

Induction of systemic acquired resistance in *Zea mays* also enhances the plant's attractiveness to parasitoids

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Abstract

Plants under attack by caterpillars emit volatile compounds that attract the herbivore's natural enemies. In maize, the caterpillar-induced production of volatiles involves the phytohormone jasmonic acid (JA). In contrast, pathogen attack usually up-regulates the salicylic acid (SA)-pathway and results in systemic acquired resistance (SAR) against plant diseases. Activation of the SA-pathway has often been found to repress JA-dependent direct defenses, but little is known about the effects of SAR induction on indirect defenses such as volatile emission and parasitoid attraction. We examined if induction of SAR in maize, by chemical elicitation with the SA-mimic benzo-(1,2,3)-thiadiazole-7-carbothioic acid *S*-methyl ester (BTH), attenuates the emission of volatiles induced by *Spodoptera littoralis* or exogenously applied JA. In addition, we determined how these treatments affected the attractiveness of the plants to the parasitoid *Microplitis rufiventris* in a six-arm-olfactometer. BTH treatment alone resulted in significant systemic resistance of maize seedlings against the pathogen *Setosphaeria turcica*, but had no detectable effect on volatile emissions. Induction of SAR significantly reduced the emission rates of two compounds (indole and (*E*)- β -caryophyllene) in JA-treated plants, whereas no such negative cross-talk was found in caterpillar-damaged plants. Surprisingly, however, BTH treatment prior to caterpillar-feeding made the plants far more attractive to the parasitoid than plants that were only damaged by the herbivore. Control experiments showed that this response was due to plant-mediated effects rather than attractiveness of BTH itself. We conclude that in the studied system, plant protection by SAR activation is compatible with and can even enhance indirect defense against herbivores.

Keywords: BTH; Cross-talk; Jasmonic acid; *Microplitis rufiventris*; Salicylic acid; *Spodoptera littoralis*; Tritrophic interactions; Volatiles

1. Introduction

Many plants release volatile compounds as a response to feeding or egg-laying by insects (reviewed in Hilker et al., 2002; Turlings et al., 2002; Dicke et al., 2003). Parasitoids and predatory arthropods are highly attracted to these plants since the emitted compounds may serve as long-range cues enabling the location of host or prey. Mechanical disruption of plant tissue by an insect instantly leads to

the emission of so-called "green-leaf volatiles" (C6 aldehydes, alcohols, and derivatives). In addition, herbivore-derived elicitors may enhance the *de novo* synthesis and systemic release of further synomones such as terpenoids, phenolic, and nitrogenous compounds (Mattiacci et al., 1995; Alborn et al., 1997, 2007; Tumlinson and Lait, 2005; Schmelz et al., 2006). The phytohormone jasmonic acid (JA) acts as a key molecule in the regulation of herbivore-induced signals (de Vos et al., 2005) and is involved in the transcription of volatile-encoding genes (Schmelz et al., 2003a; Ament et al., 2004; Gomez et al., 2005). Other plant hormones like salicylic acid (SA), ethylene, and the JA precursor 12-oxo-phytodienoic acid (OPDA) may modulate the JA-pathway (van Poecke and Dicke, 2004). Plants under attack can benefit from attracting parasitoids (van

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Loon et al., 2000; Hoballah and Turlings, 2001). Therefore, herbivore-induced volatile emission is considered to be an indirect induced plant defense (Dicke et al., 2003).

Parasitoids are important biological control agents against herbivorous insects (Stacey, 2003). Another environmental friendly control method that is used to counter pathogens is the activation of a plant's own resistance mechanisms by treating crops with synthetic non-toxic stimulants (U.S. Environmental Protection Agency;¹ Oostendorp et al., 2001). The induction of systemic acquired resistance (SAR) with benzo-(1,2,3)-thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) is a well studied example. This compound mimics to a large extent the action of the signaling molecule SA that is involved in the expression of a high number of defense genes against microbes (Morris et al., 1998; von Rad et al., 2005). However, in the field, pathogens and herbivores often attack an individual plant simultaneously or in sequence (Hatcher, 1995; Rostás et al., 2003; Stout et al., 2006). Therefore, protecting crops against diseases by using BTH could lead to a potential conflict, as both the BTH-activated SA-pathway and the JA-pathway, which is important for the defense against herbivores, are not distinct but interconnected (reviews: Heil and Bostock, 2002; Taylor et al., 2004; Bostock, 2005; Beckers and Spoel, 2006). At the molecular level, induction of the SA-pathway can suppress the synthesis of JA and thereby inhibit the expression of many JA-dependent defense genes and the production of defense compounds like proteinase inhibitors and polyphenol oxidase (Doares et al., 1995; Thaler et al., 2002a; Salzman et al., 2005). Cross-talk between both signaling pathways may lead to leaf-chewing herbivores performing better on plants that cannot mount their defenses against insects to full strength (Thaler, 2002; Thaler et al., 2002a; Cipollini et al., 2004). However, this antagonism between pathways depends on the specific plant–insect combination as investigated in cotton, maize and *Arabidopsis thaliana*, where SAR-induced by SA or BTH had no or little effect on the herbivorous insects that were studied (Bi et al., 1997; Inbar et al., 2001; van Poecke and Dicke, 2002; Danielson, 2003; Plymale et al., 2007).

So far, research on cross-talk between the SA- and JA-pathway has focused strongly on direct defenses. Little is known about the effects of inducing SA-dependent responses on JA-regulated volatile emission and consequently on the attraction of natural enemies (Dicke and Bruin, 2001). Induction of the SA-pathway alone does not usually lead to volatile emission in plants (Ozawa et al., 2000; Turlings et al., 2002; van Poecke and Dicke, 2002). An exception was found in Lima bean when treated with methyl salicylate (MeSA) in gaseous form instead of using an aqueous solution. This treatment results in the emission of MeSA, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT), which resembles the volatile blend induced by

spider mites but not by *Spodoptera exigua* caterpillars (Ozawa et al., 2000). Involvement of SA (but not cross-talk) in indirect defenses is also suggested from experiments with *Arabidopsis*. van Poecke (2002) found that *NahG*, a transgenic *Arabidopsis* that cannot accumulate SA, does not emit MeSA and the homoterpene TMTT upon volatile induction by caterpillars, which makes it less attractive to the parasitoid *Cotesia rubecula*. These findings contradict the hypothesis that SA inhibits JA-induced volatiles, since *NahG* plants should have increased JA levels. However, pleiotropic effects cannot be ruled out (van Poecke, 2002).

The induced indirect defence mechanism has been extensively investigated in the tritrophic system that comprises *Zea mays*, *Spodoptera* spp., and several of their larval endoparasitoids (e.g. Turlings et al., 1990; Turlings and Wäckers, 2004). In maize, a number of abiotic and biotic factors can modulate the quality and quantity of the herbivore-induced volatile blend, which in certain cases may have consequences for parasitoid attraction (Hoballah et al., 2002; Degen et al., 2004; Gouinguéné and Turlings, 2002). Recently, we found that infection by the leaf pathogen *Setosphaeria turcica* reduces the emission of herbivore-induced volatiles (Rostás et al., 2006). Fungal infection also resulted in SA accumulation in the plant tissue (Rostás, unpublished data) and disease symptoms could be suppressed by exogenous SA treatment of maize leaves (Yu et al., 1999). In the light of these findings it is tempting to speculate that reduced volatile emission that we observed in maize was due to negative cross-talk between the SA- and JA-pathways.

Following up on these observations, we here address the question whether chemical induction of SAR compromises the indirect defense of maize plants. We hypothesized that BTH application reduces the emission of induced volatiles due to cross-talk. Parasitoids may respond to compounds at concentrations below the level of analytical detection (Gouinguéné et al., 2005; D'Alessandro and Turlings, 2005). Therefore, we also performed a series of olfactometer experiments to test the effect of BTH pretreatment on the host location behavior of the endoparasitoid *Microplitis rufiventris*.

2. Materials and methods

2.1. General

Maize plants (*Zea mays* var. Delprim) were grown in polypropylene pots (*h*: 11 cm, \emptyset : 4 cm) containing commercial soil mix (Coop, Basel) in a climate chamber (23 °C, 60% r.h., and LD 16:8 h, 550 $\mu\text{mol}/\text{m}^2/\text{s}$).

Eggs of *Spodoptera littoralis* Bois. (Lepidoptera, Noctuidae) were supplied by Syngenta (Stein, Switzerland). Newly hatched larvae were reared in transparent plastic boxes on a wheatgerm-based artificial diet until used in the experiments. A colony of the solitary larval endoparasitoid *Microplitis rufiventris* Kok. (Hymenoptera: Braconidae) was maintained in the laboratory. For the rearing, 25 *S. littoralis* caterpillars (3–4 days) were offered to a single mated

¹ <http://www.epa.gov/opprd001/factsheets/acibenzolar.pdf>.

female (4–7 days) for 3 h in a plastic box (h : 5 cm, \varnothing : 9.5 cm). The parasitized caterpillars were kept in an incubator (25 °C, LD 16:8 h) until the parasitoids formed cocoons. The cocoons were kept in Petri dishes until adult emergence. Emerging adults were sexed and kept in plastic cages (30 × 30 × 30 cm, Bugdorm I, MegaView Ltd, Taiwan) under ambient laboratory conditions. Cages were supplied with moist cotton wool and droplets of honey.

The necrotrophic fungus *Setosphaeria turcica* (Luttrell) Leonard & Suggs (anamorph: *Exserohilum turcicum*, Dotyaceae), commonly known as Northern corn leaf blight, was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany) and cultivated on V8-Agar in darkness at laboratory conditions.

BTH (BION™) was obtained from Syngenta, Switzerland as a water-dispersible granular formulation containing 50% active ingredient.

2.2. Effect of BTH on fungal infection

Experiments were conducted to assess whether BTH, taken up systemically via the roots, would induce resistance in the leaves. We treated maize seedlings (10-days old) with 5 mM BTH dissolved in water as a soil drench. BTH application was carried out between 4 p.m. and 5 p.m. Control plants were treated with water. Forty-eight hours later all plants were infected with the fungus *S. turcica*. This time point was chosen because Morris et al. (1998) showed that in maize the strongest expression of the SA-dependent defense genes PR-1 and PR-5 was between 2 and 3 days after BTH application. Spores of *S. turcica* were harvested prior to plant inoculations. A Petri dish culture was flooded with 5 ml 0.05% aq. Tween-20 and then brushed gently with a small paintbrush in order to detach the spores from the mycelium. The density of the spore suspension was determined with an improved Neubauer chamber and adjusted to 6×10^4 spores/ml. Maize seedlings were inoculated by applying 100 μ l spore suspension to the second and third leaf, respectively. The spores were then spread homogeneously using a paintbrush. Control plants were mock-inoculated in the same manner with 0.05% aq. Tween-20. All seedlings were placed in two coolers with wet tissue papers laid out on the bottom. The plants were then kept in darkness for 16 h (5 p.m.–9 a.m.) at >90% r.h. and ambient temperatures. The following morning all plants were transferred to a climate chamber (28 °C, 60% r.h., and LD 16:8 h, 550 μ mol/m²/s). Disease symptoms were allowed to develop for 72 h. The strength of infection was calculated by scanning the diseased leaves and measuring the necrotized areas with the software Photoshop 7.0 (Adobe) and Surface (C. Thiemann, Berlin, Germany[©]).

2.3. Effect of BTH on herbivore leaf consumption

Potted maize plants (10-days old) were treated with BTH (5 mM soil drench) or water as control. Twenty-four

hours later a piece of the second-oldest leaf (5 cm) was cut off and placed into a small Petri dish (\varnothing : 55 cm) which was lined with moist filter paper. A single larva (L_2) of *S. litto-ralis* was placed into the Petri dish and left to feed for 24 h. The Petri dishes were sealed with Parafilm™ to prevent desiccation of the leaf. The consumed leaf area was calculated by scanning the leaves and measuring the missing areas with the software Photoshop 7.0 (Adobe) and Surface (C. Thiemann, Berlin, Germany[©]).

2.4. Volatile collections and chemical analyses

Volatiles from the headspace of maize seedlings were collected with a six-arm-olfactometer, a device which we used for simultaneous odor collection and testing of parasitoid host location behavior (described by Turlings et al., 2004).

In a first experiment we analyzed the headspace volatiles of plants that had received the following treatments: (i) BTH, (ii) water, (iii) BTH + herbivory, and (iv) water + herbivory. BTH was always applied as a 5 mM soil drench 24 h before herbivore treatment commenced. Single maize plants, which were kept in their pots, were placed into one of the six odor source vessels of the olfactometer. Ten caterpillars (L_2) were placed into the whorl of each seedling between 4 p.m. and 5 p.m. Volatile collections started between 9 a.m. and 10 a.m. the next day.

A second experiment was conducted to investigate the effect of BTH treatment specifically on the emission of JA-induced volatiles. BTH pretreatments were carried out as described for the first experiment. Instead of using herbivores, volatiles were induced by JA application 24 h after BTH treatment (between 4 p.m. and 5 p.m.) after scratching the abaxial side of the two oldest leaves (2 mm²) with a scalpel but without damaging the midrib. A droplet of 5 μ l JA (10 mM) was applied to each wound. The procedure (damage and JA application) was repeated the following morning between 9 a.m. and 10 a.m. on the same leaves shortly before volatile collections started.

Trapping filters were attached to each vessel consisting of glass tubes (7 cm) containing 25 mg of 80–100 mesh Super Q adsorbent (Alltech, Deerfield, Illinois, USA) that was kept in place by two fine mesh metal screens. Filtered and humidified air was pushed into the odor source vessels at a rate of 1.2 l/min/vessel originating from a central in-house compressor. Half of the air flow (0.6 l/min) was pulled through the trapping filter with a vacuum pump (ME2, Vacuubrand, Wertheim, Germany), while the other half of the incoming flow was allocated to the olfactometer choice chamber (for details see Turlings et al., 2004). Before each experiment, the traps were rinsed with 1 ml methylene chloride. Collections lasted 3 h after which the traps were removed, extracted and analyzed.

The volatile traps were eluted with 150 μ l methylene chloride after each collection and two internal standards (*n*-octane and nonyl acetate, each 200 ng in 10 μ l methylene chloride) were added to these samples. Aliquots (3 μ l) of

the samples were analyzed by gas chromatography/mass spectrometry and by GC-FID (GC: HP 6890N, MSD: Agilent 5973) equipped with a split/splitless injector and a HP-1 ms column (30 m × 0.25 mm ID, 0.25 μm film thickness). Samples were injected in pulsed splitless mode. The oven was held at 40 °C for 3 min and then programmed at 8 °C/min to 230 °C, where it was maintained for 9.5 min. Helium (24 cm/s) was used as carrier gas. Compound identities were confirmed by comparison with mass spectra of the NIST and Wiley libraries and mass spectra of commercially available standards. Quantification was based on comparisons with the internal standards. Only those compounds were quantified that were consistently found in each sample of the same treatment. The evaluated compounds comprised >90% of the total amount of the analysed volatile blends.

2.5. Effect of BTH on the host location behavior of *M. rufiventris*

A series of experiments using a six-arm-olfactometer was conducted to evaluate whether BTH application to maize seedlings had negative effects on the host location behavior of the parasitoid. In all experiments, 2–4-days-old mated female wasps were used. The parasitoids were naïve, which means that they had no contact with host insects or maize seedlings during their adult stage prior to the assays. Six wasps were removed from their cage with an aspirator and released into the central choice chamber of the olfactometer. Previous experiments had shown that female wasps do not interfere with each other in their choices (Turlings et al., 2004). The wasps initially walked up to the top, attracted by the light above the choice chamber. If an attractive odor was present, most wasps would walk into the arm from which the attractive odor emanated until their path was blocked by a stainless steel screen. Eventually, they walked up into a glass trapping bulb, where they could easily be counted and removed. Each group of insects was given 30 min to make a choice, after which they all were removed and a new group was released. Five groups of six wasps were tested on a given day. Each olfactometer experiment was replicated on a minimum of 6 days, each time with a new set of odor sources and new wasps. The position of the odor source was changed clock-wise after each day of testing to avoid position effects.

2.5.1. Experiment 1

We tested whether the odor of soil drenched with BTH would directly affect the behavior of *M. rufiventris*. Two polypropylene pots were filled with soil. One pot was drenched with 25 ml BTH (5 mM), while the other was treated with the same amount of water. The pots were left standing for 2 days after which each one was placed into an odor source vessel of the olfactometer. The remaining four vessels of the six-arm-olfactometer remained empty.

2.5.2. Experiment 2

We tested whether BTH application had plant-mediated effects on the wasp's host location behavior. Simultaneously offered odor sources consisted of (i) a maize plant treated with BTH, (ii) a maize plant damaged by caterpillars, and (iii) a plant that was subjected to both treatments. The remaining three odor vessels remained empty. Treatments were carried out as described above.

2.5.3. Experiment 3

This experiment was conducted to rule out synergistic effects between the odor of the BTH solution and herbivore-induced maize volatiles. *M. rufiventris* females were offered a choice between two maize plant damaged by caterpillars. Each of the plant-containing odor vessels also contained a pot with soil, one of which was drenched with 25 ml BTH solution and the other with the same amount of water.

2.6. Statistical analyses

Normality of data was verified after which the Student's *t*-test for independent samples was used to determine differences in the resistance of induced maize leaves against *S. turcica*. The same test was used to compare the leaf areas removed by *S. littoralis* in the feeding assay. Two-way ANOVA with 'compound' and 'treatment' as factors was used to analyze differences in volatile emissions. Data were log₁₀-transformed to meet assumptions for ANOVA. Post-hoc comparisons of treatment effects on single components were performed with LSD tests. The software package Statistica (StatSoft, Tulsa, USA) was used for the analyses. For the six-arm-olfactometer the entity computing a repetition in the statistical analysis corresponds to the response of a group of six wasps released, which was shown to follow a multinomial distribution (Ricard and Davison, 2007). As the data did not conform to simple variance assumptions implied in using the multinomial distribution, we used quasi-likelihood functions to compensate for the overdispersion of parasitoids within the olfactometer (Turlings et al., 2004). The model was fitted by maximum quasi-likelihood estimation in the software package R (<http://www.R-project.org>), and its adequacy was assessed through likelihood ratio statistics and examination of residuals (Turlings et al., 2004).

3. Results

3.1. Effect of BTH on fungal infection

Application of BTH as a soil drench induced systemic resistance in maize seedlings against the leaf pathogen *S. turcica* (Fig. 1a). The necrotic areas caused by the fungus were significantly smaller on BTH-treated plants compared to control plants (Student's *t*-test, $t = -2.68$, $p = 0.013$).

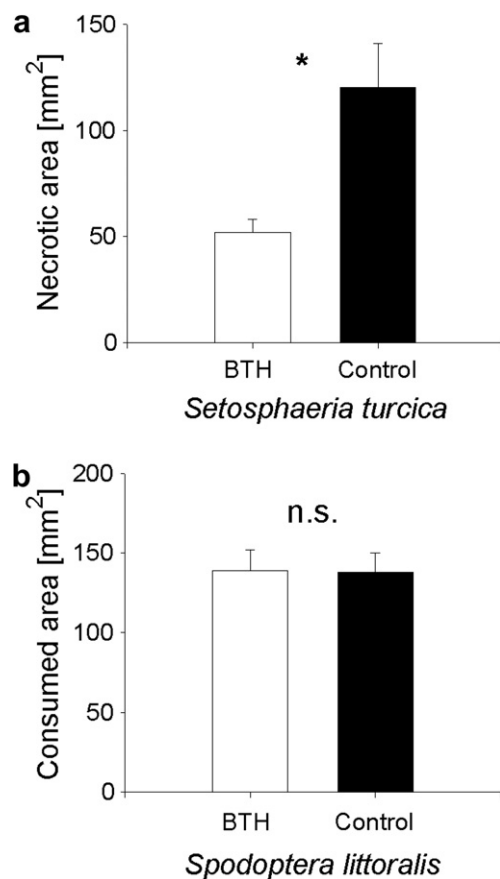


Fig. 1. Effect of BTH on the resistance of maize leaves against fungus and herbivore attack. (a) Mean (\pm SE) necrotic leaf area caused by *Setosphaeria turcica*. (b) Mean (\pm SE) leaf area consumed by caterpillars of *Spodoptera littoralis*. Asterisk denotes significant difference: * $p < 0.05$; n.s., not significant ($p > 0.05$), $N = 14$ (a), $N = 25$ (b).

3.2. Effect of BTH on herbivore leaf consumption

No significant effect of BTH was found on the amount of leaf material ingested by the herbivore *S. littoralis* (Fig. 1b). Equal amounts of maize leaf were consumed from BTH-treated plants and control plants (Student's t -test, $t = 0.10$, $p = 0.92$).

3.3. Effect of BTH on volatiles

Treatment (BTH, water, BTH + herbivory or water + herbivory) had a significant effect on volatile emissions (ANOVA, treatment: $F_{3,256} = 2414$, $p < 0.001$). However, volatile emission from BTH-treated maize seedlings did not differ significantly from water-treated plants (LSD, $p = 0.609$). Undamaged maize seedlings emitted only the oxygenated monoterpene linalool (Fig. 2). BTH application neither affected the emission rate of linalool (LSD, $p = 0.165$) nor did it induce the emission of any other volatile components. When plants were challenged by caterpillars of *S. littoralis*, the well-described (e.g. Degen et al., 2004) emission of green-leaf volatiles, terpenes and other compounds was observed (Fig. 2a). Pretreatment

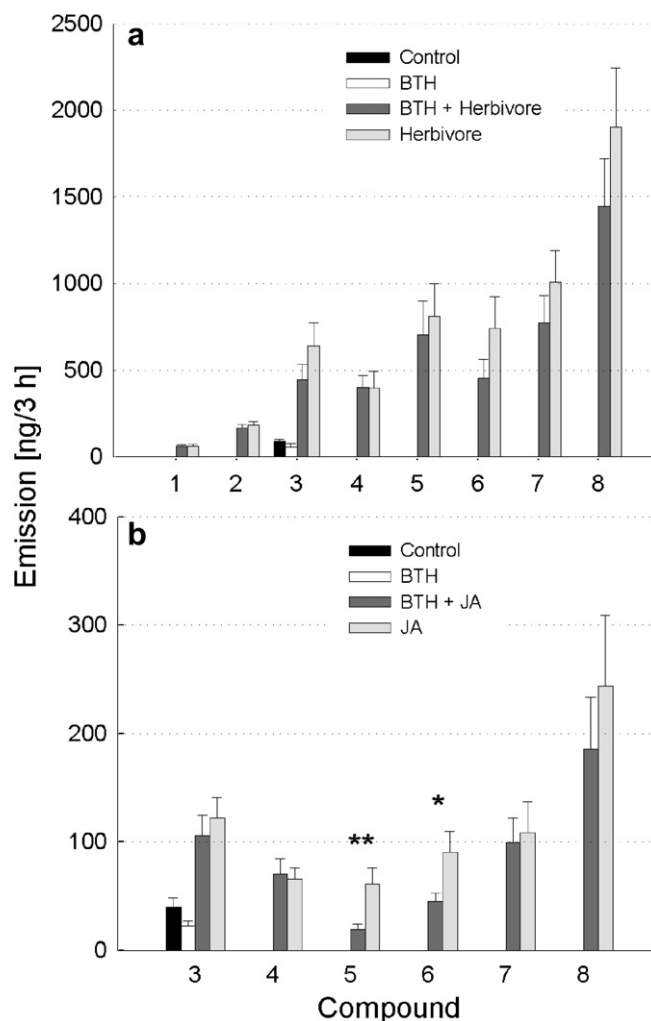


Fig. 2. Effect of BTH on the emission rate of maize volatiles induced by (a) caterpillar-feeding or (b) mechanical wounding and jasmonic acid application. 1 = (*Z*)-3-hexenal, 2 = (*Z*)-hexenyl acetate, 3 = linalool, 4 = DMNT, 5 = indole, 6 = (*E*)- β -caryophyllene, 7 = α -bergamotene, 8 = (*E*)- β -farnesene. Means (\pm SE) are given. Asterisks denote significant differences: * $p < 0.05$, $N = 6-8$.

with BTH had no significant effect on the caterpillar-induced emissions: the overall composition and quantity of the measured volatiles was the same as for plants that were only damaged by caterpillars (LSD, $p = 0.609$). Although there was a trend of lower mean emission rates for most inducible volatiles in double-treated plants, the differences were never significant.

In the experiment in which wounding and exogenous JA application replaced herbivore feeding, volatile ratios of the four treatments closely resembled those of the first experiment. Green-leaf volatiles were not considered as most of the times they were not detected. Their release requires continuous tissue damage such as carried out by feeding larvae. Again, no significant differences in emission were found between water-treated controls vs. BTH-treated plants (LSD, $p = 0.999$). However, in contrast to herbivore-induction, BTH pretreatment significantly attenuated the emission of two volatile compounds in JA-treated seed-

lings. Lower amounts of indole and trans- β -caryophyllene were found in double-treated compared to JA-treated plants (LSD, indole: $p = 0.001$; (*E*)- β -caryophyllene: $p = 0.019$). Overall, the quantities of volatiles released by JA-treated plants were approximately ten times less than for herbivore-damaged plants.

3.4. Effect of BTH on the host location behavior of *M. rufiventris*

3.4.1. Experiment 1

The response of naïve parasitoids to soil treated with BTH was tested against untreated soil and clean air (Fig. 3a). The responsiveness of the insects was low and 40% of the released wasps did not walk into any of the olfactometer arms, but remained in the central chamber. Parasitoids that did choose showed no significant preference for any one of the six arms. Soil drenched with BTH was not more attractive than untreated soil or clean air (LLM, treatment effects, $p = 0.311$).

3.4.2. Experiment 2

Females of *M. rufiventris* were highly attracted to volatiles emanating from BTH + herbivory-treated maize seedlings. This double treatment attracted many more wasps on each experimental day ($N = 8$) than any other odor source, including herbivore-attacked plants without BTH pretreatment (Fig. 3b, LLM, BTH + herbivory vs. herbivory, $p = 0.014$). Undamaged plants treated with BTH did not attract more parasitoids than control plants without any manipulation (LLM, BTH vs. control, $p > 0.05$). Herbivore-attacked maize seedlings were significantly more attractive to *M. rufiventris* than both groups of undamaged seedlings (LLM, herbivory vs. BTH, $p = 0.036$ and herbivory vs. control, $p = 0.043$).

3.4.3. Experiment 3

In contrast to experiment 2, wasps did not distinguish between the plant treatments with or without BTH (LLM, BTH-drenched soil + herbivory vs. herbivory + water-drenched soil, $p = 0.203$). However, both vessels containing caterpillar-damaged plants were highly attractive compared to empty control vessels.

4. Discussion

This study assessed whether SAR against pathogens induced by BTH application to maize seedlings would affect the plant's attractiveness to parasitoids. We expected a negative effect because several lines of evidence suggest antagonistic cross-talk between the SA- and JA-pathways (e.g. Beckers and Spoel, 2006). Furthermore, we tried to explain whether the previously observed reduction in herbivore-induced volatile emission in *S. turcica*-infected maize was due to such cross-talk (Rostás et al., 2006).

As a prerequisite, we showed that exogenous BTH application via the roots induced SAR against the widely

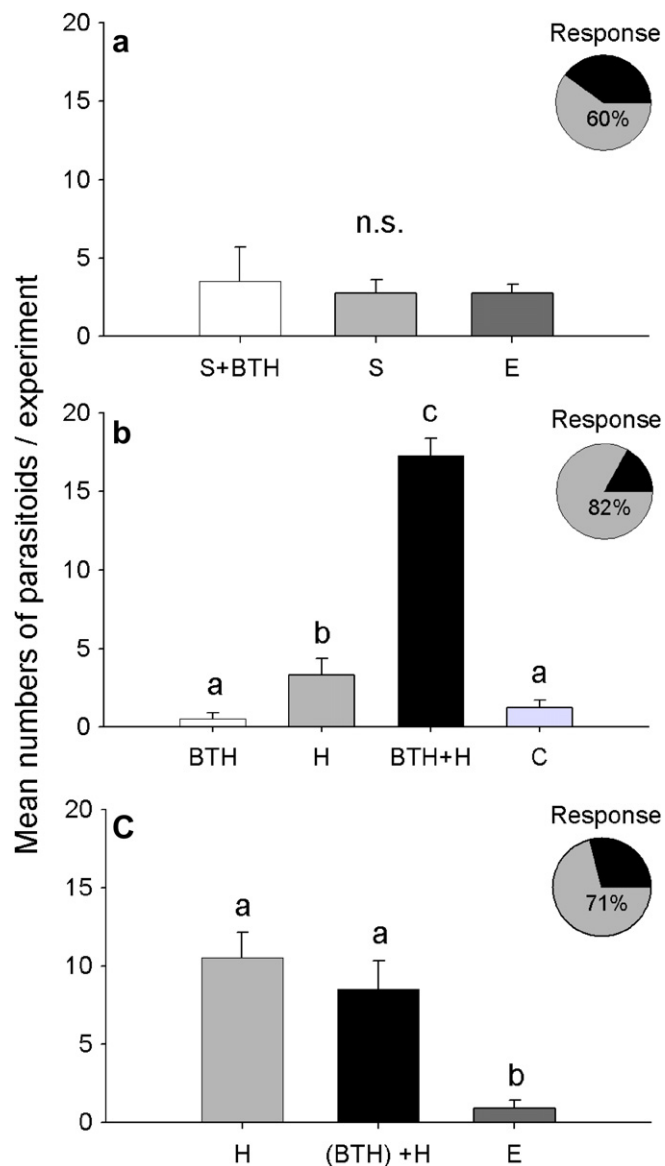


Fig. 3. Host location behavior of naïve *Microplitis rufiventris* in a six-arm-olfactometer. Bars represent mean (\pm SE) numbers of wasps making a choice for a particular odor. Wasps were allowed to choose between odors of (a) S + BTH = pot containing BTH-drenched soil, S = pot containing water-drenched soil, and E = empty control vessel (mean value of four vessels). (b) BTH = BTH-treated plant, H = herbivore-damaged plant, BTH + H = plant treated with BTH and damaged by herbivores, and C = untreated control plant (mean value of three controls). (c) H = vessel containing an herbivore-damaged plant and a pot of water-drenched soil, (BTH) + H = vessel containing an herbivore-damaged plant and a pot of BTH-drenched soil, E = empty control vessel (mean value of four vessels). Pie charts indicate percentages of wasps responding to odors. Non-responsive wasps were not considered in statistical analyses. Thirty wasps were released per experimental day. Each experiment was replicated 6–8 times. Different letters indicate significant differences; n.s., not significant ($p > 0.05$).

occurring maize leaf pathogen *S. turcica* (Fig. 1a). Timing and strength of elicitation may be crucial (Thaler et al., 2002a), therefore a relatively high concentration was chosen, combined with the time point for strongest defense gene expression (Morris et al., 1998) to obtain good resis-

tance effects. This treatment reduced disease severity by approximately 50%. The effectiveness of BTH in inducing disease resistance in maize was previously shown for downy mildew (Morris et al., 1998).

We also had to test for an effect of BTH treatment on the quality of maize leaves as food for *S. littoralis* caterpillars. Any differences in leaf consumption on BTH-treated and control plants could explain differences in volatile emissions, as the duration and the extent of the damage inflicted to a plant can determine the quality and quantity of the emitted volatile blend (Mithöfer et al., 2005). However, as in other studies on the effects of BTH on caterpillars, no differences in host plant suitability were found (Inbar et al., 2001; Thaler et al., 2002b; Plymale et al., 2007). Equal amounts were consumed from SAR-induced and control leaves, respectively, confirming the notion that the SA-pathway is not involved in direct defense against chewing herbivores (Fig. 1B).

Pretreatment of maize seedlings with BTH neither changed the composition nor the quantities of the volatiles induced by caterpillars. However, it should be noted that there was a general trend that the mean amounts of volatiles emitted by BTH + herbivory-treated plants were lower than for herbivore-treated plants without BTH. A similar trend was found for the volatiles emitted by BTH-treated plants that received an exogenously applied JA treatment and the compounds indole and (*E*)- β -caryophyllene were emitted in significantly lower amounts by plants that had received a BTH treatment in addition to the JA treatment (Fig. 2B), showing that under these conditions cross-talk between the SA- and JA-pathways may occur. It is unclear why this minor effect of cross-talk was seen in JA-induced and not in herbivore-induced plants. An explanation may be the much stronger response induced by the herbivore, which could override any effect of the SA-pathway. Induction by herbivory cannot be fully mimicked by a combination of wounding and JA application (Dicke et al., 1999), as it involves various chemical elicitors in the oral secretion and the induction of other modulating phytohormones like ethylene (Thaler, 2002; Schmelz et al., 2003b; Tumlinson and Lait, 2005). The results also suggest that the previously observed reduction in volatile emission as a consequence of *S. turcica* infection (Rostás et al., 2006) is probably due to other factors than cross-talk between the SA- and JA-signaling pathways.

From the chemical analyses described above we initially expected *M. rufiventris* to be equally attracted to herbivore-damaged maize seedlings, irrespective of SAR induction. It was surprising to find that *M. rufiventris* was much more attracted to BTH + herbivory-treated plants compared to herbivore-damaged seedlings without BTH. Evaluation of all headspace volatiles (including minor compounds) did not result in the detection of any additional herbivore-induced plant volatiles triggered by BTH. However, the BTH dispersion (applied as BION™) has a distinct odor that can be perceived by the human nose, probably resulting from small amounts of methyl disulfide and a number of chlorobenzenoids (data not shown). To test if this spe-

cific odor contributed to the enhanced attraction of BTH-treated plants we assessed the response of *M. rufiventris* to an odor-mix of normal induced maize volatiles and BTH-treated soil added in a separate pot and compared this to herbivore-induced maize volatiles only. The wasps chose equally between these two treatments, implying that BTH treatment affects the plant's volatile emission in a way that it is perceived by the wasps, but undetected by our GC-MS method. The existence of undetectable compounds that are perceived by *M. rufiventris* is also suggested by electrophysiological studies of the antennae of this parasitoid species (Gouinguéné et al., 2005). Recent evidence (D'Alessandro and Turlings, 2005, 2006) strongly suggests an important role for such minor compounds in the attraction of parasitoids, and that the presence of some of the major compounds may mask their attractiveness. Specifically indole has been shown to negatively affect attraction of *M. rufiventris* females (D'Alessandro and Turlings, 2006), which was one of the compounds that tended to be released in smaller amounts after BTH treatment (Fig. 2A).

In summary, we found that SAR induction with BTH does not interfere with the plant's direct defense against an important herbivore, but enhances the plant's indirect defense, i.e. its attractiveness to parasitoids. Further research will be necessary to determine the identity of the volatile compounds that were responsible for this effect and whether the effect is due to an increased production of attractive or a decreased production of repelling or masking compounds.

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