A mechanism for sequence specificity in plant-mediated interactions between herbivores

Wei Huang[1], Christelle A. M. Robert[1], Maxime R. Hervé[1,2], Lingfei Hu[1], Zoe Bont[1], Matthias Erb[1*]

[1] Institute of Plant Sciences, University of Bern, Altenbergrain 21, Bern 3013, Switzerland

[2] INRA, UMR1349 IGEPP, F-35653 Le Rheu, France

* Author for correspondence: matthias.erb@ips.unibe.ch, 41 31 631 8668 (Tel)

Words: Main text (5453), Introduction (1066), Materials and Methods (2125), Results (1028), Discussion (1177), Acknowledgements (57)

References: 47

Figures: 7; Supplementary figure: 1

Summary

1. Herbivore communities are shaped by indirect plant-mediated interactions whose outcomes are strongly dependent on the sequence of herbivore arrival. However, the mechanisms underlying sequence specificity are poorly understood.

2. We examined the mechanisms which govern sequence specific effects between two specialist maize herbivores, the leaf feeder *Spodoptera frugiperda* and the root feeder *Diabrotica virgifera virgifera.* In the field, *S. frugiperda* reduces *D. v. virgifera* abundance, but only when arriving on the plant first.

3. In behavioral experiments, *D. v. virgifera* larvae continued feeding on plants that they had infested prior to leaf infestation, but refused to initiate feeding on plants that were infested by *S. frugiperda* prior to their arrival. Changes in root-emitted volatiles were sufficient to elicit this sequence-specific behaviour. Root volatile and headspace mixing experiments showed that early arriving *D. v. virgifera* larvae suppressed *S. frugiperda* induced volatile repellents, which led to the maintenance of host attractiveness to *D. v. virgifera*.

4. Our study provides a physiological and behavioral mechanism for sequence specificity in plant-mediated interactions and suggests that physiological canalization of behaviorally active metabolites can drive sequence specificity and result in strongly diverging herbivore distribution patterns.

Keywords

above-below ground interactions, *Diabrotica virgifera virgifera*, induced resistance, physiological canalization, plant-herbivore interactions, *Spodoptera frugiperda*, volatile organic compounds, *Zea mays*

Introduction

Interspecific competition influences the structure, function and stability of natural and agricultural ecosystems (Loreau & de Mazancourt, 2013). For herbivorous insects, interspecific competition can occur through direct interference or through plant-mediated, indirect effects (Denno *et al.*, 1995). A growing number of studies show that plant-mediated, indirect effects are the most common form of interspecific competition between herbivores (Ohgushi, 2005; Kaplan & Denno, 2007; Xiao *et al.*, 2012; Huang *et al.*, 2013) and that they act as driving forces of herbivore community composition in nature (Kaplan & Denno, 2007; Poelman & Dicke, 2014; Stam *et al.*, 2014).

The outcome of plant-mediated interactions between herbivores is determined by a number of factors, including the identity of the attacking herbivore, the identity of the plant and the identity of the responding herbivore (Johnson & Agrawal, 2005; Wurst & van der Putten, 2007; Xiao *et al.*, 2012; Huang *et al.*, 2014). Recently, the sequence of arrival was identified as an important factor as well: Depending on which species arrives first, the effect of one herbivore on the other can change drastically. Soler *et al.* (2012) for instance observed that *Pieris brassicae* caterpillars grew bigger when feeding on *Brassica oleracea* plants that were infested by *Brevicoryne brassicae* aphids before the arrival *P. brassicae*, but not if both herbivores attacked the plant simultaneously. A recent meta-analysis on interactions between leaf and root feeding herbivores identified the sequence of arrival as a strong predictor for the directionality of effects for this type of plant-mediated interactions (Johnson *et al.*, 2012).

To date, several physiological hypotheses have been proposed that may explain sequence specificity (Erb *et al.*, 2011a; Stam *et al.*, 2014): Plant-mediated feedback-loops, overriding induction effects and physiological canalization. Plant-mediated feedback-loops occur if two herbivores sharing a host plant influence each other reciprocally (Soler *et al.*, 2012): A first arriving herbivore could then

64 influence the behavior and damage patterns of a second arriver by inducing
65 physiological changes in the plant, which, by consequence, would change the
66 plant-mediated impact of the second herbivore on the first herbivore and thereby lead
67 to sequence specific patterns. Overriding effects occur if one herbivore elicits a plant
68 response that is much stronger than the response of the other herbivore and thereby
69 determines the resulting interaction (Stam *et al.*, 2014). Physiological canalization is a
70 phenomenon where plant responses are determined by the first arriving herbivore
71 (Viswanathan *et al.*, 2007). By suppressing the response that is normally elicited by a
72 second herbivore, physiological canalization can lead to sequence-specific effects.

73 Behavioral mechanisms may also lead to sequence specificity (Erb *et al.*, 2011a;
74 Karban, 2011). Asymmetrical host acceptance for instance refers to situations where a
75 herbivore is less likely to start feeding on a new host plant than to continue feeding on
76 a colonized host. This is a common pattern for sedentary herbivores such as miners
77 and gall feeders and may lead to sequence-specific effects by modulating the behavior
78 of a herbivore differently, depending on whether it is arriving on a host plant second
79 or whether it is already established when another herbivore arrives.

80 Plant physiological and herbivore behavioral mechanisms are not mutually
81 exclusive. Asymmetrical host acceptance for instance may be favored by
82 plant-mediated feedback-loops, overriding effects or physiological canalization: For
83 example, a first arriving herbivore may negatively impact a second herbivore, which
84 may decrease the capability of second herbivore to induce volatile repellents, and in
85 turn render the plant more attractive to first herbivore. Furthermore, a first herbivore
86 may trigger strong physiological changes in the plant which may render it attractive to
87 itself irrespective of the potentially unattractive changes that are induced by a second
88 arriving herbivore. Finally, a first herbivore may change the plant's physiology in a
89 way that makes it unresponsive to the second herbivore, which may lead to the
90 suppression of an otherwise unattractive physiological change. So far, the
91 contributions of the different physiological and behavioral mechanisms and their
92 combinations to sequence specificity have not been tested experimentally. By

consequence, the drivers of sequence specificity in indirect, plant-mediated interactions are not well understood.

Here, we analyzed potential mechanisms leading to sequence-specificity by studying the effect of attack by the leaf-feeding larvae of *Spodoptera frugiperda* (J.E. Smith) on the root-feeding larvae of *Diabrotica virgifera virgifera* (LeConte) sharing maize (*Zea mays* L.) as a common host plant. Both herbivores occur on cultivated maize and its wild ancestors and cause severe damage in both agricultural and natural systems (Branson & Krysan, 1981; O'Day, 1998). They overlap spatially and temporally in the field, with their sequence of arrival varying considerably with climatic conditions and locations (Branson, 1976; O'Day, 1998). Our previous study within the same system revealed that *S. frugiperda* larvae significantly reduce the number of *D. v. virgifera* larvae feeding on maize roots in the field, but only when *S. frugiperda* larvae arrive first (Erb *et al.*, 2011a). Subsequent experiments showed that maize root systems of plants which are attacked by leaf-feeding caterpillars become highly unattractive to *D. v. virgifera* larvae, and that this effect is mediated by long and short distance host acceptance cues (Robert *et al.*, 2012a; Erb *et al.*, 2015; Lu *et al.*, 2016). In contrast, *D. v. virgifera* attack renders the plant highly attractive to conspecifics (Robert *et al.*, 2012a) and reprograms the root metabolism to become more suitable for its own development (Robert *et al.*, 2012b). Although *D. v. virgifera* decreases the performance of leaf-feeders on maize under water limiting conditions, which may lead to plant-mediated feedback loops (Erb *et al.*, 2009; Erb *et al.*, 2011b), we found no correlation between the amount of *S. frugiperda* leaf-damage and the reduction of *D. v. virgifera* performance in our previous work (Erb *et al.*, 2011a).

Based on the findings above, we hypothesized that asymmetrical host acceptance may contribute to the sequence specific interaction patterns between *D. v. virgifera* and *S. frugiperda*, and that this asymmetrical acceptance behavior may either be the result of overriding effects or physiological canalization. We therefore conducted a series of behavioral experiments to explore the impact of the sequence of arrival on host plant attractiveness and acceptability for *D. v. virgifera* larvae. We then used a

modified two-by-two-arm olfactometer to test the influence of plant volatiles on the sequence-specific behavior of *D. v. virgifera* and to distinguish between overriding effects and physiological canalization. Finally, we analyzed the changes in root volatiles elicited by the different arrival sequences to test for patterns of physiological canalization.

Materials and Methods

Plants and insects

Maize seeds (hybrid Delprim) were obtained from Delley DSP (Delley, Switzerland). They were sown individually in plastic pots (11 cm depth, 4 cm diameter) and placed in a greenhouse (26 ± 2 °C, 14 : 10 h light : dark, 55 % relative humidity). Twelve days later (henceforth called day 0), plants with three fully developed leaves were used for experiments. Eggs of *D. v. virgifera* were obtained from the USDA-ARS (Brookings, SD) and larvae were reared on freshly germinated maize plants until use. *S. frugiperda* eggs were obtained from the University of Neuchatel, (Neuchâtel, Switzerland), and the hatching larvae were reared on soy-wheat germ diet (Bio-Serv, USA) until use.

Plant treatments

To establish different feeding sequences and herbivore combinations, plants were randomly assigned to one of four treatments (Fig. 1a). (1) aboveground herbivory (AG): Twelve second-instar *S. frugiperda* larvae were added to the leaves of each plant at day 2; (2) belowground herbivory (BG): Six second-instar *D. v. virgifera* larvae were added into a hole (9 cm depth, 0.5 cm diameter) in the soil at the base of each plant at day 0; (3) belowground attack followed by aboveground attack (BG>AG): Six second-instar larvae of *D. v. virgifera* were added to each plant at day 0, and twelve second-instar larvae of *S. frugiperda* were added to each plant at day 2; (4) controls without herbivory (C). These treatments simulated a situation where *D. v. virgifera* larvae newly arrive on plants already infested with *S. frugiperda* (AG) or

where they can continue feeding on maize plants that are infested by conspecifics alone (BG) or by conspecifics that arrived prior to the arrival of the leaf feeder (BG>AG) (Fig. 1a). As *D. v. virgifera* larvae refuse to feed on plants that are previously attacked by *S. frugiperda* (Robert *et al.*, 2012a; Erb *et al.*, 2015; Lu *et al.*, 2016), an AG>BG treatment was not included in the experimental setups.

To prevent above- and belowground herbivores from escaping, the aboveground parts (leaves of maize plants) were caged with transparent 1.5L plastic bottles with their bottoms removed that were put upside down on the pots. Belowground parts (pots) were covered with aluminum foil. All plants were caged the same way regardless of herbivore treatment. Furthermore, small holes were made in the soil of each plant regardless of *D. v. virgifera* infestation. Four days after the beginning of the different treatments (day 4), the plastic bottles and *S. frugiperda* larvae were removed. Then, the responding *D. v. virgifera* larvae were introduced into the system as described below (Fig. 1b-e). Timing and herbivore densities were chosen to match earlier studies and to mimic natural occurrence patterns in the field (Erb *et al.*, 2011a; Robert *et al.*, 2012a).

Influence of sequence of arrival on host plant acceptance by D. v. virgifera

In a first set of experiments, we tested the hypothesis that *D. v. virgifera* larvae may reject roots of plants that are previously infested with *S. frugiperda*, but may continue to feed on plants on which they were able to establish a suitable feeding environment prior to the arrival of the leaf-feeder. We conducted experiments using three different setups, as described below (Fig. 1b-d).

First, we tested the behaviour of *D. v. virgifera* using a Petri dish setup which allowed for direct root contact (Robert *et al.*, 2012c) (Fig. 1b). The root systems of plants from the different treatment groups were gently washed with tap water. Plants were then paired in the following combinations: (1) C *vs* AG; (2) C *vs* BG; (3) C *vs* BG>AG. Root systems of the different plant pairs were placed on a moistened filter paper in a Petri dish (13.5 cm diameter, 2 cm depth), which had a gap (0.8 cm width,

177 2 cm height) on the side. The stems were laid into the gap, with the leaves remaining
178 outside of the Petri dish. Six second-instar larvae were then added onto the moistened
179 filter paper. The larvae could move and feed freely on the plants within the Petri dish.
180 The Petri dish was covered with aluminum foil to decrease the impact of light on the
181 roots and insects. The position of the larvae was recorded at 0.5h, 1.5h, 3h and 5h.
182 Larvae that remained on the filter paper and did not choose a plant were counted as
183 no-choice. Each treatment combination was repeated 24-36 times.

184 Second, we specifically tested the contribution of volatile cues to the observed
185 behavioral patterns. For this purpose, the same treatment combinations as in the first
186 experiment were offered to *D. v. virgifera* larvae in two-arm olfactometers as
187 described (Robert *et al.*, 2012a) (Fig. 1c). Before the beginning of the treatments,
188 plants were transplanted individually into L-shaped glass pots (11 cm depth, 5 cm
189 diameter) with a horizontal connector at a height of 0.5 cm and filled with moist sand.
190 At day 4, the horizontal connector of each glass pot was attached with one Teflon
191 connector (29 / 32 to 24 / 29 mm) which contained a fine metal screen (2300 mesh;
192 Small Parts Inc.). Then, the two Teflon connectors were linked using a glass tube (24 /
193 29 mm; length 8 cm) with a vertical access port in the middle. To keep the root
194 systems in the dark and to avoid visual cues for the larvae, the entire olfactometer was
195 covered with aluminum foil. Twenty minutes after connecting the different odor
196 sources, six second-instar *D. v. virgifera* larvae were released into the access port of
197 the glass tube. The larvae could move freely in the glass tube, but could not reach the
198 roots of the plants. After 10 min, the olfactometer was disassembled and the number
199 of larvae in each Teflon connector was recorded. Larvae that stayed in the central
200 glass tube after 10 min were recorded as no-choice. For each treatment combination,
201 18 independent replicates were carried out.

202 In a third experiment, we tested whether *D. v. virgifera* larvae are more likely to
203 leave the rhizosphere environment of infested plants, even in the absence of an
204 alternative host. For this purpose, plants were potted and infested in L-shaped glass
205 pots as described above (Fig. 1d). Then, six second-instar larvae were released

directly at the entrance of the horizontal access port of each glass pot. The access port of the horizontal connector was not sealed so the larvae could move into the soil and start feeding or try to escape from the plant through the access port. The L-pot was placed in a Petri dish filled with tap water at a height of 0.5 cm to catch escaping *D. v. virgifera* larvae without flooding the glass pot. The number of escaped larvae in the trap was recorded over 20 min. For each treatment, 12 replicates were carried out.

Plant-mediated feedback-loops

To evaluate whether belowground attack by *D. v. virgifera* changes the aboveground damage pattern by *S. frugiperda* larvae under the current experimental conditions, the leaves of plants from the different infestation treatments were collected at day 4, and total leaf area and damaged leaf area were measured for each plant using Digimizer software (MedCalc Software bvba; Mariakerke, Belgium). Eighteen replicates per treatment were carried out.

Overriding effects

To investigate whether an overriding signal may be responsible for the observed asymmetrical host acceptance of *D. v. virgifera* in the first set of experiments, we developed a two-by-two-arm belowground olfactometer that allowed us to combine the volatile headspaces from two odor sources per arm (Fig. 1e). For this purpose, the setup described above was modified as follows: Two Teflon connectors attached to glass pots were linked using a "Y" glass tube (24 / 29 mm; length 8 cm) at an angle of 60°. Then, two "Y" glass tubes were connected to a central glass tube (24 / 29 mm; length 8 cm) with a vertical access port in the middle. This modification enabled us to attach two L-shaped glass pots to each side of the release tubes and to test the preference of *D. v. virgifera* for two combinations of two mixed odor sources. The following treatment combinations were investigated using this setup: C+C *vs* C+AG; C+C *vs* C+BG, C+C *vs* AG+BG. The olfactometer was disassembled and the number of larvae in each "Y" glass tube was recorded after 10 min. We hypothesized that if *D.*

233 *v. virgifera* elicits an overriding signal, the AG+BG arms should be more attractive
234 than the C+C arm. Eighteen replicates were performed for each treatment
235 combination.

236 ***Physiological canalization***

237 To evaluate whether *D. v. virgifera* attack canalizes the root volatile response in
238 a way that suppresses responsiveness to *S. frugiperda* infestation, we collected and
239 analyzed root volatile profiles using solid-phase micro-extraction-gas
240 chromatography-mass spectrometry (SPME-GC-MS). Plants were treated as
241 described above (Fig. 1a). Crown and primary roots were then washed with tap water
242 and frozen in liquid nitrogen. Twelve plants per treatment were harvested, and the
243 roots of two plants were pooled for analysis, resulting in six biological replicates. The
244 crown and primary roots of each replicate were ground into a fine powder, and 50 mg
245 of each root type were placed in a 10 ml glass vial and sealed using Teflon tape
246 (Polytetrafluoroethylene). An SPME fiber (100 μm polydimethylsiloxane coating,
247 Supelco, USA) was then inserted into the vial for 60 min at 50°C. The incubated
248 fibers were then immediately analyzed by GC-MS (Agilent 7820A GC interfaced
249 with an Agilent 5977E MSD) following previously established protocols with a few
250 modifications (Erb *et al.*, 2011c). Briefly, the fiber was inserted into the injector port
251 at 250 °C and desorbed for 2 min. After fiber insertion, the column temperature was
252 maintained at 60 °C for 1 min and then increased to 250 °C at 5 °C min^{-1} followed by
253 a final stage of 4 min at 250 °C. The overall analysis time for each sample, including
254 oven cooling, was 45 min. Furthermore, to eliminate the impact of background peaks,
255 three glass vials without any plant material (blanks) were run using the same protocol
256 as described above. The resulting GC-MS chromatograms were processed with
257 Progenesis QI (informatics package from Waters, MA, USA) using default settings for
258 spectral alignment and peak picking. From the resulting matrix, all features which
259 were presented in more than one blank were removed, resulting in 232 features.
260 Features were assigned to individual compounds by retention time and peak shape
261 matching and identified using the NIST search 2.2 Mass Spectral Library as well as

retention time and spectral comparison with pure compounds.

Data analysis

To examine host acceptance of *D. v. virgifera* in a Petri dish experiment, the number of larvae found on different herbivory treatment groups was analyzed using a Wald test applied on a Generalized Linear Mixed Model (GLMM) with a Poisson distribution. We considered plant treatment as a fixed factor, time as covariate and the replicate as a random factor. Each plant combination (C *vs* AG, C *vs* BG and C *vs* BG>AG) was analyzed separately. Then, to compare the preference of *D. v. virgifera* between the different treatment groups, the number of larvae on infested plants (AG, BG and BG>AG) was analyzed using a likelihood ratio test applied on a Generalized Linear Model (GLM) with a Poisson distribution. The models included herbivory as fixed factor and time as a covariate. The preference of *D. v. virgifera* larvae in the olfactometer experiments and the number of escaped larvae in escape experiment were analyzed as described above. To examine whether belowground attack by *D. v. virgifera* larvae changes the aboveground damage pattern by *S. frugiperda* larvae, the relative and absolute leaf damage of *S. frugiperda* larvae was analyzed using independent sample *t*-tests (BG *vs* BG>AG). The absolute leaf damage was estimated by the sum of leaf damaged area for each plant and the relative leaf damage was calculated as the sum of leaf damaged area / the sum of total leaf area × 100 for each plant. To examine the overall differences in volatile profiles, the relative abundance of the detected features were subjected to redundancy analysis (RDA) using the different treatments as a unique explanatory variable. Monte Carlo tests with 999 permutations were then used to test for significant differences between treatments. For more detailed, compound specific analyses, the different features were assigned to individual compounds, and the relative abundance of the individual compounds, which corresponds to the sum of the signal intensities of the individual features, were analyzed by one-way ANOVAs followed by least square mean *post-hoc* tests for pairwise comparisons, including false discovery rate (FDR) corrections (Benjamini & Hochberg, 1995). All analyses were conducted using R 3.2.0 (R Foundation for

Statistical Computing, Vienna, Austria) with "car", "lme4", "lsmeans", "vegan" and "RVAideMemoire" packages (Fox & Weisberg, 2011; Bates *et al.*, 2015; Hervé, 2016; Lenth, 2016; Oksanen *et al.*, 2016).

Results

D. v. virgifera rejects S. frugiperda infested plants only when arriving second

In the Petri dish experiment, *D. v. virgifera* larvae strongly preferred the roots of control plants when offered uninfested vs. leaf-infested plants (X^2 = 30.753, P < 0.001, Fig. 2a). By contrast, the larvae showed a strong preference for roots that were previously infested with *D. v. virgifera* larvae over controls (X^2 = 69.919, P < 0.001, Fig. 2b). Roots which were infested with *D. v. virgifera* two days before the onset of *S. frugiperda* attack remained highly attractive (X^2 = 21.734, P < 0.001, Fig. 2c). The number of responding *D. v. virgifera* larvae increased with experimental time (C *vs* AG, X^2 = 5.698, P = 0.017; C *vs* BG, X^2 = 20.033, P < 0.001; C *vs* BG>AG, X^2 = 35.964, P < 0.001; Fig. 2). At the end of the experiment, 65%, 67% and 70% of *D. v. virgifera* larvae made a choice in C *vs* AG, C *vs* BG and C *vs* BG>AG, respectively. No significant interactive effects between time and treatments were found (C *vs* AG, X^2 = 3.515, P = 0.061; C *vs* BG, X^2 = 0.135, P = 0.713; C *vs* BG>AG, X^2 = 1.342, P = 0.247). Overall, more *D. v. virgifera* larvae fed on BG and BG>AG roots than on AG roots (X^2 = 38.558, P < 0.001, Fig. 2). No difference was found between the preference of *D. v. virgifera* for BG and BG>AG roots (P = 0.064, Fig. 2).

In the two-arm olfactometer experiment, similar preference patterns were observed. *D. v. virgifera* larvae showed a strong preference for control plants over *S. frugiperda* infested plants (X^2 = 8.111, P < 0.01, Fig. 3). By contrast, the larvae preferred plants that were previously infested with conspecifics over controls (X^2 = 34.177, P < 0.001, Fig. 3). Plants were infested with *D. v. virgifera* prior to *S. frugiperda* infestation remained highly attractive (X^2 = 16.849, P < 0.001, Fig. 3). In this experiment, all larvae made a choice within 10 min. Together, the larvae were

more attracted to the roots that had been infested by conspecifics alone and conspecifics that had arrived prior to the arrival of the *S. frugiperda*, while were less attracted to the roots that had been infested by *S. frugiperda* alone (χ^2 = 20.396, P < 0.001, Fig. 3). Again, BG and AG>BG treatments were not significantly different from each other (P = 0.389, Fig. 3).

When offered a single host plant, the number of escaping *D. v. virgifera* larvae differed significantly between treatments (χ^2 = 32.112, P < 0.001, Fig. 4). When offered a *S. frugiperda* infested plant, 50% of the larvae escaped from the rhizosphere within 20 min (Fig. 4). By contrast, less than 18% of the larvae left the soil of control plants or plants that were previously infested with conspecifics (Fig. 4). A similar percentage of larvae chose to remain in the rhizosphere of plants that were infested with *D. v. virgifera* prior to *S. frugiperda* attack (Fig. 4).

Plant-mediated feedback-loops are unlikely to explain D. v. virgifera behavior

There was no significant difference in relative (t = 0.055, P = 0.957) or absolute (t = 1.236, P = 0.225) damaged leaf area between plants from that were infested with *D. v. virgifera* or root herbivore free (Fig. S1). These results suggest that the interaction between *D. v. virgifera* and *S. frugiperda* is highly asymmetrical and that plant-mediated feedback-loops are unlikely to play a major role in determining sequence specific responses of *D. v. virgifera*.

D. v. virgifera does not produce an overriding attractive signal

Similarly to the two-arm olfactometer experiment, *D. v. virgifera* larvae significantly preferred to move to the side of the olfactometer containing two control plants rather than the arm leading to a control plant and an *S. frugiperda* infested plant (χ^2 = 15.446, P < 0.001, Fig. 5). The opposite was true for a combination of a control plant with a *D. v. virgifera* infested plant, which was attractive to the root feeder (χ^2 = 8.111, P < 0.01, Fig. 5). In contrast to the attractiveness of BG>AG plants observed in the two-arm olfactometer experiment however (Fig. 3), the mixed rhizosphere

volatiles from an *S. frugiperda* and a *D. v. virgifera* infested plant were highly unattractive, and significantly more larvae moved to the control side (χ^2 = 10.333, P < 0.01, Fig. 5) than to the AG+BG side. All larvae made a choice within the first 10 min. Overall, the presence of plants that were infested by *S. frugiperda* significantly repelled *D. v. virgifera* (χ^2 = 15.915, P < 0.001, Fig. 5). This experiment falsifies the hypothesis that *D. v. virgifera* triggers an overriding attractant.

D. v. virgifera feeding suppresses S. frugiperda induced root volatiles

In total, we detected 232 volatile features in the GC-MS chromatograms. Redundancy analysis revealed that *S. frugiperda* and *D. v. virgifera* attack induced different volatile blends compared to control plants and compared to each other (AG *vs* C: P = 0.008; BG *vs* C: P = 0.008; BG>AG *vs* C: P = 0.008, Fig. 6). The volatile profiles of plants that were induced by *D. v. virgifera* prior to *S. frugiperda* attack were indistinguishable from plants that were infested with *D. v. virgifera* alone (BG>AG *vs* BG: P = 0.642, Fig. 6), but both of them were significantly different from plants that were infested with *S. frugiperda* alone (BG *vs* AG: P = 0.008; BG>AG *vs* AG: P = 0.008, Fig. 6). Analysis of variance revealed twelve volatile compounds whose abundance differed significantly between treatments (Fig. 7). Pairwise comparisons showed that four of these volatiles were significantly induced by *D. v. virgifera* infestation alone (Fig. 7a-d) and two of them were significantly induced by *S. frugiperda* attack alone (Fig. 7k-l). We found no significant effect of later *S. frugiperda* attack on *D. v. virgifera* induced volatile emissions (Fig. 7). However, the induction of the *S. frugiperda* induced volatiles was suppressed by early *D. v. virgifera* infestation (Fig. 7l). This result demonstrates that *D. v. virgifera* canalizes the root volatile production and renders roots unresponsive to leaf-attack by *S. frugiperda*.

Discussion

The sequence of arrival is increasingly recognized as an important determinant

of plant-mediated indirect interactions between herbivores (Viswanathan *et al.*, 2005; Viswanathan *et al.*, 2007; Poelman *et al.*, 2008; Erb *et al.*, 2011a; Soler *et al.*, 2012; Wang *et al.*, 2014). However, the mechanisms leading to sequence specificity are not well understood. The goal of the present study was to identify the (mutually non-exclusive) behavioral and physiological mechanisms that may contribute to sequence specific effects. Our experiments show that leaf attack by *S. frugiperda* strongly reduces the attractiveness of roots for *D. v. virgifera* through changes in volatile cues. However, prior *D. v. virgifera* attack suppresses these changes and thereby maintains the attractiveness of the plants to *D. v. virgifera* larvae. This form of asymmetrical host acceptance behavior explains why S. *frugiperda* reduces the abundance and damage by *D. virgifera* in the field only when arriving first on the plant (Erb *et al.*, 2011a).

Several non-exclusive physiological mechanisms may explain why *D. v. virgifera* is repelled by *S. frugiperda* attacked plants only when arriving second. It is for instance possible that early arriving *D. v. virgifera* larvae change the behavior and induction pattern of *S. frugiperda*. However, we found no evidence for the presence of resistance feedback loops in our system: *S. frugiperda* damage remained unchanged by *D. v. virgifera* attack. Earlier studies demonstrated that *D. v. virgifera* root attack increases leaf resistance via ABA signalling under drought conditions; when plants are well watered, no negative effects of *D. v. virgifera* on *Spodoptera littoralis* growth were observed any more (Erb *et al.*, 2011b). The maize seedlings in our experiments were supplied with sufficient soil moisture, which likely prevented potential feedback loops from occurring. Another explanation for the observed behavioural patterns is that *D. v. virgifera* may induce changes that strongly increase the attractiveness of the roots and override any negative changes that are later induced by *S. frugiperda*. By mixing volatiles from different plants, we tested this hypothesis on a behavioral level. Surprisingly, we found that *D. v. virgifera* rejected the volatile mix from a combination of plants that had been infested by *D. v. virgifera* and *S. frugiperda* separately. This is in stark contrast with the strong attractiveness of plants that were

401 infested with *D. v. virgifera* and *S. frugiperda* sequentially and strongly suggests that
402 *D. v. virgifera* does not produce an overriding attractive signal.

403 On the other hand, our GC-MS analyses provide clear evidence that *D. v.*
404 *virgifera* canalizes the plant's root volatile response. Maize roots responded strongly
405 to *D. v. virgifera* attack and produced higher amounts of several volatiles, including
406 several products of the terpene synthase TPS23 which are strongly induced by *D. v.*
407 *virgifera* (Köllner *et al.*, 2008; Hiltpold *et al.*, 2011) and attract the root feeder (Robert
408 *et al.*, 2012a). These responses were not altered by later *S. frugiperda* attack. By
409 contrast, *S. frugiperda* attack induced a different set of compounds in the roots,
410 including a yet unidentified nitrophenol, and this induction was fully suppressed by
411 prior *D. v. virgifera* attack. These results demonstrate that early arriving *D. v. virgifera*
412 canalizes the root metabolism in a way that makes it unresponsive to *S. frugiperda*
413 attack. Canalization of plant responses by herbivores has been proposed to occur in a
414 number of plant-herbivore interactions (Thaler *et al.*, 2002; Viswanathan *et al.*, 2005;
415 Utsumi *et al.*, 2010). For example, Viswanathan *et al.* (2007) found that tortoise
416 beetle attack after flea beetle attack of *Solanum dulcamara* did not alter the induced
417 resistance elicited by the flea beetles. By contrast, tortoise beetle attack before flea
418 beetle attack resulted in the disappearance of induced resistance. One possible
419 explanation of canalization is negative cross-talk between signaling pathways that
420 inducing one pathway may attenuate or repress other pathways (Koornneef & Pieterse,
421 2008; Erb *et al.*, 2012). Furthermore, priority in occupying a plant resource may also
422 result in physiological canalization, as resources invested into an initial induced
423 response may be not available for investment into later induced responses (Stam *et al.*,
424 2014). In combination with the behavioral experiments, these results suggest that the
425 asymmetrical host acceptance behavior of *D. v. virgifera* is caused by physiological
426 canalization.

427 In a previous study, we found that leaf attack by *S. littoralis* leads to a slight
428 decrease in root ethylene production, and that adding ethylene back to the root system
429 restores the attractiveness of the roots to *D. v. virgifera* (Robert *et al.*, 2012a). Many

herbivores increase local ethylene emissions of their host plants (Winz & Baldwin, 2001; von Dahl & Baldwin, 2007; Schäfer *et al.*, 2011), and it is therefore possible that *D. v. virgifera* attack resulted in the reversal or canalization of the ethylene response of the roots. Unfortunately, ethylene emissions could not be measured in the current series of experiments. However, the presented findings suggest that *S. frugiperda* attack also triggers the release of repellent volatiles which are suppressed by *D. v. virgifera*. The escape experiment in particular shows that *D. v. virgifera* systematically moves away from leaf-infested plants, and it seems unlikely that a reduction in ethylene levels alone can account for this result. Furthermore, the volatile mixing experiment suggests that the volatile blend of the roots of an *S. frugiperda* attacked plant overrides the attractive signal from a *D. v. virgifera* infested root system.

In our GC-MS chromatograms, we found several volatiles which increased in the roots of *S. frugiperda* attacked plants. Elucidating their structure and bioactivity is an exciting prospect of this work. A recent paper identified methyl antranilate as a repellent for neonate *D. v. virgifera* larvae (Bernklau *et al.*, 2016). Although methyl antranilate was not among the *S. frugiperda* induced root volatiles, it provides an interesting starting point to identify the volatiles which render *S. frugiperda* attacked plants repellent to *D. v. virgifera* larvae. One aspect that should be kept in mind is that root volatiles were measured by grinding root material and sampling the headspace of the ground samples by SPME. The advantages of this technique are its sensitivity and robustness. Its disadvantage is that it may result in the detection of volatile compounds which are not actually released into the rhizosphere by intact roots. Future experiments should therefore include *in vivo* sampling techniques to confirm the release of the newly detected volatiles into the rhizosphere (Ali *et al.*, 2010; Hiltpold *et al.*, 2011).

Host location and acceptance by herbivores are key processes in plant-herbivore interactions. Our results show that physiological canalization can have a strong, sequence-specific impact on host acceptance by herbivores, which may result in

strongly diverging herbivore damage and distribution patterns in the field. Our previous work shows that the repellent effect of leaf infestation on root herbivores is highly conserved across herbivore species and maize genotypes (Lu *et al.*, 2016). Whether similar effects also occur in other plant species remains to be elucidated. Understanding the mechanisms which govern sequence specificity will allow for the integration of this phenomenon into current theory on plant-mediated interactions and will facilitate future efforts to develop predictive ecophysiological models of multi-herbivore dynamics on shared host plants.

Acknowledgements. We thank Yves Borcard (FARCE Laboratory, University of Neuchâtel) for supplying *S. frugiperda* eggs, Wade French and Chad Nielson (ARS-USDA Brookings) for supplying *D. v. virgifera* eggs and the gardeners of the IPS for their help with plant cultivation. The study was supported by the Swiss National Science Foundation (Grants Nr. 152613 and 153517 to M.E.).

Contribution. M.E., W.H. and C.A.M.R. planned and designed the research. W.H., L.H., Z.B. and C.A.M.R. carried out experiments. W.H., M.R.H. and M.E. analyzed data. M.E. and W.H. wrote the manuscript.

References

Ali JG, Alborn HT, Stelinski LL. 2010. Subterranean herbivore-induced volatiles released by citrus roots upon feeding by *Diaprepes abbreviatus* recruit entomopathogenic nematodes. *Journal of Chemical Ecology* **36**: 361-368.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**: 1-48.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B-Statistical Methodology* **57**: 289-300.

Bernklau EJ, Hibbard BE, Norton AP, Bjostad LB. 2016. Methyl anthranilate as a repellent for western corn rootworm larvae (Coleoptera: Chrysomelidae).

Journal of Economic Entomology. **109**: 1683-1690.

Branson TF. 1976. The selection of a non-diapausing strain of *Diabrotica virgifera* (Coleoptera: Chrysomelidae). *Entomologia Experimentalis et Applicata* **19**: 148-154.

Branson TF, Krysan JL. 1981. Feeding and oviposition behavior and life cycle strategies of *Diabrotica*: an evolutionary view with implications for pest management. *Environmental Entomology* **10**: 826-831.

Denno RF, Mcclure MS, Ott JR. 1995. Interspecific interactions in phytophagous insects: competition reexamined and resurrected. *Annual Review of Entomology* **40**: 297-331.

Erb M, Flors V, Karlen D, De Lange E, Planchamp C, D'Alessandro M, Turlings TCJ, Ton J. 2009. Signal signature of aboveground-induced resistance upon belowground herbivory in maize. *Plant Journal* **59**: 292-302.

Erb M, Robert CAM, Hibbard BE, Turlings TCJ. 2011a. Sequence of arrival determines plant-mediated interactions between herbivores. *Journal of Ecology* **99**: 7-15.

Erb M, Köllner TG, Degenhardt J, Zwahlen C, Hibbard BE, Turlings TCJ. 2011b. The role of abscisic acid and water stress in root herbivore-induced leaf resistance. *New Phytologist* **189**: 308-320.

Erb M, Balmer D, De Lange ES, Von Merey G, Planchamp C, Robert CAM, Röder G, Sobhy I, Zwahlen C, Mauch-Mani B *et al.* 2011c. Synergies and trade-offs between insect and pathogen resistance in maize leaves and roots. *Plant, Cell & Environment* **34**: 1088-1103.

Erb M, Meldau S, Howe GA. 2012. Role of phytohormones in insect-specific plant reactions. *Trends in Plant Science* **17**: 250-259.

Erb M, Robert CAM, Marti G, Lu J, Doyen G, Villard N, Barrière Y, French BW, Wolfender JL, Turlings TCJ *et al.* 2015. A physiological and behavioral mechanism for leaf-herbivore induced systemic root resistance. *Plant Physiology* **169**: 2884-2894.

Fox J, Weisberg S. 2011. *An R companion to applied regression*. Thousand Oaks CA,

USA: Sage.

Hervé M. 2016. *RVAideMemoire: diverse basic statistical and graphical functions. R package version 0.9-56*. URL https://CRAN.R-project.org/package=RVAideMemoire.

Hiltpold I, Erb M, Robert CAM, Turlings TCJ. 2011. Systemic root signalling in a belowground, volatile-mediated tritrophic interaction. *Plant, Cell & Environment* **34**: 1267-1275.

Huang W, Siemann E, Xiao L, Yang X, Ding J. 2014. Species-specific defence responses facilitate conspecifics and inhibit heterospecifics in above-belowground herbivore interactions. *Nature Communications* **5**: 4851.

Huang W, Siemann E, Yang X, Wheeler GS, Ding J. 2013. Facilitation and inhibition: changes in plant nitrogen and secondary metabolites mediate interactions between above-ground and below-ground herbivores. *Proceedings of the Royal Society B-Biological Sciences* **280**: 20131318.

Johnson MTJ, Agrawal AA. 2005. Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenothera biennis*). *Ecology* **86**: 874-885.

Johnson SN, Clark KE, Hartley SE, Jones TH, McKenzie SW. 2012. Aboveground-belowground herbivore interactions. A meta-analysis. *Ecology* **93**: 2208-2215.

Köllner TG, Held M, Lenk C, Hiltpold I, Turlings TCJ, Gershenzon J, Degenhardt J. 2008. A maize (*E*)-β-Caryophyllene synthase implicated in indirect defense responses against herbivores is not expressed in most American maize varieties. *The Plant Cell* **20**: 482-494.

Kaplan I, Denno RF. 2007. Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. *Ecology Letters* **10**: 977-994.

Karban R. 2011. The ecology and evolution of induced resistance against herbivores. *Functional Ecology* **25**: 339-347.

Koornneef A, Pieterse CMJ. 2008. Cross talk in defense signaling. *Plant Physiology*

146: 839-844.

Lenth RV. 2016. Least-squares means: the R package lsmeans. *Journal of Statistical Software* **69**: 1-33.

Loreau M, de Mazancourt C. 2013. Biodiversity and ecosystem stability: a synthesis of underlying mechanisms. *Ecology Letters* **16**: 106-115.

Lu J, Robert CAM, Lou Y, Erb M. 2016. A conserved pattern in plant-mediated interactions between herbivores. *Ecology and Evolution* **6**: 1032-1040.

O'Day M. 1998. *Corn insect pests: a diagnostic guide*. Missouri, USA: University of Missouri-Columbia.

Ohgushi T. 2005. Indirect interaction webs: herbivore-induced effects through trait change in plants. *Annual Review of Ecology, Evolution, and Systematics* **36**: 81-105.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2016. *vegan: Community Ecology Package. R package version 2.4-0.* URL https://CRAN.R-project.org/package=vegan.

Poelman EH, Broekgaarden C, Van Loon JJA, Dicke M. 2008. Early season herbivore differentially affects plant defence responses to subsequently colonizing herbivores and their abundance in the field. *Molecular Ecology* **17**: 3352-3365.

Poelman EH, Dicke M 2014. Plant-mediated interactions among insects within a community ecological perspective. In: Voelckel C, Jander G eds. *Annual plant reviews insect plant interactions*. New York, USA: Wiley, 309-337.

Robert CAM, Erb M, Duployer M, Zwahlen C, Doyen GR, Turlings TCJ. 2012a. Herbivore-induced plant volatiles mediate host selection by a root herbivore. *New Phytologist* **194**: 1061-1069.

Robert CAM, Erb M, Hibbard BE, Wade French B, Zwahlen C, Turlings TCJ. 2012b. A specialist root herbivore reduces plant resistance and uses an induced plant volatile to aggregate in a density-dependent manner. *Functional Ecology* **26**: 1429-1440.

Robert CAM, Veyrat N, Glauser G, Marti G, Doyen GR, Villard N, Gaillard MDP, Kollner TG, Giron D, Body M *et al.* 2012c. A specialist root herbivore exploits defensive metabolites to locate nutritious tissues. *Ecology Letters* **15**: 55-64.

Schäfer M, Fischer C, Meldau S, Seebald E, Oelmüller R, Baldwin IT. 2011. Lipase activity in insect oral secretions mediates defense responses in Arabidopsis. *Plant Physiology* **156**: 1520-1534.

Soler R, Badenes-Pérez FR, Broekgaarden C, Zheng S-J, David A, Boland W, Dicke M. 2012. Plant-mediated facilitation between a leaf-feeding and a phloem-feeding insect in a brassicaceous plant: from insect performance to gene transcription. *Functional Ecology* **26**: 156-166.

Stam JM, Kroes A, Li Y, Gols R, van Loon JJA, Poelman EH, Dicke M. 2014. Plant interactions with multiple insect herbivores: from community to genes. *Annual Review of Plant Biology* **65**: 689-713.

Thaler JS, Fidantsef AL, Bostock RM. 2002. Antagonism between jasmonate- and salicylate-mediated induced plant resistance: effects of concentration and timing of elicitation on defense-related proteins, herbivore, and pathogen performance in tomato. *Journal of Chemical Ecology* **28**: 1131-1159.

Utsumi S, Ando Y, Miki T. 2010. Linkages among trait-mediated indirect effects: a new framework for the indirect interaction web. *Population Ecology* **52**: 485-497.

Viswanathan DV, Lifchits OA, Thaler JS. 2007. Consequences of sequential attack for resistance to herbivores when plants have specific induced responses. *Oikos* **116**: 1389-1399.

Viswanathan DV, Narwani AJT, Thaler JS. 2005. Specificity in induced plant responses shapes patterns of herbivore occurrence on *Solanum dulcamara*. *Ecology* **86**: 886-896.

von Dahl CC, Baldwin IT. 2007. Deciphering the role of ethylene in plant-herbivore interactions. *Journal of Plant Growth Regulation* **26**: 201-209.

Wang M, Biere A, van der Putten WH, Bezemer TM. 2014. Sequential effects of

606 root and foliar herbivory on aboveground and belowground induced plant
607 defense responses and insect performance. *Oecologia* **175**: 187-198.
608 **Winz RA, Baldwin IT. 2001.** Molecular interactions between the specialist herbivore
609 *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana*
610 *attenuata*. IV. Insect-induced ethylene reduces jasmonate-induced nicotine
611 accumulation by regulating putrescine N-Methyltransferase transcripts. *Plant*
612 *Physiology* **125**: 2189-2202.
613 **Wurst S, van der Putten WH. 2007.** Root herbivore identity matters in
614 plant-mediated interactions between root and shoot herbivores. *Basic and*
615 *Applied Ecology* **8**: 491-499.
616 **Xiao Y, Wang Q, Erb M, Turlings TCJ, Ge L, Hu L, Li J, Han X, Zhang T, Lu J**
617 ***et al.* 2012.** Specific herbivore-induced volatiles defend plants and determine
618 insect community composition in the field. *Ecology Letters* **15**: 1130-1139.

Figure legends

Fig. 1 Overview of the experimental design and setups used in this study. (a) Experimental treatments (infestation histories). To establish different sequences of arrival, second instar *S. frugiperda* larve were added to the leaves, and second instar *D. v. virgifera* larvae were added to the roots of maize plants in different combinations. After 4 days of herbivore infestation, plants with different infestation histories were offered to *D. v. virgifera* larvae in choice and no-choice experiments and chemical analysis. AG: aboveground *S. frugiperda* larvae infestation, BG: belowground *D. v. virgifera* larvae infestation, BG>AG: belowground infestation followed by aboveground infesatation, C: control without herbivory. (b) Larval preference was measured by laying out the root systems of two plant on moist filter paper in large petri dishes. (c) Volatile-mediated larval preference was measured using a two arm belowground olfactometer. (d) Larval escape patterns were measured using a single L-shaped glass pot and a water-filled petri dish to collect the escaping larvae. (e) Volatile mixing experiments were conducted using a two arm belowground olfactometer with two volatile sources attached to each arm of the central chamber. For more details on the different treatments and setups, refer to the materials and methods section.

Fig. 2 Sequence of arrival determines root attractiveness to *D. v. virgifera*. The number of *D. v. virgifera* larvae on the roots of plants with different infestation histories was measured in Petri dish experiment. (a) *D. v. virgifera* choice between C and AG plants (n = 24). (b) *D. v. virgifera* choice between C and BG plants (n = 36). (c) *D. v. virgifera* choice between C and BG>AG plants (n = 36). AG: aboveground *S. frugiperda* larvae infestation, BG: belowground *D. v. virgifera* larvae infestation, BG>AG: belowground infestation followed by aboveground infesatation, C: control without herbivory. Values correspond to means ± 1 s.e. Asterisks indicate a significant difference in preference within each combination and time point (n.s. , non significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, GLMM). Differences in preference patterns between treatment combinations are depicted by dashed lines and asterisks on the

right of the graph (n.s., non significant; *** $P < 0.001$, GLM).

Fig. 3 Volatile cues contribute to sequence-specific preference patterns of *D. v. virgifera*. The number of *D. v. virgifera* larvae attracted to root volatiles of plants with different infestation histories was measured in two-arm olfactometers experiment. AG: aboveground *S. frugiperda* larvae infestation, BG: belowground *D. v. virgifera* larvae infestation, BG>AG: belowground infestation followed by aboveground infesatation, C: control without herbivory. Values are means ± 1 s.e. (n = 18). Asterisks indicate a significant preference within each treatment combination (** $P < 0.01$, *** $P < 0.001$; GLMM). Different letters indicate significant differences between treatment combinations ($P < 0.05$, GLM).

Fig. 4 Stay-or-leave patterns of *D. v. virgifera* are determined by the sequence of arrival. The number of *D. v. virgifera* larvae leaving from the rhizosphere of plants with different infestation histories was measured in escaping experiment. AG: aboveground *S. frugiperda* larvae infestation, BG: belowground *D. v. virgifera* larvae infestation, BG>AG: belowground infestation followed by aboveground infesatation, C: control without herbivory. Values are means ± 1 s.e. (n = 12). Different letters indicate significant differences between treatments ($P < 0.05$, GLM).

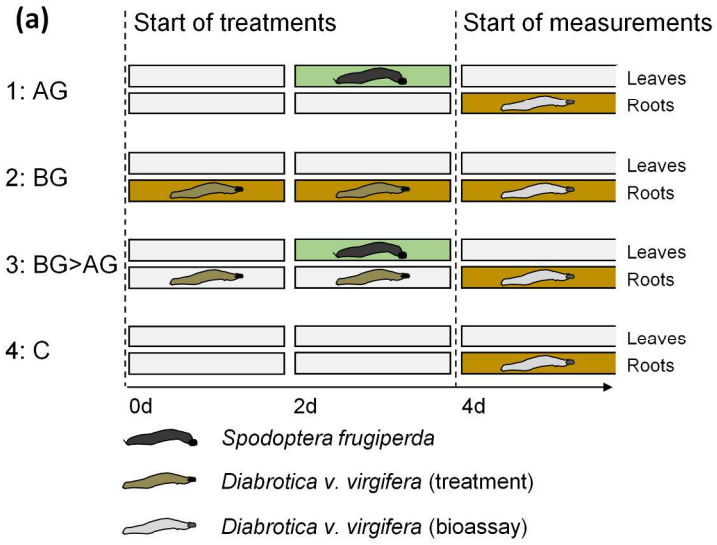
Fig. 5 Accptances of *D. v. virgifera* are determined by the additive changes in root volatiles. The number of *D. v. virgifera* larvae attracted by mixed root volatiles from plants with different infestation histories were measured in volatile-mixing experiment, with each arm containing two different volatile sources. AG: aboveground *S. frugiperda* larvae infestation, BG: belowground *D. v. virgifera* larvae infestation, C: control without herbivory. Values are means ± 1 s.e. (n = 18). Asterisks indicate a significant preference within choice combinations (**, $P < 0.01$; *** $P < 0.001$; GLMM). Different letters indicate differences in preference patterns between treatments ($P < 0.05$, GLM).

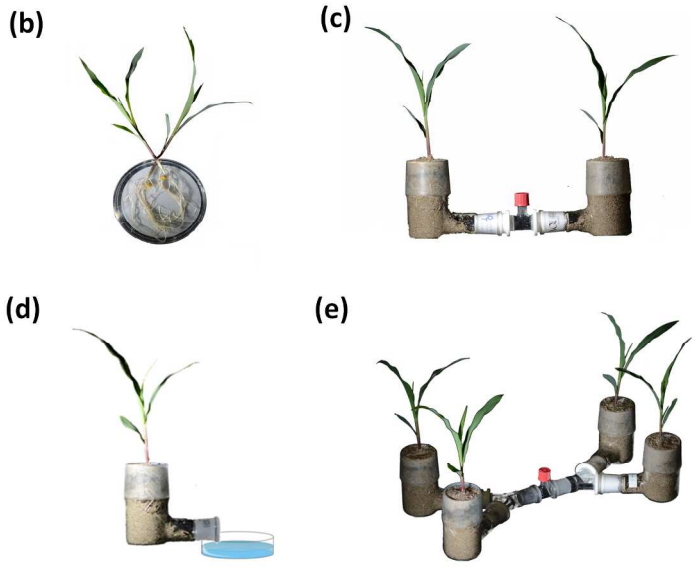
Fig. 6 Infestation by *D. v. virgifera* canalizes the volatile response of maize roots. The

675 results of a redundancy analysis (RDA) of the root volatile respones to different
676 sequences of *D. v. virgifera* and *S. frugiperda* feeding are shown. The first two axes
677 explained 53.86% and 24.36% of the total varation. AG: aboveground *S. frugiperda*
678 larvae infestation, BG: belowground *D. v. virgifera* larvae infestation, BG>AG:
679 belowground infestation followed by aboveground infesatation, C: control without
680 herbivory. Data points represent individual replicates (n = 6).

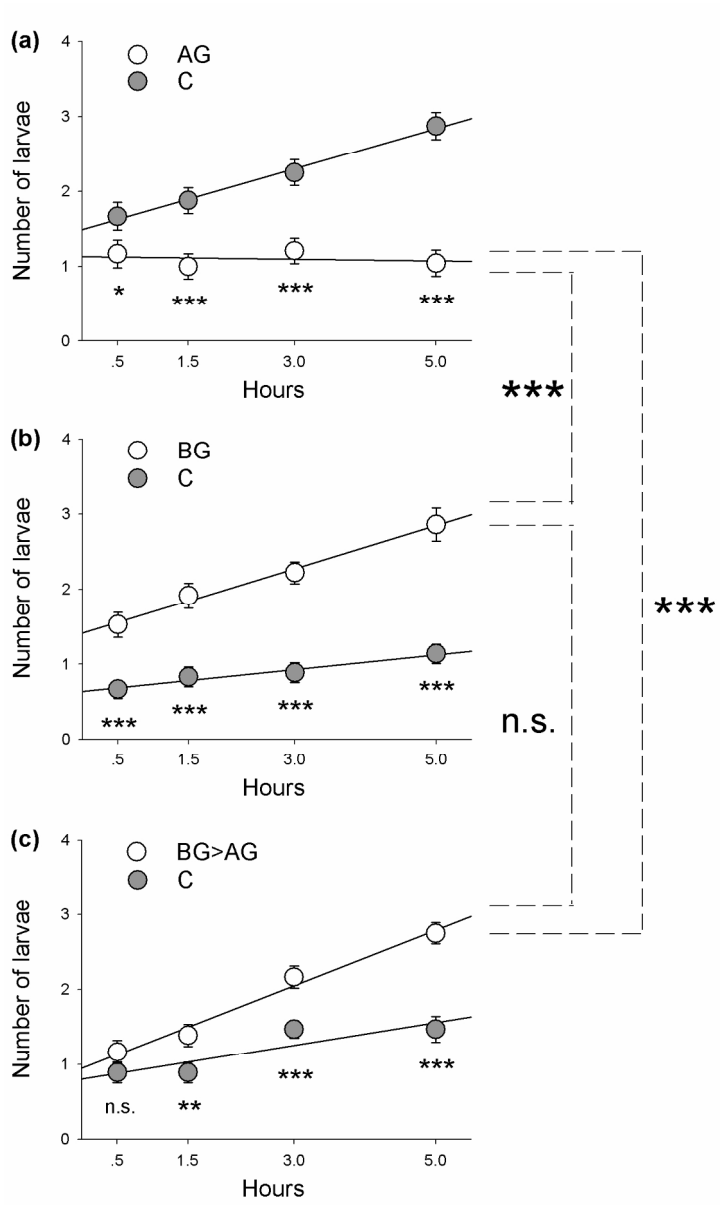
681 **Fig. 7** *D. v. virgifera* suppresses *S. frugiperda*-induced root volatiles. The relative
682 abundance of root volatile in four treatments were measured using solid phase micro
683 extraction (SPME) in combination with gas chromatograpy and mass spectrometry
684 (GC-MS). (a) *E*-β-Caryophyllene (17.33min, 189.1726 m/z), (b) Humulene (18.17min,
685 204.1966 m/z), (c) Unknown (19.30min, 503.6733 m/z), (d) Unidentified Carboxylic
686 acid (10.07min, 123.0129 m/z), (e) Unknown (19.07min, 173.0813 m/z), (f)
687 Caryophyllene oxide (21.27min, 161.1235 m/z), (g) Unknown (19.15min, 106.0578
688 m/z), (h) Ethanol acetate (15.99min, 204.1814 m/z), (i) Unknown (17.07min,
689 161.0902 m/z), (j) Unknown (25.05min, 180.0533 m/z), (k) Unknown (12.71min,
690 138.0904 m/z) and (l) Unidentifed nitrophenol (17.67min, 139.0342 m/z). AG:
691 aboveground *S. frugiperda* larvae infestation, BG: belowground *D. v. virgifera* larvae
692 infestation, BG>AG: belowground infestation followed by aboveground infesatation,
693 C: control without herbivory. Values are means ± 1 s.e. (n = 6). Different letters
694 indicate differences in relative abundance among treatments ($P < 0.05$, LM).

695 **Fig. S1.** Infestation by *D. v. virgifera* does not change aboveground damage by *S.*
696 *frugiperda* larvae. Relative and absolute leaf damage caused by *S. frugiperda* on
697 plants with and without previous infestation by *D. v. virgifera* is shown. AG:
698 aboveground *S. frugiperda* larvae infestation, BG>AG: belowground infestation
699 followed by aboveground infesatation. Values are means ± 1 s.e. (n = 18).

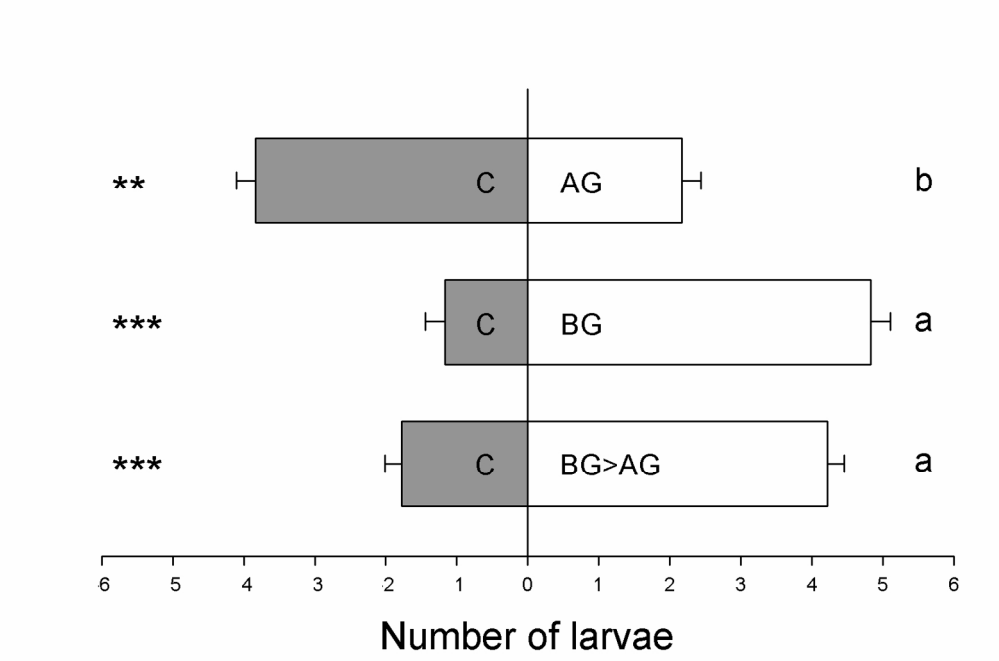




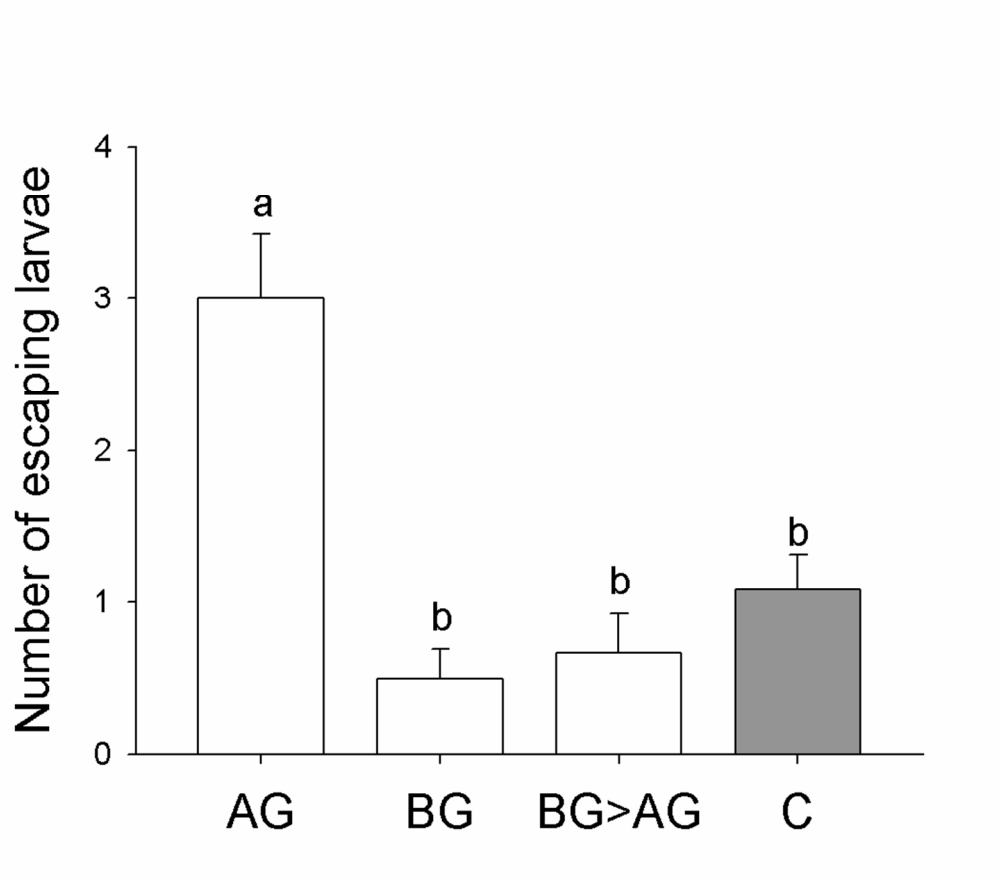
Overview of the experimental design and setups used in this study
Fig. 1
190x274mm (284 x 284 DPI)



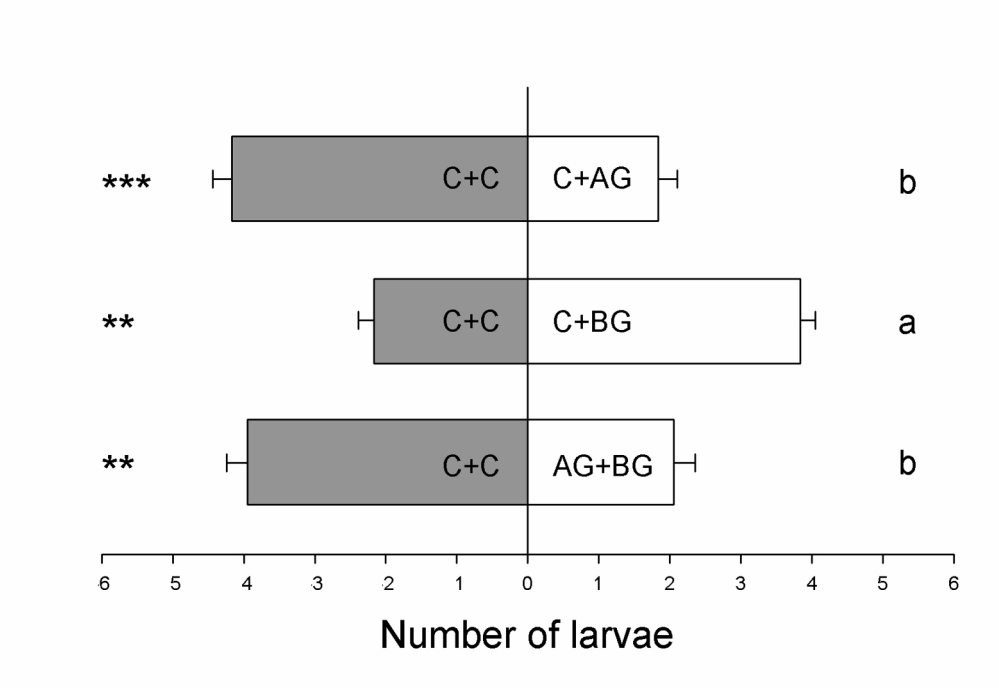
Sequence of arrival determines root attractiveness to D. v. virgifera
Fig. 2
128x216mm (300 x 300 DPI)



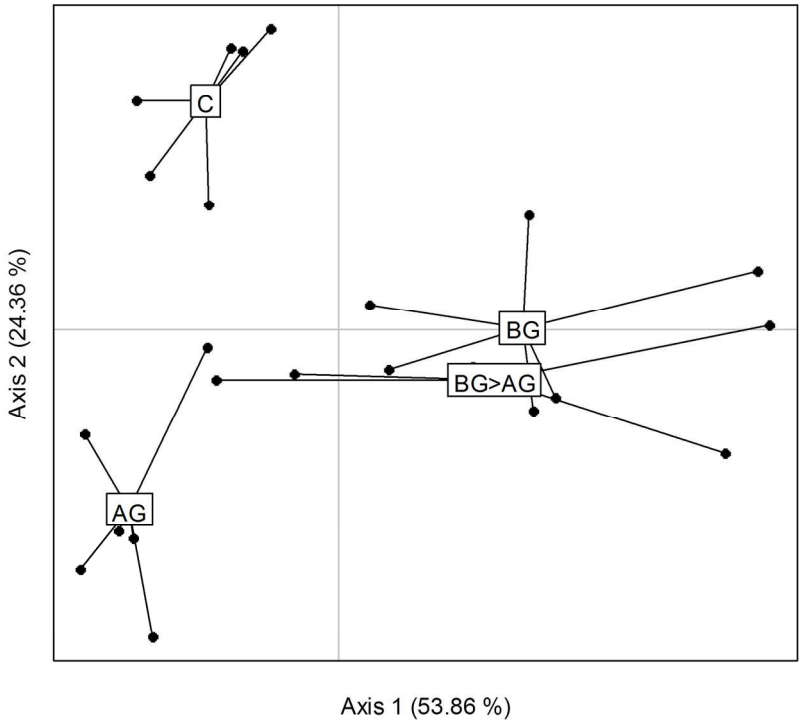
Volatile cues contribute to sequence-specific preference patterns of D. v. virgifera
Fig. 3
146x97mm (300 x 300 DPI)



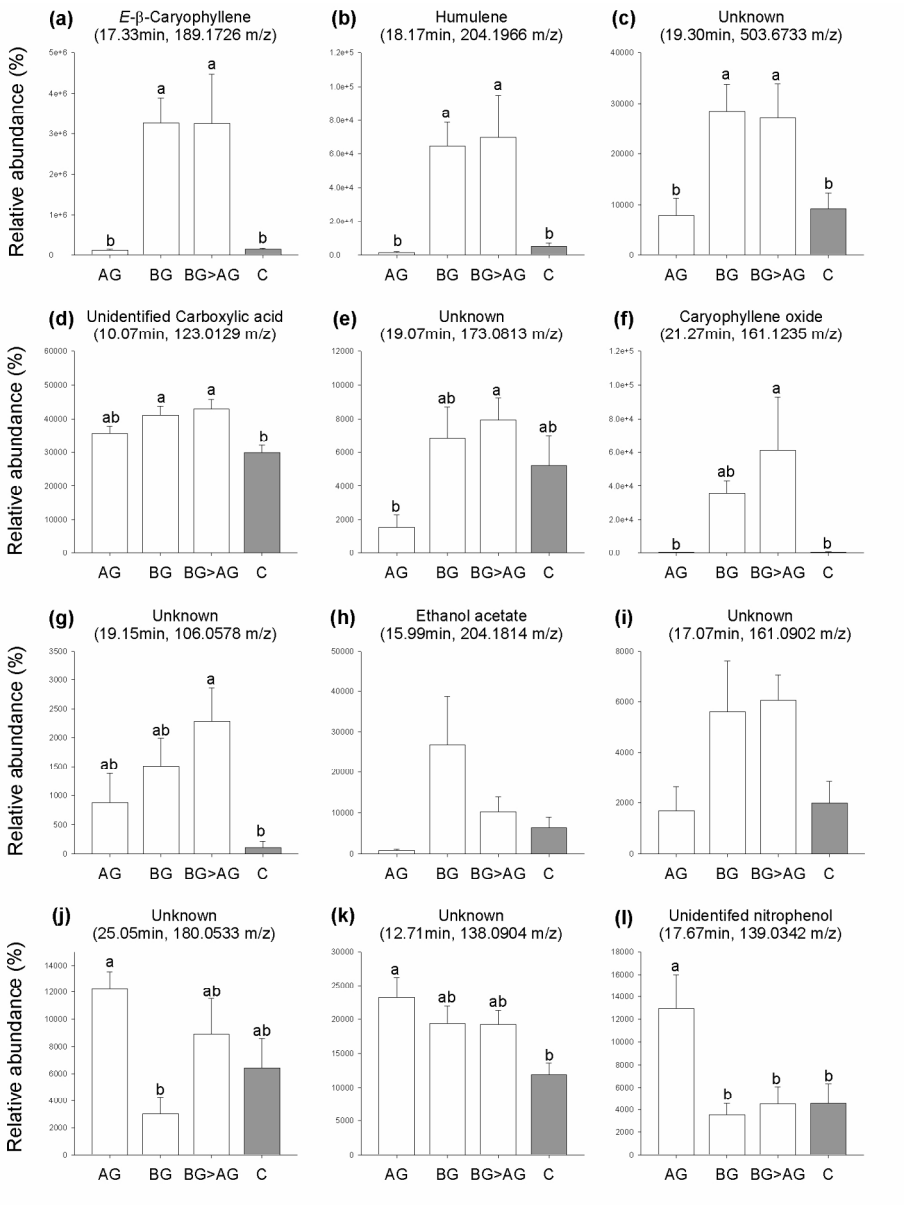
Stay-or-leave patterns of D. v. virgifera are determined by the sequence of arrival
Fig. 4
88x78mm (300 x 300 DPI)



Accptances of D. v. virgifera are determined by the additive changes in root volatiles
Fig. 5
146x101mm (300 x 300 DPI)



Infestation by D. v. virgifera canalizes the volatile response of maize roots
Fig. 6
190x142mm (300 x 300 DPI)



D. v. virgifera suppresses S. frugiperda-induced root volatiles
Fig. 7
160x213mm (300 x 300 DPI)