Plants Suppress Their Emission of Volatiles When Growing with Conspecifics

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Abstract Plant volatiles mediate interactions with herbivores, herbivore enemies, and abiotic stresses, but these interactions mostly have been studied with individual isolated plants. It is not yet known how intra- and interspecific plant competition influence volatile emission. In a greenhouse experiment, we investigated the volatile emission by red clover (*Trifolium pratense*) growing alone, with a conspecific, or with an individual of the naturally co-occurring orchard grass, *Dactylis glomerata*. The individual and combined effects of above- and below ground plant contact were investigated. When *T. pratense* grew together with a conspecific, both total and herbivore-induced emission of volatiles was significantly reduced as compared to *T. pratense* growing with *D. glomerata* or growing alone. This reduction in emission occurred despite the fact that there was a significant reduction in *T. pratense* biomass due to competition with *D. glomerata*.

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The suppression of *T. pratense* volatile emission growing next to a conspecific was a general pattern observed for all major herbivore-induced volatiles and independent of whether plants were in contact above ground, below ground, or both above- and below ground. The reduction in volatile emission from plants growing with conspecifics may serve to reduce attack by specialist herbivores and minimize exploitation of herbivore attack information by neighbors.

Introduction

Plants emit numerous volatile organic compounds from their foliage in variable amounts, but the greatest emission and highest number of emitted substances usually occur after herbivore feeding (Unsicker et al., 2009; Dicke and Baldwin, 2010). Herbivore-induced volatiles (HIVs) serve as direct defenses against the feeding and oviposition of certain insects (Unsicker et al., 2009), and also in indirect defense as cues for herbivore enemies in locating their prey (Dudareva et al., 2006). To determine the constraints acting on these defenses, researchers have studied the effect of numerous biotic and abiotic stress factors on HIV emission (Holopainen and Gershenzon, 2010; Kegge and Pierik, 2010). However, the influence of plant—plant interactions on volatile emission has not been investigated in detail, although plant competition and herbivory are considered the most important selective forces shaping natural plant communities (Crawley, 1989).

Competitive interactions between plants are driving forces in determining plant community composition and diversity (Tilman, 1994). Neighboring plants compete for resources such as light, nutrients, and water, and the competitive strength of a plant can determine its abundance and

persistence in a natural environment (Ballare, 2009; Hautier et al., 2009; Novoplansky, 2009). By reducing resources, competition may alter allocation to growth, reproduction, and defense, and thus influence the level of investment in defensive metabolites, such as volatile compounds (Herms and Mattson, 1992; Cipollini, 2004; Walters and Heil, 2007). The impact of plant competition is affected by whether interactions occur above- or below ground, and whether the competitor is a conspecific or a heterospecific (Wilson, 1988). Conspecifics may be stronger competitors than heterospecifics as they have similar resource requirements, and thus, detrimental effects of competition on individual plants often occur in monocultures (Darwin, 1859; Farrer and Goldberg, 2011). However, there also are counter-examples in the literature where inter-specific competition is stronger or of similar strength than intra-specific competition (Goldberg and Barton, 1992; Fortner and Weltzin, 2007).

Competition could affect the ability of plants to emit volatile compounds, but volatiles themselves also are involved in competition either as cues that trigger competitive responses among plants (Pierik et al., 2003) or as direct agents of competition via allelopathy (Kegge and Pierik, 2010). The potential roles of volatiles in plant—plant interactions provide further incentive to understand how these interactions alter plant volatile profiles.

We performed a greenhouse experiment to determine how volatile emission from a focal plant changes due to contact with either conspecific or heterospecific neighbors. Plants were grown together with either above ground contact, below ground contact, or both at the same time (Fig. 1). Two typical European grassland species were used that naturally co-occur, the legume *Trifolium pratense* L. (red clover) as the focal species, and the grass *Dactylis glomerata* L. (orchard grass) as the heterospecific neighbor. *Trifolium pratense* has been shown to release a complex blend of volatiles comprising terpenoids, benzenoids, and fatty acid derivatives (Kigathi et al., 2009). We asked the following questions: a) Does *T. pratense* volatile emission change when plants interact with another *T. pratense* or with *D. glomerata*? b) Does the response differ between constitutive (continuously emitted) volatiles and those induced by herbivores? c) Do the results depend on whether the interaction occurs above- or below ground? We hypothesized that both the species identity of the neighboring plant as well as the location of contact in this interaction would affect red clover volatile emission.

Methods and Materials

Plant Material and Experimental Design

Trifolium pratense and *Dactylis glomerata* seeds were purchased from Rieger Hofmann, (Blaufelden Raboldshausen,

Germany) and germinated individually in plastic trays filled with commercially available soil (Tonsubtrat®, Klassman Deilmann, Geeste, Germany, pH 5.0–6.0, N: 160–260 mg/l) in a greenhouse (day : night temperatures, 20–22 °C:18–20 °C; 30–55 % rel. humidity, 16 hr light, photosynthetically active radiation (PAR)~180 μmol m^{-2} sec^{-1}). By the middle of March 2010, 3 weeks after plant germination, individual plants were transplanted into custom designed 400 ml plastic pots ($7 \times 7 \times 8.5$ cm) filled with a 2:1 mixture of soil (Tonsubtrat®) and sand. In the root contact treatments, pots with openings on one side (5×6 cm) were connected to allow root interaction (Figure S5 c, d). Each individual plant was watered by using an automatic drip irrigation system. Plants were not supplied with additional nutrients. Two days after transplanting, the above ground part of each plant was covered with a polyethylene terephthalate (PET) bag. Depending on the type of contact, bags were either connected (20×5 cm) to allow permanent air exchange between the two plants (above ground contact), or left unconnected (no above ground contact) (Fig. S1a). Plants were arranged in five blocks in the greenhouse to control for potential light or temperature gradients. Each of these blocks included the full set of species interactions, i.e., three neighbor regimes (*T. pratense* growing singly, *T. pratense* interacting with another *T. pratense* plant or with *D. glomerata*), and two herbivory treatments ("no herbivory" and "herbivory"). Each of the intra- and inter-specific regimes included three types of contacts ("above ground", "below ground" and "full contact"), resulting in 14 treatment combinations in each of the five blocks.

Insects and Herbivore Treatment

Spodoptera littoralis Boisd. (Lepidoptera, Noctuidae) caterpillars were hatched from eggs that were provided by Syngenta (Switzerland) and reared on an artificial bean-based diet (Fontana et al., 2009) in a growth chamber at 23–25 °C with 16/8 hr L/D cycle until they reached the third instar. Two third instars (that had been starved previously for 24 hr) were allowed to feed overnight for approximately 12 hr on *T. pratense*. Caterpillar-inflicted leaf damage was approximately 5–10 % with no significant difference among treatments (Fig. S3c). The herbivores were applied 4 weeks after the plants had been introduced into the different regimes and contact treatments described above.

Volatile Collection and Analysis

The volatile compounds emitted from *T. pratense* after *S. littoralis* feeding and the volatiles emitted constitutively from undamaged control plants were collected in a dynamic headspace collection system set up *in situ* around the plants in the greenhouse under the conditions described above. New PET bags with the same dimensions (1.67 l volume)
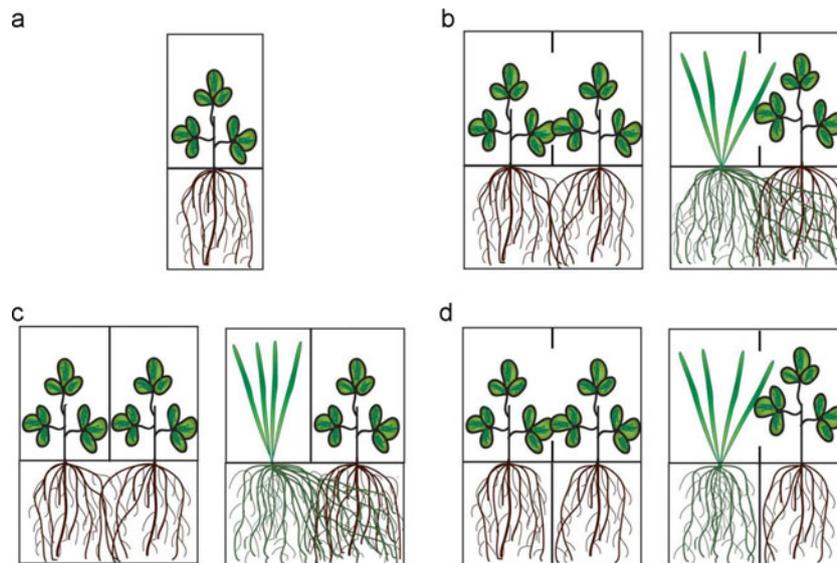
Fig. 1 Scheme of the Experiment. The focal species, *Trifolium pratense* (red clover), was grown alone, with another *T. pratense* or with *Dactylis glomerata* (orchard grass). All plants were grown in polyethylene terephthalate bags under the following treatments: **a** Control: individually grown *T. pratense* plants. **b** Full contact: plants had both above ground and below ground contact through openings in the pots and the bags. **c** Below ground contact: Plants had no above ground contact but root contact through an opening in the pots. **d** Above ground contact: plants had no root contact but had above ground contact through openings in the bags. Within each of these four contact treatments, there were 10 replicates for each species combination. Five of the replicates were subjected to herbivory by *Spodoptera littoralis* larvae. Volatiles were collected immediately after 12 hr of herbivory

as the bags under which the plants had grown were installed on each *T. pratense* plant, both herbivore-treated and control plants, just before the caterpillars were released for the herbivory treatments. Prior to volatile collection, caterpillars were removed from the plants. Charcoal-filtered air was pumped into the bags at a flow rate of 2 l min^{-1}. Outgoing air containing plant volatiles was pumped out of the bags at 0.4 l min^{-1} through a collection trap containing 40 mg Porapak adsorbent (ARS, Gainesville, FL, USA) in a Teflon tube that was inserted in the PET bag by attachment through an opening in the Plexiglas lid (Fig. S1). All volatile collections were performed between 1100 and 1600 hr. After 4 hr collection, traps were eluted with 150 μl dichloromethane that contained 1.5 μg of nonyl acetate as internal standard.

Volatiles were analyzed with a Hewlett-Packard model 6890 gas chromatograph employing the carrier gas He at 1 ml min^{-1}, splitless injection (injection temperature: 220 °C, injection volume: 1 μl), a DB-5MS column (30 m×0.25 mm, 0.25 μm film, J & W Scientific, Folsom, CA, USA), and a temperature program from 40 °C (2 min hold) to 350 °C (2 min hold) with a first gradient of 7 °C min^{-1} to 155 °C, and a second gradient of 60 °C min^{-1} to 300 °C. Coupled to the gas chromatograph was a mass spectrometer (Hewlett-Packard model 5973) with a quadrupole mass selective detector. Transfer line temperature was 270 °C, ionization potential was 70 eV, and a scan range of m/z 40–350 was employed. Volatiles emitted from *T. pratense* under different experimental conditions were identified on the GC-MS by reference spectra in the Wiley and National Institute of Standards and Technology libraries and in the literature (Adams, 2007; Joulain and König, 1998) and by comparison of retention times and mass spectra to those of standards in our collection.

For quantification, the GC was coupled to a FID detector operating at 250 °C, using the same conditions described above. As co-elution of some compounds occurred with the DB-5MS column, all samples were additionally analyzed on the GC-MS with a DB-wax column (30 m×0.25 mm, 0.25 μm film, J & W Scientific) installed. The temperature program started from 45 °C (2 min hold) to 350 °C (2 min hold) with a first gradient of 7 °C min^{-1} to 90 °C, a second gradient of 20 °C min^{-1} to 150 °C, and a third gradient of 60 °C min^{-1} to 250 °C. Quantification was accomplished by comparing the peak areas in the FID traces with that of the internal standard (nonyl acetate) calculated according to the effective carbon number concept (Scanion and Willis, 1985).

All volatiles collected were considered to be plant derived, rather than from herbivores directly, as caterpillars were removed from the plants just before the volatile collection started. Caterpillar feces were tested and did not emit measurable amounts of volatiles. Overall, we measured fewer volatile compounds than in an earlier study (Kigathi et al., 2009). This is most likely due to differences in the volatile collection methods employed. Here, plants were covered with PET bags to control competitive interactions, and so volatiles were collected using these bags, which were not as air-tight as the containers used previously. Thus, some minor volatile compounds reported in the earlier work were not detected in this experiment.

Other Plant Measurements

All plants were harvested directly after volatile collection by cutting them off 2 cm above ground level. To calculate the actual experimental leaf area loss caused by *S. littoralis* feeding, leaves from these plants were spread on a white board together with a reference area of 1 cm^2 and photographed with a digital camera (Nikon D60). All photographs were analyzed with Adobe Photoshop (Adobe Systems Inc., San Jose, CA, USA). The remaining leaf area for each plant was determined by referring to the amount of pixels in the reference area on the white board. Leaf area loss caused by herbivory was then reconstructed by using the remaining leaf area as a template. After pictures were taken, leaves, petioles, and flowers of each individual plant were separated in plastic tubes, flash frozen in liquid nitrogen, and then stored at −80 °C until they were lyophilized. The dry weights of the different plant tissues (leaves, petioles, flowers, and roots) were then measured. These were used to calculate above ground biomass and root:shoot ratio.

Statistical Analysis

Volatile compounds released from *T. pratense* were grouped into two categories for simplicity and to compare the results with other studies in the literature. We designated HIV as those compounds that increased in emission by more than 5-fold upon *S. littoralis* caterpillar herbivory, while the group of "constitutive" volatiles consisted of compounds that were already present in the headspace of *T. pratense* prior to caterpillar herbivory (Table S6). None of the constitutive volatiles increased by more than 2-fold upon herbivory. The HIVs are the same compounds described in a previous study by Kigathi and others (2009). Total volatiles were the sum of the two groups. A few (20) plants started to flower during the experiment, but the floral volatiles were determined and excluded from the analysis. The incidence of flowering did not show a pattern among treatments.

Emission data (ng g^{-1} hr^{-1}) were log-transformed to meet assumptions of normality and analyzed by using *analysis of variance* with the open source software R 2.8.1 (http://www.r-project.org/; Crawley, 2007). Since the experimental design was unbalanced, involving treatments in which plants grew without competitors (Fig. 1), analyses were performed as follows. To determine the effect of conspecific and heterospecific interaction on volatile emissions, we compared plants growing without neighbors (control) with plants growing in full contact (above and below ground) with either *D. glomerata* or *T. pratense* resulting in three interaction treatments (none, intra-specific, inter-specific) * two herbivory treatments (no herbivory, herbivory) * five replicates = 30 plants. A *two-way ANOVA* was performed

with "herbivory" and "neighbor" as the main effects, and the two way interactions as well as "block" as random effects (Fig. 1a, b). To compare the effect of above ground, below ground and full contact on volatile emission, we excluded the no neighbor treatment and analyzed all the other treatments (Fig. 1b, c, d) in a *three-way ANOVA* with "herbivory" (no herbivory vs. herbivory), "contact" (above ground, below ground or full contact) and "neighbor" (conspecific or heterospecific) as the main effects, the two and three way interactions and "block" as a random effect: two interaction treatments (inter- and intra-specific) * three contact treatments (above ground, below ground and full contact) * two herbivory treatments (no herbivory, herbivory) * five replicates = 60 plants. To determine if the levels of herbivory had a significant effect on volatile emission, an additional analysis with data from only the herbivore infested plants ("herbivory", 30 plants) was performed using the percent leaf area lost as a covariate (Table S4).

Biomass data (roots and above ground biomass) were square root transformed and herbivory data was arcsine transformed to meet the statistical assumptions. Biomass and herbivory data then were tested as a dependent variable with the same statistical testing procedure as described above for the volatile organic compounds.

Results

The interaction of the focal plant *T. pratense* with *D. glomerata* had a measurable impact on the growth of *T. pratense*. When the two species were growing in simultaneous above and below ground contact, the total biomass of *T. pratense* was reduced (32–38 %) compared to the biomass of controls growing alone ($F_{2,22} = 7.84$, $P = 0.003$). This reduction in biomass could be due to growth inhibitors released by *D. glomerata*, or be the result of competition for resources occurring upon inter-specific contact, which was not evident on intra-specific contact (Fig. S2; Table S3a). Root:shoot ratio and specific leaf area did not vary among treatments (Fig. S2b, Table S3).

The nature of the plant—plant interaction had a strong impact on volatile emission. *Trifolium pratense* plants in intra-specific contact generally emitted lower amounts of total volatiles after herbivory than plants growing in competition with heterospecifics or growing alone (Table 1; Fig. 2). The pattern was especially well defined for HIV (compounds that increased by more than 5-fold in emission rate after *S. littoralis* herbivory). For example, in intra-specific contact, *T. pratense* plants released such volatiles at a rate 50 % less than in other treatments even before caterpillar herbivory was inflicted. After herbivory, the rate of emission for plants growing with conspecifics was 80 % less than in other treatments. These patterns were consistent for all major volatiles

Table 1 Effects of different neighbor treatments on emission of volatiles from *Trifolium pratense* as analyzed by ANOVA

A)		Total		Herbivore induced		Constitutive			
	DF	F	P	F	P	F	P		
Block	4	**7.960**	**<0.01**	**5.840**	**<0.01**	**8.02**	**<0.01**		
Herbivory	1	**28.91**	**<0.01**	**70.43**	**<0.01**	**5.69**	**0.03**		
Neighbor	2	**7.16**	**<0.01**	**6.71**	**<0.01**	3.37	0.06		
Herbivory * Neighbor	2	2.64	0.10	0.48	0.62	1.15	0.25		
Residuals	19								
B)		(E)-β-ocimene		(E)-β-caryophyllene		DMNT		MeSA	
	DF	F	P	F	P	F	P	F	P
Block	14	1.92	0.14	**4.12**	**0.01**	1.09	0.38	1.02	0.42
Herbivory	1	**67.51**	**<0.01**	**103.54**	**<0.01**	**13.49**	**<0.01**	**7.61**	**0.01**
Neighbor	2	**7.13**	**<0.01**	**12.29**	**<0.01**	1.28	0.30	1.63	0.22
Herbivory * Neighbor	2	0.31	0.74	**9.80**	**<0.01**	0.39	0.68	3.14	0.07
Residuals	19								

Part A: Total volatiles emitted include herbivore induced volatiles (compounds that increased by more than 5-fold after caterpillar feeding) and constitutive volatiles (compounds already present in the absence of caterpillars). Part B shows individual results for the major herbivore-induced volatiles. Bold numbers indicate significant results. DMNT, (E)-4,8-dimethylnona-1,3,7-triene; MeSA, Methyl salicylate. Analysis includes the no neighbor, intra-specific and inter-specific neighbor treatments where contact involved simultaneous above ground and below ground interaction (Fig. 1a, b, N=30 replicates in total)

within the group of herbivore induced compounds (Table 1), including the monoterpene (E)-β-ocimene, the sesquiterpene (E)-β-caryophyllene, the C11 homoterpene (E)-4,8-dimethylnona-1,3,7-triene (DMNT), and the benzenoid methyl salicylate (MeSA) (Fig. 3). Except for (E)-β-caryophyllene, there were no significant interactions between neighbor identity and caterpillar herbivory for any of the volatile compounds (Table 1). The constitutively emitted volatiles were not significantly affected by growing with conspecific or heterospecific neighbors or growing alone.

We separately tested the effects of above ground contact, below ground contact, and simultaneous above and below ground contact on volatile emission, but found no substantial differences among these treatments in their effect on the focal plant species *T. pratense* (Fig. 4; Table S1). Compared to growing alone, volatile emission from *T. pratense* after herbivory was always reduced when the plants grew with conspecifics regardless of type of contact. However, emission after herbivory was not reduced with heterospecifics, despite the significant reduction in biomass for all *T. pratense* plants growing in below ground competition with *D. glomerata* (Figs. S2 S3).

The leaf area loss due to *S. littoralis* herbivory was similar for *T. pratense* plants in all experimental treatments (Fig. S3c, Table S3b). Plants growing in full-contact with conspecifics exhibited somewhat less experimental herbivory than plants in the other treatments but this was not significant (Fig. S3b, Table S4). When % leaf area loss was included in the analysis as a covariate (plants with herbivores only), percentage herbivory was not correlated to volatile emission (Table S4).

Discussion

Plant competition affects numerous plant processes including photosynthesis, allocation to biomass, root-shoot architecture, and overall plant fitness (Ballare, 2009; Hautier et al., 2009; Novoplansky, 2009). The strength of these effects can change depending on whether competition is intra- or inter-specific (Keddy, 2001). The results from this study showed that the emission of volatiles from a plant strongly depends on whether it interacts with a heterospecific or a conspecific neighbor. The focal plant species, *T. pratense*, emitted significantly fewer constitutive and HIVs when it was paired with a conspecific than when it grew next to the heterospecific neighbor *D. glomerata*. This phenomenon occurred despite the fact that only the heterospecific neighbor caused a significant reduction in *T. pratense* biomass, thus showing evidence of competition with *T. pratense*. The type of contact (above or below ground or both together) had no significant impact on *T. pratense* volatile emission.

Apart from our study, only two others have investigated the effects of intra- and inter-specific plant competition on volatile emission. These showed that the terpene emission of several woody Mediterranean perennials tended to increase under intra-specific competition (Ormeno et al., 2007a), but decreased or did not change under inter-specific competition (Ormeno et al., 2007b). While these results are at variance with ours, those authors measured exclusively constitutive emission, and did not investigate emission after herbivory. In addition, most of the species studied by Ormeño et al. store large quantities of terpenes in their foliage, so that
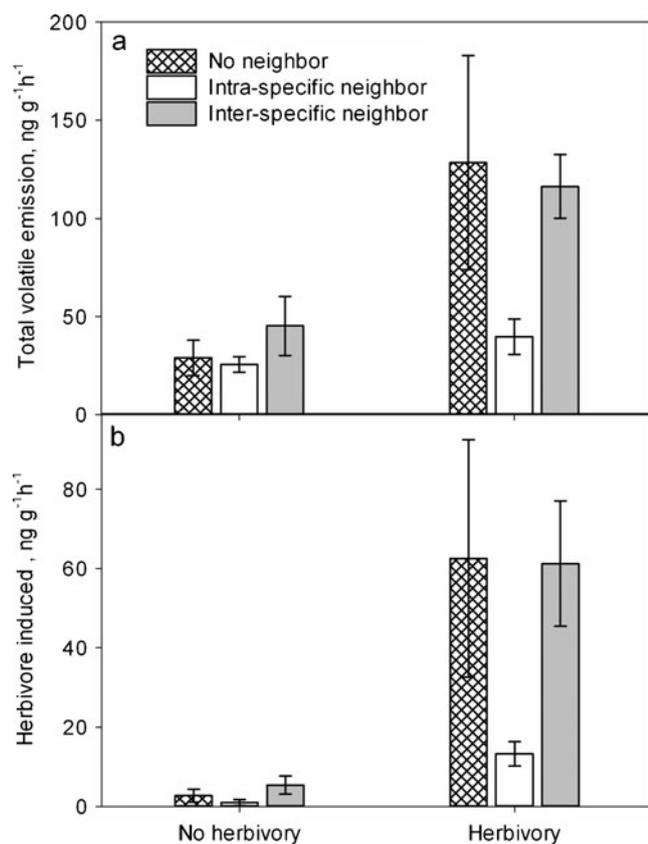
Fig. 2 Effects of Herbivory by *Spodoptera littoralis* Caterpillars, and Neighbor Identity on Volatile Emission of *Trifolium pratense*. Intraspecific interaction significantly suppressed volatile emission. Panel **a**: total volatiles; panel **b**: compounds induced at least 5-fold by herbivory (herbivore induced volatiles). Analysis includes the no neighbor, intraspecific and inter-specific treatments where competition involved simultaneous above ground and below ground contact (Fig.1a, b, $N=30$ plants in total). The effect of neighbor was significant for both total and herbivore induced emission (Table 1)
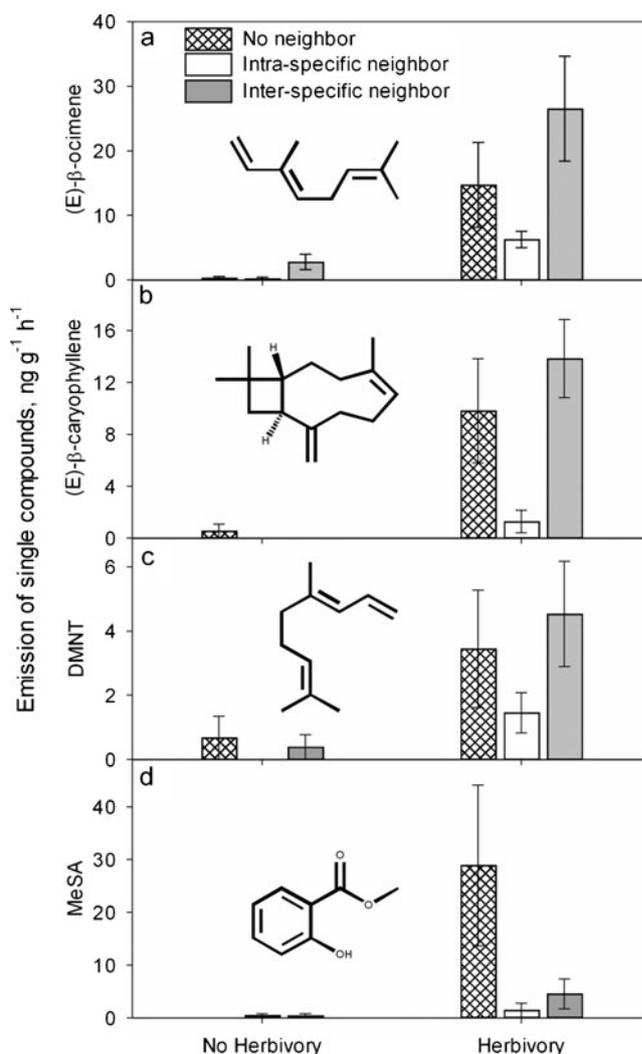

Fig. 3 Effects of Herbivory by *Spodoptera littoralis* Caterpillars and Neighbor Identity on Emission of Major Herbivore-induced Compounds of *Trifolium pratense*. Depicted are results (mean±SE, $N=30$ plants in total) expressed in ng (g dry wgt)$^{-1}$hr^{-1} for (**a**) the monoterpene (*E*)-β-ocimene, (**b**) the sesquiterpene (*E*)-β-caryophyllene, (**c**) the homoterpene (*E*)-4,8-dimethylnona-1,3,7-triene (DMNT), and (**d**) the aromatic compound, methyl salicylate (MeSA). Analysis includes the no neighbor, intra-specific, and inter-specific treatments where interaction involved simultaneous above ground and below ground contact (Fig.1a, b, $N=30$ replicates in total). Results of the statistical analysis are given in Table 1

emission is likely due to release from these storage sites rather than to *de novo* biosynthesis as in *T. pratense* and other plants that do not store terpenes (Pare and Tumlinson 1999). Thus, the biological relevance of this emission may be quite different. Neither the studies by Ormeño et al. nor our work examined volatile emission under field conditions, in the presence of multiple neighbors, or under other varying biotic and abiotic factors. Such studies are necessary before one can draw firm conclusions about the general effect of neighbors on volatile emission.

Plant volatiles, especially those that are herbivore induced, have been proposed to be involved in direct and indirect plant defense (for review see Unsicker et al., 2009). As induced defense compounds are considered to be costly (Karban and Baldwin, 1997; Walters and Heil, 2007), their production could be limited especially when plants grow in competition (Walters and Heil, 2007). This was not the case in our experiment, where volatile emission was higher in plants growing next to heterospecific neighbors (Fig. 2) that had a strong

competitive effect on *T. pratense* biomass both above and below ground (Figs. S2, S3). Thus, resource availability may not be a good explanation for the effect of plant neighbors on *T. pratense* volatile emission observed in this study. *Trifolium pratense* plants decreased their constitutive and herbivore induced volatile emission when they grew together with conspecifics even though this interaction did not result in measurable effects on biomass.

If the reduction in volatile emission from *T. pratense* growing with conspecifics is not due to reduced resources, another explanation might be that volatiles are less necessary under
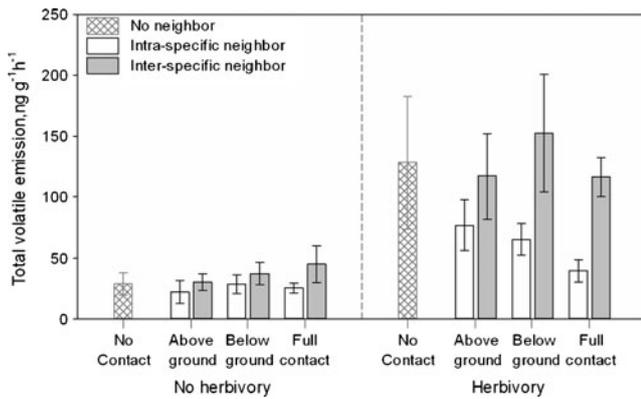
Fig. 4 Effect of Above Ground and Below Ground Contact on Volatile Emission of *Trifolium pratense* in Inter-specific and Intra-specific Interaction. Plants were grown with contact occurring either above ground only, below ground only or simultaneously above and below ground, and with either another *T. pratense* plant (intra-specific neighbor, *white bars*) or a *Dactylis glomerata* plant (inter-specific neighbor, *grey bars*, Figs. 1b–d, *N*=60 plants in total). Volatile emission is depicted as mean±SE in units of ng (g dry weight)$^{-1}$ hr^{-1}. *Grey hatched bars* represent mean emissions by *T. pratense* plants growing without any competition included in the graph for comparison. The effect of neighbor identity was significant ($F_{1,43}$=13.83; P<0.01, Table S2), with intra-specific interaction resulting in decreased emission while there was no difference whether contact was above ground, below ground or both ($F_{2,43}$=0.64; P=0.53, Table S2)

these conditions. For example, in a group of conspecifics less emission may be needed to attract herbivore enemies. Emission may even be a liability in some circumstances since volatiles that serve as defenses to some insect herbivores or attractants to herbivore enemies may also function as attractants for the herbivores themselves (Unsicker et al., 2009). In fact, plant volatiles may be particularly important as attractants for specialist herbivores (Kalberer et al., 2001; Halitschke et al., 2008). Since plants growing in monospecific stands are known to be especially susceptible to attack by specialist rather than generalist feeders (Root, 1973), the reduction of volatile emission when growing with conspecifics could indeed be an adaptation to reduce the risk of herbivory from specialist feeders.

Another way in which volatile emission could be a liability in interactions with conspecifics is if these compounds function in within-plant signaling. In recent years, herbivore-induced volatiles from damaged plant organs have been reported to activate defenses in undamaged tissues (Frost et al., 2007; Heil and Silva Bueno, 2007), but these compounds also may be perceived by neighbors, which respond by activating their own defenses (Heil and Karban, 2010). Since better defended neighbors in communities experiencing herbivore pressure may well become more vigorous competitors (Cipollini, 2007), selection may act to minimize volatile signaling in environments where it is readily intercepted. A plant surrounded by conspecific neighbors would seem at high risk to have volatile signals perceived by other plants, since conspecifics likely

employ similar signaling systems (Karban and Shiojiri, 2009). However, it has also been suggested that it could benefit an herbivore-induced plant to actively signal neighbors to activate their defenses since this might offset the fitness costs of its own induction and thus level the playing field for competitive interactions (van Dam et al. 2000). When surrounded by close relatives, signaling may be favored if it increases an individual's inclusive fitness, but the relatedness of neighboring individuals in natural communities may not be readily perceived by plants. In our experiment, plants were grown from a mixed seed batch and close genetic similarity cannot be assumed.

Plants growing with conspecific neighbors also may have less need for volatile compounds whose role is to act directly in competition as agents of allelopathy. Volatiles frequently have been reported to reduce the germination, growth, and development of neighboring plants (Arimura et al., 2010), and so may be responsible for direct competitive interactions. Since such allelopathic compounds should by design be comparatively non-toxic to the plants producing them, these substances may not have much purpose in interactions with conspecifics that have similar physiology. However, they may still have a role in inter-specific competition. In this study, it is possible that the volatiles emitted by *D. glomerata* (Table S5) may have an allelopathic effect on *T. pratense,* as the biomass of plants competing with *D. glomerata* was lower than that of plants in intra-specific competition or growing alone even when competition was solely above ground (Fig. S2).

Neighboring plants also could increase the effective emission of volatiles from the vicinity of a focal plant in a completely different manner if they happen to passively adsorb volatiles onto their foliage. Volatiles with relatively low vapor pressures, such as sesquiterpenes, have been reported to be readily adsorbed onto the foliage of neighboring plants (Himanen et al., 2010). However, this phenomenon was not evident in our experiment. First, *T. pratense* growing in above ground contact with *D. glomerata* did not emit volatiles at a significantly higher rate than *T. pratense* growing in below ground contact with this grass or than *T. pratense* growing alone (Fig. 4). In addition, *T. pratense* competing above ground with other *T. pratense* actually emitted volatiles at a lower rate than plants growing alone. Finally, the volatile blend of the two species is different (Fig. S4, Table S5,S6).

Our results suggest that the presence of conspecific vs. heterospecific neighbors has different consequences for *T. pratense* (Fig. 4), and that both above or below ground cues are sufficient for this distinction. Plants have been shown to be able to detect the presence of potential competitors from general above ground cues, such as a reduction in red:far-red light ratios or increased ethylene (Novoplansky, 2009). However, to detect the identity of competitors it is necessary to rely on more specific cues, such as the chemical profiles of

compounds exuded from the roots or released as volatiles (Kegge and Pierik, 2010). Previous workers have provided good examples of how plants detect neighbor identity by root exudates (Biedrzycki et al., 2010; Chen et al., 2012) or by aerial volatiles (Ninkovic, 2003; Runyon et al., 2006). For example, sagebrush plants respond to aerial volatiles from close but not distant relatives that suffered damage and increase their own defenses (Karban et al., 2013). Here, we demonstrated that *T. pratense* can differentiate between *D. glomerata* plants or another *T. pratense* individual, and that either above or below ground cues are sufficient for this distinction (Fig. 4). *Trifolium pratense* also has been reported to differentiate between sibs vs. non-sibs in competitive interactions, and to act competitively towards non-sibs (increased petiole elongation) but non-competitively (increased seed production) towards sibs (Lepik et al., 2012). If volatile emission serves as an agent of allelopathy, reduction in emission in the presence of close relatives can be interpreted as a way to decrease competitive interactions.

Our finding that volatile emission is lower when plants grow with conspecific as compared to heterospecific neighbors or alone may be important from an applied as well as a basic scientific perspective. In agriculture, most crops are grown in monocultures so competition is exclusively intra-specific (Andow, 1991). Generalizing from our results, volatile-mediated defenses could be inherently reduced under standard agricultural conditions, and inter-cropping may thus be recommended to increase defense against pests in addition to providing other benefits. Where inter-cropping cannot be realized, the selection of crop varieties differing in volatile output and perception could be a promising approach to maintaining defensive posture. In this context, additional research on plant-plant interactions and volatile emission may have broad significance in understanding how plant defense phenotypes are modulated in both native and agricultural ecosystems.

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