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SYSTEMIC RELEASE OF HERBIVORE-INDUCED PLANT VOLATILES BY TURNIPS INFESTED BY CONCEALED ROOT-FEEDING LARVAE *Delia radicum* L.

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Abstract—When attacked by herbivorous insects, many plants emit volatile compounds that are used as cues by predators and parasitoids foraging for prey or hosts. While such interactions have been demonstrated in several host–plant complexes, in most studies, the herbivores involved are leaf-feeding arthropods. We studied the long-range plant volatiles involved in host location in a system based on a very different interaction since the herbivore is a fly whose larvae feed on the roots of cole plants in the cabbage root fly, *Delia radicum* L. (Diptera: Anthomyiidae). The parasitoid studied is *Trybliographa rapae* Westwood (Hymenoptera: Figitidae), a specialist larval endoparasitoid of *D. radicum*. Using a four-arm olfactometer, the attraction of naive *T. rapae* females toward uninfested and infested turnip plants was investigated. *T. rapae* females were not attracted to volatiles emanating from uninfested plants, whether presented as whole plants, roots, or leaves. In contrast, they were highly attracted to volatiles emitted by roots infested with *D. radicum* larvae, by undamaged parts of infested roots, and by undamaged leaves of infested plants. The production of parasitoid-attracting volatiles appeared to be systemic in this particular tritrophic system. The possible factors triggering this volatile emission were also investigated. Volatiles from leaves of water-stressed plants and artificially damaged plants were not attractive to *T. rapae* females, while volatiles emitted by leaves of artificially damaged plants treated with crushed *D. radicum* larvae were highly attractive. However, *T. rapae* females were not attracted to volatiles emitted by artificially damaged plants treated only with crushed salivary glands from *D. radicum* larvae. These results demonstrate the systemic production of herbivore-induced volatiles in this host-plant complex. Although the emission of parasitoid attracting volatiles is induced by factors present in the herbivorous host, their exact origin remains

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unclear. The probable nature of the volatiles involved and the possible origin of the elicitor of volatiles release are discussed.

Key Words—*Trybliographa rapae*, *Delia radicum*, tritrophic interactions, host location, herbivore-induced volatiles, systemic release, root-feeders, olfactometer.

INTRODUCTION

When attacked by herbivorous arthropods, at least 23 plant species release volatiles that attract natural enemies of these herbivores (reviewed in Takabayashi and Dicke, 1996; Dicke, 1999b; Sabelis et al., 1999). Herbivore-induced plant volatiles are easily detectable and very reliable cues for foraging natural enemies, since they are produced in large amounts by plants, but only when the plants are under herbivore attack (Dicke, 1999a). Consequently, they are frequently described as the major chemical cues used by foraging parasitoids and predators in long-range host or prey location (Dicke and Sabelis, 1988; Dicke et al., 1990; Turlings et al., 1990; Vinson, 1991; Tumlinson et al., 1992, 1999; Vet and Dicke, 1992; Geervliet et al., 1994; Drukker et al., 1995; van Alphen and Jervis, 1996; Guerrieri et al., 1999; Pickett et al., 1999). In several cases, the attractive volatiles were released not only by the infested parts of plants, but also systemically by uninfested parts of infested plants, which probably further increases the detectability of the signal (Dicke et al., 1990, 1993; Turlings and Tumlinson, 1992; Potting et al., 1995; Röse et al., 1996; Cortesero et al., 1997; Guerrieri et al., 1999). In addition, a few studies have shown that uninfested plants treated with herbivore regurgitant release attractive volatiles, thereby demonstrating that the elicitor of attractive volatile release can originate from herbivores (Turlings et al., 1993; Mattiacci et al., 1994; Alborn et al., 1997). While the release of volatiles attracting parasitoids and predators appears rather widespread in the plant kingdom, in most studies, the herbivores studied to date have been leaf-feeding arthropods (Couty et al., 1999; Dicke, 1999b; Sabelis et al., 1999).

We studied the long-range plant volatiles involved in host location in a system based on a very different interaction, since the herbivore is a fly—the cabbage root fly, *Delia radicum* L. (Diptera: Anthomyiidae)—whose larvae feed on the roots of many cole plants. The parasitoid studied, *Trybliographa rapae* Westwood (Hymenoptera: Figitidae), is a specialist larval parasitoid of a few fly species belonging to the genus *Delia*. In particular, it attacks *D. radicum* L., *D. floralis* Fall., and *D. platura* Meig. (Wishart and Monteith, 1954; Wishart, 1957; Wishart et al., 1957). It is a koinobiont endoparasitoid that can develop on the three developmental stages of *D. radicum* (Jones, 1986; Tamer, 1994). All *T. rapae* hosts feed by digging galleries in the roots of their host plants (Jones, 1986). *T. rapae* females use three types of behaviors to locate host larvae buried in the plant tissues: antennal searching, ovipositor probing, and vibrotaxis (Vet and van Alphen, 1985). If larvae are located beneath the skin of the root, females

probe through the plant tissues with their ovipositor, but if larvae are concealed in the middle of the root, females slip through the galleries to reach them (Jones, 1986).

Little is known about the role of plant volatiles in host habitat location and host location of *T. rapae*. Vet (1985) and Jones (1986) have demonstrated that *T. rapae* females are attracted from a distance by volatiles emanating from *Brassica* roots and can distinguish volatiles from uninfested over infested and/or naturally decaying roots.

Because host larvae are concealed in galleries located inside buried cabbage roots, long-distance host location may be especially difficult in this parasitoid, in particular, no visual signals are available to detect damaged plants at a distance. In such a context, volatile cues may be of special importance, but they may be difficult to detect if released only by the buried root.

In the present work, we determined the attractiveness of different parts of uninfested and infested turnip plants for *T. rapae* females using a four-arm olfactometer. The possible factors triggering the emission of female-attractive volatiles were also investigated.

METHODS AND MATERIALS

Insects

The *D. radicum* culture originated from a colony maintained by the Institut National de la Recherche Agronomique (INRA) in Le Rheu, Brittany, in France.

T. rapae originated from *D. radicum* pupae collected from field Crucifera in Brittany in 1994. The strain has been refreshed yearly. The parasitoid was reared in a climate controlled room ($20 \pm 1^{\circ}$C, $60 \pm 10\%$ relative humidity, 16L:8D), on larvae of *D. radicum* fed on roots of turnip (*Brassica campestris* L.) or swede (*B. napus* L.) according to the rearing technique described in Neveu et al. (1996). After emerging from pupae, 5–10 females were kept with the same number of males in a plastic Petridish (8 cm diam. 2.5 cm high) that contained acacia honey and damp cotton wool. Food and water were regularly replenished.

All experiments were conducted with 3–6-day-old mated females. The females had no previous experience with plant or larval volatiles and had no oviposition experience. At least 1 hr before conducting the olfactometric tests, females were introduced individually in to plastic Petridishes placed in the olfactometer room.

Plants

Turnip plants were obtained from commercial roots (undetermined variety) that weighted approximately 100 g. They were placed in a climate controlled room ($20 \pm 1^{\circ}$C, $60 \pm 10\%$ RH., 16L:8D) where whole plants were grown in

350-ml plastic pots containing damp sand. Plants were watered every 2–3 days and kept for 1–2 weeks until they developed 3–10 new leaves.

Olfactometer Setup

Responses of *T. rapae* females to volatile chemicals emitted by different odor sources were measured in a four-arm olfactometer (Pettersson, 1970; Vet et al., 1983) with some modifications. Reconstituted compressed air (80% nitrogen and 20% oxygen) was circulated through a water bottle before entering the exposure chamber through the four arms. Air left the chamber through a hole in the chamber floor that was covered with a fine mesh to prevent insects from escaping the chamber. The chamber was constructed of Plexiglas. Its internal dimensions were 10 mm in height and 93 mm at its narrowest width across. The top Plexiglas plate had a central hole (12 mm diam.) closed with a plug.

Airflow in each of the four arms was adjusted with a flowmeter (Shorate 150 with glass float, Fisher-Rosemount) to 200 ml/min, thereby creating four distinct fields that were equal in size in the exposure chamber. These fields were visualized with a black background and smoke (chemical reaction of NH_4OH and HCl). The sharpest edges were produced with a flow rate of 200 ml/min through each arm. Therefore, experiments were conducted with an airflow of 200 ml/min. After circulating through the olfactometer, the air was released outside the experimental room.

To deliver the odor to one of the four fields of the olfactometer, the corresponding arm was connected with silicon tubing to a plastic box ($24 \times 18 \times 10$ cm) containing the odor source and tightly closed with adhesive plastic tape. Empty plastic boxes were connected to the control odorless fields (pure air). A circular neon light source underneath the olfactometer provided even illumination of 400 lux in the exposure chamber. The olfactometer setup was placed in a temperature controlled room ($19 \pm 2^{\circ}$C). Preliminary tests with 72 females showed that in pure air, female behavior was similar in the four fields of the olfactometer.

Bioassay Procedure

Individual female parasitoids were introduced through the top hole of the exposure chamber and placed on the fine mesh. Observations started when the female left the center and lasted for 2 min. If the female left the exposure chamber through one of the arms and entered the silicon tubing or the plastic box containing the odor source for more than 10 sec (a period after which females were never observed returning to the exposure chamber in preliminary experiments), the test was terminated and the remaining experimental time was counted as being spent in that field. Every 10–15 observations, the position of the odor field(s) was changed and the exposure chamber was washed with 95% alcohol and an antistatic chemical

(Altunet). Between odor trials, the chamber and the plastic boxes were washed with a neutral detergent (RBS 25) and rinsed with water. We measured the time spent in each field. Parasitoids were discarded if they: needed more than 5 min to leave the center; stayed only in the half of the odor field contiguous to the center; did not move for more than 60 sec; or returned for more 30 sec to the center. Insect paths in the exposure chamber were recorded with a video camera placed on top of the olfactometer.

Odor Sources

Each odor source consisted of one plant (part or whole) per plastic box. Plants were either uninfested or infested. Infested plants were obtained by depositing approximately 100 *D. radicum* eggs, 0–24 hr old with a small paintbrush. They were tested in the olfactometer 5–7 days after the infestation occurred. In all cases, only roots were infested, as *D. radicum* larvae are strictly root feeders.

When leaf volatiles were tested in the olfactometer, a special device was used to isolate the soil, the root, and the plant collar from the leaves over which the air was blown. This device consisted of a plastic cover with a 1-cm-diam. hole in the middle placed on the pot at the time of planting. Each cover was tightly closed with adhesive tape around the pot, and the hole was elongated with a vinyl sleeve (at least 3 cm in height) and Teflon tape, which covered the plant collar.

Response of T. rapae Females toward Uninfested Plants. The following odor sources were tested: uninfested whole plant (WP-UNINF); uninfested root (Rt-UNINF); and leaves from an uninfested plant (Lf-UNINF). In all cases, plants were never exposed to any larval damage.

Response of T. rapae Females toward Infested Plants. The following odor sources were tested:

1. infested root (Rt-INF): The eggs used to infest the root were deposited on the internal face of two halves of a root, placed on damp sand in a glass dish (15 cm diam.).

   For the following 2 odor sources, a root was cut in half. One half was used for UndRt-INF and the other for UndRt-UNINF.
2. Undamaged part of an infested root (UndRt-INF): One half of a root was infested. Just before the olfactometric tests, the damaged part, i.e., the part that contained the larvae, their tunnels, and any damaged tissue, was removed and the remaining part was tested.
3. Undamaged part of an uninfested root (UndRt-UNINF): The other half of the root was laid on damp sand and was left uninfested. Approximately the same part of the root as above was removed.
4. Leaves of an infested plant (Lf-INF): To infest the plant, the eggs were deposited on the top of the root.

Origin of Attractive Volatiles. The following odor sources were tested:

1. Leaves of water stressed plants (Lf-STRESS): Plants were only watered at the time of planting. At the time of the olfactometric tests, leaves were slightly withered.
2. Leaves of artificially damaged plant (Lf-ARTIF): Ten superficial cuts were made with a scalpel (about 1 cm in length, 0.5 cm in depth) at the plant's collar. To mimic feeding behavior of the root-feeder larvae, this treatment was repeated each day for 4 consecutive days. Olfactometric tests were conducted 4–6 hr after the last cuts were performed.
3. Leaves of artificially damaged plants treated with crushed larvae (Lf-ARTIF + CL): Twenty-five 5- to 7-day-old larvae were washed in distilled water, dried on filter paper, and crushed with a glass rod in a drop of water onto a slide. The liquid obtained was applied with a paintbrush on the cuts of an artificially damaged plant obtained as described above. New crushed larvae were applied to fresh artificial damage each day for 4 consecutive days.
4. Leaves of artificially damaged plants treated with larval salivary glands (Lf-ARTIF + SG): Twenty-five 5- to 7-day-old *D. radicum* larvae were dissected and their salivary glands were extracted with precision forceps and placed in a Ringer solution. Each pair of glands was torn apart, crushed with a needle, and then applied with a paintbrush on the cuts of an artificially damaged plant obtained as described above. New glands were applied to fresh artificial damage each day for 4 consecutive days.

Data Analysis

Homogeneity of times spent in fields of the olfactometer was analyzed by using ANOVA. Pairwise comparisons of time spent in each of the four fields were made by using Scheffe tests.

RESULTS

Response of T. rapae Females toward Uninfested Plants. Naive *T. rapae* females were not attracted to volatiles emanating from an uninfested turnip plant. Volatiles from either whole plants (Figure 1A) or leaves (Figure 1C) did not increase the time spent in the odor field compared to pure air ($F = 0.47$, $df = 3$, $P > 0.05$ vs. $F = 2.28$, $df = 3$, $P > 0.05$). Females preferred the pure air fields to the one odorized with an uninfested root, indicating possible avoidance ($F = 7.76$, $df = 3$, $P < 0.001$) (Figure 1B).

Response of T. rapae Females toward Infested Plants. Volatiles emanating from a root infested with *D. radicum* larvae were attractive to females (Figure 2A) ($F = 19.55$, $df = 3$, $P < 0.001$). Females also preferred volatiles from the

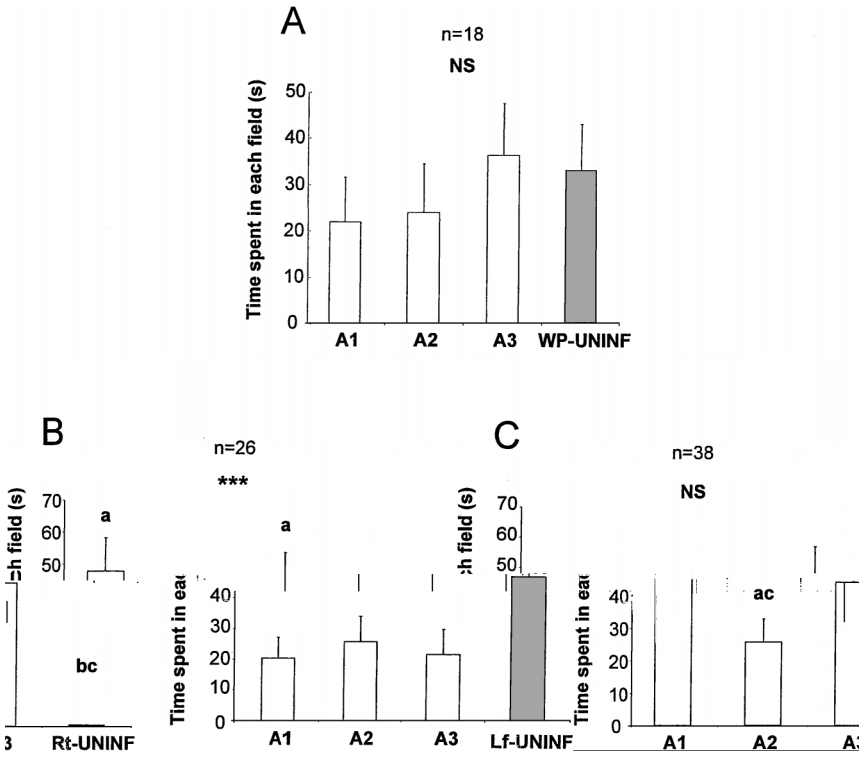

FIG. 1. Mean time spent (±SE) in each field of a four-arm olfactometer by inexperienced *T. rapae* females exposed to voltatiles from uninfested turnips. One field tested the volatiles from (A): a whole turnip (WP-UNINF), (B) a root (Rt-UNINF), or (C) leaves (Lf-UNINF). The other three fields tested pure air (A1, A2, A3). Means with different letters are significantly different (ANOVA followed by Scheffé test). NS: no significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

undamaged part of an infested root to volatiles from the undamaged part of an uninfested one (Figure 2B) ($F = 25.26$, $df = 3$, $P < 0.001$). Furthermore, they preferred volatiles from undamaged leaves of root-infested plant to volatiles from undamaged leaves of an uninfested plant (Figure 2C) ($F = 42.19$, $df = 3$, $P < 0.001$).

Origin of Attractive Volatiles. As leaves of root-infested plants were highly attractive, the factors responsible for the emission of attractive volatiles were investigated. Volatiles emanating from leaves of a water-stressed plant were not attractive (Figure 3A) ($F = 0.49$, $df = 3$, $P > 0.05$). Volatiles emanating from leaves of an artificially damaged plant were not attractive either; the females tended to prefer pure air (Figure 3B) ($F = 3.44$, 3 $df = 3$, $P < 0.05$). However, females preferred

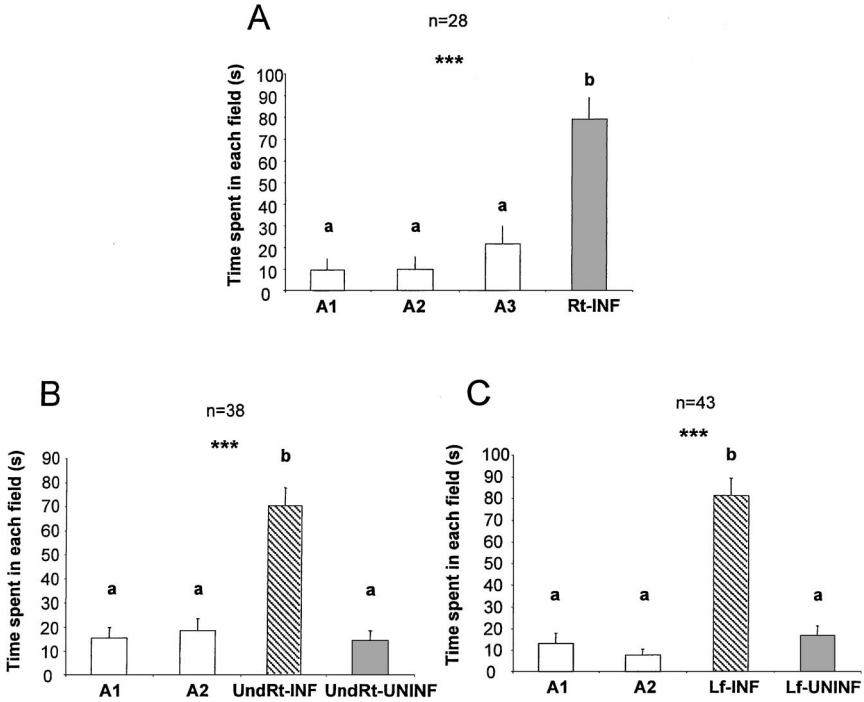

FIG. 2. Mean time spent (±SE) in each field of a four-arm olfactometer by inexperienced *T. rapae* females exposed to volatiles from infested turnips. (A) One field tested the volatiles from an infested root (Rt-INF), and the other three fields tested pure air (A1, A2, A3). (B and C) One field tested the volatiles from either a root (Rt-INF)(B) or leaves (Lf-INF)(C) of an infested turnip, and another field tested the volatiles from either a root (Rt-UNINF)(B) or leaves (Lf-UNINF)(C) of an uninfested turnip, while the other two field tested pure air (A1, A2). Means with different letters are significantly different (ANOVA followed by Scheffé test). NS: no significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

volatiles from artificially damaged plants treated with crushed larvae to volatiles from artificially damaged plants only or to pure air ($F = 26.40$, $df = 3$, $P < 0.001$) (Figure 3C). When an artificially damaged plant was treated with larval salivary glands, females were not attracted to volatiles from its leaves, when tested against volatiles from leaves of an artificially damaged plant and pure air (Figure 3D) ($F = 2.73$, $df = 3$, $P > 0.05$).

DISCUSSION

Response to Plant Volatiles. Our study demonstrated that *T. rapae* females are not attracted to volatiles emanating from uninfested turnips whether presented

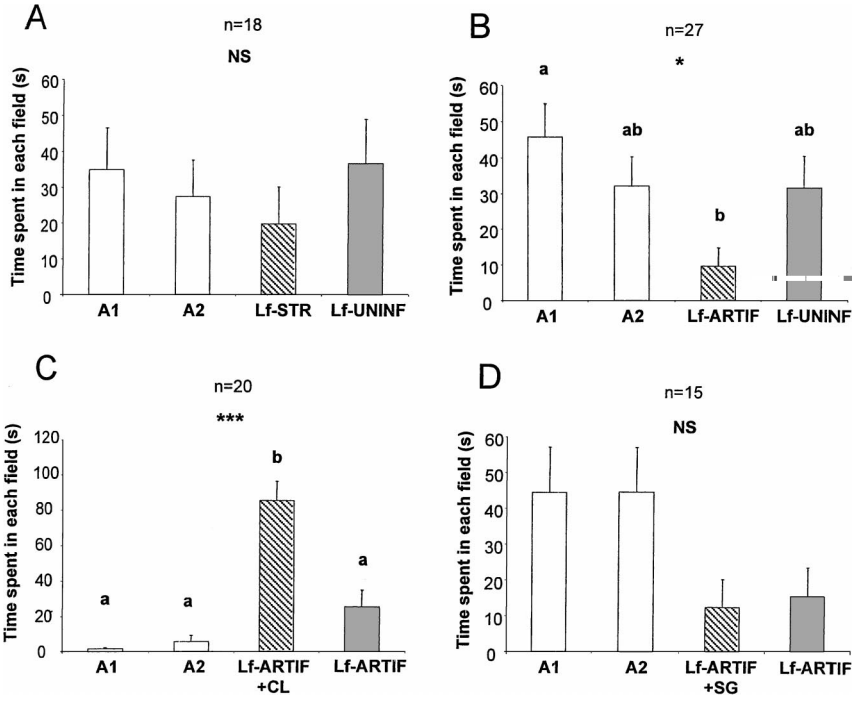

FIG. 3. Mean time spent (±SE) in each field of a four-arm olfactometer by inexperienced *T. rapae* females exposed to leafs odor of uninfested turnips submitted to different treatments. (A and B) One field tested volatiles from either a water-stressed turnip (Lf-STR)(A) or an artificially damaged turnip (Lf-ARTIF) (B), and another field tested volatiles from an uninfested plant (Lf-UNINF), while the other two fields conducted pure air (A1, A2). (C and D) One field tested the volatiles from an artificially damaged turnip treated either with crushed larvae (Lf-ARTIF+CL)(C) or salivory glands (Lf-ARTIF+SG(D), and another field tested the door of artificially damaged turnip (Lf-ARTIF); the other two fields tested pure air (A1, A2). Means with different letters are significantly different (ANOVA followed by Scheffe test). NS: no significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

as whole plants, roots, or leaves. This lack of response toward uninfested plants has been observed in other parasitoid species (Turlings et al., 1990; Guerrieri et al., 1993; Potting et al., 1995; Finidori-Logli et al., 1996; Du et al., 1996; Steidle and Schöller, 1997) and may be attributed to the low reliability of this signal to inform the parasitoid of host presence (Vet et al., 1991; Vet and Dicke, 1992).

In contrast, our results demonstrated that volatiles emanating from infested turnip roots were attractive to *T. rapae* females that are able to distinguish uninfested turnip roots from infested turnip roots.

We showed that volatiles emanating from the undamaged part of an infested root were more attractive than volatiles from an uninfested root. This demonstrates that the emission of attractive volatiles is not restricted to the infested part of the root. Moreover, attractive volatiles are emitted not only by the root itself but also by undamaged leaves of a damaged plant. Indeed, *T. rapae* females preferred volatiles from the leaves of an infested turnip to volatiles from uninfested turnip leaves. Therefore, it can be concluded that volatiles attracting *T. rapae* females are emitted systemically by plants infested by *D. radicum* root-feeding larvae. Systemic release of volatiles under herbivore attack has been demonstrated in four other plant species: lima bean (*Phaseolus lunatus*) (Dicke et al., 1990), corn (*Zea mays*) (Turlings and Tumlinson, 1992; Potting et al., 1995), cotton (*Gossypium hirsutum*) (Röse et al., 1996, Cortesero et al., 1997), and broad bean (*Vicia faba*) (Guerrieri et al., 1999). Our study demonstrates for the first time, That most herbivores attacking these plant species feed on aerial parts of plants; the emission of parasitoid-attracting volatiles from leaves of plants receive herbivore attack at the root level. Systemic release of plant volatiles is advantageous for *T. rapae* foraging females because *D. radicum* are concealed in the roots, whereas parasitoids forage for hosts in flight.

Turnips become attractive to *T. rapae* females only after being infested by *D. radicum* larvae. The volatiles involved in this attraction could be isothiocyanates, the volatiles specific to the genus *Brassica*. In this genus, the development of the turnip root fly *D. floralis*, a species close to *D. radicum*, induces chemical modifications in the plants attacked (Birch et al., 1990, 1992; Hopkins et al., 1993, 1995). In particular, larval damage to roots induces a modification in leaf glucosinolate composition: the concentration in aliphatic glucosinolates increases and the concentration in indole-based glucosinolates decreases (Birch et al., 1992). Glucosinolates are nonvolatile precursors of volatile isothiocyanates. Based on these results, it is plausible that *T. rapae* females modify leaf isothiocyanates and that this modification is induced by *Delia* spp. larval attacks on the roots. Additional chemical analysis is necessary to confirm this hypothesis, however.

Origin of Attractive Volatiles. The damage inflicted by *D. radicum* larvae on turnip roots usually generates water stress. This stress could induce the release of volatiles attracting *T. rapae*, as has been shown in lima bean (*Phaseolus lunatus*). Takabayashi et al. (1994) demonstrated that females of the acarian predator *P. persimilis* preferred volatiles emitted by water-stressed plants to those from nonstressed plants. However, in our study, volatiles emanating from leaves of a water-stressed uninfested turnip were not more attractive for *T. rapae* than volatiles from leaves of normally watered uninfested turnips. Therefore, water stress does not appear to induce the release of attractive volatiles.

Another possible factor triggering the emission of volatiles could be mechanical damage caused by *D. radicum* larvae. In many host-plant complexes, volatiles

released by an artificially damaged plant, such as green leaf volatiles, are attractive for parasitoids (Turlings et al., 1990; Whitman and Eller, 1990; McAuslane et al., 1991, Steinberg et al., 1993; Geervliet et al., 1994; Mattiacci et al., 1994; Potting et al., 1999). However, an artificially damaged turnip was not attractive for *T. rapae* females. In most cases, the release of the compounds occurs immediately after the damage and then rapidly decreases (Turlings et al., 1995). In our experiments, the damage was done made each morning for 4 consecutive days, and the olfactometric tests were performed in the afternoon of the fourth day. Consequently, green leaf volatiles were probably released in low amounts during the olfactometric tests.

et al., 1994). Therefore, we chose to apply dissected crushed salivary glands instead. The digestion of plant tissues is external in dipteran phytophagous larvae (Grassé, 1951), and it is possible that the portion of saliva collected with our method was ineffective or that the amount of saliva was insufficient to induce volatile release.

Another explanation is that the elicitor of volatile release is not present in salivary glands but in another part of the larvae, such as the digestive tract. Indeed, digestive caeca could enclose microorganisms that could induce plant release of attractive volatiles. Effects of microorganisms on volatile compound production has already been demonstrated in several studies (Croft et al., 1993 in Bruin et al., 1995; Doughty et al., 1996). In the *Cruciferae–D. radicum* system, several bacteria are involved in the decay of plant tissues that follows feeding by dipteran larvae (Johnson, 1930; Doane and Chapman, 1964). These bacteria are present in the digestive tract and in adult feces, on eggs and larvae, and inside the puparia of *D. radicum*. They are also present in the digestive tract of the figitid and staphylinid parasitoids of *D. radicum* (Johnson, 1930). The influence of these bacteria on the attraction of *T. rapae* females toward leaves remains to be investigated.

We have shown that infested turnips systemically emit herbivore-induced plant volatiles that attract the parasitoid *T. rapae*. Our next objective is to study the costs and benefits of these volatiles for the plant. As underlined by Turlings and Benrey (1998) and van der Meijden and Klinkhamer (2000), it has long been considered that plants have evolved mechanisms to actively attract natural enemies of their herbivores. If this hypothesis is true, attracting parasitoids should result in increased plant fitness. Until now, only one study has demonstrated increased fitness (seed production) when comparing a plant infested by unparasitized herbivores and one infested by parasitized herbivores (van Loon et al., 2000). However, herbivore-induced plant volatiles can be perceived and used not only by natural enemies of herbivores, but also by all other organisms present in the environment, e.g., herbivores, predators of parasitoids, hyperparasitoids, and neighboring plants, and such organisms could negatively impact plant fitness. For example, it seems that in most systems, many herbivores are attracted rather than repelled by volatiles released by infested plants. Indeed, it has been shown that herbivores can exploit these plant signals to locate their host plants and to get information on the presence of competitors and/or on the defensive state of the plant (Dicke and Vet, 1999; Dicke and van Loon, 2000). Such questions should be addressed in the *T. rapae–Cruciferae–D. radicum* complex to ascertain whether herbivore-induced volatiles are really part of the active defense system of turnip plants.

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