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Herbivore-induced emissions of maize volatiles repel the corn leaf aphid, *Rhopalosiphum maidis*

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Abstract

When maize plants, *Zea mays* L., are mechanically damaged and the damaged sites are treated with caterpillar regurgitant, the plants will release a specific blend of volatiles. It is known that these volatiles can be attractive to natural enemies of herbivores. We hypothesise that the plant volatiles constitute part of the induced plant defence and that herbivores will be affected by the odours as well. In laboratory and semi-field studies this hypothesis was tested for the aphid *Rhopalosiphum maidis* (Fitch) (Rhynchota, Sternorrhyncha, Aphididae).

In a Y-tube olfactometer significantly more aphids chose the odour of healthy, undamaged maize seedlings when tested against clean air or plants treated with regurgitant. Clean air was chosen more often when tested next to the odour of treated plants. This apparently repellent effect of the odour of treated plants was significant for winged aphids, but not for the wingless aphids.

In field experiments aphids were released in the centre of circles of eight potted maize plants. Four plants in each circle were damaged and treated with caterpillar regurgitant while the other plants were left unharmed. At different intervals after aphid release, the number of aphids was counted on each plant. Significantly fewer winged and wingless aphids were found back on treated plants than on healthy plants.

We suggest that herbivores may be repelled by the odours because they could indicate that: 1) the plant has initiated the production of toxic compounds; 2) potential competitors are present on the plant; 3) the plant is attractive to parasitoids and predators. Aphids may be particularly sensitive to induced maize volatiles because one of the major compounds emitted by the plant is (*E*)-β-farnesene, which is a common alarm pheromone for aphids. Collections and analyses of the odours emitted by crushed *R. maidis* confirmed that it too emits (*E*)-β-farnesene when stressed. The results are discussed in context of plant defence strategies and their possible exploitation for the control of pest insects.

Introduction

Several studies have demonstrated that herbivore-injured plants produce specific blends of odours which can be attractive to certain insect predators and parasitoids (Dicke, 1994; Turlings et al., 1995). Maize seedlings fed upon by caterpillars initiate the release of volatiles that are attractive to the parasitoids *Cotesia marginiventris* and *Microplitis croceipes* (Turlings et al., 1990; 1993). Seedlings that are artificially damaged and treated with the regurgitant of *Spodoptera exigua* larvae on the damaged site produced the same blend of volatiles as plants that are damaged by the caterpillars themselves. Plants with only artificial damage (no regurgitant applied) do not emit these volatiles in significant amounts (Turlings et al., 1990). Similarly, Steinberg et al. (1992) and Mattiacci et al. (1994) found that the parasitoid *Cotesia glomerata* is

attracted to volatiles emitted by Brussels sprouts after this plant is infested by *Pieris brassicae* larvae. In both cases, elicitors in the oral secretion of the caterpillar induced the plants to emit the attractants (Mattiacci et al., 1995; Alborn et al., 1997).

Thus, the importance of induced plant odours for the host seeking behaviour of parasitoids has been clearly shown, but it remains unclear what the primary function of the volatiles is. We suggest that the chemical changes in the plant are part of a defence reaction directed towards the herbivores. It may be expected that herbivores are repelled by the induced volatiles for several reasons: 1) the odours may indicate the presence of competitors, 2) the odours may indicate the production of defence compounds by the plant, 3) the odours may indicate the plants are particularly attractive to natural enemies of the herbivores. Similar arguments for repellent effects of induced plant volatiles were made by Pallini et al. (1997), but they also point out that herbivores may actually prefer already infested plants if, for instance, a previous attack weakens plant defences.

To test our hypotheses, we chose the corn leaf aphid, *Rhopalosiphum maidis*, an anholocyclic aphid that attacks various Gramineae, including maize. Evidence is accumulating that the foraging behaviour of this and other aphids are strongly affected by plant odours. Moreover, one major compound emitted by herbivore-damaged maize is (*E*)-β-farnesene. This sesquiterpene is known to be the alarm pheromone of several aphid species (Pickett & Griffiths, 1980). Here we confirm that *R. maidis*, like many other aphid species, emit (*E*)-β-farnesene when harassed (see later). Farnesenes, however, are common compounds in many plant species (Pickett & Griffiths, 1980), and the presence of (*E*)-β-farnesene, under natural conditions, does not necessarily deter settling and feeding by aphids (Pickett et al., 1992).

Volatile kairomones are also very important in host plant recognition by aphids prior to settling (Pickett et al., 1992; Visser & Taanman, 1987). Some plant volatiles may repel aphids. For example, in olfactometer experiments *Aphis fabae* was repelled by the plant-derived compounds methyl salicylate and the monoterpenoid (-)-(1R,5*S*)-myrtenal (Hardie et al., 1994). When colonising its summer host, the bird-cherry-oat aphid *Rhopalosiphum padi* L. is also repelled by methyl salicylate, which is a compound emitted by its winter host (Pettersson et al., 1994). Methyl salicylate and various terpenoids are among the compounds emitted by caterpillar-damaged maize plants (Turlings et al., 1990, 1998; Takabayashi et al., 1995) thus it can be expected that healthy, undamaged plants would be preferred by the aphid.

In the current study, the effect of induced maize volatiles on the foraging behaviour of *R. maidis* was tested in the laboratory and in the field. Under laboratory conditions, responses of individual aphids to maize odours were studied in a Y-tube olfactometer. In a field test, we compared the colonisation by *R. maidis* of volatile producing (treated) plants and untreated plants.

Materials and methods

Plant material. For all experiments, we used *Zea mays* L. var. LG11. Plants used for laboratory experiments and volatile collections were grown in climate chambers at 25 °C, 70% r.h. and 25 000 lux (Sylvania F96T12/CW/VHO) with a photoperiod of L16:D8. Maize seedlings were grown on bedding substrate 1 (Triohum 1; Klasmann, Germany) in plastic trays (22× 16 × 5 cm; 12 seeds per tray) or individually in pots (7 cm diam.). For the field experiment, plants were grown during spring and summer of 1995 in a greenhouse at the Swiss Federal Agricultural Research Station in Cadenazzo (TI) with natural light and no temperature and humidity control. They were placed in pots (18 cm diam) with local soil and fertilised (N 80.3 mg/l; P_2O_5 34.4 mg/l; K_2O 68.8 mg/l) once a week.

Aphids. A colony of the aphid *Rhopalosiphum maidis* was maintained in a climate chamber at 25 °C, 50–70% r.h. and 23000 lux (Sylvania F72T12/CW/VHO) with a photoperiod of L16:D8. The colony had been reared on barley for at least 2 years and was obtained from the Agricultural Research Station Reckenholz (Switzerland). The aphids were maintained on barley (var. Baracka) in single pots (10 cm diam.) with 10 to 15 seedlings. Plants with aphids were covered with cellophane bags (30 × 15.5) cm; quality 400 P, Celloclair AG, Liestal, Switzerland) which allows for air and humidity exchange.

Treatment of the plants. For laboratory experiments, seedlings were used when they were 8–10 days old and carried three well-developed leaves, while we used 3 week-old (9-leaf-stage) plants for the field experiment. To induce the plants to emit volatiles, herbivore

damage was simulated by scratching 1–2 cm^2 on each side of the middle vein of the surface of the underside of three leaves with a scalpel and applying 10 µl of caterpillar regurgitant on the damaged sites. Control plants were left unharmed. The caterpillar regurgitant was collected from 3rd and 4th instar *Spodoptera littoralis*, as described by Turlings et al. (1993), and kept at 5 °C for no longer than one month. *S. littoralis* larvae were obtained weekly from CIBA Insect Control (NOVARTIS), Basle (Switzerland) and were kept on an artificial diet.

Laboratory study. To test if herbivore-induced volatiles have an effect on the behaviour of foraging aphids, individual aphids were offered a choice between odours in a Y-tube-olfactometer. We used an olfactometer like the one first described by Sabelis & van de Baan (1983). It consists of glass tubes fused to each other in the shape of a 'Y'. Odour sources (maize seedlings) were placed in custom-made tubular glass chambers (0.5 l). The chambers were connected with Teflon tubes to the arms of the olfactometer. Before the air entered the odour source chambers, it was first pushed through a charcoal filter to clean the air and then water to moisten it. The flow (800 ml/min) was controlled by flowmeters (Aalborg Instruments, Monsey, New York). These airflows come together in the central tube of the olfactometer, where the odours mix. Aphids that are introduced through the central tube can make a choice between the two odours by walking into one of the arms. Preliminary experiments showed that the aphids do not walk readily on the glass of the olfactometer. This problem was solved by placing a Y-shaped brass rod in the centre of the olfactometer as described by Sabelis & van de Baan (1983). To eliminate visual distractions and to provide diffuse light, a white curtain fixed on a wooden frame was placed around the olfactometer. A spotlight was placed outside the white screen enclosure on the side of the odour sources to attract the aphids in that direction.

Treatment of the seedlings occurred at 6:00 PM and experiments were carried out the following day between 9:00 AM and 5:00 PM. The seedlings were cut immediately before the experiment and introduced into the glass chambers (two plants per chamber). During the experiments, the stems of the plants were placed into a water-filled glass vial to prevent desiccation.

Aphids were introduced individually through the entrance of the central tube with a small brush, alternating between winged and wingless aphids. After testing six aphids, the odour sources were exchanged, and after the next six aphids, new plants were placed in the odour source chambers. This was repeated 2–4 times per day. An observation ended when the aphid crossed a mark 5 cm into one of the arms or 15 min after introducing an aphid. A choice was scored only if the aphid would cross one of the marks. Aphids that did not cross a mark within the observation time were counted as 'no decision'. The aphids were offered one of three odour source combinations: healthy plants vs. clean air, treated plants vs. clean air, or treated plants vs. healthy plants. We tested for expected preferences with an one-tailed binomial test and differences between winged and wingless aphids were tested using Cochran's corrected chi-square statistics (Zar, 1984).

Field experiment This experiment was conducted at the Swiss Federal Agricultural Research Station in Cadenazzo (Switzerland) from the middle of June until the end of August 1995. Plants that were grown in a greenhouse at the research station were used when they were 3 weeks old (9-leaf stage).

Three circles (1.5 m diam.) with each 8 maize plants were placed in the form of a triangle in between maize fields. In each circle four treated plants were alternated with four unharmed plants. Plants were treated at 9:00 AM every day during 3 days. Three leaves per plant were scratched with a scalpel to damage 4 cm^2 and the damaged sites were treated with 10 µl of caterpillar regurgitant. About 7 h after the first treatment aphids were introduced into the centre of each circle by placing desiccated leaves of barley seedlings carrying aphid colonies on the ground. The number of aphids was estimated at several thousands of both winged and wingless (of all stages) individuals per circle. The aphids that moved onto the maize plants were counted at 8:00 AM and again at 2:00 PM on days 2 and 3 of each experiment. The experiments were replicated nine times at weekly intervals. The total number of aphids counted per circle on the plants on the different days were compared for healthy and treated plants using Wilcoxon's test for tied ranks (Zar, 1984).

Collection and analyses of aphid alarm pheromone. Aphids were collected from the laboratory colony and introduced into 5 ml glass vials (approximately 300 of all stages per vial). To induce the release of alarm pheromone the aphids were crushed with a glass rod. This treatment caused the aphids to secrete small droplets out of their siphunculae which contained the

alarm pheromone (Pickett & Griffiths, 1980). Immediately after this treatment, the vials with the crushed aphids were introduced into the volatile collection apparatus. The volatile collection system has been described in detail by Boevé et al. (1996). Briefly, humidified air, purified by an in-line activated charcoal filter, entered two parallel chambers consisting of glass tubes (approx. 10 cm long and 20 mm in diam.). An airflow of 500 ml/min was balanced with house air and a vacuum pump, and maintained at slightly higher than atmospheric pressure inside each chamber. Air exited each chamber through a reusable 3.7-mm-ID ×4-cm-long glass collection trap (Heath & Manukian, 1992) packed with 25 mg Super Q adsorbent (80–100 mesh) (Alltech, Deerfield, Illinois, USA), which was rinsed prior to each volatile collection with 5–10 ml methylene chloride. Volatiles were collected for 30 min, after which the collection traps were extracted immediately with 100 µl methylene chloride, and internal standards were added (200 ng of *n*-octane and nonyl acetate in 20 µl methylene chloride). Before each collection of the odour of crushed aphids, we collected from an empty glass chamber to check for impurities. The experiment was replicated six times.

Collection of plant volatiles. The procedure for collection of plant volatiles has been described by Turlings et al. (1998). We collected the volatiles from potted healthy and treated plants, whereby the plants were subjected to the same treatments as described for the olfactometer experiments. After treatment, plants were placed in a Nalophan® bag (Kalle Nalo, Wiesbaden, Germany) and the volatiles were trapped on super Q filters during a period of 2 h (Turlings et al., 1998). The filters were then extracted with 150 µl of methylene chloride and 200 ng of *n*-octane and nonyl-acetate were added as internal standards.

Chemical analyses. Of each sample, 1 µl was analysed on a 30-m × 0.25-mm-ID crosslinked methyl silicone gum (HP-1) (0.25-µm-thick film) capillary gas chromatography column combined with a 5-m × 0.25-mm HP uncoated, deactivated retention gap and a 20-cm × 0.5-mm HP uncoated, deactivated retention gap as pre-column. The Hewlett-Packard model HP 5890 II plus gas chromatograph was equipped with an automated on-column injection system (HP 7673 GC/SFC) and a flame ionisation detector. Helium (19 cm/s) was used as carrier gas. Following injection, column temperature was maintained at 40 °C for 4 min and then programmed at 5 °C/min to 200 °C. An HP Chemstation data collection program was used for collection and data processing.

Samples were also analysed by mass spectroscopy (GC-MS). The retention times and the spectra of the natural compounds were compared with those of candidate synthetic compounds. For more details see Turlings et al. (1998).

Results

Y-tube olfactometer. Wingless as well as winged *R. maidis* showed a significant preference for the odour of healthy plants when tested against clean air (Figure 1a). Both morphs moved more towards clean air when it was offered next to the odour of treated plants, but this difference was only statistically significant for winged aphids (Figure 1b).

The differential attractiveness between the odours of healthy and treated plants was most pronounced in the experiments where they were offered together as choices. Both winged as well as wingless aphids showed a significant preference for the odour of healthy seedlings (Figure 1c).

The results in Figure 1 show a tendency for wingless aphids to be more attracted to healthy plants than winged aphids, while winged aphids seem more repelled by the treated plants, but these apparent tendencies are not significant. When the ratios between 'no decisions' and 'decisions' are compared for the two morphs the winged aphids make significant fewer decisions than wingless aphids when the odour of treated plants is among the choices (Figure 1).

Field experiments. A highly variable number of the aphids released in a circle was recovered on the plants. For the wingless aphids this ranged from 27 to 881, with an average of 204 (± s.d. 230) per circle. All larval stages were counted. This may mean that we not only counted aphids that migrated onto the plants, but also larvae that were freshly deposited on the plants during the experiments. This could have affected the results, particularly towards the end of each experiment. The range for winged aphids that were recovered was 4 to 168 and the average 49 (± s.d. 44). In Figure 2 we present the relative percentages on healthy and treated plants calculated from the total number of each aphid morph on the plants per circle. The mean proportion of wingless and winged aphids was for all the observations significantly higher on healthy plants

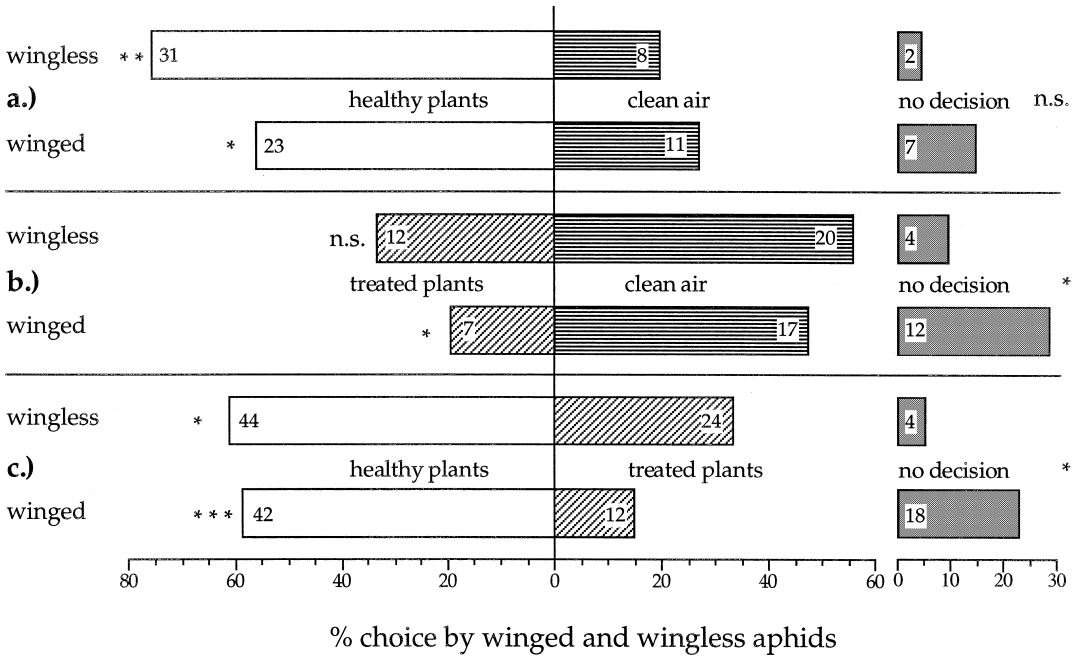

Figure 1. Choices of winged and wingless *Rhopalosiphum maidis* in the olfactometer experiment. (a) healthy plants tested next to clean air; (b) clean air tested next to plants treated with regurgitant; (c) healthy plants tested next to plants treated with regurgitant. The bars represent the percentage of tested insects that made a particular choice, the actual numbers are given in each bar. The asterisks with the choice bars indicate significant preferences (one-tailed binomial test). The asterisks with the 'no decision' bars indicate significant differences between wingless and winged in the frequency of making a choice (Cochran's corrected chi-square statistics). $^{*}P \leq 0.05$; $^{**}P \leq 0.001$; $^{***}P \leq 0.0001$ (n.s. = not significant at $\alpha = 0.05$).

than on treated plants, except for the wingless morphs on the first count after release.

Alarm pheromone. GC-MS analyses and comparison with synthetic compounds showed that (*E*)-β-farnesene was the major compound in all the collections of potential alarm pheromone from crushed *R. maidis* (Figure 3). The experiment was repeated six times and the average amount of (*E*)-β-farnesene emitted was 42.76 (±10.72) ng per 30 min. No other substances were detected in significant amounts.

Plant volatiles. We confirmed a dramatic change in odour emissions after treatment with caterpillar regurgitant (Turlings et al., 1998; Figure 3). The volatile production of treated plants was significantly higher than the production of healthy plants. We detected 20 different compounds in the odour of treated plants which were quantitatively and qualitatively different from the odour of healthy plants. These chemical analyses confirm that the aphids in the bio-assays were confronted with a relatively large amount of induced volatiles that could have affected their behaviour. (*E*)-β-farnesene was one of the predominant chemicals found in treated plants (peak 15 in Figure 3).

Discussion

Induced maize volatiles were found to influence the foraging behaviour of *R. maidis*. The strongest evidence for this comes from the olfactometer experiments where the aphids had no visual nor physical contact with the plants. The aphids, both winged and wingless, significantly preferred the odour of healthy plants over the odour of treated plants. This preference was also reflected in the results from the field experiment, where winged as well as wingless aphids were recovered more from healthy plants than from treated plants. The field data do not reveal whether the plants were differentially attractive to the released aphids or if the aphids more readily left the damaged plants after they first visited them. Moreover, in our counts we included new aphid larvae on the plants that were probably produced by the aphids that had migrated onto the plants. This too could be more a measure of plant acceptance rather than attractance.

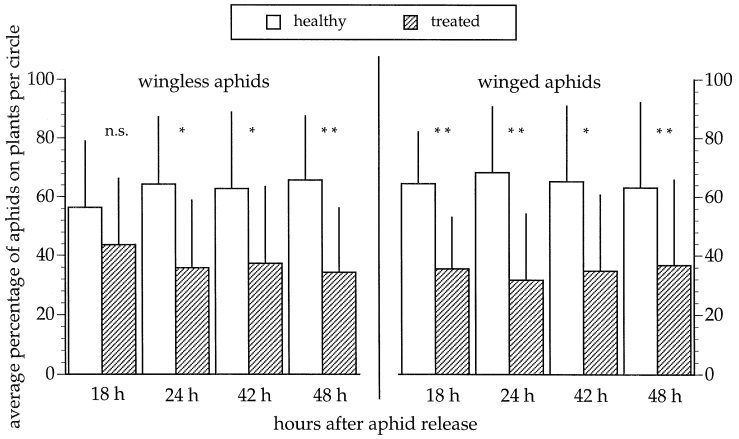

Figure 2. The average percentage (with s.d.) per circle of aphids found on treated and healthy plants at different observations after aphid release (see text for details). Significantly more aphids were found on the healthy plants (Wilcoxon's test for tied ranks, *P $\leq$ 0.05; **P $\leq$ 0.001; ***P $\leq$ 0.0001).

Other studies that have looked for effects of herbivore-induced plant volatiles on herbivores show that the responses may vary for different herbivores and different plants. Dicke (1986) found that the spider mite *Tetranychus urticae* disperses away from odours emitted by bean plants infested with conspecifics, but at a different scale, Pallini et al. (1997) showed that they are actually attracted to already infested cucumber plants. Yet, the study by Pallini et al. (1997) also showed that odours emitted from plants infested with thrips are avoided by the spider mites. By doing so, the mites may avoid competition for the same resource, but also possible predation by the thrips. Landolt (1993) found that female *Trichoplusia ni* moths are initially attracted to cotton plants damaged by conspecifics, but prefer to oviposit on undamaged plants, while the same moth avoids already infested cabbage plants altogether. In general, it appears that in the systems where induced plant volatiles are known to attract natural enemies, herbivores prefer odours from healthy plants (Turlings & Benrey, 1998). Attraction to plants that are already damaged by conspecifics is usually found for adult Coleoptera (Harari et al., 1994; Loughrin et al., 1995; Bolter et al., 1997; N. Kalberer, pers. comm.). These beetles aggregate apparently for mating purposes and/or a joint rapid exploitation of plants, which reduced exposure to plant defence compounds. It has also been proposed that aggregation helps the beetles to make more efficient use of plant secondary compounds for their own defence (Birch, 1984; Pasteels et al., 1988). These benefits may outweigh detrimental effects such as the risk of attracting natural enemies.

From collections and analyses of volatiles emitted by treated maize plants we know the identity of the induced compounds (Turlings et al., 1998; Figure 3). Some of these compounds have been shown to influence the behaviour of different aphid species. Most notably, (*E*)-β-farnesene, which is released in large amounts by treated maize plants, and is a common alarm pheromone for many aphids (Pickett et al., 1992). Bowers et al. (1972) reported that (*E*)-β-farnesene was also repellent when tested against *R. maidis*. Here we confirm that (*E*)-β-farnesene is emitted by harassed *R. maidis*. The presence of this compound in the induced volatile emissions of maize plants could be responsible for the avoidance of these plants by aphids. Gibson & Pickett (1983) reported that plant-derived (*E*)-β-farnesene from *Solanum berthaultii* repelled aphids in a similar way as does the aphid alarm pheromone. The long foliar trichomes of *S. berthaultii* produce only (*E*)-β-farnesene while the rest of the plant produces a blend of sesquiterpenes including (*E*)-β-farnesene. Repellence was only recorded when the aphids perceived the odour of the trichomes separately from the odours of the rest of the plant. *Humulus lupulus*, which emits relatively large amounts of (*E*)-β-farnesene, was not repellent to the aphid *Phorodon humuli* (Dawson et al., 1984). The same authors showed, that (*E*)-β-caryophyllene, another volatile component of the hop plant, inhibited the repellent effects of (*E*)-β-farnesene. Recently, Mostafavi et al. (1996) showed for alfalfa that only plants with high ratios of (*E*)-β-farnesene relative to (*E*)-β-caryophyllene are significantly repellent to the pea aphid and the

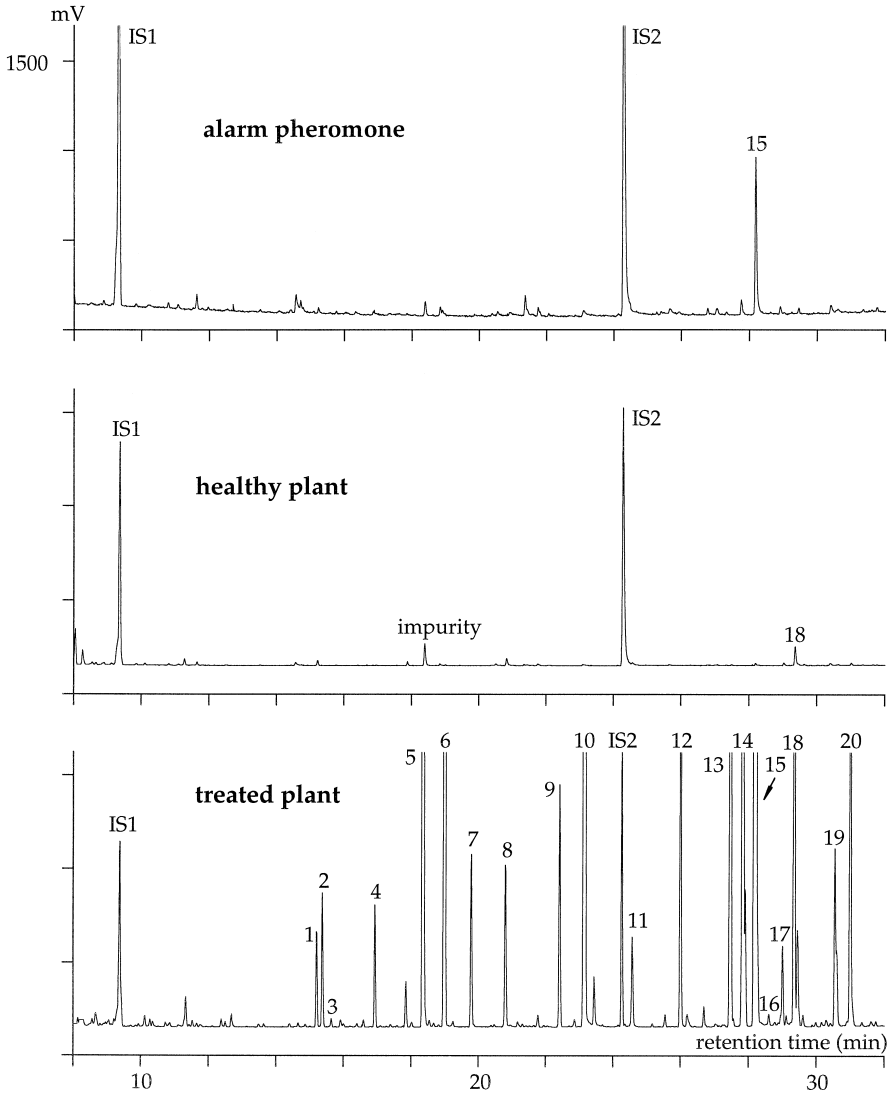

Figure 3. Chromatographic profiles of volatiles collected from crushed aphids (top), a healthy plant (middle) and a treated plant (bottom). Peak numbering: 1, β-myrcene; 2, (*Z*)-3-hexen-1-yl acetate; 3, 1-hexyl acetate; 4, (*Z*)-ocimene; 5, linalool; 6, (3*E*)-4,8-dimethyl-1,3,7-nonatriene; 7, benzyl acetate; 8, methyl salicylate; 9, phenethyl acetate; 10, indole; 11, methyl anthranilate; 12, geranyl acetate; 13, β-caryophyllene; 14, α-*trans*-bergamotene; 15, (*E*)-β-farnesene; 16, α-humulene; 17, unknown sesquiterpene; 18, (*E*,*E*)-α-farnesene; 19, (*E*)-nerolidol; 20, (3*E*, 7*E*-4,8,12-trimethyl-1,3,7,11-tridecatetraene. IS1 and IS2 represent the internal standards *n*-octane and nonyl acetate, respectively.

blue alfalfa aphid. It should be noted that the treated maize plants also emitted significant amounts of (*E*)-β-caryophyllene (Figure 3), it remains therefore unclear to what extent (*E*)-β-farnesene played a role in the observed repellence.

Some of the other induced maize volatiles are also known to be repellent to aphids. Chapman et al. (1981) reported that linalool reduced the catches of the aphid *Cavariella aegopodii* in the field. Hardie et al. (1994) showed that in olfactometer experiments methyl salicylate was repellent to the black bean aphid *Aphis fabae* and also inhibited attraction to volatiles from its host. Pettersson et al. (1994) reported that in fields treated with methyl salicylate, colonisation of the summer host by *Rhopalosiphum padi*, *Sitobion avenae* and *Metopolophium dirhodum* was significantly reduced. Methyl salicylate is a volatile released in substantial amounts from the winter host of *R. padi* (*Prunus padus*) (Pettersson et al., 1994), but it is also associated with secondary metabolite-based defence in

plants (Ward et al., 1991), and it may be advantageous for the aphids to avoid plants that produce methyl salicylate. Although all the above volatiles appear to repel aphids, we cannot generalise their impact, it is to be expected that they affect various aphid species differently.

Little is known about differential effects of plant volatiles on the behaviour of the different aphid morphs. Montgomery & Nault (1978) showed the effects of age and wing polymorphism on the sensitivity of *Myzus persicae* to the alarm pheromone (*E*)-β-farnesene. They found that alatae were most sensitive to (*E*)-β-farnesene, followed by older nymphs and adult apterae. The least sensitive were old adult apterae which were a factor 3×10^3 less sensitive than alatae. Whether this has a behavioural or a physiological (different abilities of detection in the different aphid morphs) cause is not clear and needs further investigation. The olfactometer data (Figure 1) suggest that apterae are more attracted to healthy plants, but perhaps less repelled by treated plants. In tests that included the odour of treated plants, a high number of alatae did not make a choice, perhaps they refrained from walking upwind because of the repelling odour.

From the field study, we cannot conclude that the difference in the colonisation of the treated and the healthy plants was (only) due to the induced volatiles. It is quite possible that plant treatment results in non-volatile secondary metabolites that influence the host selection behaviour of the aphids. Morse et al. (1991a) showed that artificial leaf damage on growing maize plants produced a significant increase in the concentration of the hydroxamic acid DIMBOA (2,4-dihydroxy-7-methoxy-1,4(2H)-benzoxazin-3-one) relative to undamaged control plants (max. after 2 days). Such maize leaf damage has a negative effect on the survival and growth rate of the bird-cherry-oat aphid (*Rhopalosiphum padi*) (Morse et al., 1991b).

Hydroxamic acids have also often been correlated with insect resistance in cereals. Thackray et al. (1990) found a significant correlation between hydroxamic acid levels and resistance to *Rhopalosiphum padi* and *Sitobion avenae*. However, there appears to be no correlation between hydroxamic acid levels in different maize inbred lines and resistance to *R. maidis* (Bing et al., 1990). Givovich & Niemeyer (1994) also confirmed that *R. maidis* is unaffected by hydroxamic acids in maize seedlings. While feeding, this aphid may avoid contact with these and other defence compounds because their stylets usually penetrated the plants intercellularly (Bing et al., 1991). Hence, the idea that the aphids would be repelled by induced volatiles because they indicate a chemical defence reaction in the plant is doubtful. Huber & Stringfield (1942) reported that large populations of *R. maidis* have been associated with increased numbers of the European corn borer, *Ostrinia nubilalis*, even though infestation by this stemborer increases DIMBOA concentrations up to 96% (Gutiérrez et al., 1988). At least as far as *O. nubilalis* is concerned, the aphids do not seem to avoid possible competition on the same plant. Although to a lesser extent, *O. nubilalis* also induces the emission of volatiles in maize (Turlings et al., 1998). Qualitative differences in these emissions may be important in determining their repellency of possibly attractiveness to the aphid.

Our hypothesis that induced maize odours are repellent to *R. maidis* was confirmed, but we can still only speculate on why this is so. *R. maidis* does not induce volatile emissions in maize (Turlings et al., 1998), therefore the odours probably do not indicate the presence of this aphid species on the plants. Studies with other aphids, however, present evidence for aphid-induced plant volatiles and consequently an increased attractiveness to parasitoids that attack aphids (Guerrieri et al., 1993; Micha & Wyss, 1995; Du et al., 1996). It is perhaps this increased risk of falling victim to natural enemies that the aphids avoid by selecting healthy plants. Further studies are needed to elucidate the importance of the different compounds in the induced odour of maize plants on the behaviour of *R. maidis*. This should lead to a better understanding of resistance to *R. maidis* in maize plants and perhaps reveal new opportunities to improve crop protection.

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