Plants are better protected against spider-mites after exposure to volatiles from infested conspecifics

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Abstract. When infested by herbivorous mites, cotton seedlings produce volatile cues that elicit attraction of predatory mites. Experiments were carried out to elucidate how downwind *un*infested conspecific seedlings are affected by these volatiles. It was found that the rate of oviposition of herbivorous mites was reduced on seedlings exposed to volatiles from infested seedlings. Moreover, predatory mites were attracted by exposed *un*infested seedlings. These results strongly suggest that uninfested plants are better protected against herbivore attack when exposed to airborne chemicals released by their infested neighbours.

Key words. Information transfer; communication; contamination; herbivore; spider-mite (*Tetranychus urticae*); predator; predatory mite (*Phytoseiulus persimilis*); cotton (*Gossypium hirsutum*); induced plant defence; tritrophic interactions.

Plants may defend themselves against herbivory both directly and indirectly[1]. Direct defence implies the use of physical or chemical means to reduce the impact of herbivores. Indirect defence is mediated by natural enemies of herbivores: the plant promotes the effectiveness of predators or parasitoids, thereby reducing the damage caused by their herbivorous prey. Effectiveness of natural enemies can be promoted by attracting them after herbivore damage has begun to be inflicted[1-4].

Indirect defence has been studied extensively in a tritrophic system consisting of bean plants (*Phaseolus lunatus* L.), herbivorous spider-mites (*Tetranychus urticae* Koch), and predatory mites (*Phytoseiulus persimilis* Athias-Henriot)[5,6]. Spider-mites are ravaging herbivores which, in the absence of predators, overexploit their host plants. However, when predatory mites discover a spider-mite patch, they may decimate the population of their prey. The sooner predatory mites find the herbivores, the greater will be the benefit to the plants. It was shown that bean plants can promote the discovery of herbivores by predators. When the plants are infested by spider-mites, volatile chemicals are released which attract predatory mites[5-8]. Both biological and chemical evidence strongly suggests that the plants are involved in the production of these chemicals[9]. Subsequent research has shown that many plant species other than the Lima bean respond to spider-mite damage in a similar fashion[7].

So far, all the attention has been focussed on the benefits of the predator-attracting cues for infested plants. However, since these chemicals are volatile they will also reach downwind vegetation. This fact prompted us to ask whether nearby uninfested plants might not also benefit from the airborne information released by infested conspecifics. Plants may benefit directly, through reduction in quality for the herbivores, or indirectly, through attraction of the herbivores' enemies. We examined these possibilities by assessing: [1] the ovipositional rate of *T. urticae* on uninfested cotton seedlings that either were or were not exposed to the odour emanating from cotton seedlings infested with *T. urticae*, and [2] the olfactory response of *P. persimilis* to uninfested cotton seedlings that either had been or had not been exposed to the odour from conspecifics infested with spider-mites.

We used cotton (*Gossypium hirsutum* L., var. Acala SJ-2) as a host plant for several reasons. Firstly, Karban and Carey[10] showed that population growth of *T. urticae* is reduced on new leaves of cotton seedlings whose cotyledons had been previously exposed to spider-mite feeding. The authors suggested that cotton seedlings are capable of induced direct defence against *T. urticae*. Secondly, cotton is known to release volatiles when damaged by herbivores[11]. Thirdly, Zeringue[12] showed that the concentration of phenolic compounds increased in detached, uninfested cotton leaves during exposure to odour from detached cotton leaves that were infested with a fungus. Phenolic compounds are commonly regarded as having defensive properties.

Materials and methods

Female spider-mites in the last moulting stage were collected from a stock culture on Lima bean. At 25 °C this stage lasts approximately 24 h, which ensured that the mite sample was homogeneous in age. The mites were transferred to a detached Lima bean leaf placed on moist cotton wool. Three days later the young adult females were in their ovipositional phase, and they were used in the experiments. To examine whether uninfested cotton seedlings benefit from volatile cues released by conspecifics infested with *T. urticae*, two treatments were carried out in three-compartment wind tunnels (figs 1 and 2). Each wind tunnel compartment consisted of an iron frame (75 × 50 × 35 cm), wrapped in transparent plastic foil. One side could be opened and closed by means of loose magnetic strips. A PVC tube connected two successive compartments. Two three-compartment wind tunnels were juxtapositioned in a climate room. The

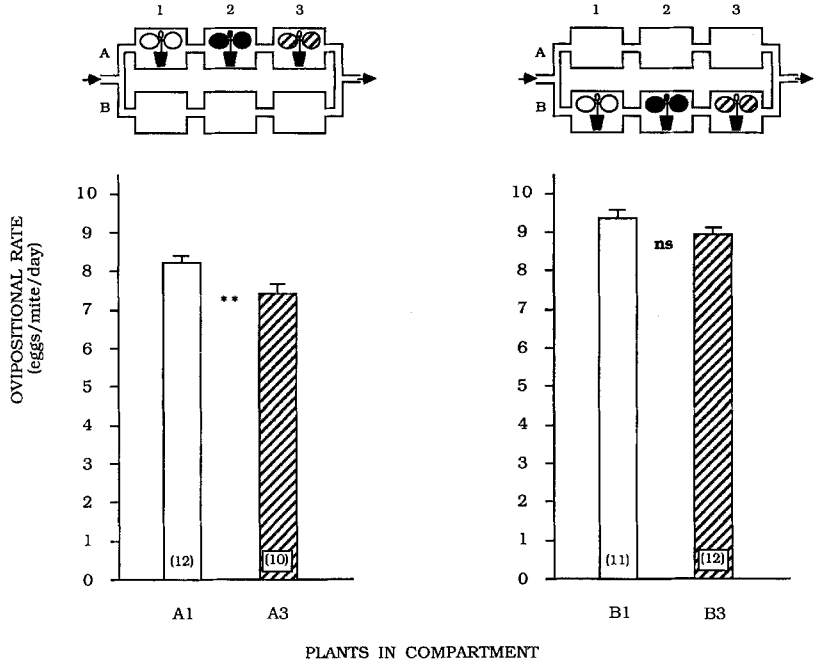

Figure 1. Mean ovipositional rate in a cohort of female spider-mites (*Tetranychus urticae*) on cotton seedlings that were previously uninfested and either were not exposed (white leaves in first compartment) or were exposed (striped leaves in third compartment) to cues from spider-mite infested conspecific seedlings (black leaves in second compartment). Histogram columns represent *overall* mean ovipositional rates. For each plant the mean ovipositional rate, based on nine mites per plant, was calculated. These ovipositional rates were averaged over all plants of the same group. The number of plants on which the overall means are based is given between brackets. Bars represent standard errors. The asterisks between two bars indicate the significance of the difference between two means (**: $0.001 < p < 0.01$; ns: not significant ($0.1 < p < 0.2$)). Small arrows in the figure indicate the direction of the air stream.

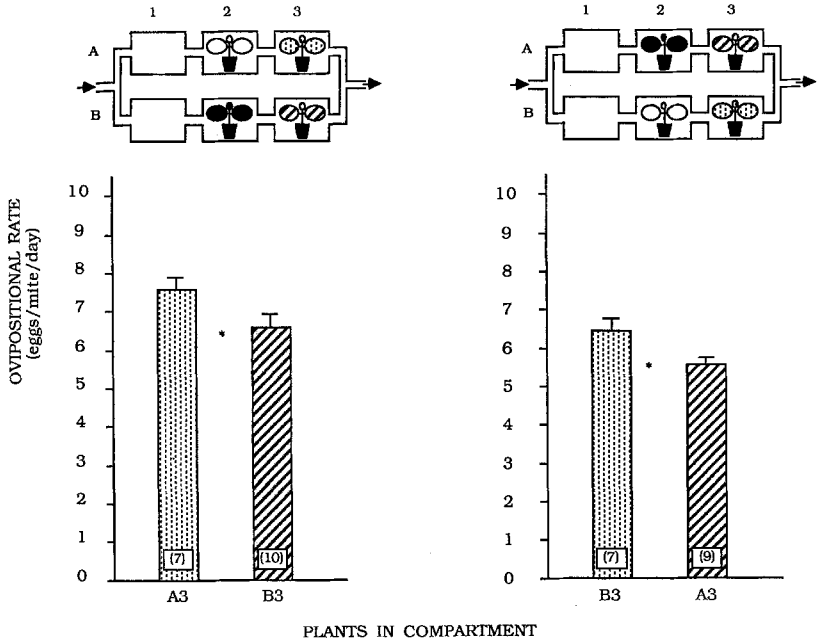

Figure 2. Mean ovipositional rate in a cohort of female spider-mites (*Tetranychus urticae*) on cotton seedlings that were previously uninfested and were exposed to cues from conspecific plants that were not infested (dotted leaves in compartment A3) or were infested (striped leaves in compartment B3) by spider-mites. The asterisk between two bars indicates the significance of the difference between two overall means (*: $0.01 < p < 0.05$). For further details see legend to fig. 1.

inlet of the wind tunnels was connected to the exterior of the room. The outlet was connected to the room's air extraction device which ensured a continuous unidirectional air stream in the wind tunnels. Climatic conditions were: 25 ± 1 °C, 70 ± 5% rh, 16L:8D photocycle.

In treatment I, both the upwind and downwind compartments of one wind tunnel contained 12 uninfested cotton seedlings. The middle compartment contained 25 cotton seedlings, infested with an ample number of *T. urticae*, varying from 30 to 100 per seedling. All plants were individually grown in 8-cm plastic pots. The plants in the upwind compartment were exposed to air from the exterior of the climate room, and served as a control. The uninfested plants in the downwind compartment were exposed to cues from both infested and uninfested plants. In the replicate series all plants were placed in the parallel wind tunnel, everything else being equal.

In treatment II, both wind tunnels were used simultaneously. In this setup each downwind compartment contained 16 uninfested cotton seedlings. The middle compartment of one wind tunnel contained 19 uninfested seedlings, whereas the middle compartment of the other wind tunnel contained 19 seedlings infested with spider-mites. Thus, one group of uninfested plants in a downwind compartment was exposed to cues from uninfested plants, whereas the other group of uninfested plants was exposed to cues from infested plants. In the replicate series plants were positioned in equivalent compartments of the parallel wind tunnel.

To investigate the impact on herbivore performance, the ovipositional rate of *T. urticae* was assessed. Nine young, ovipositing females of about the same age were placed on one leaf of each uninfested plant positioned in the upwind and downwind compartments under treatment I or in the two downwind compartments under treatment II. They were invariably placed on the first leaf following the cotyledons. A tanglefoot barrier around the petiole prevented the mites from escaping. Prior to the oviposition experiment, seedlings were exposed to airborne cues from infested conspecifics for 4–8 days. Ovipositional rates were determined over a three-day period, during which the plants remained exposed. Plants on which less than nine females were present after three days were discarded from the statistical analysis. The overall mean ovipositional rates were analysed with a t-test for comparison of two means.

To investigate the impact on predator performance the olfactory response of *P. persimilis* was studied in a Y-tube olfactometer (figs 3 and 4). We tested whether predatory mites discriminate between odours from two groups of plants, both taken from wind tunnel compartments and transferred to the olfactometer. Immediately after each trial the plants were put back in their compartments. The same plants were subsequently used to assess the ovipositional rate of *T. urticae*. The olfactory responses of *P. persimilis* to different groups of plants are represented in figs 3 and 4.

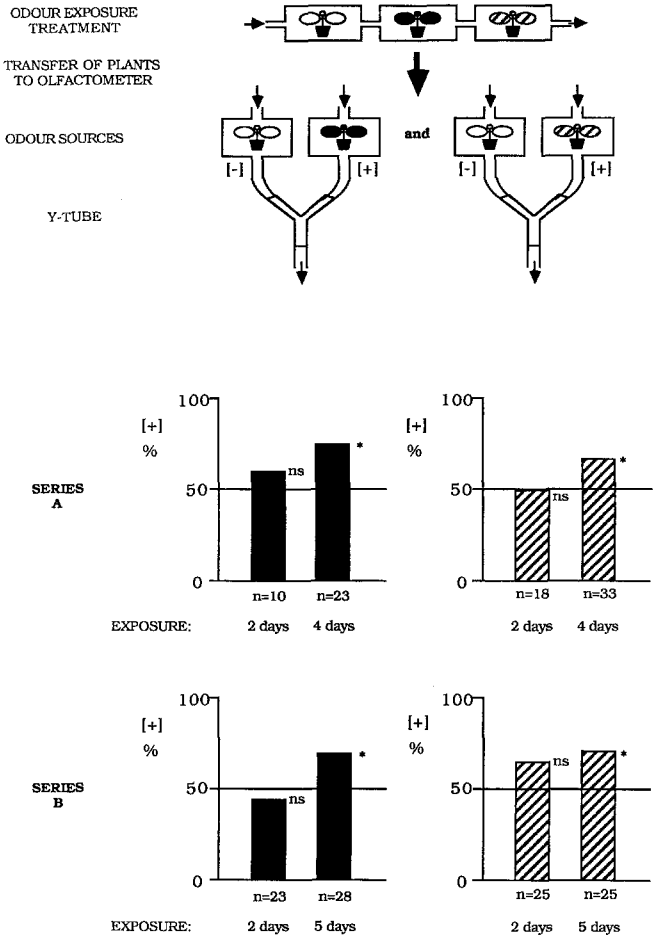

Figure 3. Differential response of *Phytoseiulus persimilis* females in a Y-tube olfactometer when offered a choice between two odour sources:
– cotton seedlings infested with spider-mites (black leaves) versus uninfested cotton seedlings (white leaves);
– uninfested cotton seedlings that were previously exposed to odour from infested seedlings (striped leaves) versus unexposed uninfested cotton seedlings (white leaves).
EXPOSURE: Duration of exposure to infested seedlings, prior to choice experiment; n: number of predators tested individually. *: $0.01 < p < 0.05$; ns: not significant. The experiments were performed twice (Series A and Series B), corresponding to the two situations shown in fig. 1.

The Y-tube consisted of a Y-shaped glass tube with an iron wire in the centre, parallel to the tube wall. Perspex boxes at the ends of both arms contained 6–8 plants as an odour source. Air was sucked out at the base of the tube and was led out of the climate room. Air speed in both arms was 0.65 ± 0.05 m/s. After each fifth mite had been tested the sources were switched. Sabelis and Van de Baan [5] for a more elaborate description of setup and procedures. The olfactometer results were analysed with a sign test (H_0: proportion of mites that prefer either odour source = 0.5; H_1: proportion of mites that prefer the odour source other than the control > 0.5). The mites that had not reached the end of one arm of the Y-tube within 5 min were omitted from the analysis.

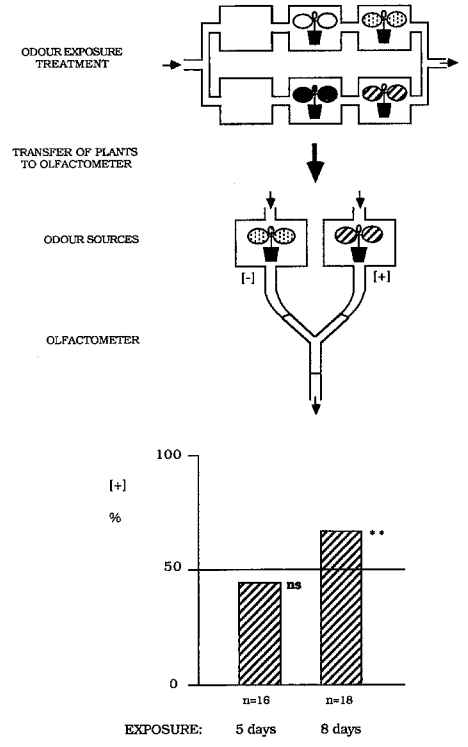

Figure 4. Differential response of *Phytoseiulus persimilis* females in a Y-tube olfactometer, when offered a choice between two odour sources: uninfested cotton seedlings that had previously been exposed to odour from uninfested cotton seedlings (dotted leaves) versus uninfested cotton seedlings that had previously been exposed to odour from spider-mite infested cotton seedlings (striped leaves). **: $0.001 < p < 0.01$. For further explanation see legend to fig. 3.

Results

The mean ovipositional rate of *T. urticae* was lower on seedlings that were exposed to cues from infested conspecifics than on control plants (figs 1 and 2). Under treatment I (fig. 1) the reduction in ovipositional rate was 0.77 egg/female/day. This reduction was statistically significant ($p = 0.0096$). In the replicate series the reduction was 0.40 egg/female/day, which was not significant ($p = 0.19$). Averaged over both series, the ovipositional rate was reduced by 0.6 egg/female/day, or 6.8%. Under treatment II (fig. 2) the reduction in ovipositional rate was significant in both series: 0.95 egg/female/day ($p = 0.047$) and 0.83 egg/female/day ($p = 0.019$). On average, it was reduced by 0.9 egg/female/day, or 12.7%. Thus, in total a reduction of ovipositional rate was found three out of four times.

The difference between both treatments for percentage reduction of oviposition may partly be caused by an effect of temperature. The air entering the wind tunnels came from outside the climate controlled room, where the temperature was lower than inside. This difference in ambient temperatures resulted in a temperature gradient along the wind tunnel. In the most downwind compartment the temperature was about 1.5 °C higher than in the most upwind compartment. Since the ovipositional rate of *T. urticae* is temperature-dependent, a control experiment was performed under treatment I in which the plants in the middle compartment remained uninfested. The ovipositional rate was 0.67 egg/female/day *higher* on the plants in the downwind compartment ($p = 0.029$). This implies that the effect of exposure to airborne cues from infested plants is likely to be underestimated in the setup for treatment I. Under treatment II there was no difference in temperature between the two downwind compartments.

At first we checked whether the predatory mites preferred the infested plants to the uninfested control plants (fig. 3). The predators showed a preference for the infested plants only at a certain level of spider-mite infestation. After two days of infestation the predators not did distinguish between the two groups of plants, but after four to five days they responded significantly (fig. 3). This response is in agreement with earlier results [5].

An entirely new result is that predatory mites were also attracted to uninfested plants that had been exposed for four to five days to volatile cues from plants infested with *T. urticae*. Again, this response was only shown after the plants had been exposed for a certain time to odour from infested conspecifics whose infestation level increased during that time. The predators did not discriminate between uninfested control plants and uninfested plants that had been exposed for two days to plants that had been infested for only two days (fig. 3). But when the plants had been exposed for four to five days, predatory mites preferred exposed uninfested plants to unexposed controls.

In the second experiment, related to treatment II, essentially the same response was shown. After exposure to infested conspecifics for five days the uninfested plants were not more attractive to the predatory mites than control plants (fig. 4). But after the plants had been exposed for eight days, the predators preferred the exposed uninfested plants to the controls. The differences in incubation time needed for a response are probably caused by the non-standardized sizes of the initial spider-mite populations on the infested plants.

In all olfactometer experiments presented thus far, the odour sources consisted of plants in plastic pots with moist soil. To be certain that the predatory mites responded to odours associated with the plants, rather than with the soil and/or the pots, we performed two small additional experiments. In the first experiment, two groups of six pots filled with moist soil were exposed to odours from either uninfested cotton seedlings or from seedlings infested with spider-mites. After eight days of exposure both groups of pots with soil were tested against each other in the olfactometer. The predatory mites showed no preference: 48% of the mites chose the olfactometer arm leading to the pots that had been exposed to the infested plants ($n = 23$; $p = 0.50$). All pots were put back in the wind tunnel compartments. After 13 days of exposure they were tested again, and predatory mites still showed no preference: 47% of the mites chose

the arm connected with the pots that had been exposed to the infested plants (n = 15; p = 0.50). Thus, this experiment gives us no reason to conclude that pots filled with moist soil are attractive by themselves.

In the second experiment, two groups of six individually-potted cotton seedlings were exposed to either uninfested or spider-mite infested cotton seedlings. After eight days of exposure, both groups were tested against each other in the Y-tube olfactometer. Predatory mites clearly preferred the potted plants that had been exposed to the infested seedlings: 86% of the mites chose the arm leading to these plants (n = 14; p = 0.0065). Immediately after the test, all plants were put back in the wind tunnel. The next day the shoots and the pots, containing the soil, were tested separately. The shoots, cut off just before the test and placed individually in small vases, were as attractive as the intact plants had been: 79% of the predatory mites chose the arm leading to the shoots that had been exposed to the infested plants (n = 14; p = 0.029). The pots and the soil, however, were not at all attractive: only 35% of the predators chose the arm leading to the pots that had been exposed to infested plants (n = 20; p = 0.13). These results give positive evidence that the odours to which predatory mites are responding are associated with the plants rather than with the pots and/or soil.

Discussion

Our experiments showed that uninfested plants that are near infested conspecifics may well be better protected against herbivory than uninfested plants that do not have infested neighbours. It is tempting to speculate on the underlying mechanism. First, it remains to be definitely proven that infested cotton plants are actually producing the signals which render the exposed plants better protected against herbivory. Production by the plants, however, seems extremely likely, by analogy with work on other plant species[3,6,9]. Secondly, we do not know the precise mode of action of the volatile cues. It is possible that some of the volatiles function as a spider-mite dispersing pheromone and induce a slower rate of feeding and egg production of the herbivore[9,20]. This could explain the reduced fecundity of spider-mites on exposed plants. However, it might be that the exposed plants are actively involved, in which case we would be dealing with plant-to-plant *communication*. This has been suggested for some plant species[13–15], but Fowler and Lawton[16] have pointed at weaknesses in experimental setups and offered alternative explanations. Recently some chemical evidence for communication between plants has been published: Zeringue[12] and Takabayashi et al.[17] showed that chemical changes occur in detached leaves exposed to cues from infested leaves. As an alternative to active response through communication, information transfer may occur passively by plant-to-plant *contamination*. The volatiles may adsorb onto waxy layers on the plant's surface and then be secondarily released at a slower rate[18,19]. It is possible that secondary release after contamination is responsible for the attraction of predatory mites.

Whatever the precise nature of the underlying mechanism, the important point is that uninfested plants are better protected after exposure to volatiles emanating from a herbivore-plant interaction. This phenomenon may influence the outcome of plant competition. In addition, when the environment is patchy, it may also contribute to the evolution of plant populations that are polymorphic in their allocation of defensive effort[21].

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