Within-plant circulation of systemic elicitor of induced defence and release from roots of elicitor that affects neighbouring plants

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Abstract

Herbivory on the above-ground plant parts can induce the production of volatiles that attract carnivorous enemies of the herbivores. These volatiles may be induced systemically in the damaged plant. In a previous study, we had shown that a systemic elicitor that induces the production of predator-attracting volatiles in lima bean plants can be collected from lima bean leaves by placing spider-mite-infested leaves with their petiole in water. However, it remained unknown to what sites this elicitor was transported within the plant. Here, we show that exposing uninfested lima bean leaves to this elicitor also results in a reduced rate of reproduction in spider mites (Tetranychus urticae). Furthermore, an elicitor can also be collected from whole lima bean plants of which one primary leaf was infested with spider mites, by detaching the other primary leaf and inserting the petiole, that is still connected to the plant, in a vial with distilled water. When uninfested lima bean leaves are incubated in this elicitor solution for three days, this results in the attraction of the predatory mite Phytoseiulus persimilis in an olfactometer. Finally, an elicitor of predator-attracting volatiles was collected from whole infested lima bean plants by placing the plants with their roots in distilled water. Uninfested lima bean plants that were subsequently placed with their roots in this water became attractive to the predatory mite P. persimilis relative to control plants that had been placed with their roots in water in which uninfested bean plants had been incubated previously. These data indicate that herbivore-infested plants may interact with undamaged neighbouring plants through chemical information that is exchanged in the soil. Important issues that need to be addressed next are discussed. The connection of above- and below-ground interactions of plants with other organisms will have important consequences for future ecological studies on multitrophic interactions. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Plants disseminate many compounds into their environment, both from their above- (Visser, 1986) and below-ground parts (Rovira, 1969). These compounds may affect other organisms such as nitrogen-fixing symbionts (Zaat et al., 1989), herbivores (Visser, 1986; Wang et al., 1997), pollinators (Schoonhoven et al., 1998) and plant-parasitic plants (Estabrook and Yoder, 1998). Damage to plant leaves or stems results in a large increase in the emission of volatiles. These volatiles can affect the behaviour of herbivorous and carnivorous arthropods (Turlings et al., 1995; Boland et al., 1999; Dicke, 1999; Sabelis et al., 1999; Dicke and Van Loon, 2000) and may cause a physiological response in the neighbouring undamaged plants (Dicke and Bruin, 2001). Thus, herbivory may result in changes in species-interactions in above-ground food webs. Recently, first data was reported which suggested that below-ground herbivory can also induce the emission of volatiles by roots, which results in the attraction of soil-dwelling organisms such as nematodes that attack the volatile-inducing herbivores (Boff et al., 2001; Van Tol et al., 2001). Thus, interactions in below-ground food webs may also be affected by herbivory.

Plant chemicals induced by above-ground herbivory are emitted systemically from damaged and undamaged leaves (Dicke et al., 1990a; Turlings and Tumlinson, 1992; Dicke, 1994; Potting et al., 1995; Rose et al., 1996) and a systemic elicitor can be collected from detached infested leaves (Takabayashi et al., 1991; Dicke et al., 1993). However, the questions that remained unstudied were how such a systemic elicitor circulates in an intact plant. Does it circulate in the above-ground plant tissues exclusively or is it also transported to the roots? And if so, is it exuded from the roots and can it affect other plants? In the literature, there is evidence that above-ground herbivory leads to changes in plant roots that affect herbivore–plant interactions (Moran and Whitham, 1990). For example, damage to tobacco leaves results in jasmonic acid mediated signalling to the roots that leads to the induction of nicotine production in the roots and subsequent transport of the nicotine to the above-ground parts (Baldwin et al., 1994; Zhang and Baldwin, 1997). Radioactively labelled compounds applied to above-ground tissues of uninfested bean plants were shown to be transferred to the soil and into neighbouring plants (Rovira, 1969). However, whether damage to above-ground plant tissues also results in an altered emission of chemicals from plant roots has remained unexplored (Van der Putten et al., 2001). Several studies on chemical information conveyance between damaged and undamaged plants have explicitly excluded the possibility for below-ground information conveyance (e.g., Baldwin and Schultz, 1983; Rhoades, 1983; Farmer and Ryan, 1990; Bruin et al., 1992; Arimura et al., 2000; Karban et al., 2000), while in one study the effects of mechanical damage to plants on characteristics of their
neighbours might be explained by below-ground information transfer (Haukioja et al., 1985).

In the present study, we investigated whether herbivory on leaves could affect neighbouring plants through chemicals emitted by the roots of the damaged plant. The system investigated consists of lima bean plants, two-spotted spider mites (Tetranychus urticae) and predatory mites (Phytoseiulus persimilis). It has been well-established that spider-mite feeding on lima bean plants results in the systemic emission of a complex blend of volatiles from the plant’s leaves (Dicke et al., 1990a,b, 1993, 1999; Koch et al., 1999). Among these volatiles are at least four compounds that mediate the attraction of the predatory mite P. persimilis (Dicke et al., 1990b). Spider-mite induced volatiles can also affect the behaviour of spider mites (Dicke, 1986) and exposure of undamaged neighbouring lima bean plants to the volatiles makes these plants attractive to the predatory mites (Dicke et al., 1990a). A water-soluble elicitor was collected from spider-mite-infested leaves by placing the leaves with their petioles in water (Dicke et al., 1993). Exposure of uninfested leaves to this elicitor results in the attraction of predatory mites. Thus, an elicitor of predator attractants is exported from spider-mite-infested lima bean leaves. However, the transport of this elicitor throughout the plant remains unknown. Furthermore, it is not known whether lima bean plants exude an elicitor through their roots that may induce volatile emission in neighbouring plants. To initiate a study of the possible interaction between damaged and undamaged plants by below-ground transfer of chemical cues, we investigated the following questions: (1) Can the elicitor of carnivore attractants be collected from whole plants with one leaf blade removed, by inserting the petiole of a leaf into a vial with water while another leaf is infested by herbivores? (2) Does the elicitor induce resistance to spider mites in lima bean leaves? (3) Does a spider-mite-infested plant disseminate an elicitor from its roots, that results in an increased attraction of predatory mites when roots of uninfested plants are exposed to this elicitor?

2. Materials and methods

2.1. Plants and mites

Lima bean plants were grown in a glasshouse at 20–30°C and 60–80% r.h. Two-spotted spider mites (Tetranychus urticae) were reared on lima bean plants in a separate greenhouse compartment under similar conditions. The predatory mites (Phytoseiulus persimilis) were reared on detached spider-mite-infested lima bean leaves at 20–30°C and 60–80% r.h.

2.2. Olfactometer

A closed-system glass Y-tube olfactometer was used to investigate the attraction of the predatory mites. For details of the set-up and procedures see Takabayashi and Dicke (1992). Adult female predatory mites that had been starved for 1–3 h were
individually introduced into the olfactometer and their choice for one of the odour sources was recorded when they reached the far end of one of the olfactometer arms. If a predator had not reached the end of one of the arms within 5 min it was recorded as 'no choice'. After five predators had been observed, the two odour sources were interchanged to adjust the potential asymmetries in the experimental arena.

The behavioural responses of the mites were statistically analysed with a binomial test to determine whether their distribution differed from 50:50. Mites that had not made a choice were excluded from the statistical analysis.

2.3. Elicitor and induced resistance

Elicitor was collected from 20 detached lima bean leaves each infested for seven days with 50 adult female spider mites by placing each of the leaves with their petiole in a vial with distilled water (13 ml), following the method described by Dicke et al. (1993). Twenty control leaves were incubated in the same way but without infestation by spider mites. The leaves originated from uninfested lima bean plants that had two primary leaves and an expanding first trifoliate leaf. One primary leaf of each plant was assigned to the treatment (infestation with 50 adult female spider mites) and the other to the control (no spider mites). The infested and control leaves were incubated in different plastic cages (66 × 66 × 100 cm), each connected to house vacuum. After seven days of infestation the leaves were discarded and the water was transferred to new vials and primary leaves from 20 uninfested lima bean plants were incubated in the vials with water. From each uninfested plant, one primary leaf was assigned to the control and one to the treatment. After three days these leaves were each infested with a single young spider-mite female (three days since the final moult). The treatment leaves were incubated in a plastic cage (66 × 66 × 100 cm) and the control leaves were incubated in a second plastic cage. The two cages—that were different from the cages in which the infested and uninfested leaves had been incubated before—were placed in the same climate room (23±1°C, 60–80% r.h., 8000 lux) and were each connected to house vacuum to avoid interference of odours between the cages. After four days of incubation the position of the spider-mite females (upper or lower side of a leaf) and the number of their eggs on the upper and lower side of the leaves were recorded.

This experiment had 22 replicates. The data for all control or treatment leaves of one replicate were taken together as one observation.

2.4. Elicitor collected from whole plant

From each of 20 lima bean plants, with two fully developed primary leaves and an expanding first trifoliate leaf, the blade of one of the primary leaves was detached and discarded. The petiole of the leaf remained attached to the plant. Each petiole was immediately inserted into a vial with distilled water (13 ml) which was closed with parafilm. The remaining primary leaves were each infested with 50 adult spider-mite females. The spider mites were prevented from emigrating from this leaf by applying Tanglefoot glue on the petiole. Twenty control plants were similarly
treated, but they were not infested with spider mites. The 20 treatment plants were incubated in two plastic cages in a climate room and the 20 control plants were incubated in two other plastic cages (66 × 66 × 100 cm) in the same climate room (23 ± 1°C, 60–80% r.h., 8000 lux). The cages were connected to house vacuum to avoid interference of odours between the cages. After seven days the primary leaves from the infested and control plants were detached from the plants and used as odour sources in the Y-tube olfactometer. The vials with water that had been attached to the infested and uninfested plants were removed from the plant. These vials with water were used to incubate primary leaves from uninfested plants in a climate room (23 ± 1°C, 60–80% r.h., 8000 lux). From each of 20 uninfested lima bean plants one primary leaf was inserted with the petiole in a vial obtained from an infested plant and the other leaf was incubated in a vial from a control plant. Treatment leaves and control leaves were incubated in separate cages. These cages were different from the ones in which the plants with vials connected to the petiole had been incubated before. After three days, these uninfested leaves were used as odour sources in the Y-tube olfactometer. The whole experiment was repeated eight times.

2.5. Elicitor collected from plant roots

Forty lima bean plants were individually grown in plastic pots in vermiculite under greenhouse conditions identical to those described for plants for the spider-mite culture. When the plants had two fully developed primary leaves and an expanding first trifoliate leaf, the plants were carefully removed from the pot with vermiculite and directly transferred to a glass bottle (250 ml) filled with distilled water. Twenty plants were assigned to the treatment and 20 to the control. On the treatment plants, the primary leaves were each infested with 50 adult female spider mites from the stock rearing. The control plants remained uninfested. The plants were incubated in a climate chamber (23 ± 1°C, 60–80% r.h., 8000 lux). Control plants and test plants were incubated in separate plastic cages (66 × 66 × 100 cm; two cages for control and two cages for test plants) that were separately connected to house vacuum to avoid contamination of control plants with volatiles from infested plants. After seven days, the plants of both groups were used as odour sources in an olfactometer test. The water from the bottles was transferred to new bottles. Uninfested lima bean plants that had grown in vermiculite under the same conditions as described before were carefully removed from the pots with vermiculite. They were immediately transferred to the bottles with water obtained from treatment and control plants and the bottles with these uninfested plants were incubated in a climate room. Plants in ‘treatment water’ and plants in ‘control water’ were incubated in different plastic containers (two containers for treatment and two containers for test plants) that were each connected separately to house vacuum. The cages were different from the cages in which the infested and uninfested plants had been incubated before.

After seven days, the leaves from the treatment and control plants were collected and used as odour sources in an olfactometer. The whole experiment was repeated three times.
3. Results

3.1. Elicitor and induced resistance

The uninfested leaves of treatment (incubated in water in which the petiole of an infested leaf had been previously inserted) and control (incubated in water in which the petiole of an uninfested leaf had been previously inserted) were incubated in separate cages. One young adult spider-mite female had been introduced per leaf. The individual leaves within a cage touched each other, and consequently the spider mites could move from one leaf to another. After four days of incubation the proportions of leaves that contained a single spider mite were similar for treatment and control: 65.9 ± 4.6 and 67.0 ± 4.2 (mean ± SE)% for the elicitor treatment and control respectively ($P = 0.92$, Wilcoxon matched pairs signed rank test). Not all spider mites were recovered after four days [average loss of mites was 12.0 ± 1.5 (mean ± SE)%, but the losses in the treatment and in the test did not differ ($P = 0.46$, matched pairs signed rank test). For each replicate, the total number of...
eggs found was divided by the total number of females recovered after four days. The majority of spider-mite females [77.0 ± 4.0 (mean ± SE)% for elicitor-treated leaves and 76.2 ± 3.5 (mean ± SE) for control leaves] and their eggs [78 ± 3.8 (mean ± SE) and 75 ± 3.2 (mean ± SE)% for elicitor and control leaves respectively] were found on the lower side of the leaves.

The spider mites’ rate of reproduction clearly differed between treatment and control leaves. In 16 out of 22 replicates the average number of eggs per female was higher on the control leaves than on the treatment leaves ($P = 0.026$, binomial test). The average number of eggs per female per four days was 45.3 ± 2.1 (SE) on the treatment leaves and 51.1 ± 2.4 (SE) on the control leaves ($P = 0.015$, Wilcoxon matched-pairs signed rank test; Fig. 1). The spider mites’ reproduction was 1.17 ± 0.07 (mean ± SE) times larger on the control than on the treatment leaves. Thus, spider mites had a higher reproductive success on leaves that were incubated in water in which uninfested leaves were incubated before, than on leaves that were incubated in water in which infested leaves had been incubated before.

Fig. 2. Collection of systemic elicitor from the petiole of the primary leaves of lima bean plants that were infested with spider mites on the other primary leaf. The elicitor solution is used to incubate uninfested leaves from an uninfested lima bean plant for three days. Subsequently, the elicitor-treated and control uninfested leaves were used as odour sources in a Y-tube olfactometer. The responses of adult female predatory mites are shown for each of the replicate experiments and for the combined data. The data were analysed with a binomial test; ‘$n$’ represents the number of predators that made a choice. The number of predators that did not make a choice is indicated on the right of the graph.
3.2. Elicitor collected through petiole from whole plant (Fig. 2)

When spider-mite-infested bean leaves were tested in the olfactometer versus uninfested leaves, 71–90% of the predators chose the odour from infested leaves in the eight replicate experiments. The data for each replicate experiment differ significantly from 50:50 (binomial test, \( \alpha = 0.05 \)). The combined results show a highly significant attraction (binomial test; \( P < 1 \times 10^{-16} \)). Leaves that had been incubated in water in which a petiole from a damaged plant had been inserted for seven days were significantly more attractive than control leaves that had been incubated in water which had been in contact with the petiole from a control plant. In this case, the majority of the predators chose for the treatment leaves in seven out of eight replicates (\( P = 0.035 \), binomial test). This preference for the treatment leaves was statistically significant in four out of eight replicates (binomial test, \( \alpha = 0.05 \)), and marginally insignificant in a fifth replicate. The overall response was highly significant (binomial test, \( P = 1.5 \times 10^{-8} \)). Thus, an elicitor of volatile production can be collected from a whole plant by inserting the petiole of a primary leaf into a vial with water.

3.3. Elicitor collected from plant roots (Fig. 3)

When spider-mite-infested bean plants were offered in the olfactometer versus uninfested plants, the majority of the predators chose the infested leaves in all three
replicates. In two replicates, the predator’s choice differed significantly from a 50 : 50 distribution, in the third replicate the choice was marginally insignificant ($P = 0.058$). The summed response was statistically significant (binomial test; $P = 1 \times 10^{-5}$). When uninfested plants that were incubated in water in which the roots of infested plants were present before were tested in the olfactometer versus control uninfested plants, the majority of the predators chose the treatment plants in all three replicates. This was statistically significant in one of the replicates and marginally insignificant in the second. The three replicates did not differ significantly (contingency table test, $P = 0.73$, df = 2) and the pooled data were significantly different from a 50 : 50 distribution (binomial test; $P = 0.007$). Thus, plants that were incubated with their roots in water in which roots of infested bean plants were incubated before were more attractive to the predators than the control plants that were incubated in water in which roots from uninfested bean plants were incubated before.

4. Discussion

A previous study showed that an elicitor can be collected from detached spider-mite-infested lima bean leaves. When fed to uninfested lima bean leaves, this elicitor resulted in an induced attraction of the predatory mite $P. persimilis$ (Dicke et al., 1993). The present study shows that, when following the same procedure, the spider mites have a reduced rate of reproduction on the elicitor-exposed uninfested leaves. The rate of reproduction is 17% higher on control leaves than on elicitor-treated leaves, which will have a considerable effect on population growth rate. The reduced rate of reproduction on elicitor-treated leaves might be the result of an alteration in spider-mite behaviour, such as an increased allocation of time to the movement at the expense of time spent feeding. It is known that spider mites respond to a high dose of odours from spider-mite-infested lima bean leaves with an avoidance response (Dicke, 1986). However, the data do not indicate that spider mite behaviour was different on treatment and control leaves: spider-mite distributions on treatment and control leaves were similar in all aspects measured. Most likely, the dose of the induced volatiles was too low to elicit dispersal behaviour. At a low dose the behaviour of the spider mites is similar to that when exposed to odours from uninfested control leaves (Dicke, 1986). Another explanation may be that the elicitor results in a change in plant secondary chemicals or nutrients. This remains to be investigated. These experiments on extraction of a systemic elicitor may be criticized on methodological grounds because detached leaves were used, in which many responses are likely to be different from those in the whole plants. For instance, being detached for some days is likely to affect many aspects of leaf physiology, although one may argue that the control leaves have undergone the same procedure. However, in order to overcome this criticism on the use of detached leaves, we did a similar experiment with whole plants and tested whether an elicitor of predator-attracting volatiles could be collected. The results clearly show that uninfested leaves that are exposed to water from a vial in which the petiole of an infested plant had
been inserted were more attractive than control leaves. Thus, this experiment supports the previous data (Dicke et al., 1993). In addition, the experiment shows that an elicitor is not only exported from an infested primary bean leaf (Dicke et al., 1993), but also arrives at the petiole of the other primary leaf of the plant. This indicates that the vascular tissue of the two primary leaves is connected in a way that allows the transport of the elicitor from one to the other. From a study on cottonwood it is known that the plumbing of a plant’s vasculature may be essential for the expression pattern of systemic plant responses, at least in woody plants (Jones et al., 1993).

Finally, we studied whether plant roots release an elicitor that can induce the production of plant volatiles in another plant. The data show that uninfested plants with their roots in water in which infested plants were standing for seven days are more attractive to predatory mites than control plants that were incubated in water in which uninfested plants were placed before. These data indicate that plants may interact with neighbouring plants through compounds released from the roots. However, several issues still need to be addressed before it can be firmly concluded that communication among lima bean plants occurs through a root-released elicitor. Some of these are presented below. (1) The experiment on an elicitor released from roots was repeated three times. In all three replicates, the majority of predators chose the odour of treatment plants. One replicate yielded a significant effect, one yielded a marginally insignificant effect and the third one was not significant (Fig. 3). Since the three replicates did not differ significantly, the data were pooled and the summed results were statistically significant. Additional replicates to confirm the present data will allow to investigate the data on a replicate basis. (2) We have collected the elicitor by placing plants with their roots in water for seven days. This may have resulted in physiological stress such as oxygen stress or changes in the microflora on the root surface with consequences for root permeability. For instance, in a study on the exudation rate of certain amino acids, a 7-fold increase was found for plants in quartz-sand over plants grown in a solution (Rovira, 1969). Therefore, the experiments should be repeated with plants in soil to investigate whether an elicitor can be extracted from the soil. For a review of the factors that may affect root exudation by intact plants see Rovira (1969). (3) During the incubation in water the plants did not receive nutrients. Visual inspection of the plants did not show any symptoms of nutrient deficiency. Yet, the deprivation of nutrients may have affected the plants’ response. Possibly, under optimal nutrient conditions the induced defence, including the production of elicitor is expressed more strongly. (4) When experiments with plants in soil support current results, both the distance on which the elicitor is emitted and its dispersal rate should be investigated. This should be done with plants in soil to avoid quantitative differences caused by an unnatural medium. Furthermore, experiments should be carried out that include a dilution effect, something that was absent from our present investigation. (5) The identity of the elicitor(s) that mediate systemic induction of plant volatiles and systemic resistance to spider mites as well as the elicitor disseminated by the roots have not yet been studied. Elucidation of their identity will unravel whether the elicitor that is exuded from
the roots is the same as the systemic elicitor. (6) Information transfer between infested and uninfested plants through airborne chemicals may represent an eavesdropping of the uninfested plant on the information with which an infested plant attracts carnivorous enemies of herbivores. However, the question is raised as to why would a plant disseminate an elicitor of volatile production into the soil in response to above-ground herbivory? This is an important question to address. Does the elicitor evoke behavioural changes in soil organisms such as herbivores and their natural enemies? A plant whose leaves are attacked may be more susceptible to below-ground herbivores because it is generally weakened, or it may be a resource that will soon be gone as a result of overexploitation by the above-ground herbivores. Therefore, the information represented by the elicitor may be exploited by various soil-inhabiting organisms in a variety of ways, e.g. they may decide to search for another plant. (7) Finally, experiments under field conditions are needed in which all other communication between plants is excluded and in which communication through the soil is allowed or blocked. So far, experiments on chemicals from plants infested by below-ground herbivores have shown that these compounds affect the behaviour of herbivore-parasitic nematodes at distances of ca. 15 cm (Boff et al., 2001; Van Tol et al., 2001). This is a distance comparable to that at which airborne communication between sagebrush and tobacco can occur (Karban, 2001). Upon the application of labelled compounds to tree stumps, it has been recorded that chemicals can be detected in other trees at distances up to ca. 8 m. Whether this occurred through root exudates or through mycorrhizal connections is not clear (Rovira, 1969). Similar experiments were also done with corn and bean plants (Rovira, 1969). Potentially, mycorrhiza also transfer plant elicitors, similar to the transfer of carbon (Simard et al., 1997). If this was to be the case, the distances over which infested plants affect neighbouring plants could be much larger than when diffusion through the soil is the only mode of dispersal of the elicitor.

Our study provides exciting options for future research. The possibility that plants communicate below ground (see also Chamberlain et al., 2001) should stimulate further investigations into the effects of the emitted information on below-ground food webs, including effects on herbivores and pathogens, natural enemies and antagonists of plant-attacking organisms, as well as plants. That above- and below-ground interactions of plants with organisms at different trophic levels are likely to be connected provides important research questions that will enhance our understanding of the ecology and evolution of plant defences (Van der Putten et al., 2001).

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References


