

RESEARCH ARTICLE

Functional Ecology



Fungal volatiles influence plant defence against above-ground and below-ground herbivory

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Abstract

1. Plants have evolved resistance traits that negatively affect attackers, and tolerance traits that sustain plant growth despite herbivore damage. These mechanisms often co-occur in a mixed-defence strategy, balancing resistance and tolerance. These plant defences can be enhanced upon interaction with soil micro-organisms.
2. Here we investigated the effects of volatiles emitted by soil-borne fungi on plant defence to insect herbivory, and on plant phenology.
3. We exposed roots of *Brassica rapa* plants to volatiles emitted by four soil-borne fungi. As a proxy of plant resistance, we assessed the performance of *Pieris brassicae*, a caterpillar feeding on leaves and inflorescences, and of *Delia radicum*, an insect root herbivore. As a proxy of plant tolerance, we compared growth of volatile-exposed plants challenged with or without insects. Additionally, we assessed the effects on plant phenology by recording bolting time and by counting the number of buds and flowers.
4. Plant exposure to fungal volatiles differentially affected plant resistance to above- and below-ground herbivory. Performance of *P. brassicae* caterpillars differed between the fungal volatile-exposed plants but was variable between experimental batches. In contrast, the effects of fungal volatiles on *D. radicum* performance were predominantly negative, indicating an increased plant resistance. Despite root consumption by *D. radicum*, root dry weight remained unchanged in infested plants compared with uninfested ones, irrespectively of the volatile exposure, suggesting compensation for the tissue loss, sometimes at the cost of undamaged above-ground tissues. When *B. rapa* plants were attacked by *P. brassicae* caterpillars, only exposure to volatiles of some fungi led to compensation for the loss of above-ground tissues consumed by the caterpillars, which differed between leaves and inflorescences. Furthermore, bolting was accelerated in response to volatiles of some fungi, resulting in more buds and flowers, which suggests a potential enhancement of plant fitness.
5. Our data show that fungal volatiles can modulate the mixed-defence strategies of *B. rapa* plants, balancing plant resistance and tolerance to above- and below-

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ground herbivory. These effects may be variable and were fungus specific. Ultimately, plant fitness may be enhanced upon root exposure to fungal volatiles.

KEYWORDS

Brassica rapa, compensatory growth, *Delia radicum*, *Pieris brassicae*, plant fitness, plant phenology, resistance, tolerance

1 | INTRODUCTION

Plants are part of complex and dynamic communities, coined as the phytobiome (Leach, Triplett, Argueso, & Trivedi, 2017). Members of the phytobiome include plant-associated micro-organisms that live inside, on the surface or adjacent to plant tissues. This plant microbiome can influence several plant phenotypic traits, altering plant interactions with their associated organisms and plant fitness (Dicke, 2016; Hassani, Durán, & Hacquard, 2018; Junker & Tholl, 2013; Philippot, Raaijmakers, Lemanceau, & van der Putten, 2013). In particular, soil and root-associated micro-organisms can play a substantial role in modulating plant defence strategies to diverse biotic and abiotic stresses, with positive or negative consequences for plant fitness (Berendsen, Pieterse, & Bakker, 2012; Choudhary et al., 2016).

Plant defence strategies consist of resistance and tolerance mechanisms that co-occur in a mixed-defence strategy (Leimu & Koricheva, 2006; Stowe, Marquis, Hochwender, & Simms, 2000), and plant colonisation by micro-organisms can affect these two components concurrently (Contreras-Cornejo, Macías-Rodríguez, del-Val, & Larsen, 2016; Hermosa et al., 2013). Microbial colonisation can condition plants to respond faster and stronger to a subsequent stress (Martínez-Medina et al., 2016; Mauch-Mani, Baccelli, Luna, & Flors, 2017) or can induce systemic plant resistance, for example, by negatively affecting the preference or performance of a herbivore or pathogen *via* plant metabolomic changes (Etalo, Jeon, & Raaijmakers, 2018; Pangesti et al., 2016; Pieterse et al., 2014; van de Mortel et al., 2012). These plant responses occur locally at the site of colonisation, but also systemically affect chemical and physical plant traits. As a consequence, root colonisation by soil micro-organisms can affect subsequent plant interactions above-ground and vice versa (Bezemer & van Dam, 2005; Pineda, Kaplan, & Bezemer, 2017; van Dam & Heil, 2010). Alongside resistance, tolerance, that is, the capability of plants to endure stresses that limit plant development, can be modulated upon microbial colonisation as well. Tolerance can be experimentally measured by determining the degree to which plant growth is affected by a given stress relative to its growth in the undamaged state (Strauss & Agrawal, 1999), and plant colonisation by micro-organisms can influence the degree of stress tolerance compared with uncolonised plants. For instance, micro-organisms can alleviate the negative effects of high salinity on plant growth by boosting photosynthetic rate (Han & Lee, 2005; Yang, Kloepper, & Ryu, 2009). These studies exemplify that plant colonisation by micro-organisms can enhance plant defence to biotic and abiotic stresses, and sustain plant fitness.

Remarkably, without direct physical contact with plants, micro-organisms, such as fungi and bacteria, can also affect plant growth and

defence through the emission of volatile organic and inorganic compounds (Kanchiswamy, Malnoy, & Maffei, 2015; Piechulla, Lemfack, & Kai, 2017; Tyagi, Mulla, Lee, Chae, & Shukla, 2018). Volatiles emitted by pathogenic and beneficial micro-organisms can promote plant growth (Casarrubia et al., 2016; Cordovez et al., 2018; Fincheira & Quiroz, 2018; Moisan et al., 2019), and accelerate plant development (Moisan et al., 2019; Sánchez-López et al., 2016), for instance by increasing nutrient uptake (Liu & Zhang, 2015) or by altering phytohormone homeostasis (Bailly & Weisskopf, 2012; Zhang et al., 2007). Microbial volatiles can also enhance plant resistance to fungal, bacterial or oomycete pathogens (Frag, Zhang, & Ryu, 2013; Jain, Varma, Tuteja, & Choudhary, 2017; Kottb, Gigolashvili, Großkinsky, & Piechulla, 2015) and to insect herbivores (Aziz et al., 2016; Cordovez et al., 2017; Moisan et al., 2019). They can do so, either directly by inhibiting the attacker's activity (Bailly & Weisskopf, 2017; Vespermann, Kai, & Piechulla, 2007) or indirectly by eliciting plant resistance (Ryu, Frag, Pare, & Kloepper, 2005; Sharifi & Ryu, 2016). Upon abiotic stresses, such as salinity or drought, microbial volatiles can improve plant tolerance and sustain plant growth (Camarena-Pozos, Flores-Núñez, López, López-Bucio, & Partida-Martínez, 2019; Han et al., 2014; Jalali, Zafari, & Salari, 2017; Liu & Zhang, 2015).

Yet, to our knowledge, it remains unknown whether microbial volatiles affect plant tolerance to insect herbivory and whether these responses are specific to the plant tissue(s) being attacked. Additionally, how plant resistance and tolerance to herbivory are concurrently modulated by microbial volatiles has not been addressed. Therefore, here, we investigated the effects of volatiles emitted by soil-borne fungi on plant tolerance and resistance to above- and below-ground insect herbivory, and on plant phenology. For this, we selected the brassicaceous plant species *Brassica rapa* and its natural herbivores: the cabbage root fly *Delia radicum* (Diptera: Anthomyiidae), whose larvae feed on *B. rapa* roots, and the large cabbage white butterfly *Pieris brassicae* (Lepidoptera: Pieridae), whose caterpillars feed on leaves and inflorescences of *B. rapa* plants. Furthermore, we selected four soil-borne fungi (*Fusarium oxysporum*, *Rhizoctonia solani*, *Ulocladium atrum* and *Phoma leveillei*) that all co-occur and interact with brassicaceous plants, and have a saprophytic phase in their cycle.

2 | MATERIALS AND METHODS

2.1 | Study system

Brassica rapa L. (Brassicaceae) is an annual plant, also known as the wild turnip. Occurring in ecosystems such as cropland, weedy

fields or roadsides, *B. rapa* is subjected to attack by insect herbivores as well as pathogens. The *B. rapa* accession used in this study originated from a wild population in Maarssen (The Netherlands). All *B. rapa* seeds were first surface-sterilised before sowing by exposure to chlorine gas for 4 hr in a desiccator and stratified at 4°C in the dark for 3–4 days (Cordovez et al., 2017).

Volatiles emitted by the four soil-borne, *F. oxysporum* f. sp. *raphani* (WCS600, provided by Utrecht University, The Netherlands), *R. solani* AG2-2 IIIb (provided by the Sugar Beet Research Institute, The Netherlands), *U. atrum* (CBS 193.67, from the Westerdijk Fungal Biodiversity Institute, The Netherlands) and *P. leveillei* (CBS 373.69, from the Westerdijk Fungal Biodiversity Institute, The Netherlands) differentially affect plant growth and resistance in vitro (Moisan et al., 2019). In the present study, all fungi were inoculated in ø 9 cm plastic Petri dishes containing one-fifth strength Potato Dextrose Agar (1/5th PDA). This medium was prepared with 7.8 g of PDA (Oxoid) and 14 g of Bacto™ Agar. The pH was set at 7. Fungi were incubated at 25°C in the dark for 7 days before the start of the exposure.

First instar caterpillars of *P. brassicae* feed on leaves, whereas later instars move to the inflorescence to feed on buds and flowers (Lucas-Barbosa, van Loon, Gols, van Beek, & Dicke, 2013). Caterpillars were reared on Brussels sprouts (*Brassica oleracea* L. var. *gemmifera* cv Cyrus) in a climate room (22 ± 2°C; L16:D8; 60 ± 10% RH). Larvae of *D. radicum* feed for most of their development on primary roots of Brassicaceae (Van Dam & Raaijmakers, 2006), which results in growth retardation or plant mortality (Shuhang, Voorrips, Steenhuis-Broers, Vosman, & van Loon, 2016). Larvae were reared on rutabaga (*Brassica napus* subsp. *napobrassica*) in a climate cabinet (20 ± 1°C; L16:D8).

2.2 | Plant exposure to fungal volatiles

To expose *B. rapa* roots to fungal volatiles in vivo, we designed a two-compartment pot system (Figure 1). One sterile *B. rapa* seed was sown in the top compartment ($h = 20$ cm, $\phi = 12.5$ cm) filled with a sterile (i.e. autoclaved twice at 121°C for 20 min with 24 hr interval in between) soil mixture (1:1 v/v, ø 4 mm sieved Horticoop potting soil:sand), whereas the test fungus (*F. oxysporum*, *R. solani*, *U. atrum* or *P. leveillei*) was grown in a ø 9 cm Petri dish enclosed in the bottom compartment ($h = 10$ cm, $\phi = 12.5$ cm). Both compartments were connected to each other by a cylinder ($h = 12.5$ cm, $\phi = 12.7$ cm), and separated by a nylon membrane of 1 µm mesh width ($\phi = 14.5$ cm) that allowed air exchange between the two compartments. Volatile exposure was initiated in a greenhouse compartment (21 ± 2°C; L16:D8; 70 ± 5% RH) with 7-day-old fungi as soon as *B. rapa* seeds were sown, and was maintained for 4 weeks, after which *B. rapa* plants had 6–8 fully developed leaves. Control plants were exposed to a Petri dish containing one-fifth PDA medium only. Petri dishes containing the fungi and control were replaced weekly with Petri dishes containing fresh 7-day-old fungi or fresh one-fifth PDA medium. A total of 30 plant replicates was prepared for each fungal volatile exposure and divided in two experimental batches, with 1 week interval.

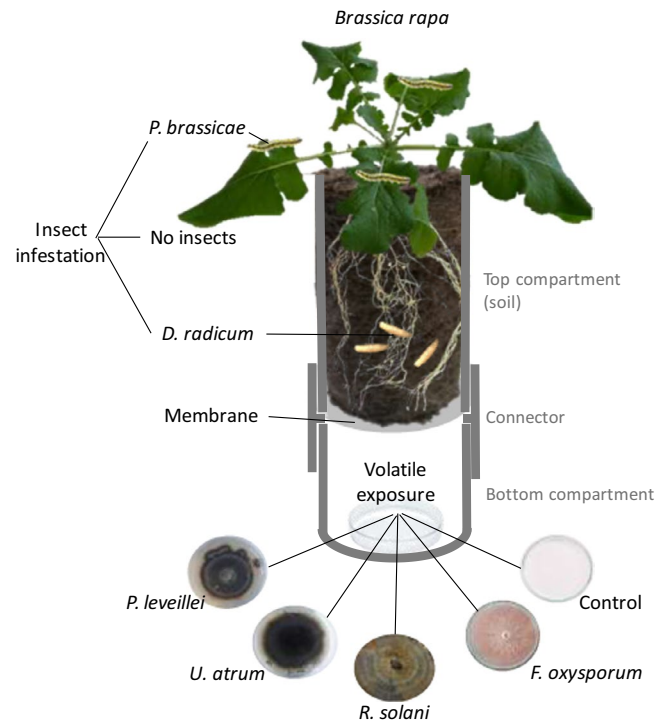


FIGURE 1 Schematic representation of the two-compartment pot system used in this study to expose roots of *Brassica rapa*, growing in soil in the top compartment, to volatiles emitted by one fungus growing in a Petri dish in the bottom compartment or by a control Petri dish. Plants were subsequently infested with either caterpillars of the above-ground herbivore, *Pieris brassicae*, or with larvae of the below-ground herbivore, *Delia radicum*; or remained uninfested (no insects)

2.3 | Plant infestation with above-ground and below-ground herbivores

After the 4-week volatile exposure, all Petri dishes were removed permanently from the bottom compartments, and the *B. rapa* plants were either infested with one of the two insect herbivores or remained uninfested. For *P. brassicae*, 20 newly hatched caterpillars were placed on the third fully expanded leaf. For *D. radicum*, 10 newly hatched larvae were placed close to the plant stem and watched until all larvae crawled down to the roots. Per experimental batch, the 15 plant replicates of each fungal volatile exposure were divided as follows: five replicates infested with *P. brassicae*, five replicates infested with *D. radicum* and five replicates remained uninfested.

2.4 | Effects of fungal volatile exposure on growth of uninfested plants

To assess the effects of fungal volatile exposure on plant growth, we measured the dry weight of 6-week-old uninfested *B. rapa* plants after the 4 weeks of exposure to fungal volatiles. Roots, leaves and inflorescences were harvested, dried at 105°C for 16 hr, and weighed. Effects of root exposure to fungal volatiles on the total plant dry weight as

well as on the dry weight of the different plant tissues were separately tested with linear models (PROC GLM in SAS v. 9.4). Fungal volatile exposure, batch, and their interactions were included in the model as fixed factors (Supporting Information; Moisan et al., 2020b). Upon a significant main effect of the fungal volatile exposures, post hoc tests were performed using the *t* distribution. We also tested the effects of fungal volatiles on plant dry weights per batch.

2.5 | Effects of fungal volatile exposure on plant growth upon above-ground and below-ground herbivory

To assess the effects of fungal volatile exposure on plant tolerance to above-ground and below-ground herbivores, we measured the dry weight of *B. rapa* plants whose roots were previously exposed to fungal volatiles and then infested with either *P. brassicae* caterpillars or with *D. radicum* larvae, and compared it to that of volatile-exposed uninfested plants. Using a mixed model (PROC MIXED in SAS), we specified an unstructured covariance matrix that allows for correlations and inequality of variances among plant tissues of the same plant (Supporting Information; Moisan et al., 2020b). Fungal volatile exposure, plant tissue, herbivory, batch, and their interactions were included in the model as fixed factors. From this model, we generated differences of least squares means of plant tissue dry weights (a) between *B. rapa* infested with *P. brassicae* and uninfested *B. rapa* plants, and (b) between *B. rapa* infested with *D. radicum* and uninfested *B. rapa* plants, within each volatile exposure (Table S1). For each plant tissue and volatile exposure, the effect of herbivory was tested by comparing the above described differences to zero, using the *t* distribution ($\alpha = 0.05$). For the visualisation of the data, we plotted the Cohen's *D* effect sizes by dividing the differences of least square means by the pooled standard deviation of each plant tissue. We ran the same analyses per batch.

2.6 | Effects of fungal volatile exposure on above-ground and below-ground herbivore performance

To assess the effects of fungal volatile exposure on plant resistance to above-ground and below-ground herbivores, we measured the performance of *P. brassicae* caterpillars and *D. radicum* larvae on plants whose roots were previously exposed to fungal volatiles. Individual fresh weight of *P. brassicae* caterpillars was assessed at 3 days post infestation (dpi) and at 7 dpi. At 3 dpi, all 20 *P. brassicae* caterpillars were recollected, and larval density was reduced by 50% to mimic natural field predation and dispersal (Lucas-Barbosa et al., 2013). For this, 10 larvae per plant were randomly selected, individually weighed and placed back on their respective plants until the second measurement at 7 dpi. Caterpillar fresh weight was ¹⁰log-transformed. Separately for the two time points, we used a linear mixed model (LMM) in R (v.3.4.0; R Development Core Team, 2011) with fungal volatile exposure, batch, and their interaction as fixed factors and plant replicate as a random factor (Supporting Information; Moisan et al., 2020b). Effects of fungal

volatile exposure on the caterpillar fresh weight were compared using approximate *F* tests with degrees of freedom calculated according to the method of Kenward and Roger (Kenward & Roger, 1997), followed by post hoc tests using the *t* distribution. We also tested the effects of fungal volatiles on caterpillar fresh weight per batch. At 14 dpi, we also scored the developmental stage (larvae or pupae) of recollected *D. radicum*, and individually weighed the recollected insects. A mixed model similar to that used for *P. brassicae* was used to analyse *D. radicum* fresh weight. Additionally, the number of recovered *D. radicum* (out of the 10 insects initially added) and the fraction of recovered *D. radicum* pupae were analysed using a beta-binomial (to handle binomial overdispersion) generalised linear model (GLM) and logit link function (Supporting Information; Moisan et al., 2020b). For this, we used the GLMMTMB package in R (Brooks et al., 2017). In this model, fungal volatiles, batch and their interaction were included as fixed factors. Upon a significant main effect of the fungal volatile exposures, likelihood ratio post-hoc tests (LRT) were performed. Furthermore, to estimate whether the insect performance was correlated with food intake or food available, we calculated Pearson correlations (a) between the average *P. brassicae* fresh weight at 7 dpi and leaf and inflorescence dry weights, (b) between *D. radicum* fresh weight, root dry weight and the number of *D. radicum* individuals recollected and (c) between average *P. brassicae* fresh weight at 7 dpi or *D. radicum* fresh weight per fungal volatile exposure and the mean of tissue dry weight difference of uninfested and infested plants ($\alpha = 0.05$).

2.7 | Effects of fungal volatile exposure on plant bolting upon herbivory

To assess the effects of fungal volatile exposure on phenology of *B. rapa* plants upon above-ground and below-ground herbivory, we recorded bolting date, as well as total count of buds and flowers of 6-week-old *B. rapa* plants. The cumulative percentage of bolting *B. rapa* plants whose roots were exposed to volatiles of different fungi and upon different herbivories was plotted against time. Time until bolting was statistically analysed in SAS using a Cox proportional hazard model (PROC PHREG; Lin & Wei, 1989), which can handle incomplete (censored) observations of plants that did not bolt within 42 days (Supporting Information; Moisan et al., 2020b). In the Cox model, bolting time was allowed to depend on fungal volatile exposure, herbivory, batch and their interactions. Total count of buds and flowers of *B. rapa* plants following root exposure to volatiles of different fungi and upon different herbivories was modelled with a GLM with a log-link and a negative binomial distribution (type 1; Hilbe, 2011). Fungal volatile exposure, herbivory, batch, and their interactions were tested as fixed factors. Upon a significant main effect of the fungal volatile exposures in one of the two models described above, LRT post hoc tests were performed. We also tested the effects of fungal volatiles, herbivory and their interaction on the number of buds and flowers per batch. To assess the relative investment of plants in bud development, the number of buds and flowers per unit of plant dry weight was calculated and analysed with an ordinary linear model. Fungal volatile

exposure, herbivory, batch and their two-way interactions were included in the models as fixed factors.

3 | RESULTS

3.1 | Effects of fungal volatile exposure on growth of uninfested plants

Fungal volatiles affected total dry weight of uninfested *B. rapa* plants (Figure 2; LM; $p = 0.039$). Growth of control plants did not differ from growth of volatile-exposed plants (Figure 2; post hoc tests; all $p > 0.050$), however, plants exposed to *F. oxysporum* volatiles were overall smaller than plants exposed to *R. solani* volatiles (Figure 2; post-hoc tests; $p = 0.032$) and to *U. atrum* volatiles (Figure 2; post hoc tests; $p = 0.003$). Fungal volatiles particularly affected leaf dry weight (Figure 2; LM; $p = 0.044$) but not root or inflorescence dry weights (Figure 2; LM; $p = 0.147$ and 0.175 , respectively). Plants exposed to volatiles from *U. atrum* had higher leaf dry weight than the control plants and the other fungal volatile-exposed plants (Figure 2; post hoc tests; all $p < 0.050$). Volatile exposure had a significant interaction with batch on the total plant dry weight (Figure 2; LM; $p = 0.022$) and leaf dry weight (Figure 2; LM; $p = 0.036$), so the effects of fungal volatiles varied between the two batches (Figure S1).

3.2 | Effects of fungal volatile exposure on plant growth upon above-ground and below-ground herbivory

Overall, herbivory by *P. brassicae* negatively affected weight of above-ground tissues. This reduction was expected as *P. brassicae*

caterpillars feed on above-ground tissues. Yet, the effects differed between tissues. When infested with *P. brassicae*, inflorescences of control plants (i.e. plants not exposed to fungal volatiles) and plants whose roots were exposed to *R. solani* volatiles weighed less than inflorescences of uninfested plants (Figure 3a; t tests; $p_{\text{control}} = 0.038$; $p_{R. solani} = 0.005$). Also, *P. brassicae*-infested plants exposed to *U. atrum* volatiles had lower leaf weight than uninfested plants (Figure 3a; t test; $p = 0.020$). However, leaf and inflorescence weights of plants whose roots were exposed to *F. oxysporum* and *P. leveillei* volatiles did not significantly differ between *P. brassicae*-infested plants and uninfested plants (Figure 3a and Table S1a; t tests; all $p > 0.050$).

Despite an overall reduction, infestation by *D. radicum* did not significantly impact root weight, irrespective of the fungal volatiles they had been exposed to (Figure 3b and Table S1b; t tests; all $p > 0.050$). However, infestation by *D. radicum* resulted in lower inflorescence weight upon exposure to *R. solani* volatiles (Figure 3b; t test; $p = 0.023$), and in lower leaf weight upon exposure to *U. atrum* volatiles (Figure 3b; t test; $p = 0.039$), compared with uninfested plants. We detected a significant interaction between batch and fungal volatiles (Table S2) thus, the effects of fungal volatiles varied between the two batches (Figure S2).

3.3 | Effects of fungal volatile exposure on above-ground and below-ground herbivore performance

Plant exposure to fungal volatiles affected *P. brassicae* caterpillar weight at 3 and 7 dpi (Figure 4; LMM; $p = 0.014$ and 0.006 , respectively). At 3 dpi, caterpillars feeding on plants whose roots were exposed to volatiles from *R. solani* or *U. atrum* were larger than those feeding on plants exposed to volatiles from *F. oxysporum* or *P. leveillei* (Figure 4; post hoc tests; $p < 0.050$). At 7 dpi, caterpillars feeding on

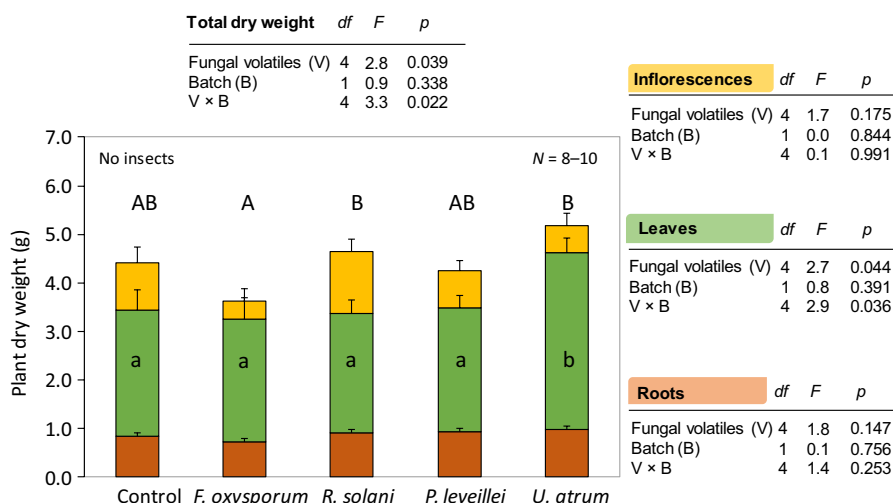


FIGURE 2 Root, leaf and inflorescence dry weight (mean + SE) of uninfested 6-week-old *Brassica rapa* plants exposed for 4 weeks to volatiles of four different fungi (*Fusarium oxysporum*, *Rhizoctonia solani*, *Phoma leveillei* and *Ulocladium atrum*). Main effects of the fungal volatile exposure, batch and their interactions were tested using linear models. 'N' indicates the number of plant replicates per treatment combination. Uppercase letters indicate pairwise differences of the total plant dry weight between the fungal volatile exposures, while lowercase letters indicate pairwise differences of the leaf dry weight between the fungal volatile exposures ($\alpha = 0.05$)

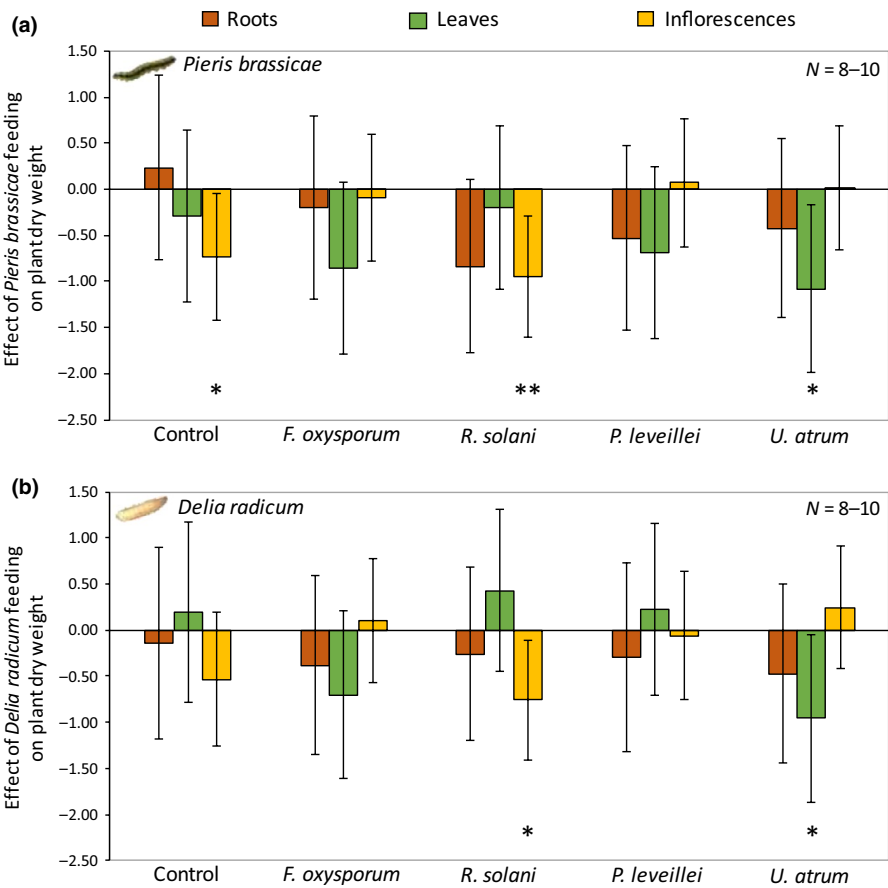


FIGURE 3 Cohen's D effect sizes [(differences of least squares means/ pooled SD) \pm CI] in root, leaf and inflorescence dry weight of (a) 6-week-old *Brassica rapa* plants infested with *Pieris brassicae* and uninfested plants, and of (b) 6-week-old *B. rapa* plants infested with *Delia radicum* and uninfested plants, when exposed to volatiles of four different fungi (*Fusarium oxysporum*, *Rhizoctonia solani*, *Phoma leveillei* and *Ulocladium atrum*). Differences of least squares means were generated using a mixed model that allows for correlations and inequality of variances among plant tissues of the same plant, and were statistically tested per plant tissue and per fungal volatile exposure using the t distribution ($*p < 0.050$; $**p < 0.010$). 'N' indicates the number of plant replicates per treatment combination. Detailed information of the least squares means can be found in Table S1

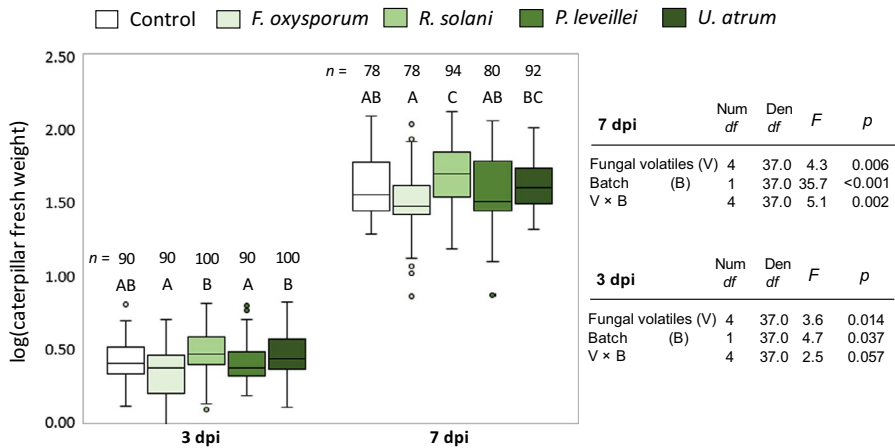


FIGURE 4 Individual fresh weight (log10) of *Pieris brassicae* caterpillars at 3 and 7 days post-infestation (dpi) when feeding on leaves and inflorescences of *Brassica rapa* plants exposed to volatiles of four different fungi (*Fusarium oxysporum*, *Rhizoctonia solani*, *Phoma leveillei* and *Ulocladium atrum*). Each box-and-whisker shows the distribution of the dataset into quartiles: the minimum, first quartile, median, third quartile and maximum. Dots show outliers. Main effects of the volatile exposure, batch and their interactions were tested per time point using a mixed model, with plant replicate as a random factor. Uppercase letters indicate overall effects of volatile exposure using post hoc tests ($p < 0.05$). 'n' indicates the number of caterpillars recollected. Each plant was infested with 10 *P. brassicae* neonates, and each volatile exposure was replicated 9–10 times

plants exposed to *R. solani* volatiles were larger than those feeding on control plants and plants exposed to volatiles from *F. oxysporum* or *P. leveillei* (Figure 4; post hoc tests; $p < 0.050$). Additionally, batch has a significant effect on caterpillar fresh weight at the two time points (Figure 4; LMM; $p = 0.037$ and 0.001 , respectively), and interacts

with fungal volatile exposure at 7 dpi (Figure 4; LMM; $p = 0.002$). Thus, the effects of fungal volatiles varied between the two batches (Figure S3). Caterpillar weight at 7 dpi was neither correlated with leaf nor inflorescence weight of *P. brassicae*-infested plants (Table S3b; Pearson correlation tests; all $p > 0.050$), nor with the difference of

weight of these plant tissues between *P. brassicae*-infested and uninfested plants (Table S3c; Pearson correlation tests; all $p > 0.050$).

Plant exposure to fungal volatiles did not affect the number of *D. radicum* we recollected (Table S4; GLM; $p = 0.058$). However, it did affect the insect developmental stage reached by the larvae (Figure 5a; GLM; $p < 0.001$). In three out of the four fungal volatile exposures tested (*F. oxysporum*, *R. solani* and *P. leveillei*), we recollected fewer pupae than in control plants (Figure 5b; LRT post hoc; all $p < 0.050$),

except for plants exposed to *U. atrum* volatiles (Figure 5a; LRT post hoc; $p = 0.723$). Fresh weight of *D. radicum* was not affected by fungal volatile exposure (Figure 5b; GLM; $p = 0.081$) and did not correlate with the number of *D. radicum* recollected (Table S3a; Pearson correlation tests; $p = 0.219$), or with the difference in root weight between *D. radicum*-infested and uninfested *B. rapa* plants (Table S3c; Pearson correlation tests; $p = 0.275$). However, insect fresh weight and the number of individuals recollected overall correlated positively with

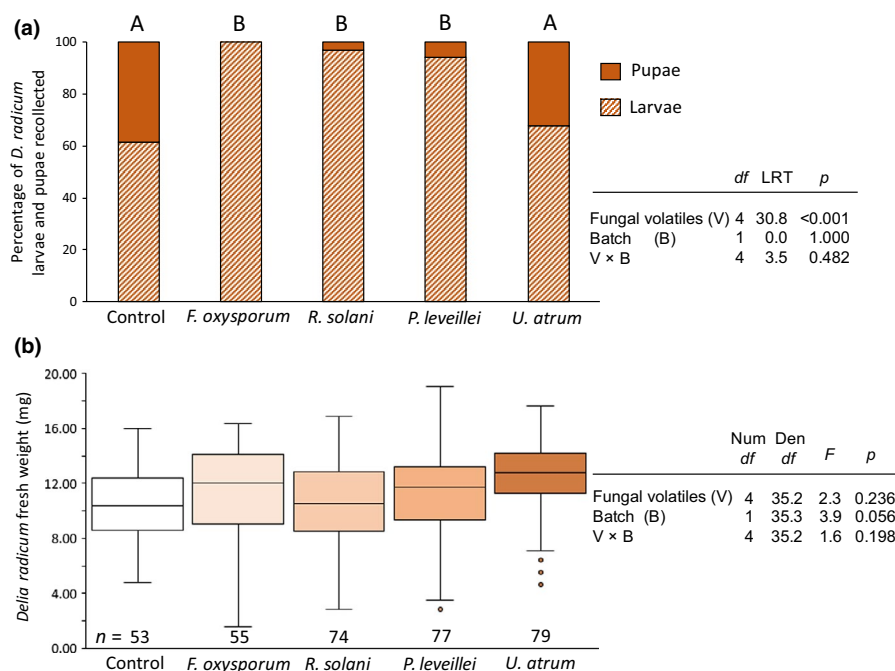


FIGURE 5 (a) Percentage of larvae and pupae of *Delia radicum* collected from roots of *Brassica rapa* plants exposed to volatiles of four different fungi (*Fusarium oxysporum*, *Rhizoctonia solani*, *Phoma leveillei* and *Ulocladium atrum*) and (b) fresh weight of the individuals. 'n' indicates the total number of individuals (pupae and larvae) recollected. Each box-and-whisker shows the distribution of the dataset into quartiles: the minimum, first quartile, median, third quartile, and maximum. Dots show outliers. For the fraction of larvae and pupae, main effects of the volatile exposure, batch, and their interaction were tested using a generalised linear model with a beta-binomial distribution, and for the insect fresh weight we used a mixed model, with plant replicate as a random factor. Uppercase letters indicate pairwise differences in percentages of larvae and pupae between fungal volatile exposures using likelihood ratio post hoc tests (LRT). Each plant was infested with 10 *D. radicum* neonates, and each volatile exposure was replicated 7–10 times

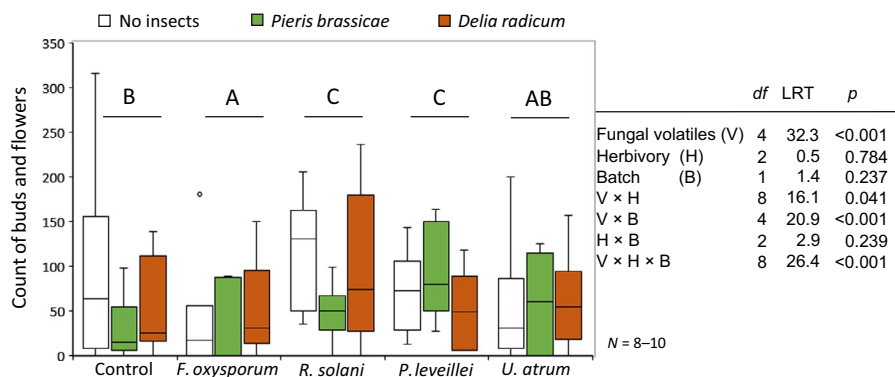


FIGURE 6 Total count of buds and flowers of 6-week-old *Brassica rapa* plants when exposed to volatiles of four different fungi (*Fusarium oxysporum*, *Rhizoctonia solani*, *Phoma leveillei* and *Ulocladium atrum*) and upon different herbivore infestations. Each box-and-whisker shows the distribution of the dataset into quartiles: the minimum, first quartile, median, third quartile and maximum. Dots show outliers. Main effects of the volatile exposure, herbivory, batch and their interactions were tested using a generalised linear model with a negative binomial distribution. Uppercase letters indicate pairwise differences between the fungal volatile exposures using likelihood ratio tests. 'N' indicates the number of plant replicates per treatment combination

root dry weight of *D. radicum*-infested plants (Table S3a; Pearson correlation test; $p = 0.023$; $p = 0.031$ respectively).

3.4 | Effects of fungal volatile exposure on plant bolting upon herbivory

Fungal volatiles affected bolting time of *B. rapa* plants (Figure S4; Cox; $p = 0.002$). Overall, plants exposed to *P. leveillei* and *R. solani* volatiles bolted faster than control plants (Figure S4; LRT post hoc; $p_{P. leveillei} = 0.012$; $p_{R. solani} = 0.018$) and plants exposed to *U. atrum* volatiles (Figure S4; LRT post hoc; $p_{P. leveillei} = 0.005$; $p_{R. solani} = 0.007$) or *F. oxysporum* volatiles (Figure S4; LRT post hoc; $p_{P. leveillei} = 0.002$; $p_{R. solani} = 0.004$). The effects of fungal volatiles on bolting time of *B. rapa* plants was not influenced by herbivore infestation (Figure S4; Cox; $p = 0.177$). In addition, fungal volatiles affected the total number of buds and flowers of *B. rapa* plants (Figure 6; GLM; $p < 0.001$) but not the number of buds and flowers per unit of plant dry weight (Figure S5; LM; $p_{volatiles} = 0.491$). On average, plants whose roots were exposed to *P. leveillei* and *R. solani* volatiles had more buds and flowers than control plants (Figure 6; LRT post hoc; $p_{P. leveillei} = 0.003$; $p_{R. solani} = 0.009$) and plants exposed to *F. oxysporum* or *U. atrum* volatiles (Figure 6; LRT post hoc; $p < 0.050$). Effects of the fungal volatiles were influenced by the experimental batch (Figure 6; GLM; $p = 0.005$), and the effects of fungal volatiles varied between the two batches (Figure S6).

4 | DISCUSSION

We found that volatiles emitted by four soil-borne fungi differentially affected *B. rapa* resistance and tolerance to herbivory by *P. brassicae* caterpillars and *D. radicum* larvae. Effects on *P. brassicae* performance varied between the different fungi and between the batches, whereas the effects on *D. radicum* performance was predominantly negative, indicating an increased plant resistance. Despite an overall reduction of root weight upon attack by the root herbivore *D. radicum*, *B. rapa* plants remained tolerant and compensated for the loss of root tissues. In contrast, attack by *P. brassicae* caterpillars led to an overall reduction of above-ground tissues and compensation varied between the tissues and between the fungal volatile exposures. Root exposure to *R. solani* or *P. leveillei* volatiles accelerated plant phenology, which resulted overall in more buds and flowers. Altogether, our data show that fungal volatiles can modulate plant mixed-defence strategy, balancing plant resistance and tolerance to above-ground and below-ground herbivory. These effects are variable and occur in a fungal-volatile-specific manner.

4.1 | Fungal volatiles influenced plant growth and tolerance upon herbivory

Exposure of *B. rapa* roots to fungal volatiles differentially affected growth of uninfested plants and insect-infested plants,

thus influencing plant tolerance. Although *D. radicum* larvae feed intensively on roots, we did not observe a reduction of root weight compared to uninfested plants, suggesting that fungal volatile-exposed plants remained tolerant and compensated for the loss of root tissues (Mesmin et al., 2019). Interestingly, upon certain volatile exposure, this compensatory growth occurred at the cost of undamaged above-ground tissues. In contrast, herbivory by *P. brassicae* caterpillars resulted in a reduction of *B. rapa* plant weight compared to uninfested plants. Nonetheless, these tissue losses differed between leaves and inflorescences and between fungal volatile exposures. These findings suggest that compensatory plant growth to herbivory may result from a reallocation of resources within the plant, for example, from above-ground tissues to roots (Núñez-Farfán, Fornoni, & Valverde, 2007; van Dam, 2009), and that plant exposure to fungal volatiles can specifically modulate this reallocation between tissues, sometimes at the cost of undamaged tissues. Yet, the effects of fungal volatiles on biomass of uninfested and insect-infested plants were variable between batches, suggesting that plant responses may be highly susceptible to the smallest variation in the fungal volatile emission. A thorough analysis of resource partitioning and allocation to storage and defence upon exposure to specific fungal volatiles will provide a better understanding of plant tolerance to herbivory following plant exposure to fungal volatiles.

4.2 | Effects of fungal volatiles on plant resistance differ between insect herbivores

Most fungal volatile exposures resulted in a reduced *D. radicum* development rate, indicating increased direct plant resistance, whereas the effects on performance of *P. brassicae* caterpillars differed between the fungi. For instance, *P. brassicae* caterpillars feeding on plants whose roots were exposed to *R. solani* volatiles were larger than those feeding on control plants and fungal volatile-exposed plants, indicating higher plant susceptibility. This finding corroborates previous studies that reported larger *Mamestra brassicae* caterpillars when feeding on *Arabidopsis thaliana* seedlings exposed to VOCs from *R. solani* (Cordovez et al., 2017; Moisan et al., 2019). As the average insect fresh weights did not correlate with the difference of biomass between infested plants and uninfested plants, we conclude that the insect performances were not correlated with the amount of plant tissues consumed. Instead, slower development and lower body mass increase of the insect may result from changes in plant chemistry and morphological traits, which can lead to chemically or structurally more resistant roots upon fungal volatile exposure. For example, root exposure to fungal volatiles may alter architecture of primary and lateral roots (Casarrubia et al., 2016; Ditengou et al., 2015; Garnica-Vergara et al., 2015), which in turn, can negatively impact the performance of root herbivores (Felkl, Jensen, Kristiansen, & Andersen, 2005; Werner, Polle, & Brinkmann, 2016). Also, plant exposure to microbial VOCs can promote the accumulation of defensive secondary

metabolites such as glucosinolates in leaves, which can diminish the performance of leaf caterpillars (Aziz et al., 2016). Levels of indole glucosinolates in the main roots can also slow down larval development (Van Dam & Raaijmakers, 2006).

For a comprehensive overview of plant resistance, it would be interesting to explore whether fungal volatiles affect behaviour of the insect herbivores. Plant exposure to fungal volatiles may have altered nutrient levels in some plant tissues, making the tissues repellent/attractive or unpalatable to the insect herbivores, thus positively or negatively impacting insect performance (Schoonhoven, Van Loon, & Dicke, 2005; Smallegange et al., 2007). As we did not monitor the position of caterpillar feeding over time, it is also plausible that fungal volatile exposure also influenced herbivore feeding preference by differentially altering the nutritional quality of leaves and inflorescences (Smallegange et al., 2007; Wetzels, Kharouba, Robinson, Holyoak, & Karban, 2016). Interestingly, the effects of fungal volatiles on *P. brassicae* performance were also influenced by the batches, which may be linked with the differential plant responses per batch, for example, plant biomass as discussed above. A thorough analysis of the chemistry of the different tissues and a daily monitoring of the insect feeding sites will further improve the understanding of the specific modulation of plant resistance to above-ground and below-ground herbivory by fungal volatiles.

4.3 | Acceleration of bolting time by fungal volatiles suggests enhancement of plant fitness

Effects of fungal volatiles on plant phenology differed between the fungi but may increase reproductive success. Plant exposure to volatiles emitted by *R. solani* or *P. leveillei* accelerated overall plant bolting and enhanced production of buds and flowers. An acceleration of bolting could be disadvantageous for the plant as *P. brassicae* caterpillars prefer to feed on inflorescences (Smallegange et al., 2007), but it can also result from an escape strategy to reproduce faster (Lucas-Barbosa et al., 2013). By producing more buds and flowers and quicker, plants increase their chance of reproductive success, which would give a clear advantage for plants surrounded by potential pathogens and challenged with an insect herbivore. Yet, acceleration of flowering seems a common plant phenomenon in response to volatiles emitted by fungi of different lifestyles, including beneficial fungi (Cordovez et al., 2017; Moisan et al., 2019; Sánchez-López et al., 2016). Thus, we hypothesise that plant responses are specific to some fungal volatiles, which consequently affect plant defences to herbivores. To further assess the effects on plant fitness, it remains to be tested whether seed set is ultimately influenced by root exposure to fungal volatiles.

5 | CONCLUSIONS

Our results show that fungal volatiles can modulate plant mixed-defence strategies, balancing plant resistance and tolerance to

above- and below-ground herbivory in a fungus-specific manner. Yet, it remains to be investigated how these results obtained in controlled conditions with single fungal isolates can be extrapolated to natural ecosystems where plant roots are exposed to volatiles emitted simultaneously or in sequence by diverse fungal and other microbial communities. In such future studies, one should also address how these responses affect subsequent plant interactions with mutualists, for instance pollinators that are essential for reproduction of obligate outcrossing plant species such as *B. rapa*.

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AUTHORS' CONTRIBUTIONS

K.M., V.C., J.M.R., M.D. and D.L.-B. planned and designed the research; K.M. and M.A. performed the bio-assays; K.M. and G.G. analysed the data; K.M., M.A., V.C., J.M.R., M.D. and D.L.-B. interpreted the data and wrote the manuscript.

DATA AVAILABILITY STATEMENT

Data are deposited in the Dryad Digital Repository <https://doi.org/10.5061/dryad.d2547d816> (Moisan et al., 2020a).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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