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Short communication

Organ identity and environmental conditions determine the effectiveness of nonhost resistance in the interaction between *Arabidopsis thaliana* and *Magnaporthe oryzae*

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SUMMARY

Mechanisms leading to nonhost resistance of plants against nonadapted pathogens are thought to have great potential for the future management of agriculturally important diseases. In this article, we report an investigation of nonhost resistance motivated by the advantages of studying an interaction between two model organisms, namely *Arabidopsis thaliana* and *Magnaporthe oryzae*. During the course of our studies, however, we discovered an unexpected plasticity in the responses of *Arabidopsis* against this ostensibly nonhost pathogen. Thus, we elucidated that certain experimental conditions, such as the growth of plants under long days at constantly high humidity and the use of high inoculum concentrations of *M. oryzae* conidia, forced the interaction in leaves of some *Arabidopsis* ecotypes towards increased compatibility. However, sporulation was never observed. Furthermore, we observed that roots were generally susceptible to *M. oryzae*, whereas leaves, stems and hypocotyls were not infected. It must be concluded, therefore, that *Arabidopsis* roots lack an effective defence repertoire against *M. oryzae*, whereas its leaves possess such nonhost defence mechanisms. In summary, our findings point to organ-specific determinants and environmental conditions influencing the effectiveness of nonhost resistance in plants.

Arabidopsis thaliana and *Magnaporthe oryzae* are both considered as model organisms for plants and phytopathogenic fungi, respectively (Ebbole, 2007; Meinke *et al*., 1998). Therefore, it was logical that researchers interested in plant–fungus interactions would combine the two organisms to take advantage of the molecular tool-boxes available for both interaction partners. Being interested in nonhost resistance and assuming that *Arabidopsis* might not be a host for *M. oryzae*, we also followed this approach. Nonhost resistance is defined as the capacity of a plant species to resist pathogens from other hosts, e.g. pathogens adapted to wheat are often unable to colonize rice plants to which they have not been successively adapted during evolution (Heath, 1980). Essentially, it seems easy to classify a plant–pathogen combination to be of the host or nonhost type simply by looking for disease symptoms. However, it is not that easy to prove the definition to be true. This is because of the impossibility of testing all genotypes of a given plant species against all genotypes of a pathogen species, and because particular experimental conditions may favour the establishment of disease over resistance, or vice versa. The latter scenario is evidenced by two recent publications on the interaction between *Arabidopsis* and the hemibiotrophic fungus *M. oryzae*, which reported host and nonhost types of interaction, respectively (Maeda *et al*., 2009; Park *et al*., 2009). For clarification, we therefore tested whether the different experimental conditions in the two studies might have influenced the outcome of the interactions and, for comparability, we used the *M. oryzae* isolate (70-15) employed in the study of Park *et al*. (2009). Both of the *M. oryzae* isolates used in the work reported here showed a nonhost interaction phenotype in Arabidopsis leaves, but were able to colonize roots. We investigated the responses of *Arabidopsis* ecotypes Est-0 and Gre-0 to inoculation with the *M. oryzae* isolate 70-15. Both combinations were rated as susceptible by Park *et al*. (2009). Using the growing conditions reported by Park *et al*. (2009) (16-h light period, 22 °C, 80% relative humidity) and drop inoculation with a spore concentration of 5×10^5 conidia/mL, macroscopic evaluation revealed chlorotic spots and necrotic lesions at 6 days post-inoculation (dpi) on the leaves of both ecotypes, although ecotype Est-0 showed more pronounced necrosis than Gre-0 (Fig. S1, see Supporting Information). Necrosis on leaves was reduced significantly when both ecotypes were grown under short-day conditions or at 65% relative humidity (Fig. S1). Similarly, spray rather than drop inoculation reduced chlorosis and necrosis symptoms on inoculated leaves (Fig. S1). Almost no disease symptoms were found on leaves of *Arabidopsis* ecotypes grown under short-day conditions (8-h light period, 22 °C) at 65% relative humidity (Fig. S1). We concluded, therefore, that prolongation of the illumination period, which generally induces flowering in

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Arabidopsis, in combination with elevated relative humidity and the application of high-density inoculum in small droplets, cause *Arabidopsis* plants to become more susceptible to *M. oryzae*. Interestingly, in our experience, Gre-0 plants did not flower under long-day conditions up to the time of inoculation, and showed less leaf necrosis relative to Est-0 plants, which, by contrast, had formed flowers by the same time point. Therefore, it may be that *Arabidopsis* plants lose the ability for adequate defence after induction of the reproductive stage. A similar phenomenon has been reported for the induction of systemic acquired resistance, which did not function in cucumber after the onset of flowering (Guedes *et al*., 1980). An influence of environmental conditions on the infection of wheat with nonadapted *M. grisea* isolates has been reported by Nga *et al*. (2009), who showed that incubation at 26 °C after inoculation can break this nonhost resistance. However, even when applying the most disease-promoting experimental conditions which allowed fungal growth, in contrast with Park *et al*. (2009), we did not observe sporulation. This suggests additional, as yet unknown, factors that also affect the capability of *Arabidopsis* to resist infection by *M. oryzae*.

Our next goal was to characterize the interaction between *Arabidopsis* and *M. oryzae* at the cellular level. Firstly, we analysed plants grown under short-day conditions and spray inoculated with a spore concentration of 2.5×10^5 conidia/mL. Microscopic investigations at 2 dpi on Col-0 wild-type (wt) plants revealed the inability of *M. oryzae* to penetrate epidermal cells (Fig. 1A,C). The same holds true for other *Arabidopsis* wt accessions, such as Est-0, Gre-0 and Ws-0 (data not shown). The lack of induced cellular defence reactions in wt plants, for example a hypersensitive response or papilla formation, might suggest a pre-penetration resistance mechanism. Secondly, we examined plants grown under long-day conditions, high humidity and spray inoculated with a high-density conidial solution (5×10^5 conidia/mL). In this case, no substantial differences were found in the success of fungal penetration in comparison with the results described above (data not shown). However, it was noticed that the application of a high-density inoculum resulted in the attack of individual epidermal cells by several appressoria, which was correlated with browning of the affected cells. The latter might be the reason for the macroscopic necrotic lesions observed at 6 dpi only on these plants (data not shown). Next, we asked whether *Arabidopsis* mutants, such as *pen2*, which are affected in penetration resistance against non-adapted powdery mildew pathogens (Lipka *et al*., 2005), might also show an altered cellular defence against *M. oryzae*. Indeed, invasion of epidermal cells of *pen2-1* mutants was frequently observed, and this occurred together with the onset of cell death, as evidenced by trypan blue staining (Fig. 1B). A quantitative assessment of *M. oryzae* infection sites on *Arabidopsis* plants without *pen* mutation (Col-0 and Col-3gL1), in comparison with *pen1-1*, *pen2-1*, *pen3-1*, *pen1-1pen2-1* and *pen2-3pen3-2* mutants, was achieved by assigning each infection site to one of four categories: (i) germination of conidia without formation of appressoria; (ii) germinated conidia with appressoria; (iii) formation of papillae beneath appressoria; and (iv) fungal hyphae in epidermal cells stained with trypan blue (Fig. 1C). In Col-0 and Col-3gL1 *Arabidopsis* genotypes, almost no cellular defence reactions were detected at sites of attempted fungal penetration. In contrast, papillae were found frequently beneath appressoria on the other genotypes, with the highest frequency on the *pen2-3pen3-2* double mutant (Fig. 1C). Penetration of *pen* mutant epidermal cells by fungal invasive hyphae caused cell death, as evidenced by trypan blue staining (Keogh *et al*., 1980). Predominantly, the latter category was found on plants carrying a mutation in the *PEN2* gene, although the overall frequency of this category was rather low (3%–6%) (Fig. 1C). Our results regarding the penetration frequency of *M. oryzae* on different *pen* mutant plants are in accordance with the data presented by Maeda *et al*. (2009). However, our data extended this study by investigating *pen* double mutants and by monitoring diverse cellular defence responses, such as the formation of papillae and the induction of cell death (Fig. 1C). Thus, we showed that papillae occurred with a frequency of 20%–30% in *pen2-1* and *pen3-1* single mutants and a frequency of 40% in *pen2-3pen3*-2 double mutants. On the basis of this observation, it could be hypothesized that *PEN2* and *PEN3* act synergistically in controlling penetration defence against *M. oryzae*. This would further support the idea of a concerted action of *PEN2* and *PEN3*, whereby the PEN2 protein enzymatically activates a toxic compound that is brought to sites of infection by the PEN3 protein (Lipka *et al*., 2005; Stein *et al*., 2006).

So far, in accordance with Maeda *et al*. (2009), our data support the view that *Arabidopsis* is a nonhost for *M. oryzae*, although environmental conditions may compromise the effectiveness of resistance, as suggested by scrutinizing the data presented by Park *et al*. (2009). To gain further insight into the key question of which factors might render *Arabidopsis* more susceptible to *M. oryzae*, we investigated root inoculations. This work was mainly driven by our observation that resistance (*R*) gene-mediated resistance, which effectively protects leaves of rice plants from being colonized by *M. oryzae*, operates less efficiently in roots (Jansen *et al*., 2006) and that, similarly in *Arabidopsis*, race-specific resistance against *Hyaloperonospora parasitica* is not operative in roots (Hermanns *et al*., 2003). Therefore, we asked the question of whether nonhost resistance, which operates in *Arabidopsis* leaves against *M. oryzae*, might also fail in a similar fashion in *Arabidopsis* roots. It must be stated, however, that infections of *M. oryzae* on rice roots are discussed controversially and gene-for-gene resistance has been reported (Sesma and Osbourn, 2004). For the root inoculation assay, *Arabidopsis* Col-0, *pen2-1pen3-1* and *pen2-1pad4-1*

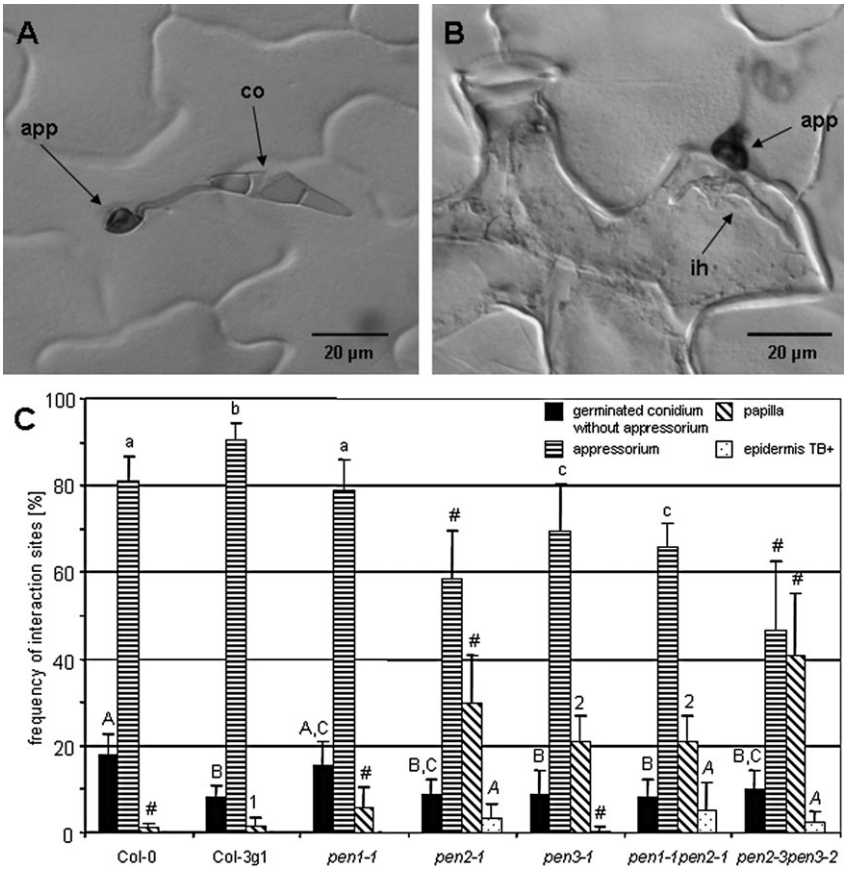

Fig. 1 Microscopy of trypan blue-stained infection sites between different *Arabidopsis* genotypes and *Magnaporthe oryzae* (isolate TH6772) at 2 days post-inoculation. (A) *M. oryzae* conidium (co) germination and appressorium (app) formation on *Arabidopsis* Col-0 plants did not induce cellular defence responses. (B) Penetration by an *M. oryzae* invasive hypha (ih) from an appressorium into an epidermal cell of a *pen2-1* mutant, which subsequently underwent cell death, as evidenced by trypan blue staining (Keogh *et al*., 1980). (C) Quantitative assessment of single-cell interaction sites on *Arabidopsis* wild-type and mutant plants. Plants were grown with an 8-h light period, at 22 °C and 65% relative humidity, and spray inoculated with *M. oryzae* isolate TH6772 (2.5×10^5 conidia/mL, 5 weeks after sowing). Individual interaction sites were grouped into different classes (see text) and the frequency for each class is given as the mean plus standard deviation per leaf. At least eight leaves were analysed per genotype and 100 infection sites were inspected per leaf. Significant differences ($P < 0.05$) for each class on different genotypes were determined using one-way analysis of variance and are indicated by different letters or numbers. Values excluded from statistical analysis because the normality test and/or equal variance test failed are indicated by #. The experiment was repeated once with a similar result.

plants were grown under sterile conditions using a method established by Hermanns *et al*. (2003). Inoculations with *M. oryzae* isolate 70-15 by the application of 3-µL droplets of spore solutions [10^5 conidia/mL in 0.1% (w/v) gelatine] were carried out 18 days after sowing. In these experiments, we avoided spray inoculation because *Arabidopsis* seedlings were cultivated on a nutrient medium on microscope slides and droplets could be placed directly on the roots, whereas spraying would have dispersed conidia ineffectively. By using a low spore concentration during this drop inoculation, single root cells were, on average, attacked by a single penetration event. Microscopic inspection of the inoculated roots revealed that *M. oryzae* formed regularly shaped appressoria at the root surface of different *Arabidopsis* wt Col-0 or mutant plants, which resulted in penetration of the fungus into root cells (Fig. 2A). The formation of melanized appressoria is a new observation not reported to date from root infection assays with *M. oryzae* on different plant species (Guimil *et al*., 2005; Jansen *et al*., 2006; Sesma and Osbourn, 2004). However, far more frequently, and in accordance with observations reported by the laboratory of Sesma and Osbourn (Sesma and Osbourn, 2004; Tucker *et al*., 2010), non-melanized hyphopodia-like structures were observed on contact of *M. oryzae* germ tubes with *Arabidopsis* roots. Hyphopodia similarly enabled entry of the fungus into root cells. Hyphopodia were

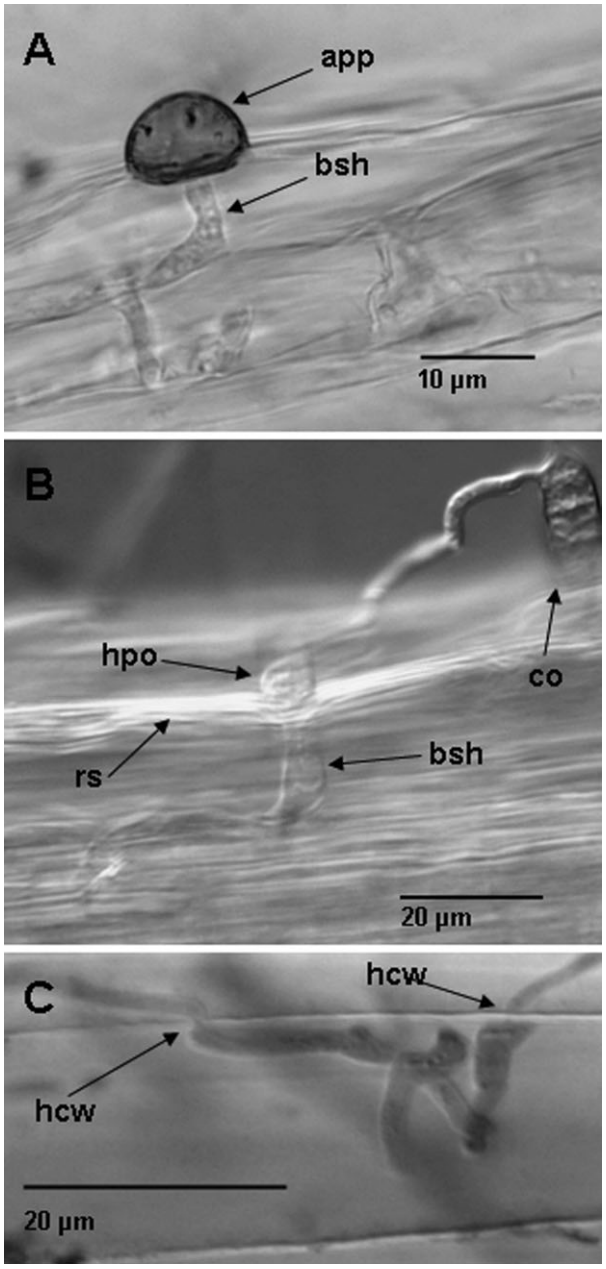

Fig. 2 *Magnaporthe oryzae* (isolate TH6772) infection sites on roots of different *Arabidopsis* genotypes at 4 days post-inoculation. (A) A melanized appressorium (app) can be seen on the surface of a *pen2-1pen3-1* root and the fungus has invaded the root tissue producing bulbous-shaped hyphae (bsh). (B) Conidium (co) of *M. oryzae* germinating on roots of *Arabidopsis pen2-1pad4-1* mutant plants and forming a hyphopodium-like (hpo) structure on contact with the root surface (rs). Bulbous invasive hyphae can be seen penetrating the underlying cell. The photograph was taken with a microscope using differential interference contrast at 4 days post-inoculation. (C) Intracellular hyphae of *M. oryzae* cross cell walls (hcw) between Col-0 root cells in a fashion similar to that observed at pit fields of leaf cells. Samples recorded in (A) and (C) were trypan blue stained before microscopic investigation. The photographs shown are representative examples of infection sites found on the respective genotypes. All infection experiments were repeated at least in triplicate with a similar outcome.

found regularly on *Arabidopsis* wt and mutant plants, such as *pen2-1pad4-1*, which is phytoalexin deficient (Fig. 2B). Recently, Heupel *et al*. (2009) reported that ERL1, an Era (*Escherichia coli* Ras)-like GTPase, is required for full root virulence of *M. oryzae* on rice. The virulence defect of a Δ*erl1* strain could be complemented by the orthologous protein from the mutualistic fungus *Glomus intraradices*, referred to as Gin1 (GTPase, intein), which is presumed to play a role in establishing compatibility with plant roots. Taken together, these data support the hypothesis that symbiotic and pathogenic fungi use conserved strategies for root infection (Heupel *et al*., 2009). Thinking ahead, it might be that today's leaf pathogens have retained the capability to invade plant roots since prehistoric times, although this feature is no longer required. Experimental evidence for this hypothesis comes from recently published results suggesting that root-infecting hyphopodia may represent a primitive form of appressoria which were acquired by pathogens later in the evolution during leaf colonization (Tucker *et al*., 2010). In turn, it might be concluded that roots, which are not generally exposed to adapted leaf pathogens, have not had the need to develop resistance against them, as has been necessary for leaves.

As yet, our microscopic investigations have not revealed the induction of any cellular defence reaction in the roots of *Arabidopsis* during invasion by *M. oryzae*, which confirms the general observation that defence pathways and resistance mechanisms described for leaf pathogens are only found to a minor degree or not at all in roots (Hermanns *et al*., 2003; Okubara and Paulitz, 2005). After the entrance of *M. oryzae* into *Arabidopsis* root tissue, the shape and bulbous growth habit of invasive hyphae closely resembled the morphology known from leaf infections on *M. oryzae*'s usual host, rice (Talbot, 2003). Recently, it has been shown that common genetic requirements control the ability of *M. oryzae* to infect leaf or root tissue of rice (Tucker *et al*., 2010). The occurrence in roots of these bulbous hyphae, which have been discussed as analogous structures to haustoria of biotrophic fungi (Wilson and Talbot, 2009), indicates a remarkably high degree of compatibility established between a particular organ of the ostensible nonhost plant *Arabidopsis* and *M. oryzae*. After penetration into the first root cell, invasive hyphae ramify and start to colonize the surrounding tissue intracellularly (Figs 2C and 3A,B). Thereby, fungal movement from cell to cell occurs in a fashion similar to that described for the colonization of rice leaves by *M. oryzae*, and is suggestive that the fungus likewise crosses cell walls at pit fields (Kankanala *et al*., 2007). The latter observation further underpins the suggestion that *M. oryzae* colonizes *Arabidopsis* roots in a manner reminiscent of growth in the leaf tissue of host plants.

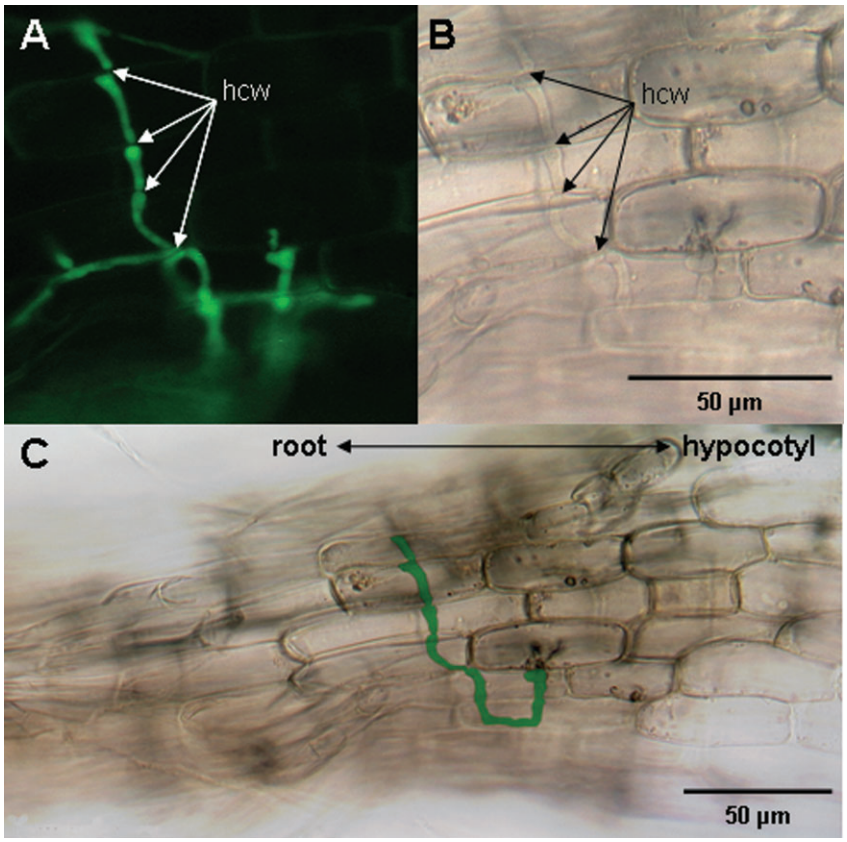

Fig. 3 *Magnaporthe oryzae* is unable to grow from colonized root tissue into the hypocotyl of *Arabidopsis* plants. Fungal hyphae of a constitutively green fluorescent protein (GFP)-expressing *M. oryzae* (70-15-GFP) isolate can be seen colonizing the root tissue of *Arabidopsis pen2-3pen3-2* plants at 4 days post-inoculation. Fungal hyphae cross root cell walls (hcw) in a fashion suggesting the utilization of pit fields. We observed that the growth of fungal hyphae was blocked at the border between roots and hypocotyl tissue. (A) and (B) show the same representative infection site using epifluorescence and bright-field microscopy, respectively. For clarity, an overview of the region, showing the intersection between root and hypocotyl tissues, is given in (C), with fungal hyphae artificially coloured green by computer.

Next, we addressed the very important question of whether *M. oryzae* might be able to overcome the nonhost resistance shown by *Arabidopsis* aerial tissues by growing from colonized roots through the hypocotyl into stems and leaves, a phenomenon observed after *M. oryzae* infection of rice roots (Sesma and Osbourn, 2004). Importantly, we observed that this was not the case. In contrast, we found that fungal hyphae which grow towards the hypocotyl are unable to cross the border between root and hypocotyl tissue, which remains free from colonization by the fungus (Fig. 3A–C). Furthermore, we elucidated that, on inoculation of hypocotyls from *Arabidopsis* wt plants, the fungus is unable to penetrate into this tissue (data not shown). Thus, nonhost resistance against *M. oryzae* seems to function in leaves, stems and hypocotyls of *Arabidopsis*, but is not active in roots. Consistently, we found that the fungus was able to penetrate hypocotyls of *pen2-3pen3-2* double mutants similarly to leaves of the same genotype (data not shown).

Finally, it must be concluded that host–pathogen interactions show an unpredictable plasticity regarding compatibility or incompatibility in relation to experimental design, which may force host/nonhost interactions into either direction. Furthermore, organ identity may greatly influence the ability of a plant to establish a host or nonhost interaction with a given pathogen.

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REFERENCES

Ebbole, D.J. (2007) *Magnaporthe* as a model for understanding host–pathogen interactions. *Annu. Rev. Phytopathol.* **45**, 437–456.

Guedes, M.E.M., Richmond, S. and Kuc, J. (1980) Induced systemic resistance to anthracnose in cucumber is influenced by the location of the inducer inoculation with *Colletotrichum lagenarium* and the onset of flowering and fruiting. *Physiol. Plant Pathol.* **17**, 229–233.

Guimil, S., Chang, H.S., Zhu, T., Sesma, A., Osbourn, A., Roux, C., Ioannidis, V., Oakeley, E.J., Docquier, M., Descombes, P., Briggs, S.P. and Paszkowski, U. (2005) Comparative transcriptomics of rice reveals an ancient pattern of response to microbial colonization. *Proc. Natl. Acad. Sci. USA*, **102**, 8066–8070.

Heath, M.C. (1980) Reactions of nonsuscepts to fungal pathogens. *Annu. Rev. Phytopathol.* **18**, 211–236.

Hermanns, M., Slusarenko, A.J. and Schlaich, N.L. (2003) Organ-specificity in a plant disease is determined independently of *R* gene signaling. *Mol. Plant–Microbe Interact.* **16**, 752–759.

Heupel, S., Roser, B., Kuhn, H., Lebrun, M.-H., Villalba, F. and Requena, N. (2009) Erl1, a novel Era-lke GTPase from *Magnaporthe oryzae*, is required for full root virulence and is conserved in the mutualistic symbiont *Glomus intraradices*. *Mol. Plant–Microbe Interact.* **23**, 67–81.

Jansen, M., Slusarenko, A.J. and Schaffrath, U. (2006) Competence of roots for race-specific resistance and the induction of acquired resistance against *Magnaporthe oryzae*. *Mol. Plant Pathol.* **7**, 191–195.

Kankanala, P., Czymmek, K. and Valent, B. (2007) Roles for rice membrane dynamics and plasmodesmata during biotrophic invasion by the blast fungus. *Plant Cell*, **19**, 706–724.

Keogh, R.C., Deverall, B.J. and Mcleod, S. (1980) Comparison of histological and physiological responses to *Phakopsora pachyrhizi* in resistant and susceptible soybean. *T. Brit. Mycol. Soc.* **74**, 329–333.

Lipka, V., Dittgen, J., Bednarek, P., Bhat, R., Wiermer, M., Stein, M., Landtag, J., Brandt, W., Rosahl, S., Scheel, D., Llorente, F., Molina, A., Parker, J., Somerville, S. and Schulze-Lefert, P. (2005) Pre- and postinvasion defenses both contribute to nonhost resistance in *Arabidopsis*. *Science*, **310**, 1180–1183.

Maeda, K., Houjyou, Y., Komatsu, T., Hori, H., Kodaira, T. and Ishikawa, A. (2009) AGB1 and PMR5 contribute to PEN2-mediated preinvasion resistance to *Magnaporthe oryzae* in *Arabidopsis thaliana*. *Mol. Plant–Microbe Interact.* **22**, 1331–1340.

Meinke, D.W., Cherry, J.M., Dean, C., Rounsley, S.D. and Koornneef, M. (1998) *Arabidopsis thaliana*: a model plant for genome analysis. *Science*, **282**, 662–682.

Nga, N.T.T., Hau, V.T.B. and Tosa, Y. (2009) Identification of genes for resistance to a *Digitaria* isolate of *Magnaporthe grisea* in common wheat cultivars. *Genome*, **52**, 801–809.

Okubara, P. and Paulitz, T. (2005) Root defense responses to fungal pathogens: a molecular perspective. *Plant Soil*, **274**, 215–226.

Park, J.-Y., Jin, J., Lee, Y.-W., Kang, S. and Lee, Y.-H. (2009) Rice blast fungus (*Magnaporthe oryzae*) infects *Arabidopsis* via a mechanism distinct from that required for the infection of rice. *Plant Physiol.* **149**, 474–486.

Sesma, A. and Osbourn, A.E. (2004) The rice blast pathogen undergoes developmental processes typical of root-infecting fungi. *Nature*, **431**, 582–586.

Stein, M., Dittgen, J., Sanchez-Rodriguez, C., Hou, B.H., Molina, A., Schulze-Lefert, P., Lipka, V. and Somerville, S. (2006) Arabidopsis PEN3/PDR8, an ATP binding cassette transporter, contributes to nonhost resistance to inappropriate pathogens that enter by direct penetration. *Plant Cell*, **18**, 731–746.

Talbot, N.J. (2003) On the trail of a cereal killer: investigating the biology of *Magnaporthe grisea*. *Annu. Rev. Microbiol.* **57**, 177–202.

Tucker, S.L., Besi, M.I., Galhano, R., Franceschetti, M., Goetz, S., Lenhert, S., Osbourn, A. and Sesma, A. (2010) Common genetic pathways regulate organ-specific infection-related development in the rice blast fungus. *Plant Cell*, **22**, 953–972.

Wilson, R.A. and Talbot, N.J. (2009) Under pressure: investigating the biology of plant infection by *Magnaporthe oryzae*. *Nat. Rev. Microbiol.* **7**, 185–195.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Influence of different environmental and inoculation conditions on the interaction between four different *Arabidopsis* ecotypes (Col-0, Est-0, Gre-0 and Ws-0) and *Magnaporthe oryzae* (isolate 70-15). All plants were inoculated with *M. oryzae* isolate 70-15 (5×10^5 conidia/mL) and symptoms were documented at 6 days post-inoculation (dpi). LD, long day (16 h light); SD, short day (8 h light); ↑ rH, high relative humidity (~80%), ↓ rH, low relative humidity (~65%); DS, disease severity; n.d., not determined.

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