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Roots under attack: contrasting plant responses to below- and aboveground insect herbivory

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Roots under attack: contrasting plant responses to below- and aboveground insect herbivory

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Summary

The distinctive ecology of root herbivores, the complexity and diversity of root-microbe interactions, and the physical nature of the soil matrix mean that plant responses to root herbivory extrapolate poorly from our understanding of responses to aboveground herbivores. For example, root attack induces different changes in phytohormones to those in damaged leaves, including a lower but more potent burst of jasmonates in several plant species. Root secondary metabolite responses also differ markedly, although patterns between roots and shoots are harder to discern. Root defences must be investigated in their own ecophysiological and evolutionary context, specifically one which incorporates root microbial symbionts and antagonists, if we are to better understand the battle between plants and their hidden herbivores.

Keywords: defensive responses, folivores, herbivores, photoassimilates, phytohormones, root feeding, secondary metabolites.

I. Introduction

It's a quarter of a century since the publication of the landmark review '*Insect Herbivory Below Ground*' by Brown and Gange (1990). This early review shed light on how plant roots were attacked by insect herbivores and provided much needed insights into the ecological consequences of such herbivory. Research in how plants respond to root attack has flourished since then and it is now very apparent that plant responses to belowground attack extrapolate poorly from our understanding of plant responses to foliar herbivory (Johnson *et al.*, 2013a). The fundamentally different nature of root attack imposes dissimilar selection pressures on plants, which in turn produces contrasting responses in terms of changes in biomass, gene expression and the production of secondary metabolites and wound hormones. Furthermore, we are now beginning to understand the extent to which the ecology of the root environment is driven by interactions between the plant and microbial symbionts, both in the rhizosphere and in the root itself (Edwards *et al.*, 2015). The complexity and diversity of plant-microbe interactions in soil provides a very different evolutionary and ecological context for plant defences against root herbivores compared with foliar herbivores (Johnson & Rasmann, 2015). Recent advances in genomic, transcriptomic, and metabolomic techniques are allowing us to unravel this complexity and its consequences for the first time (van der Heijden & Schlaeppi, 2015), whilst X-ray micro-tomography, proton-transfer reaction mass spectrometry and isotopic diet labelling have all helped to improve our knowledge of this hidden form of herbivory (Johnson *et al.*, 2013a). Hence it is timely to assess existing research on plant responses to insects feeding on belowground tissues and compare this with how plants respond to attack aboveground. We draw out contrasts and similarities between the two types of attack and address the underlying mechanisms and evolutionary drivers for these.

II. What makes root herbivores different from shoot herbivores?

Root herbivores have a number of features and life history traits that distinguish them, in most cases, from shoot herbivores (Table 1). These traits affect how they interact with plants and particularly how they damage plants, which differs from the damage inflicted by aboveground herbivores. Firstly, root herbivores are undeniably less diverse than shoot herbivores: around three quarters of insect Orders possess no root feeding species at all. Secondly, root herbivory by insects is dominated by a single feeding guild; external root chewers. This contrasts sharply with shoot herbivores, which encompass free-living chewers, borers, gall-formers, sap-feeders, miners and shredders. Thirdly, root herbivores, in general, have longer lifespans than shoot herbivores, ranging from several weeks (e.g. root flies), months (e.g. weevils), years (e.g. scarabs) to over a decade in the case of cicadas (Brown & Gange, 1990), leading to longer periods of interaction with their host plants. Fourthly, the soil environment shapes patterns of herbivory to a much greater extent than the aboveground environment influences shoot herbivory (Barnett & Johnson, 2013; Erb & Lu, 2013). Moreover, root herbivores are constantly exposed to an impressive diversity of microbes, many of which have beneficial, commensal and pathogenic relationships with host plants (Edwards *et al.*, 2015). These are likely to fundamentally affect plant suitability for herbivores far more frequently than aboveground, where microbial drivers are more limited. Living in the soil matrix also means that root herbivores are not always in physical contact with a host plant and can graze different parts of the root system which are not necessarily connected. Shoot herbivores, in contrast, are more likely to feed sequentially on connected parts of the plant and therefore are possibly more rapidly affected by induced plant defences. Finally, root herbivores are usually more aggregated than aboveground herbivores and have limited mobility, so cannot readily disperse or relocate between host plants as soil-dwelling life-stages (Brown & Gange, 1990). This probably means that plant roots come under relatively infrequent attack compared with shoot herbivory, but when they do it is acute and sustained.

Taken together, the generic description of a root herbivore is one that chews and severs plant tissue for relatively long periods of time; their herbivory is tightly linked to their immediate environment and so often occurs in tandem with abiotic stress (e.g. drought) and dynamic microbial communities; their attacks on plants are infrequent but persistent and damaging. We propose that these traits may drive the different responses of plants to above- and belowground attack.

III. Plant perception of attack and induced signalling

To respond appropriately to herbivores, plants need to perceive the attack and reconfigure their metabolism accordingly. As with shoots, plant roots respond differently to mechanical damage and herbivore attack (e.g. Lu *et al.*, 2015) and to different herbivore species (Rasmann & Turlings, 2008). To date, it remains unclear to what extent these responses are due to the specific perception of herbivores or due to different patterns of damage. A subset of plant responses are triggered by wounding alone through the recognition of damage-associated molecular patterns (Heil, 2009). Because roots are protected from abiotic mechanical stresses like wind and rain, wounding may be a more reliable indicator of herbivore attack than aboveground. At the same time, an open wound immediately comes into contact with a large number of root-associated microorganisms, many of which produce elicitors that trigger pathogen-related responses (Berendsen *et al.*, 2012). It is therefore likely that microorganisms play a much more important role in modulating herbivore recognition and responses in roots than shoots. Some herbivores are able to manipulate the root metabolism to their benefit. The most striking examples come from piercing-sucking insects that induce the formation of specific feeding structures (Kellow *et al.*, 2004). Although the mechanisms behind these forms of root manipulation are not well understood, they are insect specific and likely involve the secretion of herbivore associated molecular patterns that reprogram the root metabolism. Currently, two experimental limitations constrain our understanding of specific recognition and manipulation in below ground systems: (i) the inability to accurately mimic herbivore damage through mechanical wounding and (ii) the lack of root herbivore elicitor collection and application techniques. Overcoming these constraints will be essential to understand how roots differ from the leaves in their capacity to perceive and respond to attack.

Following herbivore recognition, plants reconfigure their metabolism through changes in phytohormonal networks (Howe & Jander, 2008). Although roots and shoots produce the same hormones, the architecture of the regulatory networks and their impact on tissue growth and development differs significantly (Acosta *et* *al.*, 2013). Jasmonates, which are widely viewed as the master regulators of plant responses to herbivores, are less inducible in the roots than the leaves. Nevertheless, roots respond to herbivore attack by increasing their jasmonate production (Lu *et al.*, 2015) and jasmonates regulate root resistance (Pierre *et al.*, 2012), suggesting that the lower jasmonic acid (JA) burst may be compensated for by higher JA sensitivity or the deployment of additional synergistic signals. Salicylic acid (SA) signalling, for instance, can buffer the JA response aboveground (Gilardoni *et al.*, 2011). In contrast to leaf herbivore attack, root herbivore attack does not seem to induce SA signalling, which again may boost JA signaling. Overall, initial experiments show that root herbivore attack induces different signal signatures compared to leaf attack. Attacked rice roots, for instance, do not increase the biosynthesis of absisic acid (ABA) and ethylene (Lu *et al.*, 2015), two important synergistic signals in the wound response of leaves (Table 2) (Bodenhausen & Reymond, 2007). This difference may be explained by the fact that both hormones strongly influence root growth and architecture; plants may therefore be able to maintain root development under herbivore attack by maintaining ABA and ethylene homeostasis. Based on the different roles of plant hormones in the roots and the first phytohormone screens, it seems clear that roots respond to attack differently to shoots, and regulate their defences through modulating their phytohormonal networks in a tissue-specific manner.

IV. Growth, photosynthetic and primary metabolite responses

Root herbivory affects patterns of growth, photosynthesis and primary metabolism in a distinct manner. Root herbivory can (i) decrease water and nutrient uptake via decreased root biomass or disruption of water and nutrient hydraulics, (ii) deplete resources that the plant is storing belowground, (iii) impose water deficits that reduce rates of photosynthesis and (iv) cause photoassimilates to be diverted belowground for root regrowth and repair. Tolerance to herbivory depends on compensatory growth, a critical way in which plants can endure attack. Compensatory growth in response to root herbivory usually occurs via lateral root proliferation (Brown & Gange, 1990), akin to increased levels of branching following stem herbivory (Stephens & Westoby, 2015), though plants find it harder to compensate, much less overcompensate, for root damage (17% of cases) compared with shoot herbivory (35-44% of cases; (Hawkes & Sullivan, 2001). Root and leaf turnover rates are not dissimilar, so it would appear that plants at least have the capacity to compensate for root attack, but can't realise it, possibly because root herbivory reduces rates of photosynthesis in plants, by about 11.7% across plant species, in contrast to shoot herbivory, which generally stimulates it (Zvereva & Kozlov, 2012).

Photoassimilates are often translocated to the roots for storage, particularly after episodes of shoot herbivory (Schultz *et al.*, 2013); do plants move primary metabolites in the reverse direction in response to root herbivory? Evidence is limited, but Robert *et al.* (2014) showed that maize plants infested with root herbivores allocated carbon to the stems as a prelude to root regrowth. Similarly, nitrogen was allocated away from roots to the shoots in knapweed (Newingham *et al.*, 2007) and the stems in milkweed (Tao & Hunter, 2013) following root attack. However, root herbivores may also manipulate their hosts to allocate primary metabolites, including carbon (Pierre *et al.*, 2012; Robert *et al.*, 2012) and phosphorus (Johnson *et al.*, 2013b) belowground to improve host plant quality (Erb *et al.*, 2013).

V. Secondary metabolite responses

The increased production of bioactive secondary metabolites enables plants to decrease leaf-herbivore damage, either by poisoning or repelling herbivores or by attracting their natural enemies. Comparative studies demonstrate that roots employ similar strategies, but through different metabolites. In maize for instance, root herbivore attack leads to the release of one predominant terpene signal which attracts natural enemies, while leaf herbivore attack triggers a complex blend of compounds that may be involved in attracting natural enemies reviewed by (Degenhardt, 2009). Also, indole glucosinolates are highly inducible in the leaves but not the roots of cruciferous plants, but roots produce higher constitutive levels of the toxic 2-phenylethyl glucosinolates (van Dam *et al.*, 2009). Despite these differences, many plants also seem to use the same types of inducible secondary metabolites to defend both leaves and roots (Kaplan *et al.*, 2008) and the review by van Dam (2009) concluded that generalizations regarding differences in root and shoot secondary metabolites are difficult to make. Several factors may contribute to the seemingly stochastic differences between leaf and root secondary metabolite responses: (i) root induced responses are regulated through different phytohormonal networks (see above), which may lead to specific physiological effects; (ii) some secondary metabolites are mobile and may be produced in one organ and then transported into the other and (iii) the rhizosphere and its microbial inhabitants may lead to selection pressures that shape root secondary metabolite profiles differently compared to the phyllosphere, or even lead to the production of defence metabolites themselves (Hartley & Gange, 2009; Johnson & Rasmann, 2015).

VI. Plant defence theories – their applicability belowground

Many theories have been put forward to address why plants display so many different defence mechanisms, how these vary in type and amount within and between plants, and how they are allocated to different plant tissues (Hartley & Jones, 1997; Stamp, 2003). However, these rarely address root tissues specifically, if indeed at all (Rasmann *et al.*, 2011) and experimental tests of these theories which involve root tissue remain sparse relative to those aboveground (Rasmann & Agrawal, 2008). Generally it has proved easier, and more common, to test the applicability of theories of defence allocation to aboveground tissue than below ground ones for several reasons. Firstly, although the situation is improving, there is still far less data on levels of secondary metabolites in roots than in foliar plant parts, affecting the application of techniques such meta-analysis to patterns of defence; many such studies do not include root-specific analyses (e.g. Endara & Coley, 2011; Massad *et al.*, 2011). Secondly, many defence allocation theories are based on trade-offs, either between growth and defence, or between limited resources, or in response to abiotic stress. We know rather less about phenotypic plasticity in response to changes in plant growth rate or resource availability in belowground tissues. It does appear that root lifespan is a less important driver of defence investment than leaf lifespan (Rasmann *et al.*, 2011) and that roots and shoots respond differently to changing environmental conditions (Gargallo-Garriga *et al.*, 2015), perhaps reflecting different growth and resource priorities. Understanding allocation patterns is likely to be particularly challenging in cases where defences are synthesised in roots but transported to shoots, such as alkaloids (van Dam, 2009): are the compounds we measure in roots being synthesised for storage, for future mobilization of defence in shoots, for direct defence of roots, or a combination of all three? Lastly, plant growth, nutrient acquisition and, as we increasingly recognise, defence against pathogens and other invading organisms, depends on interactions with beneficial microorganisms, both inside and outside root tissue (Edwards *et al.*, 2015; van der Heijden &

Schlaeppi, 2015). This, together with the huge abundance and diversity of soil microbes which plant roots encounter, sets a different evolutionary and physiological context for defence belowground: metabolites designed to attract beneficial colonisers may alter susceptibility to pathogenic organisms, whilst defence compounds produced against microbial colonisers may disrupt wound signalling, as has been found in foliar tissue (Hartley *et al.* 2015).

VII. Where next?

The exact mechanisms underpinning stochastic differences in root and shoot secondary metabolite responses remain perplexing and may include differences in phytohormonal networks, mobility of metabolites between above- and belowground organs (and *vice versa*), selection pressures reflecting the complex environment of the rhizosphere and its microbial community, or a mixture of all three. Extending our understanding of belowground defences will therefore depend on investigating responses in these three contexts. In closing, we propose three areas of research (Box 1) which relate specifically to herbivory in the rhizosphere which could provide intriguing insights into how plants respond to their hidden insect adversaries.

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Table 1. Comparison of the life history traits and ecology of root and shoot herbivores.

Insect life history traits and ecology	**Root herbivores**	**Shoot herbivores**
Diversity	Low Represented in 17% of herbivorous families[1]	High Represented in 81% of herbivorous families[1]
Feeding guild	Predominantly external chewers (c. 81%)[2]	Diverse feeding guilds
Lifespan	Long	Short
Interaction with abiotic environment	Limited variation (soil buffering) but tightly linked to patterns of herbivory	High variable; usually less influential
Exposure to the microbial environment	Constant; rhizopheric microbial community immense and highly diverse	Variable; mostly with pathogenic or endophytic fungi associated with the plant
Connectivity with host plant	Unattached and able to graze unconnected tissues	Often attached or feed sequentially on connected tissues
Distribution and mobility	Highly aggregated	Less aggregated and more mobile in many taxa

[1] Based on 257 North American insect herbivore families (Rasmann & Agrawal, 2008)
[2] Based on the experimental observations in the meta-analysis of Zvereva and Kozlov (2012)

Table 2. Phytohormone responses to shoot and root herbivory and pathogen infection. Increases in hormones indicated in green (+), decreases in red (-), no overall change or examples of both responses equally reported indicated in blue (0), untested or unknown changes indicated in white (?).

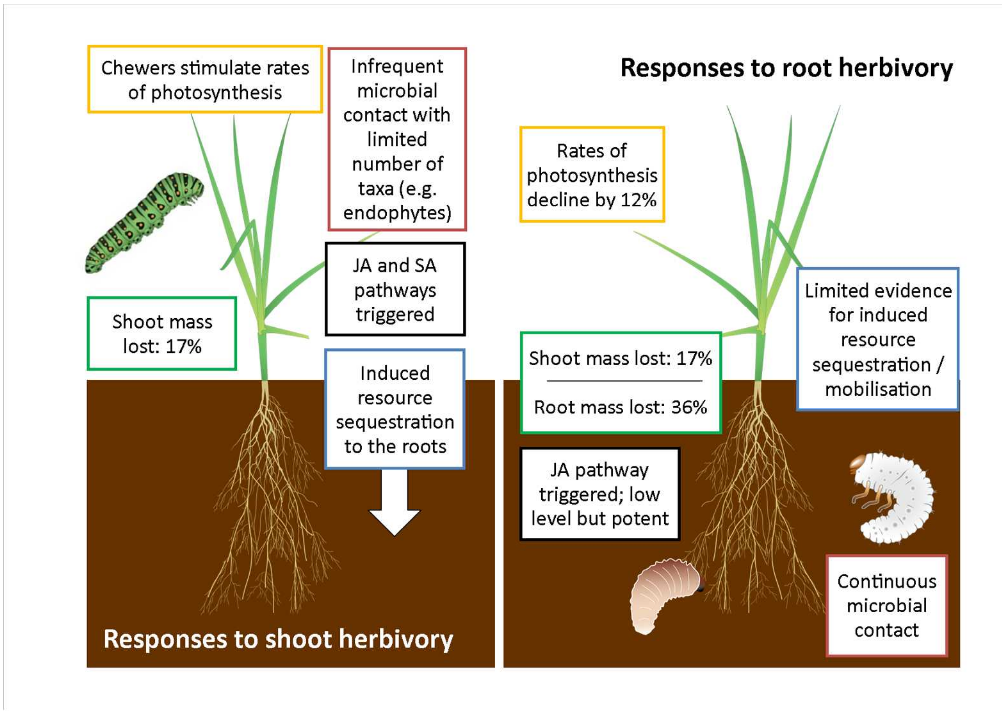
Phytohormone	**Defensive response against shoot herbivory**		**Defensive response against root herbivory**	
	Inducibility	*Resistance*	*Inducibility*	*Resistance*
Jasmonates	+	+	+	+
Abscisic acid	+	+	0/+	0**
Ethylene	+	+	0/+*	0**
Cytokinins	+	+	?	?
Salicylic acid	+	-/+	0/+	0**
Giberellins	?	-	?	?
Auxins	-/+	-/+	+	?

* Inferred from gene expression patterns; ** Exogneous application; *** Selection. Information drawn from (Erb *et al.*, 2012; Soler *et al.*, 2012; Soler *et al.*, 2013; Agtuca *et al.*, 2014; Lu *et al.*, 2015; Schäfer *et al.*, 2015)

Figure and Box Legend

Figure 1. (Draft). Salient and generic differences in plant responses to shoot and root herbivory. JA = jasmonic acid, SA = salicylic acid. Global changes in % relate to figures reported in Zvereva and Kozlov (2012).

Box 1. (Draft) We propose three distinct research areas that may feature heavily in how plants respond to belowground attack.



Salient and generic differences in plant responses to shoot and root herbivory. JA = jasmonic acid, SA = salicylic acid. Global changes in % relate to figures reported in Zvereva and Kozlov (2012).
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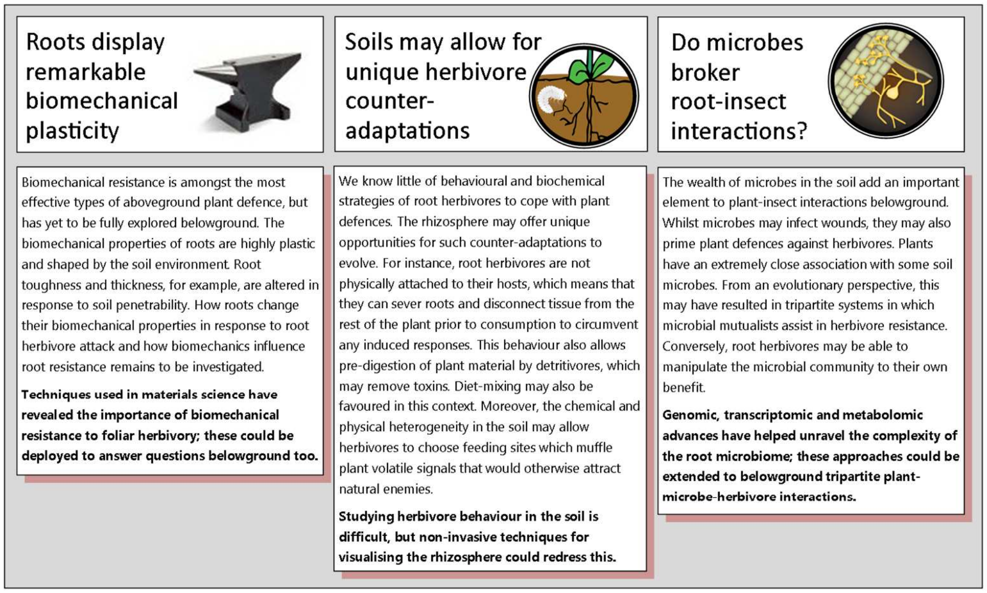

Roots display remarkable biomechanical plasticity

Biomechanical resistance is amongst the most effective types of aboveground plant defence, but has yet to be fully explored belowground. The biomechanical properties of roots are highly plastic and shaped by the soil environment. Root toughness and thickness, for example, are altered in response to soil penetrability. How roots change their biomechanical properties in response to root herbivore attack and how biomechanics influence root resistance remains to be investigated.

Techniques used in materials science have revealed the importance of biomechanical resistance to foliar herbivory; these could be deployed to answer questions belowground too.

Soils may allow for unique herbivore counter-adaptations

We know little of behavioural and biochemical strategies of root herbivores to cope with plant defences. The rhizosphere may offer unique opportunities for such counter-adaptations to evolve. For instance, root herbivores are not physically attached to their hosts, which means that they can sever roots and disconnect tissue from the rest of the plant prior to consumption to circumvent any induced responses. This behaviour also allows pre-digestion of plant material by detritivores, which may remove toxins. Diet-mixing may also be favoured in this context. Moreover, the chemical and physical heterogeneity in the soil may allow herbivores to choose feeding sites which muffle plant volatile signals that would otherwise attract natural enemies.

Studying herbivore behaviour in the soil is difficult, but non-invasive techniques for visualising the rhizosphere could redress this.

Do microbes broker root-insect interactions?

The wealth of microbes in the soil add an important element to plant-insect interactions belowground. Whilst microbes may infect wounds, they may also prime plant defences against herbivores. Plants have an extremely close association with some soil microbes. From an evolutionary perspective, this may have resulted in tripartite systems in which microbial mutualists assist in herbivore resistance. Conversely, root herbivores may be able to manipulate the microbial community to their own benefit.

Genomic, transcriptomic and metabolomic advances have helped unravel the complexity of the root microbiome; these approaches could be extended to belowground tripartite plant-microbe-herbivore interactions.

We propose three distinct research areas that may feature heavily in how plants respond to belowground attack.
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