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Article Addendum

Belowground ABA boosts aboveground production of DIMBOA and primes induction of chlorogenic acid in maize

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Plants are important mediators between above- and belowground herbivores. Consequently, interactions between root and shoot defenses can have far-reaching impacts on entire food webs. We recently reported that infestation of maize roots by larvae of the beetle *Diabrotica virgifera virgifera* induced shoot resistance against herbivores and pathogens. Root herbivory also enhanced aboveground DIMBOA and primed for enhanced induction of chlorogenic acid, two secondary metabolites that have been associated with plant stress resistance. Interestingly, the plant hormone abscisic acid (ABA) emerged as a putative long-distance signal in the regulation of these systemic defenses. In this addendum, we have investigated the role of root-derived ABA in aboveground regulation of DIMBOA and the phenolic compounds chlorogenic acid, caffeic and ferulic acid. Furthermore, we discuss the relevance of ABA in relation to defense against the leaf herbivore *Spodoptera littoralis*. Soil-drench treatment with ABA mimicked root herbivore-induced accumulation of DIMBOA in the leaves. Similarly, ABA mimicked aboveground priming of chlorogenic acid production, causing augmented induction of this compound after subsequent shoot attack by *S. littoralis* caterpillars. These findings confirm our notion that ABA acts as an important signal in the regulation of aboveground defenses during belowground herbivory. However, based on our previous finding that ABA alone is not sufficient to trigger aboveground resistance against *S. littoralis* caterpillars, our results also suggest that the ABA-inducible effects on DIMBOA and chlorogenic acid are not solely responsible for root herbivore-induced resistance against *S. littoralis*.

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Introduction

While previous studies of plant responses to herbivorous insects have mostly concentrated on locally infested tissues, it is becoming increasingly clear that the plant's metabolism also changes in distant plant parts.[1] Striking examples of systemic regulation of plant defense come from studies about the impact of root herbivory on shoot secondary metabolites.[2] In a recently conducted meta-analysis, Kaplan et al.[3] concluded that the systemic response of leaves to root herbivory is as pronounced as the local response. It seems, therefore, plausible to assume that induction of shoot defenses by root herbivory can have important consequences for aboveground attackers and multitrophic systems.[4-6] However, it remains unclear how or why this phenomenon occurs in the first place. For that reason, we set out to elucidate the physiological basis of root herbivore-induced shoot resistance in *Zea mays*.[7] Our results indicated that changes in shoot physiology upon infestation by the root herbivore *Diabrotica virgifera virgifera* are not the result of classical systemic wound signaling, but correlate with (i) a reduction in leaf water content, (ii) a local and systemic induction of abscisic acid (ABA) and (iii) a systemic increase of defense gene expression and secondary metabolite biosynthesis.[7] While we provided evidence for a role of ABA in aboveground induction of defense genes and pathogen resistance,[7] the systemic changes in secondary metabolite profiles could still be the result of either ABA-dependent or ABA-independent processes. In this study, we further examined the role of ABA in the regulation of aboveground defense metabolites. To this end, we mimicked *D. virgifera*-induced signaling by exogenous application of ABA to the roots and quantified the resulting levels of defense-related secondary metabolites in the leaves.

Root Treatment with ABA Boosts Aboveground DIMBOA Levels

A prominent secondary metabolite that increases in maize leaves during infestation by *D. virgifera* is 2,4-dihydroxy-7-methoxy-1, 4-benzoxazin-3-one (DIMBOA), a compound that has been described as a central defense metabolite in monocotyledonous plants.[8,9] The predominant form of DIMBOA in maize leaf tissue

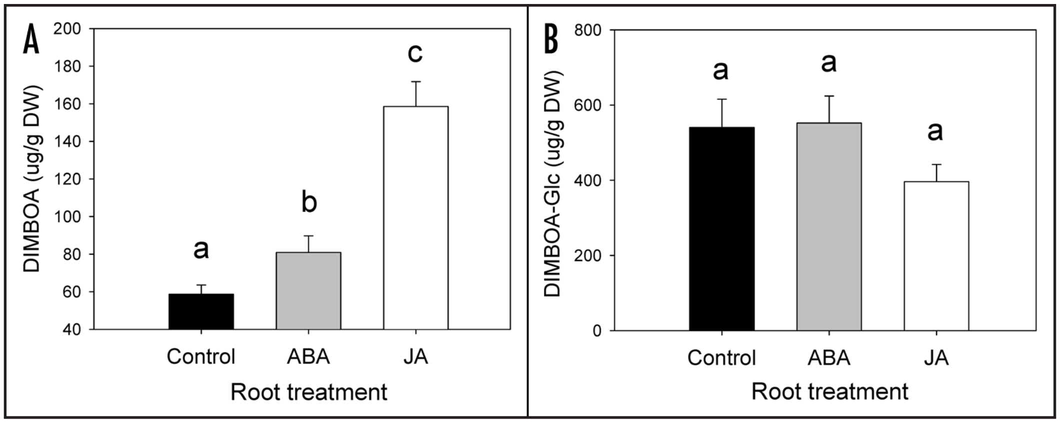

Figure 1. Impact of belowground application of absicssic acid (ABA) and jasmonic acid (JA) on aboveground production of hydroxamic acids in maize seedlings. (A) Shoot concentrations (average + SE) of DIMBOA in untreated (Control, black bars), ABA-treated (ABA, grey bars) and JA-treated (JA, white bars) plants. (B) Shoot concentrations (average + SE) of DIMBOA glucoside. Different letters indicate statistically significant differences (ANOVA post-hoc test $p < 0.05$). DW: dry weight.

is the inactive glucoside (DIMBOA-Glc), which is stored within the vacuole.[9] Although the activity of the biosynthetic pathway declines as the plant matures,[10] the plant hormone jasmonic acid (JA) has been shown to exert a stimulatory effect on DIMBOA production.[11] To investigate the impact of root-derived ABA on DIMBOA in the leaves, we collected shoot tissues of 12-day old plants at 1 day after soil-drench treatment with 300 uM ABA. We included a soil-drench treatment with 500 uM JA as a positive control treatment. Since DIMBOA production can result from hydrolysis of DIMBOA glucoside, as well as an upregulation of the entire pathway, we quantified levels of both DIMBOA and DIMBOA-Glc, as described before.[7] Shoot levels of DIMBOA-Glc were not significantly influenced after root treatment with either ABA or JA (Fig. 1). By contrast, both hormones caused a significant increase in shoot levels of the DIMBOA aglucon, indicating that these effects are caused by an upregulation of the entire biosynthetic pathway, rather than merely hydrolysis of DIMBOA-Glc. This conclusion is consistent with our finding that the *Bx1* gene, encoding the first dedicated enzyme of the DIMBOA biosynthesis pathway, was systemically induced upon infestation by the root herbivore.[7] While treatment with JA induced significantly more DIMBOA than did ABA, the systemic effect of ABA was comparable to what we had observed upon *D. virgifera* infestation (Fig. 1). Because root infestation by *D. virgifera* increases ABA and not JA in the leaves,[7] we conclude that *D. virgifera*-induced production of DIMBOA in the leaves is mediated by ABA.

Root Treatment with ABA Primes Aboveground Chlorogenic Acid Production

Apart from hydroxamic acids, phenolics are another class of secondary metabolites that have been implicated in plant resistance against insects.[12] Our earlier experiments had shown that root infestation by *D. virgifera* failed to induce any of the measured phenolic compounds in maize leaves directly. However, the induction of chlorogenic acid was more pronounced upon aboveground attack by *S. littoralis*, suggesting that below-ground herbivory primes the induction of this compound. To test the role of ABA in this systemic priming response, we soil-drenched maize seedlings over a period of two days to a total concentration of 300 μM ABA. Subsequently, leaves of 12-day old plants were exposed for 12 hours to infestation by 20 L2 *S. littoralis* larvae, after which shoot levels of chlorogenic acid were quantified. In the same tissues, we quantified levels of caffeic acid and ferulic acid. While ABA failed to increase chlorogenic acid levels directly, we observed a significant induction of this compound in ABA-drenched plants upon subsequent *S. littoralis* attack (Fig. 2). Hence, treatment of the roots with ABA, like belowground infestation by *D. virgifera*, primes maize leaves for augmented induction of chlorogenic acid. This adds to our notion that the metabolic changes observed after root herbivory are indeed mediated by ABA. However, soil-drench treatment with ABA also enhanced aboveground levels of caffeic and ferulic acid directly (Fig. 2). This is in contrast with our earlier results,[7] which revealed no effects of *D. virgifera* on these two phenolic compounds. It remains to be investigated if this difference is related to dose- or temporal-dependent effects, or whether other *D. virgifera*-inducible signals are counteracting the ABA-induced accumulation of caffeic acid and ferulic acid. Interestingly, the induction of both phenolics by ABA was no longer detectable after subsequent shoot infestation by *S. littoralis*. This suggests that the shoot herbivore is capable of suppressing induction of caffeic acid and ferulic acid in the leaves.

Relevance and Adaptiveness of ABA-Induced Changes in Leaf Chemistry

Root treatment with ABA failed to mimic *D. virgifera*-induced shoot resistance against *S. littoralis* caterpillars.[7] This strongly suggests that the ABA-inducible effects on DIMBOA and chlorogenic acid are not sufficient to explain the aboveground resistance of maize leaves against *D. virgifera*. While it is possible that these secondary metabolites have a synergistic function with other induced plant defenses, such as proteinase inhibitors,[13] alternative explanations for the phenomenon of root herbivore-induced shoot resistance have to be considered. For example, the

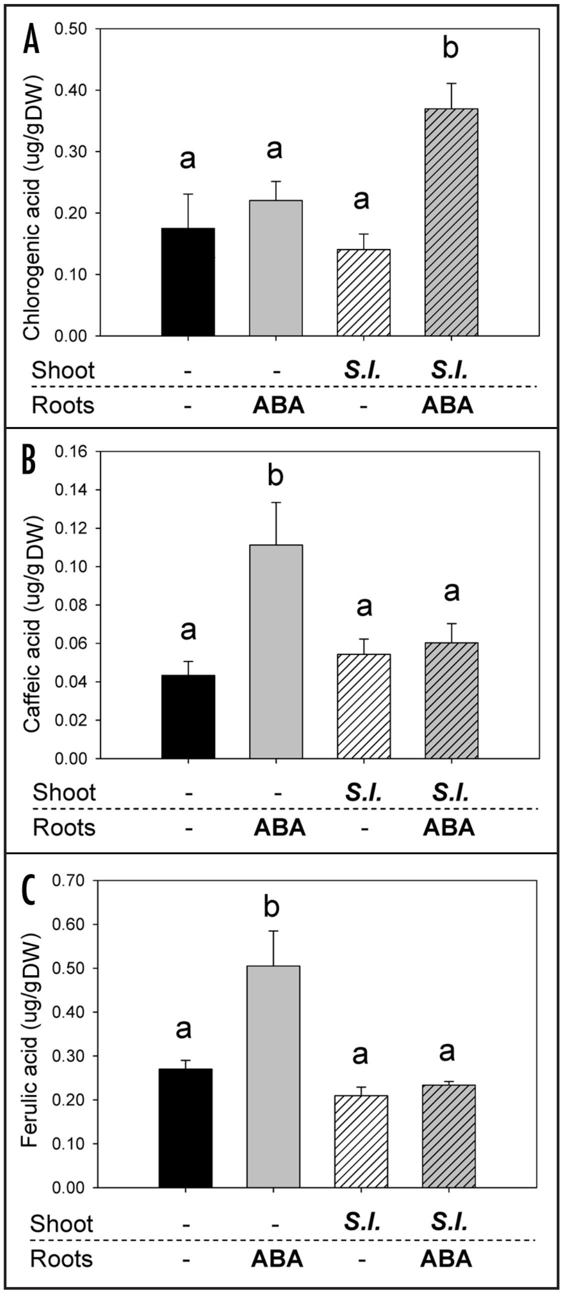

Figure 2. Impact of belowground application of ABA on aboveground induction of phenolic acids by *Spodoptera littoralis* larvae. (A) Shoot concentrations (average + SE) of chlorogenic acid in control- and ABA-treated (ABA) maize seedlings with and without subsequent shoot infestation by *S. littoralis* (*S.l.*). (B) Shoot concentrations (average + SE) of caffeic acid. (C) Shoot concentrations (average + SE) of ferulic acid. Different letters indicate statistically significant differences (ANOVA post-hoc test $p < 0.05$). DW: dry weight.

reduced water content in leaves of *D. virgifera*-infested plants could render the tissue less digestible for the shoot herbivore. Alternatively, additional root-shoot signals may be responsible for activation of ABA-independent defenses against *S. littoralis*.

Why many plants increase their investment in leaf secondary chemistry upon root herbivory has long remained elusive.[3,14] In case of maize, the changes in shoot physiology seem to be tightly linked to drought stress.[7] Interestingly, DIMBOA, as well as various phenolic compounds, have been reported to increase in water-stressed maize seedlings.[15,16] Consequently, it is possible that both classes of secondary metabolites are involved in abiotic stress tolerance. In agreement with this, some phenolic compounds have been reported to act as putative photoprotectors to limit excitation of chlorophyll during conditions of water deficit.[15] In this light, we propose that the effects of root herbivory on aboveground secondary metabolites are related to water-stress, which triggers an ABA-dependent tolerance response that includes activation and priming of protective secondary metabolites.

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