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# *Arabidopsis thaliana* model system reveals a continuum of responses to root endophyte colonization

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## ABSTRACT

We surveyed the non-mycorrhizal model plant *Arabidopsis thaliana* microscopically for its ability to form dark septate endophyte (DSE) symbioses in field, greenhouse, and laboratory studies. The laboratory studies were also used to estimate host growth responses to 34 *Periconia macrospinosa* and four *Microdochium* sp. isolates. Consistent with broad host range observed in previous experiments, field-, greenhouse-, and laboratory-grown *A. thaliana* were colonized by melanized inter- and intracellular hyphae and microsclerotia or chlamydospores indicative of DSE symbiosis. Host responses to colonization were variable and depended on the host ecotype. On average, two *A. thaliana* accessions (Col-0 and Cvi-0) responded negatively, whereas one (Kin-1) was unresponsive, a conclusion consistent with our previous analyses with forbs native to the field site where the fungi originate. Despite the average negative responses, examples of positive responses were also observed, a conclusion also congruent with earlier studies. Our results suggest that *A. thaliana* has potential as a model for more detailed dissection of the DSE symbiosis. Furthermore, our data suggest that host responses are controlled by variability in the host and endophyte genotypes.

**Key words:** *Arabidopsis thaliana*; dark septate endophytes (DSE); *Microdochium* sp.; mutualism-parasitism continuum; *Periconia macrospinosa*

## INTRODUCTION

Dark septate endophytes (DSE) are a miscellaneous group of root-colonizing fungi characterized by melanized cell walls and intracellular colonization of healthy plants (Jumpponen & Trappe 1998). Although many DSE fungi form similar morphological structures in the host roots (Jumpponen & Trappe 1998, Rodriguez *et al*. 2009), they are taxonomically unrelated, vary in ecological or physiological functions and lead to variable host responses (Addy, Piercey & Currah 2005, Alberton, Kuyper & Summerbell 2010, Newsham 2011, Tellenbach, Grünig & Sieber 2011, Knapp, Pintye & Kovacs 2012). Our earlier studies in the tallgrass prairie concluded that while grasses overall tend to be colonized to a greater extent and respond more positively to DSE colonization, forbs also range from increased to no response to decreased biomass accumulation in their response to DSE fungi (Mandyam, Fox & Jumpponen 2012).

DSE fungi are globally distributed and have been observed in more than 600 plant species across well over 100 plant families from diverse habitats, and the list of susceptible hosts increases as more studies survey plants for DSE (Jumpponen & Trappe 1998, Mandyam & Jumpponen 2005, Zhang *et al.* 2011, Knapp *et al.* 2012). However, thus far the model plant, *Arabidopsis thaliana* (L.) Heynhold., native to Europe and central Asia but now naturalized worldwide (Al-Shehbaz & O'Kane 2002), has not been surveyed or tested for its ability to form these common symbioses. A fast-growing and simple weed, *A. thaliana* is an established model and continues to provide considerable insights into plant genetics and molecular biology (Somerville & Koornneef 2002, Koornneef & Meinke 2010). *Arabidopsis thaliana* is non-mycorrhizal and no natural root mutualisms had been reported until it was shown to benefit from an association with a soil-inhabiting basidiomycete, *Piriformospora indica* Verma, Varma, Rexer, Kost & Franken (Peškan-Beghöfer *et al.* 2004). In laboratory studies, this fungus often improved plant growth or fitness, increased drought and biotic stress tolerance, and induced disease resistance (Waller *et al.* 2005, Shahollari *et al*. 2007, Shermati *et al.* 2008a, Stein *et al.* 2008, Molitor & Kogel 2009, Vandassery & Oelmüller 2009, Zuccaro *et al.* 2009, Molitor *et al*. 2011, Hilbert *et al.* 2012). These results have led to the conclusion that *P. indica* forms mutualisms with a range of hosts including *A*. *thaliana* and bears a promise to be exploited in crop protection (Qiang *et al.* 2012a). Adoption of *A. thaliana* model has also permitted a detailed dissection of molecular mechanisms underlying the *P. indica* symbiosis (Sherameti *et al.* 2008a, 2008b, Vandassery *et al*. 2008, 2009, Camehl *et al.* 2010; Lee *et al*. 2011, Khatabi *et al*. 2012, Nongbri *et al*. 2012), as well as characterization of a previously unknown colonization mechanism (Qiang *et al.* 2012b). Despite the absence of root symbioses in Brassicaceae, including *A. thaliana*, the genes that are involved in root symbioses seem to be conserved (Hayward *et al.* 2012). As a result, this model system bears a great promise in well-informed dissection of root symbioses. Our motivation in this contribution was to test whether or not *A. thaliana* would be colonized by fungi native to tallgrass prairie, would respond similarly to colonization, and could therefore serve as a model for further dissection of such DSE symbioses.

*Arabidopsis thaliana* model symbioses can permit answering many questions about obscure, but common fungal interactions (see Vandassery & Oelmüller 2009). The *Arabidopsis* model allows for expedient data accumulation and hypothesis testing. To exemplify, many *Arabidopsis* resources, including whole genome microarrays, easy access to ecotypes and/or accessions of *Arabidopsis*, mutants of many physiological pathways, and

abundant literature are available for exploitation to dissect the DSE symbiosis at the whole plant, genetic, molecular or physiological level (see Buell & Last 2010). The *Arabidopsis* Information Resource (TAIR; www.arabidopsis.org), a database for genetic and molecular data of *Arabidopsis*, indicates that over 750 accessions of *A. thaliana* have been collected around the world. These accessions are variable in form, development and physiology and routinely used to understand the complex genetic interactions underlying plant responses to pathogens, stress, or environmental conditions.

Mutualism-parasitism continuum paradigm has been used for mycorrhizal (Francis & Read 1995, Johnson, Graham & Smith 1997, Jones & Smith 2004), as well as non-mycorrhizal root and foliar endophyte associations (Saikkonen *et al*. 1998, Schulz & Boyle 2005, Schulz 2006) to account for variable host responses. Considerable uncertainty exists on whether DSE should be considered parasites, mutualists, or simply casual inhabitants of the root environment (Jumpponen 2001, Addy *et al*. 2005, Mandyam & Jumpponen 2005). Recently, Newsham (2011) conducted a meta-analysis and concluded – contrary to a previous meta-analysis (Alberton *et al.* 2010) – that the DSE symbioses should be considered mutualisms, particularly so if nitrogen was supplied in organic forms. The outcomes of the symbioses may be influenced by the variability of component fungi (Munkvold *et al*. 2004, Koch, Croll & Sanders 2006, Mandyam *et al.* 2012, Tellenbach *et al*. 2011) or host plants (Jones, Durall & Tinker 1990, Thomson *et al*. 1994, Karst, Jones & Turkington 2009, Hoeksema *et al*. 2010), as well as by abiotic variability in the availability of light or nutrients or in the stress under which the host-fungus symbiosis is evaluated (Johnson et al. 1997, Redman, Dunigan & Rodrigues 2001, Rodriguez *et al.* 2008, Johnson *et al*. 2010, Newsham 2011). Compared to better known mycorrhizal symbioses or the vertically transmitted systemic foliar endophytes, the root-associated fungal endophytes have received little attention (Rodriguez *et al.* 2009). As a result, many factors that potentially influence these symbioses remain to be substantiated. The efforts to elucidate deeper dissection of the DSE symbiosis would probably be greatly expedited by a model that could be harnessed under stringent laboratory conditions.

We aimed to test *A. thaliana* for its utility as a non-mycorrhizal model for analyses of DSE symbioses. We argue that the access to tools available for model plants far outweigh the disadvantages of remote ecological relevance in many natural systems. Our goal is to strive towards an improved understanding of the influence of host and fungal genotypes on the

outcome of the DSE symbiosis along the mutualism-parasitism continuum by using three selfed accessions of *A. thaliana* and several strains of abundant DSE fungi from a native tallgrass prairie (Mandyam, Loughlin & Jumpponen 2010). Our specific goals were to evaluate i) microscopically *Arabidopsis* colonization by the DSE fungi under field, greenhouse, and laboratory conditions; ii) *Arabidopsis* responses to a range of DSE isolates distributed across two taxa that commonly occur in a tallgrass prairie ecosystem; and iii) whether host responses vary across genotypes of conspecific fungi and/or host accessions or combinations thereof. If observed, this variability would invite selection of host-endophyte combinations that would serve to best elucidate the genetic basis for host responses to endophyte colonization. Equally importantly, selection of differently behaving symbiotic combinations might facilitate designing experiments that would improve our present understanding of why hosts respond positively to some endophytes and not to others.

## MATERIALS AND METHODS

### *Field-grown* Arabidopsis *material*

The field-grown material (18 *A. thaliana* Cvi-0 individuals) was acquired from a larger common garden experiment that included a field site in Norwich, England (Wilczek *et al.* 2009). The common garden was established at 21m × 34m fenced field site divided into 1m × 4.5m blocks as described in Wilczek *et al.* (2009). The timing of the planting was set to coincide with observed natural germination flushes. In Norwich, where winters are mild, *A. thaliana* commonly germinates in the fall, grows vegetatively through the winter, and flowers in the spring. For planting in September 2006, seeds for *A. thaliana* Cvi-0 accession were stratified in the dark at 4°C in 0.1% water agar for four days prior to sowing. Seeds were sown onto peat-based Plugits held together by a permeable, biodegradable fabric (Bulrush Horticulture Ltd.; Co. Londonderry, N. Ireland; Recipe 5919). Seedlings were germinated on the surface of moist Plugits in the greenhouse under natural photoperiod conditions and thinned to one seedling/Plugit. Temperature was set as close to current outdoor conditions as possible. Within ten days of germination, Plugits with seedlings were transplanted to the field and watered for up to a week. From then on, seedlings were left under natural conditions with no further watering and allowed to grow until harvest in February 2007 after all the flowers had opened and formed siliques. After removal of the shoot, soil surrounding the mature plant was dug up and the roots were removed gently from the soil and placed in water. After

removing soil, the roots were fixed in 3.7% formaldehyde and 15% methanol and shipped to Kansas State University for microscopy.

*Greenhouse-grown* Arabidopsis *material*

Soil was collected from an annually burned watershed in Konza Prairie Biological Station (KPBS, http://kpbs.konza.ksu.edu/, 39˚05' N, 96˚35' W) that represents a native mesic tallgrass prairie in the Flint Hills of eastern Kansas, USA. This site was selected because it is also the source of isolates used in resynthesis studies and has been shown to have high occurrence of endophytes in native plants (Mandyam & Jumpponen 2008, Mandyam *et al.* 2010). Rocks and large roots were removed and the field soil was thoroughly mixed with an equal volume of autoclaved Promix general purpose growing medium (Premier Horticulture, Quakertown, Pennsylvania, USA). A total of thirty 66mm square pots were filled with the soil and three random sets of ten pots were seeded with each of the three *A. thaliana* accessions: Columbia (Col-0), Kendallville (Kin-1) and Cape Verde Island (Cvi-0) (Lehle Seeds, Round Rock, TX, USA). The pots were transported to a greenhouse, kept in nursery flats (F1020, Hummert International, Earth City, Missouri, USA), covered with transparent plastic lids (Propagation Dome for F1020, Hummert International, Earth City, Missouri, USA), and incubated under ambient light conditions. During the first week after seeding, the pots were watered and screened for germination daily and thinned to one plant per pot. After the first week, the lids were removed and the plants watered as necessary until harvested after a total of six weeks. At harvest, the plant was removed gently from the soil and placed in water. After removing the soil, the roots were stored for microscopy in 70% ethanol.

*Confirmation of root colonization in the field and greenhouse*

To confirm field grown *A. thaliana* colonization by DSE, we screened the entire root system and recorded – but did not quantify – the presence of DSE structures in the field grown Cvi-0 accession roots. To test whether or not the *A. thaliana* accessions differed in their susceptibility to DSE from KPBS native soils, the root colonization was estimated for Col-0, Kin-1 and Cvi-0 with the gridline intersection method (McGonigle 1990). A total of one hundred intersections per root system were evaluated under 200× (Nikon Eclipse E600, Nikon Inc., Melville, New York, USA) for melanized hyphae, microsclerotia and chlamydospores in ten 1-cm root fragments. Roots were left unstained because the indicative structures are

usually melanized and the occurrence of the hyaline structures tends to be underestimated on the account of poor visibility (Barrow & Aaltonen 2001, Mandyam & Jumpponen 2005).

*Laboratory resynthesis of* Arabidopsis *DSE symbiosis*

A total of 34 *Periconia macrospinosa* and four *Microdochium* sp. isolates were used for the laboratory inoculation assays. These fungi originated from KPBS, were identified based on colony and conidial morphology plus Internal Transcribed Spacer sequencing, and ultimately confirmed to be root-associated endophytes according to Koch's postulates (Mandyam *et al.* 2010). While some isolated aspergilli and fusaria in Mandyam *et al.* (2010) were clearly pathogenic and led to plant mortality, inoculation with the *Periconia* and *Microdochium* isolates did not result in disease symptoms and were therefore selected for further studies. Fungal isolates were cultured on Difco$^{TM}$ Potato Dextrose Agar (PDA; Becton Dickinson and Co, Maryland, USA) at 25º C for 15 days prior to inoculation on *A. thaliana*.

The three *A. thaliana* accessions used for the greenhouse study were also selected for the resynthesis experiments. Seeds were cleaned and surface sterilized in 0.1% Triton-X for 30min, followed by 70% ethanol in 0.1% Triton-X for 5 min, and finally in 30% domestic bleach (6.15% in sodium hypochlorite) in 0.1% Triton-X for 5min. Seeds were then washed 4-5 times with sterile water and stratified for 3 days in 4°C. The sterilized seeds were plated on 1/10 strength Murashige Skoog basal salt mixture (MS; Sigma Aldrich, St. Louis, MO, USA) medium and allowed to germinate during a one-week incubation in the growth chamber under 12h cycle of light (ca. 250 µmol $m^{-2}$ $s^{-1}$ PAR) at 20º C. Petri dishes with 1/10 MS were prepared and after solidification one half of the medium was cut out and placed into another dish, resulting in two half plates. Seedlings were transferred to the center of the half plates. A total of ten replicates were randomly assigned to a fungal treatment and ten to its paired control (a total of twenty experimental units). The fungal treatments were inoculated with a 6mm fungal plug cored from isolates grown on PDA at 25º C for 15 days, whereas the fungus-free controls were inoculated with identical 6mm plugs cored from sterile PDA plates. The experimental systems containing the plant and either the sterile or fungus inoculated plug were sealed with parafilm resulting in a self-contained closed plate system. Some of the original pure cultures failed to revive from repeated subculturing. As a result, the isolates and their numbers varied across the accessions: 25 *Periconia* isolates were common across all three accessions, and all accessions were screened with a total of 29 isolates. All *A. thaliana*

accessions were screened with two common *Microdochium* isolates, but Col-0 was screened with a total of four, Kin-1 with three and Cvi-0 with two *Microdochium* isolates. The plants were incubated upright in the growth chamber under the above conditions, their shoots harvested five weeks after inoculation and dried at 50°C for dry weight. Roots were used for microscopic analyses, their mass was not recorded because the extraction of the fine roots from the medium proved impossible.

*Confirmation of root colonization in resynthesis*

The harvested roots were screened for presence or absence of fungal colonization under a light microscope at 200×. Microsclerotia and melanized hyphae were recorded in *Periconia* treatments, and chlamydospores in the *Microdochium* treatments, as was expected for these two endophytes (Mandyam *et al*. 2010). The fungus-free controls remained free of colonization confirming absence of contamination. As our experiment included nearly two thousand experimental units, we used a rank colonization scale: 0 indicating no colonization, 1 indicating one to two DSE structures per field of view and 2 indicating more than two DSE structures per field of view in a total of ten fields.

Arabidopsis *responsiveness to DSE colonization*

To estimate the host responses to inoculation, we used a metric more commonly known as the 'mycorrhizal dependency' (van der Heijden 2002, Klironomos 2003, Mandyam *et al.* 2012). Because we are not estimating dependency and aim to maintain a clear distinction, we refer to our metric as the "responsiveness to inoculation" or $R_{DSE}$. Use of this metric provides values that range from -1 to 1 (see Mandyam *et al.* 2012) and a framework for testing hypotheses on host responses against a null hypothesis wherein the mean response equals zero.

If the median dry weight of inoculated treatment exceeded that in fungus-free control, then

$R_{DSE}$=*[(median dry weight of inoculated treatment – median dry weight of fungus-free control treatment)/median dry weight of inoculated treatment]*

If the median dry weight of fungus-free control treatment exceeded that in the inoculated treatment, then

$R_{DSE}$=*[(median dry weight of inoculated treatment – median dry weight of fungus-free control treatment)/ median dry weight of fungus-free control treatment]*

*Statistical analyses*

The colonization estimates for the greenhouse-grown *A. thaliana* were analyzed to test for the differences in colonization among the accessions. Differences among the accessions were determined using ANOVA in PROC GLM in SAS (Version 9.1) after arcsine square root transformation.

To test for differences in colonization among the *A. thaliana* accessions in the laboratory resynthesis, the fungus-free controls were omitted. To maintain a balanced complete experimental design matrix, colonization data for only those 25 *Periconia* and two *Microdochium* isolates that were common to all accessions were included in these analyses. The endophyte species were analyzed separately. Differences among accessions were determined using a categorical response analysis in PROC CATMOD in SAS (Version 9.1).

We tested the shoot biomass responses to endophyte colonization using two strategies. i) To test whether the shoot biomass responses differed among the DSE isolates and *A. thaliana* accessions, we analyzed these data using ANOVA (PROC GLM; SAS; Version 9.1) with a model that included *A. thaliana* accession and fungal isolate main effects and their interaction for the 25 *Periconia* and two *Microdochium* isolates common to all accessions. Because our main focus in these analyses was to determine differences among isolates and accessions, only the fungal treatments were included – the paired controls were omitted. These analyses were conducted separately for *Periconia* and *Microdochium*. ii) To test whether there were any biomass differences at the level of an isolate, the fungal treatment was compared to its fungus-free control separately within each paired experiment using ANOVA (PROC GLM; SAS; Version 9.1).

Finally, we aimed to address whether or not there was an overall response to a population of fungal isolates in any of the three *A. thaliana* accessions. To do this, the $R_{DSE}$ data were analyzed separately for each of the three *Arabidopsis* accessions. We used a two-tailed t-test in PROC TTEST in SAS (Version 9.1) to test the null hypothesis that the sample was drawn from a population with a mean $R_{DSE}$ equal to zero. Since the *Microdochium* datasets were small, they were omitted from these analyses.

**RESULTS**

*DSE colonization of field-, greenhouse- and laboratory-grown* Arabidopsis

Field-collected and greenhouse-grown *A. thaliana* root samples were colonized by DSE. Of the 18 Cvi-0 field samples, twelve were colonized with melanized inter- and intracellular hyphae and some contained melanized microsclerotia or chlamydospores. The remaining six contained no DSE-indicative melanized structures. *Arabidopsis thaliana* were also colonized by DSE in Konza Prairie native soil. Root colonization tended to be low (Col-0 1.7 ± 1.6%; Cvi-0 5.2 ± 6.9%; Kin-1 2.9 ± 3.0%) and did not differ among the accessions ($F_{2,27}$ = 1.1009; $P$ = 0.3471). It is of note that only melanized structures were recorded in these analyses and the colonization is likely underestimated.

*Arabidopsis thaliana* roots, when inoculated with *Microdochium* isolates in the laboratory produced frequent intracellular chlamydospores without melanized hyphae. The colonization was high and invariable among the tested accessions (mean colonization score = 2.0 ± 0.00). *Periconia* isolates formed melanized microsclerotia in the cortex and occasionally some melanized intercellular hyphae. Colonization varied among the *A. thaliana* accessions ($X^2_{df=2}$ = 10.84; $P$ = 0.0044): Cvi-0 (mean colonization score = 1.24 ± 0.77) – the accession used in the field study and most susceptible in the greenhouse study – was the most susceptible to colonization, followed by Col-0 (0.96 ± 0.79) and Kin-1 (0.91 ± 0.65). Notably, the ranking of the colonization scores in the laboratory resynthesis study was consistent with that in the greenhouse study.

*Shoot biomass in the resynthesis study*

Shoot biomass varied among the *Periconia* isolates and *A. thaliana* accessions (Table 1). Cvi-0 obtained highest biomass among the plants inoculated with the 25 common DSE isolates, followed by Col-0 and Kin-1. Most importantly, a significant interaction term 'fungus*accession' indicated that the accessions differed in their growth responses when grown in symbiosis with different strains of *Periconia*. A similar analysis of responses to *Microdochium* colonization was also carried out (Table 1). The biomasses differed among the accessions as indicated by the significant 'accession' term. Cvi-0 had the greatest shoot biomass, followed by Kin-1 and Col-0. In contrast to *Periconia*, the *A. thaliana* shoot biomass

was not affected by the *Microdochium* strain as indicated by the non-significant 'fungus' and 'fungus*accession' terms.

In addition to overall analyses across all accessions and common isolates, each paired experiment was analyzed to test for host biomass differences between the inoculated and fungus-free treatments (Fig. 1; Table 2). Growth of Kin-1 accession was negatively impacted by two *Periconia* isolates, 21 showed no response and six increased the host biomass. One *Microdochium* had a positive effect, while the other two did not affect host biomass. In Col-0 accession, 17 *Periconia* isolates decreased shoot biomass, 12 did not have an effect, and none increased the biomass. Among the *Microdochium* isolates, one had a negative effect while the remaining three did not affect the biomass. In the Cvi-0 experiments, 13 *Periconia* isolates reduced shoot biomass, 15 had no effect, and one resulted in a positive growth response. The *Microdochium* isolates had no effect on Cvi-0 biomass.

The DSE isolates rarely had consistent effects across all accessions. Instead, different strains elicited a continuum of growth responses within an accession and the host responses substantially varied among accessions. For example, *Periconia* strains KS3055_2 and 3041_B led to host responses that ranged from negative to neutral and positive, depending on the host accession (Fig. 2). Only four of the 25 common *Periconia* isolates had the same response across all accessions, one of which (KS3087) reduced shoot biomass in all used accessions, while the remaining ones had no effect. These observations corroborate and further dissect the significant 'fungus*accession' interaction above: both host and fungal genotype influenced the outcome of the symbiosis.

*Host responsiveness to DSE colonization*

We tested the null hypothesis that the mean host response (measured by $R_{DSE}$) to a population of *Periconia* endophytes equals zero (Fig. 3). The null hypothesis was rejected for Col-0 and Cvi-0, whose overall responses to *Periconia* colonization were negative. In contrast, Kin-1 did not respond to inoculation with *Periconia, i.e.*, the symbiosis was neutral.

**DISCUSSION**

Our studies corroborate with Junker, Draeger & Schulz (2012), who inoculated *A. thaliana* with endophytes isolated from *A. thaliana,* and demonstrate that *A. thaliana* is colonized by endophytes under various experimental conditions. Including DSE isolated from *A. thaliana* grown under natural conditions in our studies would have considerably strengthened the selection of fungi. However, fungi that naturally occurred in tallgrass prairie ecosystem and unlikely to have encountered *A. thaliana* previously were capable of colonizing the model host and formed morphologies indicative of the DSE symbiosis. We exploited this observation to test the range of growth responses in a model plant.

We screened three *Arabidopsis* accessions with a selection of DSE strains representing *P. macrospinosa* and *Microdochium* sp. Junker *et al*. (2012) reported an increase in symptoms of otherwise largely asymptomatic foliar endophytes when they were inoculated back into axenically grown *A. thaliana*. The authors interpreted their observations in light of a delicate balance between virulence of naturally occurring fungi and the defenses of their hosts when grown in a system that favored the fungi but not the host. In mycorrhizal systems (Wilson & Hartnett 1998, Karst *et al.* 2009), as well as in our previous experiments with native plants and DSE fungi (Mandyam *et al.* 2012), host responses can range from negative to positive suggesting a similar delicate interplay between fungal and their host genotypes differentially modulated by the environment. In a study using strains representing four species in the *Phialocephala-Acephala* complex (PAC), Tellenbach *et al.* (2011) observed a range from neutral to antagonistic responses in Norway spruce. Such inter- and intraspecific variability has been hypothesized to be central to plant community structuring by mycorrhizal symbioses (Hartnett & Wilson 1999, van der Heijden 2002, Piculell *et al*. 2008). Similarly, the variability in host responses to DSE fungi may promote selection of compatible host-fungus mosaics as proposed for ectomycorrhizal symbioses (Piculell *et al*. 2008).

Our studies indicate that the non-mycorrhizal *A. thaliana* varies in the levels of *Periconia* colonization. In contrast, *Microdochium* isolates colonized the accessions extensively, although these conclusions were based on fewer strains. As expected based on our (Mandyam *et al.* 2010) and other (Knapp *et al.* 2012) previous studies, the microscopic observations confirmed that *Periconia* forms melanized microsclerotia in the host cortex, and that *Microdochium* invariably produces chlamydospores. This supports the utility of the *A. thaliana* model for DSE symbioses. However, *Periconia* colonization differed among the three host accessions. For arbuscular mycorrhizal fungi, Graham & Eissenstat (1999)

postulated that the host colonization is not controlled by the fungal genotype, but only by the host genotype.

Our studies provide further insight into the mutualism-parasitism paradigm for DSE fungi. The 'responsiveness' to DSE, previously used to explain the variable plant growth responses (Wilson & Hartnett 1998, Mandyam *et al.* 2012), was used to evaluate *A. thaliana* responses to DSE colonization. Our study highlights sources of variability on three levels. *First*, at a fungal population level, the *Arabidopsis*-DSE interaction was either neutral (Kin-1) or negative (Col-0 and Cvi-1; Fig. 3). These results suggest that DSE are probably weak parasites supporting some earlier conclusions (Addy *et al*. 2005, Alberton *et al*. 2010), although some strains may behave as mutualists. However, a recent meta-analysis (Newsham 2011) suggested that the DSE symbioses tended to be positive, though mainly so if nitrogen was supplied in organic forms. We used a tightly controlled laboratory system in which all nutrients were supplied in inorganic forms. *Second*, at the fungal strain level, growth responses varied within each *A. thaliana* accession. These observations corroborate those of Tellenbach *et al*. (2011), who concluded that variability in host responses is greater within species than it is among species. In our studies, *A. thaliana* accessions responded negatively, neutrally or positively to different isolates (Fig. 2). These results suggest a coupling between host and fungal genotypes as a determinant of host responses. In other words, the symbiotic outcomes are determined on a level of a genotype, not on a level of a species. *Third*, many conspecific isolates elicited a range of growth responses (Table 2). As exemplified by our results, one isolate could yield a positive, negative or neutral response, depending on the host accession.

Mycorrhizal symbiosis is generally considered beneficial to the hosts (Jones & Smith 2004). However, under some environmental conditions, the host does not respond positively. The concept of symbiotic response continuum has been used to characterize this range of outcomes (Francis & Read 1995, Johnson *et al*. 1997, Karst *et al*. 2008) or to explain the variable host responses to foliar, non-mycorrhizal or systemic endophytes (Saikkonen *et al*. 1998, Redman *et al.* 2001, Müller & Krauss 2005, Schulz & Boyle 2005, Schulz 2006, Kageyama, Mandyam & Jumpponen 2008). The outcome of an interaction likely depends on a delicate balance between the fungal virulence and host defense, both affected by genotype, physiology, nutritional status, developmental stages of the partners and environmental factors (Saikkonen *et al.* 1998, Redman *et al.* 2001, Faeth & Sullivan 2003, Schulz 2006, Junker *et*

*al.* 2012). Alternatively, this variability can be viewed as a cost-benefit ratio between the host's carbon investment to the maintenance of a symbiosis and the benefit derived from it (Johnson *et al.* 1997, Schwartz & Hoeksema 1998, Mandyam & Jumpponen 2005, Hoeksema *et al*. 2010). Our study with minimal environmental variability emphasizes the contribution of fungal and host genotypes. Few studies have documented the host and/or fungal genotypic effects (see Munkvold *et al*. 2004, Koch *et al.* 2006, Piculell *et al*. 2008, Karst *et al.* 2009, Tellenbach *et al*. 2011). Those that exist pinpoint either the host (*e.g.*, Redman *et al.* 2001, Faeth & Sullivan 2003) or the fungal (Freeman & Rodriguez 1993, Tanaka *et al*., 2006) genotype as the governing agent. Our data suggest that the outcomes are likely determined by genotypes of both the host and the fungus.

Our data highlight the context dependency of the host responses to DSE. DSE fungi, similarly to mycorrhizal fungi or grass endophytes, elicit a range of responses that are controlled by many abiotic and biotic factors. Therefore, based on the experimental evidence, Jones & Smith (2004) argue that symbioses are best defined structurally or developmentally – not based on host-derived benefit. While it has been hypothesized that mutualisms are more frequently developed between microbes and roots (Schulz & Boyle 2005), only a fraction of fungal endophytes interact positively with their hosts (Schulz 2006, Kageyama *et al*. 2008). This is supported by our study. The broad host range of DSE fungi invited and motivated our search for well-established model systems that would maximize access to tools that permit informative dissection of the host responses to fungal colonization. The *A. thaliana* responses that range from negative to positive provide the empirical setting that allows for asking questions on what controls host responses. This is particularly true as *A. thaliana* with its expedient life cycle can be harnessed to large-scale laboratory manipulations. Our studies described herein as well as those of others (Peškan-Beghöfer *et al*. 2004, Lee *et al*. 2011, Junker *et al*. 2012) highlight the potential of exploring fungal symbioses in well-controlled, axenic laboratory conditions where host-fungus interactions can be assessed in absence of environmental variability at the level of genotypes.

## CONCLUSIONS

Our studies demonstrated that *A. thaliana* forms DSE symbioses, which can be readily manipulated in the laboratory. The use of a well-established model provides a convenient tool for further dissection of the DSE symbiosis functionally, molecularly, and metabolically. Our

screening of three *A. thaliana* accessions with DSE isolates clearly indicated that both host and fungal genotypes contribute to the outcome of a symbiosis and that these outcomes may be unpredictable if only the species identities of the host and fungus are known.

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**Fig. 1.** Responses of three *A. thaliana* accessions (Col-0, diamonds; Cvi-1, circles; Kin-1, squares) to two root colonizaing fungi (*Periconia macrospinosa*, closed symbols; *Microdochium* sp. open symbols). For each paired experiment (inoculated vs. fungus-free control), the mean shoot mass of the control treatment is plotted along the x-axis and the mean shoot mass of the inoculated treatment along the y-axis. Along the dashed line, the two means are equal ($R_{DSE} = 0$); above the line, the inoculated plant mass exceeds that of the control (positive response; $R_{DSE} > 0$); and below the line the control mass exceeds that of the inoculated treatment (negative response; $R_{DSE} < 0$). Statistically significant differences (ANOVA, $\propto = 0.05$) are highlighted with black symbols.

**Fig. 2.** Responsiveness ($R_{DSE}$) of three *A. thaliana* accessions (Col-0, top; Cvi-1, middle; Kin-1, bottom) to *Periconia macrospinosa*. The experiments were ranked in ascending order for accession Kin-1 to emphasize the variable host responses. Asterisks indicate significant shoot mass differences between the fungus-free control and the inoculated treatment (ANOVA, $\propto = 0.05$). Black arrows emphasize those strains that include positive, negative and no significant response to inoculation across the three host accessions. Grey arrows identify the four strains that had consistent responses across all three accessions. The first 25 strains are common to all three accessions and five on the far right are those that were used for one or two of the three accessions.

**Fig. 3.** Distribution of the *A. thaliana* responsiveness ($R_{DSE}$) to inoculation with *Periconia macrospinosa*. The observations were grouped into ten classes at 0.2 intervals (*i.e.,* -1.0 – -0.81; -0.8 – -0.61; *etc*.). The dashed line highlights the null hypothesis ($R_{DSE} = 0$), against which the alternative hypotheses were tested. The insets provide the Student's *t*- value, p-value (ns $p > 0.05$; *** $p < 0.001$), mean and standard deviation for each of the three accessions.