Two arrays of defense strategies of Brassicaceae plants that eavesdrop on mint volatiles

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ABSTRACT

Brassica rapa can eavesdrop on volatile compounds emitted from peppermint and consequently boost its defense responses against Plutella xylostella. In the current study, to assess how B. rapa receiver plants cultivated near peppermint plants resist P. xylostella, two biological assays were conducted. The data showed that the receiver plants cultivated with peppermint exhibited lower weight gain of P. xylostella larvae, compared to that showed by the recipient plants. Moreover, a lower rate of oviposition of adult female P. xylostella was observed on B. rapa plants cultivated with peppermint plants compared to the rate on B. rapa plants cultivated with glass-enclosed peppermint plants, although receiver B. rapa plants that had been previously incubated with peppermint for 7 days and non-receiver B. rapa plants exhibited similar rates of oviposition. Taking all these findings together, we conclude that cultivation of B. rapa receiver plants with peppermint results in two arrays of defense strategies.

Introduction

Plants are able to eavesdrop on volatile organic compounds (VOCs) emitted from infested leaves to adjust their defenses (Arimura and Pearse 2017). For instance, undamaged lima bean plants can respond to VOCs transmitted from the specific nearby plants infested with spider mites by induced expression of defense genes (Arimura et al. 2000) and primed secretion of extracellular nectar, attractant cues for predatory arthropods such as ants (Heil and Silva Bueno 2007). Such plant-plant signalings have since been widely observed in many plant taxa, including trees (e.g. willow, sugar maple, poplar, and sagebrush) and grasses (e.g. cotton, maize, tobacco, and Arabidopsis) (Heil and Karban 2010). Key insights from numerous previous studies are concerned with what aspects of such eavesdropping 1) are kin-specific (Ishizaki et al. 2012; Karban et al. 2013), 2) are memorized for defense properties (Ali et al. 2013), 3) are mediated by phytohormone (jasmonate, ethylene, or salicylate) signaling and epigenetic regulation in the receiver plant cells (Engelberth et al. 2004; Ali et al. 2013), and 4) are applicable for agriculture by controlling pests and increasing production (Arimura and Pearse 2017). Accordingly, most research has especially explored whether VOCs from the damaged plants contribute to alerting undamaged neighboring plants about a threat of herbivore attack, then leading to increased fitness of the neighboring plants as a result.

It has recently been demonstrated that soybean or Brassica rapa plants cultivated near candy mint and peppermint, respectively, show induced expression of defense genes in their leaves, which leads to increased resistance towards herbivore pests in the laboratory, field, and greenhouse (Sukegawa et al. 2018). It therefore appears that undamaged crop plants can even eavesdrop on VOCs that are constitutively emitted by aromatic mint plants as a cue to elicit or prime defense. However, it remains to be elucidated how mint VOCs work to enhance defense traits in the neighboring plants against herbivores in the open greenhouse. Regarding this, we hypothesized that multiple factors, including defense responses in the neighboring, eavesdropping plants as well as direct effects of mint VOCs, for instance, that may act in the repellence of herbivores, work for the total pest control in the greenhouse. We therefore highlighted bitrophic interactions of B. rapa, crop plants planted near peppermint, with Plutella xylostella, a major herbivore pest of B. rapa. To understand this in detail, two arrays of performance assays for P. xylostella (i.e. the repellence of adult females’ oviposition and inhibition of growth of larvae on B. rapa), were carried out. Taken all together, our data show that B. rapa receiver plants are protected from P. xylostella by mint VOCs via two arrays of defense strategies.

The phenomena we have investigated here are not known to have any ecological significance. Mint plants emit a volatile profile distinct from that of Brassica plants, and the peppermint VOCs that the receiver plants seem to respond to are not representative of the herbivore-induced VOC profile of B. rapa. It should therefore be emphasized that the scope of this study was not to explore the ecological consequences but rather possible applications of mint as a companion plant for agriculture and horticulture.

Materials and methods

Plants and insects

B. rapa var. perviridis (Natsurakuten) plants were germinated and grown in soil for 14 days in a climate-controlled room at 24 ± 1°C with a photoperiod of 16 h (80 µE m⁻² s⁻¹). The light period was from 07:00–23:00. Peppermint (Mentha x piperita L.) cultivars were obtained from gardening shops and culti-
vated in the above conditions. Potted peppermint plants whose shoots were about 30 cm long were used for all the assays. In order to avoid airborne contamination, all of the Brassicaceae plants were cultivated at least 5 m away from mint plants when the plants were cultivated in the same room.

*P. xylostella* was transferred to our laboratory in 2018 from Tsukuba University. The insects were reared on *B. rapa* plants in the laboratory at 24 ± 1°C with a photoperiod of 16 h.

**Preparation of receiver *B. rapa* plants**

Five potted *B. rapa* plants were cultivated proximately (10 cm) around a potted peppermint plant in a climate-controlled room (see above) for 7 days. *B. rapa* plants cultivated without mint plants (4–5 m away from mint plants) in the same conditions served as non-receiver controls.

**Growth assays for larvae**

Third-instar *P. xylostella* larvae were weighed, and a larva with weight ranging from 1.3–1.4 mg was reared on the shoot of a potted receiver or a non-receiver control plant in a climate-controlled room (see above) for 4 days. *B. rapa* receiver plants from each of two independent sets of receiver and non-receiver plants were used.

**Oviposition assays for adult females**

Five adult females of *P. xylostella* were released in an acrylic box (60 cm x 60 cm x 60 cm) in which two *B. rapa* plants were set about 10 cm apart from a peppermint plant and two *B. rapa* plants were set about 10 cm apart from a peppermint plant covered with a glass container (2.5 L, 10 cm diameter, 30 cm deep). Another pair of two *B. rapa* receiver plants that had been cultivated without or proximately with peppermint for 7 days (see above) were set 50 cm away inside the acrylic box. After incubation in a climate-controlled room (see above) for 24 h, the number of eggs was counted under a microscope. Likewise, oviposition assays were performed on *B. rapa* receiver plants and non-receiver plants. Five independent assays were repeated for each condition tested.

**Statistical analyses**

The data were analyzed based on a Mann–Whitney U-test (Figure 1) and a generalized linear model (GLM) with Poisson distribution (Figure 2) using R version 3.4.2 with lme4 and multcomp packages (R Core Team 2017).

**Results**

To assess the defense property of receiver *B. rapa* plants that had been cultivated proximately with peppermint for 7 days, we determined the weight gain of *P. xylostella* larvae on the receiver plants. The receiver plants exhibited lower weight gain of *P. xylostella* larvae after 4 days, compared to that exhibited by non-receiver plants (Figure 1).

Next, we assessed the oviposition of adult females of *P. xylostella* on *B. rapa* plants placed proximately with peppermint plants, and found a significantly lower rate of oviposition on these *B. rapa* plants in comparison to the rate on *B. rapa* plants which was placed proximately with peppermint plant covered with a glass container to interrupt airborne interaction via mint volatiles (Figure 2A). The question then arose of whether the decrease in oviposition on *B. rapa* plants placed proximately with peppermint plants was caused by repellent and/or insecticidal activities of mint volatiles (Pohlit et al. 2011; Wubie et al. 2014) or by elevated defense properties of *B. rapa* receiver plants that had been previously incubated with peppermint for 7 days. We found that in this case, the adult females of *P. xylostella* did not show significantly lower oviposition performance on the receiver plants in comparison to non-receiver plants (Figure 2B).

**Discussion**

*B. rapa* plants cultivated with peppermint show induced expression of defense genes, and consequently these plants show less damage by natural herbivorous insects, including *P. xylostella*, in an open greenhouse (Sukegawa et al. 2018). Here we demonstrated that this decrease of pest-induced damage is at least partially due to elevated defense properties of the receiver plants, as indicated by a detrimental effect on larval growth on the receiver after the peppermint cultivated as the companion plant was removed (Figure 1). In contrast, the ovipositional repellence of adult *P. xylostella* females on *B. rapa* host plants was observed only when mint VOCs were present (Figure 2), indicating that peppermint VOCs but not receiver plants have an ability to be repellent against the herbivore. This is in accord with the fact that mint essential oils are repellent and insecticidal for herbivores, including whitely and cabbage aphids (Pohlit et al. 2011), as described above.

We have recently shown that candy mint volatiles are even attractive to carnivorous mites, *Phytoseiulus persimilis*, when compared with volatiles not only from undamaged *Phaseolus vulgaris* plants but also from *P. vulgaris* plants damaged by spider mites (Togashi et al. 2019). Taking all these findings...
together, we suggest that the use of mint plants as companion plants is an ideal platform for pest management synergistically through plant-plant signaling, repellence of herbivores, and the attraction of predatory mites, based on the mints’ multi-functions in the ecosystem.

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