

# Lack of Active Defence Responses Revealed in a Soil-free *Arabidopsis*/*Peronospora* Sterile Co-Cultivation System

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## Abstract

The molecular basis of organ specificity in plant diseases is little characterised. Downy mildew of *Arabidopsis* caused by the oomycete *Peronospora parasitica* is characteristically a leaf disease. Resistant host genotypes recognise the pathogen in a gene-for-gene dependent manner and respond with the production of H<sub>2</sub>O<sub>2</sub> and the execution of a genetically programmed hypersensitive cell death (HR). We inoculated the roots of *Arabidopsis* genotypes Col-0, Ws-0 and Wei-0 with the NOCO and WELA races of the pathogen and compared the responses with those observed in infected leaves. Combinations of incompatible genotypes of host and pathogen showed the expected responses of an oxidative burst and the HR in leaves but, surprisingly, roots showed no signs of active defence and appeared completely susceptible to all the *P. parasitica* isolates tested. RT-PCR showed that the *R* genes *RPPI* and *RPPI3*, which mediate resistance in leaves to *P. parasitica* isolates NOCO and WELA, respectively, were expressed in leaves as well as in roots. Similarly, *NDR1* and *EDSI*, two components of *RPPI*-mediated gene signalling pathways, are also expressed in both tissues. Thus, we show for the first time that expression of *R* genes and at least some of the known downstream components of the signalling cascade are not sufficient for the induction of avirulence gene-mediated defence mechanisms.

**Keywords:** defence; physiology; roots; *Arabidopsis*; *Peronospora*

## INTRODUCTION

Downy mildew of *Arabidopsis* caused by *Peronospora parasitica* is a leaf disease, although primary infections at the beginning of the annual growing season occur via the roots. Germinating oospores penetrate the root tissue and the hyphae grow into the stems and leaves where they produce conidiospores that infect the leaves of further plants (KOCH & SLUSARENKO 1990; MAUCH-MANI & SLUSARENKO 1993). This pathosystem is a “gene-for-gene” interaction where specific, generally dominant, resistance gene products in the host recognise the products of specific, complementary avirulence genes in the pathogen (FLOR 1947).

Important cellular processes occurring specifically in the context of pathogen attack can be revealed by

microscopy. Thus, it was shown that shortly after infection there is an influx of calcium ions, massive rearrangement of the cytoskeleton and movement of the nucleus and cytoplasm towards the site of pathogen ingress (for review see HEATH 2000). With the advent of transgenic *Arabidopsis* lines expressing Green Fluorescent Protein targeted to several different intracellular compartments (BOEVINK *et al.* 1998; HASEZAWA *et al.* 2000; MANO *et al.* 2002), we became interested in studying the fate of these compartments during infection by virulent or attempted infection by avirulent pathogenic oomycetes. However, we quickly realised that chlorophyll autofluorescence is a complicating factor regarding observations in leaves. Therefore, we became interested in developing a pathosystem in chloroplast-free tissues of *Arabidopsis*. Roots offer

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the advantage of being suitable for the full range of fluorescent dyes available for cell biological studies without the problem of interference from chlorophyll autofluorescence. However, soil is not a convenient medium for such root studies so we developed a sterile *Arabidopsis-Peronospora* pathosystem in Petri dishes which was suitable for microscopic studies of the infection process. Although *P. parasitica* is clearly normally regarded as a leaf-pathogen of crucifers, since oospore infection initially goes via the roots we decided to investigate the ability of *Peronospora* conidia to infect *Arabidopsis* roots. Details of the sterile co-cultivation system and first results obtained with it, are described here.

## RESULTS AND DISCUSSION

*Arabidopsis* seedlings grown from surface sterilised seed in aseptic Petri plate culture were inoculated on the cotyledons by stroking with a normally-infected (non-sterile) leaf bearing conidiophores. Further incubation under sterile conditions was maintained and those individuals showing no evidence of fungal or bacterial contamination on the agar growth medium were further propagated in a sterile co-cultivation procedure.

For microscopy, sterile conidia from the co-cultivation plates were used to inoculate sterile-grown seedlings that had been placed horizontally *in situ* on microscope slides coated by a thin film of MS agar (Figure 1). This avoided subsequent transfer of the

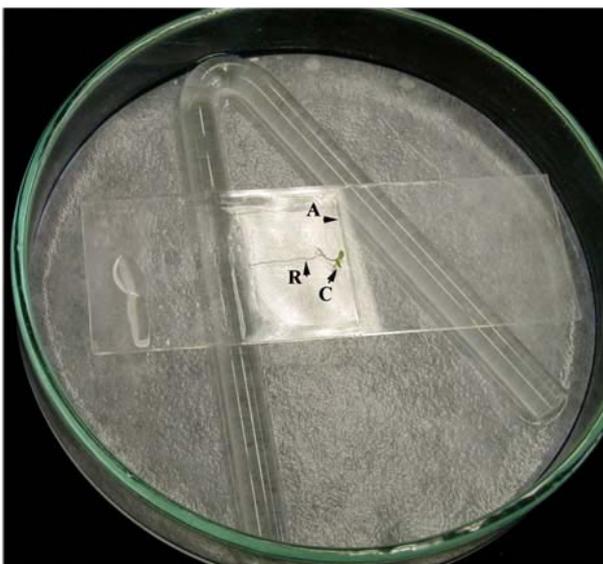


Figure 1. A sterile *Arabidopsis* growth system suitable for microscopy

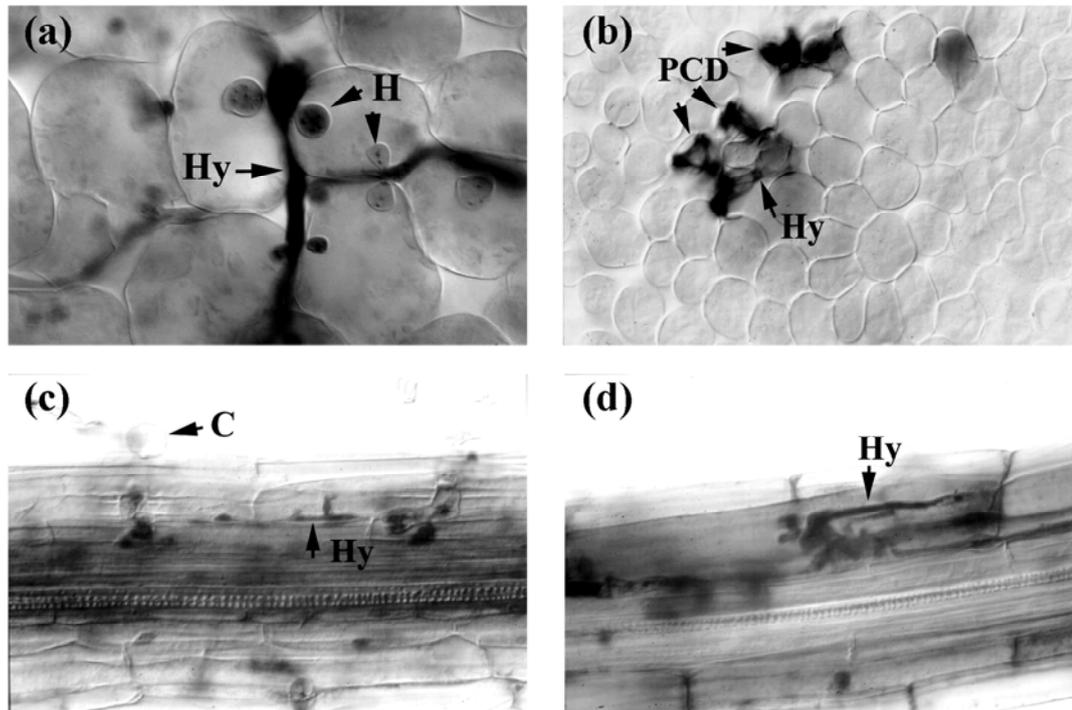
seedling to the microscope slide, which experience showed could distort the entire appearance of the infection site. Microscope slides with the inoculated seedlings were placed in a Petri dish on several layers of sterile paper wetted with sterile water and placed in an environmentally controlled light cabinet for the infection to develop. In this way, the thin agar film on the microscope slide usually remained moist for about three days. At any time point, it was now possible to study the development of the infection process by staining the seedling as well as the oomycete directly on the microscope slide with trypan blue without exerting further physical stress on the infection structures.

Five-day-old *Arabidopsis* seedlings were placed on a microscope slide covered with a thin film of agar (A) and allow to grow for another three days. Roots (R) or cotyledons (C) could be infected by placing conidiospores onto the leaves or spreading them along the root system in a water droplet. The microscope slide was placed in a Petri dish lined with wet paper to keep the relative humidity near 100% (Figure 1).

All experiments were performed with two different incompatible as well as compatible combinations of *Peronospora* isolates/*Arabidopsis* genotypes. All experiments were carried out at least 5 times with identical results.

In leaf infections, conidia were placed directly on the leaf surface, whereas in root infections conidia were spread along the roots, often resulting in a small distance from the spore to the root surface that need to be bridged by the germ tube. Typically, conidia produced germ tubes about 6 hours after inoculation and after a short period of growth these formed appressoria from which the infection hyphae penetrated into the plant tissue. In a compatible combination, intercellular hyphae and haustoria were formed. In leaves and roots there was some variation in timing to a given infection stage. Overall, the infection process in roots was very similar to that observed in leaves with respect to the type of infection structures formed. Much to our initial surprise, however, in roots combinations of host and pathogen that were ostensibly genetically incompatible led to infections indistinguishable in appearance to those seen in genetically compatible combinations (Figure 2).

This is a new observation, that defence occurring in green tissue of the same plant is absent from the root tissue. We are further investigating the basis for the observed tissue specific differences in active defence responses in the *Arabidopsis-Peronospora* pathosystem.



Leaves [panels (a) and (b)] and roots [panels (c) and (d)] were trypan blue stained 72 h after conidiospores were applied the plant. Panels (a) and (c) are from the compatible interaction between the *Arabidopsis* ecotype Wei-0 and the *Peronospora parasitica* isolate WELA. Micrographs depicted in panels (b) and (d) are from the incompatible interaction of ecotype Col-0 and the isolate WELA. Identical results were also obtained in other compatible and incompatible interactions C = conidiospores; Hy = oomycete hypha; H = haustorium; PCD = plant cell death. Scale bar = 20  $\mu$ m

Figure 2. No growth restriction of *Peronospora* occurs in roots of genetically incompatible *Arabidopsis*

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