

## ATTRACTION OF A LEAF BEETLE (*Oreina cacaliae*) TO DAMAGED HOST PLANTS

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**Abstract**—Early in spring, just after the snow melts, the leaf beetle *Oreina cacaliae* feeds on flowers of *Petasites paradoxus*. Later in spring they switch to their principle host plant *Adenostyles alliariae*. The attractiveness of short- and long-term damaged host plants was studied in a wind tunnel. The spring host *P. paradoxus* was more attractive to the beetles after it had been damaged overnight by conspecifics or artificially, but not when the plants were damaged half an hour before the wind-tunnel experiments. Contrary to *P. paradoxus*, the principle host plant, *A. alliariae* was more attractive shortly after an attack by conspecifics (half an hour before the experiment) compared to a undamaged plant, but lost its increased attractiveness when damaged overnight. The enhanced attraction of damaged plants was longer lasting in the spring host *P. paradoxus* than in the main host *A. alliariae*. Volatiles emitted by host plants were collected and gas chromatographic analyses of the odors collected showed qualitative and quantitative differences between damaged and undamaged plants. Among the volatiles recorded, green leaf volatiles and mono- and sesquiterpenes dominated. In overnight damaged *P. paradoxus* plants with an enhanced attractiveness, limonene was emitted in higher amounts. In freshly damaged *A. alliariae* leaves, more  $\alpha$ -humulene and germacrene D were emitted compared to (*E,E*)- $\alpha$ -farnesene, whereas in the less attractive *A. alliariae* plants, more (*E,E*)- $\alpha$ -farnesene was emitted compared to  $\alpha$ -humulene and germacrene D. In the field, the long lasting attraction of flowering *P. paradoxus* early in the season may facilitate mating in *O. cacaliae* after a successful overwintering.

**Key Words**—Olfaction, behavior, wind-tunnel, gaschromatography, Coleoptera, Chrysomelidae, *Oreina cacaliae*.

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## INTRODUCTION

Herbivory leads to plant injury and thus to enhanced emission of plant volatiles, which may in turn increase the attractiveness of these plants to beetles (Harari et al., 1994; Loughrin et al., 1995; Bolter et al., 1997). Host-derived volatiles can also interact with aggregation pheromones to produce synergized attraction, like that described in bark beetles, or they can be converted into pheromones, as in mountain pine beetles (Borden, 1984; Jones, 1985).

The alpine *Oreina cacaliae* (Coleoptera: Chrysomelidae) feed on two host plants at a field site in La Fouly (Val Ferret: Valais) in the Swiss Alps. In spring, just after the snow melts, the beetles feed on *Petasites paradoxus* (Asteraceae) in its flowering state. This is one of the first annual plants to occur at sun-exposed sites. Wind-tunnel experiments confirmed the attractiveness of *P. paradoxus* over a nonhost (*Tussilago farfara*) in the laboratory (Kalberer, 2000). Up to 80 individual *O. cacaliae* beetles can be found on a single flowering *P. paradoxus* plant in the field (Kalberer, personal observation). This observation led to the hypothesis that an aggregation pheromone might exist, that attracts beetles to an individual host plant. *O. cacaliae* beetles live for up to three years and generations overlap. Females are ready to copulate immediately after emergence in summer, whereas males do not mate until the following spring and summer (Rahier, unpublished results).

A second host plant, *Adenostyles alliariae* (Asteraceae), emerges three to four weeks after *P. paradoxus* and serves as summer host for the beetles. The leaves of *A. alliariae* contain pyrrolizidine alkaloids that are sequestered by *O. cacaliae* larvae and adults for their own defense (Ehmke et al., 1991; Dobler and Rowell-Rahier, 1994). *A. alliariae* has one single large leaf (0.2 × 0.3 m) in its vegetative state and occurs in patches. The leaves of adjacent plants are often overlapping, making it easy for *O. cacaliae* to walk from one plant to another. In the field, the beetles are much less aggregated on the *A. alliariae* leaves than on their spring host *P. paradoxus* (Kalberer, unpublished data). Quite often two and sometimes three beetles can be found on a single *A. alliariae* leaf, but their number rarely exceeds more than four (Kalberer, personal observation).

In relation to observations of aggregation in this beetle we tested (1) if an aggregation pheromone exists, (2) if plant injury changes the attractiveness of the host plants, and (3) if *O. cacaliae* reacts differently to short versus prolonged injury to *P. paradoxus* and *A. alliariae* plants. Different responses to already damaged *P. paradoxus* and *A. alliariae* may explain the observed differences in aggregation on these plants. Experiments to test ambulatory responses of beetles towards plant odors were conducted in a wind-tunnel. In addition, the volatiles emitted by undamaged and damaged host plants were collected, identified, and compared.

## METHODS AND MATERIALS

*The Insects.* Adults of *O. cacaliae* were collected weekly from *P. paradoxus* in spring and from *A. alliariae* in summer, near La Fouly (Val Ferret: Valais) in the Swiss Alps. To prevent stress, beetles were transported to the laboratory in cooled containers. The beetles were maintained in glass jars in an incubator at a day temperature of 16°C and a night temperature of 12°C and a 16L: 8D light regime. Beetles were used within three days of collection in experiments and were sexed using sexual dimorphism of the tarsi (Lohse and Lucht, 1994).

*The Plants.* Plants used in the experiments were dug up weekly at or near the sites where beetles were collected and immediately transplanted into 15-cm-diameter ceramic pots. Potted plants were transported to the laboratory, where they were placed near a window and kept at room temperature until use. To obtain herbivore-damaged plants, 10 adult beetles (5 males and 5 females) were placed on *P. paradoxus* and *A. alliariae* plants and allowed to feed, either for half an hour before the experiment (short-term damage) or overnight beginning at 2000 hr, and the experiments were conducted during the next day (overnight damage). *A. alliariae* was also subject to another treatment in which five last-instar *O. cacaliae* larvae were allowed to feed on a plant for half an hour before the experiment (larval short-term damage). Both host plants were also subjected to an artificial injury treatment. Artificial injury to *P. paradoxus* was inflicted by a razor blade, with which two out of several dozen flowerheads were removed together with the edge of a basal leaf surrounding the floral stalk. In *A. alliariae*, artificial injury was administered by cutting two pieces (35 × 10 mm) from the edge of the leaf with a razor blade and punching a hole (14 mm diameter) in the middle of the leaf with a cork borer. Each plant was used once as an undamaged plant and once after being damaged in wind-tunnel experiments and for odor collection.

*Wind-Tunnel.* The Plexiglas wind-tunnel (0.6 × 0.6 × 1.8 m) was surrounded by a white tent to eliminate visual distraction for the beetles. Air was pulled through the tunnel at 0.1 m/sec with the use of a tubular duct fan (RR 125 C, Melios Ventilatoren AG, Urdorf, Switzerland) and was exhausted outside the room. A charcoal impregnated fabric cleaned the air at the tunnel entrance. Room temperature in the wind-tunnel facility was 22–23°C and humidity around 55% (for details see Kalberer, 2000).

*Experimental Procedure.* The assay was designed as a dual-choice test for walking beetles. Two differently treated potted plants were placed 0.3 m apart at the upwind end of the wind-tunnel. A glass plate (1.5 × 0.5 m) was placed horizontally in the wind-tunnel at a height adjusted such that air from the volatile-emitting, upper plant parts would pass over it. The distance between the point of beetle release on the glass plate and the plants was 1 m. The glass plate was cleaned with 70% ethanol after testing 10 beetles. Each plant combination was replicated four times with

20 beetles (10 females and 10 males) per replicate. Replication took place at intervals of at least one week with different plants and different beetles. The insects were deprived of food for at least 24 hr before being tested to promote their anemotactic response (Visser and Nielsen, 1977; Miller and Strickler, 1984). Glass jars with beetles were taken out of the incubator 30 min before an experiment and left near the wind tunnel to let the beetles acclimatize to the experimental conditions. Details on the behavior of the beetles in the wind tunnel are described in Kalberer (2000).

*Wind-Tunnel Experiments Excluding Visual Cues.* To exclude visual cues, the plants were not put in the wind-tunnel but placed outside the white curtain surrounding the wind tunnel. Compressed air, humidified and purified by a charcoal filter, entered a heat-sealed Nalophan cooking bag (Kalle Nalo, Wiesbaden, Germany) containing the potted plant. Teflon tubes led the air enriched with plant odors out of the cooking bag and into the wind tunnel through two holes at the upwind end of the wind-tunnel. The ends of the tubes were taped vertically to the upwind edge of the glass plate.

*Pheromone Test.* To test for pheromonal attraction, we placed either 10 overwintered males or 10 freshly emerged females in a large mesh metallic cage and released beetles of the opposite sex. To establish a dual-choice test, we had two cages, one with beetles and one empty. The experiments were conducted with 20 beetles and replicated four times.

*Collection of Plant Volatiles.* Volatiles were collected from potted plants inside a climate chamber at 15°C, 50–60% relative humidity, and 35,000 lux. The plants were placed inside odorless polyethylene terephthalate (PET) Nalophan cooking bags. One bag opening was sealed before use with a heat sealer (TEW Electric Heating Equipment Co. Ltd., type TISH-400), the other was tied around the plant stem with a plastic tie provided with the bags. Humidified and charcoal-filtered air entered the bag via a Teflon tube at a rate of 1000 ml/min. The Teflon tube was connected to the bags by a glass tube. The glass tube entered the PET bag and was introduced through a screw cap containing a Teflon-coated O-ring of the same diameter [6 mm; for details see Turlings et al. (1998)].

A volatile collection trap, consisting of a 10-cm-long × 6-mm-diameter glass tube containing 25 mg Super Q adsorbent (as described by Heath and Manukian, 1992), was attached to the PET bag in the same way as the inlet glass tube. Air was pulled through the collection trap in the bag via Tygon tubing at a rate of 800 ml/min (Turlings et al., 1998). Volatiles were collected for 4 hr, after which each trap was rinsed with 150  $\mu$ l methylene chloride, and internal standards (400 ng *n*-octane and nonylacetate) were added before injection to a gas chromatograph (GC). Six collections per treatment were made.

*Plant Volatile Analysis.* Volatiles were analyzed with a Hewlett Packard 6890 GC equipped with an on-column injector and a flame ionization detector. Aliquots of 3  $\mu$ l were injected onto a 30 m × 0.25 mm nonpolar column (EC1, film thickness) 0.25  $\mu$ m, Alltech Associates), preceded by a 10-m × 0.25-mm deactivated

retention gap and a deactivated precolumn (30 cm × 0.53 mm, both Connex). Helium was used as the carrier gas at a flow rate of 24 cm/sec. The following temperature program was used: after a hold time of 3 min at 50°C, temperature was linearly increased at a rate of 8°C/min to 230°C and held for 9.5 min.

Data were collected and processed with ChemStation software (Hewlett-Packard). The main compounds were identified by GC-MS analysis on a Hewlett-Packard 5973 quadrupole-type mass selective detector (transfer line temp: 230°C, source temp: 230°C, quadrupole temp: 150°C, ionization potential: 70 eV, and scan range: 50 – 400 amu) coupled to the gas chromatograph described above. The compounds were identified by comparison of retention times to those of commercially available standards [nonene,  $\alpha$ -phellandrene, limonene, linalool and (*E,E*)- $\alpha$ -farnesene] and by comparison of spectra to those of the Wiley and NIST libraries.

*Behavioral Response to Collected Volatiles.* This experiment was conducted to test whether the collected volatiles elicit a behavioral response in the beetles. Samples (50  $\mu$ l each) containing the volatiles collected from healthy and artificially damaged *A. alliariae* plants, identical to the ones used for injection in the gas chromatograph, were offered on folded filter paper discs (90 mm diameter; Schleicher and Schuell, Dassel, Germany) placed on the glass plate at the upwind end of the wind-tunnel. No visual cues could help the beetles approach the odor source in the wind-tunnel.

*Statistical Analysis.* Odor preferences in the wind-tunnel were tested with a two-tailed binomial test, using the total number of beetles that made a choice for a particular odor ( $\alpha = 0.05$ ) and pooling the data of the four replicates. To analyze the quantitative differences in the plant odors emitted by undamaged and damaged plants, we used an ANOVA followed by a Bonferroni-Dunn versus control (undamaged plant) post hoc test. Collected odor data were log ( $X + 1$ ) transformed before analysis.

## RESULTS

### *Choice Experiments*

*Damaged vs. Undamaged Petasites paradoxus: Flowering State.* In choice experiments with flowering *P. paradoxus*, beetles showed a significant preference for plants damaged by overnight feeding beetles (Figure 1A,  $P < 0.001$ ) and by artificial damage the previous evening (Figure 1B,  $P < 0.01$ ) over undamaged plants. There was no difference in attractiveness between overnight damage caused by adult beetles and overnight artificial damage (Figure 1C,  $P = 0.43$ ). Beetles were not more attracted to *P. paradoxus* plants that had been damaged only half an hour before the experiment (short term attack) than they were to undamaged plants (Figure 1D,  $P = 0.43$ ).

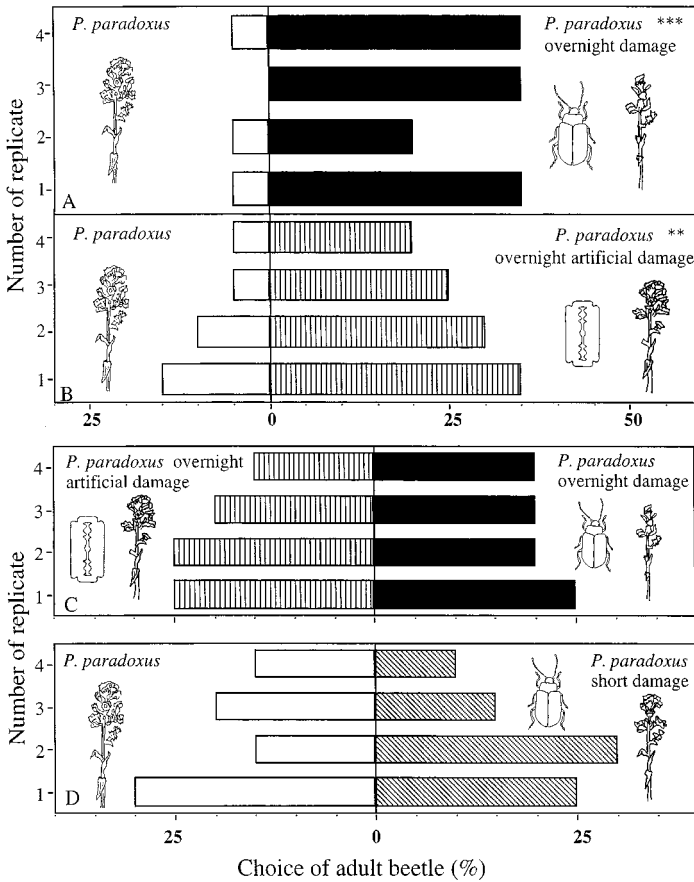


FIG. 1. Choices of *Oreina cacaliae* in wind-tunnel experiments with *Petasites paradoxus*. The bars represent the percentage of tested beetles that made a particular choice. The asterisks indicate a significant preference for a treatment (two-tailed binomial test; \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).

*Damaged vs. Undamaged. Adenostyles alliariae, Vegetative State.* Freshly damaged leaves of *A. alliariae* were more attractive to beetles than undamaged leaves, regardless whether leaf damage had been inflicted by adult or larval feeding (Figure 2A,  $P < 0.01$  and Figure 2B,  $P < 0.05$ ). Freshly damaged *A. alliariae* leaves were significantly more attractive than overnight damaged leaves (Figure 2C,  $P < 0.001$ ). Artificial fresh attack was as attractive as fresh damage caused by adult beetles feeding on the plant (Figure 2D,  $P = 0.42$ ). Plants damaged overnight by adult beetles were not more attractive than undamaged plants (Figure 2E,  $P = 0.42$ ).

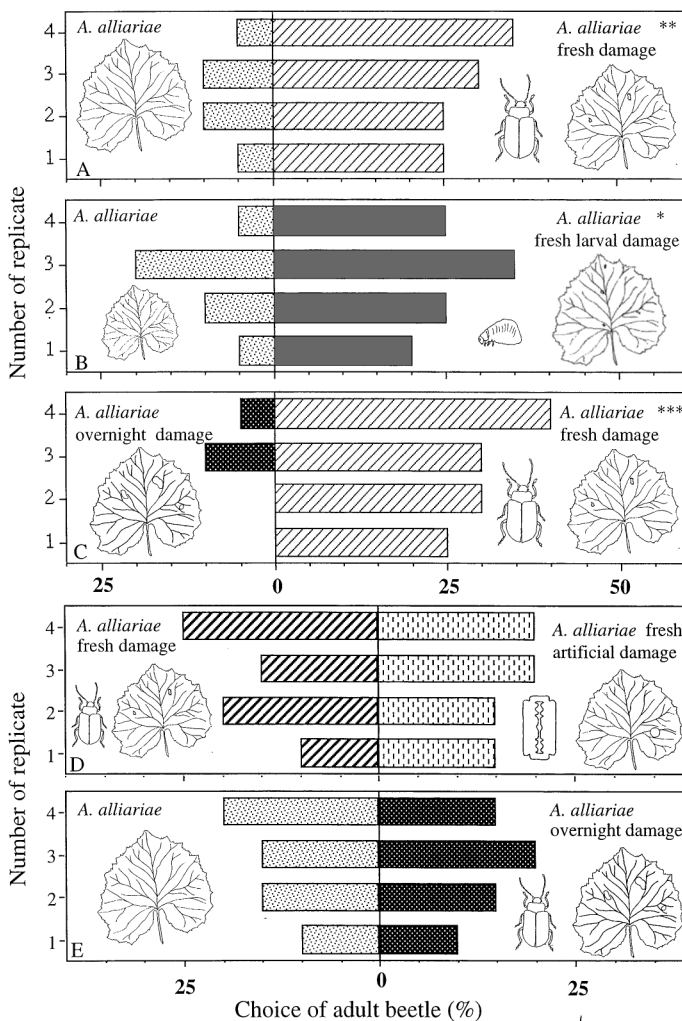


FIG. 2. Choices of *Oreina cacaliae* in wind-tunnel experiments with *Adenostyles alliariae*. The bars represent the percentage of tested beetles that made a particular choice. The asterisks indicate a significant preference for a treatment (two-tailed binomial test; \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).

*Host Odor without Visual Cues.* The tube releasing odor collected from an undamaged *A. alliariae* plant attracted significantly more beetles than the tube releasing clean air (Figure 3A,  $P < 0.05$ ). Collected headspace volatiles of a freshly damaged *A. alliariae* leaf presented on a filter paper, attracted more

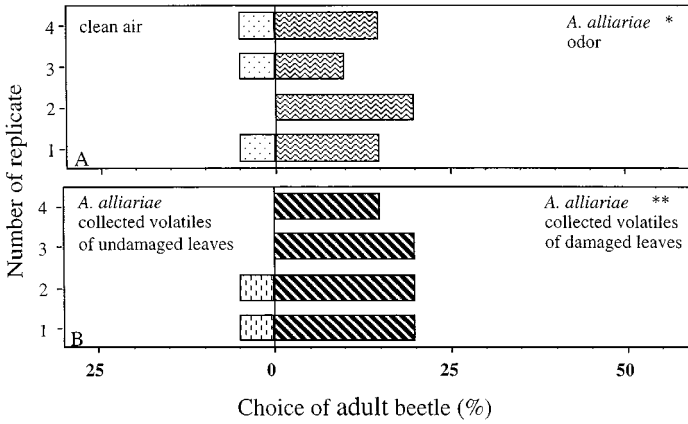


FIG. 3. (A) Choice experiments without visual cues, where the host odor entered the wind-tunnel from plants standing outside the wind-tunnel. Odor from *A. alliariae* plants attracted more beetles than pure air. (B) Collected headspace volatiles from damaged *A. alliariae* leaf put on a filter paper attracted more beetles than the collected odor of an undamaged *A. alliariae* leaf. The asterisks indicate a significant preference for a treatment (two-tailed binomial test; \* $P < 0.05$ , \*\* $P < 0.01$ ).

beetle than did collected headspace volatiles of an undamaged leaf (Figure 3B,  $P < 0.01$ ).

*Attractiveness of Conspecifics.* *O. cacaliae* beetles of one sex were not attracted by conspecifics of the other sex. Neither overwintered males nor new generation females attracted more adults of the opposite sex than did an empty cage (Figure 4,  $P = 0.34$ , both sexes). When two potted *A. alliariae* plants were added at the upwind end of the wind-tunnel, beetles still did not discriminate between the cage with conspecifics and the empty cage ( $P = 0.4$ , data not shown).

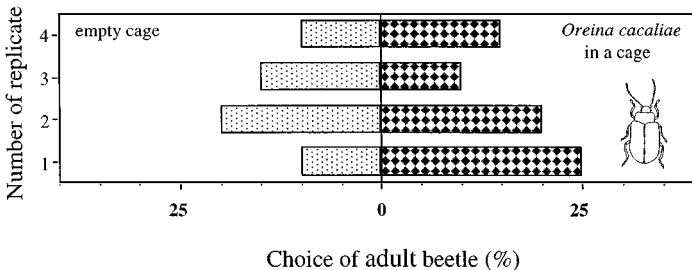


FIG. 4. *O. cacaliae* was not attracted to the smell of the opposite sex and did not approach the conspecifics hidden in a metallic cage more often than an empty cage ( $P = 0.34$ ).

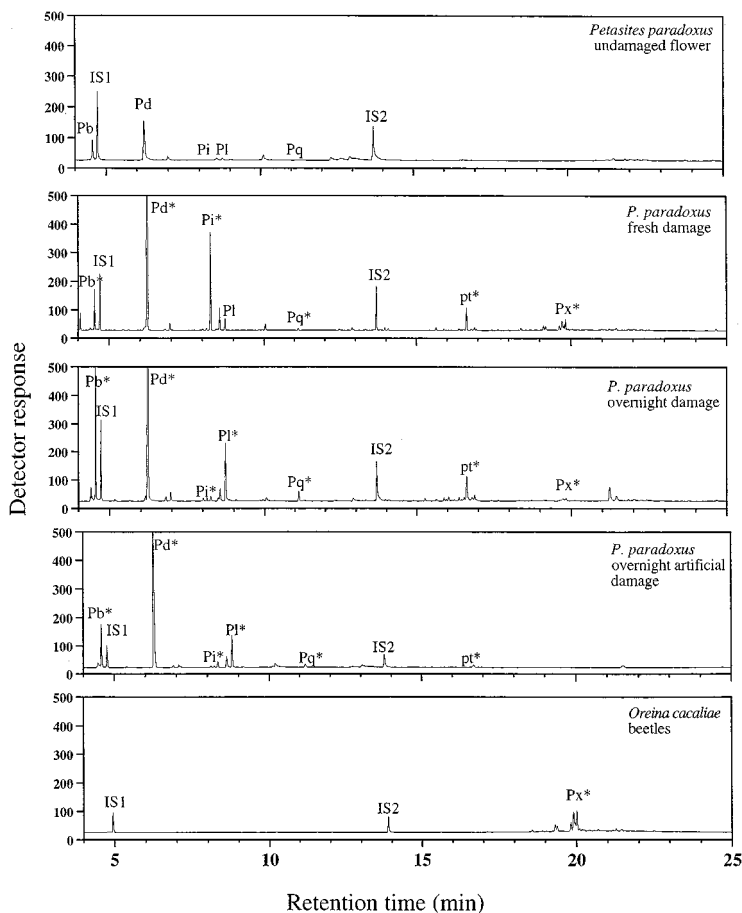


FIG. 5. Representative gas chromatographic profiles of headspace volatiles from undamaged, freshly damaged, overnight damaged and artificially overnight damaged *Petasites paradoxus* plants. The bottom chromatogram shows the odor from *Oreina cacaliae* beetles. Peak identities: (P for *Petasites*) Pb, unknown; Pd, nonene; Pi,  $\alpha$ -phellandrene; Pl, limonene; Pq, unknown; Pt, eremophyllene; Px, chromolaenin. IS1 and IS2 represent the internal standards *n*-octane and nonyl acetate, respectively. Asterisks indicate significant differences in the amount of an emitted substance as compared to a undamaged *P. paradoxus* flower.

### Analysis of Headspace Volatiles

*Petasites paradoxus* Flowering State. Representative gas chromatographic profiles of headspace volatiles collected from *P. paradoxus* plants illustrate the differences in volatile blends between undamaged and damaged plants (Figure 5).

TABLE 1. DATA OF ANOVA TEST FOR COLLECTED PLANT ODOR COMPOUNDS OF DAMAGED PLANTS COMPARED WITH UNDAMAGED CONTROL PLANTS<sup>a</sup>

Compound	MS	F	P
<i>Petasites paradoxus</i> flowering			
Unknown (Pb)	0.49	7.69	0.0013
Nonene (Pd)	1.16	7.8	0.0012
(E,E)- $\alpha$ -phellandrene (Pi)	5.81	11.7	0.0001
Limonene (Pl)	1.15	4.94	0.01
Unknown (Pq)	4.33	12.63	0.0001
Eremophylene (Pt)	4.29	23.59	0.0001
Chromolaenin (Px)	7.64	69.48	0.0001
<i>Adenostyles alliariae</i> vegetative			
(Z)-3-Hexenol (a)	2.69	3.34	0.025
Unknown (b)	1.75	3.27	0.028
(Z)-3-Hexenyl acetate (c)	1.9	3.84	0.015
Linalool (e)	0.94	7.38	0.0005
Dodecanol (h)	2.78	6.14	0.0014
$\alpha$ -Humulene (k)	0.87	7.51	0.0004
Germacrene D (n)	0.48	3.61	0.019
(E,E)- $\alpha$ -farnesene (p)	0.25	2.42	0.075

<sup>a</sup>Significant differences between treatments are indicated with an asterisk in the Figures 4 and 5.

Control collections from empty PET bags showed no background odor. The sesquiterpene  $\alpha$ -phellandrene was emitted in large amounts by freshly damaged *P. paradoxus* plants (Figure 5, peak Pi). Overnight damaged plants (adult and artificial attack) with enhanced attractiveness emitted significantly more limonene (peak Pl) than both undamaged and freshly damaged plants. The other volatile compounds—nonene, eremophylene, and two unknown substances were released in significantly larger amounts by damaged plants (either fresh or overnight damage) than by undamaged plants (Table 1, Figure 5).

*Adenostyles alliariae* Vegetative State. Representative gas chromatograms of headspace volatiles collected from *A. alliariae* leaves following different treatments are shown in Figure 6. Freshly damaged leaves with enhanced attractiveness, damaged either by adult beetles, larvae, or artificially, contained significantly larger amounts of linalool (e), dodecanol (h),  $\alpha$ -humulene (k), and germacrene D (n) than did undamaged leaves (Table 1, Figure 6). When *A. alliariae* leaves were damaged overnight by adults, they emitted additional volatiles such as (Z)-3 hexenol (a), (Z)-3-hexenyl acetate (c), (E,E)- $\alpha$ -farnesene (p), and an unknown substance (b). In undamaged and overnight damaged *A. alliariae* more (E,E)- $\alpha$ -farnesene (p) is produced compared to  $\alpha$ -humulene (k) and germacrene D (n), whereas in freshly damaged leaves (by adults, larvae, or artificially) more  $\alpha$ -humulene (k) and germacrene D (n) are emitted compared to (E,E)- $\alpha$ -farnesene (p).

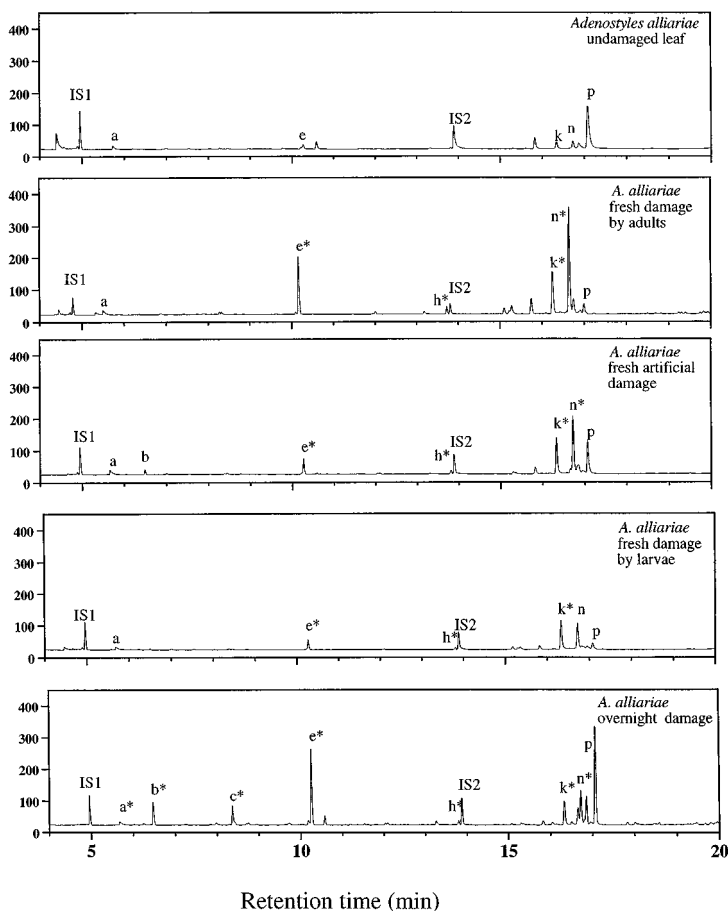


FIG. 6. Representative gas chromatographic profiles of *Adenostyles alliariae* headspace volatiles collected from leaves after various treatments. Treatments from top to bottom were: undamaged *A. alliariae* leaf, a leaf freshly damage by adult *O. cacaliae*, a leaf artificially damaged with a razor blade, a leaf freshly damaged by *O. cacaliae* larvae, and a leaf damaged overnight by *O. cacaliae* adults. Peak identities: a, (Z)-3-hexenol; b, unknown; c, (Z)-3 hexenylacetate; e, linalool; h, dodecanol; k,  $\alpha$ -humulene; n, germacrene D; and p, (E,E)- $\alpha$ -farnesene. IS1 and IS2 represent the internal standards *n*-octane and nonyl acetate, respectively. Asterisks indicate significant differences in the amount of an emitted substance as compared to a undamaged *A. alliariae* leaf.

#### Results of Beetle Odor Collections

Oreina cacaliae. Chromolaenin was the only identified substance emitted from *O. cacaliae* beetles and was also present in plant odor collections from damaged plants, where beetles had fed on the plant during collection (Figure 5).

## DISCUSSION

Wind-tunnel experiments with *O. cacaliae* and the host plants *P. paradoxus* and *A. alliariae* provide evidence that this alpine leaf beetle is more attracted to volatiles released by damaged host plants than it is to undamaged hosts. Domek and Johnson (1988) found that green June beetles feeding on ripe peach attract significantly more conspecifics than did ripe sliced fruit or beetles alone, demonstrating that beetles feeding on a host attract more conspecifics than the presence of host plants or conspecifics alone. The attraction to a general odor of wounded plants could be excluded in an experiment with *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae), where beetles were attracted to conspecifics feeding on the host plant oilseed rape, but not to conspecifics feeding on the nonhost crambe (Peng and Weiss, 1992). *O. cacaliae* beetles responded similarly to insect (larvae or adult) or artificially damaged host plants, showing that the volatile blend emitted is solely a result of plant injury and not part of the odors emitted by the insect while feeding.

Even in the absence of any visual cues characterizing its host plant in the wind tunnel *O. cacaliae* approached the host plant odor inlet more often than the clean air inlet. Nevertheless, the fact that fewer beetles responded in experiments without visual cues than in trials in which plants were placed in the wind tunnel indicates that some visual cues might help the beetles to approach a plant. In the field, the role of visual cues in host plant finding behavior is clearly less important than the role of odor cues (Kalberer, 2000).

Pasteels et al. (1994) speculated on the existence of a sexual pheromone in *O. gloriosa*, based on differences in the composition of defensive secretion, between the sexes (Eggenberger and Rowell-Rahier, 1991, 1993). We found no evidence for a sex pheromone in *O. cacaliae*. Our beetles were transported together in a pot from the field to the lab, and they may have released their defensive secretion during transport, which could have affected their subsequent responses. Keeping the beetles separate may yield different results.

Short- or long-term attack, respectively, lead to an enhanced attractiveness in one host plant of *O. cacaliae* but not in the other. The spring host *P. paradoxus* was more attractive after overnight damage, whereas *A. alliariae* had an enhanced attractiveness only after a fresh attack, but lost this enhanced attractiveness hours later. An enhanced attractiveness for overnight damaged plants similar for the one on *P. paradoxus* had been described for the Japanese beetle on crabapple leaves where overnight damaged leaves attract more beetles than undamaged leaves or leaves with fresh damage (Loughrin et al., 1995).

Collected headspace volatiles from damaged *A. alliariae* leaves were more attractive to the beetles than collected volatiles from undamaged *A. alliariae* leaves, showing that the compounds triggering a response in *O. cacaliae* had been collected. The compounds collected were analyzed in order to determine how the

composition of the odor blend changed as a result of herbivore attack. Visser et al. (1979) showed that fresh attack by Colorado potato beetles results in the emission of green leaf volatiles from injured plant cells. These volatiles are emitted by most plant species after damage as a result of the oxidative degradation of plant lipids (Visser and Avé, 1978). The compounds (*Z*)-3-hexenol and (*Z*)-3-hexenyl acetate that were released from damaged *A. alliariae* leaves after a short attack belong to this category, nevertheless they were also produced after overnight attack. Several studies have shown that the production of other volatiles is induced in the plant following the attack by a herbivore (Turlings et al., 1990, Takabayashi et al., 1994) and is the result of active chemical processes in the plant (Paré and Tumlinson, 1997). Mono- and sesquiterpenes, built via the isoprenoid pathway, belong to this category and are the most common volatiles induced by herbivore attack (Paré and Tumlinson, 1997). Nonene,  $\alpha$ -phelandrene, limonene, and eremophylene, were the compounds emitted in higher amounts by damaged *P. paradoxus* plants compared to undamaged plants. After an overnight infestation by beetles as well as after artificial damage the previous evening, *P. paradoxus* flowers released significantly more limonene than plants that were less attractive for the beetles. It is tempting to speculate that the enhanced attractiveness of overnight damaged *P. paradoxus* is due to an increased amount of limonene in the emitted plant volatiles. Nevertheless it is risky to speculate that a single compound evokes the observed response in the beetle, because several studies have shown that the blend of odor components is crucial to provoke a certain behavioral response (Visser and Avé, 1978; Saxena and Goyal, 1978).

Linalool, dodecanol,  $\alpha$ -humulene, and germacrene D were the compounds emitted in higher amounts by damaged *A. alliariae* leaves compared to undamaged leaves. In freshly damaged *A. alliariae* leaves with enhanced attractiveness, more  $\alpha$ -humulene and germacrene D were emitted compared to (*E,E*)- $\alpha$ -farnesene whereas in the less attractive *A. alliariae* undamaged and overnight-damaged plants, more (*E,E*)- $\alpha$ -farnesene was emitted compared to  $\alpha$ -humulene and germacrene D. This reverse relationship between those sesquiterpenes might be responsible for the enhanced attractiveness of freshly damaged *A. alliariae* leaves.

Most of the compounds that we found after an attack of *O. cacaliae* in its host plants have been described for damaged host plants of other systems. For instance the terpene (*E,E*)- $\alpha$ -farnesene is released by damaged crabapple and by *Psylla*-infested pear trees (Loughrin et al., 1995; Scutareanu et al., 1997), (*Z*)-3-hexenyl acetate was found to be associated with herbivory in maize (Turlings et al., 1990), and the sesquiterpene (-)-germacrene D appears to mask the attractiveness of undamaged pine trees to *Monochamus alternatus* (Coleoptera: Cerambycidae) (Yamasaki et al., 1997).

The fact that damaged host plants are more attractive than undamaged plants may promote aggregation of *O. cacaliae* on plants suitable for feeding and larval

development. The difference in the attractiveness of the two host plants with respect to time since attack corresponds nicely with field observations of beetle densities on plants. Dozens of beetles often feed on a single flowering *P. paradoxus* plant in spring, while only a few beetles (2–4) were ever observed on *A. alliariae* plants. The long-lasting attraction of flowering *P. paradoxus* early in the season may facilitate mating in *O. cacaliae*. It might be especially rewarding for the females to remate in spring with a male who successfully overwintered, to increase the fitness of their offspring (Stevens and Cauley, 1989).

*Acknowledgments*—We thank Sandrine Gouingueuné for the GC-MS analyses and Jonathan Gershenson at the Max-Planck-Institut für Chemische Ökologie in Jena, Germany, for access to equipment. Ann Fraser, Bernd Hägele, and Håkan Häggström made helpful comments on the manuscript.

#### REFERENCES

- BOLTER, C. J., DICKE, M. V. LOON, J. J. A. VISSER J. H., and POSTHUMUS, M. A. 1997. Attraction of Colorado potato beetle to herbivore-damaged plants during herbivory and after its termination. *J. Chem. Ecol.* 23:1003–1023.
- BORDEN, J. H. 1984. Semiochemical-mediated aggregation and dispersion in the Coleoptera, pp. 123–149, in T. Lewis (ed.). *Insect Communication*. Academic Press, Orlando, Florida.
- DOBLER, S., and ROWELL-RAHIER, M. 1994. Production of cardenolides versus sequestration of pyrrolizidine alkaloids in larvae of *Oreina* species (Coleoptera, Chrysomelidae). *J. Chem. Ecol.* 20:555–568.
- DOMEK, J. M., and JOHNSON, D. T. 1988. Demonstration of semiochemically induced aggregation in the green June beetle, *Cotinis nitida* (L.) (Coleoptera, Scarabaeidae). *Environ. Entomol.* 17:147–149.
- EGGENBERGER, F., and ROWELL-RAHIER, M. 1991. Chemical defence and genetic variation. *Naturwissenschaften* 78:317–320.
- EGGENBERGER, F., and ROWELL-RAHIER, M. 1993. Physiological sources of variation in chemical defense of *Oreina gloriosa* (Coleoptera: Chrysomelidae). *J. Chem. Ecol.* 19:395–410.
- EHMKE, A., ROWELL-RAHIER, M., PASTEELS J. M., and HARTMANN, T. 1991. Sequestration of ingested [<sup>14</sup>C]senecionine *N*-oxide in the exocrine defensive secretions of chrysomelid beetles. *J. Chem. Ecol.* 17:2367–2379.
- HARARI, A. R., BEN-YAKIR D., and ROSEN, D. 1994. Mechanism of aggregation behavior in *Maladera matrida* Argaman (Coleoptera: Scarabidae). *J. Chem. Ecol.* 20:361–371.
- HEATH, R. R., and MANUKIAN, A. 1992. Development and evaluation of systems to collect volatile semiochemicals from insects and plants using a charcoal-infused medium for air purification. *J. Chem. Ecol.* 18:1209–1126.
- JONES, O. T. 1985. Chemical mediation of insect behavior, pp. 311–373, in D. H. Hutson and T. R. Roberts (eds.). *Progress in Pesticide Biochemistry and Toxicology*, Vol. 5, Insecticides. Wiley, New York.
- KALBERER, N. M. 2000. Host-plant recognition, dispersal and its influence on reproduction in the alpine leaf beetle *Oreina cacaliae*. PhD thesis. University of Neuchâtel, Neuchâtel, Switzerland.
- LOHSE, G. A., and LUCHT, W. H. 1994. page 73, in Goecke and Evers (ed.). *Die Käfer Mitteleuropas* 14. Krefeld.
- LOUGHRIN, J. H., POTTER D. A., and HAMILTON-KEMP, T. R. 1995. Volatile Compounds induced by Herbivory act as Aggregation Kairomones for the Japanese beetle (*Popillia japonica* Newman). *J. Chem. Ecol.* 21:1457–1467.

- MILLER, J. R., and STRICKLER, K. L. 1984. Finding and accepting host plants, in W. J. Bell and R. T. Cardé (eds.). *Chemical Ecology of Insects*. Chapman and Hall, New York. pp. 127–155.
- PARE, P. W., and TUMLINSON, J. H. 1997. De novo biosynthesis of volatiles induced by insect herbivory in cotton plants. *Plant Physiol.* 114:1161–1167.
- PASTEELS, J. M., ROWELL-RAHIER, M., BRAEKMAN, J.-C., and DALOZE, D. 1994. Chemical defense of adult leaf beetles updated, pp. 298–301, in P. Jolivet, M. Cox, and E. Petitpierre (eds.). *Novel Aspects of the Biology of Chrysomelidae*, Kluwer, Dordrecht.
- PENG, C., and WEISS, M. J. 1992. Evidence of an aggregation pheromone in the flea beetle, *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). *J. Chem. Ecol.* 18:875–884.
- SAXENA, K., and GOYAL, N. S. 1978. Host-plant relations of the citrus butterfly *Papilio demolus* L.: Orientation and ovipositional responses *Entomol. Exp. Appl.* 24:1–10.
- SCUTAREANU, P., DRUKKER, B., BRUIN, J., POSTHUMUS, M. A., and SABELIS, M. W. 1997. Volatiles from *Psylla*-infested pear trees and their possible involvement in attracting of anthocorid predators. *J. Chem. Ecol.* 23:2241–2260.
- STEVENS, L., and CAULEY, D. E. M. 1989. Mating prior to overwintering in the important willow leaf beetle, *Plagioderma versicolora* (Coleoptera: Chrysomelidae). *Ecol. Entomol.* 14:219–223.
- TAKABAYASHI, J., DICKE M., and POSTHUMUS, M. A. 1994. Volatile herbivore-induced terpenoids in plant-mite interactions: Variation caused by biotic and abiotic factors. *J. Chem. Ecol.* 20:1329–1354.
- TURLINGS, T. C. J., TUMLINSON J. H., and LEWIS, W. J. 1990. Exploitation of herbivore-induced plant odours by host-seeking parasitic wasps. *Science* 250:1251–1253.
- TURLINGS, T. C. J., BERNASCONI, M., BERTOSSA, R., BIGLER, F., CALOZ, G., and DORN, S. 1998. The induction of volatile emissions in maize by three herbivore species with different feeding habits: possible consequences for their natural enemies. *Biol. Control* 11:122–129.
- VISSER, J. H., and AVÉ, D. A. 1978. General green leaf volatiles in the olfactory orientation of the Colorado beetle, *Leptinotarsa decemlineata*. *Entomol. Exp. Appl.* 24:538–549.
- VISSER, J. H., and NIELSON, J. K. 1977. Specificity in the olfactory orientation of the CPB. *Entomol. Exp. Appl.* 21:14–22.
- VISSER, J. H., STRATEN S. V., and MAARSE, H. 1979. Isolation and identification of volatiles in the foliage of potato, *Solanum tuberosum*, a host plant of the Colorado beetle, *Leptinotarsa decemlineata*. *J. Chem. Ecol.* 5:13–25.
- YAMASAKI, T., SATO, M., and SAKOGUCHI, H. 1997. Germacrene D: Masking substance of attractants for the cerambycid beetle, *Monochamus alternatus* (Hope). *Appl. Entomol. Zool.* 32:423–429.