

Systemic release of chemical signals by herbivore-injured corn

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ABSTRACT Corn seedlings respond to insect herbivore-inflicted injury by releasing relatively large amounts of several characteristic terpenoids and, as a result, become highly attractive to parasitic wasps that attack the herbivores. Chemical evidence showed that the induced emission of volatiles is not limited to the sites of damage but occurs throughout the plant. This evidence was obtained by comparing the release of volatiles from leaves of unharmed (control) seedlings with the release of volatiles from undamaged leaves of seedlings with two injured leaves treated with caterpillar regurgitant. Immediately after injury no differences were measured in the released volatiles, but several hours later the undamaged leaves of injured plants released the terpenoids linalool, (3E)-4,8-dimethyl-1,3,7-nonatriene, and (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene in significantly larger amounts than leaves of unharmed plants. Other volatiles that are released by herbivore-injured leaves were detected occasionally only in trace amounts from the undamaged leaves of a damaged seedling. The systemic release of volatiles by injured corn coincided with attractiveness to the parasitoid *Cotesia marginiventris*; undamaged leaves of injured plants became significantly more attractive than leaves from control seedlings. These findings show conclusively that when a plant is injured by an insect herbivore the whole plant emits chemical signals.

Recent studies have demonstrated the active role of herbivore-injured plants in the attraction of insect predators and parasitoids. Plants infested by spider mites (*Tetranychus urticae*) initiate the release of volatiles that are attractive to predatory mites (*Phytoseiulus persimilis*) (1-4). Similarly, corn seedlings under attack by noctuid caterpillars release several terpenoids that are attractive to the generalist parasitoid *Cotesia marginiventris* (5-8). Both cases suggest that plants may actively emit chemical signals that, among other things, may function as attractants to natural enemies of herbivores. If indeed the plants are actively releasing volatiles into the environment the release may be from the undamaged parts of a plant as well as from the damaged areas. Two behavioral studies indicate that such herbivore-induced systemic plant responses occur and affect the behavior of entomophagous insects; predatory mites are attracted to undamaged leaves of a spider mite-infested plant (1-3), and the parasitoid *Epidinocarsis lopezi* is attracted to undamaged leaves of mealybug-infested cassava plants (9). Although no chemical evidence for systemic volatile releases exists, it has been established that leaves do not have to be damaged in order to elicit volatile releases; when healthy lima bean leaves come in contact with wet cotton wool on which mite-infested leaves had previously lain, they will release significant amounts of the predatory mite attractants (E)- β -ocimene and (E)-4,8-dimethyl-1,3,7-nonatriene (4).

The current study was undertaken to determine whether volatile emissions occur in completely unharmed leaves of injured plants. By monitoring volatile releases by different leaves of injured corn seedlings over a 24-hr period we obtained chemical evidence for induced systemic releases of

specific volatiles by the undamaged leaves, which corresponded with an increase in attractiveness of these leaves to a generalist parasitoid.

MATERIALS AND METHODS

Treatment of Plants. Corn (*Zea mays* L., var. "Ioana sweet corn") was grown in metal trays (9 × 35 × 50 cm) in a greenhouse, ≈60 seeds per tray. Experiments were conducted with 8- to 10-day-old seedlings that carried three clearly distinguishable leaves. Treatment of the plants occurred between 9:00 and 10:30 a.m. Twenty-four seedlings were selected for treatment. Approximately 2 cm² of the surface of two leaves of each plant was scratched with a clean razor blade; immediately afterward regurgitant of a corn-fed beet armyworm larva (*Spodoptera exigua*) was rubbed over the damaged surface (see Fig. 1). This treatment is known to induce the injured leaves to release large amounts of terpenoids several hours after treatment, similar to the response induced by actual feeding by beet armyworm larvae (5). Mechanical damage was chosen over insect damage to facilitate standardizing the procedure. Of the 24 seedlings, 8 had leaves I and II damaged, 8 had leaves I and III damaged, and 8 had leaves II and III damaged (for leaf numbering, see Fig. 1).

At four different time periods after the treatment (0-1 hr, 5-6 hr, 8-9 hr, and 22-24 hr), six treated seedlings were removed from the tray by cutting them low at the stem. They were selected such that as a group they contained two of each undamaged leaf type, numbers I, II, and III. For comparison, six control seedlings (completely undamaged) were removed from the same tray. Only those that had been standing next to the treated plants were selected to ensure that possible adsorption onto the undamaged leaves of volatiles released by damaged leaves would be equal for control and treated plants. In many cases, leaves of control plants were closer to damaged leaves than were undamaged leaves of injured plants.

Immediately after they were removed from a tray, the leaves of each group of seedlings were carefully severed from the stem with a razor blade. They were then recombined into groups of two complete seedlings, each consisting of three leaves of different plants, one pair of composite seedlings with damaged leaves only (DAM), one pair with only undamaged leaves of injured plants (UND), and one pair with only leaves of unharmed plants (CONT). The severed ends were wrapped in wet cotton wool to prevent dehydration and exposure of the severed stems, and each pair of composite seedlings was immediately placed in a volatile collection chamber (7). Volatiles released by the leaves of the three treatment groups were collected for 2 hr on traps containing 25 mg of Super Q adsorbent (Alltech, Deerfield, IL) (7). The

Abbreviations: IS, internal standard; DAM leaves, damaged leaves; UND leaves, undamaged leaves of injured plants; CONT leaves, leaves of unharmed plants.

traps were then extracted with 150 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 extracts, 2.5 μ l was injected onto a Quadrex (New Haven, CT) methyl silicone column (50 m \times 0.25 mm inside diameter, 0.25- μ m film) inside a Hewlett-Packard model 5890 gas chromatograph (temperature program: 50°C, rate 5°C/min to 180°C).

Insects. Beet armyworm (*S. exigua*) larvae were obtained from the U.S. Department of Agriculture rearing facilities in Gainesville, FL. They were reared according to a previously described procedure (10).

Cocoons containing *C. marginiventris* were obtained from a colony maintained at the U.S. Department of Agriculture-Agricultural Research Service, Insect Biology and Population Management Research Laboratory, Tifton, GA. They were reared and kept as described (11, 12). Flight-tunnel tests were conducted with 3- to 5-day-old mated females 4-8 hr into the photophase.

Flight Tunnel Tests. Responses of individual *C. marginiventris* were observed in a previously described (6) flight tunnel (60 cm \times 60 cm in cross section and 2.4 m long). The following conditions were maintained inside the tunnel: airflow, 15 cm/sec; relative humidity, 55-70%; temperature, 27.5-29°C; lumination, \approx 500 lux.

To increase their responsiveness, wasps were first experienced by allowing them to contact and oviposit in host larvae on corn seedlings (6, 13) and immediately afterward released in the flight tunnel, 80 cm downwind from the corn leaves that served as odor sources. The seedlings were treated as described above. The responses of the female wasps were tested 0-1 hr, 6-8 hr, and 22-24 hr after treatment of the seedlings. They were given a choice between CONT leaves or UND leaves. On 6 different days 12 wasps were tested for each time period ($n = 72$), using two sets of plants per time period.

RESULTS

Systemic Release of Volatiles in Injured Corn. Volatiles were collected from DAM leaves as well as from UND leaves of injured corn seedlings at four time periods after injury.

Volatiles from control plants without injury were collected for comparison. Gas chromatographic analyses of the collected volatiles showed a dramatic increase in the release of previously identified terpenoids (7) by the DAM leaves over time (Figs. 1 and 2). After 24 hr the release had dropped significantly. No change in volatile release occurred in the control plants (CONT), but the UND leaves did show a significant increase in the release of linalool, (3*E*)-4,8-dimethyl-1,3,7-nonatriene, and (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene several hours after treatment (Figs. 1 and 2). (*Z*)-3-Hexen-1-yl acetate (one of the green leafy volatiles released immediately upon damage) and indole were also detected from UND leaves several hours after damage. The sesquiterpenes α -*trans*-bergamotene and (*E*)- β -farnesene were only detected from these leaves in trace amounts. Preliminary follow-up studies indicate that these compounds may be released in larger amounts under different conditions and after different time periods.

Parasitoid Responses. In addition to chemical evidence for a systemic release of volatiles, behavioral evidence was obtained by observing the responses of *C. marginiventris* in a flight tunnel. Females of this parasitoid were individually given a choice between UND and CONT leaves. Immediately after treatment of the plants (1-0 hr), the wasps showed no preference for either leaf type (Fig. 3). Yet, 5-6 hr later the wasps were significantly more attracted to UND leaves than to CONT leaves. This preference was still prevalent 24 hr after treatment (Fig. 3). The UND leaves clearly became more attractive to the wasps, but the overall response was much poorer than we previously observed for DAM leaves (5-7).

DISCUSSION

Stress-induced systemic reactions in plants are common and the resulting production and transportation of chemicals usually are proposed to function as a direct defense against herbivores and pathogens (14-18). As far as we know, no previous study has presented chemical evidence for a systemic release of volatiles by plants in response to injury. The released terpenoids and indole are likely to be defensive

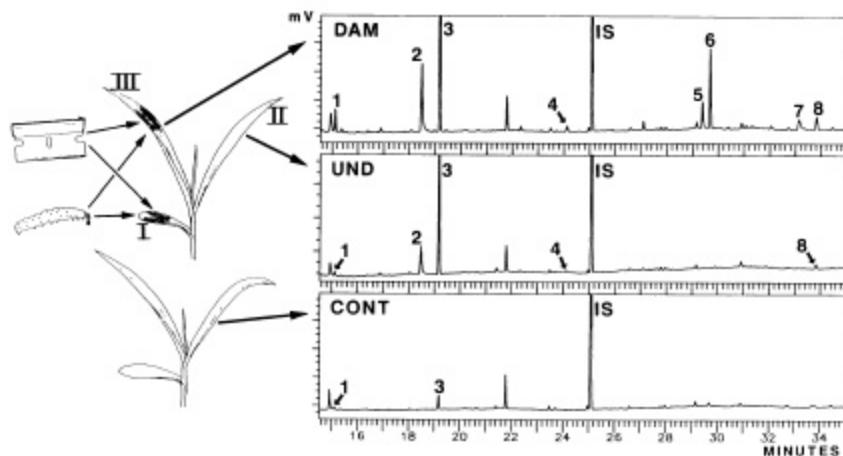


FIG. 1. Chromatographic profiles of volatiles collected from corn seedlings. Approximately 8-9 hr after treatment, volatiles were collected from leaves that had been artificially damaged and treated with caterpillar regurgitant (DAM), undamaged leaves of the seedlings that had been damaged and treated with regurgitant (UND), and control leaves of plants left unharmed (CONT). The roman numerals indicate the different leaves of a treated plant. The identities of the various compounds have been determined previously (7); they are as follows: 1, (*Z*)-3-hexen-1-yl acetate; 2, linalool; 3, (3*E*)-4,8-dimethyl-1,3,7-nonatriene; 4, indole; 5, α -*trans*-bergamotene; 6, (*E*)- β -farnesene; 7, (*E*)-nerolidol; and 8, (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene. Only one of the collections for the 8- to 9-hr time period is depicted here. Results of all collections for four different time periods are summarized in Fig. 2.

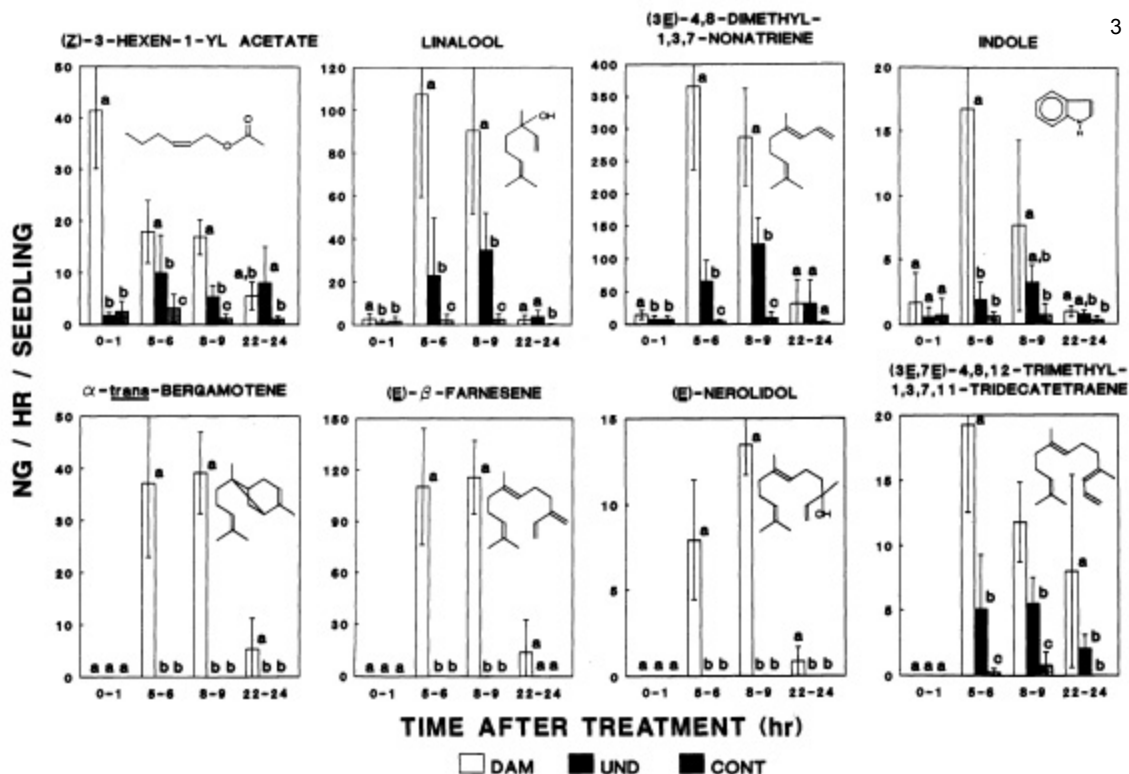


FIG. 2. Average amounts (ng/hr) of volatiles collected from corn seedling leaves 0-1 hr, 5-6 hr, 8-9 hr, and 22-24 hr after some of them had been artificially damaged and treated with caterpillar regurgitant (for details, see Fig. 1). The letters within each group of three bars indicate significant differences in average amounts released (Duncan's multiple range test after analysis of variance, $n = 6$, $P < 0.05$).

compounds or by-products of such compounds. However, in addition to initiating direct chemical defenses, herbivore-injured plants benefit indirectly by signaling information into their environment (1-5, 8, 9). The phenomenon appears to be common; several of the terpenoids released by damaged corn seedlings are also released by leaves of other plants under attack by caterpillars (13) or mites (3, 4). The chemical signals, in addition to being attractants to natural enemies of the herbivores, could also function as repellents to herbivores (3, 8). Plant-released volatiles may even affect neighboring plants (3, 19-23), but in our experiments we did not observe

any evidence for that since the emissions by the control plants were unaffected.

The observed lag in the systemic release of volatiles by corn after damage implies an active internal process. This chemical evidence, together with the behavioral data for *C. marginiventris*, is in accordance with the hypothesis that damage inflicted by herbivores induces the production of plant volatiles that serve a signal function beneficial to the plant and detrimental to the herbivores.

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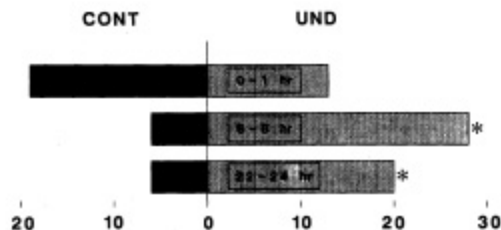


FIG. 3. Responses of *C. marginiventris* females during flight tunnel tests (6) in which they had the choice between leaves from healthy CONT plants and UND leaves. The bars and numbers indicate the total number of wasps that flew to a particular type of leaf. The asterisks indicate statistically significant preferences for UND leaves (χ^2 , $P < 0.05$). Note that <50% of the wasps flew to the leaves in each combination.

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