Gall insects can avoid and alter indirect plant defenses

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Summary

• Parasitic species can dramatically alter host traits. Some of these parasite-induced changes can be considered adaptive manipulations that benefit the parasites. Gall-inducing insects are parasites well known for their ability to alter host-plant morphology and physiology, including the distribution of plant defensive compounds. Here it was investigated whether gall-inducing species alter indirect plant defenses, involving the release of volatile compounds that are attractive to foraging natural enemies.

• Using field and factorial laboratory experiments, volatile production by goldenrod (*Solidago altissima*) plants was examined in response to attack by two gall-inducing species, the tephritid fly *Eurosta solidaginis* and the gelechiid moth *Gnorimoschema gallaesolidaginis*, as well as the meadow spittlebug, *Philaenus spumarius*, and the generalist caterpillar *Heliothis virescens*.

• *Heliothis virescens* elicited strong indirect defensive responses from *S. altissima*, but the gall-inducing species and spittlebugs did not. More significantly, infestation by *E. solidaginis* appeared to suppress volatile responses to subsequent attack by the generalist caterpillar.

• The extensive control that *E. solidaginis* apparently exerts over host-plant defense responses may reduce the predation risk for the gall inducer and the subsequent herbivore, and could influence community-level dynamics, including the distribution of herbivorous insect species associated with *S. altissima* parasitized by *E. solidaginis*.

Key words: *Eurosta*, gall, *Gnorimoschema*, herbivory, induced responses, *Solidago altissima*, volatile response.

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Introduction

Parasites often induce dramatic changes in their hosts' physiology, morphology, and even behavior (Roy, 1993; Eigenbrode *et al.*, 2002; Lacroix *et al.*, 2005). Such changes can be viewed as extended phenotypic effects of the parasite's genes and, in some cases, as adaptively evolved manipulations (Dawkins, 1982). Among parasites that attack plants, gall-inducing insects have particularly profound effects on their hosts. These insects live within plant tissues and induce tumor-like growths that provide the insects with food, shelter, and protection from natural enemies (Mani, 1992; Raman *et al.*, 2005). Like other herbivores, gall insects consume plant resources that could otherwise be used for the plant's own growth and reproduction (McCrea *et al.*, 1985; Abrahamson & Weis, 1987; Bronner, 1992), but their more far-reaching impacts on plant phenotypes are unparalleled: gall-inducing insects can change patterns of plant biomass accumulation, alter photosynthetic rates, and induce tissues that secrete a sugary 'honeydew', which attracts ants that protect the galls from natural enemies (Hartnett & Abrahamson, 1979; Washburn, 1984; McCrea & Abrahamson, 1986; Fay *et al.*, 1996; Larson, 1998). Gall-inducing insects also commonly suppress plant defenses or manipulate them for their own benefit (Abrahamson & Weis, 1987; Hartley, 1998; Nyman & Julkunen-Tiitto, 2000; Tooker *et al.*, 2002; Tooker & Hanks, 2004; Allison & Schultz, 2005). For example, the

nutritive tissues on which gall insects feed usually contain few secondary metabolites whereas the exterior of the galls often have high concentration of these toxins (Abrahamson *et al.*, 1991; Hartley, 1998; Nyman & Julkunen-Tiitto, 2000; Allison & Schultz, 2005). Galls can also influence indirect plant defenses (Izzo *et al.*, 2006; Tooker & De Moraes, 2007), which involve the emission by plants, in response to herbivore feeding, of volatile compounds that are attractive to the herbivores' natural enemies (Turlings *et al.*, 1990; Karban & Baldwin, 1997; De Moraes *et al.*, 1998). Such indirect defenses have been shown to improve plant fitness (van Loon *et al.*, 2000; Fritzsch-Hoballah & Turlings, 2001; Tooker & Hanks, 2006).

Volatile responses are commonly induced by the feeding of chewing or sucking insects or piercing–sucking mites (Turlings *et al.*, 1990; Du *et al.*, 1996; Dicke, 1999), but the indirect defensive response of plants to galling insects has not been extensively explored. At least one gall-inducing species in a native system induces volatile cues that were attractive to natural enemies (Tooker & Hanks, 2006), but others do not appear to significantly alter volatile cues (Izzo *et al.*, 2006; Tooker & De Moraes, 2007). We recently used chemical analyses to demonstrate that larvae of Hessian fly (*Mayetiola destructor*), a specialist gall midge, do not elicit indirect defensive responses from wheat plants even though feeding by generalist caterpillars elicited strong defensive responses (Tooker & De Moraes, 2007). Moreover, the presence of Hessian fly larvae led to attenuated volatile emissions from plants subsequently attacked by caterpillars, suggesting that larvae of the midge may suppress the volatile responses of galled plants (Tooker & De Moraes, 2007).

In the study described here, we explored further whether gall-inducing species tend to induce, avoid, or modify indirect defenses of their host plants. A comparative approach was used to gain insight on how distantly related herbivores species influence host-plant defenses. This was achieved by studying two gall-insect species, the tephritid fly *Eurosta solidaginis* and the gelechiid moth *Gnorimoschema gallaesolidaginis*, and the generalist xylem feeder *Philaenus spumarius*, all of which attack the same native host-plant species, *Solidago altissima*. (For biological details on the two gall-inducing species, see Leiby, 1922; Uhler, 1951; Abrahamson & Weis, 1997; Miller, 2000). We collected volatiles from galled and ungalled plants in the field to determine if gall-insect herbivory induced indirect plant defenses. We next measured levels of jasmonic acid (JA) and salicylic acid (SA) in field-collected galls and ungalled control stems. Jasmonate is often upregulated in plants in response to herbivore damage and triggers release of volatile emissions whereas SA production can also be induced by herbivory and can inhibit the influence of JA (Walling, 2000). We then conducted factorial experiments with *S. altissima* in a controlled-laboratory setting, testing the response of plants to each of the gall-inducing species in the presence and absence of a species of generalist caterpillar to determine whether plants respond differently to the two types of herbivory (i.e. galling and leaf-feeding) and their combination. Lastly, we conducted another factorial experiment replacing the gall inducer with the meadow spittlebug *P. spumarius*, which served as a quasi-control for the influence on host-plant defenses of resource depletion induced by the gall-inducing species.

Materials and Methods

Plants and insects

In April 2004, we planted *S. altissima* L. in to a mowed field at the Russell E. Larsen Agricultural Research Station (Centre Co., PA, USA; 40°42′26″ N, 77°57′13″ W), using rhizomes from a privately owned field (*c.* 2 ha) approx. 30 km from the research station (Centre Co.; 40°53′28″ N, 77°42′22″ W). This field was dominated by *S. altissima* and supported a large population of *E. solidaginis* L. and *G. gallaesolidaginis* (Riley). We selected rhizomes of plants that had developed an *E. solidaginis* gall the previous growing season to ensure that the plants were susceptible to galling, and transplanted rhizomes to our garden plot, with 2 m spacing in a 10×5 array with plants assigned randomly to position. Rhizomes were watered once just after planting, but were otherwise unattended with grass and weeds allowed to grow freely between rows.

We introduced *E. solidaginis* galls collected from the same field *c.* 1 wk after rhizome planting, scattering about 100 galls in our plot. In central Pennsylvania, adult flies typically emerge from galls in mid- to late May (Abrahamson & Weis, 1997). We could not similarly infest our plot with *G. gallaesolidaginis* because it overwinters in the egg stage with neonates locating host plants in spring (Miller, 2000). Other *Solidago* herbivores colonized the plot naturally (closest *Solidago* patch approx. 75 m away).

To grow *S. altissima* and *E. solidaginis* in a more controlled environment, we established plants in an insect-free, climate-controlled growth chamber (16 h light: 8 h dark, 22°C : 20°C; 65% relative humidity (RH)). In early April 2005, we collected *S. altissima* rhizomes from the Upper and Lower Lakes Wildlife Management Area (ULLWMA; St Lawrence Co., NY, USA; 44°35′00″ N, 75°14′58″ W), a site where host races of *E. solidaginis* develop on *S. altissima* and *S. gigantea* in sympatry (Waring *et al.*, 1990; Craig *et al.*, 1993). We collected rhizomes from plants that had developed *E. solidaginis* galls the previous growing season and stored them at 4°C until use (*c.* 8 wk). We then washed rhizomes to remove soil, cut them into 5-cm lengths, and planted them in shallow trays with peat-based, general-purpose potting soil (Pro-Mix BX; Premier Horticulture Inc., Quakertown, PA, USA). When the ramets were 10-cm tall (*c.* 2 wk after planting), we transplanted them into pots (16 cm diameter, 16.5 cm tall, soil as already described). One week after transplanting, we released approx. 100 adult *E. solidaginis* (approximately equal number of males and females) into the growth chamber to establish galls. These adult flies were obtained from galls collected at

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ULLWMA in early April 2005 and maintained at −20°C before rearing adult flies in an incubator (14 h light : 10 h dark, 21°C : 19°C; 60% RH).

To grow *S. altissima* in a growth chamber for experiments with *G. gallaesolidaginis* and spittlebugs, we collected rhizomes in early spring 2006 from the same old field that served as the source of plant material for our garden plot. We collected plants that had developed *G. gallaesolidaginis* galls the previous year and cleaned, planted, and transplanted rhizomes (as already described). To establish *G. gallaesolidaginis* galls in the growth chamber, we collected mature galls containing pupae (indicated by the presence of an exit bung; Miller, 2000) from local old fields (Centre Co.) in mid-August 2005. We placed galls in rearing cages (30 × 30 × 30 cm), which remained outdoors but were sheltered from direct sunlight and precipitation. In late August and September adult moths emerged, mated and laid eggs. Cages with eggs remained outdoors until February when they were placed inside a cold room (4°C) for storage (approx. 8 wk). One week after planting rhizomes, we gathered eggs from the cold room into plastic Petri dishes, which we put in an incubator (16 h light : 8 h dark, 25°C, 65% RH) for hatching. Eggs began hatching in 7 d and we released neonates on young *S. altissima* ramets in flats (< 10 cm tall; as already described). Caterpillar galls began to be evident on stems 7–10 d post release. To obtain meadow spittlebugs, *P. spumarius* (L.), for growth chamber-based experiments, we collected early instar nymphs from *S. altissima* (Centre Co., PA) in early June 2006 and stored them at 4°C until use (< 2 h).

To induce volatile responses from *S. altissima*, some of the plants used in our growth-chamber based experiments were exposed to feeding by the generalist caterpillar *Heliothis virescens* (Fabricius). This species does not regularly feed on *Solidago*, but will do so if starved and so can be used as a tool to elicit plant defensive responses (Tooker & De Moraes, 2007). Moreover, the scenario established by allowing *H. virescens* to feed upon *S. altissima* would not be too different from a host plant reacting to a generalist caterpillar exploring its food options when preferred host plants are not present. *Heliothis virescens* larvae were reared from eggs in an incubator (16 h light : 8 h dark, 22°C : 20°C; 65% RH) on an artificial casein-based diet. Third-instar caterpillars were starved for 24 h before being placed on *S. altissima* plants and two caterpillars were used per pot.

Field-based volatile collections and analysis

Volatiles were collected from plants in our garden plot during summer 2004. Five genets of *S. altissima* were selected that developed an *E. solidaginis* gall on only one ramet and had at least one other ramet of approximately equal size that was free of any galls. The galled portion of the ramet (gall diameter approx. 1–2 cm) was enclosed in a nylon oven-cooking bag (Reynolds, Richmond, VA, USA) supported by a cylindrical wire cage (approx. 10 cm tall, 12 cm diameter), enclosing approx. 15 cm of stem and *c.* 15–30 leaves. (These plastic bags are effective for collecting volatiles and compare favorably to glass chambers (Stewart-Jones & Poppy, 2006).) We used a comparable portion of an ungalled ramet from the same genet as a control, collecting from both ramets simultaneously. Ungalled ramets from the same genets were appropriate controls because they have the same genotype and plants with more than one ramet do not produce a systemic volatile response when attacked by chewing insects (J. F. Tooker & C. M. De Moraes, unpublished). Air was drawn through the bags (0.5 l min^{-1}) with vacuum pumps (Model 2522B-01; Welch Vacuum Technology, Inc., Skokie, IL, USA), which were run on a deep-cycle marine battery through a power inverter; airflow meters adjusted the flow. Air entering the bags was scrubbed with activated charcoal. Air was drawn from the bagged ramet for 15-min periods through a 25-mg column of the polymeric adsorbent SuperQ (Alltech Associates, Deerfield, IL, USA). It was decided to use 15-min collection periods because this enabled collection of substantial amounts of compounds (see later; 0.2–39 μg per collection across our field collections) and longer collections can result in accumulation of contaminants from the nylon bags (J. F. Tooker, pers. obs.). Volatiles were collected from these five genets (i.e. five galled and five ungalled ramets) on four dates (14 and 21 July, 20 August, and 24 September 2004), when galls were full-sized (Abrahamson & Weis, 1997). Collections were made between 10 : 00–15 : 00 h and weather on all days was partly sunny to sunny with air temperatures between 25°C and 30°C.

Volatiles were also collected from *S. altissima* plants galled by *G. gallaesolidaginis* caterpillars; however, this species was not present in our common garden in 2004 so volatiles were collected from the same old field that served as our source for rhizomes for the garden and *G. gallaesolidaginis* pupae (as already described). Samples were collected (as already described) from galled ($n = 13$; gall size: 1–2-cm diameter; 3–5-cm long) and adjacent ungalled ($n = 11$) ramets of similar size; ramets were selected which appeared to have low levels of leaf herbivory. Ramets were 45–60 cm tall and volatiles were collected on three different dates (20 May, 26 May and 4 June 2004), sampling each ramet just once. The collections were completed between 12 : 00 and 15 : 00 h when the weather ranged from partly cloudy to cloudy and air temperature ranged from 23°C to 30°C.

Jasmonate and salicylate analyses

To measure levels of JA and SA in galled and ungalled tissues, *E. solidaginis*- and *G. gallaesolidaginis*-infested ramets were collected ($n = 10$ and $n = 20$, respectively) from a local old field (Centre Co.; 40°53′17″ N, 77°46′29″ W), immediately freezing samples in liquid nitrogen. Ramets with little other herbivore damage and no other galls (*S. altissima* is host to at least 15 gall-inducing species; Felt, 1940; Gagné 1989) were selected. At the same time, samples from adjacent undamaged

ramets of approximately the same size were also collected. These samples were taken from the same height as the adjacent gall and were approx. 5 cm long. Collections were made in May for *G. gallaesolidaginis* and August for *E. solidaginis* when galls were approximately the same size as those used for volatile collections in the field and laboratory (see below).

In the laboratory, galls and ungalled stems were dissected on dry ice, collecting samples of the tissue lining the interior and exterior of galls and ungalled stems (approx. 30 mg of tissue per sample). Samples were collected directing into FastPrep tubes (Qbiogene, Carlsbad, CA, USA) containing 1 g of Zirmil beads (1.1 mm; Saint-Gobain ZirPro, Mountainside, NJ, USA) and frozen at −80°C until processing. A previously described method was used to extract and detect JA and SA (Schmelz *et al.*, 2003, 2004). Briefly, carboxylic acids were derivatized to methyl esters, which were isolated using vapor-phase extraction and analysed by GC-MS with isobutane chemical ionization using selected-ion monitoring. The amounts of JA and SA were quantified using 100 ng of each of the internal standards dihydro-JA and [$^2H_6$]SA (CDN Isotopes, Pointe-Claire, Quebec, Canada). Dihydrojamonic acid was derived from methyl dihydrojamonate (Bedoukian Research Inc., Danbury, CT, USA), which was subjected to alkaline hydrolysis. To confirm the identity of methyl jasmonate and methyl salicylate recovered from the samples, extracts were analyzed by gas chromatography (GC)–mass spectrometry (MS) with electron ionization, comparing retention times and spectra with that of pure compounds.

Laboratory-based volatile collections

To collect volatiles from *S. altissima* grown in the growth chamber, three separate experiments were conducted with *E. solidaginis*, *G. gallaesolidaginis*, and spittlebugs using a closed push–pull system (Analytical Research Systems, Inc, Gainesville, FL, USA). Filtered air was pushed (2.5 l min^{-1}) through Teflon tubing and a Teflon cap into a glass cylinder (46.5 cm tall, 8 cm diameter), which enclosed a ramet of *S. altissima* (each rhizome produced a single ramet in the growth chamber). The cylinder rested on a two-piece Teflon base, which lay on the rim of the plant pot and had a hole for the plant stem to pass. Plant stems were wrapped in cotton where they passed through the hole to plug the gap between stem and base. Plant volatiles were collected from 6 : 00–22 : 00 h by pulling air continuously from the cylinder through side ports (0.8 l min^{-1}) across 25-mg beds of SuperQ.

For the *E. solidaginis* experiment, plant volatiles were collected from galled and ungalled plants subjected to one of four randomly assigned treatments: uninfested control ($n = 5$), *H. virescens* feeding ($n = 7$), *E. solidaginis* feeding ($n = 6$) or *H. virescens* and *E. solidaginis* feeding ($n = 6$). At the time of collection, plants were approx. 70 d old, 20–27 cm tall (approx. 13–20 cm of plants were enclosed in the glass chambers) and galls (approx. 1–2 cm diameter) were approx. 40 d old. For the experiment with *G. gallaesolidaginis*, volatiles were collected from plants subjected to one of four randomly assigned treatments ($n = 4$ for each treatment): uninfested control, *H. virescens* feeding, *G. gallaesolidaginis* feeding or *H. virescens* and *G. gallaesolidaginis*. At the time of collection, plants were approx. 55 d old and 20–50-cm tall (galling can severely stunt plant height; Hartnett & Abrahamson, 1979) and galls were approx. 40 d old (approx. 1–2 cm diameter × 2.5–4.5 cm long). Preliminary experiments indicated that *H. virescens* would feed on gall tissue, therefore, for these factorial experiments with *E. solidaginis* and *G. gallaesolidaginis*, the galls were wrapped in Teflon tape, denying caterpillars access to galls. To control for the influence of tape equal lengths of ungalled stems were also wrapped with tape.

Because gall-inducing insects may influence their host plants via active control of plant physiology as well as through the sapping effects of parasitism (Abrahamson & Weis, 1987; Hartley, 1998), a factorial experiment was also conducted with *H. virescens* and the spittlebug *P. spumarius*, a xylem feeder commonly found on *S. altissima*. Similar to a galling insect, *P. spumarius* can drastically and negatively influence growth of *S. altissima* by removing plant sap and its constituents (Meyer & Whitlow, 1992; Meyer, 1993), but their piercing–sucking mode of feeding would not appear to cause extensive plant damage like a chewing herbivore, and thus might not elicit substantial release of plant volatiles. We, therefore, included spittlebugs as a control for the sapping influence of a parasite-induced sink on plant defensive responses. For our experiment, field-collected spittlebugs (as already described) had been placed on plants 15 h before our collections. Volatiles were collected from plants subjected to one of four randomly assigned treatments ($n = 4$ for each treatment): uninfested control, *H. virescens* feeding, spittlebug feeding or *H. virescens* and spittlebug feeding. At the time of collection, plants were approx. 40 d old and 20–30-cm tall.

Volatile analyses

For all volatile collections, SuperQ® traps were eluted with 150 µl of dichloromethane and added to each sample 200 ng *n*-octane and 400 ng nonyl acetate as internal standards. Samples were injected in 1-µl aliquots into an Agilent model 5890 gas chromatograph fitted with a flame ionization detector, using a splitless injector held at 220°C. The column (HP-1, 15 m × 0.25 mm × 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA) was maintained at 35°C for 30 s, then ramped 2°C min^{-1} to 130°C, and ramped again at 20°C min^{-1} to 220°C. Quantifications of compounds were made relative to the nonyl acetate standard using ChemStation software (Agilent Technologies, Wilmington, DE, USA). Enantiomeric compositions of α-pinene, camphene, β-pinene, limonene, and linalool were resolved using a chiral column (Rt-β Dexsm; 30 m × 0.25 mm × 0.25 µm film thickness; Restek Corp., Bellefonte, PA, USA). We used the same gas chromatograph

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as already described, but the injector was held at 230°C and the chiral column was maintained at 35°C for 30 s, then ramped 5°C min^{-1} to 220°C. Identifications of volatile components and their enantiomers were made with GC-MS in electron ionization mode comparing retention times and spectra with that of pure compounds.

Leaves appear to be the primary source of nonfloral volatiles released by plants (Karban & Baldwin, 1997); therefore, after each collection we measured the length and width of each leaf contained within collection chambers (i.e. nylon bags or glass cylinders) was measured to calculate amounts of volatile compounds released by plants per unit leaf area. The area of an individual leaf was best described using the equation for area of an ellipse and the calculated leaf areas for each plant were summed, generating a total leaf area collected per plant (cm^2). The amount of volatiles released per plant was also calculated independent of leaf area in case galls themselves released volatiles.

Statistical analyses

For all univariate analyses, volatiles were log-transformed to normalize data and stabilize variance. In the field experiment on *E. solidaginis*, repeated measures ANOVA was used to test for differences between galled and ungalled plants across sampling dates. Pairwise differences between all sampling dates were assessed with a Tukey's HSD means separation test (Sokal & Rohlf, 1995; Statistix, 2003). For field collections with *G. gallaesolidaginis*, the total volatiles (untransformed) released between galled and ungalled plants were compared using Student's *t*-test (Sokal & Rohlf, 1995; Statistix, 2003). In the field experiments, individual components of the volatile blends were not statistically analysed because these plants were subject to damage by other herbivores and we could not be certain that compounds were influenced only by the presence of *E. solidaginis* or *G. gallaesolidaginis*. For JA and SA data, two-way ANOVA was used for each compound testing the effect of each gall-inducing species (present or absent) and tissue type (interior or exterior) and their interaction on levels of JA and SA.

For each growth chamber experiment, a two-way ANOVA was also used to test for the main effects of gall species (present or absent) and herbivore species (present or absent) and their interaction on total volatile production. Because gall-inducing species can influence stereochemistry of plant compounds (Tooker *et al.*, 2002, Tooker & Hanks, 2004), ANOVA was also used to assess the influence of these same effects on enantiomeric composition (proportions) of the five chiral monoterpenes (i.e. α-pinene, camphene, β-pinene, limonene, and linalool) produced by *S. altissima*. Even though we standardized our analyses 'per unit leaf area', we also used ANOVA to compare the total leaf area across the four different treatments to determine whether volatiles within each experiment were sampled from comparable amounts of leaf tissue. For ANOVA results suggesting trends for the influence of treatments, *post-hoc* power analysis was conducted to determine the strength of the statistical tests (Statistica, 2006).

Multivariate statistics were used to analyse volatile production because *S. altissima* emitted large numbers of compounds (Table 1), many of which are derived from similar pathways and responded similarly to treatments. We began by analysing each of the three factorial experiments separately. The volatile data did not meet assumptions of multivariate normality and linearity even when transformed. Therefore, nonparametric, permutational analysis of variance for balanced and unbalanced designs (PERMANOVA and DISTLM, respectively; Anderson, 2004, 2005) were used to test for the main effects of gall and herbivore species (present or absent) and their interaction. Analyses were conducted on Bray–Curtis distance with 9999 permutations. These methods work well with small sample sizes, which typically can challenge more conventional analyses (Gonzalez & Manly, 1998, Anderson, 2004).

Although the three growth chamber experiments were conducted at different times and on plants of different ages, we still felt that it would be valuable to compare the effect of the two gall inducers and the spittlebug on volatile release by statistically comparing the results across the three experiments. Because of the substantial differences in their phenologies, it would not be realistic to conduct a single experiment where are all three parasite species were tested concurrently. The data from each experiment were transformed to *Z*-scores because there were significant differences among the three experiments in volatiles released per unit of leaf area. The *Z*-score transformation results in a standard deviation of one and a mean of zero for each experiment (Sokal & Rohlf, 1995), which facilitates cross-experiment comparisons and precludes a test for the main effect of 'experiment' (*E. solidaginis*, *G. gallaesolidaginis*, spittlebug experiments). A permutation-based MANOVA was conducted on these *Z*-scores to test for the effects of 'parasite' (i.e. *E. solidaginis*, *G. gallaesolidaginis*, spittlebugs or none), '*H.* virescens' (present or absent), and all two- and three-way interactions between/among 'parasite', '*H. virescens*' and 'experiment.'

To visualize the multivariate data, ordination biplots were constructed for each of the three growth chamber experiments and the three experiments combined (conducted on *Z*-scores). The biplots were generated from distance-based redundancy analyses (Db-RDA) constrained by the factors of the factorial experiments and present information complementary to the permutational analyses of variance, displaying relative importance of treatment factors for individual volatile components that were significantly associated with the defined axes of the biplots. Bray–Curtis dissimilarity was used as the distance measure, using the Lingoes method to correct for negative eigenvalues (Legendre & Anderson, 1999), and the biplots were scaled by the inter-compound (i.e. inter-species) correlations. Db-RDA was conducted using a combination of DistPCoA (Legendre & Anderson, 1998) and CANOCO (version 4.5;

Table 1 Volatile compounds released by *Solidago altissima* plants used in factorial experiments with *Eurosta solidaginis*, *Gnorimoschema gallaesolidaginis*, and spittlebugs subjected to four treatments

	Experiment											
	E. solidaginis (Es)				*G. gallaesolidaginis* (Gg)				Spittlebug (Sp)			
Compound	Control	Hv[a]	Es	Es/Hv	Control	Hv	Gg	Gg/Hv	Control	Hv	Sp	Sp/Hv
Unknown1	–	–	–	–	–	–	–	–	0.7 ± 0.7	42.5 ± 13.7	1.8 ± 1.1	87 ± 38
(*E*)-2-hexanal[bc]	–	1.7 ± 1.4	–	4.0 ± 3.0	0.04 ± 0.04	0.21 ± 0.08	–	–	1.3 ± 1.3	16 ± 5.9	–	10.7 ± 2.9
(*Z*)-3-hexen-1-ol[bc]	2.1 ± 1.2	6.1 ± 3.0	0.8 ± 0.8	21.3 ± 10.6	–	5.9 ± 1.4	–	3.8 ± 1.5	662 ± 474	1472 ± 1087	23.8 ± 13.7	806 ± 579
α-Pinene[bc]	119 ± 54	503 ± 143	101 ± 53	272 ± 96	0.78 ± 0.21	20.1 ± 5.9	1.9 ± 0.7	54 ± 27	30 ± 9	1676 ± 307	90 ± 28	2583 ± 941
Camphene[bc]	13.6 ± 5.5	34 ± 12	25 ± 14	27 ± 9	0.12 ± 0.07	2.4 ± 0.64	0.33 ± 0.14	2.3 ± 0.8	4.7 ± 1.8	117.6 ± 45.8	21.8 ± 8.1	135 ± 49
β-Pinene[bc]	197 ± 94[b]	644 ± 173	115 ± 42	232 ± 92	1.33 ± 0.68	100 ± 60	3.53 ± 2.44	149 ± 96	69 ± 21	2140 ± 791	87.8 ± 27.3	2273 ± 1032
Myrcene[bc]	33 ± 9	320 ± 55	44.5 ± 9.2	151 ± 51	0.19 ± 0.12	25 ± 9	0.65 ± 0.19	45 ± 21	17.8 ± 6.7	2627 ± 1764	28 ± 9	1338 ± 420
(*Z*)-3-HAC[bcd]	34 ± 10	135 ± 46	43 ± 15	116 ± 25	–	0.73 ± 0.34	–	0.43 ± 0.31	66 ± 47	1472 ± 1087	24 ± 18	806 ± 579
β-Phellandrene[bc]	57 ± 34	109 ± 48	19.2 ± 9.4	141 ± 49	0.88 ± 0.66	9.9 ± 5.5	1.24 ± 0.66	3.0 ± 2.0	9.6 ± 5.4	336 ± 325	33 ± 14	227 ± 191
Limonene[bc]	81 ± 32	643 ± 181	106 ± 29	229 ± 94	0.32 ± 0.16	22 ± 6	1.08 ± 0.72	42 ± 21	35 ± 12	987 ± 371	48 ± 13	2381 ± 1523
(*E*)-β-ocimene[bc]	26 ± 9	95 ± 28	25 ± 11	61 ± 35	0.39 ± 0.19	2.5 ± 0.8	1.09 ± 0.71	5.8 ± 1.9	46 ± 9	1131 ± 864	96 ± 56	314 ± 95
Linaloold	8.2 ± 3.8	19.4 ± 5.9	20 ± 9.0	13.1 ± 4.6	0.12 ± 0.07	2.0 ± 0.6	0.53 ± 0.48	1.4 ± 0.43	9.9 ± 3.0	587 ± 533	7.0 ± 5.5	111 ± 65
Nonatriene[bce]	90 ± 23	157 ± 50	49 ± 16	81 ± 22	0.33 ± 0.24	5.5 ± 1.1	0.72 ± 0.64	1.8 ± 0.2	19.3 ± 9.3	2036 ± 1517	37 ± 7	579 ± 267
Methyl salicylate	67 ± 34	153 ± 91	55 ± 40	43 ± 21	0.18 ± 0.12	0.62 ± 0.10	1.2 ± 1.2	0.43 ± 0.25	–	37 ± 21	16.9 ± 10.6	6.1 ± 3.6
β-Caryophyllene[bc]	10 ± 7.3	69 ± 20	8.4 ± 4.1	32 ± 15	0.6 ± 0.6	2.7 ± 0.6	0.62 ± 0.5	5.3 ± 2.4	20.1 ± 15.6	1117 ± 923	15 ± 7.1	371 ± 182
α-Humulene	7.6 ± 5.2	11.9 ± 4.5	–	2.5 ± 1.6	0.08 ± 0.08	0.67 ± 0.1	0.08 ± 0.08	0.80 ± 0.35	3.6 ± 2.3	105 ± 76	6.0 ± 6.0	38.1 ± 17.8
Germacrene D[bc]	56 ± 23	406 ± 120	17.3 ± 6.9	269 ± 116	0.27 ± 0.17	24 ± 9	0.85 ± 0.54	43 ± 15	11.9 ± 3.0	629 ± 223	44.3 ± 19.6	785 ± 117
Unknown sesquit1[f]	–	–	–	–	–	–	–	–	22.8 ± 14.4	2728 ± 2684	34 ± 19	1202 ± 1180
Unknown sesquit2[f]	–	–	–	–	–	–	–	–	16.9 ± 14.2	223 ± 125	43 ± 40	66 ± 33
β-Farnescene	4.2 ± 3.6	3.3 ± 1.8	7.0 ± 5.2	0.8 ± 0.8	1.04 ± 0.60	0.88 ± 0.38	–	15.2 ± 14.3	50.1 ± 6.9	100 ± 63	16.3 ± 9.5	54 ± 28
α-Farnescene	8.2 ± 5.2	134 ± 100	–	33 ± 24	–	1.05 ± 0.05	–	0.93 ± 0.43	4.2 ± 2.6	233 ± 193	14.1 ± 10.3	76 ± 39
Nerolidol	13.6 ± 6.6	17.1 ± 7.2	3.5 ± 2.3	5.8 ± 3.7	0.08 ± 0.08	0.38 ± 0.10	–	0.45 ± 0.22	1.97 ± 1.14	86 ± 60	–	29 ± 10
Tridecatetraene	15.9 ± 7.7	7.4 ± 3.9	12.3 ± 5.7	15.6 ± 8.7	0.25 ± 0.15	0.64 ± 0.35	0.55 ± 0.49	0.63 ± 0.32	4.8 ± 2.8	13.1 ± 7.5	4.5 ± 2.6	11.2 ± 8.8

Results are presented as means (± SE; ng h^{-1} cm^{-2}; data shown untransformed). [a]*Helithois virescens*; compound also present in field experiment with [b]*E. solidaginis* or [c]*G. gallaesolidaginis*; [d](*Z*)-3-hexenyl acetate; [e](3*E*)-4,8-dimethyl-1,3,7-nonatriene; [f]unknown sesquiterpene.

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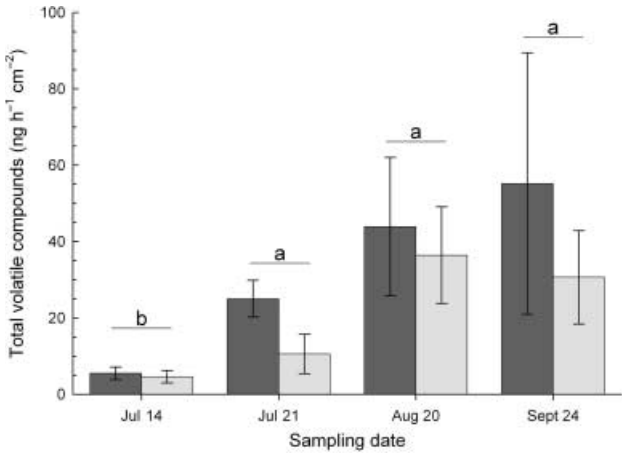

Fig. 1 Mean total (± 1 SE) amounts of volatile compounds produced on four sampling dates during summer 2004 by galled (dark gray bars) and ungalled (light gray bars) ramets of field-grown *Solidago altissima*. Data shown are untransformed. No significant differences were found between galled and ungalled plants, but sampling dates marked with different letters are statistically different (see text for results of repeated measures ANOVA; Tukey's HSD $P < 0.05$).

ter Braak & Šmilauer, 2002); biplots were produced using CANODRAW (version 4.12; ter Braak & Šmilauer, 2002).

Data are presented as means ± 1 SE throughout, except where stated otherwise.

Results

Field-based volatile collections

Field collections from plants grown in our garden plot revealed that ungalled ramets of *S. altissima* and those galled by *E. solidaginis* emitted similar total amounts of volatiles on each of the four sampling dates (Fig. 1; repeated measures ANOVA $F_{1,38} = 0.50$, $P = 0.50$). Volatile production increased over the course of the summer, and plants produced significantly more total volatiles during the last three sampling dates than on the first (Fig. 1; repeated measures ANOVA $F_{3,38} = 11.8$, $P < 0.0001$). Field collections from natural populations of *S. altissima* infested with *G. gallaesolidaginis* also revealed that galled and ungalled ramets produced similar amounts of total volatiles (ungalled: 23.2 ± 6.1; galled 30.7 ± 8.0 ng h^{-1} cm^{-2}; $t = 0.74$, df = 22, $P = 0.47$). Both *E. solidaginis*- and *G. gallaesolidaginis*-galled plants consistently emitted the same 13 compounds at similar levels (Table 1). All the compounds detected in field collections were a subset of the compounds detected with our more intensive sampling of growth chamber-grown plants (Table 1).

Jasmonate and salicylate analyses

Eurosta solidaginis galls and control ramets had similar levels of JA (Fig. 2a; overall ANOVA $F_{3,36} = 0.36$, $P = 0.78$) and the main effects of gall, tissue, and the gall × tissue interaction did not significantly influence JA content (gall vs ungalled: $F_{1,36} = 0.26$, $P = 0.61$; tissue from interior or exterior of stems: $F_{1,36} = 0.76$, $P = 0.39$; gall × tissue interaction: $F_{1,36} = 0.13$, $P = 0.72$). Levels of SA in *E. solidaginis* galls and control stems did, however, show significant differences (Fig. 2b; overall ANOVA $F_{3,36} = 4.47$, $P = 0.01$) with tissue and the gall × tissue interaction having significant influences (tissue: $F_{1,36} = 4.4$, $P = 0.04$; interaction: $F_{1,36} = 8.5$, $P = 0.006$). On its own the effect of gall status was insignificant ($F_{1,36} = 0.01$, $P = 0.90$). The significant influence of tissue and the gall × tissue interaction was driven mostly by a large difference in the SA content of the interior and exterior of ungalled stems (Fig. 2b; Tukey's HSD $P = 0.005$). No similar difference existed for the interior and exterior of galled stems (Fig. 2b; Tukey's HSD $P = 0.94$), suggesting that *E. solidaginis* moderated SA production particularly in exterior tissue. An apparent difference between the SA content of the interiors of galls and ungalled stems was not borne out statistically (Fig. 2b; Tukey's HSD $P = 0.25$), but may be suggestive of a biologically meaningful trend.

Jasmonate and salicylate levels from *G. gallaesolidaginis*-infested and control ramets of *S. altissima* gave results similar to those found for galls of *E. solidaginis* (Fig. 2c,d; *G. gallaesolidaginis* overall ANOVA, JA: $F_{3,71} = 0.11$, $P = 0.95$; SA: $F_{3,71} = 9.3$, $P < 0.0001$). Jasmonic acid levels were similar across *G. gallaesolidaginis* galls and ungalled ramets ($F_{1,71} = 0.16$, $P = 0.69$), interior and exterior tissue ($F_{1,71} = 0.01$, $P = 0.98$) and their interaction ($F_{1,71} = 0.17$, $P = 0.68$). Conversely, salicylate levels were significantly influenced by the presence of a *G. gallaesolidaginis* gall ($F_{1,71} = 7.7$, $P = 0.007$), tissue identity ($F_{1,71} = 15.8$, $P = 0.0002$) and their interaction ($F_{1,71} = 5.2$, $P = 0.03$). However, these significant differences were largely driven by a discrepancy in the SA levels in the interior and exterior of ungalled stems (Fig. 2d; Tukey's HSD $P = 0.0003$). Interior and exterior of *G. gallaesolidaginis*-infested stems had levels of SA that were similar to each other (Fig. 2d; Tukey's HSD $P = 0.61$) again suggesting that the galler moderated SA production in tissue on the exterior of their gall.

Growth chamber-based volatiles collections: total volatiles

The growth chamber-based experiments with *E. solidaginis* revealed significant effects of both *H. virescens* (ANOVA $F_{1,23} = 21.9$, $P = 0.0001$) and *E. solidaginis* (ANOVA $F_{1,23} = 4.5$, $P = 0.047$) on total volatile production of *S. altissima*, but the interaction of the two herbivores was not significant (Fig. 3a; ANOVA $F_{1,23} = 2.3$, $P = 0.14$). It must be noted, however, that the statistical power of this test was low ($F_{1,20} = 4.4$, Power [Π] = 0.17) and we would have needed a total sample size of *c.* 45 to detect a significant influence of the interaction ($P < 0.05$). Caterpillar damage to apical buds and leaves caused a significant increase in total emissions relative to plants without caterpillars whereas galling by *E. solidaginis* also

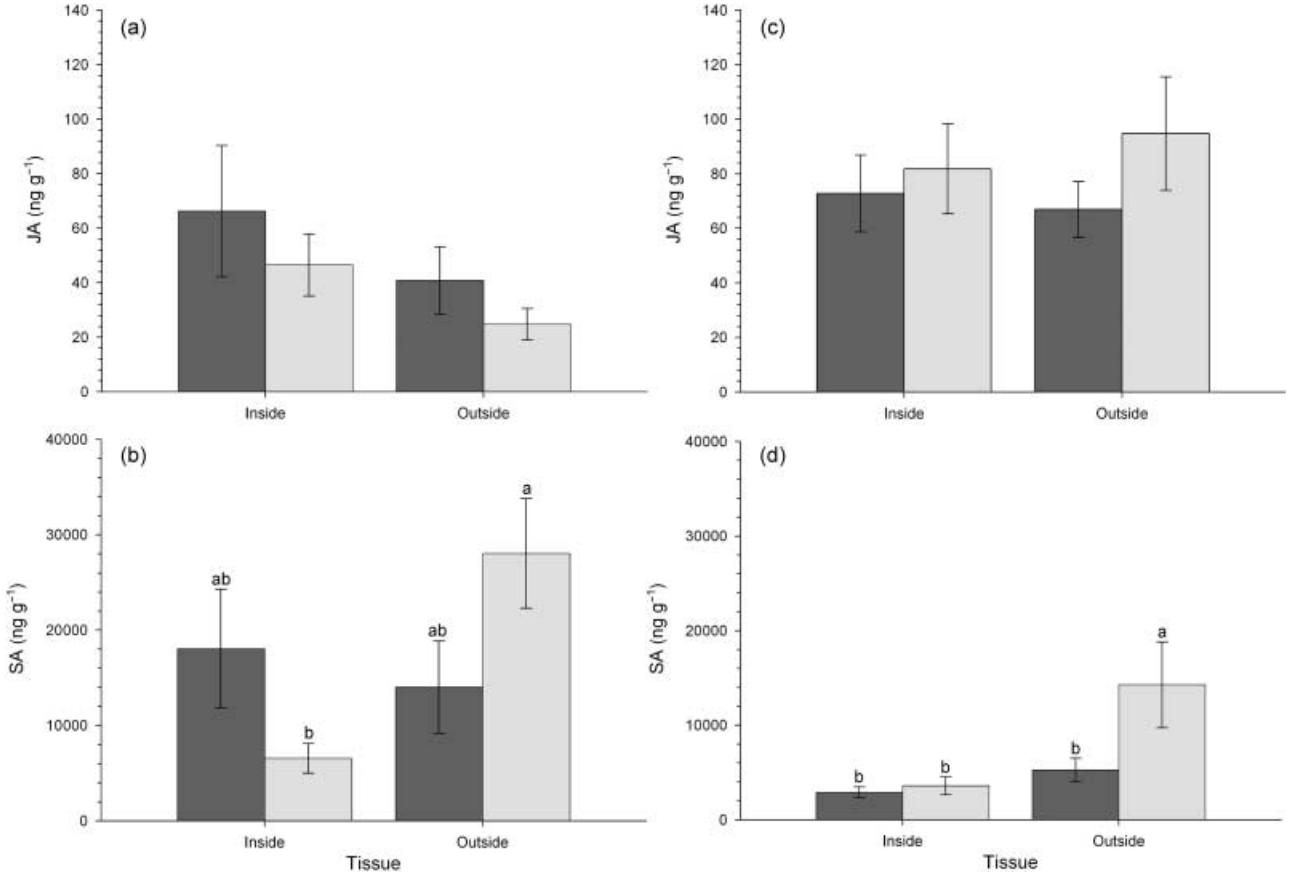

Fig. 2 Levels (mean ± 1 SE) of jasmonic (JA) and salicylic (SA) acids in interior and exterior tissue from ungalled (light gray bars) control stems of *Solidago altissima* and stems galled by *Eurosta solidaginis* (dark gray bars, a and b, respectively) and *Gnorimoschema gallaesolidaginis* (dark gray bars, c and d, respectively). Bars marked with different letters are statistically different (Tukey's HSD $P < 0.05$; data are shown untransformed; see text for details on statistics).

significantly influenced volatile production, but galled plants emitted statistically lower amounts of total volatiles than those without galls (Fig. 3a).

The factorial experiment with *G. gallaesolidaginis* also showed a significant effect of *H. virescens* on total volatile production following damage to apical buds and leaves (ANOVA $F_{1,15} = 12.1$, $P = 0.005$); however, unlike *E. solidaginis*, *G. gallaesolidaginis* did not significantly influence total volatile production (Fig. 3b; ANOVA $F_{1,15} = 0.60$, $P = 0.45$). Feeding by both caterpillar species did not yield a significant interaction (ANOVA $F_{1,15} = 0.44$, $P = 0.52$). Our factorial experiment with spittlebugs again revealed a significant influence of *H. virescens* on total volatile production (Fig. 3c; ANOVA $F_{1,15} = 6.2$, $P = 0.03$), but neither spittlebugs nor the interaction of *H. virescens* and spittlebugs resulted in significant effects (Fig. 3c; spittlebugs: ANOVA $F_{1,15} = 0.20$, $P = 0.67$; interaction: $F_{1,15} = 0.15$, $P = 0.71$). Within each factorial experiment, *H. virescens* appeared to cause equal damage to leaves and apical buds of plants subjected to the two *H. virescens*-infested treatments.

When all three factorial experiments were compared directly using *Z*-scores, *H. virescens* was the only factor that significantly influenced total volatile production in a univariate analysis ($F_{1,9} = 42.8$, $P = 0.001$), although the three-way interaction of the *E. solidaginis* experiment × *H. virescens* × parasite approached significance ($F_{1,9} = 3.71$, $P = 0.061$), suggesting that *E. solidaginis* influenced total volatile production differently than the other two parasites.

Growth chamber-based volatiles collections: abundance of volatile constituents

Multivariate analysis of the abundance of the volatile constituents in our factorial experiment with *E. solidaginis* revealed that feeding by *H. virescens* significantly altered volatile production ($F_{1,23} = 6.02$, $P = 0.001$), whereas the effects of *E. solidaginis* and the interaction of the two herbivores were not significant (*E. solidaginis*: $F_{1,23} = 0.84$, $P = 0.47$; *H. virescens* × *E. solidaginis* interaction: $F_{1,23} = 1.51$, $P = 0.17$). In the biplot that resulted from the RDA of the abundance of volatile constituents, the first canonical axis was most strongly correlated with presence or absence of *H. virescens* while the second axis was most strongly correlated with the interaction of *E. solidaginis*

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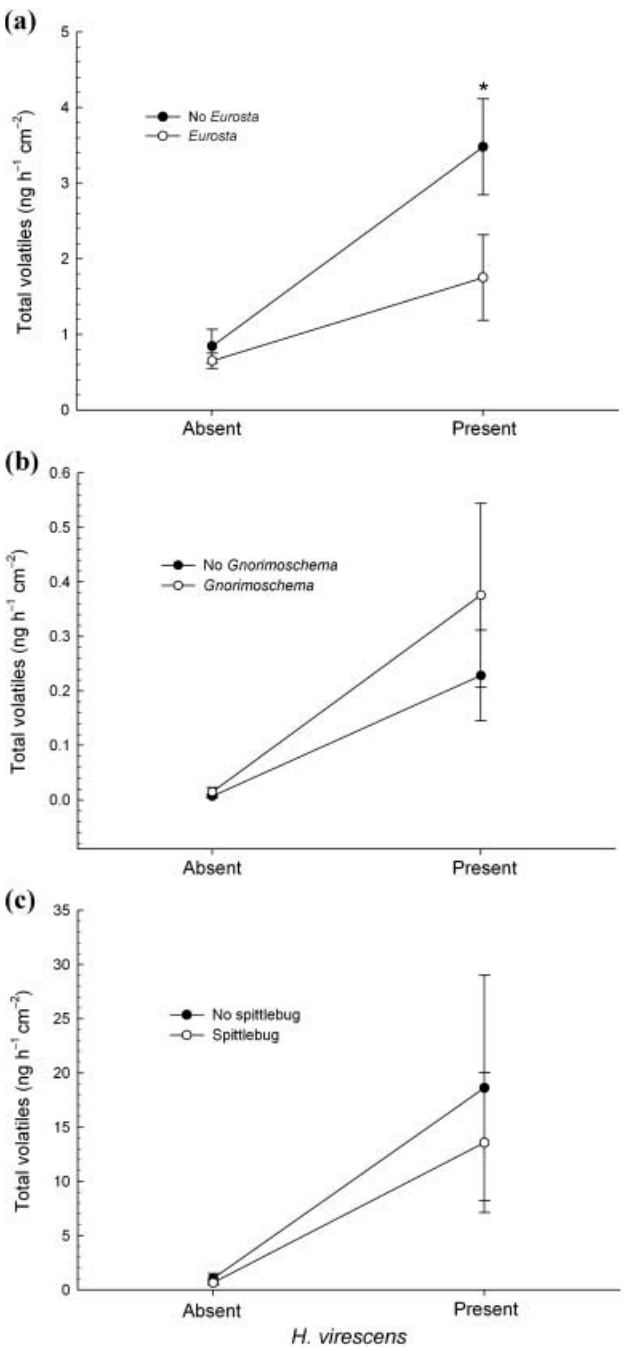


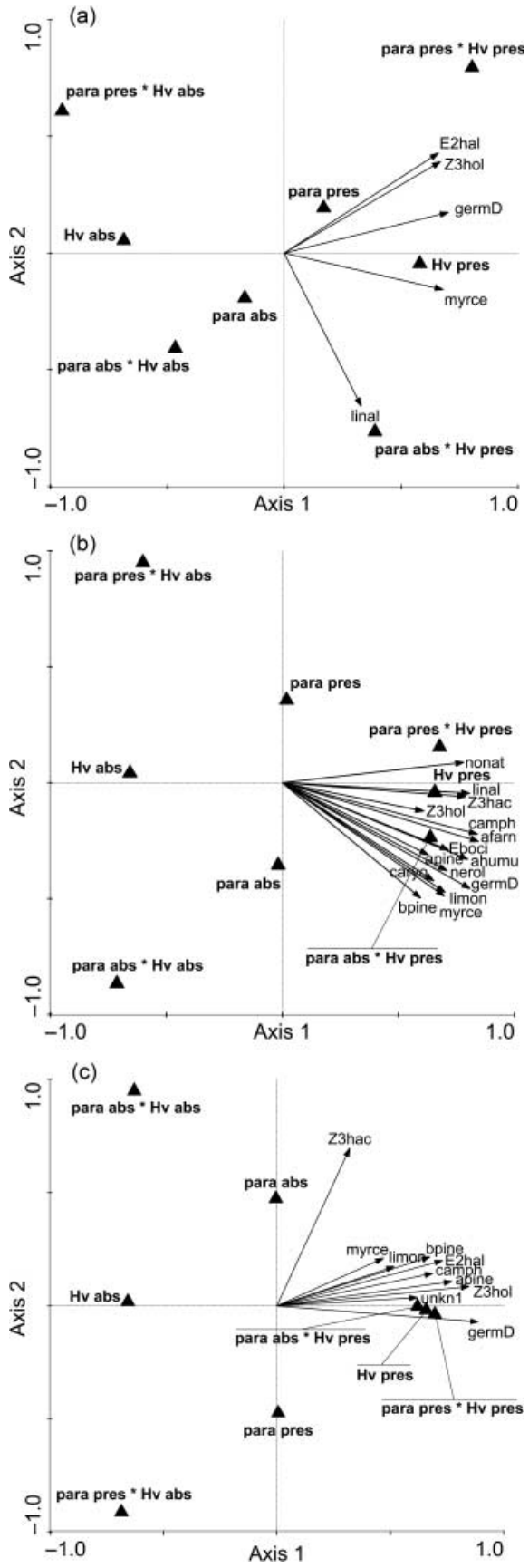
Fig. 3 Total amounts (mean ± 1 SE) of volatile compounds produced during three factorial experiments with *Solidago altissima* infested by *Heliothis virescens* (presence/absence) crossed by the presence or absence of either (a) *Eurosta solidaginis*, (b) *Gnorimoschema gallaesolidaginis* or (c) spittlebugs. Data shown are untransformed and means marked with an asterisk are statistically different (see text for ANOVA details).

and *H. virescens* (Fig. 4a; for details on how to interpret biplots, see Appendix). The position of treatment factors and interaction terms in the RDA biplot illustrated that most of the volatiles significantly associated with the two axes were influenced most strongly by the combination of both *E. solidaginis* and *H. virescens* ('para pres × Hv pres') followed by *H. virescens* alone (Fig. 4a; 'Hv pres'), indicating that *E. solidaginis* substantially altered the defensive responses of *S. altissima* to subsequent herbivory by *H. virescens*. This notion is further supported by quantitative data showing that the *H. virescens* and *E. solidaginis* combination appeared to alter production of individual volatiles relative to *H. virescens* by itself (Table 1).

Our multivariate analysis of the abundance of volatile compounds produced in our factorial experiment with *G. gallaesolidaginis* yielded results similar to those of the experiment with *E. solidaginis*. Again, *H. virescens* feeding significantly increased volatile release ($F_{1,15} = 8.68$, $P = 0.0002$), while the effects of *G. gallaesolidaginis* and the interaction of the two herbivores were not significant (*G. gallaesolidaginis*: $F_{1,15} = 0.46$, $P = 0.84$; *G. gallaesolidaginis* × *H. virescens*: $F_{1,15} = 0.64$, $P = 0.65$). These statistical results were evident in the RDA biplot of volatile abundance (Fig. 4b), which clearly shows that *H. virescens* feeding, when considered separately ('H.v. present') or in the absence of *G. gallaesolidaginis* ('Gnor absent × H.v. present'), was the factor that most strongly influenced the production of most compounds (Fig. 4b). The interaction of *G. gallaesolidaginis* and *H. virescens* had the strongest influence on only one compound (Fig. 4b; nonatriene), a result that indicates that *G. gallaesolidaginis* did not influence the response of *S. altissima* to *H. virescens* as strongly as *E. solidaginis* did.

Multivariate analysis of volatile abundance for our factorial experiment with spittlebugs revealed that feeding by *H. virescens* was again a significant effect ($F_{1,15} = 10.5$, $P = 0.0002$) and the influence of spittlebugs and the interaction of spittlebugs and *H. virescens* were not significant (spittlebugs: $F_{1,15} = 0.79$, $P = 0.54$; spittlebugs × *H. virescens*: $F_{1,15} = 0.78$, $P = 0.55$). Similarly, the RDA biplot for volatile abundance revealed that presence of *H. virescens* was the effect that most strongly influenced production of individual volatiles whether spittlebugs were present or not (Fig. 4c), a pattern that differed from the strong influence of *E. solidaginis* on volatiles following *H. virescens* damage.

Within each of the three growth chamber experiments, the chiral compounds α-pinene, camphene, β-pinene, limonene and linalool showed no significant differences in the composition of their '+' and '−' enantiomers between the four treatments (data not shown; for all comparisons ANOVA $P > 0.05$). Even though the analysis of volatile production was standardized by dividing by total leaf area, the galled and ungalled plants sampled had similar leaf areas (field: e.g. 14 July, *E. solidaginis* 998.1 ± 198.9 cm^2, control 737.8 ± 124.6; e.g. 24 September: *E. solidaginis* 1031.0 ± 144.0, control 943 ± 160.7; ANOVA $P > 0.05$; growth chamber: e.g. control 1162 ± 426, *H. virescens* 1529 ± 394, *E. solidaginis* 708.2 ± 466, *E. solidaginis* and *H. virescens* 1538 ± 426, ANOVA $F_{3,23} = 0.78$, $P > 0.05$). Also, analyses of amounts of volatiles released per plant independent



of leaf area generated results very similar to those already presented that were determined 'per unit leaf area' and, consequently, are not shown.

Multivariate analysis of *Z*-scores across the three experiments again revealed that only *H. virescens* significantly altered abundance of individual volatile compounds ($F_{1,9} = 20.8$, $P = 0.001$). The RDA plot revealed only one significant axis, the presence/absence of *H. virescens*, which significantly influenced abundance of 18 of the volatile constituents with mycrene being most strongly affected (Fig. 5).

Discussion

Gall insects and spittlebugs do not induce indirect plant defenses

The generalist caterpillar *H. virescens* induced strong changes in the total volatiles released by *S. altissima*, demonstrating that our plants were capable of mounting indirect defensive responses following herbivory as has been found for numerous arthropod-plant systems (e.g. Turlings *et al.*, 1990; Du *et al.*, 1996; De Moraes *et al.*, 1998; Dicke, 1999). By contrast, field- and laboratory-based collections revealed that neither *E. solidaginis* nor *G. gallaesolidaginis* induced significant increases in volatile emissions from *S. altissima* (Figs 1, 3a,b; Table 1). It should be noted that while field collections from *E. solidaginis* galls may have appeared to elicit a volatile response from *S. altissima* (Fig. 1) any relevant differences should have also been detected in our more intensive and better-controlled growth-chamber collections, but these revealed no differences between galled and ungalled plants in the total amounts of volatiles produced, the individual compounds released or their quantity (Fig. 3a; Table 1). Like the gall insects, the meadow spittlebug *P. spumarius* also failed to induce a volatile response (Fig. 3c). The failure of these three parasite species to trigger indirect plant defenses is noteworthy given the strong

Fig. 4 Correlation biplots resulting from distance-based redundancy analysis on abundant volatile constituents collected from *Solidago altissima* subject to *Heliothis virescens* and one of three plant parasites in a factorial design: (a) *Eurosta solidaginis*, (b) *Gnorimoschema gallaesolidaginis*, (c) spittlebugs. Plants were classified by four experimental factors, the presence and absence of *H. virescens* ('Hv pres', 'Hv abs') or the parasites ('para pres', 'para abs'), and the interaction of *H. virescens* and parasites ('Hv * para'). Axes 1 and 2 explain 12.3 and 2.8, 40.0 and 3.3, and 42.8 & 5.7% of the variance for *E. solidaginis*, *G. gallaesolidaginis*, and spittlebug biplots, respectively. Volatile compounds displayed on biplots (i.e. arrows) are significantly correlated with the axes after a Bonferroni adjustment ($\alpha = 0.002$). See text for details on statistical analyses and how to interpret the biplots (afarn, α-farnescene; ahumu, α-humulene; apine, α-pinene; bpine, β-pinene; bcary, β-caryophyllene; camph, camphene; E2hal, (E)-2-hexanal; Eboic, (E)-β-ocimene; germD, germacrene D; limon, limonene; linal, linalool; myrce, myrcene; nerol, nerolidol; nonat, nonatriene; unkn1, unknown1; Z3hac, (Z)-3-HAC; Z3hol, (Z)-3-hexen-1-ol).

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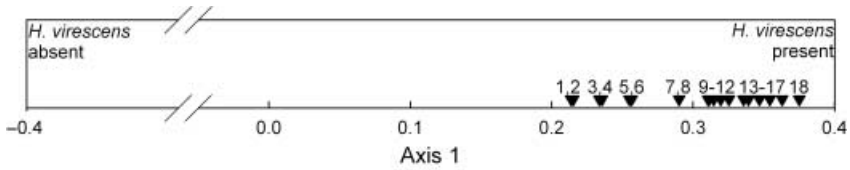

Fig. 5 Axis 1 ordination scores resulting from distance-based redundancy analysis of *Z*-scores representing abundance of volatile constituents collected from *Solidago altissima* in all three factorial experiments with *Eurosta solidaginis*, *Gnorimoschema gallaesolidaginis*, and spittlebugs. Axis 1, the only significant axis, explains 12.4% of the variance and the displayed volatile compounds are significantly correlated with the axis after a Bonferroni adjustment ($\alpha = 0.002$). See text for details on statistical analyses and how to interpret the plots. Compounds labeled as follows: 1, unknown sesquiterpene2; 2, (*E*)-2-hexanal; 3, unknown sesquiterpene1; 4, linalool; 5, nerolidol; 6, (*Z*)-3-hexen-1-ol; 7, nonatriene; 8, germacrene D; 9, limonene; 10, α-pinene; 11, (*Z*)-3-hexenyl acetate; 12, α-humulene; 13, α-farnescene; 14, (*E*)-β-ocimene; 15, camphene; 16, β-pinene; 17, β-caryophyllene; 18, myrcene.

negative influence that they can have on their host plants (Abrahamson & Weis, 1987; Meyer, 1993).

It is unclear why spittlebugs do not elicit a volatile response despite having robust mouthparts that can cause significant damage to plant tissue (Crews *et al.*, 1998). Other piercing–sucking arthropod species, such as mites, aphids, bugs and thrips do trigger volatile responses (Du *et al.*, 1996; Dicke, 1999; Rodriguez-Saona *et al.*, 2002; Delphia *et al.*, 2007). Others, however, feed without damaging plant cells and as a result do not induce volatile defenses, perhaps because they trigger SA-based defenses rather than jasmonate-based defenses, which lead to increased volatile release (Turlings *et al.*, 1998; Walling, 2000; Rodriguez-Saona *et al.*, 2003). The failure of *S. altissima* to release volatiles in response to *E. solidaginis* and *G. gallaesolidaginis* contrasts with the results of a previous study, which reported that a specialist parasitoid was attracted to host-plant volatiles induced by a cynipid gall wasp (Tooker & Hanks, 2006). The current results, however, corroborate previous findings with Hessian fly, a gall midge whose larvae do not trigger a volatile response from wheat (Tooker & De Moraes, 2007).

At least five, potentially interacting, mechanisms may explain the failure of host plants to produce volatiles in response to gall-insect feeding. First, gall-inducing species may avoid detection. Both *E. solidaginis* and *G. gallaesolidaginis* fed on tissue that they induce the plant to produce (Beck, 1953; Abrahamson & Weis, 1987, 1997), and it is conceivable that these tissues (or the larvae themselves) lack biochemical precursors necessary to elicit a volatile response (De Moraes & Mescher, 2004). Second, as already mentioned, gall insects may trigger salicylate-based defenses that interact antagonistically with the JA-based defenses that lead to increased volatile emissions (Walling, 2000). Other gall-inducing species are known to induce a SA-mediated defensive response (Ollerstam & Larsson, 2003), but neither *E. solidaginis* nor *G. gallaesolidaginis* showed significant increases in SA (or JA) production inside their galls. It should be noted however that despite statistical nonsignificance the interior of *E. solidaginis* galls contained approximately three times the SA content of the interior of ungalled stems. This apparent difference may be biologically meaningful and deserves further investigation. Unexpectedly, both gall-inducing species moderated SA levels of the exterior of their galls relative to ungalled stems and further research will be necessary to understand the repercussions of this alteration. Third, stem tissue of *S. altissima* may be less reactive than leaves and thus respond weakly or not at all. However, stem tissues of other plant species are capable of responding to herbivory by elevating volatile production (Karban & Baldwin, 1997; Turlings *et al.*, 1998; Ngumbi *et al.*, 2005). Fourth, larvae may deprive plants of the energy and resources needed to mount a volatile response. Gall insects are parasitic on their hosts, sapping energy and nutrients that would otherwise be used for normal functioning, growth, or reproduction (Abrahamson & McCrea, 1986; Abrahamson & Weis, 1987). Fifth, gall insects may actively suppress the defensive response of their host plants. Gall insects, including *E. solidaginis*, are known to manipulate plant secondary metabolites (Abrahamson *et al.*, 1991; Hartley, 1998; Tooker *et al.*, 2002; Tooker & Hanks, 2004; Allison & Schultz, 2005) and to downregulate some host-plant defenses (Nyman & Julkunen-Tiitto, 2000). Thus, it would not be surprising to find that *E. solidaginis* and/or *G. gallaesolidaginis* can exert some control over plant-volatile emissions.

Eurosta solidaginis alters indirect defensive responses to subsequent herbivory

While *E. solidaginis* alone triggered few changes in host-plant volatiles, its influence on volatile induction became evident in the presence of *H. virescens*. Although the interaction of *E. solidaginis* × *H. virescens* was not significant in our multivariate analysis of the abundance of volatile constituents ($P = 0.17$), the RDA biplot revealed that the interaction of these two herbivore species was the factor most strongly influencing production of some volatile compounds (Fig. 4a). The strength of this interaction is further supported by: the significant effect of *E. solidaginis* dampening total volatile production in response to *H. virescens* feeding (Fig. 3a); and the marginally significant three-way interaction *E. solidaginis* experiment × *H. virescens* × parasite, which influenced total

volatile production in our univariate *Z*-score analysis across the three factorial experiments. Neither spittlebugs nor *G. gallaesolidaginis* appear to have influenced total volatile production of *S. altissima* in response to *H. virescens* as did *E. solidaginis* (Fig. 3), providing further evidence that *E. solidaginis* exerted a unique degree of control over host-plant chemistry. *Eurosta solidaginis* induces a complex gall with four distinct tissue layers that are well-integrated into *S. altissima* stems (Beck, 1947; Weis *et al.*, 1989), perhaps allowing maggots to exert a larger degree of control over their host plant than the relatively simple stem swellings induced by *G. gallaesolidaginis* (Beck, 1953).

We do not know the mechanism by which the presence of *E. solidaginis* dampens volatile responses to caterpillar herbivory. Differential feeding by *H. virescens* on *E. solidaginis*-galled and ungalled plants also does not appear to explain our results, as damage appeared equal between the two *H. virescens*-infested treatments. The reduced volatile response to caterpillar feeding might simply be a byproduct of gall induction if, for example, *E. solidaginis* feeding triggers SA-based defenses that negatively influence subsequent jasmonate-mediated defenses triggered by caterpillars as already discussed (Walling, 2000). Our data on this point are equivocal: as already mentioned, concentrations of SA inside galls were three times greater than those inside ungalled stems; however, high variance rendered this apparent different insignificant and further research will be necessary to determine the level of SA inside galls and its potential role. Nevertheless, increased SA concentrations may be an inadequate explanation for our results because of the lack of downregulation following damage by spittlebugs, which, as piercing–sucking insects, might also be expected to trigger SA-based defenses (Walling, 2000).

Given the disparate volatile responses of *S. altissima* to *H. virescens* in the presence of *E. solidaginis* and spittlebugs, we hypothesize that the apparent control exerted by *E. solidaginis* over volatile emission is regulated by the gall fly maggot, because the sapping influence of spittlebugs did not alter volatile production following *H. virescens* damage to the same degree as *E. solidaginis*. We must note, however, that even though spittlebugs appear to be good controls for the negative influence of resource removal (Meyer, 1993), they may not be perfect 'controls' and our results might have been different using another species of plant parasite.

It is not obvious what benefit *E. solidaginis* might derive from altering plant-volatile emissions to subsequent herbivory. Gall insects may benefit from minimizing the chemical cues available to foraging natural enemies, even if those cues are induced by other herbivores. Other plant parasites are capable of manipulating volatile emissions of their hosts to suit their own interests (Eigenbrode *et al.*, 2002; Tooker *et al.*, 2002; Tooker & Hanks, 2004). Generalized suppression of volatile responses is especially likely to be adaptive if the presence of galls alters volatile responses to feeding by other herbivores in characteristic ways that might provide cues to specialist parasitoids, which have been shown to exploit subtle differences in the composition of volatile blends to locate particular hosts (De Moraes *et al.*, 1998).

Concentrations of volatile compounds emitted by plants (e.g. terpenes) can be strongly correlated with in-leaf concentrations of these toxic compounds, which also act directly against feeding herbivores (Fraenkel, 1959, Köllner *et al.*, 2004). If *E. solidaginis* truly downregulates plant defensive responses to subsequent herbivory, one might therefore expect other herbivorous insect species to benefit from the attenuated defensive response and the resultant decreased apparency gained by feeding on galled plants. Such altered defenses could influence insect-community composition if insect species differed in their sensitivity to, or preference for, the defensive terpenoids analysed, resulting in *E. solidaginis*-infested plants harboring a different suite of herbivorous insect species from that of ungalled plants. In fact, six species of insects commonly found on *S. altissima* are positively correlated with *E. solidaginis*-infested plants whereas three are negatively correlated (Maddox & Root, 1990). Such associations potentially could reflect a range of responses to changes induced by *E. solidaginis*, with the preference of individual species influenced by their predilection for stressed or healthy plants. Further research would obviously be needed to address mechanisms that might drive these relationships, but it is reasonable to suppose that gall-induced changes in host-plant chemistry may play a role in structuring community composition.

In conclusion, herbivory by *E. solidaginis*, *G. gallaesolidaginis* and the spittlebug *P. spumarius* did not induce significant releases of volatile chemicals as has been shown for other plant species being attacked by arthropod herbivores (Turlings *et al.*, 1990; Du *et al.*, 1996; De Moraes *et al.*, 1998; Dicke, 1999; Kessler & Baldwin, 2001; Rasmann *et al.*, 2005). This lack of a volatile response is notable because both spittlebugs and gall insects can have strong negative effects on their host plants (Hartnett & Abrahamson, 1979; Abrahamson & Weis, 1987; Meyer, 1993). Gall insects, on the other hand, exert considerable control over their host plants and might be expected to minimize any host-plant defensive response that renders them vulnerable to natural enemies. *Gnorimoschema gallaesolidaginis* and *E. solidaginis* in well-developed galls are not associated with increased JA production and may avoid triggering the indirect defenses of host plants, but the significant influence of *E. solidaginis* on the response of *S. altissima* to *H. virescens* herbivory suggests that *E. solidaginis* may actively suppress plant-volatile responses as has been shown for other plant-defense mechanisms (Nyman & Julkunen-Tiitto, 2000). Establishing the role of SA in these interactions requires further study. Such an ability to downregulate volatile production corroborates previous findings with Hessian fly (Tooker & De Moraes, 2007) and adds to the list of host-plant traits that gall insects are able to manipulate. While the fitness benefit gained by *E. solidaginis* is unclear, gall fly maggots would seem to be less vulnerable to natural enemies if their host plants were less

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apparent than others in the population. Further research will be necessary to determine exactly why *S. altissima* fails to produce indirect defenses in response to gall formation, whether *E. solidaginis* actively suppresses the response and whether other insect herbivore species are sensitive to any such changes.

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References

Abrahamson WG, McCrea KD. 1986. The impacts of galls and gallmakers on plants. *Proceedings of the Entomological Society of Washington* **88**: 364–367.

Abrahamson WG, McCrea KD, Whitwell AJ, Vernieri LA. 1991. The role of phenolics in goldenrod ball gall resistance and formation. *Biochemical Systematics and Ecology* **19**: 615–622.

Abrahamson WG, Weis AE. 1987. Nutritional ecology of arthropod gall makers. In: Slansky F Jr, Rodriguez JG, eds. *Nutritional ecology of insects, mites, spiders, and related invertebrates*. New York, NY, USA: Wiley & Sons, 238–258.

Abrahamson WG, Weis AE. 1997. *Evolutionary ecology across three trophic levels: goldenrods, gallmakers, and natural enemies*. Princeton, NJ, USA: Princeton University Press.

Allison SD, Schultz JC. 2005. Biochemical responses of chestnut oak to a galling cynipid. *Journal of Chemical Ecology* **31**: 151–166.

Anderson MJ. 2004. *DISTLM v.5: a FORTRAN computer program to calculate a distance-based multivariate analysis from a linear model*. Auckland, NZ: University of Auckland, Department of Statistics.

Anderson MJ. 2005. *PERMANOVA: a FORTRAN computer program to calculate a distance-based multivariate analysis from a linear model*. Auckland, NZ: University of Auckland, Department of Statistics.

Beck EG. 1947. *Some studies on the Solidago gall caused by* Eurosta solidaginis *Fitch*. Ph.D. thesis. Ann Arbor, MI, USA: University of Michigan.

Beck EG. 1953. The nature of the stimulus in the *Solidago* gall induced by the larva of *Gnorimoschema gallaesolidaginis*. *Brookhaven Symposia in Biology* **6**: 235–251.

ter Braak CJF. 1994. Canonical community ordination: Part 1 Basic theory and linear methods. *Ecoscience* **1**: 127–140.

ter Braak CJF, Šmilauer P. 2002. *CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination*, 4.5 edn. Ithaca, NY, USA: Microcomputer Power.

Bronner R. 1992. The role of nutritive cells in the nutrition of cynipids and cecidomyiids. In: Shorthouse JD, Rohfritsch O, eds. *Biology of insect-induced galls*. New York, NY, USA: Oxford University Press, 118–140.

Craig TP, Itami JK, Abrahamson WG, Horner JD. 1993. Behavioral evidence for host-race formation in *Eurosta solidaginis*. *Evolution* **47**: 1696–1710.

Crews LJ, McCully ME, Canny MJ, Huang CX, Ling LEC. 1998. Xylem feeding by spittlebug nymphs: some observations by optical and cryo-scanning electron microscopy. *American Journal of Botany* **85**: 449–460.

Dawkins R. 1982. *The extended phenotype*. Oxford, UK: WH Freeman & Co.

De Moraes CM, Mescher MC. 2004. Biochemical crypsis in the avoidance of natural enemies by an insect herbivore. *Proceedings of the National Academy of Sciences, USA* **101**: 8993–8997.

De Moraes CM, Lewis WJ, Pare PW, Alborn HT, Tumlinson JH. 1998. Herbivore-infested plants selectively attract parasitoids. *Nature* **393**: 570–573.

Delphia CM, Mescher MC, De Moraes CM. 2007. Induction of plant volatiles by herbivores with different feeding habits and the effects of induced defenses on host-plant selection by thrips. *Journal of Chemical Ecology* **33**: 997–1012.

Dicke M. 1999. Are herbivore-induced plant volatiles reliable indicators of herbivore identity to foraging carnivorous arthropods? *Entomologia Experimentalis et Applicata* **91**: 131–142.

Du YJ, Poppy GM, Powell W. 1996. Relative importance of semiochemicals from first and second trophic levels in host foraging behavior of *Aphidius ervi*. *Journal of Chemical Ecology* **22**: 1591–1605.

Eigenbrode SD, Ding H, Shiel P, Berger PH. 2002. Volatiles from potato plants infected with potato leafroll virus attract and arrest the virus vector, *Myzus persicae* (Homoptera: Aphididae). *Proceeding of the Royal Society of London, Series B* **269**: 455–460.

Fay PA, Hartnett DC, Knapp AK. 1996. Plant tolerance of gall-insect attack and gall-insect performance. *Ecology* **77**: 521–534.

Felt EP. 1940. *Plant galls and gall makers*. Ithaca, NY, USA: Comstock Publishing.

Fraenkel GS. 1959. Raison d'être of secondary plant substances. *Science* **129**: 1466–1470.

Fritzsch-Hoballah ME, Turlings TCJ. 2001. Experimental evidence that plants under caterpillar attack may benefit from attracting parasitoids. *Evolutionary Ecology Research* **3**: 553–565.

Gagné RJ. 1989. *The plant-feeding gall midges of North America*. Ithaca, NY, USA: Comstock Publishing.

Gonzalez L, Manly BFJ. 1998. Analysis of variance by randomization with small data sets. *Environmetrics* **9**: 53–65.

Hartley SE. 1998. The chemical composition of plant galls: are levels of nutrients and secondary compounds controlled by the gall former? *Oecologia* **113**: 492–501.

Hartnett DC, Abrahamson WG. 1979. The effect of stem gall insects on life history patterns in *Solidago canadensis*. *Ecology* **60**: 910–916.

Izzo TJ, Julião GR, Almada ED, Fernandes GW. 2006. Hiding from defenders: localized chemical modification on the leaves of an Amazonian ant-plant induced by a gall-making insect (Diptera: Cecidomyiidae). *Sociobiology* **48**: 417–426.

Karban R, Baldwin IT. 1997. *Induced responses to herbivory*. Chicago, IL, USA: University of Chicago Press.

Kessler A, Baldwin IT. 2001. Defensive function of herbivore-induced plant volatile emissions in nature. *Science* **291**: 2141–2144.

Köllner TG, Schnee C, Gershenzon J, Degenhardt J. 2004. The sesquiterpene hydrocarbons of maize (*Zea mays*) form five groups with distinct developmental and organ-specific distributions. *Phytochemistry* **65**: 1895–1902.

Lacroix R, Mukabana WR, Gouagna LC, Koella JC. 2005. Malaria infection increases attractiveness of humans to mosquitoes. *PLoS Biology* **3**: e298 doi:10.1371/ journal.pbio.0030298.

Larson KC. 1998. The impact of two gall-forming arthropods on the photosynthetic rates of their hosts. *Oecologia* **115**: 161–166.

Legendre P, Anderson MJ. 1998. *Program* DISTPCOA. Montreal, Canada: Département de Sciences Biologiques, Université de Montréal.

Legendre P, Anderson MJ. 1999. Distance-based redundancy analysis: testing multi-species responses in multi-factorial ecological experiments. *Ecological Monographs* **69**: 1–24.

Leiby RW. 1922. Biology of the goldenrod gall-maker *Gnorimoschema gallaesolidaginis* Riley. *Journal of the New York Entomological Society* **30**: 81–94.

van Loon JJA, de Boer G, Dicke M. 2000. Parasitoid-plant mutualism: parasitoid attack of herbivore increases plant reproduction. *Entomologia Experimentalis et Applicata* **97**: 219–227.

Maddox GD, Root RB. 1990. Structure of the encounter between goldenrod (*Solidago altissima*) and its diverse insect fauna. *Ecology* **71**: 2115–2124.

Mani MS. 1992. Introduction to Cecidology. In: Shorthouse JD, Rohfritsch O, eds. *Biology of insect-induced galls*. New York, NY, USA: Oxford University Press, 3–7.

McCrea KD, Abrahamson WG. 1986. Nutrient and biomass allocation in *Solidago altissima*: effects of two stem gallmakers, fertilization, and ramet isolation. *Oecologia* **68**: 174–180.

McCrea KD, Abrahamson WG, Weis AE. 1985. Goldenrod ball gall effects on *Solidago altissima*: ^{14}C translocation and growth. *Ecology* **66**: 1902–1907.

Meyer GA. 1993. A comparison of the impacts of leaf- and sap-feeding insects on growth and allocation of goldenrod. *Ecology* **74**: 1101–1116.

Meyer GA, Whitlow TH. 1992. Effects of leaf and sap feeding insects on photosynthetic rates of goldenrod. *Oecologia* **92**: 480–489.

Miller ME. 2000. A comparative taxonomic-natural history study of eight Neartic species of *Gnorimoschema* that induce stem galls on Asteraceae, including descriptions of three new species (Lepidoptera: Gelechiidae). In: *Thomas Say Publication in Entomology: Monographs*. Lanham, USA: Entomological Society of America.

Ngumbi EN, Ngi-Song AJ, Njagi ENM, Torto R, Wadhams LJ, Birkett MA, Pickett JA, Overholt WA, Torto B. 2005. Responses of the stem borer larval endoparasitoid *Cotesia flavipes* (Hymenoptera: Braconidae) to plant derived synomones: laboratory and field cage experiments. *Biocontrol Science and Technology* **15**: 271–279.

Nyman T, Julkunen-Tiitto R. 2000. Manipulation of the phenolic chemistry of willows by gall-inducing sawflies. *Proceedings of the National Academy of Sciences, USA* **97**: 13184–13187.

Ollerstam O, Larsson S. 2003. Salicylic acid mediates resistance in the willow *Salix viminalis* against the gall midge *Dasineura marginemtorquens*. *Journal of Chemical Ecology* **29**: 163–174.

Raman A, Schaefer CW, Withers TM. 2005. Galls and gall-inducing arthropods: an overview of their biology, ecology and evolution. In: Raman A, Schaefer CW, Withers TM, eds. *Biology, ecology, and evolution of gall-inducing arthropods*. Enfield, CT, USA: Science Publishers, 1–33.

Rasmann S, Köllner TG , Degenhardt J, Hiltpold I, Toepfer S, Kuhlmann U, Gershenzon J, Turlings TCJ. 2005. Recruitment of nematodes to insect damaged maize roots. *Nature* **434**: 732–737.

Rodriguez-Saona C, Crafts-Brandner SJ, Canas LA. 2003. Volatile emissions triggered by multiple herbivore damage: Beet armyworm and whitefly feeding on cotton plants. *Journal of Chemical Ecology* **29**: 2539–2550.

Rodriguez-Saona C, Crafts-Brandner SJ, Williams L, Paré PW. 2002. *Lygus hesperus* feeding and salivary gland extracts induce volatile emissions in plants. *Journal of Chemical Ecology* **28**: 1733–1747.

Roy BA. 1993. Floral mimicry by a plant pathogen. *Nature* **362**: 56–58.

Schmelz EA, Engelberth J, Alborn HT, O'Donnell P, Sammons M, Toshima H, Tumlinson JH. 2003. Simultaneous analysis of phytohormones, phytotoxins, and volatile organic compounds in plants. *Proceedings of the National Academy of Sciences, USA* **100**: 10552–10557.

Schmelz EA, Engelberth J, Tumlinson JH, Block A, Alborn HT. 2004. The use of vapor phase extraction in metabolic profiling of phytohormones and other metabolites. *The Plant Journal* **39**: 790–808.

Sokal RR, Rohlf FJ. 1995. *Biometry*, 3rd edn. New York, NY, USA: WH Freeman.

Statistica. 2006. *User manual, release 8.0*. Tulsa, OK, USA: StatSoft.

Statistix. 2003. *User manual, release 8.0*. Tallahassee, FL, USA: Analytical Software.

Stewart-Jones A, Poppy GM. 2006. Comparison of glass vessels and plastic bags for enclosing living plant parts for headspace analysis. *Journal of Chemical Ecology* **32**: 845–864.

Tooker JF, De Moraes CM. 2007. Feeding by Hessian fly [*Mayetiola destructor* (Say)] larvae does not induce plant indirect defences. *Ecological Entomology* **32**: 153–161.

Tooker JF, Hanks LM. 2004. Stereochemistry of host plant monoterpenes as mate location cues for the gall wasp *Antistrophus rufus*. *Journal of Chemical Ecology* **30**: 473–477.

Tooker JF, Hanks LM. 2006. Tritrophic interactions and reproductive fitness of the prairie perennial *Silphium laciniatum* Gillette (Asteraceae). *Environmental Entomology* **35**: 537–545.

Tooker JF, Koenig WA, Hanks LM. 2002. Altered host plant volatiles are proxies for sex pheromones in the gall wasp *Antistrophus rufus*. *Proceedings of the National Academy of Sciences, USA* **99**: 15486–15491.

Turlings TCJ, Bernasconi M, Bertossa R, Bigler F, Caloz G, Dorn S. 1998. The induction of volatile emissions in maize by three herbivore species with different feeding habits: possible consequences for their natural enemies. *Biocontrol* **11**: 122–129.

Turlings TCJ, Tumlinson JH, Lewis WJ. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* **250**: 1251–1253.

Uhler, LD. 1951. Biology and ecology of the goldenrod gall fly, *Eurosta solidaginis* (Fitch). *Memoir of the Cornell University Agricultural Experiment Station* **300**: 3–51.

Walling LL. 2000. The myriad plant responses to herbivores. *Journal of Plant Growth Regulation* **19**: 195–216.

Waring GL, Abrahamson WG, Howard DJ. 1990. Genetic differentiation among host-associated populations of the gallmaker *Eurosta solidaginis* (Diptera: Tephritidae). *Evolution* **44**: 1648–1655.

Washburn JO. 1984. Mutualism between a cynipid gall wasp and ants. *Ecology* **65**: 654–656.

Weis AE, Wolfe CL, Gorman WL. 1989. Genotypic variation and integration in histological features of the goldenrod ball gall. *American Journal of Botany* **76**: 1541–1550.

Appendix

Interpretation of distance-based redundancy analysis biplots

In the Db-RDA biplots (e.g. Fig. 4), the spatial ordination of samples is laid out in two-dimensional space. The origin represents the grand mean and distance from the origin indicates relative strength or importance of the treatments (i.e. 'H.v. absent', '*Eurosta* present', etc.) whereas distance between points estimates their dissimilarity in volatiles (χ^2 distance; ter Braak, 1994). The length of arrows represents how strongly the variable is related to the displayed ordination whereas the arrows themselves can be interpreted as axes of ordination for particular compounds (e.g. Fig. 4a, germacrene D). The position

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of the treatment relative to the axes represented by each arrow indicates rank importance of each factor. For example, in Fig. 4a if the arrow for 'germacrene D' was extended in both directions and perpendicular lines were dropped from the treatment factors and the interaction terms (e.g. 'Eurosta present', 'H.v. present', 'Eurosta present × H.v. present', etc.) to the 'germacrene D' arrow, the plot reveals that the production of germacrene D was most strongly influenced by the interaction of *E. solidaginis* presence and *H. virescens* presence followed by *H. virescens* presence, *E. solidaginis* presence, the interaction of *E. solidaginis* absence and *H. virescens* presence, etc. Moreover, because the factor '*Eurosta* absence' is on the left side of the origin (i.e. the mean), these factors were associated with less than average production of the compounds. Further details on the interpretation of biplots can be found elsewhere (e.g. ter Braak, 1994).

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