

- 1 *Running head:* Leaf attack triggers root herbivore avoidance
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- 4 *Research area:* Signaling and response

5 **A physiological and behavioral mechanism for leaf-herbivore induced systemic root resistance**

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21 *One-sentence Summary:* Leaf-herbivore attack changes free and conjugated phenolic acids in maize
22 roots and thereby triggers an avoidance response in a specialist root herbivore.

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35 *Abstract*

36 Indirect plant-mediated interactions between herbivores are important drivers of community
37 composition in terrestrial ecosystems. Among the most striking examples are the strong indirect
38 interactions between spatially separated leaf and root feeding insects sharing a host plant. Although leaf-
39 feeders generally reduce the performance of root herbivores, little is known about the underlying
40 systemic changes in root physiology and the associated behavioral responses of the root feeders. We
41 investigated the consequences of maize leaf-infestation by *Spodoptera littoralis* caterpillars for the root-
42 feeding larvae of the beetle *Diabrotica virgifera*, a major pest of maize. *Diabrotica virgifera* strongly
43 avoided leaf-infested plants by recognizing systemic changes in soluble root components. The avoidance
44 response occurred within 12 hours and was induced by real and mimicked herbivory, but not wounding
45 alone. Roots of leaf-infested plants showed altered patterns in soluble free and soluble conjugated
46 phenolic acids. Biochemical inhibition and genetic manipulation of phenolic acid biosynthesis led to a
47 complete disappearance of the avoidance response of *D. virgifera*. Furthermore, bioactivity guided
48 fractionation revealed a direct link between the avoidance response of *D. virgifera* and changes in
49 soluble conjugated phenolic acids in the roots of leaf-attacked plants. Our study provides a physiological
50 mechanism for a behavioral pattern which explains the negative effect of leaf-attack on a root feeding
51 insect. Furthermore, it opens up the possibility to control *D. virgifera* in the field by genetically
52 mimicking leaf-herbivore induced changes in root phenylpropanoid patterns.

53 *Introduction*

54 Insect herbivores constantly compete for plants as primary terrestrial source of organic carbon and
55 nitrogen (Denno et al., 1995). Consequently, resource competition is thought to be a major determinant
56 of the distribution and abundance of insects in natural and agricultural systems (Begon et al., 2006).
57 Recent evidence suggests however that in many cases, insect herbivore competition may not follow the
58 traditional theoretical assumptions of direct interference and/or resource exploitation, but may be
59 determined by indirect plant mediated effects (Kaplan and Denno, 2007; Poelman et al., 2008). Among
60 the most striking examples of indirect plant mediated interactions is the interplay between root and leaf
61 feeding insects (Blossey and Hunt-Joshi, 2003): Despite their non-overlapping feeding niches, leaf and
62 root herbivores determine each other's performance through shared host plants (Bezemer and Van Dam,
63 2005). While root feeders can have positive or negative effects on leaf feeders (Van Dam and Heil,
64 2011), the effect of leaf-herbivores on root-consumers is predominantly negative (Johnson et al., 2012,
65 but see Huang et al., 2014).

66 Despite the increasing number of examples demonstrating negative effects of leaf attack on root
67 herbivores (Tindall and Stout, 2001; Blossey and Hunt-Joshi, 2003; Soler et al., 2007; Gill et al., 2011),
68 the mechanisms underlying this form of systemic induced resistance remain poorly understood (Erb et
69 al., 2008; Rasmann and Agrawal, 2008). *Pieris brassicae* for instance was found to increase
70 glucosinolate levels in the roots, which correlated with a reduced survival of the root feeder *Delia*
71 *radicum* (Soler et al., 2007). Understanding why root feeders perform worse on leaf-infested plants
72 would allow for more detailed investigations regarding the adaptive and evolutionary context of the
73 phenomenon and may allow for its exploitation in agriculture, for instance by triggering root resistance
74 through targeted leaf treatments.

75 A promising system to study the mechanisms and agroecological consequences of plant-mediated
76 interactions between herbivores is maize and its associated pests. In the field, maize is attacked by a
77 suite of herbivores, including leaf-feeders, stem-borers and root-feeders. The highly specialized root-
78 feeding larvae of the western corn rootworm *Diabrotica virgifera virgifera* cause significant plant
79 damage and yield loss in the United States and Eastern Europe. Earlier studies demonstrated that *D.*
80 *virgifera* attack increases leaf resistance against *Spodoptera* spp. by triggering drought stress responses
81 (Erb et al., 2011b; Erb et al., 2009). In the opposite direction, leaf-feeding by *Spodoptera* caterpillars
82 reduces *D. virgifera* growth and development in a sequence-specific manner in the laboratory and the
83 field (Gill et al., 2011; Erb et al., 2011c). *D. virgifera* was subsequently demonstrated to avoid leaf-
84 infested plants by detecting and responding to a reduction in root ethylene emissions (Robert et al.,
85 2012). However, it remains unclear whether non-volatile chemical changes in the roots of leaf-infested
86 maize plants affect *D. virgifera* foraging and performance. In this study, we explored the hypothesis that
87 leaf infestation by *Spodoptera* spp. caterpillars trigger a short range avoidance response in *D. virgifera*.
88 Through a combination of bioactivity guided fractionation of root extracts, biochemical and molecular

89 manipulation, we show that systemic changes in soluble phenylpropanoid derivatives trigger a strong
90 avoidance response in *D. virgifera*. We furthermore demonstrate that this avoidance response is
91 mediated by systemic internal signals and is triggered specifically by herbivory, suggesting that *D.*
92 *virgifera* actively and specifically recognizes and avoids leaf-infested plants.

93 *Results*

94 ***Diabrotica virgifera* specifically recognizes and avoids leaf-infested plants**

95 To test whether *D. virgifera* is able to distinguish between infested and non-infested plants in the soil,
96 we offered maize seedlings that were infested in the leaves by *Spodoptera littoralis* or herbivore-free to
97 the root feeding larvae of *D. virgifera* in a two-arm below ground system (Robert et al., 2012) (Fig. 1).
98 After 48 h of foraging activity, significantly more larvae were recovered on control plants than infested
99 plants (Fig. 1A). As root-volatiles may mediate *D. virgifera* foraging behavior (Robert et al., 2012), we
100 conducted an additional experiment in which root systems of leaf-infested and non-infested plants were
101 intertwined and offered to *D. virgifera* together in a single petri dish, so that larvae could not distinguish
102 between the root systems of individual plants by using volatiles as long-distance cues. Again, *D.*
103 *virgifera* showed a pronounced preference to feed on non-infested plants (Fig. 1B) indicating that
104 changes in root volatiles are not necessary to trigger the avoidance response. A time course revealed that
105 the avoidance response started 24 h after the beginning of leaf attack by *S. littoralis* and was most
106 pronounced after 48 h (Fig. 1C). To test whether *D. virgifera* responds specifically to herbivore-induced
107 changes in the plants, we wounded leaves and treated a subset of them with *S. littoralis* regurgitate,
108 which induces a plant response similar to real herbivory (Erb et al., 2009). Wounding and leaf-removal
109 did not trigger an avoidance response (Fig. 1D). By contrast, adding regurgitate to the wounds elicited
110 a behavioral response similar to real *S. littoralis* attack, demonstrating that *D. virgifera* specifically
111 recognizes leaf-infested plants. The response to a single, artificial elicitation event started 12 h after
112 treatment and subsided between 24 and 48 h (Fig. 1E), suggesting a slow and transient change in root
113 chemistry upon a single leaf elicitation. To understand whether internal leaf-to-root signals are
114 responsible for the elicited behavior or whether signals pass externally from the above ground
115 atmosphere through the rhizosphere, we sealed off the soil and root system from the above ground
116 atmosphere with an air-tight agarose/aluminum seal so that the only shoot-root contact was via the plant
117 interior. *Diabrotica virgifera* responded by avoiding *S. littoralis* infested plants irrespective of direct
118 contact between the phyllosphere and the rhizosphere (Fig. 1F), demonstrating that a systemic change
119 in the roots mediated by internal signaling is responsible for the reduction in attractiveness of the roots.
120 To evaluate whether the systemic changes are due to water-soluble or non-soluble substances, we
121 obtained liquid fractions from the roots and mixed them with agarose to test the feeding preference of
122 *D. virgifera* in an agarose cube choice assay. *D. virgifera* larvae preferred to feed on control fractions
123 over leaf-induced fractions (Fig. 1G), showing that non-structural chemical changes in the roots are
124 sufficient to explain the observed behavior.

125 **Leaf infestation changes root phenylpropanoid accumulation**

126 As phenolic compounds have been associated with changes in root herbivore performance in other plant
127 species (Johnson et al., 2011), we hypothesized that changes in the phenylpropanoid pathway may be
128 responsible for the change in *D. virgifera* behavior. To evaluate whether leaf infestation changes root
129 phenolic acids, we analyzed crown and primary roots of leaf-infested plants by HPLC-MS/MS. Based
130 on the results of our choice experiments, we focused on soluble rather than cell-wall bound phenolic
131 acids. As soluble phenylpropanoids can be conjugated to proteins and other metabolites and may thereby
132 escape detection (Nicoletti et al., 2013), we subjected soluble extracts to acid and basic hydrolysis to
133 release ester and ether-bound soluble phenolic acids. Both hydrolysis protocols resulted in the release
134 of significant quantities of phenolic acids. Compared to free phenolic acids, which were found in
135 concentrations between 1 and 70 ng*g FW, soluble hydrolyzed phenolic acids were up to 100 times
136 more abundant in the roots, with concentrations ranging from 0.1 to 8 µg*g FW (Fig. 2). Primary and
137 crown roots differed in their phenylpropanoid patterns, with primary roots containing higher amounts
138 of basic hydrolysable caffeic acid, free and acid hydrolysable ferulic acid and acid hydrolysable sinapic
139 acid. Primary roots also had lower concentrations of basic hydrolysable *p*-coumaric acid, acid
140 hydrolysable caffeic acid and basic hydrolysable ferulic acid than crown roots. Leaf-infestation by *S.*
141 *littoralis* reduced the concentrations of all basic and acid hydrolysable phenolic acids as well as free
142 caffeic acid in the roots (Fig. 2). By contrast, we observed a small but consistent average increase in
143 soluble ferulic acid in the roots of leaf-infested plants. Pairwise comparisons revealed that different
144 phenolic acids were reduced in primary and crown roots, even though the overall trends stayed the same
145 and no significant interactions between root type and leaf treatment were detected by two-way ANOVA
146 ($p>0.05$).

147 **Manipulating the phenylpropanoid pathway disrupts *Diabrotica virgifera* host choice**

148 To test whether the leaf-herbivore induced changes in root phenolic acids are responsible for the reduced
149 attractiveness of maize roots to *D. virgifera*, we performed a series of manipulative experiments (Fig.
150 3). First, we treated maize roots with piperonylic acid (PA), which inhibits the conversion of cinnamic
151 acid to *p*-coumaric acid through competitive inhibition of the cinnamate 4-hydroxylase C4H (Schalk,
152 1998). To confirm the efficacy of the treatment, we measured cinnamic acid accumulation in the roots
153 following PA application. As expected, we observed a strong accumulation of soluble free and
154 conjugated cinnamic acid (Fig. S1). Furthermore, we observed a slight reduction in free sinapic acid.
155 However, contrary to what has been reported in other plant species (Schalk, 1998; Naseer et al., 2012)
156 we did not observe a depletion of *p*-coumaric acid, caffeic acid or ferulic acid. Soluble acid hydrolysable
157 caffeic acid, ferulic acid and sinapic acid even increased in concentration in C4H inhibited plants,
158 suggesting that they are formed and induced by PA through a C4H independent pathway, for instance
159 through the production of *p*-coumaric acid from tyrosine (Rösler et al., 1997). As the PA treatment
160 significantly changed the synthesis of free and conjugated phenolic acids, we concluded that this
161 treatment is nevertheless suitable to gain first insights into the potential involvement of this metabolite

162 class in leaf-herbivore induced root resistance. When *D. virgifera* larvae were offered a choice between
163 buffer-treated control and *S. littoralis* infested plants, they showed the usual preference for control
164 plants. However, when C4H was inhibited, no choice was observed any more (Fig. 3A). A similar result
165 was obtained with plants that were elicited by wounding and application of regurgitate (Fig. 3B). To
166 understand this pattern in more detail, we complemented inhibited and non-inhibited control and induced
167 plants with a 5.5 mM solution of *p*-coumaric acid. *P*-coumaric acid complementation in the absence of
168 induction did not elicit a preference response in *D. virgifera* (Fig. 3C). However, complementing a C4H
169 inhibited, leaf-induced plant restored the preference pattern of the larvae, suggesting that C4H dependent
170 *p*-coumaric acid is necessary for the repellent effect of the roots, and that induction by leaf-herbivory is
171 specifically required to elicit this response.

172 In maize, several mutants have been characterized that are defective in their capacity to produce *p*-
173 coumaric acid derived phenolic acids and lignin (Halpin et al., 1998). We used two brown-midrib
174 mutants, bm1 and bm3, to further understand the importance of phenolic acid derivatives for *D. virgifera*
175 host choice (Fig. 4). Bm1 is defective in cinnamyl alcohol dehydrogenase (CAD) activity required to
176 convert phenolic aldehydes into their alcoholic forms (Halpin et al., 1998). The bm3 mutant has a
177 defective caffeic acid *O*-methyl transferase (COMT), which is necessary for the production of sinapic
178 acid type phenolics and lignin (Vignols et al., 1995). Both mutations exert feedback effects on
179 phenylpropanoid biosynthesis (Guillaumie et al., 2007). Our own analyses showed that, compared to the
180 near isogenic wild type line F2 (Guillaumie et al., 2007), the bm1 mutant is depleted in most soluble
181 free phenolic acids, but over-accumulates soluble hydrolysable ferulic acid and sinapic acid, while the
182 bm3 mutant is depleted in free phenolics without showing an over-accumulation of hydrolysable
183 compounds (Fig. S2). Furthermore, both mutants accumulated slightly higher levels of caffeic acid. No
184 phenotypic differences in root system architecture were observed between wild type and mutant lines
185 (Fig. S3). Lignin levels at the seedling stage are low, as most lignin deposition occurs after the end of
186 internode elongation. (Müse et al., 1997, Riboulet et al., 2009). When given a choice between *S. littoralis*
187 infested and control F2 wild type plants, *D. virgifera* exhibited a strong preference for the controls. In
188 both bm1 and bm3 mutants however, *D. virgifera* was no longer able to distinguish leaf-infested from
189 control plants (Fig. 4A). When leaves were elicited by wounding and regurgitate, *D. virgifera* chose the
190 control side in the F2 and bm1 background, but did not show any preference in the bm3 mutant any
191 more (Fig. 4B). The differential preference between real and simulated herbivory in the bm1 mutant was
192 confirmed in a supplementary experiment that directly compared the two treatments (Fig. S4). These
193 data confirm that an intact phenylpropanoid pathway is required for the negative effect of leaf-herbivory
194 on root attractiveness. Furthermore, they illustrate that Bm1 is required for *D. virgifera* to recognize *S.*
195 *littoralis* infested, but not artificially elicited plants.

196 **Bioactivity-guided fractionation associates *D. virgifera* choice with differential accumulation of**
197 **conjugated phenolic acids**

198 To further confirm the role of phenolic acids in leaf-herbivore induced root resistance, we collected
199 soluble root fractions from control and *S. littoralis* infested plants, redissolved them in 50 % MeOH and
200 fractionated them further by reverse-phase semi-preparative HPLC. Each fraction was then tested for
201 activity by mixing it with agarose and offering it to *D. virgifera* in a choice assay (Fig. 5). Two non-
202 polar fractions (VIII & IX) were identified to exhibit activity and elicit a significant preference for
203 control over *S. littoralis* infested extracts (Fig. 5A). As conventional metabolomics fingerprinting by
204 ultra-high performance liquid chromatography-time of flight-mass spectrometry (UHPLC-TOF-MS)
205 did not reveal any differentially accumulating peaks in the active fractions (Fig. S5), we conducted a
206 second fractionation run and analyzed the active fraction for free and hydrolyzable phenolic acids by
207 HPLC-MS/MS. This approach enabled us to separate conjugated phenolic acids in intact form and assess
208 their abundance in each fraction individually through hydrolysis. Free phenolic acids were mostly
209 contained in the polar fractions (Fig. 5B), while conjugated phenolics occurred across the entire polarity
210 gradient (Fig. 5C-D). In the bioactive fraction VIII-IX, hydrolysis revealed a herbivore-induced increase
211 in acid hydrolysable and a decrease in basic hydrolysable *p*-coumaric acid (Fig. 5C-D). Decreasing
212 concentrations of free and hydrolysable phenolic acids were observed in several inactive fractions. These
213 data support the hypothesis that leaf herbivory changes the pattern of phenolic acid conjugates in the
214 roots, and that these changes are associated with a decreased attractiveness of the roots for *D. virgifera*.

215 *Discussion*

216 Although leaf-herbivory often reduces the fitness of root-feeders (Blossey and Hunt-Joshi, 2003;
217 Johnson et al., 2012), the physiological and behavior mechanisms behind this phenomenon are poorly
218 understood. Our results link systemic changes in conjugated phenolic acids to a strong avoidance
219 response of a root feeder and thereby provide a physiological and behavioral explanation for the reduced
220 abundance of *D. virgifera* larvae on the roots of leaf-attacked plants.

221 In the field, *D. virgifera* commonly co-occurs with many lepidopteran leaf-feeders, including
222 *Spodoptera spp.* (O'Day, 1998). Previous studies show that feeding by *S. frugiperda* on the leaves
223 reduces the survival of late-arriving *D. virgifera* larvae in the roots (Erb et al., 2011c; Gill et al., 2011),
224 especially in the upper layers of the rhizosphere (Erb et al., 2011d). As *D. virgifera*, which is highly
225 specialized on maize (Clark and Hibbard, 2004), can migrate up to 1 m in the soil to find new host plants
226 (Hibbard et al., 2003), it is conceivable that it may have developed the capacity to assess the quality of
227 different plant roots. *In vitro* assays have demonstrated that maize root extracts are strong arrestants of
228 *D. virgifera* larvae (Bernklau and Bjostad, 2005), and that monosaccharides as well as free and galactose-
229 linked fatty acids (monogalactosyldiacylglycerols) stimulate their feeding (Bernklau et al., 2015;
230 Bernklau and Bjostad, 2008). Interestingly, it has also been demonstrated that contact with an inferior
231 host plants (e.g. *Glycine max*) changes the behavior of *D. virgifera* neonates from localized to wider-
232 ranging search behavior (Strnad and Dunn, 1990). Our experiments show that if given a choice, *D.*

233 *virgifera* larvae can also assess qualitative differences within genotypes and avoid inferior leaf-infested
234 plants.

235 Theoretically, *D. virgifera* may use different cues to avoid leaf-infested plants. Possibilities include
236 direct cues from the phyllosphere like leaf-volatiles, larvae or their frass, changes in root exudates or
237 root volatile patterns, modification of the root-associated bacterial community, structural changes on the
238 root surface and changes in the root metabolite profile. In an earlier study, we found that *D. virgifera*
239 can use changes in volatile organic compounds to avoid leaf-infested plants (Robert et al., 2012). The
240 experiments presented here show that, in addition, changes in soluble root chemicals are sufficient to
241 dramatically reduce the attraction of *D. virgifera*. The following findings supports this conclusion: First,
242 *D. virgifera* distinguished infested from non-infested plants even when the roots of the two types plants
243 are tightly intertwined and presented together in the same volatile headspace and when above-ground
244 cues were physically blocked by isolating the soil with agar and aluminium foil. Second, the preference
245 was maintained in liquid extracts of the root metabolome, even after evaporation and re-solubilization.
246 Fourth, the active metabolites could be separated from non-active compounds by fractionation using
247 conventional reverse phase HPLC. Interestingly, the preference patterns were less strong when using
248 root extracts compared to intact roots. It is therefore possible that short-range volatile and non-volatile
249 cues act in a synergistic manner.

250 Despite the evidence pointing to stable, soluble root chemicals as causal factors in the interaction, our
251 earlier attempts to identify root metabolites that respond to leaf-infestation through a UHPLC-TOF
252 based metabolomics approach did not yield any clear candidate features (Marti et al., 2013). Using more
253 targeted methods, we here provide several lines of evidence that phenolic acid conjugates can play a
254 central role in mediating the preference of *D. virgifera* larvae for non-infested plants: First, our profiling
255 assays demonstrate that the abundance of several hydrolysable phenolic acids in the roots decreases in
256 leaf-infested plants. Second, chemical and molecular interference with phenolic acid biosynthesis led to
257 the disappearance of the differential preference exhibited by *D. virgifera*. Third, the bioactive fraction
258 of root extracts contained significant amounts of hydrolysable phenolic acids, of which the abundance
259 strongly changed with leaf infestation. However, despite the presented evidence, several open questions
260 regarding the biosynthesis, regulation and identity of the foraging cues remain. Phenolic acids, a
261 majority of which is derived from coumaric acid, can be conjugated to other phenylpropanoids, sugars,
262 proteins, fatty acids and terpenoids (Cheynier et al., 2013; Quideau et al., 2011; Hoff et al., 1994; Koetter
263 et al., 1994; Shimizu and Ohta, 1960), resulting in a large number of possible soluble and insoluble
264 structures, many of which are biologically active (Cheynier et al., 2013). Our fractionation/hydrolysis
265 approach reveals that conjugated phenolic acids are both highly abundant and diverse. Orthogonal
266 approaches, including for instance hyphenated NMR will be necessary to identify the actual metabolites
267 that are recognized by *D. virgifera*. Phenylpropanoid derivatives are known to serve as signalling
268 molecules (Brown, 2001) and enzymatic co-factors (Šukalović et al., 2005). Furthermore, despite the

269 bioactivity of our HPLC fractions pointing at a direct effect, the possibility that changes in *D. virgifera*
270 preference are not due to changes in phenolic acid content, but rather due to other metabolites that are
271 regulated by phenolic acids, cannot be fully excluded at this point (Maag et al., 2015). Another
272 interesting observation concerns the fact that the bm1 mutation made it impossible for *D. virgifera* to
273 distinguish control from *S. littoralis* infested plants, but still allowed it to distinguish control from
274 artificially elicited seedlings. This finding suggests that the application of oral secretions to wounded
275 leaves does not fully mimick the systemic changes in the roots that are elicited by real herbivory. Further
276 experiments will be necessary to determine whether the intensity and speed of wounding or labile
277 elicitors in the oral secretions of *S. littoralis* are responsible for this remarkable degree of specificity.

278 Host plant selection in phytopagous insects is a key process shaping plant-insect interactions. While
279 much is known about how leaf-feeders find and choose their food source (Bernays and Chapman, 1994),
280 it remains poorly understood how root herbivores accomplish this task. So far, it was not known if root
281 herbivores might escape plant-mediated competition with aboveground feeders by specifically
282 recognizing systemic changes in the roots of leaf-infested plants. Our experiments show that root-
283 feeding *D. virgifera* larvae actively engage in host selection, and that leaf-herbivory specifically
284 influences their host choice by altering phenylpropanoid patterns in the roots. This implies that above-
285 ground herbivores may have a strong effect on the distribution and abundance of soil-dwelling
286 organisms via systemic changes in root metabolites and could thereby shape entire below-ground food
287 webs.

288 From an applied point of view, our findings open up two potential strategies to improve the management
289 of one of the world's most damaging maize pests: First, by altering root phenylpropanoid biosynthesis,
290 *D. virgifera* may be tricked into feeding on inferior (i.e. leaf-infested) host plants, which may reduce its
291 performance and overall damage in the field. Second, it may eventually be possible to mimic leaf-
292 infestation at a genetic level and thereby produce maize plants that are avoided by *D. virgifera* larvae.
293 The currently available bm mutants may be a good starting point to assess whether changes in
294 phenylpropanoid and lignification patterns can be used to alter the behavior of *D. virgifera* and reduce
295 its damage under field conditions. Root-specific silencing of the corresponding genes could be a next
296 step to harness the positive effect of these alterations without compromising the resistance of the plants
297 to leaf pests and pathogens.

298 *Material and methods*

299 **Plants and insects**

300 Maize plants (*Zea mays*) were grown as described previously (Erb et al., 2011c). Unless otherwise
301 indicated, the hybrid Delprim (Delley DSP, Delley, Switzerland) was used for experiments. The brown
302 midrib mutants bm1 and bm3 were bred at INRA Lusignan as described (Barriere *et al.*, 2004). Plants
303 for experiments were 10-12 days old and had 2-3 fully expanded leaves. The herbivores *Diabrotica*
304 *virgifera virgifera* and *Spodoptera littoralis* were reared following previously established protocols

305 (Robert et al., 2012). Third-instar *D. virgifera* and second-instar *S. littoralis* larvae were used in all
306 experiments. All plants were covered with 1.5L PET bottles as described (Erb et al., 2011a) to prevent
307 leaf herbivore escape.

308 **Root herbivore choice patterns in the soil**

309 To assess the choice of *D. virgifera* when exposed to leaf-infested and herbivore-free plants, we used
310 several different behavioral setups. First, we developed a system composed of two L-shaped glass pots
311 to assess *D. virgifera* choice in the soil. The pots were 5 cm in diameter and 10 cm deep. At the bottom
312 of the pots, an open glass tube (4 cm long, 1.5 cm inner diameter) extended the rhizosphere system
313 horizontally. The lowest 2 cm of the pots (including the glass tubes) was filled with soil, before
314 individual plants, together with the soil and sand medium from their cultivation system, were transferred
315 carefully into the vessels. After 24 hours of acclimatization in a temperature and light-controlled
316 environment (23°C, 16:8h L/D, 90µmol/m²), 1.5 l PET bottles with their bottoms removed were attached
317 to the glass pots upside down. Half of the plants were then infested with 20 second instar *S. littoralis*
318 larvae over 48h, while the other half was left herbivore free. After this period, during which the leaves
319 were damaged, but still had ample leaf biomass (>50%), 6 second instar *D. virgifera* larvae were
320 introduced into the horizontal glass tubes (3 on each side). The openings of the glass tubes of a control
321 and a leaf-infested plant were then connected and sealed using plastic film (n=15). In this way, the root
322 herbivores had access to the differentially treated plants via a 10 cm glass tube filled with soil. *D.*
323 *virgifera* larvae were left to move freely between the two plants for 48 h, after which the system was
324 disassembled, and the position of the root herbivores was recorded.

325 **Root herbivore choice patterns with superposed root systems**

326 To assess whether *D. virgifera* can use tactile cues to distinguish leaf-infested from uninfested plants,
327 we developed a petri dish assay. First, maize seedlings were treated in their normal growth environment
328 (see below). Plants were then removed from their pots and the roots were gently washed under a stream
329 of warm water. The root systems of two plants (control vs. treatment, see below) were laid out on a
330 moist filter paper embedded in a large petri dish (12cm diameter). Roots were mixed to create a random
331 pattern of roots from the two plants. The petri dish had a cavity on the side, into which the stems were
332 laid, leaving the leaves of the plant free in the air. Six second instar *D. virgifera* larvae were then
333 introduced into the dish, which was sealed with its lid and laid out on an experimental bench supplied
334 with plant growth lights (23°C, 16:8h L/D, 90µmol/m²). To guarantee moisture saturated air around the
335 exposed roots, water-drenched paper tissue was wrapped around the petri dish, followed by a layer of
336 aluminum foil to shade the roots from light. The position of the larvae was recorded 30 min, 1 h, 2 h 3
337 h and 4 h after introduction into the choice arena. Using this setup, eleven different experiments were
338 conducted. 1. Maize seedlings were infested with 20 second instar *S. littoralis* larvae for 48h. The
339 herbivores were removed after this period, and roots from a control and an infested plants were offered
340 to *D. virgifera* (n=19). 2. Plants were infested in the same manner, but offered to *D. virgifera* at different

341 time points ranging from 8 to 48 h (n=12). 3. Leaf-herbivory was simulated using three different
342 treatments: i) scratching of about 1 cm² of leaf-tissue 6 times over 48h, until all leaves were damaged,
343 ii) additional application of 10 µl of *S. littoralis* regurgitant to the scratched surfaces, as described in
344 (Erb et al., 2009), iii) removal of leaf area by sequentially cutting off 50% of each leaf over 48h. All
345 treatments started at the lowest leaf and ended with the youngest, freshly developed leaf, which
346 corresponds to the order of attack by *S. littoralis* (Köhler et al., 2014). Plants of each treatment were
347 paired with untreated control plants and the superposed roots were offered to *D. virgifera* larvae (n=12).
348 4. Leaves were induced by scratching and application of *S. littoralis* regurgitate and offered to the larvae
349 at different time points after induction (n=12-14). 5. Plants were induced by *S. littoralis*, but the root
350 system and soil were sealed off from the aboveground environment of the plant by pouring a 2cm layer
351 of solidifying agar (2% Agarose in H₂O, 45°C) onto the soil in the pots, resulting in an air-tight seal
352 around the stem. Furthermore, the stem was sealed off by two layers of tightly wrapped aluminum foil,
353 ensuring that the stem was the only physical connection between leaves and roots. After removing the
354 agar-seal and aluminum from the plants, roots were washed and exposed to *D. virgifera* as described
355 above. A control set of plants without seal was included in the assay (n=12). 6. The potential effect of
356 phenylpropanoids was investigated by C4H inhibition with piperonylic acid (PA). Plants were either
357 infested with 20 *S. littoralis* larvae or left uninfested for 48 h. Half of the control and infested plants
358 were treated with PA (Sigma Aldrich, Saint-Louis, MO, USA) by adding 10 mL of a 75 µM PA in 10
359 % EtOH solution to the soil every 24 h over the 48 h infestation period (three times in total). The other
360 half of the plants were treated with buffer (10 % EtOH). After this time, the preference of six *D. virgifera*
361 larvae for control or leaf infested plants within PA or buffer treated plants was evaluated (n=12). 7. The
362 same setup as for experiment 6 was used, with the only difference that the plants were elicited by
363 repeated wounding and regurgitate application over 48 h (n=12). 8. PA treated plants were
364 complemented with 5.5mM *p*-coumaric acid (Sigma-Aldrich, St.-Louis, MO). In this experiment, we
365 simultaneously tested the preference of *D. virgifera* as follows (n=23 for each experiment): (i) control
366 versus leaf-elicited plants, both treated with PA, (ii) control versus leaf elicited plants, both treated with
367 PA and complemented with *p*-coumaric acid (CA), (iii) control plants treated with buffer versus control
368 plants complemented with CA, both treated with PA, (iv) control plants treated with buffer versus
369 control plants complemented with CA. Maize leaves were induced as described above. Control plants
370 remained undamaged. PA treatment was performed as described above. Complementation with *p*-
371 coumaric acid was achieved by watering the plants with 10 mL of a 5.5 mM CA in 1 % EtOH solution
372 every 24 h over 48 h (two times in total). Non complemented plants were watered with 10 mL 1 % EtOH
373 solution. 9. The choice of *D. virgifera* was evaluated for 3 different maize genotypes: The near isogenic
374 line F2 and the mutants bm1 and bm3 (Guillaumie *et al.*, 2007). Plants were infested with *S. littoralis*
375 as described (n=12-13). 10. The preference of *D. virgifera* was tested in the three genotypes using
376 artificial induction by wounding and regurgitate as described above (n=12). 11. In the last choice

377 experiment, the choice of *D. virgifera* on *S. littoralis* infested and artificially induced bm1 mutants was
378 compared directly using conditions and treatments as described above (n=15).

379 **Root herbivore choice patterns with plant extracts**

380 To test whether *D. virgifera* can detect leaf-herbivore induced, systemic metabolic changes in root
381 extracts, we conducted a separate experiment using root extracts in agarose. For this, roots of control
382 plants and plants infested with 20 *S. littoralis* larvae were removed from the pots, washed gently and
383 flash frozen in liquid nitrogen. After grinding the roots to a fine powder with mortar and pestle in liquid
384 nitrogen, the root material was centrifuged (2 min at 17'500 g), and the supernatant was recovered and
385 stored at -80°. In this way, we recovered about 50 % of the root fresh mass in liquid form. During our
386 assays, we found that filling root material into a 5 ml syringe tube and pressing it with the plunger was
387 equally effective and much faster to extract root liquid, and we used this technique for large scale
388 isolation (see below). For behavioral assays, agarose solutions were prepared (2 % agarose in H₂O). Just
389 before solidification of the solutions (45°C), we added different root extracts and stirred the mix. The
390 solutions were then poured into petri dishes and left to solidify. From the different gels, cubes (5x5x5
391 mm) were cut and placed into new petri dishes (2 per dish with different treatments). Six second instar
392 *D. virgifera* larvae were then introduced to each dish, and the dishes were placed in a humidity-
393 controlled phytotron (23°C, 95 % r.h., no light). For 4 h, the position of the root herbivores in the dishes
394 was recorded every 30 minutes. Using this procedure, we offered cubes containing extracts from control
395 and infested plants (diluted 1:1 in water) to the root herbivores (n=15). In a second experiment, we tested
396 10 fractions of control and induced root extracts from 400 plants for each treatment obtained from semi
397 preparative HPLC runs (see below). For each pair of fractions, we evaporated the solvents, resuspended
398 the fractions in 10 µl AcN and diluted the dissolved fractions in 0.5 mL agarose (4%). This mixture was
399 then diluted with 0.4 mL H₂O to reach concentrations that were equivalent to root concentrations and
400 the 50 % dilution of crude root extracts in agarose. To provide a metabolite background for the choice
401 experiments, 125 µL crude root extract of non-infested plants was added to each test fraction. *S. littoralis*
402 choice was then assessed for each pair of fractions (n=15).

403 **Fractionation of root extracts by semi preparative HPLC**

404 To facilitate the identification of the metabolites that are used by *D. virgifera* larvae to distinguish
405 between leaf-damaged and control plants, we carried out two fractionation runs using semi preparative
406 reverse-phase HPLC (Marti et al., 2013). For the choice assay, extracts from roots of 400 control and
407 400 *S. littoralis* infested plants were lyophilized to yield 50 mg dry matter per treatment. The extracts
408 were redissolved in 500 µl 50 % MeOH (v/v) and fractionated using a semi-preparative C18 column
409 (C18, 250 × 10 mm i.d., 5 µm, XBridge™, Waters, UK) connected to a Varian 9012 Solvent Delivery
410 System operating at a flow rate of 10 ml*min⁻¹. Injection volume was 250 µl. The solvent gradient
411 started with 90 % H₂O and 10 % can (both with 0.1 % v/v formic acid) for two minutes, followed by a
412 ramp to 50:50 over 20 minutes and a ramp to 5:95 over 70 minutes. The 5:95 mix was held for 12

413 minutes, followed by a post equilibration at 90:10 for 10 minutes. Fractions were collected at 6 minute
414 intervals between 2 and 62 minutes. After pooling the fractions from 2 different runs per treatment, they
415 were lyophilized and evaporated to dryness under nitrogen flow before being redissolved for biological
416 experiments. Dry weights for the different fractions were between 0.1 and 0.9 mg. For phenolic acid
417 analysis, pools of 18 control, 18 *S. littoralis* infested and 18 artificially induced plants were fractionated
418 using the same setup, with the only difference that instead of 11 fractions, 5 different fractions were
419 collected, with fraction VII-IX corresponding to the bioactive window as determined by biological
420 experiments.

421 **Metabolomic fingerprinting of active root fractions**

422 Metabolic fingerprinting of the active fractions was carried out as described previously (Marti et al.,
423 2013). Briefly, the fingerprints of each extract was obtained using a short UPLC BEH C18 Acquity
424 column (50 × 1.0 mm i.d., 1.7 µm) (Waters, MA, USA). The mobile phase consisted of 0.1 % formic
425 acid (FA) in water (phase A) and 0.1 % FA in acetonitrile (phase B). The linear gradient program was
426 as follows: 98 % A over 0.2 minutes, to 100 % B over 4.9 minutes, held at 100 % B for a further 1.1
427 min, then returned to initial conditions (98 % A) in 0.1 min for 1.1 minutes of equilibration before next
428 analysis. The flow rate was 0.3 ml/min; column temperature was kept at 40°C. Detection was performed
429 by TOF-MS (LCT Premier, Waters, MA, USA) in W-mode in both electrospray (ESI) negative (NI) and
430 positive (PI) ion modes in independent runs over a m/z range of 100-1000 Da. The MS was calibrated
431 using sodium formiate, and leucine enkephalin was used as an internal reference. The injection volume
432 was 1 µl and all samples were diluted at 0.5 mg/mL. The recorded profiles were normalized to 1000
433 counts, and peaks were extracted using MZmine v 2.12 (Pluskal et al., 2010) followed by univariate data
434 analysis with Microsoft Excel.

435

436 **Root phenolic acid profiling of leaf infested plants**

437 Soluble free and hydrolysable root phenolic acids were profiled in three different experiments. To
438 evaluate changes in root phenolic acids upon leaf infestation, maize seedlings were infested with 20 *S.*
439 *littoralis* larvae. Control plants remained uninfested. After 48 h, maize primary and crown roots were
440 collected separately, washed in a stream of tap water, immediately frozen in liquid nitrogen and stored
441 at -80° C until use (n=12). The extraction procedure was adapted from de Ascensao and Dubery (2003).
442 Briefly, all samples were ground in nitrogen to a fine powder using a mortar and a pestle. 600 µL of 100
443 % MeOH was added to 100 mg of root powder, vortexed and centrifuged at 17'500 rpm for 20 min at
444 4°C. The supernatant was collected and used for the next extraction steps. For each biological replicate,
445 extracts of 3 plants were pooled and separated into 3 aliquots: (i) 1mL aliquots were evaporated to
446 dryness under a flow of nitrogen (Glas-Col, CAT No. 099A EV9624S, Terre Haute, USA) and
447 resuspended in 50 µl 50% MeOH for the analysis of free phenolic acids. (ii) 50 µL aliquots were mixed
448 with 50 µL of concentrated HCl (37%, Sigma Aldrich, Saint-Louis, MO, USA) and heated for 1 hour at
449 80° C for acid hydrolysis. 1 mL diethyl ether was added, and the organic phase was collected and

450 evaporated to dryness under a flow of nitrogen (Glas-Col, CAT No. 099A EV9624S, Terre Haute, USA)
451 before resuspension in 50 μ L 50% MeOH. (iii) 50 μ L aliquots were mixed with 100 μ L 2M NaOH
452 (Sigma Aldrich, Saint-Louis, MO, USA) and left to stand for 3 h at ambient temperature for basic
453 hydrolysis. The samples were then mixed with 50 μ L concentrated HCl (37%, Sigma Aldrich, Saint-
454 Louis, MO, USA) and 1 mL diethyl ether (Glas-Col, CAT No. 099A EV9624S, Terre Haute, USA), and
455 the organic phase was recovered and evaporated under a nitrogen stream (Glas-Col, CAT No. 099A
456 EV9624S, Terre Haute, USA) before resuspension in 50 μ L 50 % MeOH. The three different types of
457 extracts were then analyzed by HPLC as described below

458 **HPLC-MS analysis of phenolic acids**

459 Chromatography was conducted on an Agilent 1260 Infinity HPLC system (Agilent Technologies,
460 Boeblingen, Germany) coupled to an API 5000 tandem mass spectrometer (MS) (Applied Biosystems,
461 Darmstadt, Germany). Briefly, the separation was achieved on a Zorbax Eclipse XDB-C18 column (50
462 x 4.6 mm, 0.5 μ m; Agilent, Santa Clara, CA, USA) using formic acid (1%, Fisher Scientific, Geel,
463 Belgium) in water and acetonitrile (Fisher Scientific UK, Loughborough, Leics, UK) as mobile phases
464 A and B respectively. The employed elution gradient was as follow: 0 to 0.5 min, 10% B; 0.5 to 4 min,
465 10% to 90% B; 4 to 4.02 min, 90% to 100%, 4.02 to 4.5 min, 100% B; 4.5 to 4.51 min, 10%; and 4.51
466 to 7 min, 10 % B. The flow rate of the mobile phase was of 1.1 mL.min⁻¹. The column temperature was
467 maintained at 25 ° C. The instruments parameters were optimized with infusion of pure standards
468 (Sigma-Aldrich, St.-Louis, MO). The ion spray voltage was of -4500 eV. The turbo gas temperature was
469 of 700 °C. Nebulizing gas was set at 60 c, curtain gas at 25 c, heating gas at 60 c and collision gas at 7
470 c. Multiple reaction monitoring was used to measure the parent ion \rightarrow product ion transitions as
471 described in Table S1. Data acquisition and processing was performed on Analyst 1.5 software (Applied
472 Biosystems, Darmstadt, Germany). Dilution series of standard mixtures of each phenolic acid
473 (purchased from Sigma-Aldrich, St.-Louis, MO) were used for quantification. Peak areas of *Cis* and
474 *trans* isomers were summed up for quantification.

475 **Data treatment and statistical analysis**

476 To test the preference of *D. virgifera* in the two-arm belowground system, we used the statistical
477 procedure outlined previously (GLM with quasi-poisson distribution to take into account overdispersal,
478 followed by analysis of variance (ANOVA)) (Robert et al., 2012) using R (version 3.2.1.). To assess
479 larval choice in petri dish assays, a choice differential was calculated from each replicate by subtracting
480 the average number of larvae on the control side from the average number of larvae on the treatment
481 side. The differentials were then compared against the null hypothesis (equal preference for both sides,
482 resulting in a differential of 0) using ANOVA in R. Differences in phenolic acid profiles were evaluated
483 by ANOVAs followed by Holm-Sidak Post Hoc tests in Sigma Plot 12.5.

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487 *Figure legends*

488 **Fig. 1:** *The root herbivore Diabrotica virgifera specifically avoids leaf-infested plants by recognizing*
489 *systemic changes in soluble root components.* A. Preference of *D. virgifera* larvae for roots of control
490 vs. leaf-infested plants in a two-arm below ground choice system (n=15). B. Preference for roots of
491 control vs. leaf-infested plants with washed root systems in a petri dish setup (n=19). C. Preference
492 patterns after different durations of leaf-infestation (n=12). D. Preference for roots of damaged plants
493 with and without defence elicitation by application of *S. littoralis* regurgitant (n=12). E. Preference time
494 course using a single leaf-induction event (n=12-14). F. Preference for roots of control and leaf-infested
495 plants with and without direct contact between the rhizo- and phyllosphere (n=12). G. Preference for
496 root extracts of control and leaf-infested plants using agarose cubes (n=15). Preference is expressed as
497 % choice corresponding to the proportions of independent replicates in which a given preference was
498 observed (No choice: A-F: <10%; G: 21%). Stars indicate significant differences between treatments
499 (*p<0.05, **p<0.01, ***p<0.001).

500 **Fig. 2:** *Leaf-infestation alters soluble free and conjugated phenolic acids in the roots.* Average
501 concentrations of different phenolic acids in control roots (grey bars) and roots of leaf-infested plants
502 (purple bars) are shown for crown and primary roots (\pm SE). Shading indicates a significant overall
503 treatment effect determined by analysis of variance (p<0.05). Stars indicate significant pairwise
504 differences between treatments within root types (Holm-Sidak post-hoc tests: *p<0.05, **p<0.01,
505 ***p<0.001).

506 **Fig. 3:** *Manipulating the biosynthesis of phenolic acids through cinnamate 4-hydroxylase inhibition*
507 *leads to the disappearance of the D. virgifera avoidance response towards leaf-infested plants.* A.
508 Preference for roots of buffer treated and C4H-inhibited control and *S. littoralis* infested plants (n=12).
509 B. Preference for roots of buffer treated and C4H-inhibited control and artificially induced plants (n=12).
510 C. Preference for roots of buffer treated, C4H-inhibited and *p*-Coumaric acid (CA) complemented
511 control and artificially induced plants. C4H was inhibited by application of the selective inhibitor
512 piperonylic acid (n=23). Preference is expressed as % choice corresponding to the proportions of
513 independent replicates in which a given preference was observed (No choice: <10%). Stars indicate
514 significant differences between treatments (*p<0.05, **p<0.01, ***p<0.001).

515 **Fig. 4:** *Genetic modification of the phenylpropanoid pathway leads to the disappearance of the D.*
516 *virgifera avoidance response towards leaf-infested plants.* A. Preference of *D. virgifera* for roots of
517 control and leaf-infested wild type (WT) plants, cinnamyl alcohol dehydrogenase (CAD) (bm1) mutant
518 plants and Caffeic acid O-methyl transferase (COMT) (bm3) mutant plants (n=12-13). B. Preference of
519 *D. virgifera* for roots of control and artificially induced WT, bm1 and bm3 plants (n=12). Preference is

520 expressed as % choice corresponding to the proportions of independent replicates in which a given
521 preference was observed (No choice: <10%). Stars indicate significant differences between treatments
522 (*p<0.05, **p<0.01, ***p<0.001).

523 **Fig. 5:** Bioactivity-guided fractionation links changes in conjugated phenolic acids with *D. virgifera*
524 preference patterns. A. Preference of *D. virgifera* for fractions of root extracts of control and leaf-
525 infested plants (n=15). The polarity gradient of the fractionation setup is shown in purple. B.
526 Concentrations of free phenolic acids in root extracts of control and leaf-induced plants across the
527 polarity gradient. Note that analyzed fractions cover the range of two fractions of experiment A. C.
528 Concentrations of soluble, acid hydrolysable phenolic acids. D. Concentrations of soluble, basic
529 hydrolysable phenolic acids. Shaded areas correspond to the bioactive fraction.

530 *Supplemental data*

531 **Fig. S1:** Cinnamate 4-hydroxylase inhibition alters the accumulation of soluble free and conjugated
532 phenolic acid in the roots.

533 **Fig. S2:** Genetic modification of the phenylpropanoid pathway alters the accumulation of soluble free
534 and conjugated phenolic acid in the roots.

535 **Fig. S3:** Genetic modification of the phenylpropanoid pathway does not alter root architecture of maize
536 seedlings.

537 **Fig. S4:** The *bm1*-dependent preference pattern of *D. virgifera* differs between *S. littoralis* infested and
538 artificially elicited plants.

539 **Fig. S5:** Metabolomic fingerprints of active root fractions

540 **Table S1.** Multiple reaction monitoring parameters for phenolic acid analysis.

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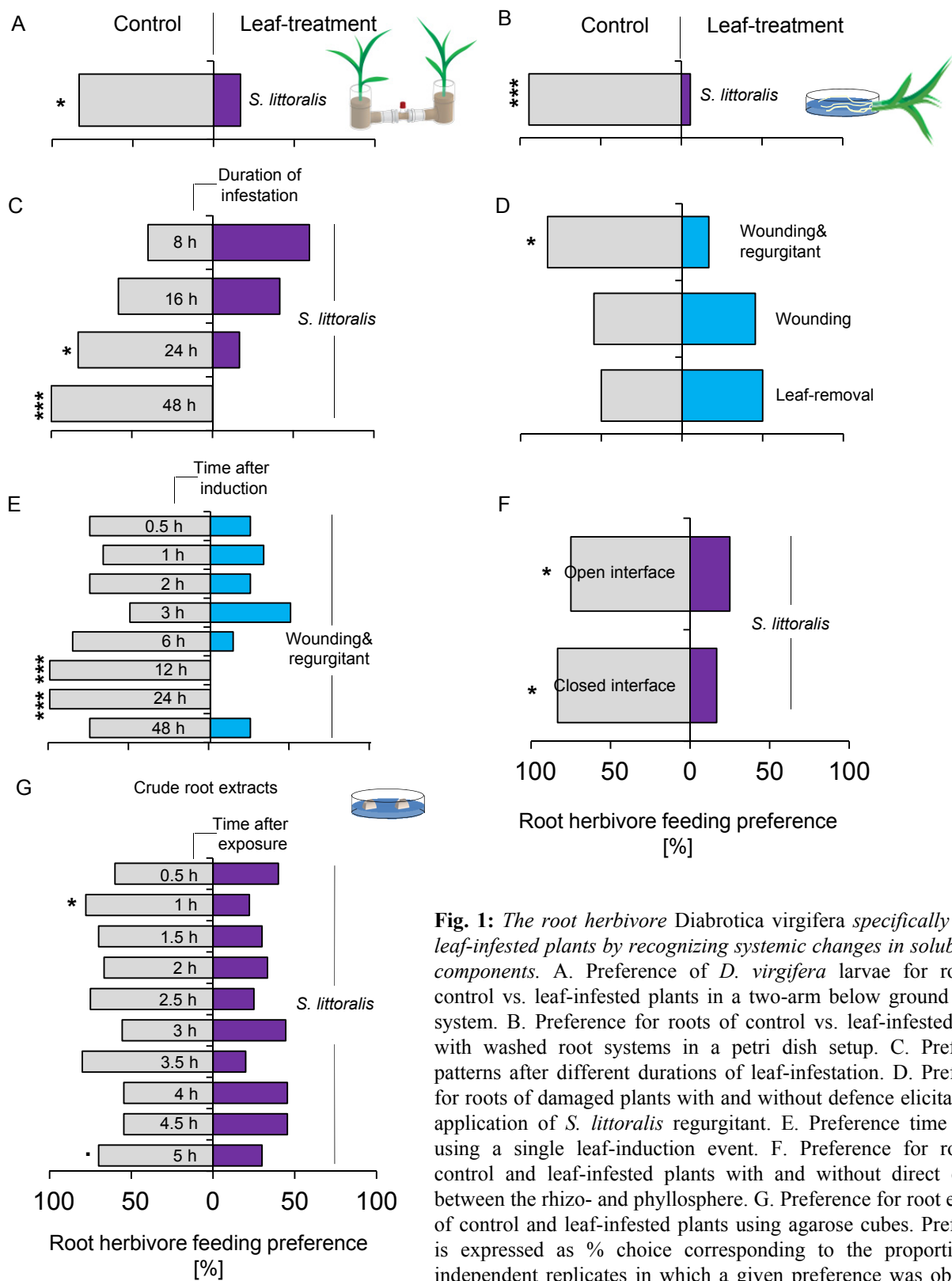


Fig. 1: The root herbivore *Diabrotica virgifera* specifically avoids leaf-infested plants by recognizing systemic changes in soluble root components. A. Preference of *D. virgifera* larvae for roots of control vs. leaf-infested plants in a two-arm below ground choice system. B. Preference for roots of control vs. leaf-infested plants with washed root systems in a petri dish setup. C. Preference patterns after different durations of leaf-infestation. D. Preference for roots of damaged plants with and without defence elicitation by application of *S. littoralis* regurgitant. E. Preference time course using a single leaf-induction event. F. Preference for roots of control and leaf-infested plants with and without direct contact between the rhizo- and phyllosphere. G. Preference for root extracts of control and leaf-infested plants using agarose cubes. Preference is expressed as % choice corresponding to the proportions of independent replicates in which a given preference was observed. Stars indicate significant differences between treatments (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

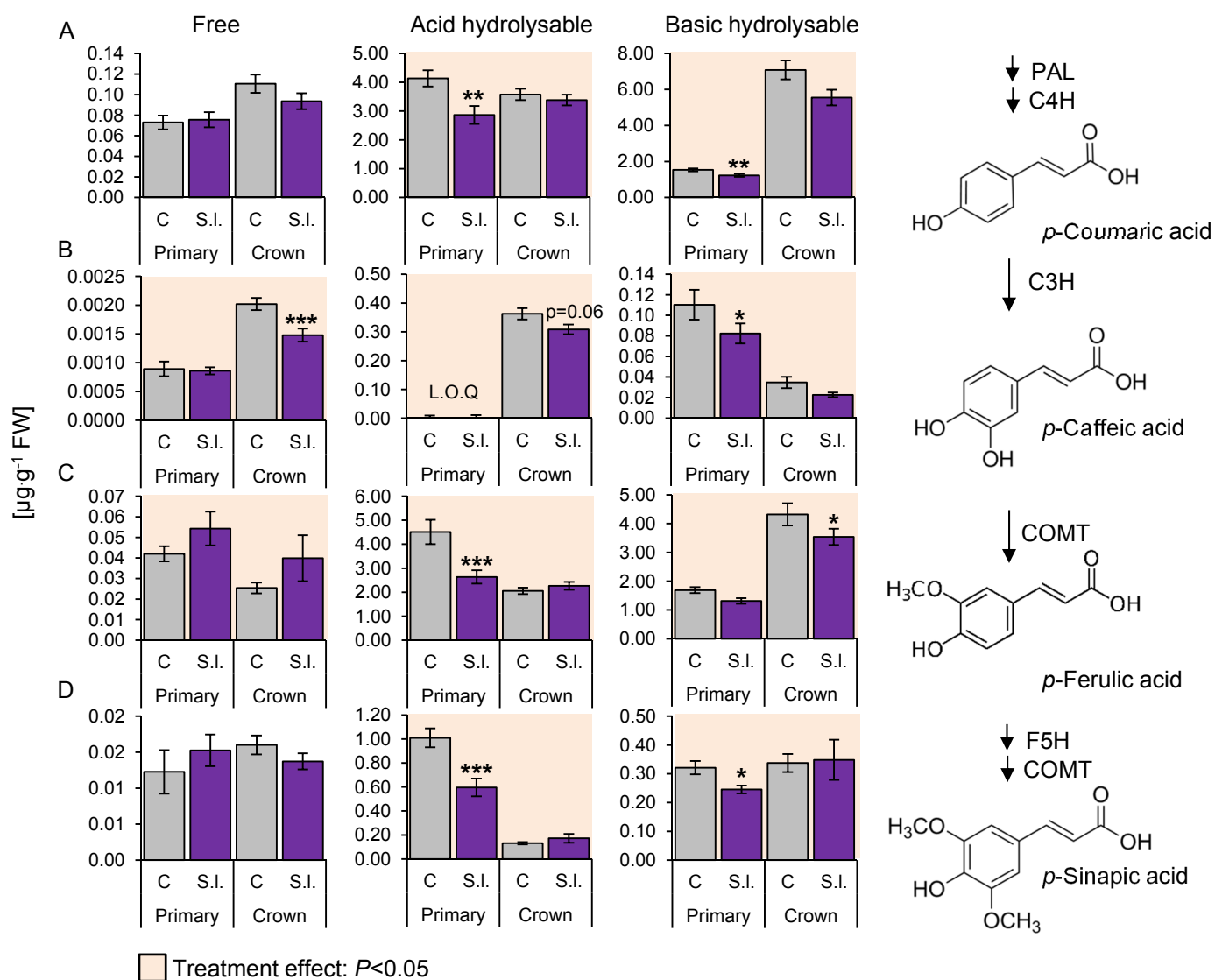


Fig. 2: Leaf-infestation alters soluble free and conjugated phenolic acids in the roots. Average concentrations of different phenolic acids in control roots (grey bars) and roots of leaf-infested plants (purple bars) are shown for crown and primary roots (\pm SE). Shading indicates a significant overall treatment effect determined by analysis of variance ($p < 0.05$). Stars indicate significant pairwise differences between treatments within root types (Holm-Sidak post-hoc tests: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

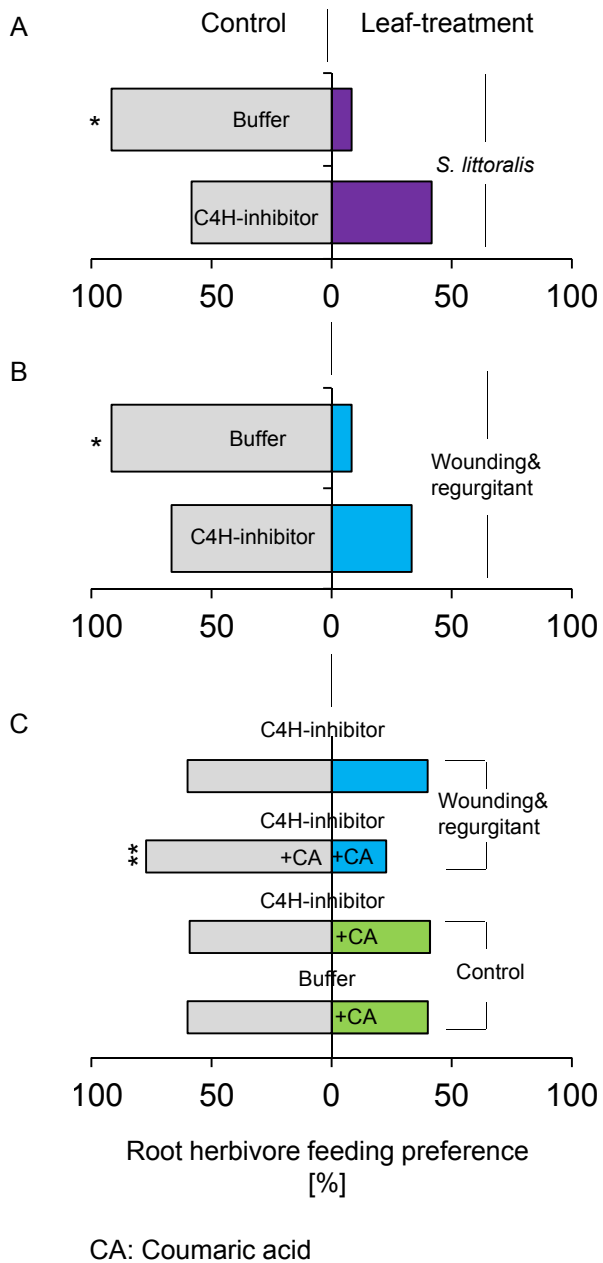


Fig. 3: Manipulating the biosynthesis of phenolic acids through cinnamate 4-hydroxylase inhibition leads to the disappearance of the *D. virgifera* avoidance response towards leaf-infested plants. A. Preference for roots of buffer treated and C4H-inhibited control and *S. littoralis* infested plants. B. Preference for roots of buffer treated and C4H-inhibited control and artificially induced plants. C. Preference for roots of buffer treated, C4H-inhibited and *p*-Coumaric acid (CA) complemented control and artificially induced plants. C4H was inhibited by application of the selective inhibitor piperonylic acid. Preference is expressed as % choice corresponding to the proportions of independent replicates in which a given preference was observed. Stars indicate significant differences between treatments (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

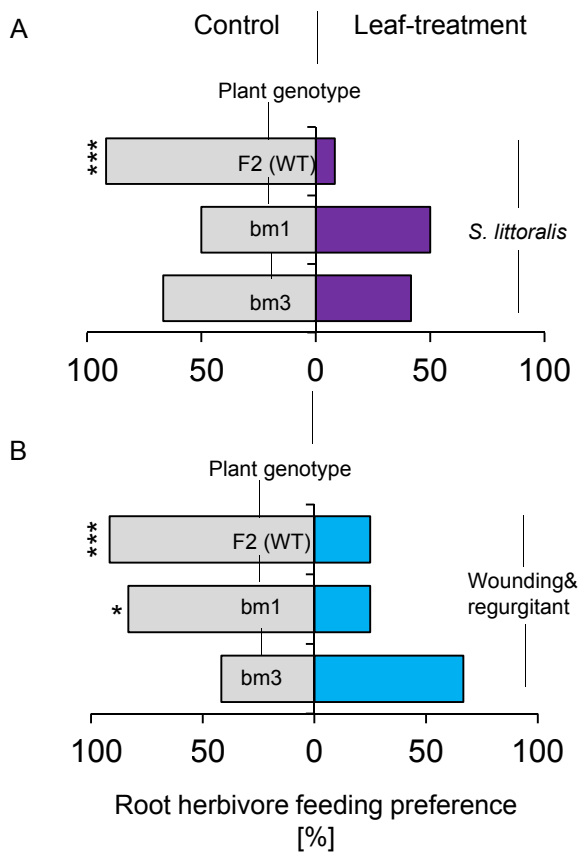


Fig. 4: Genetic modification of the phenylpropanoid pathway leads to the disappearance of the *D. virgifera* avoidance response towards leaf-infested plants. A. Preference of *D. virgifera* for roots of control and leaf-infested wild type (WT) plants, cinnamyl alcohol dehydrogenase (CAD) (bm1) mutant plants and Caffeic acid O-methyl transferase (COMT) (bm3) mutant plants. B. Preference of *D. virgifera* for roots of control and artificially induced WT, bm1 and bm3 plants. Preference is expressed as % choice corresponding to the proportions of independent replicates in which a given preference was observed. Stars indicate significant differences between treatments (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

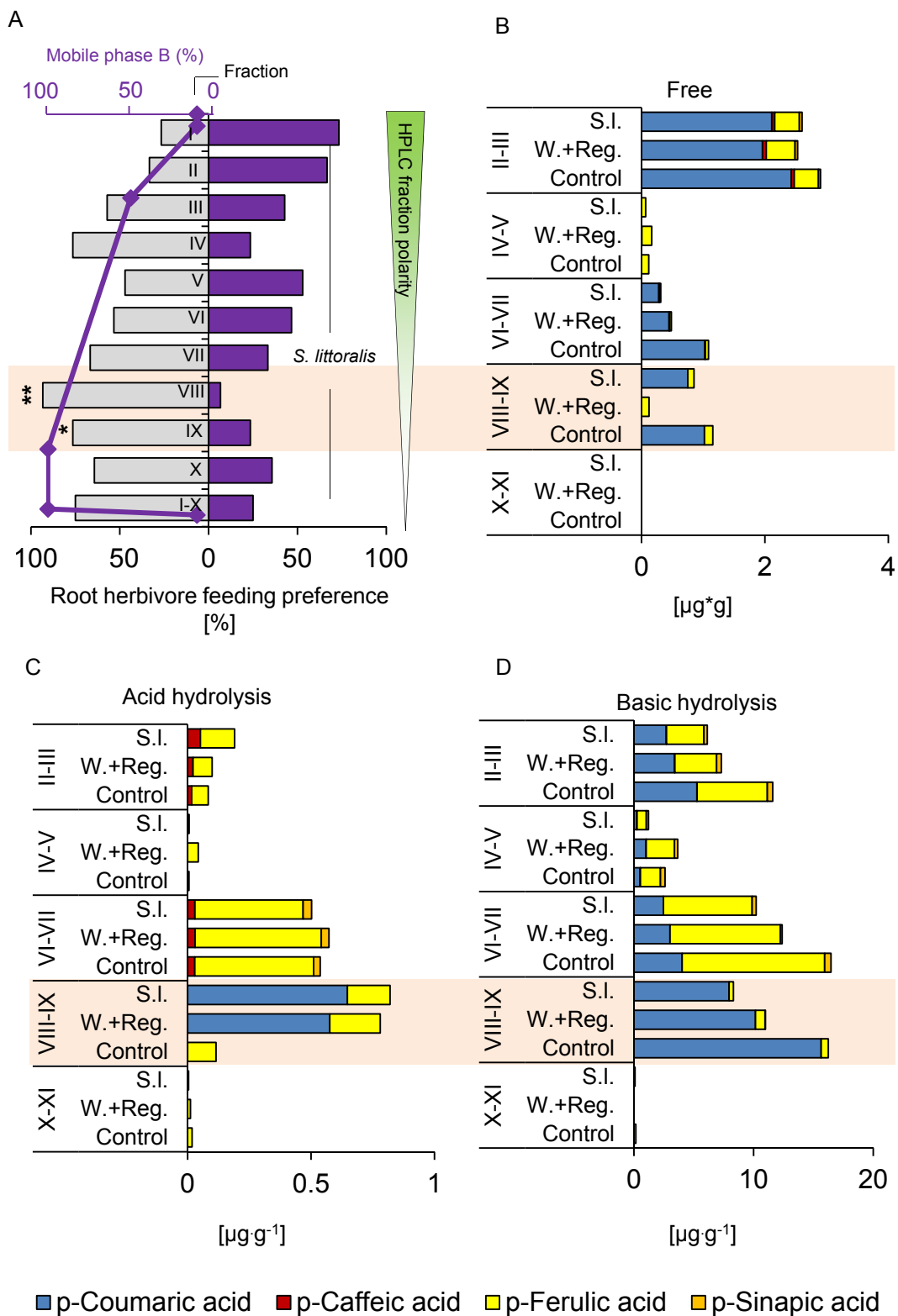
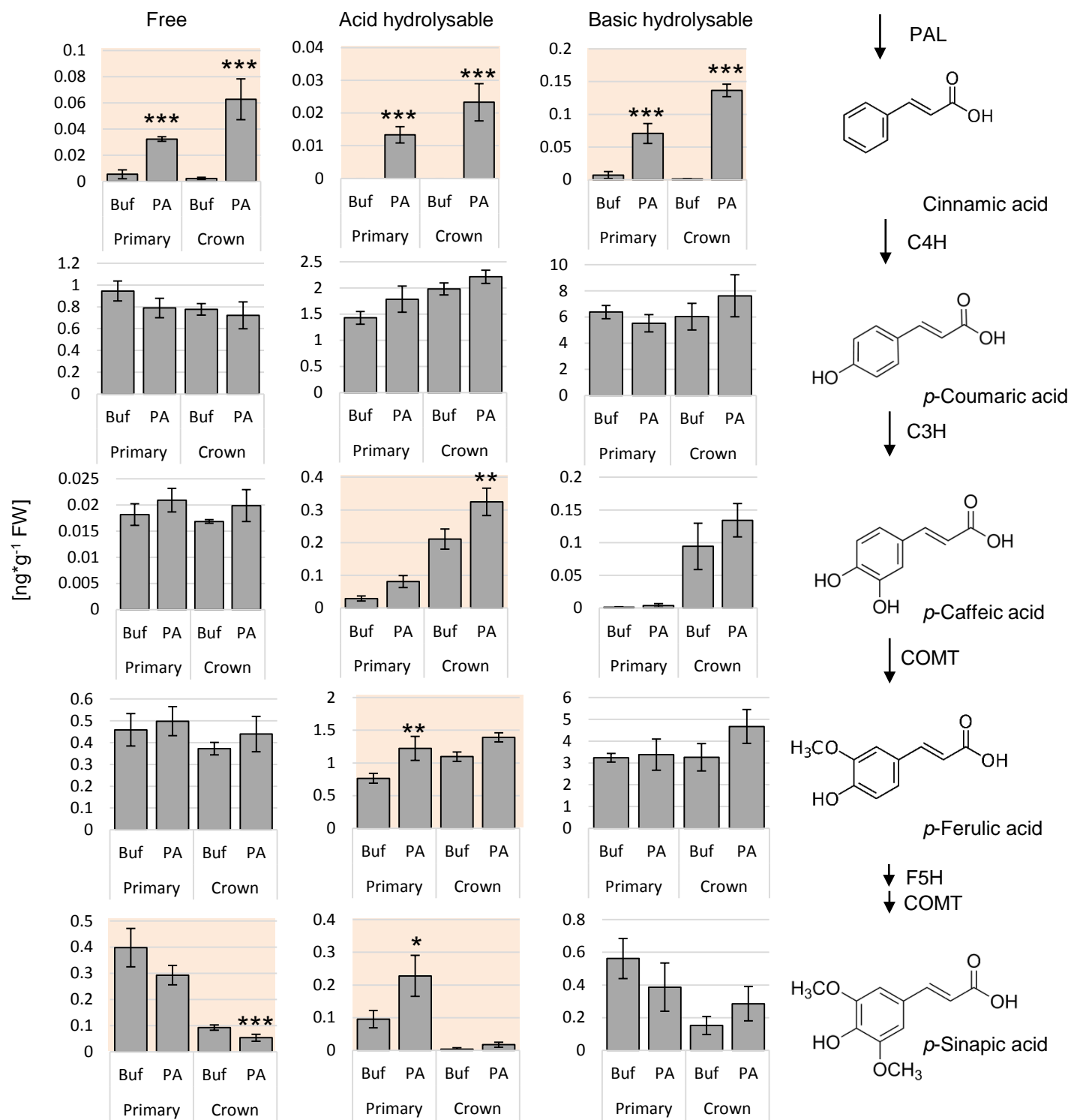


Fig. 5: Bioactivity-guided fractionation links changes in conjugated phenolic acids with *D. virgifera* preference patterns. A. Preference of *D. virgifera* for fractions of root extracts of control and leaf-infested plants. The polarity gradient of the fractionation setup is shown in purple. B. Concentrations of free phenolic acids in root extracts of control and leaf-induced plants across the polarity gradient. C. Concentrations of soluble, acid hydrolysable phenolic acids. D. Concentrations of soluble, basic hydrolysable phenolic acids. Shaded areas correspond to the bioactive fraction.



□ Treatment effect: $P < 0.05$

Fig. S1: Cinnamate 4-hydroxylase inhibition alters the accumulation of soluble free and conjugated phenolic acid in the roots. Average concentrations of different phenolic acids in buffer treated roots (Buf) and piperonylic acid treated, C4H inhibited roots (PA) are shown for crown and primary roots (\pm SE). Shading indicates a significant overall treatment effect determined by analysis of variance ($p < 0.05$). Stars indicate significant pairwise differences between treatments within root types (Holm-Sidak post-hoc tests: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

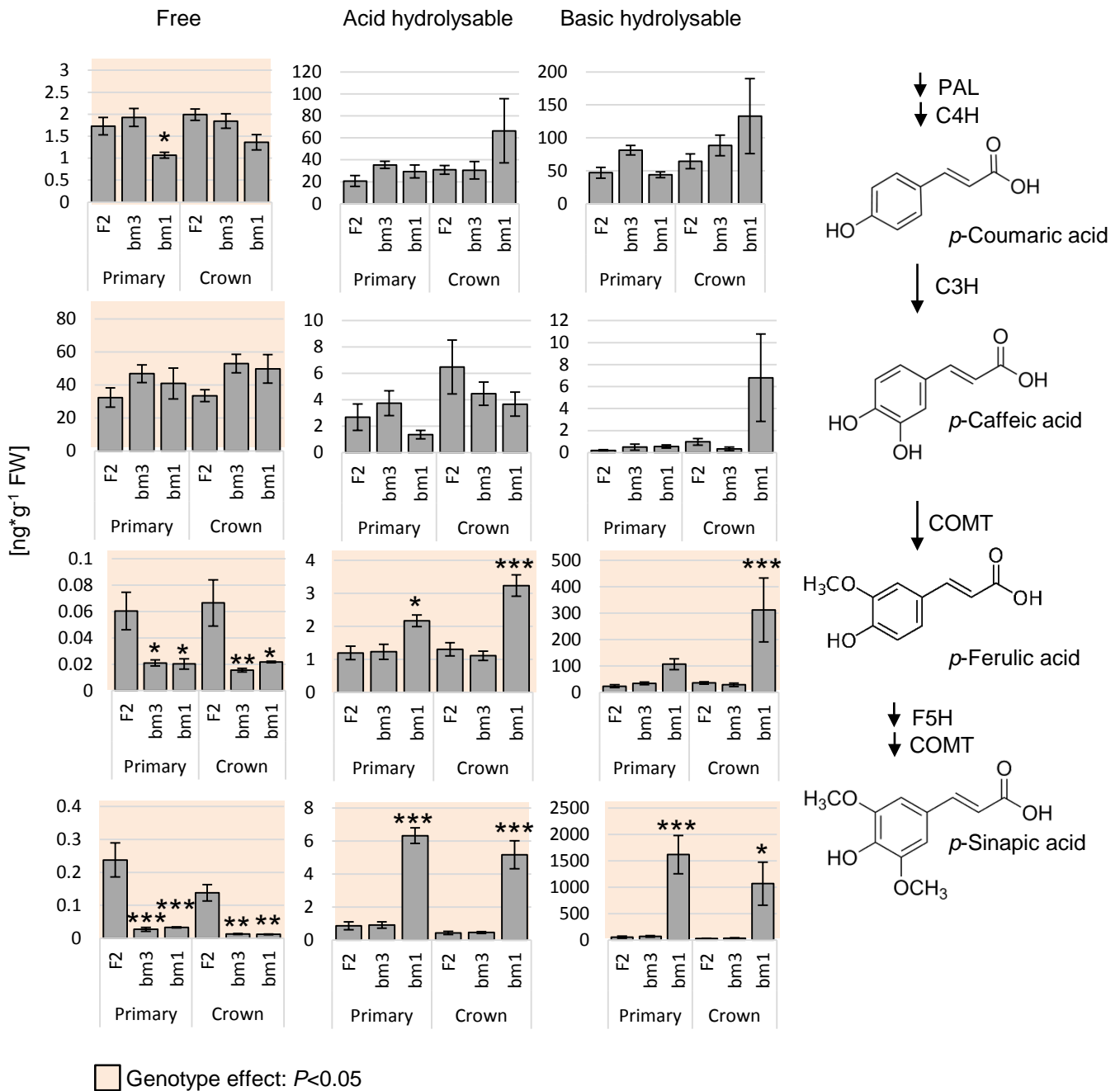


Fig. S2: Genetic modification of the phenylpropanoid pathway alters the accumulation of soluble free and conjugated phenolic acid in the roots. Average concentrations of different phenolic acids wild type (F2), bm1 and bm3 mutants are shown for crown and primary roots (\pm SE). Shading indicates a significant overall treatment effect determined by analysis of variance ($p < 0.05$). Stars indicate significant pairwise differences between treatments within root types (Holm-Sidak post-hoc tests: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

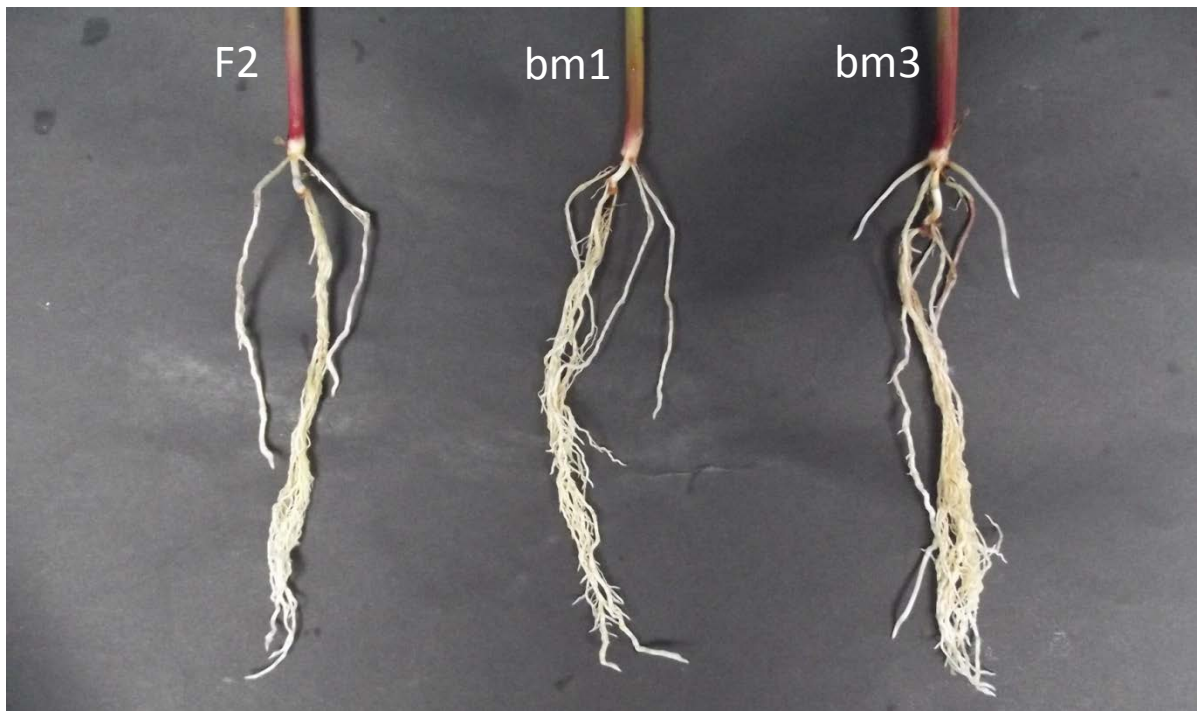


Fig. S3: *Genetic modification of the phenylpropanoid pathway does not alter root architecture of maize seedlings.* Pictures of wild type (F2), bm1 and bm3 roots of 12-day-old maize seedlings are shown.

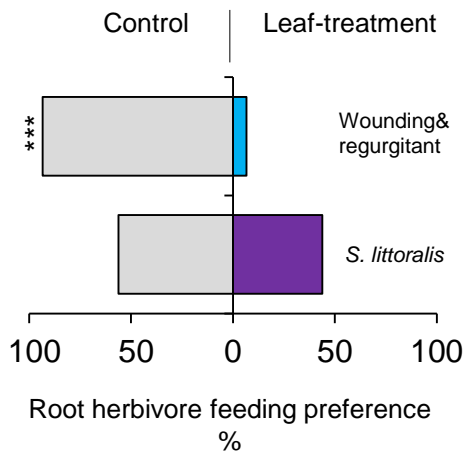


Fig. S4: The *bm1*-dependent preference pattern of *D. virgifera* differs between *S. littoralis* infested and artificially elicited plants. The preference for roots of leaf-induced *bm1* mutant plants is shown. Stars indicate significant differences between treatments (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

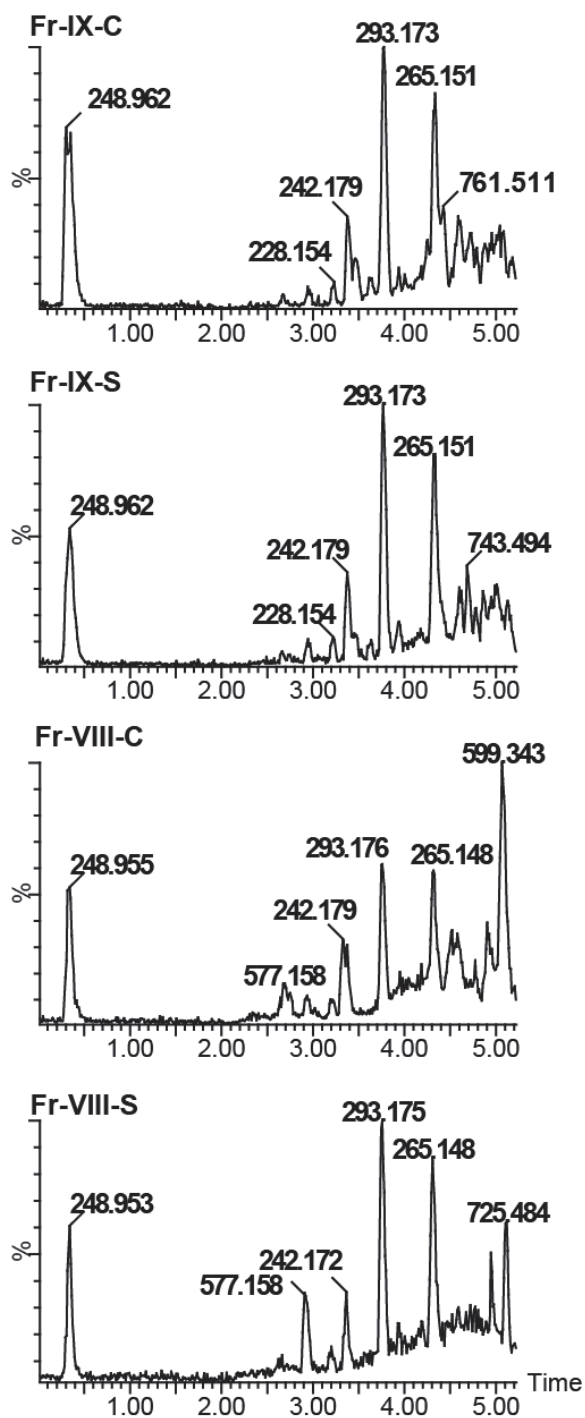


Fig. S5: Metabolomic fingerprints of active root fractions. LC-TOFMS chromatograms in ESI- of fraction VIII and IX from roots of control (C) and *S. littoralis* infested plants (S). Each chromatogram was blank-subtracted and normalized to 1000 counts. Peak extraction followed by univariate data analysis did not reveal any clear differences in ESI- or ESI+ modes.

Table S1. Multiple reaction monitoring parameters for phenolic acid analysis. Q1: Parent ion → Q3: product ion; mass to charge ratio [m/z]. ID: compound name; DP: Declustering potential; CE: Collision energy.

Q1 mass (Dalton)	Q3 mass (Dalton)	ID	DP (Volts)	CE (Volts)
147	102.8	Cinnamic acid	-65	-16
163	118.9	Coumaric acid	-60	-20
179	134.9	Caffeic acid	-55	-22
193.1	133.9	Ferulic acid	-75	-22
223	149	Sinapic acid	-65	-26