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Intraspecific Variation in Plant Defense Alters Effects of Root Herbivores on Leaf Chemistry and Aboveground Herbivore Damage

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Abstract Root herbivores can indirectly affect aboveground herbivores by altering the food quality of the plant. However, it is largely unknown whether plant genotypes differ in their response to root herbivores, leading to variable defensive phenotypes. In this study, we investigated whether root-feeding insect larvae (*Agriotes* sp. larvae, wireworms) induce different responses in *Plantago lanceolata* plants from lines selected for low and high levels of iridoid glycosides (IG). In the absence of wireworms, plants of the "high-IG line" contained approximately twofold higher levels of total IG and threefold higher levels of catalpol (one of the IG) in leaves than plants from the "low-IG line," whereas both lines had similar levels of IG in roots. In response to wireworms, roots of plants from both lines showed increased concentrations of catalpol. Leaves of "low-IG line" plants increased catalpol concentrations in response to wireworms, whereas catalpol concentrations of leaves of "high-IG line" plants decreased. In contrast, glucose concentrations in roots of "low-IG" plants decreased, while they increased in "high-IG" plants after feeding by wireworms. The leaf volatile profile differed between the lines, but was not affected by root herbivores. In the field, leaf damage by herbivores was higher in wireworm-induced compared to noninduced "low-IG" plants and lower in wireworm-induced compared to noninduced "high-IG" plants, despite induction of catalpol in leaves of the "low-IG" plants and reduction in "high-IG" plants. This pattern might arise if damage is caused mainly by specialist herbivores for which catalpol may act as feeding stimulant rather than as deterrent. The present study documents for the first time that intraspecific variation in plant defense affects the outcome of plant-mediated interactions between root and shoot herbivores.

Keywords *Plantago lanceolata* · Wireworms · Iridoid glycosides · Volatiles · Herbivory · Induced defense · Belowground–aboveground interactions

Introduction

Plants are attacked by and respond to both shoot and root herbivores. However, while induced plant responses to aboveground herbivores are well-documented (Karban and Baldwin 1997), knowledge about induced responses to root herbivores and consequences for aboveground plant–herbivore interactions is scarce (Van der Putten et al. 2001; Van Dam et al. 2003). Root and shoot herbivores can indirectly affect each other by changing the food quality of their common host plant. Root feeding by insect larvae can induce systemic plant defensive responses that affect aboveground herbivores (Bezemer et al. 2003, 2004; Soler et al. 2005; Van Dam et al. 2005). Root-feeding insect larvae may also affect water uptake of plants, thus

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leading to drought stress symptoms that influence aboveground herbivores (Masters et al. 1993; Poveda et al. 2005). Plant-mediated interactions between root and shoot herbivores likely depend on the plant and herbivore species, as well as on abiotic factors (Wurst and Van der Putten 2007). However, it is unknown if genetic variation in plant defense traits influences belowground-aboveground interactions.

Plants display genetic variation in traits that influence the performance of herbivores. These resistance traits may respond to abiotic and biotic environmental factors that lead to variable defensive phenotypes (Agrawal and Karban 1999). So far, it is largely unknown whether the plant genotype affects the outcome of plant-mediated interactions between root and shoot herbivores. Moran and Whitham (1990) documented that plant resistance to leaf galling abolishes the negative impact of a leaf galling aphid on a root-feeding aphid on *Chenopodium album*. However, it remained unclear whether this effect was mediated by a difference in plant response or by the smaller number of leaf galling aphids on the resistant plants. In general, the way in which a plant responds to herbivores is expected to differ among genotypes and can be considered a trait that is subject to selection. Since root-associated soil organisms may interact with the plant before shoots emerge, root-induced plant responses might have a strong impact on aboveground plant–herbivore interactions and even on higher trophic levels such as predators and parasitoids (Bezemer and Van Dam 2005).

In this study, we investigated the effects of coleopteran root herbivores (*Agriotes* sp. larvae, Elateridae) on secondary plant chemistry of two full-sib families of *Plantago lanceolata* (Plantaginaceae) from lines that were selected for low and high levels of defensive compounds (iridoid glycosides [IG]). IG are known to deter generalist insect herbivores (Bowers and Puttick 1988) and pathogens (Marak et al. 2002a). However, they are used as feeding and oviposition stimulants by specialist herbivores (Bowers 1983). Production of IG can be induced both by herbivores (Darrow and Bowers 1999) and pathogens (Marak et al. 2002b).

Additionally, we investigated whether the production of aboveground volatiles differed between the plant lines and between root herbivore-induced and noninduced plants. While IG may serve as direct defense compounds against herbivores, leaf volatiles may attract carnivorous enemies of herbivores as an indirect defense mechanism (Dicke 1999). Recently, insect root herbivores were shown to change the release of leaf volatiles, which affects aboveground parasitoid behavior (Soler et al. 2007). In a field assay, we monitored leaf damage of root herbivore-induced and noninduced plants of the two lines. The results indicate that plant responses to root herbivory differ between plant lines leading to different defensive phenotypes that aboveground herbivores have to deal with.

Methods and Materials

Experiments were performed with seeds of two full-sib families of *Plantago lanceolata* L. from lines artificially selected for low and high levels of IG (Marak et al. 2000). After four generations, leaf IG concentrations of the "low-IG line" were in average twofold to fourfold lower than of the "high-IG," depending on environmental conditions (Marak et al. 2000, 2003; Biere et al. 2004). The "low-IG" and "high-IG" full-sib families used in the current experiments were the offspring of crosses between two (self-incompatible) parents from the "low-IG line" and between two parents from the "high-IG line", respectively.

On 19 May 2006, seeds of the two *Plantago* lines were surface-sterilized with potassium hypochlorite solution (1%) and sown on wet paper in Petri dishes in the greenhouse (16 h light, 20°C/25°C night/day temperature). Twelve days after sowing, germinated plants were transplanted into seedling trays filled with gamma-sterilized (25 kGy) experimental soil. The experimental soil was a loamy, sandy mineral soil (N=0.13%, C=2.1%, C/N=16.7) that had been sieved through a 0.5-cm mesh.

Experimental Set-Up A total of 48 pots (11.5 cm height, 13 cm diameter) filled with 1,300 g nonsterile experimental soil were placed in a greenhouse with 16 h light and 20°C/25°C night/day temperature. *P. lanceolata* plants with two to three leaves (except the cotyledons) were planted from the seedling trays into the pots on 13 June (day 1 of the experiment). Half the pots were planted with one plant from the "low-IG line," the other half with one plant from the "high-IG line." Two weeks later, two *Agriotes* larvae (fresh biomass added: mean=45.14 mg, SE=1.62) were added to half of the pots of each IG line. *Agriotes* larvae (wireworms) are abundant root herbivores in grasslands, feed on a wide range of plant species, and have a long life cycle with the larval stage taking 4–5 years (Staley et al. 2007). The set-up resulted in a full factorial experimental design with six replicates per treatment and harvest time (two harvests).

The pots were watered with 50 mL demineralized H_2O every second day and redistributed randomly within the greenhouse every second week. In week 8 of the experiment, the stalks were cut and frozen at −80°C to prevent a possible influence of inflorescence odor on the subsequent leaf volatile measurements.

In week 9, the volatile organic compounds (VOC) of 40 experimental plants were measured (ten replicates per

treatment; replicates of harvest 1 and 2 were combined). Plants were placed under 17 L bell-shaped glass cylinders (30 cm height, 24.5 cm diameter) in a controlled climate cabinet (21°C, 72% relative humidity). The glass cylinders were supplied constantly with 300 mL pressurized air (Hoekloos, NL) from the top. The air was cleaned by a Zero Air generator to remove hydrocarbons (Parker Hannifin, Tewksbury, MA, USA). Plant VOC were collected from a potted plant by using a steel trap filled with 150 mg Tenax TA and 150 mg Carbopack B. The trap was connected to a vacuum pump (flow rates 200 mL/min). Traps were removed after 1 h from the pump and kept for approximately 1 week at 5°C until analysis. For control, we measured background VOC profiles from empty glass cylinders (N=2) and VOC profiles from a pot with soil but without a plant inside the cylinder (N=2).

In week 10, half of the plants (N=24) were harvested. Wireworms were collected and weighed during root washing. Since wireworms might have affected colonization of roots by arbuscular mycorrhizal fungi (AMF) with subsequent effects on leaf chemistry and herbivore damage (Gange et al. 1994; Gange 2001; Gange and West 1994), random subsamples of approximately 1.5 g fresh roots were taken, stored in ethanol (50%), stained, and assessed for mycorrhizal fungi colonization (for details see Wurst et al. 2004). Shoots and roots were frozen at −80°C, freeze-dried, weighed, and subsamples were ground for analyses of N and C, glucose, and IG.

The other half of the pots (N=24) was placed in a randomized block design outside on a meadow to expose them to natural herbivory from June until November (field experiment). Fourteen weeks later, these plants were harvested. The leaves of these plants were counted, and their size and extent of herbivore damage (area of holes eaten from the leaf area) were recorded with WinFOLIA (Regent Instruments, Sainte-Foy, Canada). The leaves and roots were frozen at −80°C, freeze-dried, and weighed.

Chemical Analyses Volatiles (VOC) were desorbed from the steel traps by using an automated thermodesorption unit (model Unity, Markes, Pontyclun, UK) at 200°C for 10 min (He flow 30 mL/min) and focused on a cold trap (−10°C). After 1 min of dry purging, trapped volatiles were introduced into the gas chromatograph–mass spectrometer (model Trace, ThermoFinnigan, Austin, TX, USA) by heating the cold trap for 3 min to 270°C. Split rate was set to 1:4, and the column used was a 30-m×0.32-mm ID RTX-5 Silms, film thickness 0.33 μm. Temperature raised from 40°C to 95°C at 3°C/min, then to 165°C at 2°C/min, and finally to 250°C at 15°C/min. The VOC were detected by the mass spectrometer operating at 70 eV in EI mode. Mass spectra were acquired in full scan mode (33–300 AMU, three scans per second). Compounds were identified (a) by their mass spectra by using deconvolution software (AMDIS) and comparison with mass spectra from NIST 98 and Wiley 7th edition spectral libraries, (b) by their linear retention indices and comparison with values reported in the literature (C.A. Hordijk, personal database), and (c) by analyzing reference substances commonly reported to be detected in plant volatile profiles (octanal, nonanal, decanal, limonene, benzylcyanide, *cis*-3-hexenyl acetate, *cis*-3-hexen-1-ol, dimethyl trisulfide [Sigma-Aldrich, St Louis, MO, USA], methyl salicylate [Merck, Darmstadt, Germany], *cis*-3-hexenyl isobutyrate [Oxford Chemicals, Harlow, Essex, UK], and 4,8-dimethyl 1,3,7-nonatriene [kindly provided by Dr. TA Van Beek, Wageningen University, The Netherlands]) and comparing their mass spectra with those of the compounds detected. Peak areas of identified compounds were integrated by the Xcalibur software (Version 1.3, Finnigan). To exclude potential interference by coeluting compounds, specific quantifier ions were carefully selected for each individual compound of interest. In general, these quantifier ions were similar to the most intense model ions extracted from the raw mass spectrum by AMDIS. The integrated absolute signal of the quantifier ion(s) were used for comparison between the treatments. Peak areas in each sample were divided by the total volume in milliliters (calculated as total volume= $(\text{flow}_{\text{begin}}+\text{flow}_{\text{end}})/2\times$sampling time in minutes) that was sampled over the trap. This procedure corrects for differences in flow rates over individual traps that arose during the sampling procedure and minor difference in sampling times between replicate sets.

IG and glucose from 25 mg freeze-dried and ground root samples were extracted overnight with 70% methanol. The concentrations of the IG aucubin and catalpol were analyzed using a Dionex (Sunnyvale, CA, USA) BioLC equipped with a GP50 gradient pump, a Carbopac PA1 anion-exchange guard (2×50 mm) and analytical column (2×250 mm), and an ED50 electrochemical detector for pulsed amperometric detection. Compounds were eluted with NaOH (1 M) and ultrapure water (10%:90%). Retention times were 3.5 and 5.0 min for aucubin and catalpol, respectively. Concentrations were analyzed with Chromeleon Software Release 6.60 (Dionex). Contents of total N and C in leaves and roots were measured by using a C/N analyzer (Flashea Series1112, Interscience, Breda, NL, USA).

Statistical Analyses Data were analyzed by factorial analyses of variance (ANOVA) in a general linear model (GLM, Statistica 6.0, Statsoft) with the class factors "*Plantago* line" and "wireworms." The data were tested for normality (Kolmogorov–Smirnov one-sample test) and homogeneity of variances (Levene test) and log-transformed or arcsine transformed (for percentage data) if necessary. Volatiles

were analyzed by a redundancy analysis (RDA) followed by a Monte Carlo 999 permutation test in CANOCO for Windows 4.5. The linear method RDA was justified by the length of the gradients being shorter than 3.0 in a detrended canonical correspondence analysis (Lepš and Šmilauer 2003). Only volatiles that were not detected in the background controls (i.e., empty glass cylinders) and were detected in at least 70% of the ten replicates of one treatment were used for statistical analysis (Table 1).

Results

Harvest 1 (Greenhouse Experiment) Leaf biomass was greater in plants from the "high-IG line" ($F_{(1,\ 20)}$=21.74, P<0.001) (Fig. 1), while plants from the "low-IG line" produced more inflorescences ("low-IG line": mean=2.50, SE=0.26; "high-IG line": mean=1.00, SE=0.17; $F_{(1,\ 44)}$= 23.53, P<0.001) and higher reproductive biomass ("low-IG line": mean=0.41 g dry weight, SE=0.03; "high-IG line": mean=0.15 g dry weight, SE=0.02; $F_{(1,\ 37)}$=33.74, P< 0.001). Note, however, that the inflorescences were cut in week 8 (prior to VOC analyses), while the leaves and roots were harvested in week 10 (after VOC analyses). Overall, the total aboveground biomass did not differ between the lines. Plants from the "high-IG line" had a greater root biomass ($F_{(1,\ 20)}$=8.00, P<0.05), resulting in a greater total biomass than plants from the "low-IG line" ($F_{(1,\ 20)}$=7.18, P<0.05). Wireworms did not affect the biomass of roots and inflorescences, but enhanced the leaf biomass ($F_{(1,\ 20)}$= 7.46, P<0.05) and the total shoot biomass ($F_{(1,\ 20)}$=6.64, P<0.05) (Fig. 1). The effects of wireworm feeding on plant biomasses did not depend on the *Plantago* line.

Carbon concentration (percent dry weight) in leaves was slightly but significantly higher in plants from the "high-IG line" (mean=41.62, SE=0.23) than in plants from the "low-IG line" (mean=40.73, SE=0.34; $F_{(1,\ 20)}$=4.79, P<0.05). Nitrogen concentrations in leaves (mean=1.15, SE=0.11) and roots (mean=0.89, SE=0.16) and carbon concentrations in roots (mean=37.65, SE=0.98) were not affected by the plant line or by wireworms. Glucose concentration in shoots (mean=0.55%, SE=0.07) was unaffected by the treatments, while glucose concentration in roots increased when wireworms fed upon the "high-IG line," but decreased in the "low-IG line" (interaction between *Plantago* line and wireworms: $F_{(1,\ 20)}$=6.86, P<0.05; Fig. 2).

Catalpol concentration (percent dry weight) in leaves was higher in the "high-IG line," but only in the absence of wireworms. When wireworms were present, plants from the "high-IG line" reduced leaf catalpol concentrations, while plants from the "low-IG line" showed enhanced concentration of this IG in leaves (interaction between *Plantago* line and wireworms: $F_{(1,\ 20)}$=9.21, P<0.01; Fig. 3). The same pattern was observed for the concentration of total IG (aucubin+catalpol) in leaves (interaction between *Plantago* line and wireworms: $F_{(1,\ 20)}$=6.36, P<0.05). The leaf aucubin concentration did not differ significantly between treatments (mean=2.86%, SE=0.34), but tended to show the same pattern (interaction between *Plantago* line and wireworms: $F_{(1,\ 20)}$=3.62, P=0.07). In roots of both lines, the catalpol concentration was enhanced in the presence of wireworms ($F_{(1,\ 20)}$=4.85, P<0.05; Fig. 3). The concentrations of aucubin (mean=2.52%, SE=0.18) and total IG (mean=2.89%, SE=0.19) in roots were not significantly affected by wireworms or plant line.

Colonization of roots by AMF (mean=41.17%, SE= 2.24) was not affected by plant line or wireworms. Recovery rates of wireworms at harvest 1 were as follows: 37.5% as larvae, 12.5% as pupae, and 12.5% as adult beetles. In 83.33% of the wireworm-treated pots, at least one *Agriotes* individual was recovered. Since the *Agriotes* specimens recovered were of different life stages, no potential effect of the plant line on wireworm weight could be measured.

Table 1 Volatiles of *Plantago lanceolata* that were detected in ≥70% of the ten replicates of at least one treatment and not present in the background controls

Terpenes (RI)	Green leaf volatiles (RI)	Others (RI)
cis-β-ocimene (1048)	*cis*-3-hexen-1-yl-acetate (1010)	2-Methyl furan (0604)
(3-*Trans*)-4,8-dimethyl-1,3, 7-nonatriene (1117)	1-Hexyl-acetate (1015)	Heptanoic acid (1081)
		2-Nonanone (1091)
β-elemene (1385)		Octanoic acid (1184)
cis-caryophyllene (1408)		Nonanoic acid (1274)
Trans-α-bergamotene (1429)		
Unknown sesquiterpene (1434)		
Unknown sesquiterpene (1439)		
Trans-β-farnesene (1454)		
(δ or γ)-Cadinene (1511)		
Germacrene A (1494)		

RI retention index

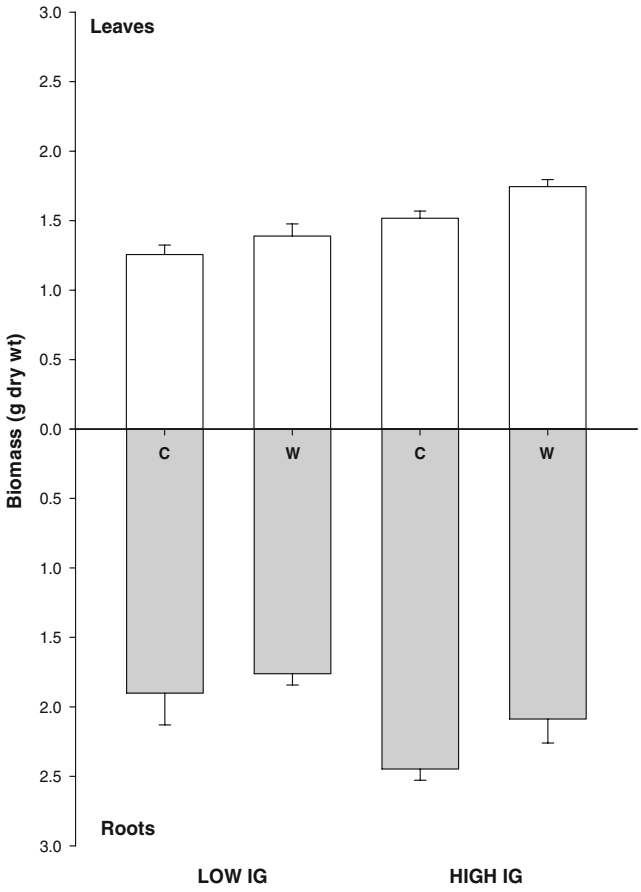

Fig. 1 Effects of plant line (low IG and high IG) and wireworms (*C* without wireworms, *W* with wireworms) on the biomass (mean+SE) of roots (*gray*) and leaves (*white*) of *Plantago lanceolata*. For the significant main and interaction effects of ANOVA, see text

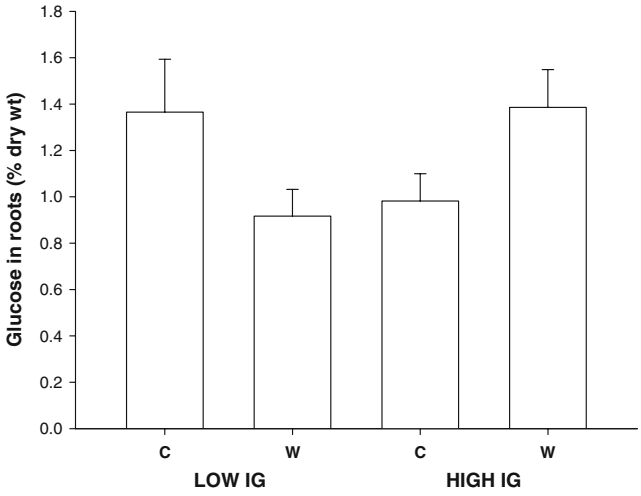

Fig. 2 Effects of plant line (low IG and high IG) and wireworms (*C* without wireworms, *W* with wireworms) on the glucose concentration (mean+SE) in roots of *Plantago lanceolata*. Significant interaction between *Plantago* line and wireworms ($F_{(1,\ 20)}$=6.86, P<0.05), ANOVA

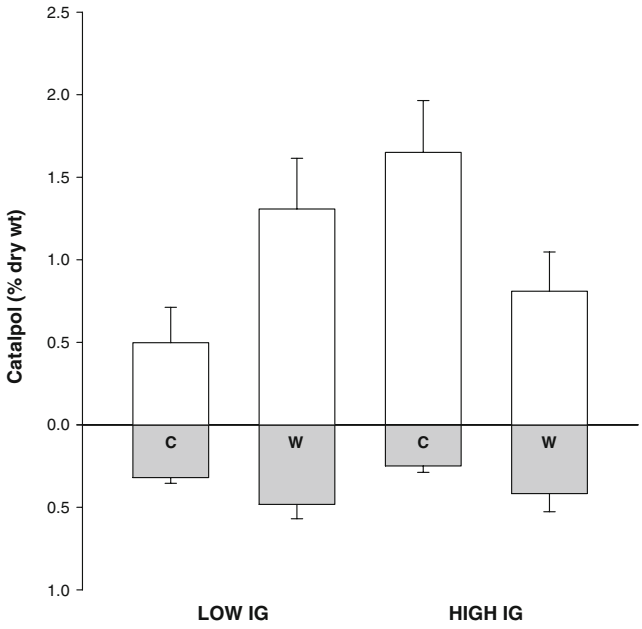

Fig. 3 Effects of plant line (low IG and high IG) and wireworms (*C* without wireworms, *W* with wireworms) on the concentration of catalpol (mean+SE) in shoots (*white*) and roots (*gray*) of *Plantago lanceolata*. For the significant main and interaction effects of ANOVA, see text

Volatiles The RDA showed that the environmental variable "*Plantago* line" explained 14.5% of the total variability in volatile emission (999 Monte Carlo permutations test: F= 6.42, P<0.01; Fig. 4). No effects of wireworms or an interaction between wireworms and *Plantago* line on the volatile blends were detected. Germacrene A and β-elemene were only detected in "high-IG" plants. Cadinene (either the gamma- or the delta-isomer), an unknown sesquiterpene with retention index 1434, and *cis*-3-hexen-1-yl-acetate were omitted in higher quantities from "high-IG" plants. When corrected for leaf biomass, the emission of *cis*-3-hexen-1-yl-acetate and cadinene did not differ between the plant lines, while the unknown sesquiterpene was still emitted in higher quantities from "high-IG" plants.

Harvest 2 (Field Experiment) After more than 3 months in the field, the "low-IG line" and the "high-IG line" did not differ in root (mean=2.12 g, SE=0.07), remaining leaf (mean=1.07 g, SE=0.05), and total biomass (mean=3.19 g, SE=0.10) and were no longer affected by the wireworms. While the number of leaves (mean=24.96, SE=2.03) did not differ between lines, the rosette and average leaf area of plants from the "high-IG line" were 32% and 40% larger than rosette and average leaf area of plants from the "low-IG line," respectively (rosette area: $F_{(1,\ 20)}$=4.44, P<0.05; leaf area: $F_{(1,\ 20)}$=7.38, P<0.05). Total leaf damage (measured as the hole area within the leaf area) of the plants was enhanced in wireworm-induced "low-IG" plants,

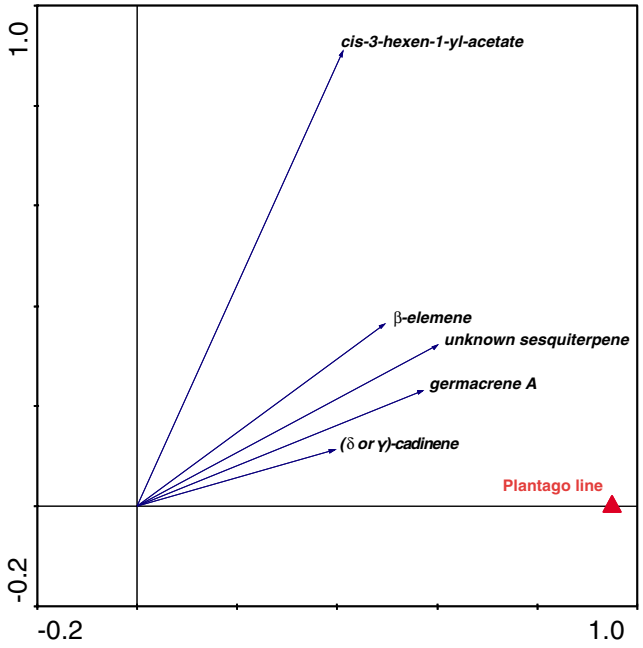

Fig. 4 Species–environment biplot from RDA summarizing differences in the leaf volatile compounds between the *Plantago* lines studied. The substrates shown are well-fitted by the sample scores on the first (*horizontal*) ordination axis, i.e., at least 14.5% of their variability is explained by the *Plantago* line. The second (*vertical*) ordination axis represents the residual variance

but tended to be reduced in "high-IG" plants affected by wireworms (interaction between *Plantago* line and wireworms: $F_{(1,\ 20)}=5.35$, $P<0.05$; Fig. 5). From the added wireworms, 12.5% were recovered as larvae and 4.2% as imagines.

Discussion

Both *Plantago* lines responded to wireworms by increasing the levels of catalpol in roots, thus indicating a defense response upon root herbivory. Induction of secondary metabolites in roots due to root feeding by insect larvae has been reported for a number of plant and herbivore combinations (Birch et al. 1992; Bezemer et al. 2003, 2004; Borowicz et al. 2003; Van Dam and Raaijmakers 2006) and might be a general plant response similar to aboveground induction due to shoot herbivores (Karban and Baldwin 1997). Irrespective of plant line, wireworms had no significant effect on root biomass, but led to an increase in leaf biomass, pointing to compensatory shoot growth (Wurst and Van der Putten 2007). However, the indirect effect of wireworms on leaf chemistry differed between plant lines. While the "low-IG line" showed an increase of catalpol concentration in leaves in response to wireworms, the "high-IG lines" responded with a reduction. By contrast, wireworm feeding led to a reduction of glucose concentration in roots of the "low-IG line," but to an increase of glucose concentration in the roots of the "high-IG line." Whether the contrasting patterns observed for catalpol and glucose are related to a trade-off response in the plants is unclear. The carbon and nitrogen concentrations and the AMF colonization of roots were not affected by wireworms. Consistently, another insect root herbivore (*Otiorhynchus sulcatus*—Coleoptera: Curculionidae) did not affect AMF colonization of roots (Gange et al. 1994; Gange 2001)

The leaf volatile profiles differed between the plant lines, but were not affected by the wireworms. In contrast, Soler et al. (2007) reported changes in leaf volatile profiles of *Brassica nigra* due to root herbivory by *Delia radicum*. The "high-IG line" emitted higher amounts of cadinene and higher amounts of an unknown terpene and the green leaf volatile *cis*-3-hexen-1-yl-actetate. Germacrene A and β-elemene were only detected in the volatile blend of plants from the "high-IG line." The higher emission of the green leaf volatile *cis*-3-hexen-1-yl-acetate and the terpene cadinene could be explained by the greater leaf biomass of the "high-IG line." The other terpenes were emitted only or to a greater extent by the "high-IG" plants, irrespective of the leaf biomass. This might be considered a characteristic trait of the "high-IG line," possibly leading to enhanced indirect defense by attraction of parasitoid or predators. Thus, we found no evidence for a trade-off between direct and indirect defenses in *P. lanceolata*.

Plants from the two lines differed in a number of traits. As expected, noninduced plants of the "high-IG line" contained twofold higher levels of total IG and threefold higher levels of catalpol in the leaves, but levels of these

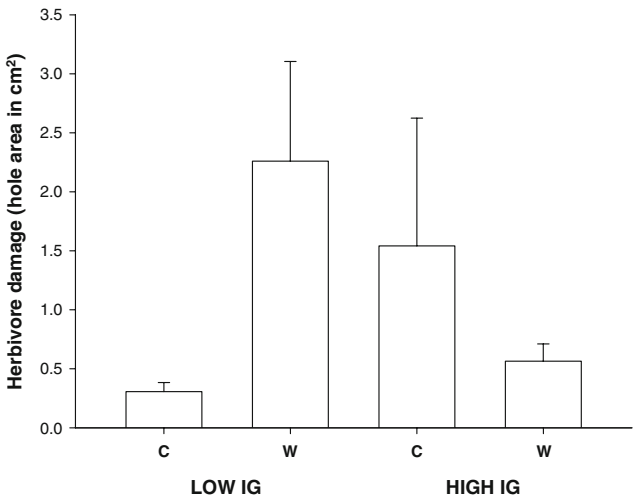

Fig. 5 Effects of plant line (low IG and high IG) and wireworms (*C* without wireworms, *W* with wireworms) on leaf damage by herbivores (mean+SE; measured as total hole area in square centimeter) of *Plantago lanceolata* in the field. Significant interaction between *Plantago* line and wireworms ($F_{(1,\ 20)}=5.35$, $P<0.05$), ANOVA

compounds in roots were similar between lines. Leaf carbon concentration was generally higher in the "high-IG line," while the carbon concentration in roots and the nitrogen concentration in roots and shoots were not affected by the plant line. As in previous studies (Marak et al. 2003), plants from the "high-IG line" produced fewer flower stalks, but produced more root and leaf biomass than plants from the "low-IG line." Former studies also did not detect costs in terms of vegetative biomass for plants producing high levels of IG (Bowers and Stamp 1992; Adler et al. 1995; Marak et al. 2003).

After placing half of the experimental plants for more than 3 months in the field, the root and remaining leaf biomass of the plant lines did not differ and were not affected by the wireworm treatment anymore. However, the rosette area and average leaf area of plants from the "high-IG line" was still greater compared to the "low-IG line." Leaf damage by naturally occurring herbivores was enhanced in wireworm-induced plants from the "low-IG line." Interestingly, aboveground herbivore damage was reduced in wireworm-induced plants from the "high-IG line." During the field experiment, shoot herbivores such as flea beetles, caterpillars, cicadas, and snails were observed on the plants. Since the "low-IG line" increased leaf catalpol concentrations, while the "high-IG line" decreased them after root herbivory, one of the possible explanations for the observed pattern in leaf damage could be the attraction of specialist herbivores to plants containing higher levels of catalpol (Bowers 1983; Bowers and Puttick 1988). For example, flea beetles (*Longitarsus*) are specialist herbivores of plants containing IG (Willinger and Dobler 2001) and might use these compounds as feeding stimulants.

In summary, the two *Plantago* lines examined both showed compensatory leaf growth and enhanced catalpol concentrations in roots in response to wireworm feeding. However, other physiological plant responses to wireworms differed between the "low-IG line" and the "high-IG line." An interaction between *Plantago* line and root herbivory was found for the catalpol concentration in leaves and the glucose concentration in roots. The different responses of the plant lines to root herbivory led to different aboveground defensive phenotypes which herbivores had to deal with. In the field, greater leaf damage was observed on plants with higher leaf catalpol concentration, possibly caused by some specialist herbivores. The present study documents for the first time that intraspecific variation in plant defense can affect the outcome of plant-mediated interactions between root and shoot herbivores.

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