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The underestimated role of roots in defense against leaf attackers

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Plants have evolved intricate strategies to withstand attacks by herbivores and pathogens. Although it is known that plants change their primary and secondary metabolism in leaves to resist and tolerate aboveground attack, there is little awareness of the role of roots in these processes. This is surprising given that plant roots are responsible for the synthesis of plant toxins, play an active role in environmental sensing and defense signaling, and serve as dynamic storage organs to allow regrowth. Hence, studying roots is essential for a solid understanding of resistance and tolerance to leaf-feeding insects and pathogens. Here, we highlight this function of roots in plant resistance to aboveground attackers, with a special focus on systemic signaling and insect herbivores.

Roots as an underappreciated part of the defensive system of plants

One reason why plants are so successful in colonizing the Earth is that they can directly use abundantly occurring molecules in the atmosphere and the earth surface layer to sustain their growth and development [1]. To do so, they have developed two distinct but integrated structural elements: shoots and leaves to access to the world above the surface, and roots to gain entry into the soil [2]. Many organisms, including pathogens and insect herbivores, attack plants to obtain the organic matter they produce [3]. To withstand these attacks, plants again have evolved multiple resistance and tolerance strategies, including the synthesis of defensive proteins [4–6], toxins [7–9], volatile attractants and extrafloral nectar [10,11], as well as the reallocation of resources upon attack [12,13]. The ecological relevance and potential for use in pest control of these defensive tactics has motivated scientists to unravel the underlying mechanisms. In the past, there has been a particular emphasis on the mechanisms underlying the response of plant leaves upon attack [14]. Roots, despite their integral role in plant defense in some species [15,16], have largely been ignored, which again has led to an unbalanced understanding about the role of aboveground parts in plant defense compared with belowground tissues [17]. It is commonly thought that plant–insect interactions can be entirely understood by investigating leaves. However, roots are being rediscovered as dynamic storage organs that could help plants to tolerate aboveground herbivory [12,13], and research on root–herbivore and root–microbe interactions emphasizes the influence of belowground tissues on aboveground physiology and resistance [18–25]. Furthermore, a dramatic effect of shoot insect infestation on the root transcriptome was found (Figure 1; M. Erb, PhD Thesis, University of Neuchâtel, 2009), adding to the increasing evidence for the existence of a defensive shoot–root–shoot loop in plant-defense reactions (Figure 2).

Here, we discuss the current state of knowledge about the importance of roots as: (i) biosynthetic origin of defensive compounds; (ii) environmental sensors and root–shoot signal emitters; as well as (iii) dynamic storage organs of primary metabolites. Based on these functions, we argue that future research on plant responses against leaf herbivores, as well as pathogens should include belowground tissues.

Synthesis of aboveground defenses by roots

Roots are increasingly recognized to synthesize secondary metabolites involved in leaf defenses (Table 1). Nicotine, for instance is synthesized in the roots of tobacco plants [26], where it is loaded into the xylem, translocated into the shoots and stored in the vacuoles of leaves [27,28]. In response to insect attack, root nicotine synthesis increases even further, leading to augmented concentrations aboveground [29] and increased herbivore resistance (Figure 2) [7]. In addition, other plant species have been shown to produce alkaloids in the roots and transport them into the leaves. Tropane alkaloids, for example are synthesized in the roots by various Solanaceae including nightshades and *Datura* spp. [30]. Reciprocal grafts with other species result in alkaloid patterns that are dependent on the rootstock rather than the foliage of the chimeras [31]. Another example are the pyrrolizidine alkaloids (PAs) in the Asteraceae [32]. In ragwort species in particular, the senecionine N-oxide produced in the roots is the backbone alkaloid structure that is distributed within the plant and subsequently modified in the target cells to yield species-specific PA patterns [32]. Although many defensive alkaloids from different plant families show this clear root–shoot pattern, it is important to note that a considerable number of alkaloids are produced aboveground [30,33].

Besides alkaloids, plants might also produce other leaf defenses belowground. Indirect evidence for this comes from pharmaceutical studies using co-cultures of hairy roots and shoots of plants. Umelliferone, the precursor of many furocoumarins (FCs), for example is produced in significant quantities by the roots of bishopsweed (*Ammi majus* L.) [34]. When a hairy root culture of this plant is

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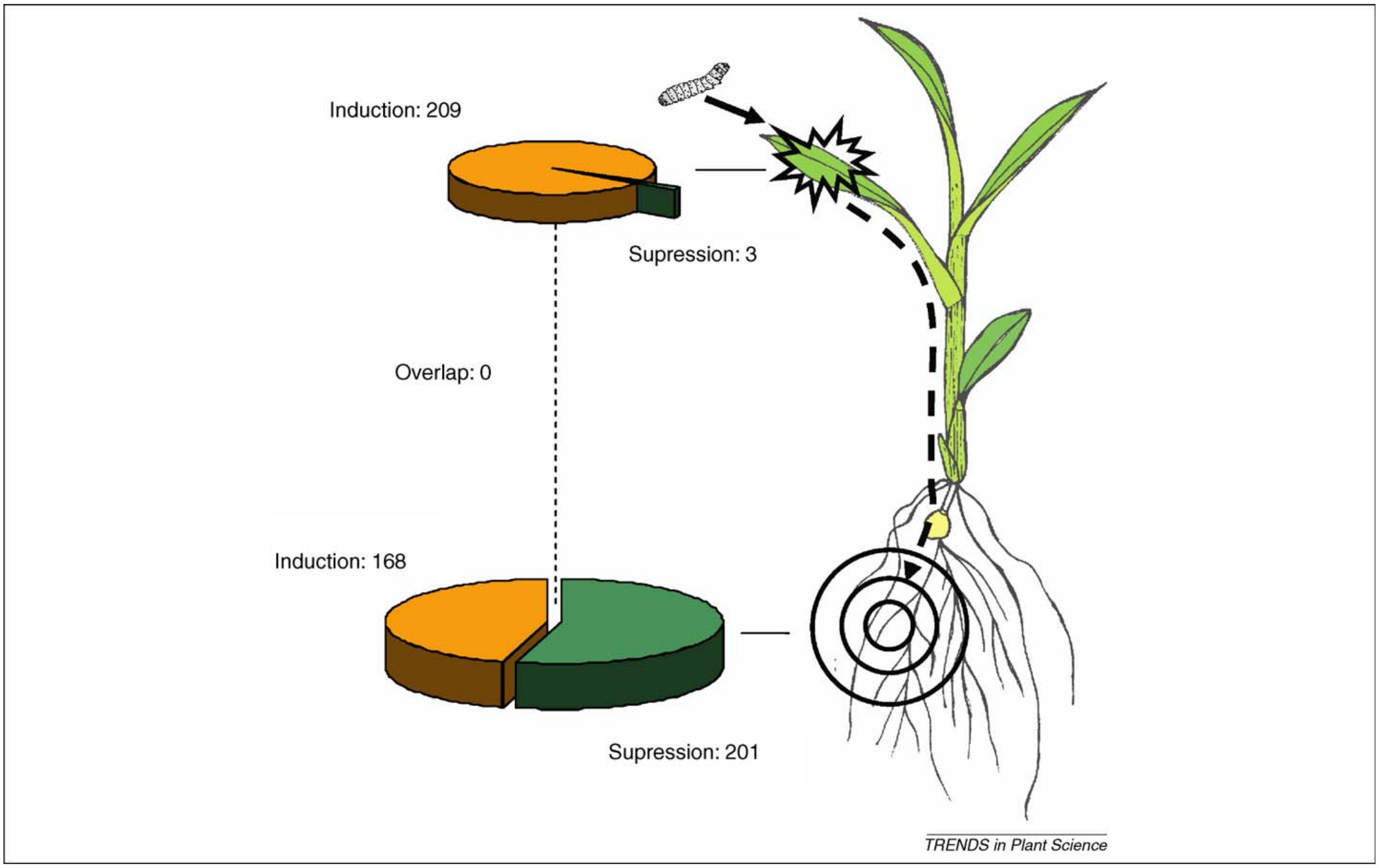

Figure 1. Transcriptional changes in shoots and roots of maize plants attacked by the leaf herbivore *Spodoptera littoralis*. Maize seedlings were infested with 20 L2 larvae for 18 h, after which the roots and shoots were harvested and analyzed using whole genome 57K maize oligo arrays. Four biological replicates (each with two technical replications) were performed, and genes with a fold change >2 at a p-value of <0.01 were selected for analysis. Shoot infestation resulted in the induction of 209 (orange) and the suppression of three transcripts (green) aboveground. The resulting systemic response in the roots was even more pronounced, with 168 induced and 201 suppressed transcripts. The surface area of the pie charts represents the relative number of changed transcripts. There was no overlap in transcriptional changes between the two tissues, indicating that the root response is profoundly different from the local reaction of the plant (M. Erb, PhD thesis, University of Neuchâtel, 2009; methodological details and thesis reprint available upon request).

cultivated together with shoots of the common rue (*Ruta graveolens*), the production of the FC xanthotoxin by *R. graveolens* shoot cells is greatly increased [34], implying that the plant can use the root precursor to synthesize specific shoot FCs. Even some defensive proteins seem to be synthesized in the roots of shoot-attacked plants. The maize (*Zea mays*) cysteine protease Mir1-CP, a protein that disrupts the periotrophic matrix of herbivores [35], increases in the lumen of root metaxylem vessels 24 h after aboveground attack by the fall armyworm (*Spodoptera frugiperda*) [36]. Furthermore, this protein accumulates in smaller amounts at herbivore-feeding sites in the leaves when roots are excised [36]. Although further evidence is needed to substantiate this hypothesis, available data suggest that the synthesis and transport of Mir1-CP from roots accounts for its accumulation aboveground (Figure 2).

A recent meta-analysis demonstrated that overall root attack by herbivores increases secondary metabolite concentrations in the leaves to a similar extent as locally in the roots [37]. The long list of defensive metabolites includes glucosinolates [19,38–40] and hydroxamic acids [41], both of which are not only active against insects but also against pathogens [42,43]. Although some of the documented effects might be a result of systemic root–shoot defense signaling (as discussed below), other effects might be caused by the secondary metabolites themselves that are synthesized in roots and then transported to leaves [22]. These studies, therefore, provide a basis for further research on possible root-derived defense compounds with a role in leaf defenses. In this context, it is noteworthy that even well known secondary metabolites such as glucosinolates are increasingly recognized as mobile elements within plants [44,45].

Why exactly plants synthesize such a variety of shoot defenses in their roots is unclear. It has been proposed that this strategy could be valuable under conditions where the attackers destroy large parts of the foliage, because the biosynthetic site of defense remains protected belowground [46]. Furthermore, because leaves are often not directly connected via the vascular system [47,48], sending a signal down into the roots via the phloem that triggers xylem-mobile defensive elements could enable the plant to induce defenses systemically. This might be an especially valuable strategy for plants that are strongly connected in their xylem vessel structure, as opposed to plants that exhibit a sectorial design (with axially restricted root–shoot pathways) [49]. Another possibility why it might be advantageous to assemble certain secondary metabolites in the roots is the availability of precursors; roots

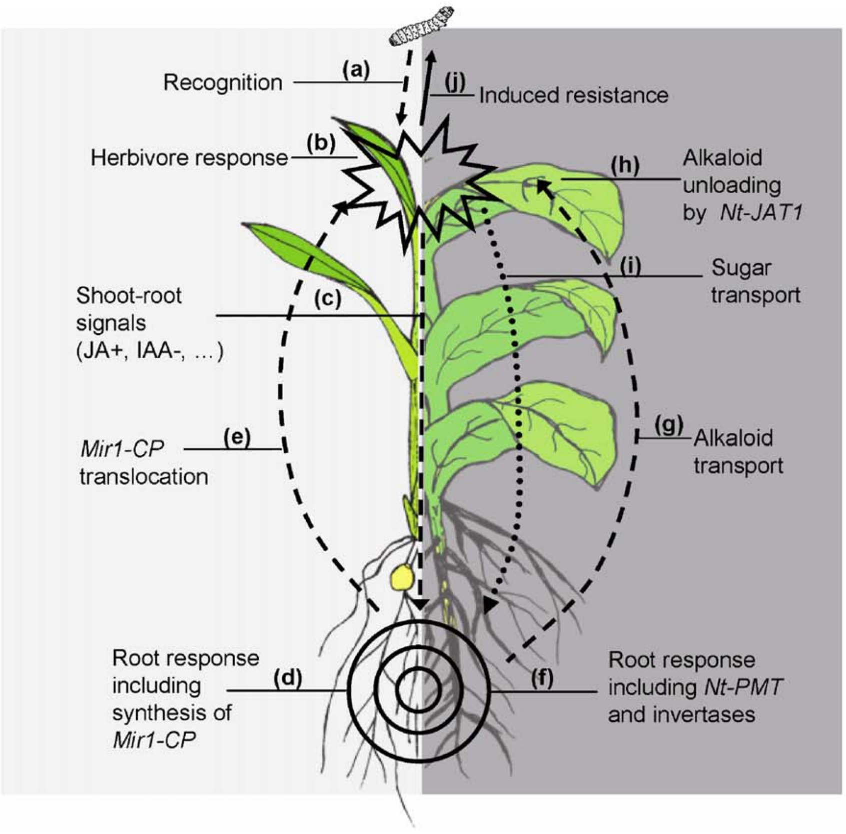

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Figure 2. The shoot–root–shoot loop in maize and tobacco. A composite figure of maize (left) and tobacco (right) is shown. **(a)** The plant recognizes the herbivore via specific elicitors. **(b)**. Local defenses are activated, including *Na-GAL83* (implicated in resource allocation) in tobacco. **(c)** Shoot–root signals are deployed. Possible candidate signals include jasmonic acid (JA; increase in concentration) [50] and the auxin indole-3-acetic acid (IAA; decrease in concentration) [52]. **(d)** A reorganization of the root metabolism takes place in maize, including possibly increased synthesis of the protease Mir1-CP [36]. **(e)** Signals and defensive compounds resulting from processes c–d travel from the roots to the shoot [36]. **(f)** The *N*-methyltransferase responsible for nicotine synthesis [28] and invertases increasing assimilate flow into the roots [20] are induced in tobacco. **(g)** Alkaloids are transported into leaves. **(h)** Alkaloids are unloaded and deposited in the vacuoles by the transport protein *Nt-JAT1* [27]. **(i)** Assimilate flow into roots increases [20]. **(j)** The resulting plant response involving roots increases herbivore resistance [7] and tolerance [12].

have ready access to nitrogen containing compounds in the soil, whereas carbohydrates are produced in photosynthetically active tissues. Indeed, both alkaloids and Mir1-CP contain nitrogen, providing some support to this hypothesis (Table 1). Further research should aim at developing a testable theory explaining the spatial separation of biosynthesis and action for some plant toxins.

The induction of synthesis of root defense compounds on shoot herbivory has led to attempts to identify the responsible shoot–root signals. In tobacco, early work focused on the role of jasmonates as mobile elements. It was reported that shoot jasmonic acid (JA) concentrations increase locally 30 min after wounding of woodland tobacco (*Nicotiana sylvestris*) leaves, and 90 min later in the roots [29]. By exogenous application of ^{14}C labeled JA, it was confirmed that shoot-to-root transport of jasmonates can occur and that the dynamics of root nicotine induction matches a possible role of JA as the long-distance signal [50]. In accordance with this, it was found that in hybrid poplar, several shoot-inducible transcripts are also induced in roots after leaf damage [51], indicating downward transport of a wound-inducible signal. Interestingly, a recent study reported that the increase in leaf-nicotine in cultivated tobacco (*Nicotiana tabacum*) was more pronounced after removal of the shoot apex than after mechanical leaf damage [52]. Removing the apex, contrary to mechanically damaging a leaf, did not seem to increase root JA pools strongly. Moreover, accumulation of nicotine

Table 1. Root-derived plant toxins implicated in leaf defense

Compound	Active against	Nitrogen containing	Inducible	Root–shoot pattern substantiated by experiments	Refs
Nicotine	Insects/ (pathogens)[b]	Yes	Yes	Yes	[26,29]
Tropane alkaloids	Insects	Yes	Yes	Yes	[30,31]
Pyrrolizidine alkaloids	(Insects)	Yes	Yes	Yes	[32]
Furocoumarins	Insects/ pathogens	No	Yes	Partially	[34]
Mir1-CP	Insects	Yes	Yes	Partially	[36]
Terpenoid aldehydes[a]	Insects/ pathogens	No	Yes	No	[37,93]

[a] We included terpenoid aldehydes because they have been cited repeatedly as an example of a root derived shoot secondary metabolite.

[b] Parentheses denote circumstantial evidence.

was inhibited by the application of 1-naphthylacetic acid, an auxin homolog. It was therefore concluded that shoot-derived auxin is likely to be a major negative regulator of root–nicotine biosynthesis and that removal of its biosynthetic tissue (the apex) by a herbivore might activate root toxin production [52]. The fact that auxins seem to suppress wound-induced JA [53] suggests a close interplay between the two potential shoot-to-root signals. Interestingly, in *Z. mays*, it seems that the shoot-to-root signal upon Egyptian cotton leafworm (*Spodoptera littoralis*) attack in leaves is dissimilar to systemic shoot signals, as there is no overlap between induced genes in leaves and roots at the whole genome level 18 h after infestation (Figure 1). We furthermore found that JA, salicylic acid (SA) and abscisic acid (ABA) are not induced in the roots 48 h after shoot attack [41]. Although future research will need to confirm this by including a more extended time course, current results clearly suggest the involvement of alternative shoot–root signals in *Z. mays*.

Roots as environmental sensors and root–shoot signal emitters

Roots are in intimate contact with their environment and can thereby perceive a variety of abiotic factors. Changes in these factors can lead to root–shoot signaling influencing leaf defenses. Water logging, for example results in the accumulation of 1-aminocyclopropane-1-carboxylic acid in roots, which is transported to leaves and converted to ethylene (ET) [54]. ET synergizes activation of JA-dependent defense responses against leaf-feeding insects and necrotrophic pathogens [55,56] and thereby serves as a positive regulator for insect and disease resistance [57,58]. By contrast, ABA is classically associated with water-limiting conditions in the rhizosphere. Such conditions can result from drought stress but also from root insect attack [41,59]. Belowground synthesis and root–shoot transport of ABA then causes a reorganization of leaf metabolism that can profoundly alter plant defense and resistance [57,60,61]. Interestingly, potassium (K) starvation in thale cress (*Arabidopsis thaliana*) results in an increase of JA-dependent genes in shoots [62]. This suggests that root–shoot signals deployed after changes in availability of K to roots can also impact aboveground defenses [63]. Although these examples only represent a small fraction of what is known about the effects of soil abiotic factors on aboveground defenses [64], they imply that roots influence shoot metabolism actively via hormonal signaling rather than only passively via reduced nutrient or water supply.

Plant roots are in constant contact with soil-borne microorganisms such as plant growth promoting rhizobacteria (PGPRs) and mycorrhizal fungi, which are known to affect leaf metabolism and resistance. In this regard, one of the best studied examples is the positive effect of certain PGPRs on leaf resistance against necrotrophic pathogens [65] and insect herbivores [66], a phenomenon termed "induced systemic resistance" (ISR). ISR in *A. thaliana* seems to function via priming of leaves for an enhanced JA/ET response [67,68]. Transcriptional profiling of roots and shoots show that rhizobacteria directly suppress several genes in both tissues [67,68]. Although this is not entirely consistent with the priming hypothesis based on the perception of an initial mild stress [48], it might nevertheless serve as a starting point to uncover the elusive root–shoot signal emanating from colonized roots. Interestingly, evidence is increasing that arbuscular mycorrhizal fungi (AMF) can have similar effects on aboveground resistance as ISR [69,70] and it has been proposed that the two phenomena share a similar mechanistic basis [24,69]. However, it was recently shown for barrel clover (*Medicato truncatula*) that root colonization by the AMF *Glomus intraradices*, unlike ISR, directly induces a variety of defense related genes in the shoot [71]. In this system, the induced resistance against the pathogen *Xanthomonas campestris* [71] is thus likely to be the result of an aboveground defense inducing signal deployed by the roots upon colonization by AMF. It has also been shown that colonization by AMF and ectomycorrhizal fungi can benefit herbivores [72,73], as well as biotrophic pathogens [74], and can reduce production of extrafloral nectar [75]. Although these negative effects on plant resistance have been attributed to several factors including nutrient supply and pathogen lifestyle, they demonstrate that roots also have the capacity to lower the defensive investment of aboveground plant parts.

Taken together, the examples listed here provide compelling evidence for the importance of roots as active modulators of shoot defenses and resistance against herbivores and pathogens. Some root–shoot signals have been described but others still remain to be discovered, and we argue that unraveling the intricacies of this "root–shoot information highway" will considerably improve our understanding of plant defensive processes. In this context, known local signals induced by AMF colonization such as JA [76], ABA and indole-3-acetic acid (IAA) [77] can provide interesting starting points.

The importance of roots for plant tolerance

Roots, by being inaccessible to aboveground attackers, can serve as storage organs for assimilates that enable regrowth after herbivore attack. Whereas perennials often accumulate nutrients belowground over the growing season to endure less favorable periods [78,79], several plant species actively increase their root nutrient pools upon leaf attack. This includes *Z. mays* after grasshopper infestation [80], perennial ryegrass (*Lolium perenne*) after defoliation [81], poplar after methyl jasmonate (MeJA) treatment [13] and coyote tobacco (*Nicotiana attenuate*) after wounding and treatment with tobacco hornworm (*Manduca sexta*) regurgitant [12]. Moreover, nitrogen allocation to roots has been observed after MeJA treatment of alfalfa (*Medicago sativa*) [82].

Assimilate partitioning via the phloem is mainly regulated by osmotic differences between source and sink tissues [83]. Increased nutrient allocation to the roots after herbivore attack can thus be achieved by changes in phloem import and export patterns [84] or by conversion of osmotically active primary metabolites [85]. Using ^{13}C labeling and invertase activity measurements it has been shown that, after leaf feeding by *M. sexta*, *N. tabacum* increases sugar-cleaving enzyme activity belowground, contributing to assimilate repartitioning [20]. Similar results have also been reported for *N. attenuata* [12].

Although these examples demonstrate that roots are not only passive receivers of storage products but also active organs changing their sink strength after leaf attack, the mechanisms by which roots mediate aboveground tolerance to leaf herbivores are still poorly understood. Analyses of transcriptional changes upon aboveground insect infestation have facilitated the discovery of key mechanisms involved in plant tolerance, such as the SnRK kinase *GAL83*, an SNF1-related protein kinase involved in root–shoot resource allocation [86]; yet, there are no data available on corresponding changes in the root transcriptome for any of the common model organisms such as *A. thaliana*. Expanding the available methodology to profile transcriptional changes in roots upon leaf attack has the potential to change this.

Interestingly, we found that there is not only an induction of a variety of transcripts in the roots upon shoot attack but also a pronounced suppression of an equal number of genes in the roots (Figure 1). This suggests that many metabolic processes are downregulated belowground when defense mechanisms are induced in leaves. In accordance with this, a rapid and transient reduction of root growth after shoot herbivore attack occurs in *N. attenuata* [87]. It remains to be investigated if this reduced investment into growth is a part of the strategy of plants to “turn down its spatial expansion and strengthen its function as a safe retreat” [87] or if it helps to liberate additional resources to boost leaf defenses.

The active role of roots in plant tolerance against leaf herbivory clearly implies shoot–root communication. First attempts to gain insight into possible signals that are involved show that carbon partitioning in *N. attenuata* is independent of the octadecanoid pathway, as plants expressing the lipoxygenase gene *NaLOX3* in antisense direction were not impaired in this response [12]. This was unexpected because the octadecanoid pathway is a central component of the signaling network mediating plant responses against insects [14] and necrotrophic pathogens [88]. Further research is required to look for alternative shoot–root signals, the discovery of which will improve understanding of the role of roots in plant tolerance to aboveground herbivory. Known shoot–root signals such as auxins [89] and other phloem-borne molecules including proteins [90] and small RNAs [91] are candidates for such investigations. Furthermore, it has been suggested that the reduction of root growth after leaf wounding could be mediated by a hydraulic signal [87]. Interestingly, sucrose is not only a simple assimilate that is moved to roots for tolerance purposes upon insect attack but also by a potential shoot–root signal [92]. Some root responses discussed above might therefore be regulated via this molecule.

Concluding remarks and future research

Our survey of current literature shows that roots are important for the defensive strategies of plants against leaf attackers. Roots can be “senders” of shoot-modulating factors (e.g. upon changes in water availability, root herbivore attack or colonization by microbes), as well as “receivers” (e.g. of assimilates for tolerance purposes). Roots are also active participants in the “shoot–root–shoot loop” in the defensive system of the plant (Table 1). This loop involves the transmission of signals from the attacked tissue to the belowground organs, their metabolic reconfiguration (Figure 1) and a subsequent translocation of defensive compounds upwards (Figure 2). Nicotine alkaloids and the protease Mir1-CP are two examples illustrating the effectiveness of the loop and it can be assumed that other induced defensive metabolites and proteins are produced via the same route. Full elucidation will depend on a better understanding of root metabolism and its plasticity upon shoot attack. We conclude that research aimed at unraveling and utilizing the currently underestimated role of roots in aboveground defenses is much needed. The three most pressing questions for future research are as follows:

Firstly, it will be important to specifically address how roots respond to aboveground attack by herbivores and pathogens. This can be done swiftly by applying already established methodology such as transcriptome profiling or metabolomics from the leaves to the roots, which should show if the dynamic response of roots after aboveground attack, as shown in Figure 1, is indeed a widespread phenomenon in the plant kingdom.

Secondly, research into the identification of shoot–root and root–shoot signals should be intensified. Elucidating which signals regulate the exchange of information between roots and shoots will facilitate future efforts to describe and test root–shoot cross effects and might harbor potential applications in plant protection.

Thirdly, a general ecological theory will need to be developed to explain why plants involve their roots in aboveground defenses and why they enhance leaf-resistance upon contact with root-feeding insects and soil-dwelling microorganisms. Understanding the physiological processes as outlined above will provide the means to test these hypotheses experimentally.

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