SHORT COMMUNICATION

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Volatile interaction between undamaged plants affects tritrophic interactions through changed plant volatile emission

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Abbreviations: HIPVs, herbivore-induced plant volatiles; TMTT, (3*E*, 7E)-4, 8, 12-trimethyl-1, 3, 7, 11-tridecatetraene

Volatile interactions between unattacked plants can lead to changes in their volatile emissions. Exposure of potato plants to onion plant volatiles results in increased emission of 2 terpenoids, (*E*)-nerolidol and TMTT. We investigated whether this is detectable by the ladybird *Coccinella septempunctata*. The odor of onion-exposed potato was significantly more attractive to ladybirds than that of unexposed potato. Further, a synthetic blend mimicking the volatile profile of onion-exposed potato was more attractive than a blend mimicking that of unexposed potato. When presented individually, TMTT was attractive to ladybirds whereas (*E*)-nerolidol was repellent. Volatile exchange between unattacked plants and the consequent increased attractiveness for ladybirds may be a mechanism that contributes to the increased abundance of natural enemies in complex plant habitats.

Plants release volatile organic compounds in the course of their normal physiological activities. These volatiles may be received by neighboring plants, which makes plant–plant interaction via volatiles a continuous and dynamic process. Plant volatiles have an important role in mediating multi-trophic interactions; between plants, phytophagous insects, and herbivore natural enemies. The emission of volatiles from plants is significantly increased under stress conditions, caused by abiotic,[1,2] or biotic factors.[3,4] These volatile chemicals released by plants are available as signals for neighboring plants. For example, volatiles from damaged plants induce responses in neighboring undamaged plants, changing their volatile emission[5] and making them less attractive to herbivores[6] and more attractive to herbivore natural enemies.[7] However, it has been shown that volatile interaction between unattacked plants[8] can also occur, reducing attractiveness of the receiving plants to insect herbivores.[9-11] Further, volatile interaction between unattacked plants can also lead to attraction of predatory insects, despite the absence of prey feeding on the plants.[8,12,13]

Increasing diversity of plant species, or even the presence of different genotypes of the same plant species within an environment, has an impact on the abundance of phytophagous insects and their natural enemies.[9,13-15] Despite numerous studies on these effects, knowledge of the underlying mechanisms is still limited. Recently we have shown that volatile interaction between unattacked plants can significantly change the volatile profile of potato plants after exposure to volatile chemicals from onion, making them less attractive for the aphid *Myzus persicae*.[16] In the field, it was found that migration of *M. persicae* into an intercrop, where onion plants were grown alongside the potato, was significantly reduced compared to potato grown in pure stand. The study found around 4 times greater concentrations of the terpenoids (*E*)-nerolidol and TMTT in the headspace of potato plants previously exposed to volatiles from onion compared with the headspace of unexposed plants. This showed for the first time that volatile chemical exchange between unattacked plants can cause responding plants to change their volatile profiles. In subsequent laboratory experiments, a synthetic volatile blend mimicking the headspace of potato plants previously exposed to onion was significantly less attractive to *M. persicae* than a blend based on headspace of unexposed potato, and (*E*)-nerolidol and TMTT repelled aphids when tested individually, showing similar behavioral responses to the odor of living plants.

If volatile interaction between unattacked plants leads to reduced numbers of herbivores, could such communication contribute to understanding of the mechanisms underlying the increased abundance of their natural enemies in botanically diverse habitats? A number of studies have examined the effects of HIPVs on the behavior and effectiveness of natural

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enemies including parasitic wasps,[17-19] predatory mites,[4,20,21] and ladybirds.[22-25] In nature, however, not every plant is attacked by herbivores, particularly not in species rich habitats where the number of phytophagous insects is reduced, and few studies have examined the effects of volatile interaction between undamaged plants on the behavior of herbivore natural enemies.[8,12,13]

In the present study we investigated whether volatile interaction between unattacked onion and potato had an effect on the 7-spot ladybird *Coccinella septempunctata*, an important natural enemy of aphids. Plants were grown in a greenhouse maintained at 18–22 °C with a light regime of L16:D8. Ladybirds were fed on different aphid species and pollen under same conditions as the test-plants. Olfactory responses of ladybirds were measured using a 2-way airflow olfactometer.[8] When plants were used as an odor source, 5 different treatment arrangements were designed: a potato plant that had been previously exposed to an onion plant compared with an unexposed potato plant; an odor mixture of potato and onion compared with an odor mixture of 2 potato plants; an unexposed potato plant compared with an onion plant; an unexposed potato plant compared with soil without a plant; an onion plant compared with soil without a plant. Exposures were made in a series of "2-chamber cages."[16]

To investigate ladybird olfactory response to the synthetic chemical blends that mimicked the volatile profiles of onion-exposed and unexposed potatoes, and also their behavioral activity to the compounds (*E*)-nerolidol and TMTT, we conducted dose-response olfactometer experiments based on previous odor collections from the plants.[16] Ladybird response to the synthetic blend of exposed potato plants was tested against the synthetic blend of unexposed potato plants. Test concentrations were 1/100, 1/10, 1x, 10x, and 100x the concentration of volatiles collected from plants. The chemicals were tested against redistilled n-hexane in 5 different concentrations: 0.01ng, 0.1ng, 1ng, 10ng, and 100ng. Wilcoxon matched pairs test was used for comparisons of the number of ladybird visits in each olfactometer arm.[26]

We found that potato exposed to volatiles from onion was more attractive to ladybirds than unexposed potato ($Z = 2.19$, $P = 0.03$, $n = 16$) (**Fig. 1**). Further, a synthetic chemical blend that mimicked the volatile profile of onion-exposed potato was significantly more attractive for ladybirds than a synthetic blend mimicking unexposed potato ($Z = 2.2$, $P = 0.03$, $n = 22$) at the highest concentration tested (**Fig. 2**). It has been shown previously that *M. persicae* behaved in the opposite way; odor of exposed potato was less attractive than unexposed potato, and a synthetic blend based on exposed potato headspace was repellent at the highest concentration tested.[16] It is interesting that these relatively minor changes in the volatile profile are detectable by 2 polyphagous insects such as *M. persicae* and *C. septempunctata*.

Ladybirds in the present study were strongly attracted by the mixture of potato and onion compared with potato alone ($Z = 2.16$, $P = 0.03$, $n = 14$) (**Fig. 1**). Responses to odor mixtures have been shown previously in this ladybird. In laboratory and field experiments with different varieties of barley, ladybirds preferred a specific combination of barley varieties over single varieties alone,[12,13] and also responded positively to mixtures of barley and weeds, both in the field and with odors in the laboratory,[8] suggesting a preference for foraging in species-rich habitats.

In the present study, ladybirds did not prefer odor of potato or onion over soil alone, suggesting that volatiles of the single healthy plants were not attractive for them. However, ladybirds did prefer onion when given a choice between odor of onion and potato ($Z = 2.09$, $P = 0.04$, $n = 20$) (**Fig. 1**). Plant volatiles absorbed on neighboring plant surfaces can considerably change the volatile profile of the exposed plant with their re-emission.[27] In our previous study, chemical analysis of the plant headspace showed that the 2 terpenoids released in higher amounts by onion-exposed potato were not detectable in the headspace of onion itself,[16] suggesting the insect responses were not affected by absorption and re-release of onion volatiles from the surface of potato plants.

We tested the olfactory responses of ladybirds to (*E*)-nerolidol and TMTT, terpenoids emitted in greater concentrations by potato after exposure to volatiles from onion.[16] A concentration of 10ng/µl TMTT (dosed at volume of 10µl on small piece of filter paper) was significantly more attractive for ladybirds than the control (n-hexane) ($Z = 2.37$, $P = 0.02$, $n = 18$) (**Fig. 3**). Interestingly, a concentration of 1 ng/µl (*E*)-nerolidol was repellent to ladybirds ($Z = 2.16$, $P = 0.03$, $n = 15$) (**Fig. 4**). (*E*)-nerolidol is known to repel aphids[16,28] and to attract predatory mites,[29] but had no influence on the behavior of the hover fly *Episyrphus balteatus* whose larvae are natural enemies of aphids.[28] Individual HIPV components can increase predator attraction both independently and when individually enhanced within plant blends.[7]

The effects of TMTT and (*E*)-nerolidol as individual components or in blends have been studied mainly in predatory mites.[20,29] *Coccinella septempunctata* is an important predator of aphids, strongly attracted by volatiles of attacked plants, but knowledge of the role of these HIPV components on their behavior, alone or in blends, is lacking. It has been previously shown that ladybirds are attracted by changes in volatile profiles resulting from chemical interaction between unattacked barley plants[8,12,13] and the present study supports these findings and gives a potential explanation of the phenomenon. Our results show that *C. septempunctata* is attracted by the synthetic blend of onion-exposed potato, and by TMTT, one of the components of the blend.[16] Although (*E*)-nerolidol is repellent for ladybirds when presented alone, it was present in higher amounts in the blend released by onion-exposed potato, which was attractive. This supports the idea that some volatiles have different effects on insect behavior when encountered alone or together with other compounds in blends.[30]

Ladybirds were attracted to potato plants exposed to onion neighbor volatiles, whereas aphids were repelled.[16] It may seem non-adaptive for ladybirds to be attracted to a plant that had reduced attraction to aphids. However, ladybird immigration into crop fields is not always correlated with aphid abundance.[31] The ladybird is polyphagous and may benefit from locating habitats with increased plant diversity, and thus increased prey diversity. Further, a previous study has shown that while plant–plant volatile exchange can reduce aphid numbers on a plant, ladybirds

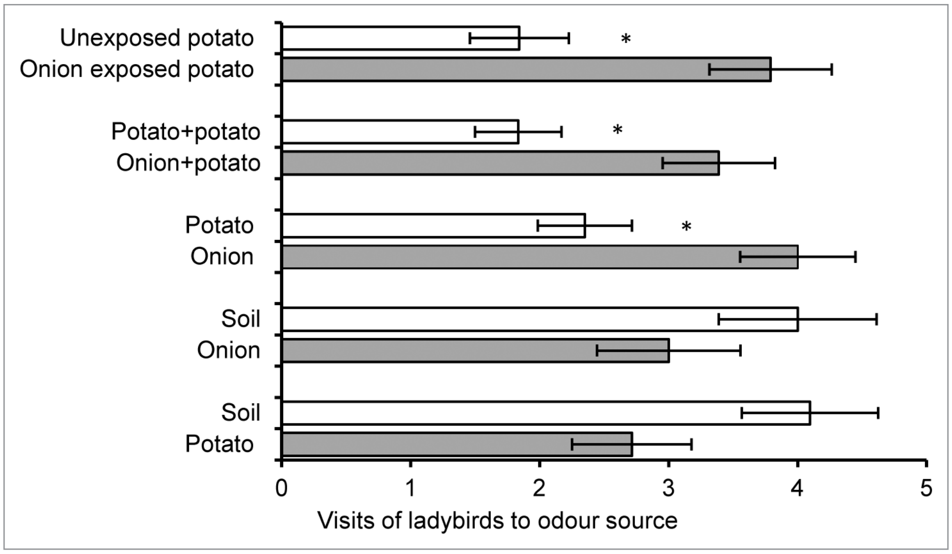

Figure 1. Ladybird olfactory responses to volatiles from plants. Error bars indicate ± SEM. Asterisks indicate statistical significance levels of * $P \le 0.05$ (Wilcoxon matched pairs test).

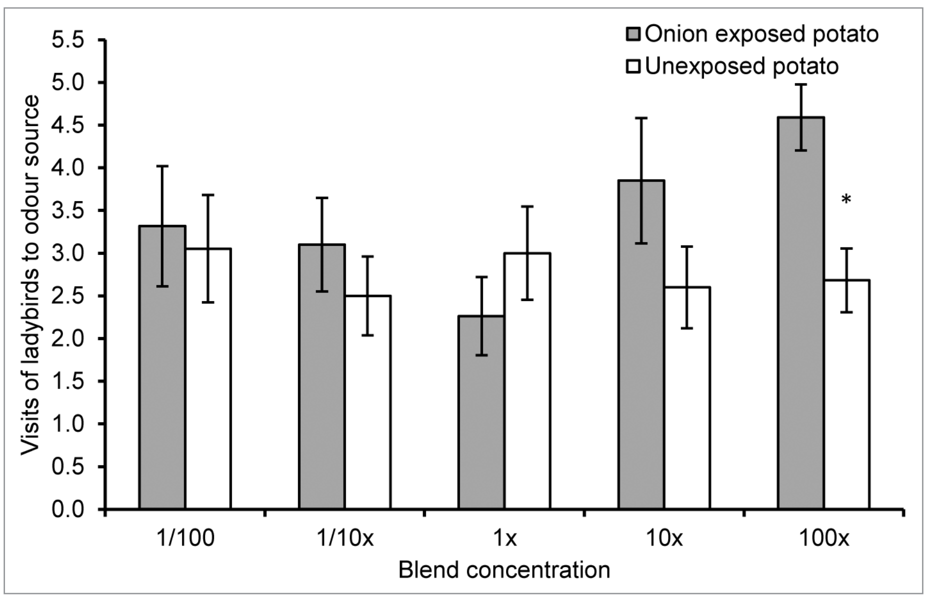

Figure 2. Ladybird olfactory responses to synthetic blends of volatile organic compounds of potato plants that had been previously exposed (treatment) and unexposed (control) to onion plants. Synthetic blends were at 1/100, 1/10, 1, 10, and 100 times the original concentration of volatiles identified in potato headspace. Error bars indicate ± SEM. Asterisks indicate statistical significance levels of * $P \le 0.05$ (Wilcoxon matched pairs test).

consume more aphids on exposed than on unexposed plants.[12] Thus the adaptive significance of predator responses to chemical interaction between undamaged plants is likely to depend on several factors and this type of plant interaction might be an underlying mechanism in plant diversity systems, effecting not only phytophagous insects but also their natural enemies.

A number of studies have shown that complex plant habitats can decrease the occurrence of phytophagous insects and increase that of their natural enemies.[14] Studies of tri-trophic interactions usually address the importance of plant chemical compounds in regulating insect herbivore species richness and the abundance of natural enemies. The present study provides additional evidence that even communication between healthy plants has a strong impact on predatory insects, and suggests that volatile interaction between undamaged plants leading to changes in volatile profiles may be a mechanism contributing to these observations. These findings may have practical importance for the development of habitat manipulation strategies, e.g., intercropping, that reduce insect pests and increase the abundance of their natural enemies.

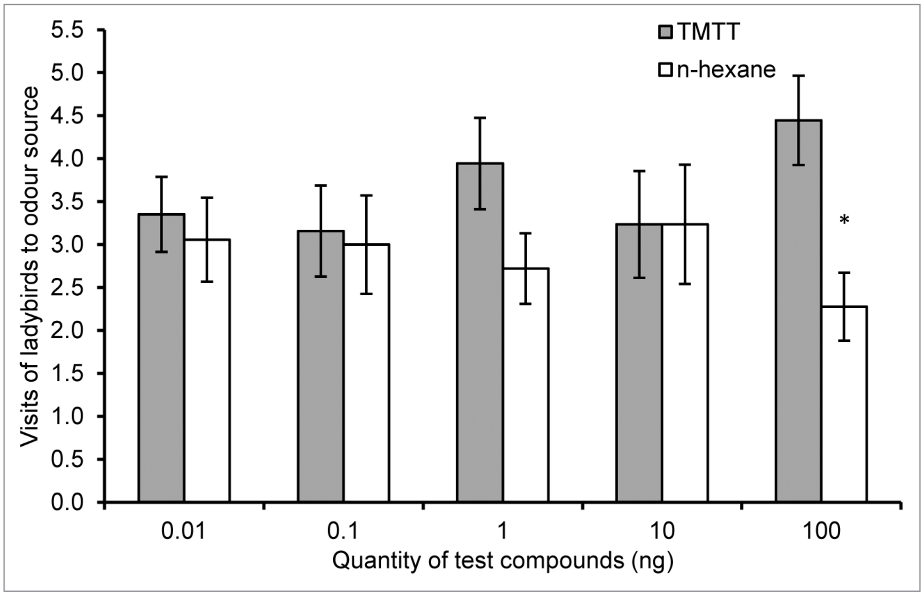

Figure 3. Ladybird olfactory responses to (3E, 7E) 4, 8, 12-trimethyl-1, 3, 7, 11-tridecatetraene (TMTT), a terpenoid released in higher amounts from potato plants after exposure to volatiles from onion plants, vs. n-hexane controls. Error bars indicate ± SEM. Asterisks indicate statistical significance levels of * $P \leq 0.05$ (Wilcoxon matched pairs test).

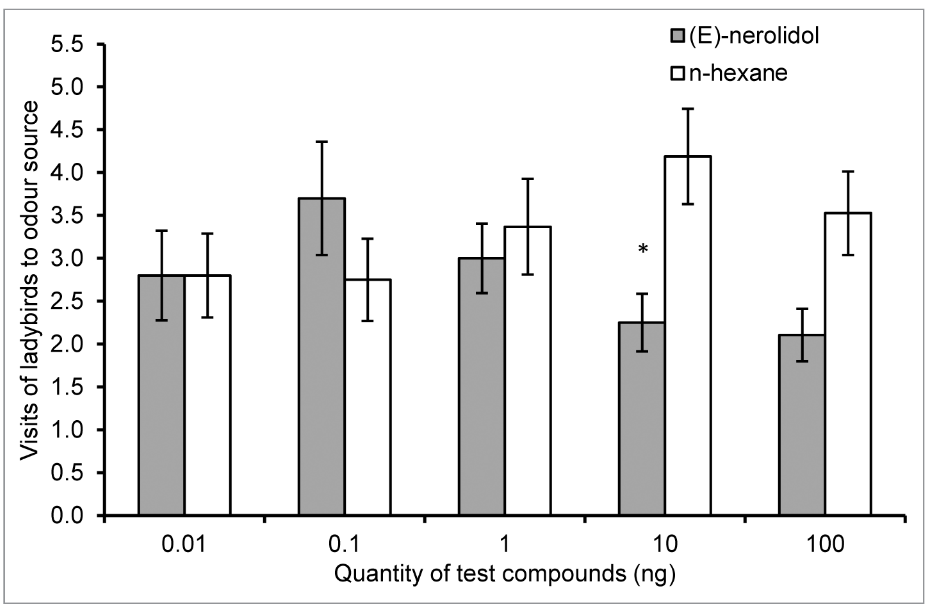

Figure 4. Ladybird olfactory responses to (*E*)-nerolidol, a terpenoid released in higher amounts from potato plants after exposure to volatiles from onion plants, vs. n-hexane controls. Error bars indicate ± SEM. Asterisks indicate statistical significance levels of * $P \leq 0.05$ (Wilcoxon matched pairs test).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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