

Plant integrity

An important factor in plant-pathogen interactions

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The effect of plant integrity and of above- and below-ground defense signaling on plant resistance against pathogens and herbivores is emerging as a subject of scientific research. There is increasing evidence that plant defense responses to pathogen infection differ between whole intact plants and detached leaves. Studies have revealed the importance of aboveground-belowground defense signaling for plant defenses against herbivores, while our studies have uncovered that the roots as well as the plant integrity are important for the resistance of the potato cultivar Sarpo Mira against the hemibiotrophic oomycete pathogen *Phytophthora infestans*. Furthermore, in the Sarpo Mira-*P. infestans* interactions, the plant's meristems, the stalks or both, seem to be associated with the development of the hypersensitive response and both the plant's roots and shoots contain antimicrobial compounds when the aerial parts of the plants are infected. Here, we present a short overview of the evidence indicating the importance of plant integrity on plant defense responses.

Plants possess a dynamic, innate immune system that responds to and protects against different herbivores and pathogens.^{1,2} Upon exposure to harmful organisms, plants activate local and systemic defenses that increase their tolerance or resistance to the threat.^{3,4} These mechanisms involve the participation of defensive metabolites and proteins whose synthesis, distribution and accumulation is, in part, orchestrated by plant hormones signaling.

Until recently, research efforts devoted to study plant responses to pathogens and herbivores that attack the aerial parts of plants were almost entirely focused on investigating leaves or shoots independently.⁵ As a consequence, detached leaves and shoots (de-rooted plants) have been widely used for studying plant-herbivore/pathogen interactions.⁶⁻¹⁰ The role of plant roots on systemic defense mechanisms has been largely unappreciated and discrepancies between results obtained using whole plants or plant parts have been attributed to experimental differences.¹¹⁻¹⁴ Researchers have only lately begun to search for

whole-plant responses and recent evidence suggests that many defense responses are systemic and involve a communication between the aboveground (AG) plant tissues and the roots [belowground (BG) tissues].¹⁵⁻¹⁷

Evidence is emerging that plant defense responses to pathogen infection differ between whole intact plants and detached leaves. In a review from 2007, Lieberei¹⁸ discussed the importance of having the inoculated leaves attached to the mother plants when studying the defense of rubber trees (*Hevea* spp.) against the necrotrophic fungus *Microcyclus ulei*. According to Lieberei,¹⁸ leaves are metabolic sink tissues and are dependent on the energy balance of the mother plant for a long time. Energy-dependent synthesis requires the transport of assimilates into the leaves. Therefore, it will be expected that resistance-screening experiments performed with detached leaves will lead to different results from those with leaves attached to plants.¹⁸ Processes that are involved in pathogen defense, such as cinnamic acid synthesis, changes in pool sizes of amino acids, and the active synthesis of scopoletin, lignin and glycosides, will be retarded or even stopped in detached leaves due to exhaustion of energy-delivering compounds.¹⁸

Also studies of *Arabidopsis thaliana*-*Colletotrichum* interactions showed differences between the defense responses of detached leaves compared with that of attached leaves.¹⁹ The infection of detached *A. thaliana* leaves with the hemibiotrophic pathogen *Colletotrichum* led to atypical symptoms that appeared uncoupled from usual plant defense response pathways and more closely associated with responses involved in plant senescence.¹⁹ The differences between attached and detached leaves were also reflected in the differential expression of pathogenesis-related genes.¹⁹

Several reports demonstrated significant differences in defense responses of detached compared with attached leaves in the potato (*Solanum tuberosum*)-*Phytophthora infestans* interaction.^{12-14,20,21} These differences were attributed to the experimental setup^{12,13} or to the presence of a specific *R* gene.^{14,21} In our recent study, we showed that not only the visual symptoms of the infection differed between attached and detached leaves, but also the expression of pathogenesis-related genes, such as the acidic and basic chitinases (*ChtA* and *ChtB*) and *PR-1*, was more highly induced by the pathogen in leaves of whole plants than in detached leaves at early time points.²⁰ Interestingly, we had previously observed that these genes were induced earlier in resistant plants than in

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susceptible ones.²² Future studies will indicate whether the difference in resistance between whole intact plants and detached leaves are limited to individual hemibiotrophic/necrotrophic-plant interactions or if it is a more general process in plant-pathogen interactions.

Most of the studies on the signaling between AG and BG plant responses concerned plant-herbivore interactions. It has been shown that roots subjected to herbivore attack, mechanical damage or jasmonic/salicylic acids (JA/SA) application promote an increase in the levels of shoot defenses (reviewed by Erb et al.²³). Likewise, shoot herbivory can induce the synthesis of defense compounds in roots, and this effect has also been observed in shoots treated with JA/SA. An example can be found in the genus *Nicotiana*, in which, upon the leaf damage, nicotine production is induced in the roots and is then transported to AG tissues, providing foliar protection against further herbivore attacks.^{24,25}

In our study, we addressed the impact of roots on the resistance of the highly resistant potato cultivar Sarpo Mira against the hemibiotrophic oomycete pathogen *P. infestans* and found that roots were indeed important to achieve full resistance.²⁰ Our findings indicate that compounds with antimicrobial activity against *P. infestans* accumulate in both the leaves and roots of the resistant plant cultivar, and do not occur in measurable amounts in the susceptible potato variety Bintje.²⁰ Though the accumulation happens differently in AG and BG tissues, the leaves of the resistant cultivar had a measurable antimicrobial activity prior to the inoculation with *P. infestans*, while the antimicrobial activity in the roots was only detected once the AG parts of the plants were challenged with the pathogen.²⁰

The importance of the roots in establishing an efficient foliar resistance to the pathogen was confirmed by grafting experiments using shoots and roots of the susceptible and resistant potato cultivars.²⁰ Although it seems that the plant shoot system plays the main role in the *P. infestans* resistance of Sarpo Mira, full foliar resistance was achieved only when the resistant shoots were grafted to the resistant roots.²⁰ Other approaches using parts of the plant with or without roots also highlighted the significance of plant integrity in the defensive response to the oomycete pathogen.²⁰

The establishment of full foliar resistance against *P. infestans* clearly implies shoot-root communication. Erb and colleagues²⁶ have proposed mechanisms that account for the role of roots in the foliar resistance against herbivores and which could apply to explain the role of roots in the resistance of potato against *P. infestans*. Severe pathogen infection can partially destroy the AG tissues also in resistant cultivars. In that situation, the

induced production of antimicrobial compounds in BG tissues and their subsequent delivery to the shoot could be a valuable strategy to counteract the pathogen infection. In addition to the production of antimicrobial compounds, the roots would then provide assimilates to enable re-growth of the plant.²⁶ In view of the evidence, we proposed a model of AG-BG signaling during *P. infestans* infection of potato plants where the shoot needs to mobilize a signal or an active compound to or through the roots to achieve complete resistance.²⁰

Few signaling molecules have been proposed to be important in the plant-herbivore/pathogen interactions though their roles are still not fully elucidated and almost certainly alternative shoot-root signals await to be discovered. One known shoot-root signal molecule is auxin (Indole-3-acetic acid; IAA).²⁷ Auxin can inhibit the growth of *P. infestans* in vitro and in detached potato leaves.²⁸ Auxin has also been suggested to be a signal molecule in the potato-*P. infestans* interaction, acting through the regulation of the enzyme glutathione S-transferase (GST).²⁹ The inhibition or modification of GST activity by auxin could modulate the necrosis of host tissue in the vicinity of infection sites.²⁹ This controlled cell death is typical of the hypersensitive response (HR) and a common symptom of the late blight disease in potato cultivars carrying *R* genes. Our latest study²⁰ also suggested that the number of HR lesions increased with increasing number of meristems, which are rich sources of auxin.³⁰ The leaves with meristems also included short stalks and therefore the effect of stalks and meristems cannot be separated. No HR lesions were observed in the absence of meristems and stalks.²⁰ Altogether, it is tempting to speculate that auxin may well be the signal, or one of the signals, responsible for the enhanced resistance observed in Sarpo Mira plants, an hypothesis yet to be tested.

In addition to the more studied defense responses at the level of each single cell,^{2,31} we are now starting to recognize a new layer of the plant innate immune system that implies the coordination of different plant organs to achieve a more efficient defense response. It will be extremely interesting to further comprehend the mechanisms behind this AG-BG signaling.

Since oomycete pathogens are widespread and responsible for major plant diseases, knowledge about whole-plant coordinated defense responses could contribute to the development of more resistant plants and novel pest control strategies.

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