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Pashalidou, FG

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1 **Plant volatiles induced by herbivore eggs prime defenses and mediate shifts in the**
2 **reproductive strategy of receiving plants**

4 Foteini G. Pashalidou^{1,2}, Lisa Eyman¹, James Sims¹, James Buckley¹, Nina E. Fatouros³,
5 Consuelo M. De Moraes¹, Mark C. Mescher^{1*}

7 ¹ Department of Environmental Systems Science, ETH Zürich, 8092, Zürich, Switzerland

8 ² UMR Agronomie, INRAE, AgroParisTech, Universite Paris-Saclay, 78850, Thiverval-
9 Grignon, France

10 ³ Biosystematics Group, Wageningen University, Droevedaalsesteeg 1, 6708 PB
11 Wageningen, Netherlands

13 Email addresses: foteini.pashalidou@inra.fr , lisa.eymann@gmx.ch ,
14 james.sims@usys.ethz.ch , james.buckley@env.ethz.ch , nina.fatouros@wur.nl,
15 consuelo.demoraes@usys.ethz.ch, mark.mescher@usys.ethz.ch

16 * corresponding author: mcmescher@usys.ethz.ch, tel: [+41 44 632 39 30](tel:+41446323930)

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36 **ABSTRACT**

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

48

49 **INTRODUCTION**

50 Plants face inevitable trade-offs when investing in defense versus growth and
51 reproduction, as well as among defense traits that may be effective against different classes of
52 antagonists (Zangerl & Rutledge 1996; Cipollini & Heil 2010). Where the risk of
53 encountering particular antagonists is unpredictable, these trade-offs may be mitigated via
54 inducible defenses that postpone the commitment of resources until a plant comes under
55 attack (Karban & Myers 1989; Agrawal 1999; Orrock *et al.* 2015); however, this strategy
56 entails a period of vulnerability during the time required to effectively deploy defenses
57 following induction (Karban 2011). Plants can shorten this period by adjusting their defense
58 phenotypes in response to early warning cues—detected prior to attack—that provide
59 information about the risk of encountering herbivores or pathogens (Dicke & Baldwin 2010;
60 Hilker & Fatouros 2015; Mescher & De Moraes 2015). It is now clear that the recognition of

61 such cues is widespread and that plant responses to such cues often entail defense priming
62 rather than full-scale defense induction (van Hulten *et al.* 2006; Frost *et al.* 2008a; Kim &
63 Felton 2013; Conrath *et al.* 2015; Dicke 2016; Hilker *et al.* 2016). Nevertheless, our
64 understanding remains limited both with respect to the range of early warning cues that plants
65 recognize and the nature of their responses.

66 Work on plant responses to the threat of herbivore attack has identified a range of
67 cues that appear to prime plant defenses. These include cues directly associated with
68 herbivores, such as herbivore movement on leaf surfaces (Peiffer *et al.* 2009), the presence of
69 insect eggs on plant tissues (Beyaert *et al.* 2012; Bandoly *et al.* 2016; Hilker & Fatouros
70 2016; Lortzing *et al.* 2019), and olfactory cues emitted by herbivores, such as pheromones
71 (Helms *et al.* 2013; Helms *et al.* 2017; Bittner *et al.* 2019). In addition, plants may respond to
72 indirect cues that reveal information about the presence of herbivores, most notably
73 herbivore-induced volatile emissions from plant tissues that are already under attack, which
74 have been shown to prime plant defenses in a wide range of systems (Heil & Kost 2006;
75 Frost *et al.* 2008a; Dicke & Baldwin 2010; Karban *et al.* 2014) and which appear to play a
76 signaling function within plants (Frost *et al.* 2007; Heil & Silva Bueno 2007), as well as
77 serving as cues for neighboring plants of the same or different species (Karban & Maron
78 2002; Kessler *et al.* 2006; Zhang *et al.* 2019).

79 The specific cues responsible for priming are often not known, although individual
80 herbivore-induced plant volatile compounds responsible for priming have been identified in a
81 handful of systems (Frost *et al.* 2008b; Sugimoto *et al.* 2014; Erb *et al.* 2015), as has an
82 herbivore-emitted compound that primes defenses in goldenrod (Helms *et al.* 2013; Helms *et*
83 *al.* 2017). The compounds identified by these studies are diverse, suggesting that plants can
84 potentially detect a wide range of chemical cues. In addition to herbivore-induced volatiles,
85 other indirect cues may provide plants with reliable information about the risks or impacts of

86 future herbivory. For example, there is evidence that abiotic factors, such as exposure to
87 heavy metals or changes in weather conditions, can alter the sensitivity of defenses to future
88 herbivore attack (Holopainen & Gershenson 2010; Winter *et al.* 2012; Pezzola *et al.* 2017).

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

97 In a few systems early warning cues have been shown to elicit responses that enhance
98 plant resistance against herbivores via direct or indirect defense mechanisms. For example,
99 tomato plants can convert the volatile (Z)-3-hexenol from damaged neighboring plants to (Z)-
100 3-hexenylvicianoside, a form of chemical defense that directly reduces herbivore
101 performance (Sugimoto *et al.* 2014). Meanwhile, plants infested with herbivore eggs emit
102 volatile compounds that attract egg or early-stage larval parasitoids, an effective indirect form
103 of defense (Hilker & Fatouros 2015), and egg infestation itself has been associated with an
104 enhanced physiological defense response upon herbivore feeding (Bandoly *et al.* 2016;
105 Lortzing *et al.* 2019), perhaps mediated by the detection of chemical cues in the egg-glue or
106 in oviduct secretions covering the eggs (Hilker & Fatouros 2015).

107 While most work on plant responses to early warning cues has focused on defense,
108 plant responses to herbivore feeding often include changes in growth or life-history traits that
109 can enhance plant tolerance or otherwise mitigate the fitness impacts of herbivory (Strauss &
110 Agrawal 1999; Agrawal 2000; Carmona *et al.* 2011; Garcia & Eubanks 2019). For example,

111 plants can invest more resources in vegetative growth and reproduction (Garcia & Eubanks
112 2019), or accelerate flowering and fruit production (a strategy referred to as reproductive
113 escape; Lucas-Barbosa *et al.* 2013). There is also evidence that plants can respond to severe
114 herbivore attack by deferring reproduction to future growing seasons and shifting current
115 investment to resistant belowground tissues (Schwachtje *et al.* 2006). It is therefore plausible
116 that cues detected prior to attack might elicit similar changes in plant traits not directly
117 related to defense. To date, however, only a few studies have documented changes in plant
118 growth or reproductive traits in response to cues that provide an early warning of herbivore
119 attack. For example, sagebrush exposed to cues from damaged neighbors increased
120 production of inflorescences and lateral branches at the expense of vertical growth (Karban *et*
121 *al.* 2012; Karban 2017), while tobacco plants exposed to damaged (manually clipped)
122 sagebrush neighbors produced more flowers and seeds than plants with undamaged neighbors
123 (Karban & Maron 2002). Exposure to the putative sex pheromone of a gall inducing fly was
124 also shown to increase short-term growth in goldenrod plants (Yip *et al.* 2017).

125 The current study explores how the annual brassicaceous plant *Brassica nigra* responds
126 to early warning cues associated with the presence of a specialist herbivore, *Pieris brassicae*.
127 Herbivory by *P. brassicae* can impose high fitness costs on *Brassica nigra*, which has
128 therefore evolved specialized defense responses (Blatt *et al.* 2008). These include responses
129 to oviposition, which has been shown to prime *B. nigra* defenses under both greenhouse and
130 field conditions (Pashalidou *et al.* 2013; Pashalidou *et al.* 2015a; Pashalidou *et al.* 2015b;
131 Pashalidou *et al.* 2015c). Plants infested with *P. brassicae* eggs were also found to flower
132 earlier and produce significantly more seeds than control plants (Lucas-Barbosa *et al.* 2013;
133 Pashalidou *et al.* 2015b) and to emit volatiles that recruit larval parasitoids (Pashalidou *et al.*
134 2013; Pashalidou *et al.* 2015b). Recent studies in other systems have also reported effects of
135 volatiles induced by herbivore oviposition, including enhanced parasitoid attraction in

136 teosinte (Mutyambai *et al.* 2016) and decreased herbivore recruitment in poplar (Guo *et al.*
137 2019). In light of these findings, we hypothesized that it might be adaptive for *B. nigra* plants
138 themselves to detect and respond to volatile cues associated with the presence of *P. brassicae*
139 eggs.

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

156

157 MATERIALS & METHODS

158 Plants and insects

159 This study examines two brassicaceous plant species, the wild annual *Brassica nigra*,
160 and the cultivated perennial *Brassica oleracea*. *Brassica nigra* is native to many European

161 countries (Lauber & Wagner, 2012). Seeds for this study were provided by the Centre of
162 Genetic Resources in Wageningen, the Netherlands (accession number: CGN06619). The
163 seeds were collected from plants grown in field sites around Wageningen, which were exposed
164 to wild pollinators; these seeds were then used to grow plants in the greenhouse for our
165 experiments. *Brassica oleracea* var. *capitata* (white cabbage) is endemic to the southern and
166 western coast of Europe and is now grown worldwide as an agricultural crop. White cabbage
167 seeds from the commercial variety “ESCAZU” (seed lot 2875500) were provided by Syngenta
168 Crop Protection AG (Basel, Switzerland). Plants of both species were grown in standard
169 potting soil (Substrat 2, Klasmann-Deilmann GmbH, Germany) in a climate chamber under
170 warm, long-day conditions (light 24°C, dark 22°C, L16h-D8h, 60% relative humidity).

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

176

177 **Generating the experimental treatments**

178 For each of the subsequent experiments with the two *Brassica* species, we used four
179 “priming” treatments (Fig. 1, Table S1), including: (i) plants infested with *P. brassicae* eggs
180 (E); (ii) plants receiving volatiles from egg-infested neighbors (Re); control receivers
181 exposed to volatiles from uninfested neighbors at close proximity (Rc); and unmanipulated
182 controls (C). Plants in the first treatment (E) were individually placed in cages with ~50
183 mated *P. brassicae* females and left until at least 40 eggs were laid (~15 minutes). Excess
184 eggs were gently removed with a fine brush (uninfested emitters were also brushed), and the
185 remaining eggs stayed on the plant until larval emergence (five days later). Plants in the

186 second treatment (Re) were placed ~15cm from plants in treatment E (for volatile exposure)
187 but removed prior to larval emergence. Control receiver plants (Rc) were similarly placed
188 ~15cm away from uninfested plants. Unmanipulated control plants (C) were grown farther
189 apart (>25cm) to minimize effects of volatile exposure. After five days, but prior to larval
190 emergence, plants in these four treatments were moved to random positions, and the spacing
191 between plants was increased to ~25cm. After larvae hatched on E plants, they were removed
192 and then used for subsequent damage treatments (with or without larvae, as discussed below)
193 (Fig. 1). Unique plants sets were used for each experiment described below.

194

195 ***Larval performance bioassays***

196 The effect of exposure to oviposition-induced volatiles on plant defenses in both *Brassica*
197 species was assessed via larval performance assays on non-flowering plants. Ten plants from
198 each priming treatment (E, Re, Rc and C) received 10 neonate *P. brassicae*. On days three and
199 seven following the initiation of feeding, larval mass was measured on a microbalance
200 (accuracy +/- 1µg; Mettler- Toledo AG, Greifensee, Switzerland) as described in Pashalidou et
201 al. (2013, 2015a, c).

202

203 ***Volatile collection and analysis***

204 For both *Brassica nigra* and *Brassica oleracea*, we collected volatiles from plants
205 exposed to the four priming treatments (N=12 per treatment) and with or without larval
206 damage. For damage treatments, ten L1 larvae were placed on E, Re and Rc plants. Due to
207 logistical constraints (and because our previous assays showed no effect of priming treatment
208 Rc on larval performance) we collected volatiles only from damaged Rc plants and used C
209 plants as undamaged controls. Larvae were placed on the adaxial side of the 3rd highest leaf.
210 One damaged plant was excluded from the damaged Re treatment because of unrelated

211 damage. Volatile collections were made one day prior to larval emergence and two hours after
212 the initiation of larval feeding. Pots were wrapped in foil to minimize plastic contaminants.
213 Two connecting metal plates were closed around the plant stem (with a hole for the stem to
214 pass), and cotton was used to seal gaps. A 30 L glass dome was carefully placed over the leafy
215 parts of the plant, with openings for incoming and outgoing air, which was filtered through
216 activated charcoal, pulled through the chamber at a rate of 150 ml/min for 4 h, and collected in
217 a stainless-steel cartridge containing 200 mg of Tenex TA (20/35 mesh; CAMSO, Houston,
218 TX, USA). Due to space limitations, volatile collections were conducted in three blocks. After
219 volatile collection, the aboveground parts of the plant were cut and weighed.

220 Volatile compounds were eluted from the filter using 150 µL of internal standard
221 solution (2 ng/µL octane and 4 ng/µL nonyl acetate in dichlormethane) and the eluant was
222 analysed by gas chromatography-mass spectrometry (GC-MS). Two µL of the eluant was
223 injected with an automatic Agilent injector 7693 autosampler (Santa Clara, CA, USA) to an
224 Agilent 7890B GC (Santa Clara, CA, USA) with a pulsed splitless inlet at 250°C, which was
225 held for 2min and then analyzed on the connected MS Agilent 5977A. Compounds were
226 quantified and identified as described in supplementary methods (Appendix S1). Volatile
227 emissions per plant were calculated as mean peak area divide by both the fresh weight of
228 foliage (in grams) and by 10⁴ the *n* of samples.

229

230 ***Testing effects of exposure to individual volatile compounds***

231 Because the emission of cumene was significantly elevated on egg-infested plants for
232 *B. oleracea* (Table S2), we also explored the defense priming effects of this compound on *B.*
233 *oleracea* and *B. nigra*. Unfortunately, we were unable to similarly test the effect of β-thujene—
234 a compound showing elevated emissions following egg infestation in *B. nigra* (Table S3)—as
235 we could not obtain this compound. We made a cumene solution containing 156µg/ml of

236 synthetic cumene (Sigma-Aldrich) in hexane, a concentration approximating the mean daily
237 emission of an egg-infested plant with a fresh aboveground mass of 200g. Over a five-day
238 period, 50µL of this solution was applied daily to sleeve-stopper septa (Sigma-Aldrich) placed
239 at a distance of 15cm from focal plants (treatment Cu; Table S1). The septa were placed at the
240 height of the receiver's apical meristem to simulate elevated cumene emission from an egg-
241 infested plant. Control plants were similarly exposed to 50µL hexane (treatment He). Each of
242 the 10 replicate plants per treatment was infested with 10 neonate larvae after exposure to
243 cumene for five days, and larvae were weighed three and seven days after placement.

244

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

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

260 cycle, ripe seeds were collected from each plant and measured with a seed counter (elmor c3
261 version 1.1, Switzerland). Germination rates were measured as in Pashalidou et al 2015b.

262

263 **Statistical analyses**

264 Caterpillar weights were log-transformed to satisfy normality and the effect of different
265 treatments analyzed with a General Linear Model (GLM) using restricted maximum likelihood
266 (REML). Germination rates, as well as numbers of flowers and seeds, were also log-
267 transformed to satisfy normality and analysed with a LMM using REML (Bates *et al.* 2014)
268 with R Software version 3.3.1. Treatments were defined as fixed effects, and inter-plant
269 variation in reproductive/growth traits was modeled using a random effect of plant replicate.
270 The significance of differences between treatments was evaluated with a Tukey's post hoc test.

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

280

281 **RESULTS**

282 **Oviposition-induced plant volatiles prime plant defenses**

283 Larvae feeding on *B. oleracea* plants previously exposed to eggs (E) or to volatiles from egg-
284 infested plants (Re) had significantly lower biomass after three and seven days feeding than

285 larvae feeding on control plants (C), or those exposed to volatiles from control plants (Rc) (3
286 days: $F = 11.1$, $P < 0.001$, 7 days: $F = 13.4$, $P < 0.001$, Appendix S2a,b, Fig. 2a). Similarly,
287 larvae feeding on *B. nigra* plants were also negatively affected by plant exposure to eggs or
288 volatiles from egg-infested plants (3 days: $F = 24.3$, $P < 0.001$, 7 days: $F = 43.6$, $P < 0.001$,
289 Appendix S3a, b, Fig. 2c).

290

291 **The presence of eggs on leaves upregulates specific volatile compounds**

292 In total, 39 volatile compounds were detected for *Brassica nigra* and 40 for *Brassica oleracea*
293 across all experimental treatments and time points. Principal components analysis of volatile
294 emissions did not show clear multivariate divergence among treatments (Fig. S1). In *Brassica*
295 *oleracea*, most compounds (39/40) did not significantly differ among treatments after
296 controlling for multiple testing using Bonferroni correction (Table S2); however, cumene was
297 emitted in significantly higher amounts from egg-infested (E) plants than from plants in any
298 other treatment (t -value = 2.46, $df = 25$, $P = 0.02$, Fig. 3a). In *Brassica nigra*, a different
299 compound, β -thujene, was the single compound emitted in significantly larger amounts from
300 plants induced by egg deposition (E) compared to all other treatments (t -value=2.36, $df = 34$,
301 $P = 0.024$; Fig. 3b; Table S3).

302

303 **Cumene primes defences in *B. oleracea***

304 Because our volatile analyses identified clear effects of eggs on volatile emissions, we next
305 explored the effects of the relevant compounds on defense priming. We found that *P. brassicae*
306 larvae feeding on *B. oleracea* plants previously exposed to cumene, the single compound
307 upregulated by *P. brassicae* eggs in this plant species, had significantly lower biomass than
308 larvae feeding on plants exposed to hexane alone after 7 days of feeding (after 3 days: $F = 0.6$,
309 $P = 0.42$; after 7 days: $F = 7.1$, $P = 0.02$; Appendix S4a,b; Fig. 2b). By contrast, larvae feeding
310 on *B. nigra* plants previously exposed to cumene exhibited no significant differences in

311 biomass from larvae feeding on plants exposed only to hexane at either time point (3 days: $F =$
312 $0.8, P = 0.35$; 7 days: $F = 1.7, P = 0.18$; Appendix S5a,b; Fig. 2d). It thus appears that cumene
313 functions as a species-specific priming signal in *B. oleracea*. β -thujene, the compound similarly
314 upregulated by eggs on *B. nigra* plants, may play a similar role for that species; however, we
315 were unable to test the effects of β -thujene on priming, as this compound could not be
316 commercially obtained and was challenging to synthesize.

317

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

319 Compared to undamaged controls (C), undamaged *Brassica nigra* plants infested with eggs (E)
320 and those exposed to volatiles from egg-infested plants (Re) produced significantly higher
321 numbers of flowers ($F = 10.6, P < 0.001$; Appendix S6; Fig. 4a), fewer leaves ($F = 4.64, P =$
322 0.002 ; Appendix S7; Fig. 4b), and more seeds ($F = 8.3, P < 0.001$; Appendix S8; Fig. 4c),
323 indicating a possible shift in growth-reproduction strategy. No similar effects were observed
324 among damaged treatments, although there was a trend toward greater flower production by
325 damaged E and Re plants compared to damaged control plants (Fig. 4). Germination rates of
326 resulting seeds did not significantly differ between treatments ($F = 1.75, P = 0.14$; Fig. 4d).

327

328 **DISCUSSION**

329 Our results show plant resistance to herbivory can be enhanced by prior exposure to
330 volatile emissions from plant tissues infested with herbivore eggs, suggesting that oviposition-
331 induced plant volatiles may play a role in priming plant defenses similar to that of other early
332 warning cues directly and indirectly associated with the presence of herbivores. However, we
333 also found that exposure to oviposition-induced volatiles elicited changes in life history traits
334 not directly related to defense (Fig. 5). Specifically, we observed a shift from investment in
335 growth to reproduction in *B. nigra* plants exposed to oviposition-induced volatiles similar to

336 that seen in plants directly infested with eggs, in both the current study and previous work
337 (Pashalidou *et al.* 2015b). These findings thus have implications, which we discuss in more
338 detail below, for understanding both the breadth of early warning cues to which plants respond
339 and the nature of those responses.

340

341 ***Oviposition-induced volatiles prime anti-herbivore defenses***

342 Defense priming has previously been reported in response to direct cues from
343 herbivores, including egg deposition (Hilker & Fatouros 2015; Bandoly *et al.* 2016; Lortzing
344 *et al.* 2019), as well as indirect cues in the form of damage-induced volatiles from neighboring
345 plants (Engelberth *et al.* 2004; Ton *et al.* 2007; Frost *et al.* 2008a; Mescher & De Moraes 2015).

346 The current results suggest that oviposition-induced volatiles can also prime plant defenses;
347 this finding complements other recent work showing that oviposition-induced volatiles also
348 play a communicative function in indirect plant defense by serving as foraging cues for natural
349 enemies of herbivores, as well as herbivores themselves. For example, both egg and larval
350 parasitoids showed a stronger preference for maize plants exposed to volatiles of plants infested
351 by eggs of the herbivore *Chillo partellus* than control plants (Mutyambai *et al.* 2016).
352 Ovipositing herbivores were also seen to avoid poplar plants exposed to volatiles from egg-
353 infested neighbors (Guo *et al.* 2019).

354 We found that the presence of eggs on leaves upregulated levels of different terpenoid
355 compounds for each of our two focal species: β -thujene for *B. nigra* and cumene for *B.*
356 *oleracea*. Furthermore, we found that exposure to cumene alone was sufficient to prime
357 defenses in *B. oleracea*. We were unfortunately unable to obtain β -thujene for testing; however,
358 cumene did not prime defenses in *B. nigra*, suggesting that there is species-level specificity in
359 oviposition-induced volatile signaling systems. A number of studies have now identified
360 specific volatile cues induced by feeding insects that act as a priming stimuli for neighboring

361 plants (Engelberth *et al.* 2004; Kost & Heil 2006; Frost *et al.* 2008b; Sugimoto *et al.* 2014; Erb
362 *et al.* 2015), yet few studies have assessed the potential generality or specificity of these volatile
363 cues across different species. For example, exposure to herbivore-induced indole clearly
364 altered volatile profiles emitted by maize, but had a much weaker effect on cotton (*Gossypium*
365 *hirsutum*) or cowpea (*Vigna unguiculata*) (Erb *et al.* 2015). The current results suggest that
366 volatile signals associated with defense priming can vary even among closely related plant
367 species responding to the same herbivore antagonist. It would be interesting to test whether the
368 specific compounds implicated here (cumene and β -thujene) might also serve as important cues
369 for herbivores and their natural enemies, particularly as *P. brassicae* females have been shown
370 to use oviposition-induced volatile cues to avoid plants harboring conspecific eggs (Fatouros
371 *et al.* 2012).

372 As in other systems where volatile cues produced by conspecifics prime defenses, it is
373 unclear whether oviposition-induced volatile cues function primarily in interactions within or
374 between plants and, to the extent that plants do respond to cues from neighbors, whether this
375 represents between-plant communication or merely eavesdropping (Heil & Silva Bueno 2007;
376 Dicke & Baldwin 2010). *Brassica* spp. often grow in dense stands across which herbivores,
377 including later instars of specialists like *Pieris brassicae*, can readily move (Lucas-Barbosa *et*
378 *al.* 2013; Pashalidou *et al.* 2013), suggesting that defense priming (or shifts in reproductive
379 strategy, as discussed below) in response to herbivore-associated cues from neighboring plants
380 might be adaptive. Furthermore, because these plants often grow in close proximity to close
381 relatives, such responses might also enhance the inclusive fitness of the emitter. On the other
382 hand, as the young gregarious larvae of *Pieris brassicae* do not move to neighboring plants
383 until they reach later larval stages (Lucas-Barbosa *et al.* 2013), such cues might not always
384 indicate that herbivory is imminent; however, such cues might still serve to indicate increased
385 risk that the receiving plants will also be targeted for oviposition.

386

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

388 In addition to priming or inducing defenses, plants exposed to the threat of herbivory
389 can alter their reproductive strategies to minimize impacts on fitness (Strauss & Agrawal 1999;
390 Lucas-Barbosa *et al.* 2013). We found that plants exposed to oviposition-induced volatiles from
391 neighboring plants produced more flowers and seeds, but fewer leaves, than control plants, a
392 response similar to that previously reported for plants directly exposed to eggs (Lucas-Barbosa
393 *et al.* 2013; Pashalidou *et al.* 2015b). To our knowledge, no previous studies have reported
394 shifts in plant reproductive strategy following exposure to oviposition-induced volatiles.
395 However, there is evidence for life history shifts in response to damage-induced volatiles in a
396 few systems; for example, tobacco plants with clipped sagebrush neighbors produced more
397 flowers and seeds than plants with unclipped neighbors (Karban & Maron 2002).

398 The increased seed production observed in undamaged *B. nigra* plants exposed to
399 oviposition-induced volatiles (or to eggs) was lost when larvae were allowed to feed on the
400 plants, although a trend towards greater flower production persisted—we did not measure seed
401 mass in the present study, but previous field data revealed no differences in seed mass between
402 uninfested plants and plants infested with *P. brassicae* eggs and larvae, despite differences in
403 seed number (Pashalidou *et al.* 2015b). The fitness implications of the observed reproductive
404 shifts are thus difficult to interpret, especially as even herbivory itself did not reduce seed
405 production in damaged control plants (i.e., those not exposed to eggs or oviposition-induced
406 volatiles). However, it appears that the onset of herbivory by the specialist *P. brassicae* (or
407 exposure to herbivore-associated cues) mediates a shift to reproduction in *B. nigra*, perhaps
408 with implications for fitness not captured in the context of our controlled greenhouse studies.
409 In a previous field study (Pashalidou *et al.* 2015b), we did observe increased seed production
410 by plants primed by eggs (relative to unprimed plants), perhaps due to higher parasitism rates

411 documented for *P. brassicae* larvae feeding on these plants—which in turn might be explained
412 by longer larval development times on primed plants (Pashalidou et al., 2015a).

413 We did not directly assess flowering phenology, but evidence from previous work in
414 this system suggests that the presence of *P. brassicae* eggs on leaves induces faster flowering
415 relative to control plants (Pashalidou et al. 2013), which might serve as another means of
416 reproductive escape from herbivory (Lucas-Barbosa et al. 2013). Plants are widely known to
417 alter their flowering phenology in response to a wide range of abiotic and biotic stressors
418 (Kazan & Lyons 2016), and such responses might plausibly help plants maintain their
419 reproductive output in the face of herbivore attack, either by maximizing reproduction prior to
420 tissue loss, delaying reproduction until the threat of herbivory has passed (particularly in the
421 case of herbivores that feed directly on flowers), or shifting reproductive effort from the male
422 to female function (i.e., via pollen transfer). The alternative hypothesis that the observed
423 increase in flower production reflects manipulation by the herbivore, *P. brassicae*, which
424 frequently feeds on flowers, is not supported by our previous findings that prior exposure to
425 eggs, which has effects on both defense and life history similar to those reported here for
426 exposure oviposition induced volatiles, had positive effects on plant fitness in the field while
427 negatively affecting caterpillar performance (Pashalidou et al. 2015a,b).

428

429 CONCLUSION

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

436 herbivory can extend to plant traits not directly related to defense.

437

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441

442 CONFLICTS OF INTEREST

443 The authors state no conflicts of interest

444

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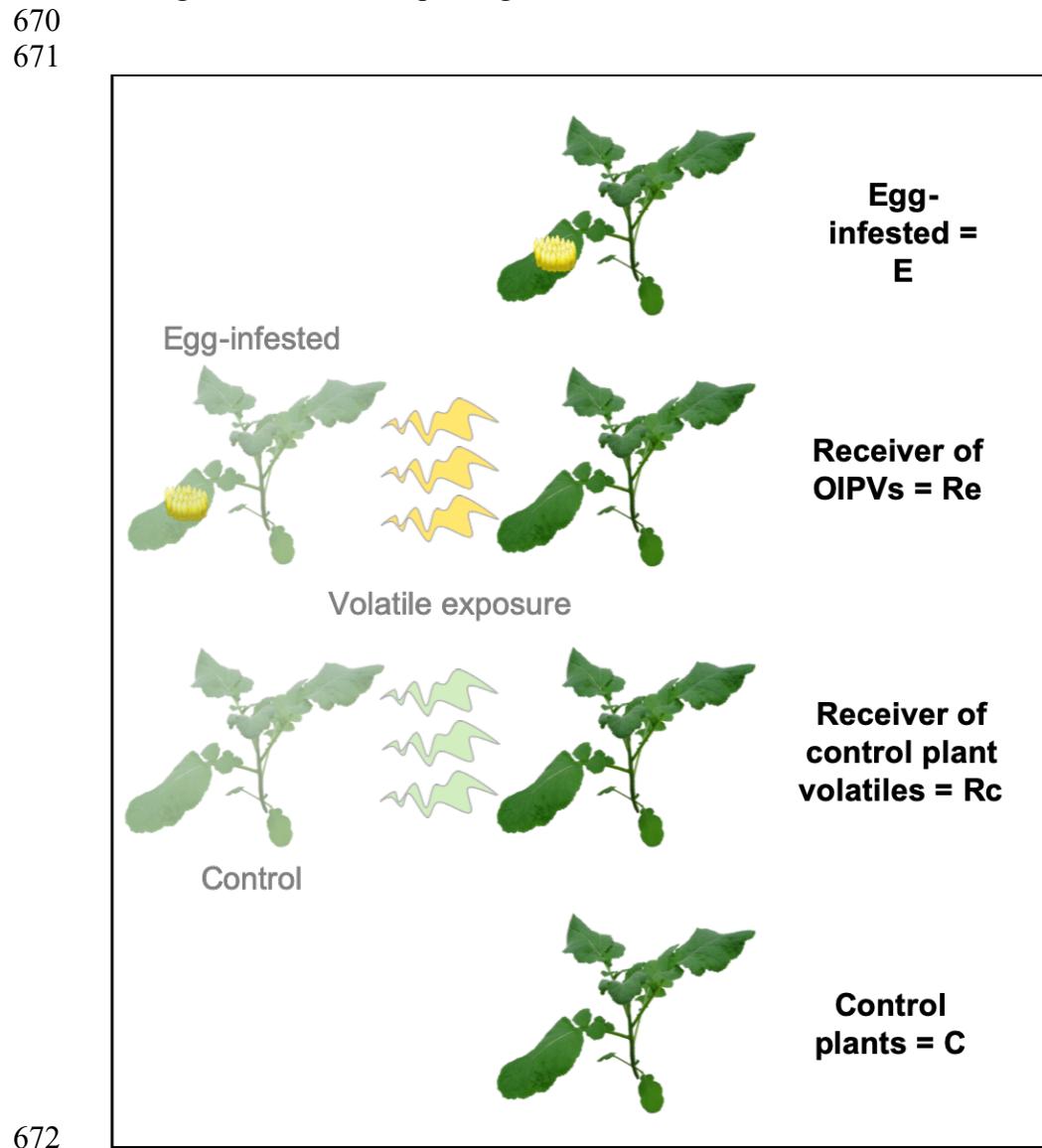
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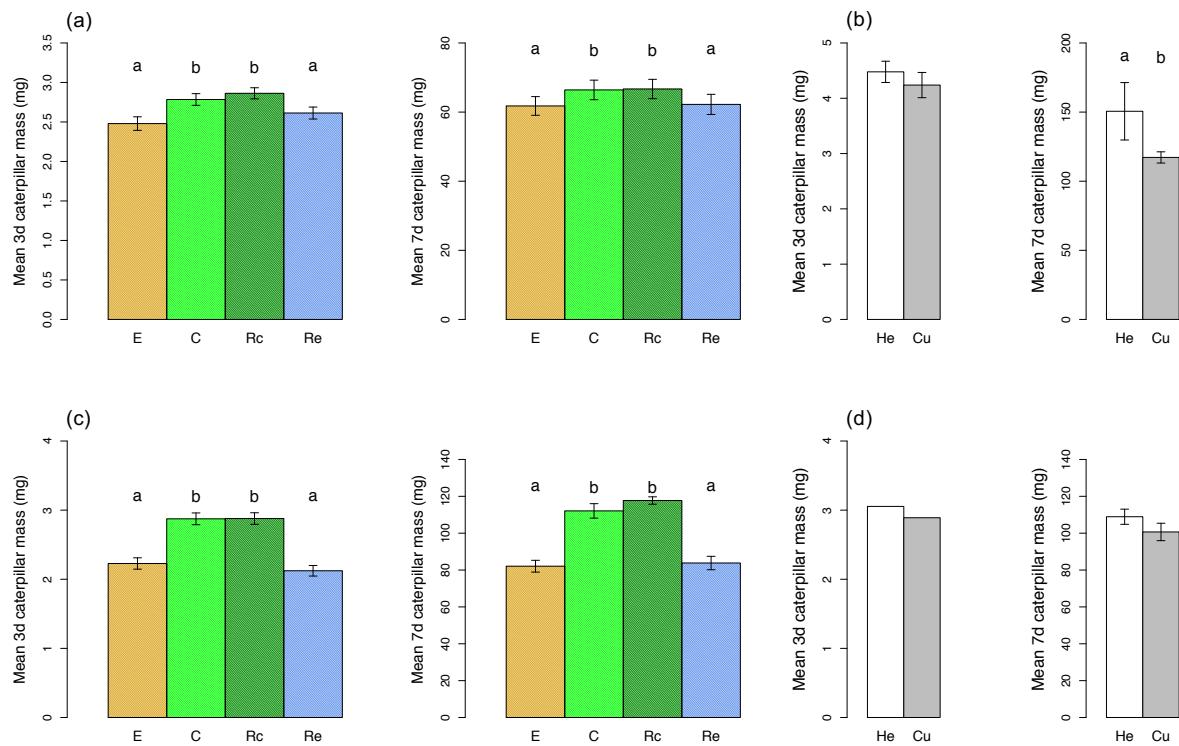
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662 **FIGURES**
663 **Figure 1:** The different treatments used throughout this study were generated through exposure to
664 direct egg infestation, oviposition-induced volatiles or volatiles from neighboring control plants.
665 Control plants were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



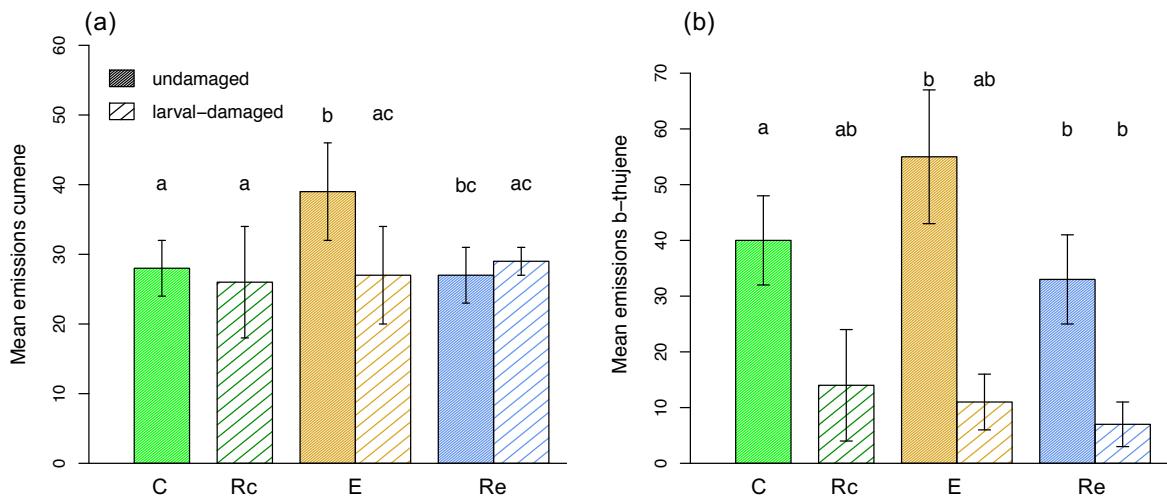
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674 **Figure 2.** Effects of exposure to oviposition-induced plant volatiles or cumene on plant
 675 palatability to a specialist herbivore. As a proxy for relative investment in defense, caterpillar
 676 weight was measured three and seven days after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



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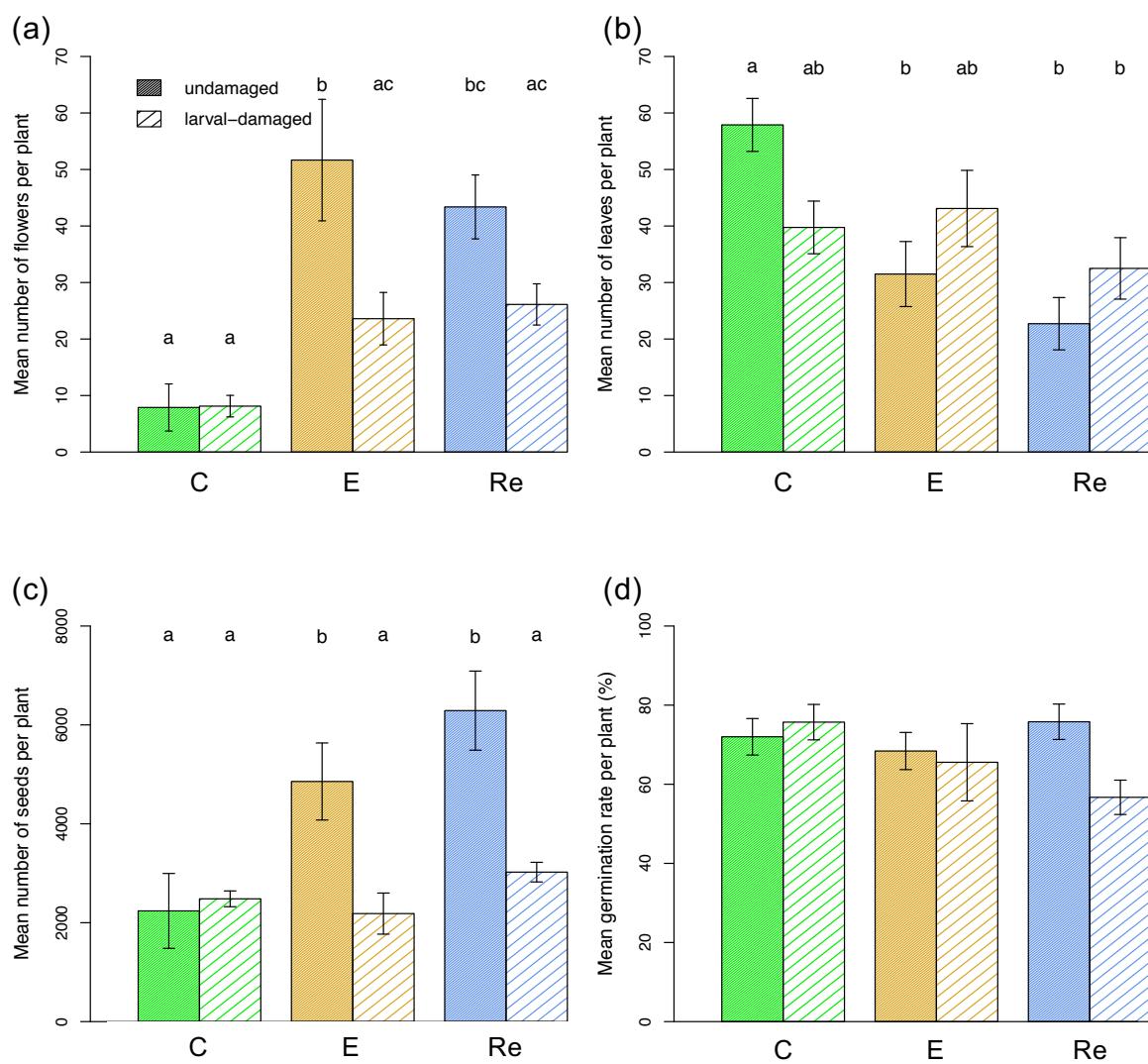
684 **Figure 3:** Variation in emission of the volatile compound significantly induced by egg
 685 infestation in each of the *Brassica* species. (a) Cumene emissions were significantly elevated
 686 for egg-infested *Brassica oleracea* plants. (b) β -thujene emissions were significantly elevated
 687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



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

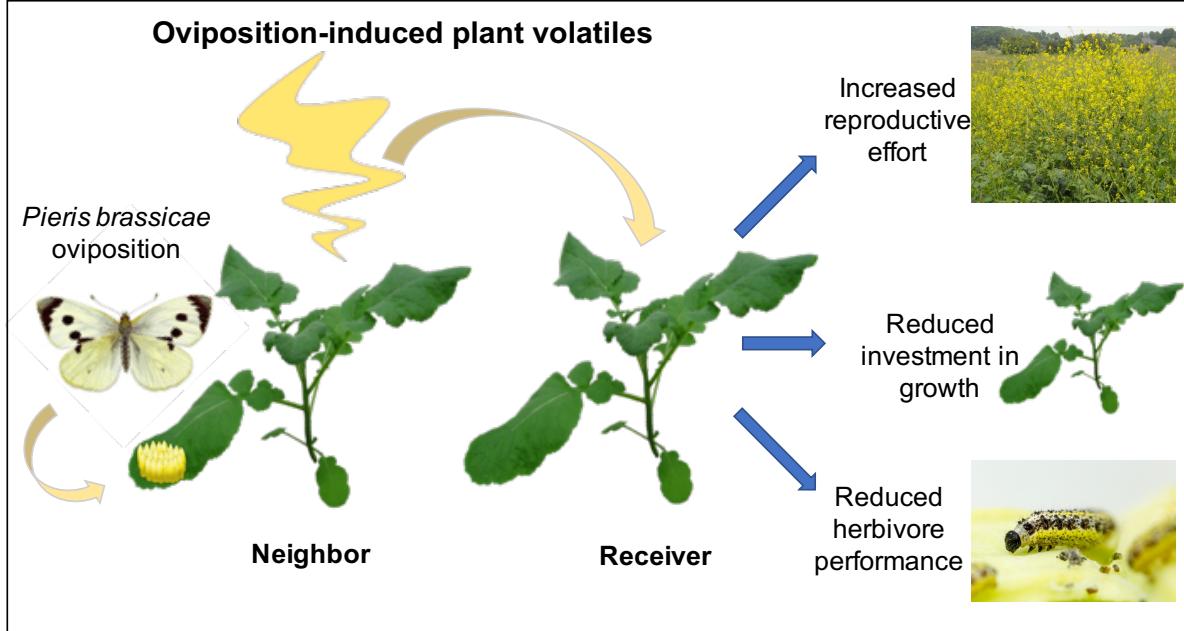
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708 **Figure 5:** Oviposition-induced plant volatiles prime defenses in *Brassica nigra*, but also elicit
709 changes in life history strategy, including a shift from investment in growth to reproduction.
710 Photo credit: Rupesh Kariyat.

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