cold trap at -90 °C (M-16200, Chrompack, the Netherlands) and subsequently analysed on a Supelcowax-10 capillary column (60×0.25 mm i.d., 0.25 mm film thickness). The temperature programme of the gas chromatograph was 40 °C (4 min) to 140 °C (at 3 °C/min) to 270 °C (at 6 °C/min). The column was connected to a Finnigan MAT95 mass spectrometer, operated in the 70 eV EI mode with scanning from mass 24–400 at 1.05 s cycle time.

Compounds were identified by comparison of mass spectra with those in the Wiley Library (McLafferty & Stauffer 1989) and our own library of natural products (M. A. Posthumus, unpublished data), and by comparison of retention times with our database of retention indices based on authentic samples. Quantification was based on the average response of a mixture of 10 selected natural compounds run in a separate trial (1000 counts corresponded to 70 ng in all samples). We standardized the volatile data for the fresh plant weight of each sample.

Three replicate samples were taken from each of the four treatments, except that one replicate each of the sweet-control and bitter-infested plants was lost for reasons beyond our control. We analysed the data for the total volatiles produced and the number (diversity) of different peaks detected using two-way ANOVAs with plant variety (fixed effect) and mite infestation (fixed effect) as main effects. We then conducted a similar multivariate ANOVA using the same factors to examine effects on the three dominant volatile peaks. The dominant peaks were determined simply by ranking the volatiles in terms of highest mean production across all treatments.

Indirect effects of cucurbitacins: performance of predators

To assess the quality of spider mites feeding on bitter and sweet plants as food for predatory mites, we conducted an experiment with single female predatory mites in 8 mL vials with *ad libitum* spider mite eggs from mites grown on bitter or sweet plants. Spider mite eggs were collected using methods described in detail by Scriven & McMurtry (1971). Briefly, infested cucumber leaves were put in a water bath with a few drops of soap to dissolve the mite webbing and release the mites into suspension. The water was then passed through a series of four mesh-lined containers. The mesh size sequentially decreased to catch unwanted debris, adult female mites, immature and male mites, and eggs, respectively. The rinsed eggs were transferred from the container and collected on filter paper by using a Buchner funnel. A small sheet of filter paper containing approximately 50 eggs was placed in each vial with a single predatory mite from a laboratory colony. After 48 h we counted the number of predatory mite eggs laid in the vial. At the end of the experiment there were tens of mite eggs left uneaten in all vials. This experiment was conducted twice, with $n = 12$, for each treatment and for each trial. The data were analysed using a two-way ANOVA with treatment and trial as factors and the number of predatory mite eggs laid as the response variable.

RESULTS

Effect of cucurbitacins on spider mite performance

Our population of herbivorous spider mites was unaffected by the presence of cucurbitacins (mean \pm SE eggs laid: bitter 192 ± 6 , sweet 178 ± 7 , d.f. = 62, $t = 1.545$, $P = 0.127$). Given that mite fecundity is strongly related to feeding damage on plants ($r^2 = 0.806$, $F_{1,69} = 287.056$, $P < 0.001$, data from Agrawal *et al.* 2002), we conclude that bitter and sweet plants receive the same level of damage by our population of spider mites. Additionally, we detected no obvious differences in the amount of webbing or speckling on the leaves.

Indirect effects of cucurbitacins: attraction of predators to plants

In the release–recapture experiments conducted to test if bitter and sweet plants infested with spider mites differentially attracted predatory mites, we found that infested plants attracted $\approx 20\%$ of the predators released, whereas uninfested plants attracted $\approx 5\%$ of the predators (Fig. 2, Table 1). In addition, the significant plant variety by infestation interaction indicates that the bitter and sweet varieties were

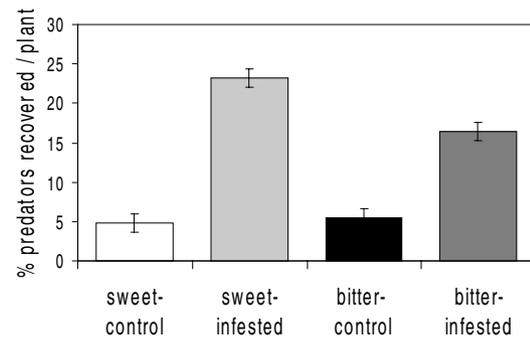


Figure 2 Effects of cucumber variety (bitter or sweet) and infestation by spider mites on the percent recapture of predatory mites per plant 8 h after the predators had been released. Data shown are mean \pm SE and have been adjusted for plant size. Note that the sum of the percentages equals 50% because there were two replicate plants of each treatment in each trial.

Table 1 Analysis of variance for effects of cucumber variety (bitter or sweet), infestation by spider mites, trial and plant mass on the percentage recapture of predatory mites per plant. Plant position was not included in the final model because it did not explain a significant level of variation

Source	d.f.	ms	<i>F</i>	<i>P</i>
Variety	1	0.447	0.319	0.576
Infestation	1	10.912	7.782	0.008
Variety \times infestation	1	6.047	4.312	0.045
Trial	5	1.507	1.075	0.390
Plant mass	1	8.569	6.111	0.018
Error	38	1.402		

differentially attractive when damaged (Table 1). Inspection of the means (Fig. 2) shows that whereas infested bitter plants attracted 16% of the predators, infested sweet plants attracted 23% of the predators. Across all experimental trials, bitter infested plants attracted 60 predatory mites, whereas sweet-infested plants attracted 82 (37% more predators). This difference is probably not caused by differences in the level of plant damage (see above) or differences in plant size, since this was included in the statistical model as a covariate (Table 1). Plant varieties did not systematically vary in plant size ($F_{1,46} = 0.678$, $P = 0.414$).

Indirect effects of cucurbitacins: volatiles

We trapped volatiles from 10 samples (two plants each) and found that bitter plants that produced nonvolatile cucurbitacins also emitted over five times the volatiles (standardized for plant fresh weight) and nearly two times as many different volatile compounds compared to sweet plants

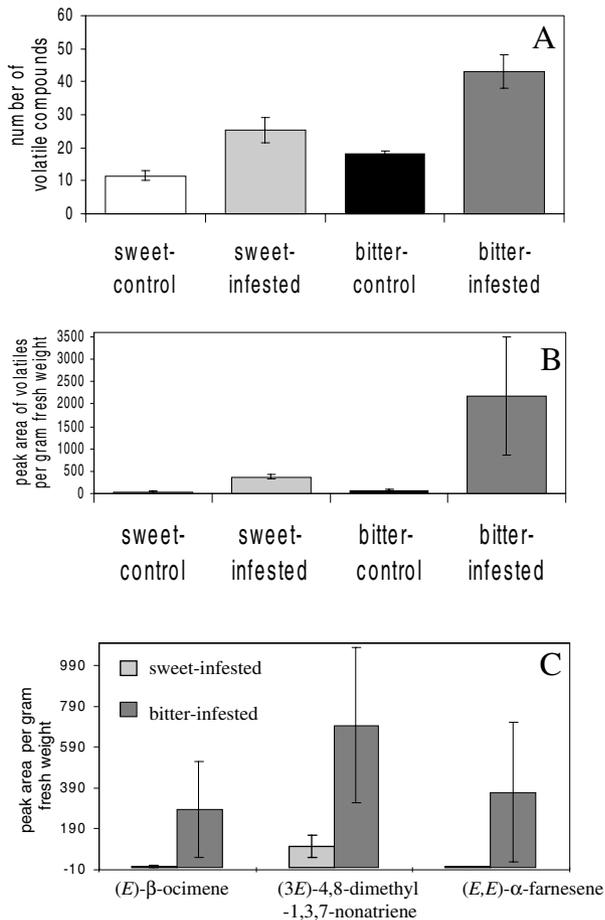


Figure 3 Analysis of the volatile compounds emitted from bitter and sweet cucumber plants as influenced by spider mite herbivory. (A) the diversity of volatile compounds, (B) the total amount of volatiles, and (C) the three dominant volatile compounds. Values for the sweet and bitter control plants in panel C are not given because there were no detectable levels of these volatiles. Values for sweet-infested plants were also low, and were undetectable where no bar is shown. Mean \pm SE is shown.

(Fig. 3, Table 2). For both of these measures of volatile production, mite infestation also had significant effects, increasing total volatiles 22-fold and more than doubling the diversity of compounds emitted across both plant varieties. In neither case was there a significant interaction between plant variety and infestation ($P \approx 0.13$, Table 2), although our power to detect such an interaction was quite low.

We next analysed the effects of our treatments on the three dominant volatile peaks that were emitted from plants using MANOVA. The dominant peaks were determined by examining the highest mean volatile production per gram fresh weight across treatments and were identified as (*E*)- β -ocimene, (*3E*)-4,8-dimethyl-1,3,7-nonatriene, and (*E,E*)- α -farnesene, each known to be attractive to predaceous

Table 2 Analysis of variance for effects of cucumber variety (bitter or sweet) and infestation by spider mites on (A) the number (i.e. diversity) of plant volatile compounds produced and (B) the total amount of volatiles (peak area) produced relative to fresh plant weight. (C) Multivariate analysis of variance for effects of treatments on the three dominant volatile compounds released by plants that are known attractants of predators [(*E*)- β -ocimene, (*3E*)-4,8-dimethyl-1,3,7-nonatriene, and (*E,E*)- α -farnesene]

Source	d.f.	ms	<i>F</i>	<i>P</i>
A				
Variety	1	350.417	14.894	0.008
Infestation	1	904.817	38.457	0.001
Variety \times infestation	1	74.817	3.180	0.125
Error	6	23.528		
B				
Variety	1	2.248	8.254	0.028
Infestation	1	17.345	63.698	< 0.001
Variety \times infestation	1	0.841	3.090	0.129
Error	6	0.272		
C				
Variety	3, 4	0.187†	5.795	0.061
Infestation	3, 4	0.100†	12.027	0.018
Variety \times infestation	3, 4	0.187†	5.795	0.061

†Wilks' λ .

arthropods (Dicke *et al.* 1990; Scutareanu *et al.* 1997). These three volatile compounds were not detectable in undamaged sweet or bitter plants and also had very low emissions in mite-infested sweet plants (Fig. 3, Table 2). Thus, the dominance of these compounds in the volatile analysis is driven primarily by their presence in bitter plants infested with mites ($P \approx 0.06$, Table 2).

Given the reduced attraction of predators to bitter-infested plants compared to sweet-infested plants, we examined the volatile profile for any known repellents of mites that may have been induced. Takabayashi *et al.* (1994) reported that production of volatile oximes by cucumber plants is associated with repellency against *P. persimilis*. We thus examined the effects of variety and mite infestation on the production of oximes. Indeed, the undamaged plants of both varieties produced virtually no oximes, whereas the bitter-infested plants produced three times the oximes as sweet-infested plants (mean \pm SE, sweet-infested: 1103 \pm 156, bitter-infested: 2948 \pm 1048 peak area per gram fresh weight; plant variety by infestation interaction term: $F_{1,6} = 5.160$, $P = 0.063$).

Indirect effects of cucurbitacins: performance of predators

To test if predatory mites suffer negative effects of feeding on mite prey raised on bitter vs. sweet plants, we conducted experiments in the absence of plant material to isolate the

effects of differences in the prey. Over the course of 2 days, predatory mites laid twice as many eggs when feeding on 'sweet eggs' compared to 'bitter eggs' (mean \pm SE fecundity, sweet eggs: 1.591 ± 0.352 , bitter eggs: 2.944 ± 0.468 , treatment: $F_{1,37} = 4.871$, $P = 0.034$). The effect of trial ($F_{1,37} = 9.629$, $P = 0.004$) simply indicates an overall difference in the number of eggs laid between trials (females were younger in the second trial); there was no treatment-trial interaction ($P = 0.410$). There were plenty of prey left in all replicates, indicating that the negative effects of plants on predators were not caused by prey availability.

DISCUSSION

Trade-offs between direct and indirect defence

Ecological costs of plant defence occur when plant traits that reduce herbivory have a negative consequence for the plant through interactions with other species. These types of effects have been increasingly reported in the literature and can take the form of attraction of specialist or generalist herbivores (Da Costa & Jones 1971; Bolter *et al.* 1997; Agrawal *et al.* 1999a), increased susceptibility of plants to pathogens (Felton *et al.* 1999; Thaler *et al.* 1999), reduced pollination (Strauss *et al.* 1996, 1999), and negative effects on predators of herbivores (Campbell & Duffey 1979; Thaler 1999). In the extreme case, high ecological costs of defence may cause plants to avoid investment in defence altogether, or switch to alternative strategies (e.g. tolerance). For example, such ecological costs may favour the evolution of inducible defences because the trade-off between defence and other traits is only expressed when the defence trait is needed (Agrawal & Karban 1999). In this study we show a novel cost of direct chemical defence: reduced attraction of predators of herbivores on defended plants compared to genetically similar undefended plants.

Knowledge of plant ecophysiology allows for mechanistic hypotheses on where and why costs may be expected (Halitschke *et al.* 2000). For example, the jasmonate biochemical cascade, which is triggered by herbivore feeding in many plants, is recognized to mediate both the direct and indirect defence pathways in tomato plants; it was therefore not expected that tomato plants exhibit a qualitative trade-off between direct and indirect defence (Thaler 1999). In the cucumber system, some of the direct and indirect defence compounds are of terpenoid origin: both the cucurbitacins (C30 triterpenoids, a direct defence in the bitter plants) and one of the major volatiles (the C11 homoterpene (3*E*)-4,8-dimethyl-1,3,7-nonatriene, an indirect defence compound mainly in bitter plants) are derived from C15 precursors (farnesyl pyrophosphate, FPP) (Gershenson & Croteau 1992). Thus, there is the potential for a physiological trade-off between direct and indirect defence. In this admittedly

oversimplified model, we speculate that FPP is simply diverted to direct or indirect defence; clearly this biochemical pathway may intersect with others and FPP may be employed for other functions as well. Our results indicate very little volatile production in sweet plants (Fig. 3). Thus, we hypothesize that genes regulating cucurbitacin production are upstream of the FPP because both cucurbitacins and volatiles were strongly influenced by the bitter genes. In this case, no physiological trade-off should be expected for defence precursors.

Some theory based on (1) ecological redundancy and (2) the negative effects of direct defence on natural enemies of herbivores has predicted an ecological trade-off in direct and indirect plant defence, although limited evidence supports these hypotheses (Steward & Keeler 1988; Dicke 1999). For example, cotton plants are polymorphic for the production of pigment glands that contain toxic terpenoid aldehydes. Glandless plants are susceptible to caterpillars (Parrott *et al.* 1989) and emit minimal volatiles when attacked (McAuslane & Alborn 1998); glanded cotton plants that are infested with herbivores release terpenoid volatiles that are attractive to the natural enemies of the herbivores (De Moraes *et al.* 1998). As in the cucumber system, direct and indirect defence in cotton plants may be linked because of their common terpenoid origin (Gershenson & Croteau 1992). Given the empirical evidence, the logic behind a proposed ecological trade-off can be comfortably reversed: plants that are not subject to herbivores should employ neither direct nor indirect defences. As long as direct and indirect defences do not completely negate each other's effect, a positive correlation between the two may be expected to be beneficial to plants. Nonetheless, our data indicate an ecological trade-off under the circumstances employed.

Plant volatiles and attraction of predators

The three dominant volatiles released by our bitter cucumber plants ((*E*)- β -ocimene, (3*E*)-4,8-dimethyl-1,3,7-nonatriene, and (*E,E*)- α -farnesene) have been previously demonstrated to be attractive to predators of herbivores. In particular, olfactometer experiments showed that *P. persimilis* was attracted to (*E*)- β -ocimene and (3*E*)-4,8-dimethyl-1,3,7-nonatriene (Dicke *et al.* 1990), and *Anthrenus* sp. was attracted to (*E,E*)- α -farnesene (Scutareanu *et al.* 1997). Herbivore-damaged cucumber plants also produced methyl salicylate, another volatile compound that is attractive to predators (Dicke *et al.* 1990). Induction of methyl salicylate is previously unreported for cucumber plants and its positive association with cucurbitacin production deserves further investigation given its distinct biosynthetic origin (phenyl propanoid pathway). Uninfested plants of both varieties did not produce detectable levels of methyl salicylate, whereas bitter-infested plants produced 15 times

the quantity (mean \pm SE, sweet-infested: 16 ± 10 , bitter-infested: 232 ± 187 peak area per gram fresh weight) compared to sweet-infested plants. Nevertheless, fewer predators were found on bitter-infested plants than sweet-infested plants. Thus our results present a paradox: bitter plants produce much higher quantities of known attractants of predators of herbivores yet were less attractive to predators in our experiments.

At least two nonexclusive possibilities may explain our results of lower predator attraction to plants producing more volatiles. First, the rearing history of predators may influence their subsequent choices (Takabayashi & Dicke 1992; Shimoda & Dicke 2000), including making formerly attractive compounds repellent to predators (Drukker *et al.* 2000a,b). As in many other experiments (e.g. Janssen 1999), in the current study, predatory mites were well-fed (*ad libitum*) until 1 h before release in our arenas. These predators were reared on a commercial (sweet) variety of cucumber leaves, which may have biased the predatory mites to be attracted to sweet cucumber leaves in our release–recapture experiments. However, because of the poor quality of herbivorous mites on bitter plants, if predators were reared on bitter plants we predict that they would be even less attracted to bitter-infested plants because of the negative experience of feeding on poor quality prey. This possibility illuminates the fact that our system is non-equilibrium and that the players do not share a long evolutionary history. Behavioural responses to previous experience (i.e. associative learning) or evolutionary change could eventually shift the balance of effects of volatile production on attraction of predators.

An alternative possibility for reduced predator attraction to bitter plants compared to sweet plants is that there may be repellent compounds in the blend of volatiles induced in bitter plants. Indeed, the diversity of compounds was nearly twice as high in bitter plants compared to sweet plants, although the attractive or repellent nature of most of these volatiles is unknown. Our volatile samples contained 2-methylbutanal O-methyloxime, 3-methylbutanal O-methyloxime-(*E*)v(*Z*), and 3-methylbutanal O-methyloxime-(*E*) + (*Z*), which had been previously implicated as repellent compounds released by mite-infested cucumber plants (Takabayashi *et al.* 1994). These oximes were of the most abundant volatiles released by bitter-infested plants (nearly 3000 units peak area per gram fresh weight). However, definitive evidence that oximes are repellent to predators is lacking, and the ultimate effects of such ‘repellent’ compounds after learning or adaptation by predators is unknown.

The ratios of particular volatile compounds may also tip the balance from attraction to repulsion. For example, Dicke *et al.* (1990) demonstrated that when the relative amount of (*E*)- β -ocimene to (*Z*)- β -ocimene was high (90% or greater)

these volatiles were attractive to *P. persimilis*; when this ratio was lower (60% and below), the volatiles were repellent. In the current study (*Z*)- β -ocimene was only detectable in the bitter-infested plants, which is suggestive that it may be interfering with the attraction of predators. However (*Z*)- β -ocimene comprised only 2% of the total β -ocimene, which may be too low to have a negative effect.

Plant defence and predator performance

Spider mites, like other generalist herbivores, are typically not thought to sequester plant toxins as a defence against their predators (see Dyer 1995). Yet several lines of evidence suggest that predators may be affected by the diet of spider mites. In the current experiment, predatory mites fed with spider mite eggs from adults raised on bitter plants were less fecund than predatory mites fed with spider mite eggs from sweet plants. This effect could be caused by reduced feeding on, or toxicity of the bitter eggs, but not to food limitation. Omnivorous thrips, which also consume mite eggs, showed a preference for consuming eggs from adults reared on control cotton plants over plants that were induced by previous herbivory (Agrawal *et al.* 1999b; Agrawal & Klein 2000). In both of these results, it is perhaps irrelevant whether the mites are actively sequestering plant toxins, because the net effect is that the eggs they produce are likely to be protected from predation. Supporting the sequestration hypothesis, De Moraes & McMurtry (1987) show that *P. persimilis* fed with adult herbivorous mites reared on a toxic plant gained less weight than mites reared on a preferred host. These results were consistent even when the herbivorous mites were starved for 24 h prior to predation trials, suggesting that plant toxins were incorporated into the herbivore’s body, and were not merely in the gut. From the plant’s perspective, producing toxins that negatively impact natural enemies of herbivores is an additional ecological cost of defence.

CONCLUSION

We present evidence for an ecological trade-off between direct and indirect defence for cucumber plants. Plants that produce cucurbitacins are typically protected from herbivory by many herbivores, but are less attractive to predators of tolerant herbivores in greenhouse common gardens. In these semi-natural arenas, blind predaceous mites travelled at least 600 body lengths and preferred sweet-infested plants over bitter-infested plants. Moreover, predators were less fecund when consuming herbivores on such defended plants compared to plants free of cucurbitacins. Although we can only conclude that we detected an ecological cost of defence with the particular organisms employed in our experiments, circumstantial evidence points to repellent

compounds (oximes) as an agent of this cost. The consequences of reduced fecundity of predators feeding on mites fed with bitter plants could lead to evolutionary change or experience-based behavioural shifts in the predators. Thus, the ultimate costs of defence may shift as the organisms in a tritrophic system interact over time (Agrawal 2001).

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