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11. Some progress has been made in that direction in models of storm-surge phenomena, as reported in (7).
12. We thank D. S. Dunbar for letting us use his

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Exploitation of Herbivore-Induced Plant Odors by Host-Seeking Parasitic Wasps

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Corn seedlings release large amounts of terpenoid volatiles after they have been fed upon by caterpillars. Artificially damaged seedlings do not release these volatiles in significant amounts unless oral secretions from the caterpillars are applied to the damaged sites. Undamaged leaves, whether or not they are treated with oral secretions, do not release detectable amounts of the terpenoids. Females of the parasitic wasp *Cotesia marginiventris* (Cresson) learn to take advantage of those plant-produced volatiles to locate hosts when exposed to these volatiles in association with hosts or host by-products. The terpenoids may be produced in defense against herbivores but may also serve a secondary function in attracting the natural enemies of these herbivores.

MOST STUDIES ON THE SIGNIFICANCE of herbivore-induced production of secondary metabolites in plants focus on the direct ecological interactions between plants and the herbivores that feed on them (1–3). Only a few investigators (4–6) have suggested active interactions between herbivore-damaged plants and the third trophic level of insect parasitoids and predators. There are many examples of these insects being attracted to plant odors (7), but only recently have studies indicated an active involvement of plants (5, 6). Dicke and co-workers presented the first convincing evidence for an active release of volatiles by herbivore-infested plants that attract natural enemies of the herbivorous attackers (6). As yet, no herbivore-specific factor that induces characteristic changes in plants, used by foraging entomophagous insects, has been pinpointed.

It is common that parasitic wasps learn to respond to specific odors that are associated with their hosts (8). The often observed flexibility in these responses has been attributed to the variability in space and time of reliable cues that may best guide the wasps to available hosts (9). Their ability to learn should allow parasitoids to distinguish among odors of plants with different types

of damage, thus enabling them to focus on plants damaged by potential hosts. Chemical responses evoked in plants by herbivorous hosts may therefore play an important role in host-habitat location by parasitoids. We report that herbivore-inflicted injury induces plants to release volatile terpenoids. The plant response is greatly enhanced by the oral secretions of caterpillars and is exploited by the parasitic wasp *C. marginiventris*, which uses the terpenoids as cues to locate hosts.

In flight tunnel trials, females of the parasitoid *C. marginiventris* are attracted to the

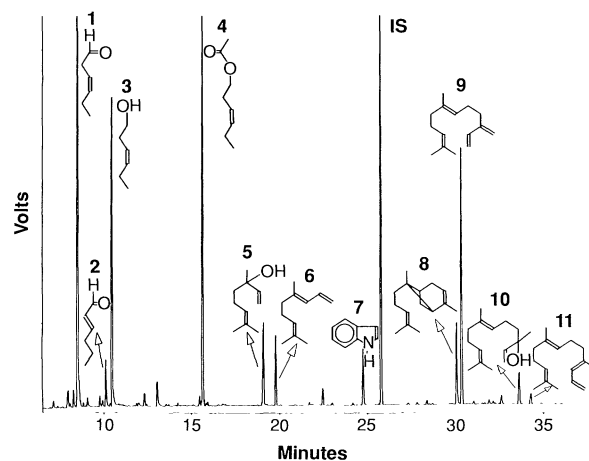
odors emanating from a complex of host larvae feeding on corn (*Zea mays* L., var. "Toana sweet corn") seedlings (10). Of the three main components of a complete plant-host complex, the damaged plants, and not the host larvae or their feces, are the main source of the volatiles that attract the parasitoid (10).

Volatiles from a complete plant-host complex consisting of beet armyworm larvae (BAW), *Spodoptera exigua* (Hübner), that were feeding on corn seedlings inside an all-glass collection system (11) were collected in traps containing Super Q adsorbent (12). Gas chromatographic analyses of methylene chloride washes of the traps revealed the consistent presence of eleven compounds (Fig. 1). The first four most volatile compounds were identified as leafy aldehydes, an alcohol, and an acetate, commonly found in the leaves of many plants (13). The remaining compounds were, except for indole, all terpenoids.

All the identified compounds are released by the caterpillar-damaged seedlings and not by the caterpillars themselves nor by something in their feces or other by-products (14). Additional volatile collections, however, revealed that the larger terpenoids, particularly α -trans-bergamotene, (*E*)- β -farnesene, and (*E*)-nerolidol, were only released by leaves that had been damaged by caterpillars for several hours. Plants subjected to caterpillar damage for 2 hours released the larger terpenoids only in minute amounts immediately afterward. The following day, however, large amounts of these compounds could be detected (Fig. 2).

Fast growing plants like corn invest much of their energy in growth and little in de-

Fig. 1. A chromatographic profile of the volatiles collected from a complex of BAW caterpillars feeding on corn seedlings. The identified compounds are 1, (*Z*)-3-hexenal; 2, (*E*)-2-hexenal; 3, (*Z*)-3-hexen-1-ol; 4, (*Z*)-3-hexen-1-yl acetate; 5, linalool; 6, (3*E*)-4,8-dimethyl-1,3,7-nonatriene; 7, indole; 8, α -trans-bergamotene; 9, (*E*)- β -farnesene; 10, (*E*)-nerolidol; and 11, (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene. For this particular collection, 15 early third instar caterpillars were allowed to feed on three 2-week-old greenhouse-grown corn seedlings. After 14 hours of feeding, the seedlings together with the caterpillars were transferred into the collection apparatus (11). Volatiles were collected for 2 hours in traps containing 25 mg of Super Q adsorbent (12). The traps were then extracted with 200 μ l of methylene chloride and an internal standard (IS) in 50 μ l of methylene chloride (*n*-nonyl-acetate, 20 ng/ μ l) was added. Of the extract 2.5 μ l was injected onto a Quadrex methyl silicone column (50 m by 0.25 mm inside diameter, 0.25 μ m film) inside a Varian model 3700 gas chromatograph. Temperature program: 50°C, rate 5°C/min to 180°C. Compounds were identified by mass spectroscopy and, where necessary, by NMR spectroscopy. Their identities were confirmed with synthetic versions of the candidate compounds (14).



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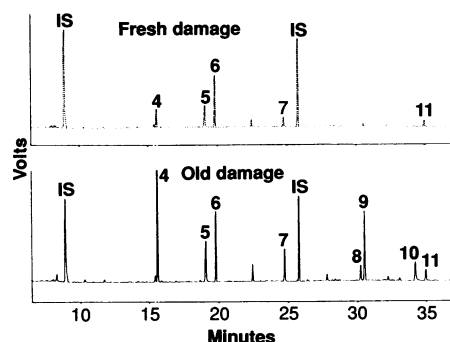


Fig. 2. Chromatographic profiles of collected volatiles from three corn seedlings damaged by BAW caterpillars over 2 hours. The volatiles were collected either just after damage took place (fresh damage) or 16 hours later on the following day (old damage). The collection procedure was the same as described in the legend to Fig. 1. No BAW larvae were present during the collections. Note that compounds 1 through 3 were not detected; these compounds only show up in significant amounts when the caterpillars are actively damaging the plants (14). Peak numbers correspond with the numbers and compounds given with Fig. 1. Each internal standard (*n*-octane and *n*-nonyl acetate) represents 1 μ g.

fense. When under herbivore attack, however, their flexible defense expressions will allow a fast induced production of carbon-based defensive chemicals (3). We suspect that the terpenoids released by corn seedlings serve in a direct defense against herbivores. In addition, their volatility and high turnover rate should make them reliable indicators of the presence of hosts for parasitoids. They would be even more reliable if the induced response is specific for damage inflicted by herbivores. This was tested.

Ten third instar BAW caterpillars were allowed to feed on three corn seedlings for 2 hours (late afternoon). During the same period, six other seedlings were damaged artificially with a razor blade, whereby the damage done by the caterpillars was roughly mimicked. Three of the artificially damaged corn seedlings, as well as three undamaged seedlings, were treated with the regurgitated gut contents of other corn-fed BAW caterpillars (15). The following morning, volatiles were collected from all four treatment groups for 2 hours. Striking differences in terpenoid and indole release were found among the collections when analyzed by gas chromatography (Fig. 3). Again, leaves with larval damage released the compounds in relatively large amounts. Seedlings that only underwent artificial damage released far less. The artificially damaged seedlings that were treated with caterpillar regurgitant released the most dominant compounds and in amounts similar to those found for the larval-damaged seedlings. The control seedlings, undamaged and treated with regurgi-

tant, released virtually no detectable amounts of volatiles.

Other seedlings that were subjected to these treatments were tested for attractiveness to experienced (16) *C. marginiventris* females in a flight tunnel (17). In two-choice tests, the insects strongly preferred the leaves with larval damage over leaves with just artificial damage (Fig. 4). The artificially damaged leaves that had been treated with caterpillar regurgitant were clearly preferred over the leaves with only artificial damage. When given the choice between leaves with larval damage and artificially damaged leaves treated with regurgitant, the females showed no preference (Fig. 4C). Fewer females flew to undamaged leaves treated with regurgitant than to artificially damaged leaves (Fig. 4D). This is in agreement with the observation that artificial damage alone does result in the release of some terpenoids (Fig. 3) and shows that the regurgitant by itself did not elicit attraction. The results strongly indicate that the observed plant response is greatly enhanced by the feeding of BAW caterpillars. It involves not only damage, but requires a factor in the regurgitant (most likely in the saliva) of the caterpillars as well. Whether this factor involves enzymes, microorganisms, or something else has yet to be determined.

We also found that volatiles collected from BAW larvae feeding on corn were attractive to the parasitoid when applied on a piece of paper (14). An equivalent synthetic blend containing all 11 compounds was attractive as well (14). Response depended on an insect's experience: females that had experienced BAW caterpillars feeding on corn (16) responded more to a natural blend than to a synthetic blend. Females that had experienced only a synthetic blend responded equally well to the synthetic and natural blend (14).

It is clear that experience plays a major role in the behavior of the parasitoids (16), and the preference for the terpenoid-releasing plants may have been learned during the preflight experience. Again, this demonstrates the highly flexible host-searching behavior exhibited by these insects that allows

them to learn odors closely associated with their hosts.

Many herbivores have developed variable levels of resistance to plant-produced chemicals, making those chemicals less effective in direct defense. Attraction of the natural enemies of herbivores, however, may result in an additional advantage to the plants, thereby maintaining selection pressures that favor the production of these chemicals in the observed high quantities. Cost-benefit anal-

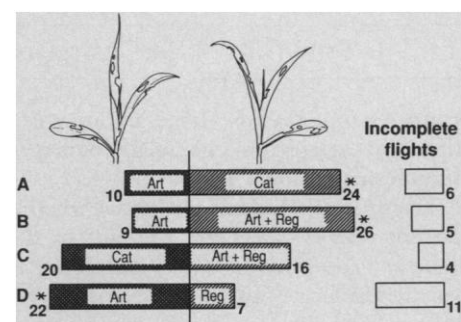


Fig. 4. Responses during two-choice flight tunnel tests (17) by experienced *Cotesia marginiventris* females to corn seedlings that underwent various treatments. A day after the leaves were treated, females had the opportunity to choose between the odors released by seedlings with (A) artificial damage (Art) or caterpillar damage (Cat); (B) artificial damage (Art) or artificial damage treated with regurgitant (Art + Reg); (C) caterpillar damage (Cat) or artificial damage treated with regurgitant (Art + Reg); (D) artificial damage (Art) or no damage treated with regurgitant (Reg). On five different days eight females were tested to each combination ($n = 40$). The open bars represent the females that did not fly to the odor sources. Total numbers are given with each bar. Asterisks indicate statistically significant preferences for a particular odor (χ^2 test, $P < 0.05$).

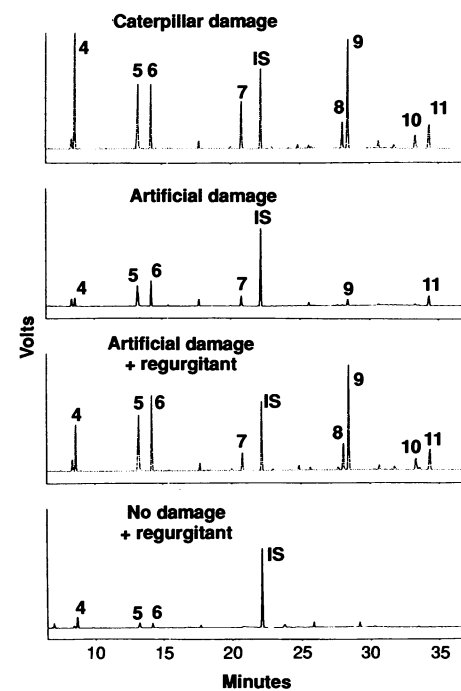


Fig. 3. Chromatographic profiles of corn seedlings with natural caterpillar damage, or with various artificial treatments. The day before collections took place, the seedlings were either damaged by BAW caterpillars for 2 hours (caterpillar damage), artificially damaged with a razor blade during the same period (artificial damage), artificially damaged and treated with caterpillar regurgitant (15) (artificial damage + regurgitant), or left undamaged but treated with regurgitant (15) (no damage + regurgitant). The collection procedure was the same as described in the legend to Fig. 1. Peak numbers correspond to the numbers and compounds given in Fig. 1.

yses concerning plant defensive strategies should consider how plants can safeguard themselves against severe herbivore injury by attracting predators or parasitoids (18). The terpenoids are reliable cues for the parasitoids because they are closely associated with herbivore damage and they are released even during the frequent pauses in eating by the caterpillars (Fig. 2). We do not yet know whether the induced reaction is limited to the damaged sites, or whether it is systemic as has been shown in other studies (19, 20).

Our results indicate an active release of chemicals by plants that is exploited by host-searching parasitoids. It is likely that the terpenoids and indole are involved in other types of interactions as well. They may, for example, act as oviposition deterrents for herbivorous insects searching for sites to deposit their eggs or function in communication between plants (20, 21). More knowledge about the injury-dependent production of airborne semiochemicals by plants may point to new possibilities for biological control of pest insects.

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11. The glass collection chambers, similar to those described by J. H. Cross [*J. Chem. Ecol.* **6**, 781 (1980)], consisted of two parts. Purified, humidified air entered the first part through a 4-cm-long 0.25-inch outer diameter inlet, which connected onto a wider section (6 cm long; 3 cm inner diameter) that contained a glass frit. After passing through the frit the air entered the second part (15 cm long; 3 cm inner diameter) which contained the odor source. The second part tapered into a 4-cm long, 0.25-inch outer diameter, outlet. Both parts had fitting ball joints that were clamped together. Super Q (12) traps were connected to the outlet with brass Swagelok fittings containing teflon ferules. Air passed through the chambers at a rate of 300 ml/min. The air was pushed in at the inlet side and pulled at the outlet, such that the pressure inside the system was slightly higher than outside. See T. C. J. Turlings *et al.* (14) for details.
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15. The leaves were treated by grabbing a caterpillar with a pair of forceps, pinching the head region with another pair until regurgitation was induced. The regurgitant was then immediately rubbed over a site on a seedling that had just previously been damaged. All artificially damaged sites on three of the seedlings were treated this way. As a control equal amounts of regurgitant were rubbed over the leaves of three seedlings that did not receive any type of damage.
16. Before release in the tunnel (17) each test insect was given a 20-s contact experience with a plant-host complex of BAW caterpillars on corn. Experience on host-infested leaves dramatically increases the subsequent responses by *C. marginiventris* females to host-related odors in olfactometric bioassays [T. C. J. Turlings, J. H. Tumlinson, W. J. Lewis, L. E. M. Vet, *J. Insect Behav.* **2**, 217 (1988); T. C. J. Turlings, J. W. A. Scheepmaker, L. E. M. Vet, J. H. Tumlinson, W. J. Lewis, *J. Chem. Ecol.* **16**, 1577 (1990)].
17. Responses of individual *C. marginiventris* females to odors were tested in a Plexiglas flight tunnel. Dimensions of the tunnel were 60 cm by 60 cm in cross section and 2.4 m long. Air was pulled through the tunnel at 0.15 m/sec and was exhausted through a 30-cm flexible pipe with a fan. More details on the tunnel are given by F. J. Eller, J. H. Tumlinson, and W. J. Lewis [*Environ. Entomol.* **17**, 745 (1988)]. Seedlings used as odor sources were placed 20 cm apart and 30 cm from the tunnel floor approximately 80 cm upwind from the insect release point. After the females were experienced (16), they were released into the tunnel from a glass funnel (10). Their responses and choices were recorded. If a female, after three trials, had not flown all the way to a source the flight was considered incomplete.
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Increase of the Catalytic Activity of Phospholipase C- γ 1 by Tyrosine Phosphorylation

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Phospholipase C- γ 1 (PLC- γ 1), an isozyme of the phosphoinositide-specific phospholipase C family, which occupies a central role in hormonal signal transduction pathways, is an excellent substrate for the epidermal growth factor (EGF) receptor tyrosine kinase. Epidermal growth factor elicits tyrosine phosphorylation of PLC- γ 1 and phosphatidylinositol 4,5-bisphosphate hydrolysis in various cell lines. The ability of tyrosine phosphorylation to activate the catalytic activity of PLC- γ 1 was tested. Tyrosine phosphorylation in intact cells or in vitro increased the catalytic activity of PLC- γ 1. Also, treatment of EGF-activated PLC- γ 1 with a tyrosine-specific phosphatase substantially decreased the catalytic activity of PLC- γ 1. These results suggest that the EGF-stimulated formation of inositol 1,4,5-trisphosphate and diacylglycerol in intact cells results, at least in part, from catalytic activation of PLC- γ 1 through tyrosine phosphorylation.

SINCE IT WAS RECOGNIZED THAT INOSITOL 1,4,5-trisphosphate (Ins 1,4,5-P₃) and diacylglycerol (DAG) are important intracellular second messengers in hormonal regulation of various cellular functions (1, 2), considerable effort has been invested in dissecting the molecular events that underlie receptor modulation of phosphoinositide-specific phospholipase C

(PLC) activity. A variety of receptor-PLC coupling systems are modulated by bacterial toxins, aluminum fluoride, and analogs of guanosine triphosphate (GTP) reagents that are believed to modify the actions of guanine nucleotide binding proteins (G proteins). As a result, it has been suggested that these hormones modulate PLC activity by a G protein-mediated mechanism (3). In con-