

Effects of Plant Vascular Architecture on Aboveground–Belowground-Induced Responses to Foliar and Root Herbivores on *Nicotiana tabacum*

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Abstract Herbivores induce systemic changes in plant traits, and the strength of these induced responses is often associated with the degree of vascular connectivity that links damaged and undamaged plant tissues. Although this phenomenon is known to occur aboveground in leaves, it is unknown whether or not leaf–root induction similarly follows the vascular architecture of plants. To test for this possibility, we manipulated foliar and root herbivory on tobacco (*Nicotiana tabacum*) by the leaf-chewing insect *Spodoptera exigua* and the root-galling nematode *Meloidogyne incognita*. Subsequent changes in secondary chemistry (alkaloids and phenolics) were measured in leaves and roots that were orthostichous (vertically aligned) and nonorthostichous (opposite) from the herbivore-damaged tissues. Aboveground caterpillar herbivory elicited stronger secondary chemical responses in orthostichous compared with nonorthostichous plant tissues, although the magnitude of this difference was greater in leaves than roots. However, belowground nematode herbivory did not affect the secondary chemistry of tobacco leaves, despite inducing strong local responses in roots. Thus, plant vascular architecture can mediate the magnitude of systemic induction in roots as well as in leaves, with stronger responses in tissues that are more closely aligned. As a result, herbivores

that co-occur on the same sector of plant (both aboveground and belowground) may be more likely to affect one another via induced responses than herbivores that occur on plant tissues sharing fewer resources.

Keywords Aboveground–belowground interactions · Induced plant responses · Orthostichy · Plant sectoriality · Root herbivory · Vascular architecture

Introduction

Herbivore-induced plant responses are often systemic in nature, but their expression is not uniformly distributed across undamaged tissues (Karban and Baldwin 1997; Orians 2005). Instead, herbivore feeding typically elicits a response that varies quantitatively among plant parts, with some leaves responding strongly and others not at all (Stout et al. 1996; Viswanathan and Thaler 2004; Shelton 2005). Such within-plant variation in the magnitude of induction generates fine-scale patterns of plant resistance, and this heterogeneity has important ecological implications for plant–herbivore interactions at large (Denno and McClure 1983; Hunter and Price 1992; Orians et al. 2002).

The most widely cited explanation for leaf-to-leaf variation in the strength of systemic induction is that leaves are differentially interconnected with other leaves via the plant vascular system (Orians 2005). As a result, many phytochemicals, including the signals and resources required for launching plant defense responses, are restricted in their translocation patterns (Davis et al. 1991; Arnold and Schultz 2002; Arnold et al. 2004). Although it has long been known that sectorial transport influences the allocation of carbon (i.e., sugars) between plant sources and sinks (Watson and Casper 1984; Sprugel et al. 1991; Vuorisalo and Hutchings 1996), the

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impact of plant sectoriality on induced metabolic responses to herbivory has only recently been described (Jones et al. 1993; Mutikainen et al. 1996; Orians et al. 2000; Schittko and Baldwin 2003; Viswanathan and Thaler 2004). In these cases, leaves that share the strongest vascular connections (i.e., orthostichous pairs) also tend to transmit the strongest induced responses (Orians 2005; but see Frost et al. 2007 for within-plant volatile signaling).

The consequences of plant vascular architecture for induction have, thus far, only been emphasized in responses that are both elicited and expressed in foliar tissues. In fact, recent studies demonstrate that induced responses span the aboveground and belowground systems (van Dam et al. 2003; Bezemer and van Dam 2005, 2006; Kaplan and Denno 2007). Thus, root herbivory can induce changes in leaf secondary chemistry (Bezemer et al. 2004; Soler et al. 2005; van Dam et al. 2005; van Dam and Raaijmakers 2006; Kaplan et al. 2008), and leaf herbivory can similarly induce changes in root chemistry (Soler et al. 2007). Because resource sharing between leaves and roots is known to be sectorial in certain plants (Orians et al. 2004; Bledsoe and Orians 2006; Zanne et al. 2006), vascular architecture may influence aboveground–belowground induction patterns in a manner analogous to orthostichous leaves. Yet, to our knowledge, this possibility has never been tested.

If leaf–root induction is indeed sectorial, as is often the case with leaves, this has important ecological implications for understanding the causes of heterogeneity in plant defense traits and their extended effects on consumers. Importantly, leaf and root herbivores are known to be patchily distributed in the aboveground and belowground environments, and thus herbivory on only part of the plant is likely to be ubiquitous under natural growing conditions (Denno and McClure 1983; Ettema and Wardle 2002). When root feeders, for example, predominantly damage one sector of a root system (likely given the poor dispersal capabilities of most belowground consumers), then potential indirect effects on foliar herbivores may be restricted to the same sector of plant aboveground. Similarly, foliar herbivores that damage one or a few leaves may be more likely to affect resource quality for belowground consumers that occur on adjacent root tissue, potentially contributing to the inherent patchiness of soil communities (Ettema and Wardle 2002).

We experimentally manipulated foliar and root herbivory on tobacco (*Nicotiana tabacum*) by using a split-root design and quantified the effects of plant vascular architecture on aboveground–belowground-induced chemical responses.

Methods and Materials

Tobacco plants (var. MD 609) were propagated from seed in a greenhouse (seeds were cultivated in a standard potting

mix described below and maintained in a mist room). After 9 week of growth, seedlings were transplanted to pots containing a sterilized growing medium [50% sand, 50% potting mix (SunGro LC1 and professional blend; sphagnum peat moss, bark, perlite, vermiculite, and clay)]. Plants were supplemented with nutrients weekly by applying a soluble fertilizer (20:10:20 NPK) and were maintained at 23–27°C under natural light conditions. A split-root technique was used whereby the roots of all seedlings were divided, and each of the two halves was transplanted into separate, but adjacent, 2-gal pots. Thus, each plant possessed a single root system divided equally between two independent growth environments. Plants were acclimated to these growing conditions for an additional 3 week until use in experiments. At such time, plants remained in the rosette stage of growth and possessed four to five fully expanded leaves and at least five additional developing sink leaves.

Experiment 1—Leaf–Root Vascular Connectivity Vertically aligned leaves and roots are predicted to be more strongly connected via the vascular system than leaves and roots on opposing sides of the plant. To test this hypothesis, we employed a dye tracer technique that used the above described split-root plants (Orians et al. 2000; Viswanathan and Thaler 2004). The first fully expanded leaf on each plant ($N=18$) was excised with a razor blade, and an Eppendorf tube filled with a 0.025% w/v solution of rhodamine-B dye (Sigma, St. Louis, MO, USA) was inserted onto the petiole. After 48 h, the main tap root was dissected by cutting a cross section, and the location of the dye was visually assessed relative to the excised leaf. The intensity of dye staining in the vascular tissue was compared between the root half that occurred closest to the labeled leaf and the remaining half of the root that was opposite from the labeled leaf. We then recorded whether the intensity of vascular dye was greater in the orthostichous root segment, the nonorthostichous root segment, or that there was no difference between the two root segments.

Experiment 2—Aboveground Herbivory Foliar herbivory was manipulated by applying beet armyworm caterpillars, *Spodoptera exigua*, to selected leaves. Beet armyworms are a polyphagous species that are known to feed and induce responses on tobacco (Voelckel and Baldwin 2004). Caterpillars were obtained from a biological supply company (Benzon Research, Inc., Carlisle, PA, USA) and reared on artificial diet until they reached the third instar. Fine-mesh sleeve cages were placed over leaves and fastened to the petiole with a twist tie to ensure that caterpillars only damaged the leaf assigned to the foliar herbivory treatment.

Thirty split-root plants were used; half of these were randomly assigned to caterpillar defoliation ($N=15$), while

the other half served as undamaged controls. Damage regimes were initiated 21 day after seedlings were transplanted into pots and thus in the rosette stage of growth. The first fully expanded leaf that was orthostichous (i.e., vertically aligned) with one of the two root sections (i.e., pots) was assigned to caterpillar damage. Three third-instar *S. exigua* were placed in sleeve cages and remained for 72 h, during which time they removed 20–50% of the leaf area. Control plants also received sleeve cages, but no caterpillars were added. After 48 h (5 day after the initiation of damage regimes) leaves and roots were harvested for analysis of secondary metabolites. This temporal scale is known to coincide with peak concentrations of nicotine and other induced tobacco secondary compounds following caterpillar herbivory (Keinänen et al. 2001).

Two recently expanded sink leaves (i.e., within 1 week of emergence) were collected from each plant—one leaf that was orthostichous with the caterpillar-damaged leaf and a second leaf that was opposite from the damaged leaf. Rosette-stage tobacco plants follow a 3/8 phyllotaxis whereby each new leaf emerges at a 135° angle from the previous leaf (Allard 1942; Jones et al. 1959; Schittko and Baldwin 2003). Therefore, the leaf that is eight positions higher on the plant from the damaged leaf is vertically aligned. Additionally, root tissue (only fine roots) was harvested from both pots in each plant for chemical analysis.

Tissue samples were frozen immediately in liquid N₂. Secondary chemicals with known antiherbivore properties were extracted and analyzed by high-performance liquid chromatography (HPLC; see Keinänen et al. 2001) on a reverse-phase C18 column (Gemini C18, 150×4.6 mm; Phenomenex). Concentrations of identified alkaloids (nicotine) and phenolics (chlorogenic acid and rutin) were quantified by using calibration curves prepared from commercially available standards. Caffeoyl putrescine is not commercially available and was quantified as chlorogenic acid equivalents as described by Keinänen et al. (2001). A further phenolic compound was putatively identified as 7-methyl esculin by comparison with published retention time and mass spectral data (Vereecke et al. 1997). HPLC–mass spectrometry (MS) was performed under identical HPLC conditions as described above, with the exception of trifluoroacetic acid replacing the phosphoric acid in the mobile phase. MS was performed on a Varian 1200 triple quadrupole instrument (Varian, Palo Alto, CA, USA). Under positive electrospray ionization conditions (needle voltage 4.8 kV; capillary voltage 35 V; drying gas 18.5 psi per 200°C, nebulizer gas 51 psi), the compound produced a [M+H]⁺ at *m/z* 355. Collision-induced dissociation at a 10-V collision energy yielded a dominant fragment ion at *m/z* 193 corresponding to the 7-methyl esculin fragment or a loss of a hexose (Vereecke et al. 1997). Concentrations of nonnicotine alkaloids and 7-

methyl esculin were calculated from peak areas at 254 nm (for alkaloids) and 320 nm (for 7-methyl esculin).

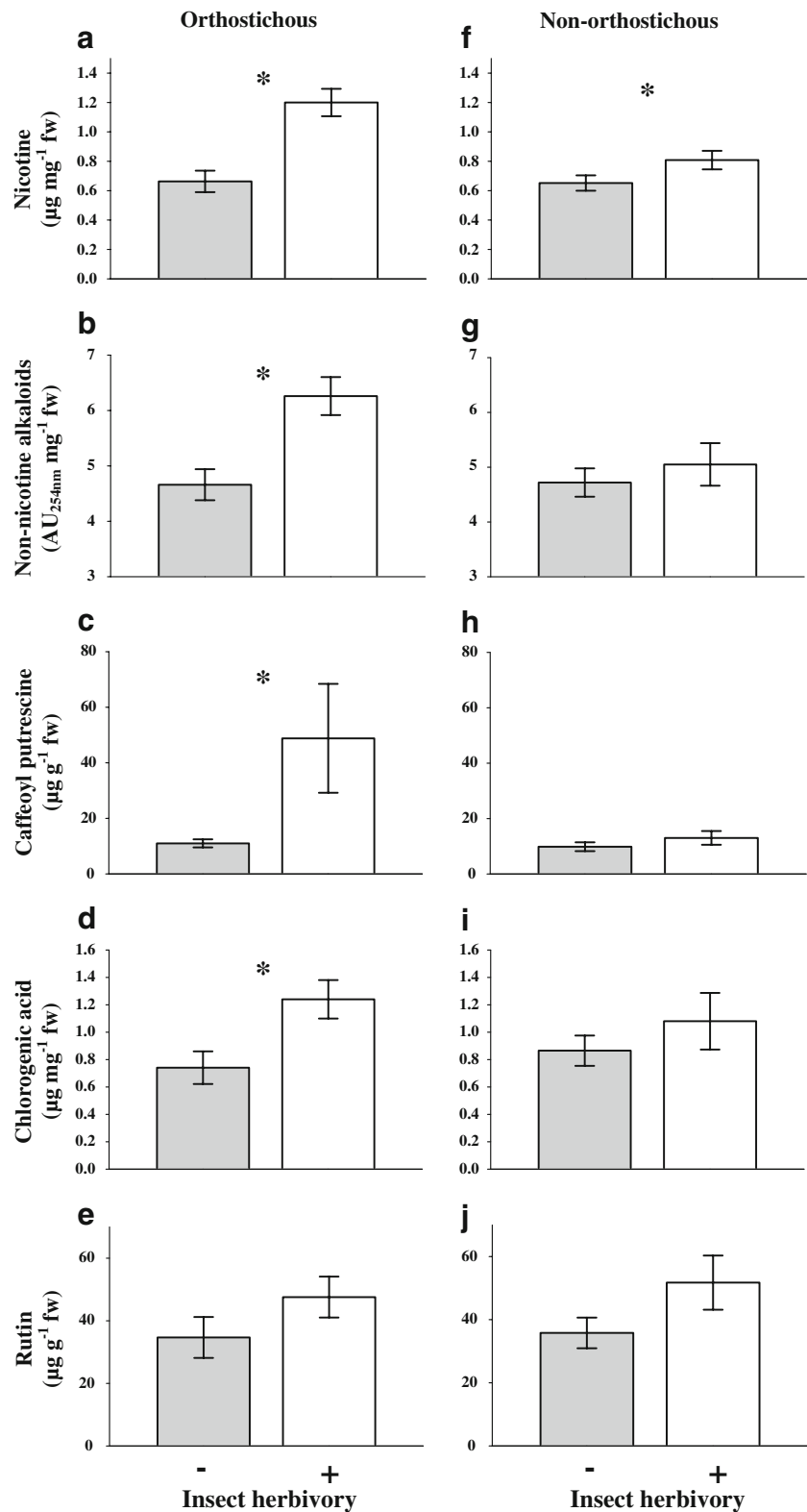
Experiment 3—Belowground Herbivory Root herbivory was manipulated by inoculating tobacco roots with the nematode *Meloidogyne incognita*. This polyphagous species is a sedentary endoparasite that induces a gall at feeding sites (Trudgill and Block 2001). Nematode cultures originated from local agricultural fields and were reared on tobacco prior to use in experiments. Nematode eggs were harvested from the roots of heavily infested plants by using a modified version of the Hussey and Barker (1973) extraction procedure. Galled roots were cut into 2-cm-long segments, placed into a 250-ml flask containing 100 ml of a 0.6% NaOCl dilution, and subsequently poured through nested sieves (250- μ m-pore sieve on top of a 25- μ m-pore sieve). The resulting eggs were counted and applied to the roots of experimentally inoculated seedlings at transplant. Each seedling assigned to the root herbivory treatment received ~100,000 *M. incognita* eggs. This density falls well within the range documented for *M. incognita*-infested tobacco fields (Barker and Lucas 1984) and also corresponds with inoculum levels used in prior studies on *M. incognita*–tobacco interactions (Hanounik and Osbourne 1975, 1977; Barker and Weeks 1991; Wheeler et al. 1991; Vovlas et al. 2004).

Thirty split-root plants were used—half were assigned to the nematode root herbivory treatment (*N*=15), while the other half acted as control plants. On nematode-treated plants, roots from only one of the two pots were inoculated with nematodes. Thus, in the root herbivory treatment, only half of the root system was galled by nematodes—the other half was not. Twenty-one days after nematode addition, plant leaves and roots were harvested for secondary chemical analysis. This time scale was used because *M. incognita* requires at least 3 week to complete gall development, and this also coincides with prior experimental work on nematode-induced responses in tobacco (Kaplan et al. 2008). Two recently expanded sink leaves were harvested from each plant (nematode root herbivory is known to induce secondary chemical changes in tobacco sink leaves; see Kaplan et al. 2008), one leaf that was orthostichous (i.e., vertically aligned) with the pot containing nematode-inoculated roots and a second leaf that was opposite from the inoculated root section. On control plants, the two leaves were chosen to align with each of the two pots as in the nematode treatment. Secondary plant chemicals were quantified in leaf and root tissue samples as described above (see Exp. 2). Roots in each pot were visually inspected to assess the efficacy of treatment and confirmed that nematode-induced galls only occurred on roots that were inoculated (i.e., there was no migration of juveniles between neighboring pots). Moreover, nematode-inoculated roots were heavily galled

and displayed no evidence of strong resource competition. Roots from each pot were dried (at 60°C for 72 h) and weighed to assess the impact of nematode herbivory on belowground biomass.

Statistical Analyses A *G* test was used to determine whether orthostichy affected patterns of dye accumulation in roots (Sokal and Rohlf 1994). To assess the impact of plant vascular architecture on induced responses to foliar and root herbivory,

Fig. 1 Effects of aboveground caterpillar herbivory on the expression of secondary plant chemicals in orthostichous (*A–E*) and nonorthostichous (*F–J*) tobacco leaves, including nicotine, nonnicotine alkaloids, caffeoyl putrescine, chlorogenic acid, and rutin (means±SE). Asterisks denote significant differences between means ($P<0.05$)



we used multivariate analysis of variance (MANOVA; Proc glm; statistical analyses were performed using SAS, version 9.1; SAS Institute, Inc. 2001), followed by univariate analyses of variance for each secondary chemical measured (Proc mixed). Separate analyses were performed to quantify the impact of aboveground caterpillar herbivory on orthostichous and nonorthostichous leaves and roots, as well as the effect of belowground nematode herbivory on orthostichous and nonorthostichous leaves and roots. Blocks (i.e., spatial groupings of control and herbivory plants) were considered as a random effect in the model. Because the position of the caterpillar-damaged leaf in Experiment 2 varied along the stem (i.e., we were restricted to using the leaf that was growing directly above one of the two pots), we used regression analyses (Proc glm) with leaf number as the predictor variable and root chemistry as the response variable to test for potential relationships between leaf position and belowground induction. *T* tests were used to compare root dry mass on plants with and without nematodes. Data were transformed (square root and log transformations) as needed to meet assumptions of normality and homogeneity of variances.

To compare directly the overall magnitude of secondary chemical induction in orthostichous vs. nonorthostichous plant tissues, effect sizes were calculated (Hedges' *d*) for the impact of caterpillar herbivory on leaf and root chemistry, and the effect of nematode herbivory on leaf chemistry (Rosenberg et al. 2000). Mixed-effects categorical models were then used to compare effect sizes in orthostichous and nonorthostichous plant tissues. For each group, a mean effect

size was calculated and reported with 95% bootstrap confidence intervals. Effect sizes greater than zero indicate that herbivory elicited an overall increase in the concentration of secondary plant chemicals. Between-group heterogeneity (Q_B) was tested against a χ^2 distribution to determine whether plant vascular architecture affected the magnitude of aboveground and belowground induction.

Results

The appearance of dye in tobacco roots was affected strongly by leaf position ($G=24.95$, $df=1$, $P<0.001$). In all cases ($N=18$), greater dye accumulation was found in roots that were vertically aligned with the labeled leaf than in roots that were opposite.

Foliar herbivory elevated the secondary chemistry of tobacco leaves that were both orthostichous (Figs. 1 A–E) and nonorthostichous (Figs. 1 F–J) with the caterpillar-damaged leaf (Table 1). However, the impact of induction was more apparent in leaves sharing strong vascular connections. Four of the five secondary chemicals measured (nicotine, nonnicotine alkaloids, caffeoyl putrescine, and chlorogenic acid) were induced to higher levels in orthostichous leaves (Figs. 1 A–D), compared with only one of five (nicotine) in nonorthostichous leaves (Fig. 1 F).

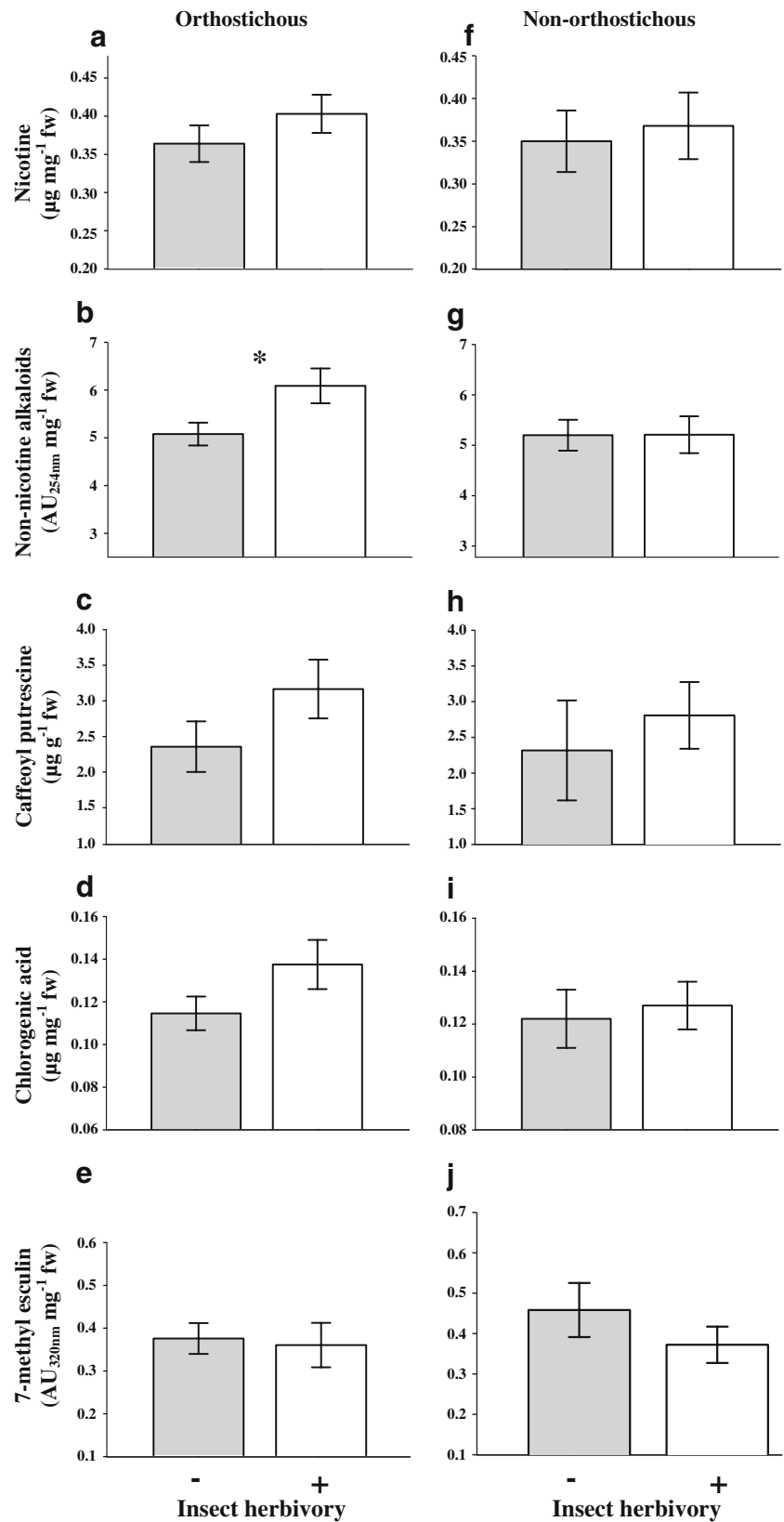
Similar results were found when comparing the effects of aboveground herbivory on root chemistry in vertically

Table 1 The effects of aboveground and belowground herbivory on the expression of secondary plant chemicals in orthostichous and nonorthostichous leaves and roots of tobacco

Herbivory	Tissue	Compound	Orthostichous			Nonorthostichous		
			<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
(A) Caterpillar aboveground	Leaf	MANOVA	5, 22	8.98	<0.001	5, 24	3.02	0.030
		Nicotine	1, 12	19.43	<0.001	1, 14	7.83	0.014
		Nonnicotine alkaloids	1, 12	13.65	0.003	1, 14	0.79	0.389
		Caffeoyl putrescine	1, 12	9.09	0.011	1, 14	0.99	0.336
		Chlorogenic acid	1, 12	7.19	0.020	1, 14	0.11	0.750
		Rutin	1, 12	2.32	0.154	1, 14	2.49	0.137
(B) Caterpillar aboveground	Root	MANOVA	5, 23	2.33	0.075	5, 18	0.63	0.681
		Nicotine	1, 13	1.88	0.194	1, 10	0.07	0.791
		Nonnicotine alkaloids	1, 13	9.09	0.010	1, 10	0.00	0.979
		Caffeoyl putrescine	1, 13	2.64	0.128	1, 10	0.72	0.415
		Chlorogenic acid	1, 13	4.01	0.066	1, 10	0.09	0.765
		7-methyl esculin	1, 13	0.48	0.499	1, 10	4.33	0.064
(C) Nematode belowground	Leaf	MANOVA	5, 23	0.89	0.505	5, 23	0.48	0.784
		Nicotine	1, 13	1.29	0.276	1, 13	0.71	0.416
		Nonnicotine alkaloids	1, 13	0.01	0.935	1, 13	0.09	0.769
		Caffeoyl putrescine	1, 13	0.01	0.910	1, 13	0.00	0.957
		Chlorogenic acid	1, 13	0.05	0.821	1, 13	0.55	0.470
		Rutin	1, 13	1.43	0.254	1, 13	0.27	0.609

Significant effects ($P<0.05$) are bolded for emphasis

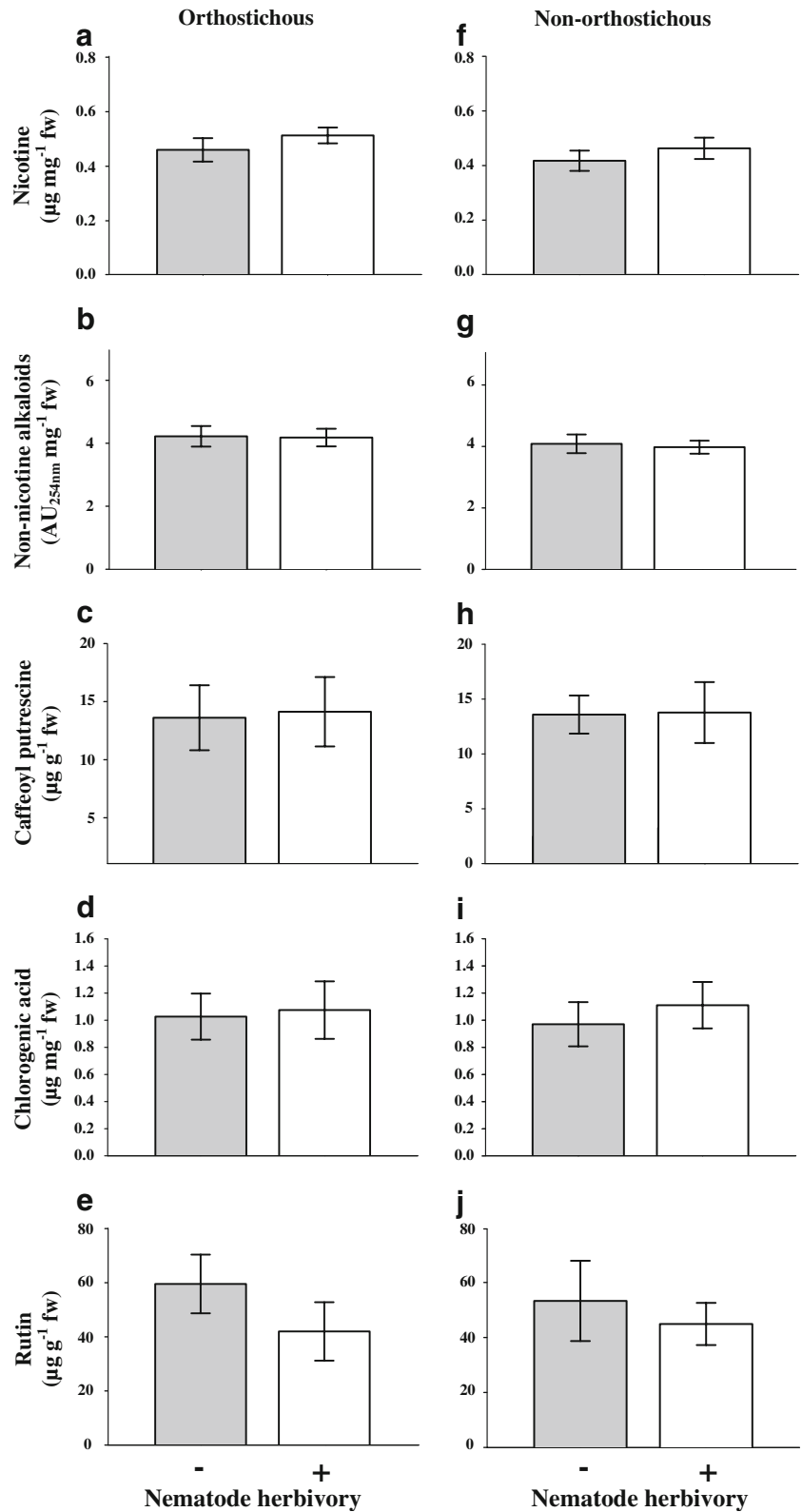
Fig. 2 Effects of aboveground caterpillar herbivory on the expression of secondary plant chemicals in orthostichous (*A–E*) and nonorthostichous (*F–J*) tobacco roots, including nicotine, nonnicotine alkaloids, caffeoyl putrescine, chlorogenic acid, and 7-methyl esculin (means±SE). Asterisk denotes significant differences between means ($P<0.05$)



aligned (Figs. 2 A–E) vs. opposing (Figs. 2 F–J) root sections (Table 1). Caterpillar feeding elevated the below-ground concentration of certain compounds (e.g., non-nicotine alkaloids; Fig. 2 B), and there was a trend for

herbivory to affect overall secondary chemical expression in orthostichous roots (MANOVA: $P=0.075$). In contrast, aboveground induction did not affect the chemistry of roots that were opposite from the insect-defoliated leaf (Figs. 2

Fig. 3 Effects of belowground nematode herbivory on the expression of secondary plant chemicals in orthostichous (A–E) and nonorthostichous (F–J) tobacco leaves, including nicotine, nonnicotine alkaloids, caffeoyl putrescine, chlorogenic acid, and rutin (means±SE)



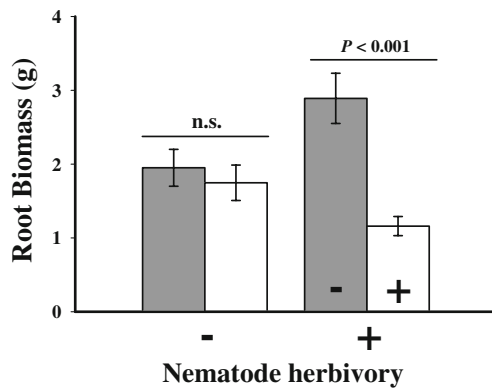


Fig. 4 The impact of nematode root herbivory on belowground plant biomass. The bars on the right denote plants that were experimentally inoculated with nematodes, whereas the group of bars to the left represent plants that were not exposed to nematode herbivory. A positive sign indicates which of the two split-root sections received nematodes. *n.s.* = nonsignificant

F–J). Moreover, there was no relationship for either root type between the position (age) of the caterpillar-damaged leaf and root chemistry ($P > 0.1$ for all secondary metabolites measured).

Nematode root herbivory had no impact on leaf chemistry in either of the two leaf tissue types (Table 1), with nonsignificant effects of nematodes on secondary compounds in both orthostichous (Figs. 3 A–E) and nonorthostichous (Figs. 3 F–J) leaves. However, nematodes induced local changes (i.e., those measured in the roots of pots inoculated with nematodes) in the secondary chemistry of tobacco roots (MANOVA: $F_{5, 18} = 9.24$, $P < 0.001$), including nicotine ($F_{1, 22} = 19.54$, $P < 0.001$), nonnicotine alkaloids ($F_{1, 22} = 30.93$, $P < 0.001$), caffeoyl putrescine ($F_{1, 22} = 37.14$, $P < 0.001$), chlorogenic acid ($F_{1, 22} = 25.56$, $P < 0.001$), and 7-methyl esculin ($F_{1, 22} = 37.20$, $P < 0.001$). In all cases, the concentrations of secondary chemicals were higher in the roots of nematode-damaged plants.

Although nematodes did not affect total root biomass (i.e., pooled belowground dry weights from both split-root

sections; $F_{1, 28} = 0.49$, $P = 0.489$), the effects of nematode herbivory on roots were apparent within each split-root pot (Fig. 4). The mass of the two root sections in control pots were not different from one another ($t = 0.60$, $P = 0.555$), but root mass in nematode-inoculated pots was >50% less than in the opposite pot of the same plant ($t = 4.69$, $P < 0.001$). This effect was driven partly by nematodes reducing root biomass relative to control plants (average control pot = 1.85 ± 0.18 g, nematode-inoculated pot = 1.16 ± 0.13 ; $F_{1, 28} = 10.00$, $P = 0.004$) and also by plants compensating for nematode herbivory by increasing root biomass in the nematode-free pot of plants assigned to root herbivory (average control pot = 1.85 ± 0.18 g, nematode-free pot of root herbivory plants = 2.89 ± 0.34 g; $F_{1, 28} = 7.36$, $P = 0.011$).

The overall magnitude of phytochemical induction differed between orthostichous and nonorthostichous plant tissues for one of the three comparisons, i.e., the impact of caterpillar herbivory on leaf chemistry ($Q_B = 4.90$, $df = 1$, $P = 0.027$). In this case, the effect size for responses in the orthostichous leaf was >2× as large as in the nonorthostichous leaf (Fig. 5 A). The difference in root responses to foliar herbivory was nearly significant ($Q_B = 2.90$, $df = 1$, $P = 0.089$), with moderately strong responses in vertically aligned roots (effect size = 0.45) and virtually no response in opposing root sections (effect size = 0.02; Fig. 5 B). However, aboveground responses to root herbivory were equally weak in both leaf tissue types ($Q_B = 0.03$, $df = 1$, $P = 0.864$; Fig. 5 C).

Discussion

Vascular architecture mediates herbivore-induced plant responses that are both elicited and expressed in leaves, but whether vascular connections similarly shape patterns of induction linking leaves with roots is unknown. In agreement with previously published accounts (Orians et al. 2000; Schittko and Baldwin 2003; Viswanathan and Thaler

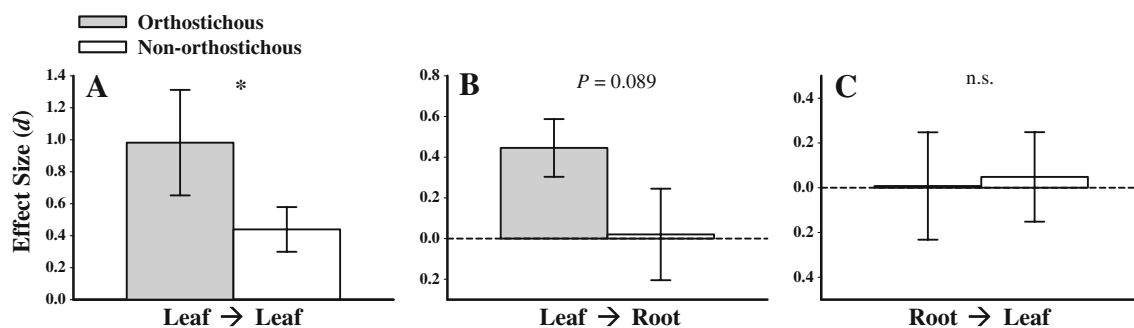


Fig. 5 The magnitude of herbivore-induced responses in orthostichous vs. nonorthostichous plant tissues, including (A) leaf responses to foliar herbivory, (B) root responses to foliar herbivory, and (C) leaf responses to root herbivory (means \pm 95% bootstrap confidence

intervals). Effect sizes (Hedges' d) were calculated to summarize the cumulative secondary chemical response to herbivory in leaves and roots. Asterisk denotes significant differences between means ($P < 0.05$). *n.s.* = nonsignificant

2004), we found that the magnitude of phytochemical induction by foliar herbivory was most pronounced in leaves that share strong vascular connections with the caterpillar-damaged leaf (Figs. 1 and 5 A). However, we also document the novel finding that vascular architecture can similarly mediate the effects of foliar herbivory on certain root chemicals (e.g., nonnicotine alkaloids), with stronger induction in roots that are vertically aligned with insect-defoliated leaves (Figs. 2 and 5 B).

Because studies that measure the impact of leaf herbivory on root chemistry do not account for vascular architecture, whereas studies on aboveground induced responses often do, the magnitude of root responses to foliar induction may be underestimated in the literature. For example, in a recently published meta-analysis that merged data from multiple independent studies, evidence was found that foliar induction elicits a relatively weak response in root tissue (Kaplan et al. 2008). This outcome may in part be driven by the lack of control over leaf–root connectivity in previous studies. For this reason, we urge investigators in future studies of aboveground–belowground induction to consider plant sectoriality in the design of experiments. However, leaf responses to foliar herbivory were nevertheless stronger than root responses in the present study, despite controlling for vascular architecture in both cases (leaf–leaf effect size = +0.982, leaf–root effect size = +0.446). Also, the ontogenetic stage of belowground tissues (e.g., fine vs. tap roots) is likely to affect whether or not induced responses are detected in roots (Van Dam and Raaijmakers 2006).

Despite the fact that our experiments support the hypothesis that leaf–root induction follows the vascular architecture of plants, our results also indicate that this phenomenon is more apparent and potentially more important aboveground. Caterpillar damage induced higher concentrations of four of five secondary chemicals measured in orthostichous leaves (compared with only one of five in nonorthostichous leaves), whereas one of five chemicals were induced to higher levels in orthostichous roots (compared with zero of five in nonorthostichous roots). As a result, the overall magnitude of sectorial induction was significant among leaves ($P=0.027$) but nonsignificant for roots ($P=0.089$).

Surprisingly, root herbivory did not alter the subset of secondary chemicals that we measured in tobacco leaves (Fig. 3), making it difficult to evaluate the consequences of vascular architecture (Fig. 5C). This outcome was unexpected because in prior experiments strong foliar responses to nematode (*M. incognita*) root herbivory were documented with the same tobacco system and under similar environmental and ecological conditions (Kaplan et al. 2008). More specifically, we previously found that nematodes induced a 40% reduction in leaf nicotine and elevated foliar concentrations of phenolics and diterpene glycosides

(two to four times) above control levels. Thus, nematode herbivory in only half of the root system appears to elicit very different aboveground responses when compared with plants experiencing widespread herbivory distributed throughout the entire root system. The quantity of tissue damaged affects the strength of herbivore-induced responses in leaves (see Table 4.5 in Karban and Baldwin 1997). Therefore, lower levels of root herbivory may lead to lower levels of aboveground induction. Because nematodes damage alkaloid biosynthetic sites and interfere with foliar expression (Hanounik and Osborne 1977; Barker and Weeks 1991; Kaplan et al. 2008), another potential explanation is that plants compensate for root damage on one side of the plant by increasing alkaloid production on the other undamaged side (Fig. 4). Moreover, compensatory root growth may come at the expense of foliar chemical responses if plants are limited in their allocation of resources towards growth and defense.

Foliar and root herbivores are often patchily distributed on plant leaves and roots (Denno and McClure 1983; Ettema and Wardle 2002). As a result, their systemic effects on plant defense traits are likely to be heterogeneous. Given the recent emphasis on linkages between aboveground and belowground biota (Wardle et al. 2004; Wardle 2002), our findings provide new mechanistic insight into the ecological circumstances in which foliar and root herbivores affect both plant chemistry and potentially other co-occurring herbivores. Additional studies are needed to assess the relative importance of sectorial induction between leaves and other plant tissues.

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References

- ALLARD, H. A. 1942. Some aspects of the phyllotaxy of tobacco. *J. Agric. Res.* 64:49–55.
- ARNOLD, T. M., and SCHULTZ, J. C. 2002. Induced sink strength as a prerequisite for induced tannin biosynthesis in developing leaves of *Populus*. *Oecologia* 130:585–593.
- ARNOLD, T., APPEL, H., PATEL, V., STOCUM, E., KAVALIER, A., and SCHULTZ, J. C. 2004. Carbohydrate translocation determines the phenolic content of *Populus* foliage: a test of the sink-source model of plant defense. *New Phytol.* 164:157–164.
- BARKER, K. R., and LUCAS, G. B. 1984. Nematode parasites of tobacco, pp. 213–242, in W. R. Nickle (ed.). *Plant and Insect Nematodes* Marcel Dekker, New York.
- BARKER, K. R., and WEEKS, W. W. 1991. Relationships between soil and levels of *Meloidogyne incognita* and tobacco yield and quality. *J. Nematol.* 23:82–90.
- BEZEMER, T. M., and VAN DAM, N. M. 2005. Linking aboveground and belowground interactions via induced plant defenses. *Trends Ecol. Evol.* 20:617–624.

- BEZEMER, T. M., WAGENAAR, R., VAN DAM, N. M., VAN DER PUTTEN, W. H., and WÄCKERS, F. L. 2004. Above- and below-ground terpenoid aldehyde induction in cotton, *Gossypium herbaceum*, following root and leaf injury. *J. Chem. Ecol.* 30:53–67.
- BLEDSE, T. M., and ORIANS, C. M. 2006. Vascular pathways constrain ^{13}C accumulation in large root sinks of *Lycopersicon esculentum* (Solanaceae). *Amer. J. Bot.* 93:884–890.
- DAVIS, J. M., GORDON, M. P., and SMIT, B. A. 1991. Assimilate movement dictates remote sites of wound-induced gene expression in poplar leaves. *Proc. Natl. Acad. Sci. U. S. A.* 88:2393–2396.
- DENNO, R. F., and MCCLURE, M. S. 1983. Variable Plants and Herbivores in Natural and Managed Systems. Academic, New York.
- ETTEMA, C. H., and WARDLE, D. A. 2002. Spatial soil ecology. *Trends Ecol. Evol.* 17:177–183.
- FROST, C. J., APPEL, H. M., CARLSON, J. E., DE MORAES, C. M., MESCHER, M. C., and SCHULTZ, J. C. 2007. Within-plant signaling via volatiles overcomes vascular constraints on systemic signaling and primes responses against herbivores. *Ecol. Lett.* 10:490–498.
- HANOUNIK, S. B., and OSBORNE, W. W. 1975. Influence of *Meloidogyne incognita* on the content of amino acids and nicotine in tobacco grown under gnotobiotic conditions. *J. Nematol.* 7:332–336.
- HANOUNIK, S. B., and OSBORNE, W. W. 1977. The relationship between population density of *Meloidogyne incognita* and nicotine content of tobacco. *Nematologica* 23:147–152.
- HUNTER, M. D., and PRICE, P. W. 1992. Playing chutes and ladders—heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology* 73:724–732.
- HUSSEY, R. S., and BARKER, K. R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Rep.* 57:1025–1028.
- JONES, C. G., HOPPER, R. F., COLEMAN, J. S., and KRISCHIK, V. A. 1993. Control of systemically induced herbivore resistance by plant vascular architecture. *Oecologia* 93:452–456.
- JONES, H., MARTIN, R. V., and PORTER, H. K. 1959. Translocation of ^{14}C carbon in tobacco following assimilation of ^{14}C carbon dioxide by a single leaf. *Ann. Bot. London* 23:493–510.
- KAPLAN, I., and DENNO, R. F. 2007. Interspecific interactions in phytophagous insects revisited: a quantitative assessment of competition theory. *Ecol. Lett.* 10:977–994.
- KAPLAN, I., HALITSCHKE, R., KESSLER, A., SARDANELLI, S., and DENNO, R. F. 2008. Constitutive and induced defenses to herbivory in above- and belowground plant tissues. *Ecology* 89:392–406.
- KARBAN, R., and BALDWIN, I. T. 1997. Induced Responses to Herbivory. The University of Chicago Press, Chicago.
- KEINÄNEN, M., OLDHAM, N. J., and BALDWIN, I. T. 2001. Rapid HPLC screening of jasmonate-induced increases in tobacco alkaloids, phenolics, and diterpene glycosides in *Nicotiana attenuata*. *J. Agric. Food Chem.* 49:3553–3558.
- MUTIKAINEN, P., WALLS, M., and OVASKA, J. 1996. Herbivore-induced resistance in *Betula pendula*: the role of plant vascular architecture. *Oecologia* 108:723–727.
- ORIANS, C. 2005. Herbivores, vascular pathways, and systemic induction: facts and artifacts. *J. Chem. Ecol.* 31:2231–2242.
- ORIANS, C. M., POMERLEAU, J., and RICCO, R. 2000. Vascular architecture generates fine scale variation in systemic induction of proteinase inhibitors in tomato. *J. Chem. Ecol.* 26:471–485.
- ORIANS, C. M., ARDON, M., and MOHAMMAD, B. A. 2002. Vascular architecture and patchy nutrient availability generates within-plant heterogeneity in plant traits important to herbivores. *Am. J. Bot.* 89:270–278.
- ORIANS, C. M., VAN VUUREN, M. M. I., HARRIS, N. L., BABST, B. A., and ELLMORE, G. S. 2004. Differential sectoriality in long-distance transport in temperate tree species: evidence from dye flow, ^{15}N transport, and vessel element pitting. *Trees* 18:501–509.
- ROSENBERG, M. S., ADAMS, D. C., and GUREVITCH, J. 2000. MetaWin: Statistical Software for Meta-analysis, Version 2.0. Sinauer, Sunderland.
- SCHITTKO, U., and BALDWIN, I. T. 2003. Constraints to herbivore-induced systemic responses: bidirectional signaling along orthostichies in *Nicotiana attenuata*. *J. Chem. Ecol.* 29:763–770.
- SHELTON, A. L. 2005. Within-plant variation in glucosinolate concentrations of *Raphanus sativus* across multiple scales. *J. Chem. Ecol.* 31:1711–1732.
- SOKAL, R. R., and ROHLF, F. J. 1994. Biometry. Freeman, New York.
- SOLER, R., BEZEMER, T. M., VAN DER PUTTEN, W. H., VET, L. E. M., and HARVEY, J. A. 2005. Root herbivore effects on above-ground herbivore, parasitoid and hyperparasitoid performance via changes in plant quality. *J. Anim. Ecol.* 74:1121–1130.
- SOLER, R., BEZEMER, T. M., CORTESERO, A. M., VAN DER PUTTEN, W. H., VET, L. E. M., and HARVEY, J. A. 2007. Impact of foliar herbivory on the development of a root-feeding insect and its parasitoid. *Oecologia* 152:257–264.
- SPRUGEL, D. G., HINCKLEY, T. M., and SCHAAP, W. 1991. The theory and practice of branch autonomy. *Annu. Rev. Ecol. Syst.* 22:309–334.
- STOUT, M. J., WORKMAN, K. V., and DUFFEY, S. S. 1996. Identity, spatial distribution, and variability of induced chemical responses in tomato plants. *Entomol. Exp. Appl.* 79:255–271.
- TRUDGILL, D. L., and BLOK, V. C. 2001. Apomictic, polyphagous root-knot nematodes: exceptionally successful and damaging biotrophic root pathogens. *Annu. Rev. Phytopath.* 39:53–77.
- VAN DAM, N. M., and BEZEMER, T. M. 2006. Chemical communication between roots and shoots: towards an integration of aboveground and belowground induced responses in plants, pp. 127–143, in M. Dicke, and W. Takken (eds.). Chemical Ecology: from Gene to EcosystemSpringer, Dordrecht.
- VAN DAM, N. M., and RAAIJMAKERS, C. E. 2006. Local and systemic induced responses to cabbage root fly larvae (*Delia radicum*) in *Brassica nigra* and *B. oleracea*. *Chemoecology* 16:17–24.
- VAN DAM, N. M., HARVEY, J. A., WÄCKERS, F. L., BEZEMER, T. M., VAN DER PUTTEN, W. H., and VET, L. E. M. 2003. Interactions between aboveground and belowground induced responses against phytophages. *Basic Appl. Ecol.* 4:63–77.
- VAN DAM, N. M., RAAIJMAKERS, C. E., and VAN DER PUTTEN, W. H. 2005. Root herbivory reduces growth and survival of the shoot feeding specialist *Pieris rapae* on *Brassica nigra*. *Entomol. Exp. Appl.* 115:161–170.
- VERECKE, D., MESSENS, E., KLARSKOV, K., DE BRUYN, A., VAN MONTAGU, M., and GOETHALS, K. 1997. Patterns of phenolic compounds in leafy galls of tobacco. *Planta* 201:342–348.
- VISWANATHAN, D. V. and THALER, J. S. 2004. Plant vascular architecture and within-plant spatial patterns in resource quality following herbivory. *J. Chem. Ecol.* 30:531–543.
- VOELCKEL, C., and BALDWIN, I. T. 2004. Generalist and specialist lepidopteran larvae elicit different transcriptional responses in *Nicotiana attenuata*, which correlate with larval FAC profiles. *Ecol. Lett.* 7:770–775.
- VOVLAS, N., SIMOES, N. J. O., SASANELLI, N., DOS SANTOS, M. C. V., and ABRANTES, I. M. D. 2004. Host-parasite relationships in tobacco plants infected with a root-knot nematode (*Meloidogyne incognita*) population from the Azores. *Phytoparasitica* 32:167–173.

- VUORISALO, T., and HUTCHINGS, M. J. 1996. On plant sectoriality, or how to combine the benefits of autonomy and integration. *Vegetatio* 127:3–8.
- WARDLE, D. A. 2002. *Communities and Ecosystems: Linking the Aboveground and Belowground Components*. Princeton University Press, Princeton.
- WARDLE, D. A., BARDGETT, R. D., KLIRONOMOS, J. N., SETÄLÄ, H. H., VAN DER PUTTEN, W. H., and WALL, D. H. 2004. Ecological linkages between aboveground and belowground biota. *Science* 304:1629–1633.
- WATSON, M. A., and CASPER, B. B. 1984. Morphogenetic constraints on patterns of carbon distribution in plants. *Annu. Rev. Ecol. Syst.* 15:233–258.
- WHEELER, T. A., BARKER, K. R., and SCHNEIDER, S. M. 1991. Yield-loss models for tobacco infected with *Meloidogyne incognita* as affected by soil moisture. *J. Nematol.* 23: 365–371.
- ZANNE, A. E., LOWER, S. S., CARDON, Z. G., and ORIANI, C. M. 2006. ^{15}N partitioning in tomato: vascular constraints versus tissue demand. *Funct. Plant Biol.* 33:457–464.