In Defense of Roots: A Research Agenda for Studying Plant Resistance to Belowground Herbivory[1]

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Interest in root biology research is experiencing a dramatic increase. From a microphytocentric perspective, the availability of Arabidopsis (*Arabidopsis thaliana*) mutants, along with a sequenced genome, has led to valuable insights into root biochemistry, development, and other functions (Flores et al., 1999; D'Auria and Gershenzon, 2005). From an ecological perspective, belowground processes are now recognized as essential components of ecosystem productivity and stability (Van der Putten, 2003; Wardle et al., 2004). The surface area of roots can far exceed that of aerial parts, thus providing tremendous resources for microbes, nematodes, and arthropods in the soil, and these organisms are now recognized as drivers of plant diversity and ecosystem functioning (De Deyn and Van der Putten, 2005). Here we address one of the most neglected aspects of root biology, namely, the chemistry of plant defense against belowground herbivores. Focusing on secondary compounds present in roots and drawing some parallels with aboveground plant tissues, we outline preliminary patterns in root defense and propose a research agenda for future work.

BACKGROUND ON BELOWGROUND HERBIVORY

Root-feeding insects play an important role in both agricultural and natural ecosystems (Blossey and Hunt-Joshi, 2003; Wardle et al., 2004). Through physiological and physical changes of roots, belowground herbivores have the potential to shape plant communities (De Deyn et al., 2003), belowground microorganism and macroorganism communities (Wardle, 2006), as well as aboveground arthropod communities (Bezemer and van Dam, 2005). When attacked by herbivores, plants defend themselves through a continuum of defensive strategies, including direct resistance (chemical or mechanical traits that reduce herbivory), indirect resistance (traits enhancing the action of enemies of herbivores), or tolerance (i.e. regrowth). Although most of the theory developed to predict variation in plant defense investment was based on interactions aboveground, relatively little attention has been paid to similar interactions belowground. Is this due to a general bias against studies on underground herbivory (out of sight, out of mind?), or is belowground herbivory relatively negligible compared with aboveground herbivory?

Although there are currently no global comparisons of aboveground versus belowground herbivory, the few studies that have simultaneously examined their relative impact indicate that belowground herbivory rivals that of aboveground damage in terms of effects on plant fitness (Brown and Gange, 1989; Maron, 1998). For example, the per area biomass of cicadas (root xylem feeders) in eastern deciduous forests of North America is the highest of any terrestrial animal and their damage is substantial enough to reduce tree growth (Karban, 1980). To assess diversity of belowground feeders, we conducted a survey of all North American families of insects (Borror et al., 1989). Only 17% of the 257 families with herbivorous species (as adults or juveniles) contain species of root feeders (including chewers, sap suckers, and gall makers), compared with 92% of the families containing aboveground herbivores (including leaf, stem, flower, and seed feeders, gall makers, and miners, but not including flower visitors such as honeybees or other pollinators). We are not aware of estimates of the relative abundance of belowground and aboveground herbivores. Thus, we tentatively conclude that although root feeders may be less diverse than aboveground feeders, their impacts appear to be quite strong. Greater attention needs to be paid to the relative abundances of aboveground and belowground herbivores, and the fitness consequences of herbivory on different plant parts, separating roots, shoots, and reproductive structures.

CHEMICAL DEFENSES IN ROOTS

It is currently unclear if there are patterns associated with phytochemical investment aboveground versus belowground. In Table I we present an initial survey of major compound classes that are known to be effective defenses against herbivores. We focused primarily on studies that presented both root and shoot chemistry simultaneously from undamaged plants.

Table I. *Concentrations of defensive phytochemical compounds in roots and shoots from studies that simultaneously investigated both plant parts*

Although not comprehensive, we attempted to broadly survey plant families and classes of chemical compounds. Data are mostly from mature leaf and root tissues.

Family	Plant Species	Compound	Root	Shoot	Unit	R/S[a]	Citation
Apiaceae	Wild parsnip	Furanocoumarin	0.02	0.52	μg/mg dry wt	0.04	Zangerl and Rutledge (1996)
Apocynaceae	*Asclepias nivea*	Cardenolide	0.26	0.48	μg/mg dry wt	0.53	S. Rasmann and A.A. Agrawal (unpublished data)
Asteraceae	*S. jacobaea*	Pyrrolizidine alkaloid	0.88	1.68	mg/g dry wt	0.53	Hol et al. (2004)
	Taraxacum officinale	Triterpene	0.29	0.59	% of mg dry wt	0.50	Akashi et al. (1994)
Betulaceae	*Betula papyrifera*	Phenolic	1.08	1.21	mg/g dry wt	0.89	Mattson et al. (2005)
Brassicaceae	Arabidopsis	Glucosinolate	22.52	19.40	μmol/g dry wt	1.16	Petersen et al. (2002)
	Barbarea vulgaris	Glucosinolate	33.17	33.33	% mol dry wt	1.00	Agerbirk et al. (2003)
	B. oleracea	Glucosinolate	14.63	4.88	μmol/g dry wt	3.00	van Dam et al. (2004)
	Radish	Glucosinolate	0.48	0.26	μmol/g fresh wt	1.86	Hara et al. (2000)
Chenopodiaceae	Spinach	Phytoecdysteroid	38.79	124.94	μg/g dry wt	0.31	Schmelz et al. (1999)
Euphorbiaceae	Cassava	Cyanogenic glucoside	77.63	0.77	μmol/g fresh wt	101.02	Jorgensen et al. (2005)
Fabaceae	*Lupinus argenteus*	Alkaloid	0.47	3.26	mg/g dry wt	0.14	Adler and Wink (2001)
Malvaceae	*Gossypium herbaceum*	Terpenoid aldehyde	8,460.10	149.44	μg/g dry wt	56.61	Bezemer et al. (2004)
Orchidaceae	*Phalaenopsis hybrids*	Pyrrolizidine alkaloid	28.00	10.50	mg/g fresh wt	2.67	Frolich et al. (2006)
Pinaceae	*Picea sitchensis*	Polyphenol	5.20	4.00	% g dry wt	1.30	Wainhouse et al. (1998)
	P. sitchensis	Resin	2.10	3.70	% g dry wt	0.57	
	P. sitchensis	Tannin	10.49	4.87	% g dry wt	2.15	
Plantaginaceae	*Plantago laceolata*	Iridoid glycoside	1.50	1.39	% dry wt	1.08	Marak et al. (2002)
Poaceae	*Secale cereale*	Hydroxamic acid	3.93	19.31	mmol/kg fresh wt	0.20	Collantes et al. (1999)
	Maize	Hydroxamic acid	18.70	80.80	% g fresh wt	0.23	Collantes et al. (1998)
Polygonaceae	*Rumex acetosa*	Phenolic	0.31	0.07	μmol/g fresh wt	4.52	Tolra et al. (2005)
Solanaceae	*Nicotiana sylvestris*	Alkaloid	20.24	3.57	% μg fresh wt	5.67	Ohnmeiss and Baldwin (2000)
	Solanum carolinense	Polyphenol	0.17	0.03	mg/g dry wt	5.89	Walls et al. (2005)
	S. carolinense	Glycoalkaloid	3.50	0.03	μmol/g fresh wt	114.21	

[a]R/S indicates a comparison of root and shoot chemistry, where 1 indicates equivalent concentrations, and values above or below 1 represent higher or lower concentrations of phytochemicals in the roots compared with the shoots, respectively.

Three major points emerge from the survey. First, compounds present in aboveground tissues are most often also found in roots. Second, roots appear to be unambiguously defended by phytochemicals given the substantial investment there. And finally, there is no consistent bias across species of differential allocation to aboveground versus belowground tissues. Returning to the question at the beginning of the section, we would answer that roots do not express constitutive chemical defenses more or less strongly than shoots. Nonetheless, differential allocation apparently depends on plant family, species, and genotype (Collantes et al., 1999; Agerbirk et al., 2003; Hol et al., 2004), age of plant (Hara et al., 2000; Frolich et al., 2006), and ontogenetic stage of the tissues (Ohnmeiss and Baldwin, 2000; Walls et al., 2005). Moreover, variability between root and shoot defensive chemical concentration can be explained by abiotic factors such as atmospheric CO_2, N_2, light, and site of origin (Wainhouse et al., 1998; Mattson et al., 2005).

Using a series of mechanistic and evolutionary approaches, we propose six major factors that may shape the relative composition of root versus shoot chemistry: site of production and mobility of the chemicals, damage history of an individual plant (induced responses), signaling pathways, damage history of a population (evolutionary response), domestication, and phylogenetic history. The importance of each factor and future directions are outlined below.

Site of Production and Mobility of Phytochemicals as Constraints in Defense Allocation

The site of production of defensive secondary metabolites is variable in plants, depending on the nature of the compound and the plant species. For example, in several Solanaceous species, alkaloids are synthesized in the roots and are transported into aboveground parts of plants. Hence, the pattern of alkaloids found in the leaves is determined by production in the roots. When reciprocal grafts were established between a nicotine-producing and a tropane alkaloid-producing species, the type of alkaloids found in the leaves were determined by the rootstock (Waller and Novacki, 1977). Similarly, Hartmann (1999) has also shown that the precursor of highly toxic pyrrolizidine alkaloids in the Asteraceae (senecionine *N*-oxide) is produced in roots, and only after translocation to the shoot is biochemical diversification achieved. Given the frequently observed higher accumulation of the transported compounds in roots compared with shoots (especially in undamaged tissues; Table I), it is unclear whether this differential initial allocation serves any adaptive function (i.e. the need for high constitutive root defense). Thus, the consequences of high constitutive levels of root compounds that are mobile (or not mobile) need to be investigated, and the inducibility of such compounds retained in roots represents an additional important and unexplored area.

Vascular constraints in shoots result in unequal systemic induction of plant secondary metabolites in different aboveground plant parts after elicitation at one site of attack (Frost et al., 2007). Similar vascular constrains are well known in the relationship between belowground and aboveground allocation of nutrients and secondary metabolites (Orians et al., 2002). The well-documented examples of signal molecules traveling from one damaged leaf to others via direct vascular connections begs the question of similar constraints in aboveground induction after root herbivory in particular zones of the rhizosphere. Aboveground and belowground signaling mediated by hormones is briefly discussed below and also by Erb et al. (2008).

Finally, site specificity in defense expression may be generated by gene differentiation and expression. Chen et al. (2004) report on an Arabidopsis terpene synthase gene mainly responsible for the production of 1,8-cineole in the roots. The most closely related gene is a flower-specific synthase that catalyzes the formation of a set of monoterpenes very similar to those produced by the root synthase, but its major products are myrcene and (E)-β-ocimene, not 1,8-cineole. This study suggested that divergence of organ expression patterns and product specificity is potentially part of an ongoing evolutionary processes. The function of terpene production in Arabidopsis roots is unknown, although it could be related to belowground tritrophic interactions (Rasmann et al., 2005) or interactions with microbes.

Damage History (Induced Responses)

Induction of defensive compounds following herbivore attack is generally thought of as a way in which plants maximize their fitness depending on the site and probability of attack (Karban et al., 1997; Agrawal, 1998; Kessler and Baldwin, 2001). In this regard, roots should not differ from aboveground tissues. For example, in potato (Solanum tuberosum) roots and tubers, wounding and jasmonate treatment elicit lipoxygenases transcript accumulation (Geerts et al., 1994). Perhaps the best studied root induction system is that of spinach (Spinacia oleracea). Phytoecdysteroids present in spinach increase after damage by a root herbivore, mechanical damage, or jasmonate application (Schmelz et al., 1998, 1999, 2002), and, interestingly, only treatment of roots, not shoots, increased the chemical concentration in the roots (Schmelz et al., 1998); see Table II. This suggests a level of organ specificity and compartmentalization of the aboveground and belowground signaling pathways. Moreover, Schmelz et al. (2002) have shown that phytoecdysteroids decrease the survivorship and establishment of the root-feeding fungus gnat (Bradysia impatiens), and result in higher root and shoot biomass of induced plants compared with controls. Moreover, maize (Zea mays) plants, when attacked by the leaf herbivore Spodoptera littoralis, emit a conspicuous blend of volatile organic compounds in the leaves but not in the roots; likewise, root feeding induces the plant to produce the sesquiterpene (E)-β-caryophyllene in the roots, and none or very little in the leaves (Rasmann and Turlings, 2007). Thus, root herbivory itself may alter patterns of defense investment aboveground and belowground, and potentially even reverse the constitutive ratio of defense investment (Table II).

Optimal defense theory predicts that plants should invest the most defense in plant parts with the greatest fitness value and those that are most likely to be attacked (Stamp, 2003). That is, plant parts that are likely to be attacked should contain high constitutive levels of defense, whereas other parts that have similar fitness value but are rarely attacked should rely more on inducible defenses. Zangerl and Rutledge (1996) tested this hypothesis and showed that roots of wild parsnip (Pastinaca sativa) contained the lowest constitutive levels of xanthotoxin, but were highly inducible, whereas leaves and flowers having a high probability of attack invested more in constitutive xanthotoxin and were less inducible than roots.

Although aboveground and belowground responses are sometimes independent (Bezemer et al., 2003, 2004), there is growing evidence for interactions between the two. For example, Brassica nigra plants showed a clear systemic increase in shoot glucosinolate levels in response to Delia radicum feeding on roots; although a congener, Brassica oleracea did not show this pattern. In both species, however, a similar local increase in indole glucosinolate levels was found in roots at the feeding site of the larvae (van Dam and Raaijmakers, 2006). Maize seedlings, when simultaneously attacked aboveground by the noctuid moth S. littoralis and belowground by the chrysomelid beetle Diabrotica virgifera virgifera, showed a reduction of attraction of the aboveground parasitoid Cotesia marginiventris and the belowground predatory nematode Heterorhabditis megidis, compared with singly damaged plants (Rasmann and Turlings, 2007). Aboveground and belowground studies are showing a complex path of communication between root and shoot tissues, and the nature and mode of signaling between the soil organs and leaves is a rapidly expanding area of research (Erb et al., 2008).

Finally, some defensive traits may only be expressed in one plant part, even though the expression may be influenced by the state of other plant parts. Our recent unpublished work shows that latex is only produced aboveground in common milkweed (Asclepias syriaca) and no latex is found in damaged roots. Despite the strong compartmentalization, root herbivory diminishes the production and exudation of latex in the leaves (S. Rasmann and A.A. Agrawal, unpublished data). Whether this is due to loss of turgor pressure or a response to defense signaling has yet to be investigated.

Signaling Pathways of Plant Defense Induction Underground

Induction of plant resistance is generally mediated by three major signaling compounds, jasmonic acid, salicylic acid, and ethylene, and studies on aboveground herbivory have shown that the different pathways interact

Table II. *A survey of chemical induction in plant roots when attacked by herbivores (LHe and RHe represent leaf and root herbivores, respectively), treated with plant hormones (LHo or RHo), or mechanically damaged (LMe or RMe)*

Family	Plant Species	Induction Agent	Hormone[a]	Herbivore[b]	Feeding Mode	Chemical Involved	Effect[c]	Citation
Amaranthaceae	Spinach	RMe, RHo, RHe	MJ, MSA	*Otiorhynchus sulcatus*	Chewing	Phytoecdysteroids	+	Schmelz et al. (1998, 1999)
	Spinach	RHe, RHo	MJ	*B. impatiens* r	Chewing	Phytoecdysteroids	+	Schmelz et al. (2002)
Apiaceae	Wild parsnip	RMe	–	–	–	Furanocumarin	+	Zangerl and Rutledge (1996)
Asteraceae	*S. jacobaea*	LHe, RMe	–	–	–	Pyrolizidine alkaloids	±	Hol et al. (2004)
Brassicaceae	Arabidopsis	LMe, RMe, RHe	JA	*B. impatiens*	Chewing	Gene expression		McConn et al. (1997)
	B. oleracea	LHo, RHo	JA, SA	–	–	Glucosinolate	±	van Dam et al. (2004)
	B. oleracea	RHe	–	*D. radicum* r	Chewing	Glucosinolate	±	van Dam and Raaijmakers (2006)
	B. nigra	LHo, RHo	JA, SA	–	–	Glucosinolate	±	van Dam et al. (2004)
	B. nigra	RHe	–	*D. radicum* r	Chewing	Glucosinolate	±	van Dam and Raaijmakers (2006)
Euphorbiaceae	Cassava	RHe	–	*Cyrtomenus bergi* r	Piercing	Hydrogen cyanide	+	Riis et al. (2003)
Malvaceae	*G. herbaceum*	LHe, RHe, RMe	–	*Agriotes lineatus*	Chewing	Terpenoid aldeheide	+	Bezemer et al. (2004)
	G. herbaceum	LHe, RHe	–	*A. lineatus* r	Chewing	Terpenoids	+	Bezemer et al. (2003)
Poaceae	Maize	RHe	–	*Diabrotica virgifera* r	Chewing	Terpenoid	+	Rasmann et al. (2005)
Solanaceae	Tomato	LHo	JA	*Meloidogyne* sp. r	Piercing	–	N/A[d]	Cooper et al. (2005)
	Potato	RHo, RMe	MJA	–	–	Lipoxygenase	+	Geerts et al. (1994)
	S. carolinense	LHo	JA	–	–	Glycoalkaloids	+	Walls et al. (2005)
	N. sylvestris	LMe	–	–	–	Nicotine	+	Baldwin et al. (1994)
	N. attenuata	Ho	JA	–	–	Nicotine	+	Baldwin (1998)
Vitaceae	*Vitis vinifera*	LHo	JA	*Daktulosphaira vitifolia* r	Piercing	–	N/A	Omer and Granett (2000)

[a]MJ, Methyl jasmonate; JA, jasmonic acid; MSA, methyl salicylate; or SA, salicylic acid. [b]The letter "r" after the herbivore species indicates that resistance (e.g. insect growth rate) was measured. [c]The effect ("+" or "−") indicates the overall increase or decrease in compound concentration after induction treatment. [d]N/A, Not applicable.

with each other to construct a pattern of independent and networked defense responses (Heidel and Baldwin, 2004). How hormones are translocated from their site of origin to other plant parts will likely play a key role in shoot-to-root communication (Atkins and Smith, 2007). Some signaling molecules follow phloem ducts to reach root cells (Sachs, 2005), but how a damaged root alerts the leaves to mobilize or synthesize defensive compounds still remains unclear. An example of this can be a pool of signaling molecules such as the precursors of jasmonic acid in cell membranes, already present in the belowground organs, which, after damage, are readily transported aboveground (Gatehouse, 2002). Although little is known about aboveground and belowground movement of hormones in response to root herbivory, evidence for their general importance is mounting. McConn et al. (1997) showed that Arabidopsis mutants deficient in the jasmonate precursor linolenic acid are extremely susceptible to the root chewing fungus gnat *B. impatiens*, and that resistance is partially restored by methyl jasmonate application to the leaves. Very few studies have analyzed accumulation of gene transcripts in response to root mechanical damage or feeding, but initial results indicate that the three major signaling pathways mentioned above are involved with the elicitation of root responses (Salzman et al., 2005; Puthoff and Smigocki, 2007).

Microevolution of Root Defenses

Like all other adaptations, root defenses may evolve if heritable variation in their allocation affects fitness.

This has barely been studied and little direct evidence indicates that natural selection has shaped root defenses. Nonetheless, root defensive chemistry is certainly heritable (Myszewski et al., 2002; Hol et al., 2004). For example, both constitutive and induced production of pyrrolizidine alkaloids in the roots of *Senecio jacobaea* are heritable (Hol et al., 2004). The maintenance of genetic variation in defense is thought to be driven by benefits and costs of these traits. Evidence for benefits of root defense comes primarily from the agricultural literature (see discussion of spinach above and the next section on domestication). In the only study on costs, the fitness effect of resource allocation to tannin production in the roots of *Populus deltoids* was estimated as reduced aboveground tree growth, and costs were detected in only one out of three common garden experiments (Kosola et al., 2004). Thus, costs and benefits of root defense represent an opportunity for future research. Given population level variation in root herbivory and its impacts (Blossey and Hunt-Joshi, 2003; Maron and Kauffman, 2006), we predict that local adaptation to root herbivory (i.e. genetically differentiated plant populations) represents an excellent tool to study costs and benefits of allocation to root defense.

Domestication Favors Specific Plant Defensive Traits

Root herbivores are among the most harmful insects attacking crop plants (e.g. corn root worm, *Diabrotica* spp.), and the handful of species known to attack roots of the major crop plants dominate the literature on

belowground resistance (Blossey and Hunt-Joshi, 2003). Although specific knowledge on human selection for root defenses is poorly documented, strong direct selection on root chemistry has occurred in root crops to increase palatability to humans (radish [*Raphanus sativus*], potato, cassava [*Manihot esculenta*], etc.) and to enhance resistance to pests. For example, *Delia* flies damaging *Brassica* spp. (oilseed rape [*Brassica napus*], kale [*B. oleracea*], swede [*Brassica napobrassica*], canola [*B. napus*]) roots affect plant performance and commercial yields. Since the discovery of the defensive role of glucosinolates in reducing herbivory, breeding efforts have led to increased belowground concentrations of these compounds in *Brassica* crops (Potter et al., 2000). Additionally, in maize, strains bred with naturally occurring high levels of hydroxamic acids showed improved resistance to corn rootworm, more biomass, and had better yield compared with varieties with lower levels of hydroxamic acids (Assabgui et al., 1993).

Selection on crop plants can also lead to inadvertent changes in belowground chemical defenses. We recently discovered the presence of *Diabrotica*-induced (*E*)-β-caryophyllene in maize roots, which attracts predatory nematodes (Rasmann et al., 2005). However, this trait was apparently lost in North American lines of maize, whereas it is still present in European varieties (Rasmann et al., 2005). The reactivation of the gene responsible for (*E*)-β-caryophyllene production in American lines could lead to a more efficient tritrophic battle against *Diabrotica*.

Phylogenetic Analyses

Although selection and domestication are powerful approaches to studying the microevolution of plant defense, macroevolutionary (or phylogenetic) studies have recently been advocated for studying broader patterns (Agrawal, 2007). Few phylogenetic analyses have addressed belowground defensive chemistry, and further assessments will improve our knowledge of: (1) the biosynthetic origin of defenses (Liscombe et al., 2005), (2) phylogenetic conservatism in defense allocation (Wink, 2003), (3) potential trade-offs among defensive strategies (Agrawal and Fishbein, 2006), and (4) phyletic patterns indicative of coevolution (Ehrlich and Raven, 1964). Our unpublished data on cardenolides in the roots of milkweed plants (*Asclepias* spp.) have shown over 8-fold variation in the constitutive allocation among 34 species, and thus a high level of evolutionary lability in this trait. This result suggests that root defenses may rapidly evolve as species-specific adaptations to belowground herbivores (Agrawal, 2004).

CONCLUSION

There are tremendous opportunities in the study of root defense, especially those that meld ecological and evolutionary context with mechanistic approaches. Why do Arabidopsis roots produce volatile terpenes? Are the alkaloids produced in Aster roots providing frontline defense against root herbivory or are they

simply produced and stored underground? How might increasing root defense alter aboveground signaling in plant-herbivore-parasitoid interactions? And finally, if root herbivores are truly less diverse than foliar herbivores, is there anything fundamentally different in the chemically mediated coevolutionary interactions occurring in the rhizosphere? We have made some progress, but there is a lot to be discovered about resistance to belowground herbivory.

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