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Fungal Infection Reduces Herbivore-Induced Plant Volatiles of Maize but does not Affect Naïve Parasitoids

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Abstract Plants attacked by insects release volatile compounds that attract the herbivores' natural enemies. This so-called indirect defense is plastic and may be affected by an array of biotic and abiotic factors. We investigated the effect of fungal infection as a biotic stress agent on the emission of herbivore-induced volatiles and the possible consequences for the attraction of two parasitoid species. Maize seedlings that were simultaneously attacked by the fungus *Setosphaeria turcica* and larvae of *Spodoptera littoralis* emitted a blend of volatiles that was qualitatively similar to the blend emitted by maize that was damaged by only the herbivore, but there was a clear quantitative difference. When simultaneously challenged by fungus and herbivore, the maize plants emitted in total 47% less of the volatiles. Emissions of green leaf volatiles were unaffected. In a six-arm olfactometer, the parasitoids *Cotesia marginiventris* and *Microplitis rufiventris* responded equally well to odors of herbivore-damaged and fungus- and herbivore-damaged maize plants. Healthy and fungus-infected plants were not attractive. An additional experiment showed that the performance of *S. littoralis* caterpillars was not affected by the presence of the pathogen, nor was there an effect on larvae of *M. rufiventris* developing inside the caterpillars. Our results confirm previous indications that naïve wasps may respond primarily to the green leaf volatiles.

Keywords *Cotesia marginiventris* · Cross effects · Fungus · Induced indirect defense · *Microplitis rufiventris* · Parasitoids · *Spodoptera littoralis* · Tritrophic interactions · Volatiles · *Zea mays*

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Introduction

Plants have evolved a broad spectrum of inducible defense mechanisms to resist damaging insects and pathogens (Karban and Baldwin, 1997). Induced plant defenses may act directly against herbivores or microorganisms, and comprise mechanisms as diverse as the strengthening of plant cell walls, hypersensitive cell death, or the production of toxic and deterrent substances. In addition, plants may employ indirect defenses for instance by emitting volatile compounds in response to feeding or oviposition by arthropods (Dicke and Sabelis, 1988; Hilker and Meiners, 2002). These volatiles can serve as long-range signals for parasitoids and predators, by indicating the presence and location of their often inconspicuous prey.

A central question in the study of indirect plant defenses is how specific the plant-provided volatile cues are (Vet and Dicke, 1992; Dicke, 1999; Turlings et al., 2002). It has been argued that ideally volatile cues should not only be easy to detect but, should also be specific enough to provide information on the identity of the herbivore and its suitability as a host or prey (Vet and Dicke, 1992). Plants can vary considerably in the volatile blends they emit, both in terms of the chemical composition (quality) and in the quantity of volatile compounds. Depending on what factors cause it, this variability may either interfere with or enhance the specific information that the signals contain. Evidence has been found for both, existence and absence of specificity, depending on the studied system (reviewed by Dicke, 1999). At least in some plant–herbivore systems it has been shown that different insect species and instars may elicit different odor blends, resulting in preferential attraction of natural enemies to plants on which their specific (De Moraes et al., 1998; Guerrieri et al., 1999) or preferred host stages (Takabayashi et al., 1995) were feeding. It remains to be resolved, however, how this specificity in plant signals can exist in the face of considerable variability in induced volatile blends caused by other factors. Major differences in the chemical composition can be found among different plant species (Turlings et al., 2002), but within a species genotypic effects have also been found to be important (Loughrin et al., 1995; Peacock et al., 2001; Gouinguené et al., 2001; Hoballah et al., 2002). Moreover, abiotic factors such as humidity, temperature, light intensity, light cycle, and nutrient availability all can have an effect on the quantity and the quality of herbivore-induced plant odors (Gouinguené and Turlings, 2002).

An additional complicating aspect is that plants live in environments in which they face the possibility of multiple, synchronous attacks by insects and pathogens. Host finding with the aid of plant-provided volatiles may prove difficult for parasitoids if plant pathogens significantly alter the chemical composition of herbivore-induced odor blends. Pathogen-derived or pathogen-induced odors could mask the host-induced blend or reduce the emission of important compounds. On the other hand, odor blends resulting from simultaneous herbivore and pathogen attack may provide useful information for natural enemies if their hosts are less or better suitable due to poorer or better development on a diseased plant. So far, cross effects between plant-feeding insects and microorganisms have been investigated almost exclusively in the context of induced direct defenses (Hatcher, 1995; Agrawal et al., 1999; Rostás et al., 2003). Whether induced indirect defense against herbivores is compromised by pathogen attacks remains almost unexplored, with the exception of the studies conducted by Cardoza et al. (2002, 2003a,b), who found a significant effect of fungus infection on direct and indirect defenses in peanut plants (see Discussion).

At the biochemical level, pathogen infection commonly induces the salicylic acid (SA) defense pathway, whereas insect attack triggers a defense based on jasmonic acid (JA).

Cross talk between these pathways, however, may occur (Felton and Korth, 2000; Thaler et al., 2002; Devoto and Turner, 2003). It is conceivable that the induction of the SA pathway may also interact with the JA-dependent induction of volatiles in response to herbivory. If so, a change in the emission of many compounds should be the result, and members of the third trophic level may adapt their responses to optimize the exploitation of the signals.

The induced indirect defense mechanism has been extensively investigated in the tritrophic system maize, *Spodoptera* spp., and several larval endoparasitoids (Turlings et al., 1995; Alborn et al., 1997; Hoballah et al., 2002). Feeding by *Spodoptera* larvae leads to the release of green leaf volatiles and induces the accumulation of JA and ethylene. These phytohormones are responsible for the emission of indole, terpenoids, and other compounds (Schmelz et al., 2003a,b; Ruther and Kleier, 2005). Parasitic wasps are highly attracted to these odors (Turlings et al., 1990), and this attraction may benefit the attacked plant (Hoballah and Turlings, 2001). In contrast to some plants, the induction of the SA pathway does not lead to any detectable release of volatiles in maize (Turlings et al., 2002; Van Poecke and Dicke, 2004). Because of the available information on caterpillar-induced emissions of maize and its attractiveness to parasitoids, this tritrophic system lends itself well to studies on the effects of pathogen infection on indirect defense.

In this study, we assessed the effect of the necrotrophic fungus *Setosphaeria turcica* (Leonard et Suggs) on the emission of maize volatiles induced by *Spodoptera littoralis* (Boid.), and the consequences for the third trophic level. The ascomycete *S. turcica* causes the foliar disease known as northern corn leaf blight and is a serious problem for maize growers worldwide (Borchardt et al., 1998). The fungus cooccurs with *S. littoralis* and its parasitoid *Microplitis rufiventris* (Kok.) in Egypt and the countries of the Middle East (Gerling, 1969; Hegazi, 1977; CAB International, 1988). It also cooccurs with *Cotesia marginiventris* in the United States and Latin America (CAB International, 1988; Molina-Ochoa et al., 2003). This parasitoid species uses *S. exigua* and *S. frugiperda* larvae as hosts. These two hosts trigger the release of the same inducible compounds in maize when compared to *S. littoralis* (Turlings et al., 1995; Hoballah, 2001). In a six-arm olfactometer, we tested the responses of the parasitoids *M. rufiventris* and *C. marginiventris* (Cresson) to odors of maize seedlings attacked either by the fungus, the herbivore, or by both. Simultaneously, all odor blends were sampled and subsequently analyzed for comparison. Furthermore, the performance of *S. littoralis* feeding on fungus-infected maize plants and the performance of its natural enemy, *M. rufiventris*, developing in larvae that fed on diseased maize was evaluated.

Methods and Materials

General Methods

Maize (*Zea mays* var. Delprim) plants were grown in polypropylene pots (11 cm high, 4 cm diam) containing commercial soil mix (Coop, Basel) in a climate chamber (23°C, 60% r.h., and 16:8 hr L/D, 550 μmol m^{-3} sec^{-1}). This maize variety was chosen because it is partially resistant to *S. turcica*. Stronger and/or faster induced responses to fungal attack as well as less necrotic tissue that does not produce volatiles can be expected from partially resistant compared to susceptible varieties. In addition, "Delprim" is a variety that emits exceptionally high amounts of herbivore-induced volatiles.

Eggs of *S. littoralis* (Lep., Noctuidae) were supplied by Syngenta (Stein, Switzerland). Newly hatched larvae were reared in transparent plastic boxes on a wheat germ-based artificial diet until used. Colonies of the solitary endoparasitoids *M. rufiventris* and *C. marginiventris* (both Hym., Braconidae) were maintained in the laboratory. For the rearing, 25 *S. littoralis* caterpillars (3–4 d) were offered to a single mated female (4–7 d) for 3 hr in a plastic box (5 cm high, 9.5 cm diam). The parasitized caterpillars were kept in an incubator (25°C, L:D 16:8 hr) 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, Taichung, Taiwan) in the same incubator (*C. marginiventris*) or under ambient laboratory conditions (*M. rufiventris*). Cages were supplied with moist cotton wool and droplets of honey.

The necrotrophic fungus *S. turcica* (anamorph: *Exserohilum turcicum*, Dothideaceae) was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany) and cultivated on V8 agar in darkness under laboratory conditions.

Plant Inoculation and Volatile Induction

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 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^{-1}. Maize seedlings (7 d) were inoculated by applying 100 µl spore suspension to the second and third leaves, respectively. Spores were spread homogeneously with a paintbrush. Control plants were mock-inoculated in the same manner with 0.05% aq. Tween 20. All seedlings were placed into two cool boxes with wet tissue papers laid out on the bottom. Plants were kept in darkness for 16 hr (17:00–09:00) at >90% r.h. and ambient temperatures. The following morning, all plants were transferred to a climate chamber (23°C, 60% r.h., and L/D 16:8 hr, 550 µmol m^{-2} sec^{-1}). Disease symptoms were allowed to develop for 72 hr after which the plants were used in the experiments. Strength of infection was calculated by scanning the diseased leaves ($N = 9$) and measuring the necrotized areas with Photoshop 7.0 (Adobe) and Surface (© C. Thiemann, Berlin, Germany). On average, 11% of the leaf surface was visibly affected by *S. turcica*. This was the highest achievable disease rate for the partially resistant variety Delprim in the seedling stage.

Volatile Collections

Volatiles from maize seedlings were collected with a six-arm olfactometer, a device allowing for simultaneous odor collection and testing of parasitoid host location behavior (described by Turlings et al., 2004). A single maize plant was placed into one of the six odor source vessels of the olfactometer. 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, IL, USA) that was kept in place by two fine mesh metal screens (described by Heath and Manukian, 1992). Filtered and humidified air was pushed into the odor source vessels at a rate of 1.2 l min^{-1} $vessel^{-1}$ originating from a central in-house compressor. Half of the air flow (0.6 l min^{-1}) was pulled through the trapping filter with a vacuum pump (ME2, Vacuubrand, Wertheim, Germany), whereas the other half was allocated to the olfactometer choice chamber. Before each experiment, traps were rinsed with 1 ml methylene chloride. Collections lasted 3 hr after which traps were removed, extracted, and analyzed.

Chemical Analysis

Volatile traps were eluted with 150 μl methylene chloride after each collection, and two internal standards (*n*-octane for green leaf volatiles and nonyl acetate for terpenoids and others, each 200 ng in 10 μl methylene chloride; all chemicals were purchased from Sigma-Aldrich, Germany) were added to these samples. Aliquots (3 μl) of the samples were analyzed by gas chromatography/mass spectrometry (GC: HP 6890 N, MSD: Agilent 5973) equipped with a split/splitless injector and an 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 sec^{-1}) was used as carrier gas. Compound identities were confirmed by comparison with mass spectra of the National Institute of Standards and Technology (NIST) library and mass spectra of commercially available standards. Quantification of compounds was based on comparison with the internal standards. Only those compounds that were reliably found in each sampled plant of the same treatment were quantified. The evaluated compounds comprised >90% of the total amount of the analyzed volatile blends.

Host Location Behavior of Parasitoids

Attraction of *M. rufiventris* and *C. marginiventris* was assessed in the six-arm olfactometer, and simultaneously a part of the volatiles released from the plants was collected for subsequent analyses. Mated 2- to 4-d-old female wasps were used in all experiments. Insects were naïve in a sense that they had no contact to host insects or plants during the adult stage. Six wasps were removed from the 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). Wasps initially walked up to the top, attracted by the light above the choice chamber. Most would walk into an arm with an attractive odor until the 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 8 d with a new set of plants ($N = 8$). The position of the plants was changed clockwise after each day of testing. Different sets of plants were used for each parasitoid species. *M. rufiventris* and *C. marginiventris* were tested to simultaneously presented odors of: (1) three undamaged maize plants, (2) one herbivore-damaged maize plant, or (3) one fungus-infected maize plant, or (4) one herbivore-damaged/fungus-infected maize plant. The three undamaged plants were alternated with attacked plants and arranged in a circle. Herbivory treatment was achieved by transferring the plants into the odor source vessels and then placing ten 2nd instars of *S. littoralis* into the whorl of a healthy or infected maize plant (10 d old) the evening (17:00) before an experiment was performed. All other plants were placed in the olfactometer at the same time. The subsequent olfactometer assays were carried out between 09:00 and 13:00 hours.

Development of *S. littoralis* on Fungus-Infected Maize

The performance and mortality of *S. littoralis* caterpillars on *S. turcica*-infected maize plants was tested in two separate experiments. In the first assay, fifteen 4-d-old caterpillars

were selected for equal weight (2.6 ± 0.15 mg) and then placed singly into the whorl of a potted maize seedling. The host plants had previously been inoculated with the fungus or mock-inoculated with Tween 20. Symptoms of the fungus were present on the first three leaves, but not on the youngest fourth leaf. This reflects the heterogenic distribution of fungal infection in nature allowing the herbivore to choose between locally and systemically induced leaves. A cellophane bag (Celloclair, Liestal, Switzerland) over each plant prevented caterpillars from escaping while permitting gas exchange. After 5 and 10 d of feeding, larvae were weighed and placed on a new host plant. Finally, the pupal weight was calculated 2 d after pupation. In another experiment, we measured leaf consumption and survival of *S. littoralis* on infected and healthy leaves. A group of 10 neonate caterpillars was placed into each of 15 Petri dishes (9 cm diam) with moist filter paper. As a food source, each group of caterpillars received either a piece of (1) *S. turcica*-infected leaf bearing symptoms (third leaf), (2) healthy leaf (third leaf), (3) symptom-free leaf (fourth leaf), or (4) healthy leaf (fourth leaf). All Petri dishes were kept in an incubator (25°C, 16:8 hr L/D). The leaf area removed by *S. littoralis* was evaluated as described above for lesion area measurement after 2 d, and the number of surviving caterpillars was recorded.

Development of *M. rufiventris* inside Caterpillars Feeding on Fungus-Infected Maize

We assessed whether *S. turcica* infection had an indirect effect on the parasitoid *M. rufiventris*. Two groups of either five fungus-infected or five healthy maize plants were placed into four insect rearing tents (Bugdorm 2, Megaview, Taiwan). Ten neonate *S. littoralis* caterpillars were transferred onto each plant with a small paintbrush and allowed to feed for 3 d. All caterpillars were collected from the plants and placed into a transparent plastic box. From this pool, 20 caterpillars were randomly chosen and placed in a Petri dish into which a female parasitoid was introduced. The wasp was allowed to oviposit into six caterpillars. Wasp and parasitized caterpillars were then removed and the procedure was repeated five times with new wasps and herbivores. This yielded 36 parasitized caterpillars from fungus-infected and healthy maize plants, respectively. The *S. littoralis* larvae were allowed to continue to feed on the same type of plant they had originated from, either infected or healthy maize plants, for 6 d, i.e., 2 d before the first parasitoid hatched from its host. Caterpillars were then individually placed in Petri dishes (5.5 cm diam) containing a piece of filter paper and artificial diet. After emergence, the hatching rate, survivorship, pupal weight, developmental time, and longevity of *M. rufiventris* were measured. Parasitoid longevity was assessed by measuring the time from egg deposition until the adult died. Adults were supplied with water but were not fed.

Statistics

Data obtained from the six-arm olfactometer were analyzed by modified G-statistics for comparison of log linear models based on a quasi-Poisson distribution and thus fitted to overdispersed data. The software package R (http://stat.ethz.ch/CRAN/) was used. For detailed explanations, see Turlings et al. (2004). Two-way ANOVA with treatment and compound as main effects was performed for comparison of volatiles. Herbivore and parasitoid performances were also compared by using Student's *t*-test for independent samples. However, numbers of surviving *S. littoralis* on healthy and diseased plants were analyzed by Mann–Whitney *U* test, and hatching rate and survivorship of parasitoids were assessed via chi square tests.

Results

Effect of *S. turcica* on Volatile Emission

Fungal infection had a quantitative but no detectable qualitative effect on the odor bouquet of maize seedlings, i.e., no new compounds were detected (Fig. 1). Healthy and fungus-infected maize seedlings, both exclusively released linalool at the same rate (Newman–Keuls test after ANOVA, Treatment effect: $P = 0.794$). In contrast, caterpillar feeding triggered the release of large amounts of green leaf volatiles, monoterpenes, indole, and sesquiterpenes. Plants double-treated with *S. turcica* and *S. littoralis* emitted the same blend as plants damaged by *S. littoralis* alone, but most of the induced volatiles were found in significantly lower amounts. Fungus- and herbivore-damaged plants released 48% less of the total amount of volatiles than herbivore-only damaged plants (Newman–Keuls test after ANOVA, Treatment effect: $P < 0.001$). Compounds not reduced in their amounts due to pathogenic infection were (*Z*)-3-hexenal, (*Z*)-3-hexen-1-yl-acetate, and β-caryophyllene (Newman–Keuls test after ANOVA, Compound × Treatment, P values > 0.05). The ratios between a given volatile compound and the total emission did not differ significantly between double-treated and herbivore-only treated plants with one exception: fungal infection lowered the indole/total amount ratio from 14% to 5% ($t = 3.467$; $P = 0.002$).

Host Location Behavior of Parasitoids

Parasitic wasps *M. rufiventris* and *C. marginiventris* preferred the odor of plants that were damaged by *Spodoptera* larvae when compared with healthy (*M. rufiventris*: estimate ± SE: −2.72 ± 0.31, $P < 0.001$; *C. marginiventris*: estimate ± SE: −2.33 ± 0.23, $P < 0.001$) and fungus-infected maize (*M. rufiventris*: estimate ± SE: −2.45 ± 0.45, $P < 0.001$; *C. marginiventris*: estimate ± SE: −3.10 ± 0.54, $P < 0.001$) (Fig. 2). However, they did not

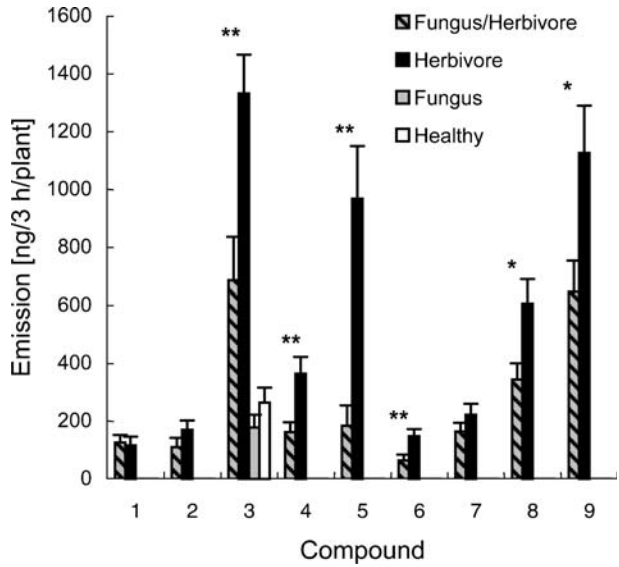

Fig. 1 Plant volatiles emitted from seedlings of *Zea mays* (var. Delprim) after single or combined herbivore feeding and fungal infection. Means ± SE are shown. $N = 10–13$. Asterisks denote significant differences between treatments: *$P < 0.05$, **$P < 0.01$, n.s. = not significant. Newman–Keuls test after two-way ANOVA. 1 = (*Z*)-3-Hexenal; 2 = (*Z*)-3-hexen-1-yl acetate; 3 = linalool; 4 = (3*E*)-4,8-dimethyl-1,3,7-nonatriene; 5 = indole; 6 = geranyl acetate; 7 = β-caryophyllene; 8 = (*E*)-α-bergamotene; 9 = (*E*)-β-farnesene

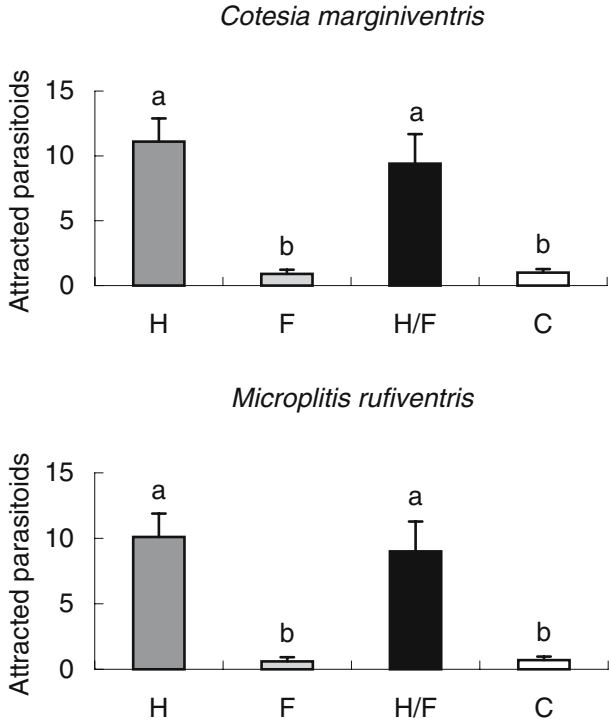

Fig. 2 Responses of naïve female parasitoids to odors emanating from: H = herbivore-infested, F = fungus-infected, H/F = herbivore- and fungus-attacked, or C = healthy maize seedlings in a six-arm olfactometer. The experiments were replicated on 8 d with different sets of plants for each parasitoid species. Per day 30 wasps were released in groups of six. Responding wasps: 80% of *Cotesia marginiventris*, 81% of *Microplitis rufiventris*. Bars represent mean number of parasitoids (±SE) per experimental day responding to an odor source. Significant differences between treatments are indicated by different letters above bars ($P < 0.05$)

respond differently to the odors of plants that had been damaged by *S. littoralis* alone and those that had been inoculated with *S. turcica* in addition (*M. rufiventris*: estimate ± SE: −0.12 ± 0.18, $P = 0.522$; *C. marginiventris*: estimate ± SE: −0.14 ± 0.16, $P = 0.377$). No significant differences in preference were found between fungus-infected and healthy maize seedlings (*M. rufiventris*: estimate ± SE: 0.27 ± 0.51, $P = 0.597$; *C. marginiventris*: estimate ± SE: −0.77 ± 0.56, $P = 0.172$).

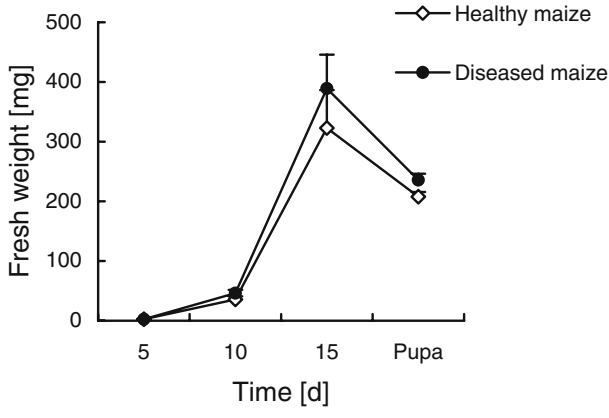

Fig. 3 Performance of *Spodoptera littoralis* on healthy or *Setosphaeria turcica*-infected maize plants. One caterpillar fed on a single plant. All plants were exchanged after 5, 10, and 15 d of feeding. Means ± standard errors are given. No significant differences were found between treatments. Student's *t*-test for independent samples

Table 1 Performance of *Spodoptera littoralis* (L_1) on *Setosphaeria turcica*-infected or healthy maize

	Fungus[a]		Healthy[a]		P[c]
	Local	Systemic	Local	Systemic	
Area fed [mm^2][b]	102 ± 7.0	139 ± 11.0	103 ± 9.3	125 ± 15.4	n.s.
Surviving larvae/leaf	9.6 ± 0.17	9.2 ± 0.27	9.4 ± 0.20	9.6 ± 0.15	n.s.

[a] Ten caterpillars were kept on detached leaves (local = second leaf, systemic = fourth leaf) for 2 d.

[b] Means and standard errors are given for fed leaf areas (N = 14–15).

[c] n.s. = Not significant, $P > 0.05$. Student's t-test for independent samples and Mann–Whitney U test.

Development of *S. littoralis* on Fungus-Infected Maize

Feeding on fungus-infected whole maize plants had no negative impact on the development of *S. littoralis* when compared with caterpillars feeding on healthy plants. No significant differences in larval fresh weights (after 5 d: $t = -1.666$, $P = 0.116$; 10 d: $t = -1.209$; $P = 0.245$, 15 d: $t = -1.21$; $P = 0.245$) or pupal fresh weights ($t = -2.071$, $P = 0.056$) were found at any time point of measurement (Fig. 3). Also, the duration of development was not affected by pathogen infection ($t = 0.355$, $P = 0.728$). Neonate larvae survived equally well on locally or systemically induced leaves of infected maize as on the corresponding leaves from healthy plants (local: $U = 166$, $Z = -0.137$, $P = 0.891$; systemic: $U = 109.5$, $Z = 0.954$, $P = 0.340$) (Table 1). Caterpillars on healthy leaves did not consume significantly different amounts of leaf material than those on diseased leaves (local: $t = 0.219$, $P = 0.828$; systemic: $t = -0.900$, $P = 0.376$).

Development of *M. rufiventris* inside Caterpillars Feeding on Fungus-Infected Maize

No indirect effect of *S. turcica* on the development of *M. rufiventris* was found (Table 2). Larvae of the parasitoid developed equally well in *S. littoralis* caterpillars feeding on infected maize as in caterpillars feeding on control plants. Hatching rate ($\chi^2 = 1.19$, $P = 0.276$), developmental speed (egg–pupa: $t = -0.398$, $P = 0.693$), pupal weight ($t = -1.692$, $P = 0.097$), and survivorship ($\chi^2 = 0.89$, $P = 0.345$) did not differ between treatments.

Table 2 Performance of *Microplitis rufiventris* in caterpillars of *Spodoptera littoralis* that had been reared on either fungus-infected or healthy maize plants

	Fungus	Healthy	P[d]
Hatched pupae[a] [%]	69	80	n.s.
Hatched adults[b] [%]	47	58	n.s.
Developmental time (egg to pupa) [d]	8.7 ± 0.14	8.7 ± 0.12	n.s.
Pupal weight[c] [mg]	3.2 ± 0.04	3.2 ± 0.04	n.s.
Longevity[c] (egg to adult) [d]	25.9 ± 1.10	26.3 ± 0.57	n.s.

[a] Parasitoid larvae that left the host and pupated outside.

[b] Adult parasitoids emerging from cocoon.

[c] Means and standard errors are given.

[d] Chi square test and Student's t-test for independent samples. $N = 36$. n.s. = not significant.

Discussion

The results demonstrate that fungal infection had an impact on the emission of caterpillar-induced plant volatiles. Herbivore-damaged maize seedlings emitted lower amounts of the most abundant volatiles if they were previously infected by *S. turcica.* Pathogen infection alone did not result in the emission of detectable amounts of any compound other than linalool, which is also emitted by healthy plants. Typical fungus volatiles, such as 3-octanone, were not found. We also found no methyl salicylate that could have resulted from the induction of the SA pathway by *S. turcica* (Rostás et al., unpublished), despite using more sensitive methods (MS in single ion mode) to detect this compound. Although it was not possible to achieve a higher infection rate in this maize variety, it is doubtful that a heavier disease rate would have led to the emission of additional volatiles: treatment with a high dose (5 mM) of the SA mimic BTH did not lead to any differences in volatile emission (Rostás et al., unpublished data). Attenuated volatile emission due to double infestation has also been reported by Rodriguez-Saona et al. (2003), but in their case both plant antagonists were insects. It was found that cotton plants damaged by caterpillars of *S. exigua* emitted 60% less volatile compounds if simultaneously infested by the phloem-feeding insect *Bemisia argentifolii*. This is noteworthy because herbivores with a sucking–piercing–feeding mode can induce the SA pathway and, consequently, plant responses that are comparable with defenses against pathogens (Walling, 2000). Concerning the underlying mechanism, we hypothesize that fungal infection could reduce plant volatile emission as a result of the negative cross talk between the pathogen-induced SA pathway and herbivore-induced JA signaling. This antagonistic interaction has been shown for direct defenses in several plants (Fidantsef et al., 1999; Preston et al., 1999; Thaler et al., 2002).

Our observations contrast with reports on the only other plant–fungus–herbivore system investigated so far: in peanut plants. the emission of volatiles induced by *Spodoptera exigua* was not attenuated by the fungus *Sclerotium rolfsii*. However, methyl salicylate (MeSA), an attractive compound for a number or natural enemies (James, 2003a,b; de Boer and Dicke, 2004), was emitted by fungus-infected and double-attacked plants (Cardoza et al., 2002, 2003a,b). This difference in odor emission between maize and peanut is also reflected in the interactions with the second and third trophic level. On peanut plants, *S. exigua* eat more leaf tissue and perform better when the plant is diseased, thus leading to increased volatile emission (Cardoza et al., 2002). More individuals of *C. marginiventris* were found to land on fungus- and herbivore-attacked peanut plants than on plants infested by *S. exigua* alone (Cardoza et al., 2002, 2003a,b). These observations suggest that the wasp's response may be adaptive. However, it needs to be verified whether *C. marginiventris* performs better in *S. exigua* feeding on diseased compared to healthy plants.

In our study, neither *C. marginiventris* nor *M. rufiventris* preferred herbivore-damaged plants to double-treated maize seedlings, although the latter emitted less volatiles. We expected both parasitoid species to be more attracted by plants that were damaged by the herbivore alone, as they emitted about 50% more in total than maize seedlings attacked by both antagonists. Both *C. marginiventris* (Turlings et al., 2004) and *M. rufiventris* (C. Tamó, personal communication) respond in a dose-dependent manner in the six-arm olfactometer. As a general rule, the stronger the volatile emission, the stronger the attraction of the wasps. However, the odor blends in our experiments were not directly comparable to blends offered in dose–response assays where all compounds in the odor blends were equally reduced or increased. In contrast, in double-damaged maize not every compound was significantly reduced: the green leaf volatiles (*Z*)-3-hexenal and (*Z*)-3-hexen-1-yl acetate, as well as the sesquiterpene β-caryophyllene, were not affected by *S. turcica*

infection. The unaltered emission of green leaf volatiles in double-treated plants and their significance for both parasitoid species could be a sufficient explanation as to why the wasps did not change their preference. Hoballah (2001) and D'Alessandro and Turlings (2005) found that green leaf volatiles from maize are important attractants for naïve *M. rufiventris* and, in particular, for *C. marginiventris*. For the latter species, green leaf volatiles and/or related compounds were more attractive than induced terpenoids, as long as wasps had had no oviposition experience in the presence of induced maize odors. Further experiments will have to reveal whether *C. marginiventris* will respond differently to herbivore-infested maize plants with or without fungal infection after associative learning.

Alternatively, the unaltered responsiveness of wasps to caterpillar-damaged plants with fungus infection may be adaptive in the sense that there appears to be no selection pressure on female *M. rufiventris* to avoid *S. turcica*-infected maize. The offspring of *M. rufiventris* developed equally well in caterpillars feeding on healthy or *S. turcica*-infected maize leaves. It is conceivable that for this reason the wasps mainly use those volatiles for host location that are not affected by the fungus. To our knowledge, this is the first study to relate the potential impact of a phytopathogen to parasitoid performance.

Our research shows (1) that fungal infection is another factor that leads to variability in herbivore-induced odor emissions, and (2) that certain variability in the odor bouquet does not necessarily disrupt the mutualistic relationship between parasitoid wasps and plants.

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