

LETTER

Plant volatiles induced by herbivore eggs prime defences and mediate shifts in the reproductive strategy of receiving plants

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Abstract

Plants can detect cues associated with the risk of future herbivory and modify defence phenotypes accordingly; however, our current understanding is limited both with respect to the range of early warning cues to which plants respond and the nature of the responses. Here we report that exposure to volatile emissions from plant tissues infested with herbivore eggs promotes stronger defence responses to subsequent herbivory in two *Brassica* species. Furthermore, exposure to these volatile cues elicited an apparent shift from growth to reproduction in *Brassica nigra*, with exposed plants exhibiting increased flower and seed production, but reduced leaf production, relative to unexposed controls. Our results thus document plant defence priming in response to a novel environmental cue, oviposition-induced plant volatiles, while also showing that plant responses to early warning cues can include changes in both defence and life-history traits.

Keywords

Brassica, cues, defence, fitness, herbivore performance, herbivore-induced plant volatiles, oviposition-induced plant volatiles, *Pieris brassicae*, priming.

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INTRODUCTION

Plants face inevitable trade-offs when investing in defence versus growth and reproduction, as well as among defence traits that may be effective against different classes of antagonists (Zangerl & Rutledge 1996; Cipollini & Heil 2010). Where the risk of encountering particular antagonists is unpredictable, these trade-offs may be mitigated via inducible defences that postpone the commitment of resources until a plant comes under attack (Karban & Myers 1989; Agrawal 1999; Orrock *et al.* 2015); however, this strategy entails a period of vulnerability during the time required to effectively deploy defence following induction (Karban 2011). Plants can shorten this period by adjusting their defence phenotypes in response to early warning cues – detected prior to attack – that provide information about the risk of encountering herbivores or pathogens (Dicke & Baldwin 2010; Hilker & Fatouros 2015; Mescher & De Moraes 2015). It is now clear that the recognition of such cues is widespread and that plant responses to such cues often entail defence priming rather than full-scale defence induction (van Hulst *et al.* 2006; Frost *et al.* 2008a; Kim & Felton 2013; Conrath *et al.* 2015; Dicke 2016; Hilker *et al.* 2016). Nevertheless, our understanding remains limited both with respect to the range of early warning cues that plants recognise and the nature of their responses.

Work on plant responses to the threat of herbivore attack has identified a range of cues that appear to prime plant defences. These include cues directly associated with herbivores, such as herbivore movement on leaf surfaces (Peiffer *et al.* 2009), the presence of insect eggs on plant tissues

(Beyaert *et al.* 2012; Bandoly *et al.* 2016; Hilker & Fatouros 2016; Lortzing *et al.* 2019) and olfactory cues emitted by herbivores, such as pheromones (Helms *et al.* 2013; Helms *et al.* 2017; Bittner *et al.* 2019). In addition, plants may respond to indirect cues that reveal information about the presence of herbivores, most notably herbivore-induced volatile emissions from plant tissues that are already under attack, which have been shown to prime plant defences in a wide range of systems (Heil & Kost 2006; Frost *et al.* 2008a; Dicke & Baldwin 2010; Karban *et al.* 2014) and which appear to play a signalling function within plants (Frost *et al.* 2007; Heil & Silva Bueno 2007), as well as serving as cues for neighbouring plants of the same or different species (Karban & Maron 2002; Kessler *et al.* 2006; Zhang *et al.* 2019).

The specific cues responsible for priming are often not known, although individual herbivore-induced plant volatile compounds responsible for priming have been identified in a handful of systems (Frost *et al.* 2008b; Sugimoto *et al.* 2014; Erb *et al.* 2015), as has a herbivore-emitted compound that primes defences in goldenrod (Helms *et al.* 2013; Helms *et al.* 2017). The compounds identified by these studies are diverse, suggesting that plants can potentially detect a wide range of chemical cues. In addition to herbivore-induced volatiles, other indirect cues may provide plants with reliable information about the risks or impacts of future herbivory. For example, there is evidence that abiotic factors, such as exposure to heavy metals or changes in weather conditions, can alter the sensitivity of defences to future herbivore attack (Holopainen & Gershenson 2010; Winter *et al.* 2012; Pezzola *et al.* 2017).

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As noted above, the detection of early warning cues by plants often does not lead to full-scale defence induction, but rather to defence priming, which enables faster deployment of induced defences following subsequent attack (Frost *et al.* 2008a; Kim & Felton 2013; Douma *et al.* 2017). For example, defence priming in response to both herbivore-induced plant volatiles and the volatile emissions of herbivores themselves have been shown to prime defence induction via the jasmonic acid pathway (Frost *et al.* 2008b; Helms *et al.* 2017). It is currently unclear whether similar underlying processes mediate the defence priming responses to disparate cues observed in different plant systems.

In a few systems, early warning cues have been shown to elicit responses that enhance plant resistance against herbivores via direct or indirect defence mechanisms. For example, tomato plants can convert the volatile (Z)-3-hexenol from damaged neighbouring plants to (Z)-3-hexenylvicinoside, a form of chemical defence that directly reduces herbivore performance (Sugimoto *et al.* 2014). Meanwhile, plants infested with herbivore eggs emit volatile compounds that attract egg or early stage larval parasitoids, an effective indirect form of defence (Hilker & Fatouros 2015), and egg infestation itself has been associated with an enhanced physiological defence response upon herbivore feeding (Bandoly *et al.* 2016; Lortzing *et al.* 2019), perhaps mediated by the detection of chemical cues in the egg-glue or in oviduct secretions covering the eggs (Hilker & Fatouros 2015).

While most work on plant responses to early warning cues has focused on defence, plant responses to herbivore feeding often include changes in growth or life-history traits that can enhance plant tolerance or otherwise mitigate the fitness impacts of herbivory (Strauss & Agrawal 1999; Agrawal 2000; Carmona *et al.* 2011; Garcia & Eubanks 2019). For example, plants can invest more resources in vegetative growth and reproduction (Garcia & Eubanks 2019), or accelerate flowering and fruit production (a strategy referred to as reproductive escape; Lucas-Barbosa *et al.* 2013). There is also evidence that plants can respond to severe herbivore attack by deferring reproduction to future growing seasons and shifting current investment to resistant belowground tissues (Schwachtje *et al.* 2006). It is therefore plausible that cues detected prior to attack might elicit similar changes in plant traits not directly related to defence. To date, however, only a few studies have documented changes in plant growth or reproductive traits in response to cues that provide an early warning of herbivore attack. For example, sagebrush exposed to cues from damaged neighbours increased the production of inflorescences and lateral branches at the expense of vertical growth (Karban *et al.* 2012; Karban 2017), while tobacco plants exposed to damaged (manually clipped)-sagebrush neighbours produced more flowers and seeds than plants with undamaged neighbours (Karban & Maron 2002). Exposure to the putative sex pheromone of a gall-inducing fly was also shown to increase short-term growth in goldenrod plants (Yip *et al.* 2017).

The current study explores how the annual brassicaceous plant *Brassica nigra* responds to early warning cues associated with the presence of a specialist herbivore, *Pieris brassicae*. Herbivory by *P. brassicae* can impose high fitness costs on

Brassica nigra, which has therefore evolved specialised defence responses (Blatt *et al.* 2008). These include responses to oviposition, which has been shown to prime *B. nigra* defences under both greenhouse and field conditions (Pashalidou *et al.* 2013; Pashalidou *et al.* 2015a; Pashalidou *et al.* 2015b; Pashalidou *et al.* 2015c). Plants infested with *P. brassicae* eggs were also found to flower earlier and produce significantly more seeds than control plants (Lucas-Barbosa *et al.* 2013; Pashalidou *et al.* 2015b) and to emit volatiles that recruit larval parasitoids (Pashalidou *et al.* 2013; Pashalidou *et al.* 2015b). Recent studies in other systems have also reported effects of volatiles induced by herbivore oviposition, including enhanced parasitoid attraction in teosinte (Mutymbai *et al.* 2016) and decreased herbivore recruitment in poplar (Guo *et al.* 2019). In the light of these findings, we hypothesised that it might be adaptive for *B. nigra* plants themselves to detect and respond to volatile cues associated with the presence of *P. brassicae* eggs.

We therefore aimed to determine whether *B. nigra* plants respond to oviposition-induced volatile cues and to characterise the effects of such cues on both plant defence and life-history traits. To achieve this, we first tested whether defence priming by oviposition-induced volatiles from neighbours results in reduced larval performance on focal plants (a proxy for defence resistance). Next, we sampled the headspace of control and egg-infested plants to identify potential oviposition-induced volatile cues emitted by *Brassica nigra* (as well as the related species *Brassica oleracea*, a perennial commercial crop plant) and tested the effects of exposure to a key compound found to be upregulated by the presence of eggs. Finally, we assessed whether oviposition-induced volatiles from neighbours can elicit changes in *B. nigra* life-history traits similar to those previously observed in response to direct egg deposition. Our results demonstrate that exposure to oviposition-induced volatiles reduces the subsequent performance of *P. brassicae* larvae and also results in apparent shift from growth to reproduction (exposed plants produced more flowers and seeds but fewer leaves than unexposed controls). These findings confirm that plants use oviposition-induced volatiles as an early warning cue of herbivory, and that exposure to these volatiles influences both defence-related and life-history traits.

MATERIALS AND METHODS

Plants and insects

This study examines two brassicaceous plant species, the wild annual *Brassica nigra* and the cultivated perennial *Brassica oleracea*. *Brassica nigra* is native to many European countries (Lauber & Wagner, 2012). Seeds for this study were provided by the Centre of Genetic Resources in Wageningen, the Netherlands (accession number: CGN06619). The seeds were collected from plants grown in field sites around Wageningen, which were exposed to wild pollinators; these seeds were then used to grow plants in the greenhouse for our experiments. *Brassica oleracea* var. *capitata* (white cabbage) is endemic to the southern and western coast of Europe and is now grown worldwide as an agricultural crop. White cabbage seeds from

the commercial variety 'ESCAZU' (seed lot 2875500) were provided by Syngenta Crop Protection AG (Basel, Switzerland). Plants of both species were grown in standard potting soil (Substrat 2, Klasmann-Deilmann GmbH, Germany) in a climate chamber under warm, long-day conditions (light 24 °C, dark 22 °C, L16h-D8h, 60% relative humidity).

Herbivore assays were conducted with larvae of the large cabbage white butterfly, *Pieris brassicae* (Lepidoptera: Pieridae), a globally distributed species that specialises on plants in the Brassicaceae family. Butterflies used in this experiment were collected in Switzerland and reared under similar conditions as the plants (light 24 °C, dark 22 °C, L16h-D8h, 60% relative humidity).

Generating the experimental treatments

For each of the subsequent experiments with the two *Brassica* species, we used four 'priming' treatments (Fig. 1, Table S1), including: (1) plants infested with *P. brassicae* eggs (E); (2) plants receiving volatiles from egg-infested neighbours (Re); control receivers exposed to volatiles from uninfested neighbours at close proximity (Rc); and unmanipulated controls (C). Plants in the first treatment (E) were individually placed

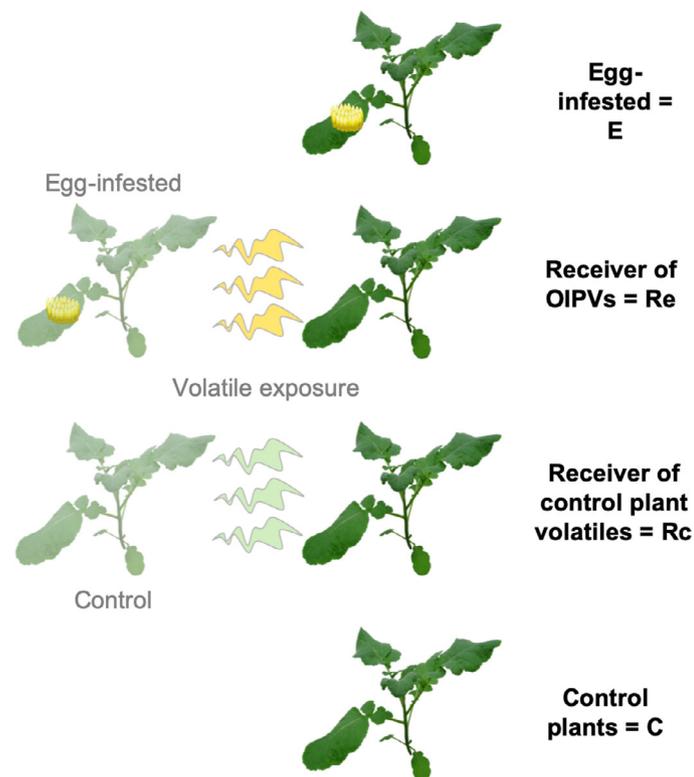


Figure 1 The different treatments used throughout this study were generated through exposure to direct egg infestation, oviposition-induced volatiles or volatiles from neighbouring control plants. Control plants were exposed to neither volatiles from other plants nor eggs. Plants were maintained under each of these conditions for 5 days (until just before larval emergence) before use in different experiments. To generate the damaged and undamaged treatments, larvae were added to subsets of these plants and allowed to feed for a total of 7 days. The shorthand treatment codes used throughout the manuscript are given.

in cages with *c.* 50 mated *P. brassicae* females and left until at least 40 eggs were laid (*c.* 15 min). Excess eggs were gently removed with a fine brush (uninfested emitters were also brushed), and the remaining eggs stayed on the plant until larval emergence (5 days later). Plants in the second treatment (Re) were placed *c.* 15 cm from plants in treatment E (for volatile exposure) but removed prior to larval emergence. Control receiver plants (Rc) were similarly placed *c.* 15 cm away from uninfested plants. Unmanipulated control plants (C) were grown farther apart (> 25 cm) to minimise the effects of volatile exposure. After 5 days, but prior to larval emergence, plants in these four treatments were moved to random positions, and the spacing between plants was increased to *c.* 25 cm. After larvae hatched on E plants, they were removed and then used for subsequent damage treatments (with or without larvae, as discussed below; Fig. 1). Unique plants sets were used for each experiment described below.

Larval performance bioassays

The effect of exposure to oviposition-induced volatiles on plant defences in both *Brassica* species was assessed via larval performance assays on non-flowering plants. Ten plants from each priming treatment (E, Re, Rc and C) received 10 neonate *P. brassicae*. On days 3 and 7 following the initiation of feeding, larval mass was measured on a microbalance (accuracy $\pm 1\mu\text{g}$; Mettler-Toledo AG, Greifensee, Switzerland) as described in Pashalidou *et al.* (2013, 2015a,c).

Volatile collection and analysis

For both *Brassica nigra* and *Brassica oleracea*, we collected volatiles from plants exposed to the four priming treatments ($N = 12$ per treatment) and with or without larval damage. For damage treatments, 10 L1 larvae were placed on E, Re and Rc plants. Due to logistical constraints (and because our previous assays showed no effect of priming treatment Rc on larval performance) we collected volatiles only from damaged Rc plants and used C plants as undamaged controls. Larvae were placed on the adaxial side of the third highest leaf. One damaged plant was excluded from the damaged Re treatment because of unrelated damage. Volatile collections were made 1 day prior to larval emergence and 2 h after the initiation of larval feeding. Pots were wrapped in foil to minimise plastic contaminants. Two connecting metal plates were closed around the plant stem (with a hole for the stem to pass), and cotton was used to seal gaps. A 30 L glass dome was carefully placed over the leafy parts of the plant, with openings for incoming and outgoing air, which was filtered through activated charcoal, pulled through the chamber at a rate of 150 ml/min for 4 h, and collected in a stainless-steel cartridge containing 200 mg of Tenex TA (20/35 mesh; CAMSO, Houston, TX, USA). Due to space limitations, volatile collections were conducted in three blocks. After volatile collection, the aboveground parts of the plant were cut and weighed.

Volatile compounds were eluted from the filter using 150 μL solution (2 ng/ μL octane and 4 ng/ μL nonyl acetate, as internal standards, in dichloromethane) and the eluant was analysed by gas chromatography–mass spectrometry (GC-MS). Two μL of the eluant was injected with an automatic Agilent injector 7693 autosampler (Santa Clara, CA, USA) to

an Agilent 7890B GC (Santa Clara, CA, USA) with a pulsed splitless inlet at 250 °C, which was held for 2 min and then analysed on the connected MS Agilent 5977A (De Moraes *et al.* 2001). Compounds were quantified and identified as described in supplementary methods (Appendix S1). Volatile emissions per plant were calculated as mean peak area divided by both the fresh weight of foliage (in grams) and by 10^4 the n of samples.

Testing effects of exposure to individual volatile compounds

Because the emission of cumene was significantly elevated on egg-infested plants for *B. oleracea* (Table S2), we also explored the defence priming effects of this compound on *B. oleracea* and *B. nigra*. Unfortunately, we were unable to similarly test the effect of β -thujene – a compound showing elevated emissions following egg infestation in *B. nigra* (Table S3) – as we could not obtain this compound. We made a cumene solution containing 156 $\mu\text{g/ml}$ of synthetic cumene (Sigma-Aldrich) in hexane, a concentration approximating the mean daily emission of an egg-infested plant with a fresh aboveground mass of 200 g. Over a 5-day period, 50 μL of this solution was applied daily to sleeve-stopper septa (Sigma-Aldrich) placed at a distance of 15 cm from focal plants (treatment Cu; Table S1). The septa were placed at the height of the receiver's apical meristem to simulate elevated cumene emission from an egg-infested plant. Control plants were similarly exposed to 50 μL hexane (treatment He). Each of the 10 replicate plants per treatment was infested with 10 neonate larvae after exposure to cumene for 5 days, and larvae were weighed on days 3 and 7 after placement.

Testing effects of egg-induced volatiles on plant growth and reproduction

To test whether priming by oviposition-induced volatiles altered plant reproductive output we focused on *Brassica nigra*, as this annual species has been previously shown to respond to egg infestation through changes in reproductive phenology. We produced new plants using six treatments described in previous sections (C, E and Re with and without larval damage; Fig. 1, Table S1), omitting Rc plants which were similar to C plants in previous assays. Larvae were allowed to feed freely until pupation, with the larval number reduced from ten to three at the third instar stage to avoid complete defoliation. When larvae neared pupation, plants were covered with a fine net (to prevent larvae from leaving the plant), which was removed following pupation (plant treatments without larvae were similarly covered). We recorded the number of leaves and flowers present 3 weeks after the first flower appeared on each plant. Once all plants were flowering, commercial bumblebees (Biobest, Switzerland) were introduced for 3 weeks to ensure pollination; previous work indicates that bumblebees do not discriminate between undamaged plants and those with either *P. brassicae* eggs or feeding damage on leaves/flowers (Lucas-Barbosa *et al.* 2013). After plants had completed their life cycle, ripe seeds were collected from each plant and measured with a seed counter (elmor c3 version 1.1, Switzerland). Germination rates were measured as in Pashalidou *et al.* 2015b.

Statistical analyses

Caterpillar weights were log-transformed to satisfy normality and the effect of different treatments analysed with a general linear model (GLM) using restricted maximum likelihood (REML). Germination rates, as well as the numbers of flowers and seeds, were also log-transformed to satisfy normality and analysed with a LMM using REML (Bates *et al.* 2014) with R Software version 3.3.1. Treatments were defined as fixed effects, and interplant variation in reproductive/growth traits was modelled using a random effect of plant replicate. The significance of differences between treatments was evaluated with a Tukey's *post hoc* test.

A principal component analysis on the raw chemical data was performed with the statistical software JMP® 11.1.1 of SAS Institute. The effects of the single compounds (mean-centred and log-transformed) were tested with a GLM using REML in R statistical software, with treatment as a fixed factor and repetition as a random factor. For compounds with significant treatment effects, the Tukey-Anscombe plot was used to check for normality of the error, and the Q-Q plot was used to check for normal distribution of residuals. *P*-values were corrected for multiple comparisons with the Bonferroni adjustment. If the log transformation did not manage to satisfy the assumption of the GLM a nonparametric Wilcoxon/ Kruskal–Wallis Test (Rank Sums) was performed in JMP.

RESULTS

Oviposition-induced plant volatiles prime plant defences

Larvae feeding on *B. oleracea* plants previously exposed to eggs (E) or to volatiles from egg-infested plants (Re) had significantly lower biomass after 3 and 7 days of feeding than larvae feeding on control plants (C), or those exposed to volatiles from control plants (Rc; 3 days: $F = 11.1$, $P < 0.001$; 7 days: $F = 13.4$, $P < 0.001$; Appendix S2a,b; Fig. 2a). Similarly, larvae feeding on *B. nigra* plants were also negatively affected by plant exposure to eggs or volatiles from egg-infested plants (3 days: $F = 24.3$, $P < 0.001$; 7 days: $F = 43.6$, $P < 0.001$; Appendix S3a, b; Fig. 2c).

The presence of eggs on leaves upregulates specific volatile compounds

In total, 39 volatile compounds were detected for *Brassica nigra* and 40 for *Brassica oleracea* across all experimental treatments and time points. Principal component analysis of volatile emissions did not show clear multivariate divergence among treatments (Fig. S1). In *Brassica oleracea*, most compounds (39/40) did not significantly differ among treatments after controlling for multiple testing using Bonferroni correction (Table S2); however, cumene was emitted in significantly higher amounts from egg-infested (E) plants than from plants in any other treatment (t -value = 2.46, d.f. = 25, $P = 0.02$; Fig. 3a). In *Brassica nigra*, a different compound, β -thujene, was the single compound emitted in significantly larger amounts from plants induced by egg deposition (E) compared

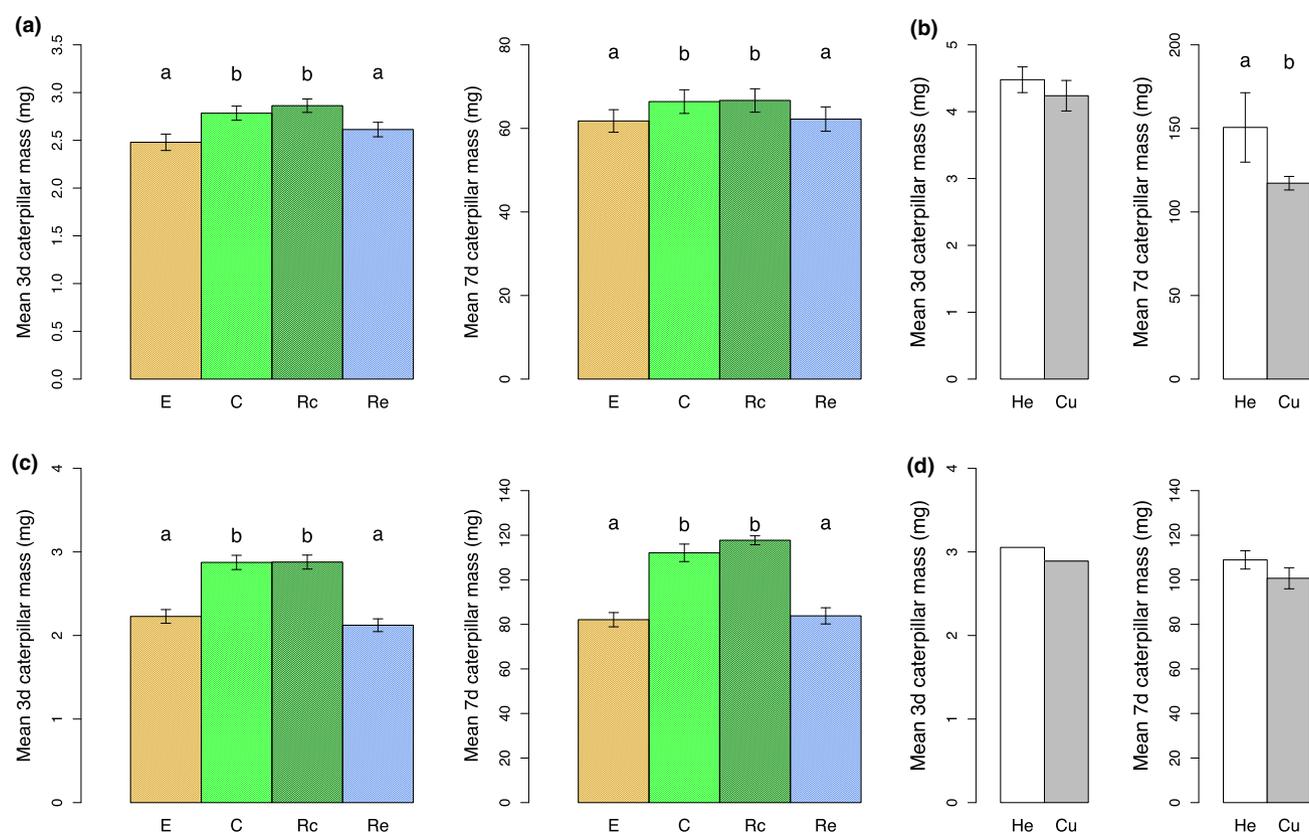


Figure 2 Effects of exposure to oviposition-induced plant volatiles or cumene on plant palatability to a specialist herbivore. As a proxy for relative investment in defence, caterpillar weight was measured 3 and 7 days after feeding on *Brassica oleracea* (a and b) and *Brassica nigra* (c and d). Bars represent mean larval biomass ($\pm 1 \times SE$); $n = 10$ plants per treatment. Treatments in plots (a and c): egg-infested plants (E); control plants (C); receiver of volatiles from control plants (Rc); receiver of egg-infested plant volatiles (Re). Treatments in plots (b and d): receiver of hexane solvent (He); and receiver of cumene emissions (Cu). Letters indicate significant differences among treatments (as assessed by Tukey's test).

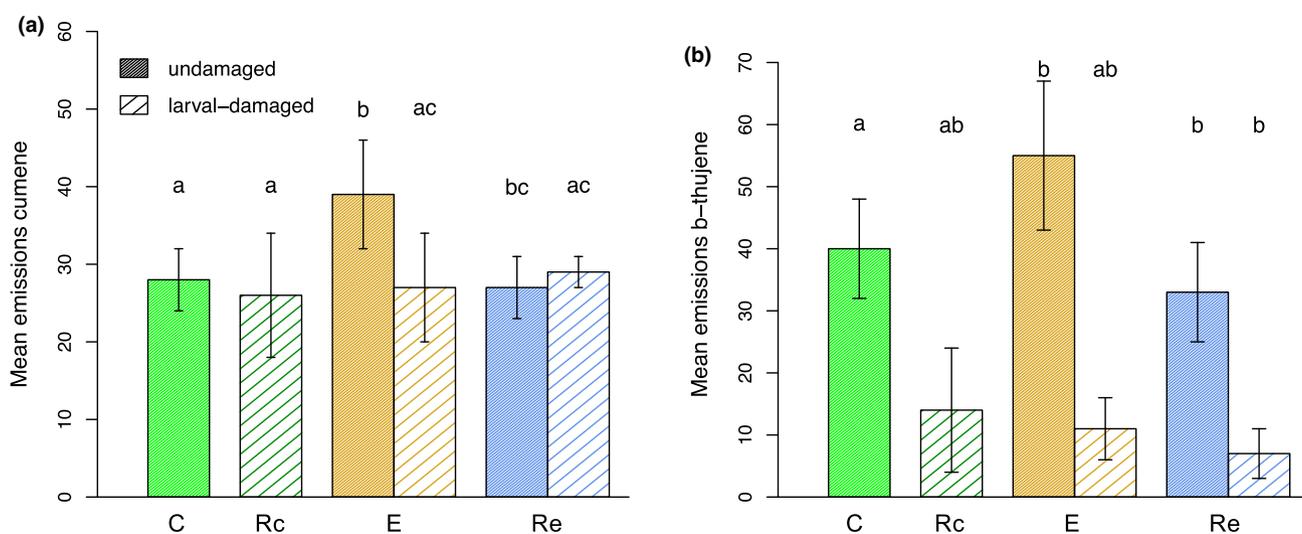


Figure 3 Variation in emission of the volatile compound significantly induced by egg infestation in each of the *Brassica* species. (a) Cumene emissions were significantly elevated for egg-infested *Brassica oleracea* plants. (b) β -Thujene emissions were significantly elevated for egg-infested *Brassica nigra* plants. Mean volatile emissions per treatment were calculated as mean peak area $\pm 1 \times SE$ / g fresh weight of foliage divided by 10^4 the n of samples. Treatments: control plants (C); receiver of volatiles from control plants (Rc); egg-infested plants (E); receiver of egg-infested plant volatiles (Re). Cross hatching indicates where plants were additionally exposed to larval feeding for 7 days. Letters indicate significant differences among treatments (Tukey's test, $P < 0.05$).

to all other treatments (t -value = 2.36, d.f. = 34, P = 0.024; Fig. 3b; Table S3).

Cumene primes defences in *B. oleracea*

Because our volatile analyses identified clear effects of eggs on volatile emissions, we next explored the effects of the relevant compounds on defence priming. We found that *P. brassicae* larvae feeding on *B. oleracea* plants previously exposed to cumene, the single compound upregulated by *P. brassicae* eggs in this plant species, had significantly lower biomass than larvae feeding on plants exposed to hexane alone after 7 days of feeding (after 3 days: F = 0.6, P = 0.42; after 7 days: F = 7.1, P = 0.02; Appendix S4a and b; Fig. 2b). By contrast, larvae feeding on *B. nigra* plants previously exposed to cumene exhibited no significant differences in biomass from larvae feeding on plants exposed only to hexane at either time point (3 days: F = 0.8, P = 0.35; 7 days: F = 1.7, P = 0.18; Appendix S5a and b; Fig. 2d). It thus appears that cumene functions as a species-specific priming signal in *B. oleracea*. β -Thujene, the compound similarly upregulated by eggs on *B. nigra* plants, may play a similar role for that species; however, we were unable to test the effects of β -thujene on priming, as this compound could not be commercially obtained and was challenging to synthesise.

Oviposition-induced plant volatiles shift plant reproductive strategies in *B. nigra*

Compared to undamaged controls (C), undamaged *Brassica nigra* plants infested with eggs (E) and those exposed to volatiles from egg-infested plants (Re) produced significantly higher numbers of flowers (F = 10.6, P < 0.001; Appendix S6; Fig. 4a), fewer leaves (F = 4.64, P = 0.002; Appendix S7; Fig. 4b) and more seeds (F = 8.3, P < 0.001; Appendix S8; Fig. 4c), indicating a possible shift in growth-reproduction strategy. No similar effects were observed among damaged treatments, although there was a trend towards greater flower production by damaged E and Re plants compared to damaged control plants (Fig. 4). Germination rates of resulting seeds did not significantly differ between treatments (F = 1.75, P = 0.14; Fig. 4d).

DISCUSSION

Our results show plant resistance to herbivory can be enhanced by prior exposure to volatile emissions from plant tissues infested with herbivore eggs, suggesting that oviposition-induced plant volatiles may play a role in priming plant defences similar to that of other early warning cues directly and indirectly associated with the presence of herbivores. However, we also found that exposure to oviposition-induced volatiles elicited changes in life-history traits not directly related to defence (Fig. 5). Specifically, we observed a shift from investment in growth to reproduction in *B. nigra* plants exposed to oviposition-induced volatiles similar to that seen in plants directly infested with eggs, in both the current study and previous work (Pashalidou *et al.* 2015b). These findings thus have implications, which we discuss in more detail below,

for understanding both the breadth of early warning cues to which plants respond and the nature of those responses.

Oviposition-induced volatiles prime anti-herbivore defences

Defence priming has previously been reported in response to direct cues from herbivores, including egg deposition (Hilker & Fatouros 2015; Bandoly *et al.* 2016; Lortzing *et al.* 2019), as well as indirect cues in the form of damage-induced volatiles from neighbouring plants (Engelberth *et al.* 2004; Ton *et al.* 2007; Frost *et al.* 2008a; Mescher & De Moraes 2015). The current results suggest that oviposition-induced volatiles can also prime plant defences; this finding complements other recent work showing that oviposition-induced volatiles also play a communicative function in indirect plant defence by serving as foraging cues for natural enemies of herbivores, as well as herbivores themselves. For example, both egg and larval parasitoids showed a stronger preference for maize plants exposed to volatiles of plants infested by eggs of the herbivore *Chillo partellus* than control plants (Mutymbai *et al.* 2016). Ovipositing herbivores were also seen to avoid poplar plants exposed to volatiles from egg-infested neighbours (Guo *et al.* 2019).

We found that the presence of eggs on leaves upregulated the levels of different terpenoid compounds for each of our two focal species: β -thujene for *B. nigra* and cumene for *B. oleracea*. Furthermore, we found that exposure to cumene alone was sufficient to prime defences in *B. oleracea*. We were unfortunately unable to obtain β -thujene for testing; however, cumene did not prime defences in *B. nigra*, suggesting that there is species-level specificity in oviposition-induced volatile signalling systems. A number of studies have now identified specific volatile cues induced by feeding insects that act as a priming stimuli for neighbouring plants (Engelberth *et al.* 2004; Kost & Heil 2006; Frost *et al.* 2008b; Sugimoto *et al.* 2014; Erb *et al.* 2015), yet few studies have assessed the potential generality or specificity of these volatile cues across different species. For example, exposure to herbivore-induced indole clearly altered volatile profiles emitted by maize, but had a much weaker effect on cotton (*Gossypium hirsutum*) or cowpea (*Vigna unguiculata*; Erb *et al.* 2015). The current results suggest that volatile signals associated with defence priming can vary even among closely related plant species responding to the same herbivore antagonist. It would be interesting to test whether the specific compounds implicated here (cumene and β -thujene) might also serve as important cues for herbivores and their natural enemies, particularly as *P. brassicae* females have been shown to use oviposition-induced volatile cues to avoid plants harbouring conspecific eggs (Fatouros *et al.* 2012).

As in other systems where volatile cues produced by conspecifics prime defences, it is unclear whether oviposition-induced volatile cues function primarily in interactions within or between plants and, to the extent that plants do respond to cues from neighbours, whether this represents between-plant communication or merely eavesdropping (Heil & Silva Bueno 2007; Dicke & Baldwin 2010). *Brassica* spp. often grow in dense stands across which herbivores, including later instars of specialists like *Pieris brassicae*, can readily move (Lucas-

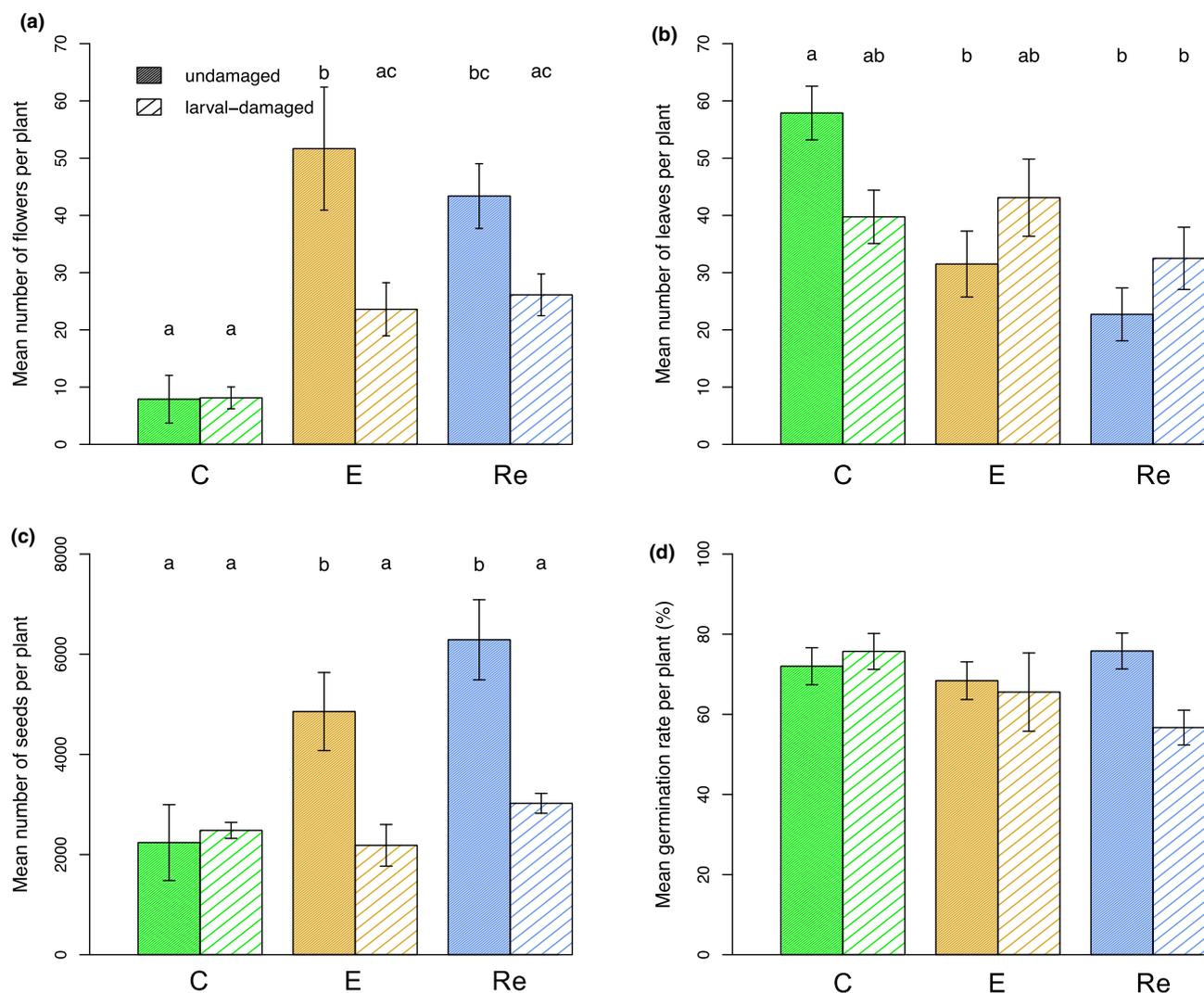


Figure 4 Effect of exposure to *P. brassicae* oviposition and oviposition-induced volatiles on reproduction and fitness traits of *Brassica nigra*, including: (a) Mean flower number; (b) mean leaf number; (c) mean number of seeds produced; (d) mean germination rate. Error bars represent \pm one standard error. Treatments: control plants (C); egg-infested plants (E); receiver of egg-infested plant volatiles (Re). Cross hatching indicates where plants were additionally exposed to feeding larvae (until larval pupation). Letters indicate significant differences among treatments, with 10 replicate plants per treatment (60 in total).

Barbosa *et al.* 2013; Pashalidou *et al.* 2013), suggesting that defence priming (or shifts in reproductive strategy, as discussed below) in response to herbivore-associated cues from neighbouring plants might be adaptive. Furthermore, because these plants often grow in close proximity to close relatives, such responses might also enhance the inclusive fitness of the emitter. In contrast, as the young gregarious larvae of *Pieris brassicae* do not move to neighbouring plants until they reach later larval stages (Lucas-Barbosa *et al.* 2013), such cues might not always indicate that herbivory is imminent; however, such cues might still serve to indicate increased risk that the receiving plants will also be targeted for oviposition.

Oviposition-induced volatile cues mediate changes in life-history strategy

In addition to priming or inducing defences, plants exposed to the threat of herbivory can alter their reproductive strategies

to minimise the impacts on fitness (Strauss & Agrawal 1999; Lucas-Barbosa *et al.* 2013). We found that plants exposed to oviposition-induced volatiles from neighbouring plants produced more flowers and seeds, but fewer leaves, than control plants, a response similar to that previously reported for plants directly exposed to eggs (Lucas-Barbosa *et al.* 2013; Pashalidou *et al.* 2015b). To our knowledge, no previous studies have reported shifts in plant reproductive strategy following exposure to oviposition-induced volatiles. However, there is evidence for life-history shifts in response to damage-induced volatiles in a few systems; for example, tobacco plants with clipped sagebrush neighbours produced more flowers and seeds than plants with unclipped neighbours (Karban & Maron 2002).

The increased seed production observed in undamaged *B. nigra* plants exposed to oviposition-induced volatiles (or to eggs) was lost when larvae were allowed to feed on the plants, although a trend towards greater flower production

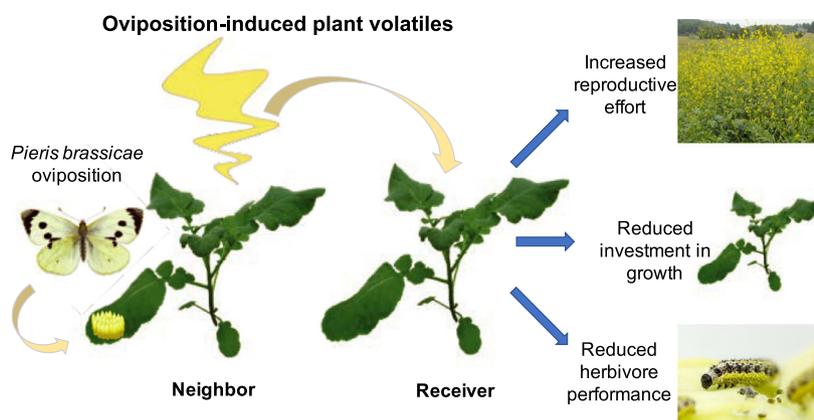


Figure 5 Oviposition-induced plant volatiles prime defences in *Brassica nigra*, but also elicit changes in life-history strategy, including a shift from investment in growth to reproduction.

persisted – we did not measure seed mass in the present study, but previous field data revealed no differences in seed mass between uninfested plants and plants infested with *P. brassicae* eggs and larvae, despite differences in seed number (Pashalidou *et al.* 2015b). The fitness implications of the observed reproductive shifts are thus difficult to interpret, especially as even herbivory itself did not reduce seed production in damaged control plants (i.e. those not exposed to eggs or oviposition-induced volatiles). However, it appears that the onset of herbivory by the specialist *P. brassicae* (or exposure to herbivore-associated cues) mediates a shift to reproduction in *B. nigra*, perhaps with implications for fitness not captured in the context of our controlled greenhouse studies. In a previous field study (Pashalidou *et al.* 2015b), we did observe increased seed production by plants primed by eggs (relative to unprimed plants), perhaps due to higher parasitism rates documented for *P. brassicae* larvae feeding on these plants – which in turn might be explained by longer larval development times on primed plants (Pashalidou *et al.*, 2015a).

We did not directly assess flowering phenology, but evidence from previous work in this system suggests that the presence of *P. brassicae* eggs on leaves induces faster flowering relative to control plants (Pashalidou *et al.* 2013), which might serve as another means of reproductive escape from herbivory (Lucas-Barbosa *et al.* 2013). Plants are widely known to alter their flowering phenology in response to a wide range of abiotic and biotic stressors (Kazan & Lyons 2016), and such responses might plausibly help plants maintain their reproductive output in the face of herbivore attack, either by maximising reproduction prior to tissue loss, delaying reproduction until the threat of herbivory has passed (particularly in the case of herbivores that feed directly on flowers), or shifting reproductive effort from the male to female function (i.e. via pollen transfer). An alternative hypothesis that the observed increase in flower production may reflect manipulation by *P. brassicae*, a specialist herbivore that frequently feeds on flowers, is not supported by our previous findings that prior exposure to eggs – which has effects on both defence and life history similar to those

reported here for exposure oviposition-induced volatiles – had positive effects on plant fitness in the field while negatively affecting caterpillar performance (Pashalidou *et al.* 2015a,b).

CONCLUSION

Our results demonstrate that plants can detect volatiles produced by egg-infested plants in their vicinity and that these volatiles not only prime defences in the receiver plant, but also elicit striking changes in life-history strategies, namely a rapid shift from growth to reproduction that may enhance fitness via reproductive escape. These findings complement other recent studies showing that cues other than damage-induced volatiles can prime plant defences. In addition, they show that plant responses to early warning signals associated with herbivory can extend to plant traits not directly related to defence.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository: (<https://doi.org/10.5061/dryad.m0cfxpp0m>)

AUTHORSHIP

FGP, JB, NEF, MCM and CDM designed the study; FGP, JS and LE conducted the experiments; JS, LE and CDM conducted the chemical analyses, FGP and LE conducted the

statistical analyses; FGP wrote the first draft of the manuscript and all the authors contributed significantly to revisions.

DATA ACCESSIBILITY STATEMENT

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.m0cfxpp0m>.

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