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Septate endophyte colonization and host responses of grasses and forbs native to a tallgrass prairie

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Abstract Native tallgrass prairies support distinct dark septate endophyte (DSE) communities exemplified by *Periconia macrospinosa* and *Microdochium* sp. that were recently identified as common root symbionts in this system. Since these DSE fungi were repeatedly isolated from grasses and forbs, we aimed to test their abilities to colonize different hosts. One *Microdochium* and three *Periconia* strains were screened for colonization and growth responses using five native grasses and six forbs in an in vitro system. Previously published data for an additional grass (*Andropogon gerardii*) were included and reanalyzed. Presence of indicative inter- and intracellular structures (melanized hyphae, microsclerotia, and chlamydospores) demonstrated that all plant species were colonized by the DSE isolates albeit to varying degrees. Microscopic observations suggested that, compared to forbs, grasses were colonized to a greater degree in vitro. Host biomass responses varied among the host species. In broad comparisons, more grass species than forbs tended to respond positively to colonization, whereas more forb species tended to be non-responsive. Based on the suspected differences in the levels of colonization, we predicted that tallgrass prairie grasses would support greater DSE colonization than forbs in the field. A survey of field-collected roots from 15 native species supported this hypothesis. Our study supports the "broad host range" of DSE fungi, although the differences in the rates of colonization in the laboratory and in the field suggest a greater compatibility between grasses and DSE fungi. Furthermore, host responses to DSE range from mutualism to parasitism, suggesting a genotype-level interplay between the fungi and their hosts that determines the outcome of this symbiosis.

Keywords Dark septate endophytes (DSE) · Mycorrhizal dependency · Mutualism–parasitism continuum

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Introduction

Dark septate endophytic (DSE) fungi are a common component of the microbial communities colonizing healthy plant roots (Mandyam and Jumpponen 2005, 2008). Although several studies have focused on DSE and documented their abundance in different habitats, many aspects of their ecology remain unknown. Much of the present understanding of DSE symbiosis, diversity, and ecological significance is based on limited number of taxa (Addy et al. 2005; Mandyam and Jumpponen 2005). Recent studies from grasslands have provided valuable information about DSE including estimates of their abundance, their temporal and seasonal variability, as well as their community composition (Mandyam and Jumpponen 2008; Herrera et al. 2010; Mandyam et al. 2010).

In the tallgrass prairie ecosystem, DSE fungi are a major component and colonize a large proportion of the root system in mixed plant communities equaling colonization by arbuscular mycorrhizal (AM) fungi (Mandyam and Jumpponen 2008). To identify the dominant DSE fungi in this system, Mandyam et al. (2010) repeatedly isolated and successfully resynthesized DSE formed by *Periconia macrospinosa* Lefevbre and Johnson and *Microdochium* sp. expanding the list of potential DSE fungi. The abundance of the DSE fungi is not limited to tallgrass prairies as indicated by the colonization of *Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths across a broad

geographical range (Herrera et al. 2010). *B. gracilis* not only supports an abundance of DSE fungi (Porras-Alfaro et al. 2007; Khidir et al. 2010), but seems to be colonized by novel DSE communities with a large Pleosporalean component (Porras-Alfaro et al. 2008).

Despite the recent broad studies on the ecology and abundance of DSE fungi, many aspects—including their host range—remain largely unknown. DSE fungi have been proposed to possess a broad host range based on the number of hosts they can colonize in laboratory experiments or on the number of plant species from which DSE fungi have been isolated (Jumpponen and Trappe 1998). For example, *Phialocephala fortinii* Wang and Wilcox colonizes at least eight plant species and has been isolated from as many as 29 plant species (Jumpponen and Trappe 1998; Jumpponen 2001). Fernando and Currah (1996) isolated *Leptodontidium orchidicola* Sigler and Currah from ten species at a Canadian site. Similarly, a DSE isolate from *Ranunculus adoeus* A. Gray produced endophytic structures when inoculated onto *Zea mays* L. (Schadt et al. 2001). Taken together, these observations support the hypothesis that DSE have a broad host range, which has remained to be explicitly tested under controlled experimental conditions.

Host responses to DSE fungi are uncertain and currently under debate (Jumpponen 2001; Addy et al. 2005; Mandyam and Jumpponen 2005; Alberton et al. 2010; Newsham 2011). Recent meta-analyses of the limited number of available studies have produced conflicting results. Alberton et al. (2010) concluded that, while host growth responses to DSE fungi were variable, they tended to be on average negative. In contrast, broader and more detailed meta-analyses conducted by Newsham (2011) found that DSE inoculation increased host biomass, particularly when inoculation was conducted either in a system with no additional inorganic N or when majority of the N had been supplied in organic forms. These conflicting meta-analyses emphasize the considerable variability in host responses to DSE fungi but also that, like arbuscular mycorrhizas and ectomycorrhizas, the DSE symbioses may be context-dependent (Karst et al. 2008; Hoeksema et al. 2010). In addition to the inter- and intraspecific variability of the plants (Piculell et al. 2008; Karst et al. 2009) and fungi (Munkvold et al. 2004) addressed here, the differences in host responses may be attributable to environmental conditions and modulated by shade, drought, salinity, and nutrient depletion (Johnson et al. 1997; Kageyama et al. 2008; Rodriguez et al. 2008; Hoeksema et al. 2010).

In the present studies, we used controlled laboratory resyntheses to explore the specificity and effects on host responses of two DSE fungi, *P. macrospinosa* and *Microdochium* sp. commonly isolated from a native tallgrass prairie (Mandyam et al. 2010). We inoculated five native grasses and six forbs and included additional previously published data for the grass *Andropogon gerardii* Vitman (Mandyam et al. 2010). Specifically, we aimed to (1) microscopically confirm root colonization of the 12 target plant species by four strains of two DSE fungi (three strains of *P. macrospinosa* and one of *Microdochium* sp.) and (2) evaluate the effects of DSE colonization on host biomass. Led by the results of these resyntheses, we validated our laboratory observations and tested hypotheses on whether or not the observed greater DSE colonization of grasses in vitro could be confirmed in field-collected material.

Materials and methods

Site description

Fungal strains and plant materials were obtained from Konza Prairie Biological Research Station (KPBS; 39°05′ N, 96°35′ W), which represents a mesic native tallgrass prairie in the Flint Hills of eastern Kansas, USA. This site spans 3,487 ha and has remained undisturbed by agriculture. The vegetation is dominated by *A. gerardii*, *Sorghastrum nutans* (L.) Nash., *Schizachyrium scoparium* (Michx.) Nash, and *Panicum virgatum* L. (see Towne 2002 for a complete list of vascular plants at KPBS). The soil parent material is chert-bearing limestone, and the soil bulk density is 1.0 g/cm^3. January mean temperature is −3°C (range −9°C to 3°C), and the July mean is 27°C (range 20°C to 33°C). Annual precipitation is 835 mm, of which about 75% occurs in the growing season. For the field survey of root colonization, samples were collected from two annually spring burned lowlands and two infrequently (every 20 year burn) burned watersheds to account for management and geographic variability in fungal colonization.

Resynthesis with native prairie plants

One *Microdochium* sp. (KS0012) and three *P. macrospinosa* strains (KS0019, KS0045, and KS0100) previously isolated and identified by Mandyam et al. (2010) from KPBS were used for in vitro experiments. In these laboratory experiments, we tested the fungal compatibility with native prairie plants by resynthesizing fungal structures (microsclerotia and chlamydospores, Mandyam et al. 2010) indicative of the DSE symbiosis. We analyzed a total of 12 native species (Table 1) for their in vitro colonization and growth responses. Five grass species (*B. gracilis*, *Elymus canadensis* L., *P. virgatum*, *S. scoparium*, *S. nutans*) were selected for the resynthesis experiments. We also include and re-analyze *A. gerardii* data

Table 1 List of plant species used for testing host range of DSE fungi

Family	Species	Field study		Resynthesis
		May	July	
Asclepiadaceae	*Asclepias syriaca* (C_3)	7	6	16
Asteraceae	*Achillea millefolium* (C_3)	8	6	N/A
	Ambrosia artemisiifolia (C_3)	8	8	N/A
	Artemesia ludoviciana (C_3)	3	8	N/A
	Echinacea angustifolia (C_3)	N/A	N/A	27
	Helianthus maximilianii (C_3)	N/A	N/A	31
	Solidago missouriensis (C_3)	N/A	8	N/A
Fabaceae	*Baptisia australis* (C_3)	N/A	N/A	16
	Dalea purpurea (C_3)	N/A	N/A	56
	Lespedeza capitata (C_3)	N/A	6	N/A
Malvaceae	*Sphaeralcea* sp. (C_3)	6	N/A	N/A
Plantaginaceae	*Plantago patagonica* (C_3)	8	N/A	N/A
Poaceae	*Andropogon gerardii* (C_4)[a]	3	7	59[a]
	Bouteloua curtipendula (C_4)	N/A	8	N/A
	Bouteloua gracilis (C_4)	N/A	5	36
	Buchloe dactyloides (C_4)	N/A	5	N/A
	Elymus canadensis (C_3)	N/A	N/A	72
	Panicum virgatum (C_4)	N/A	N/A	67
	Poa pratensis (C_3)	6	4	N/A
	Schizachyrium scoparium (C_4)	5	8	72
	Sorghastrum nutans (C_4)	6	8	57
Violaceae	*Viola* sp. (C_3)	N/A	N/A	21

Numbers indicate the total number of experimental units included in the field and in vitro resynthesis studies. The numbers for the field study identify number of samples in May and July

N/A plants were not available for the study component

[a] Data from (Mandyam et al. 2010)

published earlier (Mandyam et al. 2010) for comparison with this broader selection of taxa. In addition to these six native grasses, we selected six forbs (*Asclepias syriaca* L., *Baptisia australis* (L.) Br. ex Aiton, *Echinacea angustifolia* DC, *Dalea purpurea* Vent, *Helianthus maximilianii* Schrad, *Viola* sp.). Grass seeds (provided by Richard Wynia at the United States Department of Agriculture Natural Resources Conservation Service) were surface-sterilized in 70% alcohol for 30 min followed by 30% bleach for 20 min. Forb seeds (W. Atlee Burpee and Co., Warminster, PA, USA) were sterilized in alcohol for 10 min followed by 30% bleach for 10 min. Sterilized seeds were germinated on 1/10th strength Murashige–Skoog (MS) basal salt medium (Sigma, St. Louis, MO, USA) for a week in a growth chamber under 12 h cycle of light (ca. 250 μmol/m^{-2} s^{-1} PAR at 20°C). Seeds of some plant species were repeatedly contaminated by seed-borne fungal endophytes, and due to the unavailability of a large number of sterile seeds, some resynthesis experiments became unbalanced and some fungal treatments were omitted (Fig. 1; Table 1).

The resynthesis system was set up as described in Mandyam et al. (2010). In brief, the system consisted of Petri plates with MS plant growth media and sealed with parafilm. Germling roots were placed inside the plate so that the shoots grew outside through a slit cut in both the lid and the plate. After seedling stabilization for 4 days, 6-mm plugs cut from an actively growing margin of a colony on potato dextrose agar (PDA) were used for inoculation, and plates were incubated for 6 weeks. Fungus-free controls were inoculated with similar sterile, fungus-free PDA plugs. Initially, each treatment received 15 replicates and was incubated in the growth chamber under the above conditions. Omission of contaminated experimental units led to unbalanced experimental designs at harvest. Where possible, shoots of all 15 replicates and roots of ten replicates were harvested, dried at 50°C, and their dry weight recorded. Roots from the remaining five replicates were used for microscopic observation to confirm colonization in the *Periconia* and *Microdochium* treatments or absence of contamination in the fungus-free controls. Total biomass and root/shoot ratio were calculated.

Confirmation of root colonization in resynthesis roots

Root samples from five replicates were used for screening presence/absence of DSE structures. Micro-

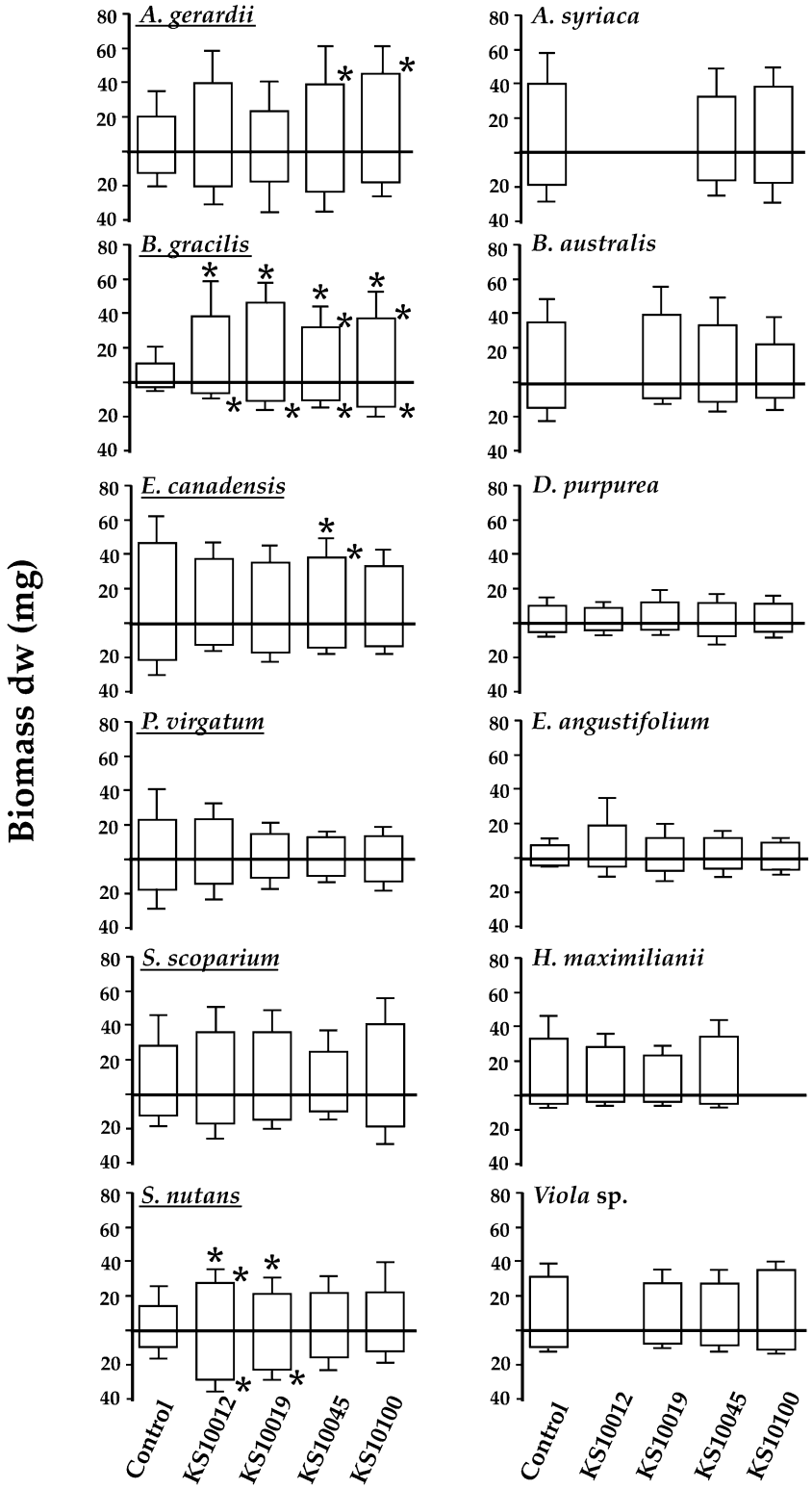

Fig. 1 Host responses (dry weight milligrams; mean±1 standard deviation) to five inoculation treatments (fungus-free control, *Microdochium* sp. (strain KS10012) and *P. macrospinosa* (strains KS10019, KS10045, and KS10100)). Complete host binomials are listed in Table 1 and grasses are *underlined* for clarity. Note that some fungal treatments were omitted for species that had limited seed availability and/or carried seed-borne contaminants. Shoot biomass above the *x*-axis; root biomass below *x*-axis. *Asterisks* on the top of, next to the shoot, and next to the root biomass values indicate treatment difference in total, shoot, root biomasses from the control based on Dunnett's test at α=0.05. There were no differences in root/shoot ratios

sclerotia and melanized hyphae were recorded in the *Periconia* treatments and chlamydospores in the *Microdochium* treatment (Mandyam et al. 2010). Because most DSE structures are melanized, the roots were observed without staining. The agar medium dried in some treatments with fast growing host species after 6 weeks of incubation. Removal of these root systems was difficult and estimation of the percent root length colonized (%RLC) omitted.

Plant responses to DSE colonization

To gain a better understanding of the relative host responses to DSE fungi, we modified the "mycorrhizal dependency," a metric occasionally used in determining plant responsiveness to mycorrhizal symbiosis (Wilson and Hartnett 1998; Klironomos 2003). Since use of the term "dependency" for DSE symbiosis is likely inaccurate, we use "responsiveness" (R) to describe DSE colonization as a means to assist in evaluating variable plant growth responses:

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

$$R = [(\text{median dry weight of inoculated treatment} - \text{median dry weight of fungus} - \text{free control treatment}) / \text{median dry weight of inoculated treatment}]$$

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

$$R = [(\text{median dry weight of inoculated treatment} - \text{median dry weight of fungus} - \text{free control treatment}) / \text{median dry weight of fungus} - \text{free control treatment}]$$

Because our data were often skewed and non-normally distributed, we chose to use median dry weights instead of means to estimate R. A great advantage of this metric is that it normalizes the responses to inoculation treatments so that the observations are distributed from −1 to 1 and can be tested for a null hypothesis of R equaling zero for no response. This metric also simply illustrates the host responses to inoculation: Values greater than zero indicate positive responses, values lesser than zero negative responses.

Field sample collection from KPBS

To test the hypothesis that grasses are more heavily colonized by DSE fungi than forbs, we analyzed 15 species collected from KPBS (Table 1). Roots from up to eight individuals of commonly occurring grasses (*A. gerardii*, *Bouteloua curtipendula* (Michx.) Torr., *B. gracilis*, *Buchloe dactyloides* (Nutt.) Engelm, *Poa pratensis* L., *S. scoparium*, *S. nutans*) and forbs (*Achillea millefolium* L., *Ambrosia artemisiifolia* L., *Artemesia ludoviciana* Nutt., *A. syriaca*, *Lespedeza capitata* Michx., *Plantago patagonica* Jacq., *Sphaeralcea* sp., *Solidago missouriensis* Nutt.) were randomly sampled in late May and late July in 2004. Since no green plants for some of the early-season forbs (e.g., *P. patagonica*) could be located or identified in the July sampling, or some late season grasses (e.g., *B. dactyloides*, *B. gracilis*, *B. curtipendula*) and forbs (e.g., *L. capitata*, *S. missouriensis*) be located or identified in the May sampling, the numbers of species and samples varied across the samplings. Consequently, the colonization data were analyzed separately for the two sampling occasions. The final, complete data matrices consisted of 43 forb and 20 grass samples in May and 42 forb and 45 grass samples in May for a total of 150 samples. A plant shoot was collected to assure sampling of correctly identified, attached roots. Roots were washed free of soil under running tap water. Cleaned roots were transported to the laboratory for further processing.

Staining and microscopy of field-collected roots

Roots adhering to the shoots were cut to 1-cm fragments and cleared by autoclaving (5 min; 121°C; 15 psi) in 2.5% potassium hydroxide followed by several washes with water and neutralization with acetic acid. To observe AM and DSE colonization in the sampled roots, one random half of the cleared roots was immersed in Trypan blue (Philips and Hayman 1970), another in Sudan IV (Barrow and Aaltonen 2001), and autoclaved for 4 min followed by several washes in water. The stained roots were allowed to destain in acidic glycerol (500 ml glycerol, 450 ml H_2O, 50 ml HCl) overnight.

Colonization in the stained roots was estimated by magnified intersection method (McGonigle et al. 1990) at ×200 magnification for total colonized root length (%RLC). For each plant, ten randomly selected roots (1 cm) were used for quantification and AM (i.e. presence of any AM structure—AM hyphae, vesicles, arbuscules, or coils) and DSE (i.e. presence of any DSE structure—melanized

septate hyphae and microsclerotia) colonization in ten intersections per segment were recorded for a total of 100 intersections.

Statistical analysis of resynthesis data

The mean total, shoot, and root biomass plus root/shoot ratio of the inoculated treatments for each plant species were compared to the controls using Dunnett's test in JMP (Version 7.02, SAS Institute, Cary, NC, USA). To equalize variances, the biomasses and root/shoot ratios were log-transformed prior to analyses. The responsiveness (R) was analyzed across the broad functional groupings (grasses vs. forbs) using one-way ANOVA. Two-tailed Student's t tests were used to test whether or not the mean of R differed from zero for any one species. To account for the multiple comparisons, Bonferroni-corrected, conservative values for significance are also provided for these analyses.

Statistical analyses of AM and DSE colonization at Konza Prairie

DSE data were not normally distributed and variances were not homogeneous (Levene's test: $P<0.05$), whereas the AM data were normally distributed and the variances were homogeneous. To correct for these violations of the assumptions for ANOVA, all %RLC values were transformed by arcsine of the square root and analyzed with ANOVA in JMP. Pairwise differences, when necessary, were determined by Tukey's honestly significant difference (HSD) with $\alpha=0.05$.

Results

Root colonization in the resynthesis study

All tested hosts were colonized by *Microdochium* sp. (KS0012) and two *Periconia* strains (KS0045 and KS0100). The third *Periconia* strain (KS0019) colonized the hosts sparsely indicating variability in the ability of *Periconia* strains to colonize hosts. *Microdochium* sp. produced abundant chlamydospores in the cortex and root hairs in all tested plants. *Periconia* (KS0045, KS0100) produced melanized microsclerotia and intercellular hyphae in the grasses similar to those observed in *A. gerardii* resynthesis reported earlier (Mandyam et al. 2010), but colonized the forbs only sparsely. Regrettably, we did not record the %RLC for the resynthesis studies and are unable to provide statistical inference for these observations. One *Periconia* (KS0045) sporulated frequently on the roots and produced melanized, septate conidiophores with black, echinulate spores characteristic to the taxon.

Host responses to inoculation in the resynthesis study

Overall, grasses were more responsive to DSE inoculation treatments than forbs, although two of the six analyzed native grasses (*P. virgatum* and *S. scoparium*) were non-responsive (Fig. 1). Compared to the fungus-free control, *Periconia* isolates KS0045 and KS0100 increased *A. gerardii* shoot biomass by 79.8% and 110.3%, respectively, whereas the other two strains had no effect (Mandyam et al. 2010). Inoculation with all four fungal strains increased *B. gracilis* total biomass (221.8–325.0%) and root biomass (291.1–460.5%) compared to the fungus-free control; these biomass responses did not alter the root/shoot ratios. In *E. canadensis*, total biomass was unaffected by three of the four strains, but was reduced 30.4% by *Periconia* strain KS0045. In *S. nutans*, *Microdochium* sp. inoculation increased total (131.1%), shoot (92.5%), and root (191.1%) biomass and one of the three *Periconia* strains (KS0019) increased total (90.9%) and root (135.7%) biomass. However, root/shoot ratio in none of the treatments differed from the control. In contrast to grasses, none of the fungal treatments affected any of the forbs (Fig. 1). The shoot and root weights as well as root/shoot ratios were similar to those in controls across all inoculation treatments.

Plant responsiveness to DSE colonization

Overall, grasses mostly responded positively to DSE colonization, whereas forbs were mostly unresponsive (Fig. 2; Table 2). However, there were no differences between the grasses and forbs overall: R values for forbs were not different from those for grasses, regardless of whether the strains were analyzed separately ($F<2.66$; $P>0.1472$) or the data combined ($F_{1,\ 42}=2.69$; $P=0.1086$). Accordingly, these apparent differences were driven by species identities rather than broad monocot vs. dicot groupings. For grasses, R was greater than zero in sixteen of the 24 observed plant–fungus trials (Table 2). Based on Bonferroni-corrected two-tailed Student's t tests for hypothesis H_0: $R=0$ at $\alpha=0.05$, two of the six grasses (*B. gracilis*, *S. nutans*; Fig. 2) responded positively inoculation, whereas one responded negatively (*E. canadensis*; Fig. 2). Based on R, *B. gracilis* was most responsive and *P. virgatum* was ranked last. In contrast to grasses, forbs tended to be relatively unresponsive to inoculation: R was greater than zero for only eight of the 19 successfully completed trials (Table 2). None of the six forbs had an R different from zero (two-tailed Student's t tests for H_0: $R=0$ at $\alpha=0.05$ after Bonferroni correction).

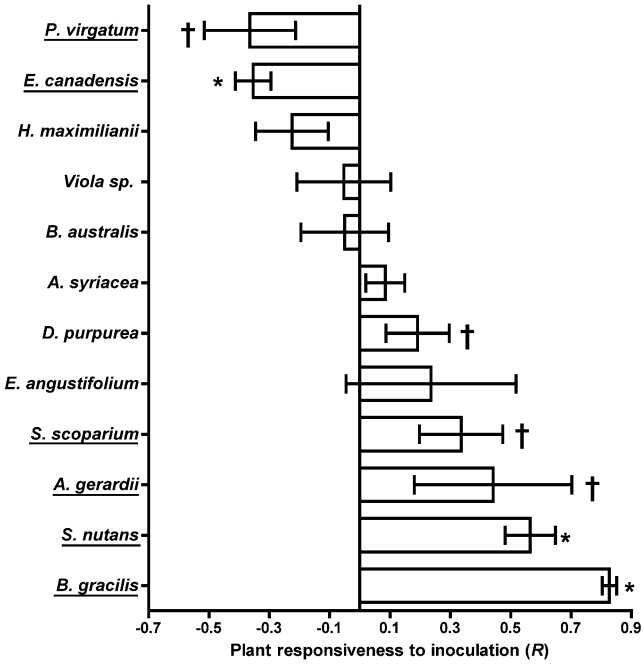

Fig. 2 Host responsiveness (R; mean±1 standard deviation) to inoculation with four strains of DSE fungi. Complete host binomials are listed in Table 1 and grasses are *underlined* for clarity. Responsiveness metric R was modified from that described in Klironomos (2003). The *symbols* indicate significant two-tailed Student's t tests for H_0: $R=0$ at $\alpha=0.05$ with (*asterisk*) and without (*cross*) the conservative Bonferroni correction

Endophyte colonization in field-collected grasses and forbs

To compare the colonization of native forbs and grasses by AM and DSE fungi, we analyzed the two types of root-associated fungi separately in early growing season in May and at peak growing season in July. The separate analyses were necessary because the species compositions between the two sampling events differed. Overall, the colonization tended to be highly variable and differed in only few of the 15 species (Fig. 3). However, in the early-season sampling in May, AM colonization in forbs was 11.2% greater than in grasses ($F_{1,\ 63}=8.10$; $P=0.0058$). In contrast to AM, DSE colonization in May was 20.7% greater in grasses than in forbs ($F_{1,\ 63}=6.97$; $P=0.0107$). However, it is of note that these differences are mainly driven by variability and individual species, not by grass vs. forb differences. For example, two forbs (*A. syriaca* and *Sphaeralcea* sp.) and the common C4 grass (*S. scoparium*) had consistently high AM colonization that was greater than that of one forb (*A. ludoviciana*) and two grasses (*A. gerardii* and *S. nutans*; Fig. 3). In general, pairwise differences as indicated by Tukey's HSD were few among the species analyzed in May (Fig. 3). To exemplify, two grasses (*S. scoparium* and *S. nutans*) had significantly ($\alpha=0.05$) greater DSE colonization than *Sphaeralcea* sp. and *A. gerardii*, whereas the other species did not differ from any of these.

In July, additional plant species were sampled because their identification was enabled by presence of reproductive parts that were absent in May. In this sampling, grasses were 11.8% more heavily colonized by AM ($F_{1,\ 86}=8.08$; $P=0.0056$) and 34.6% more heavily colonized by DSE ($F_{1,\ 86}=39.28$; $P<0.0001$) than forbs (Fig. 3). Similar to the sampling in May, variability in AM colonization was high and differences between the species few. Two grasses (*P. pratensis* and *S. scoparium*) were more heavily

Table 2 Plant response (R) to inoculation by four strains of DSE fungi

Plant species	*Microdochium* sp. KS0012	*Periconia macrospinosa* KS0019	*Periconia macrospinosa* KS0045	*Periconia macrospinosa* KS0100	Mean response
Grasses					
Andropogon gerardii	0.55	0.05	0.56	0.60	0.44
Bouteloua gracilis	0.84	0.84	0.79	0.84	0.77
Elymus canadensis	−0.29	−0.36	−0.43	−0.33	−0.35
Panicum virgatum	−0.14	−0.48	−0.41	−0.43	−0.37
Schizachyrium scoparium	0.38	0.36	0.14	0.46	0.34
Sorghastrum nutans	0.67	0.59	0.48	0.53	0.57
Forbs					
Asclepias syriaca	N/A	N/A	0.13	0.04	−0.09
Baptisia australis	N/A	0.02	0.05	−0.22	−0.15
Dalea purpurea	0.06	0.19	0.20	0.32	0.19
Echinacea angustifolia	−0.17	0.46	0.39	0.25	0.23
Helianthus maximilianii	−0.25	−0.33	−0.09	N/A	−0.15
Viola sp.	N/A	−0.14	−0.14	0.13	−0.15

N/A missing data

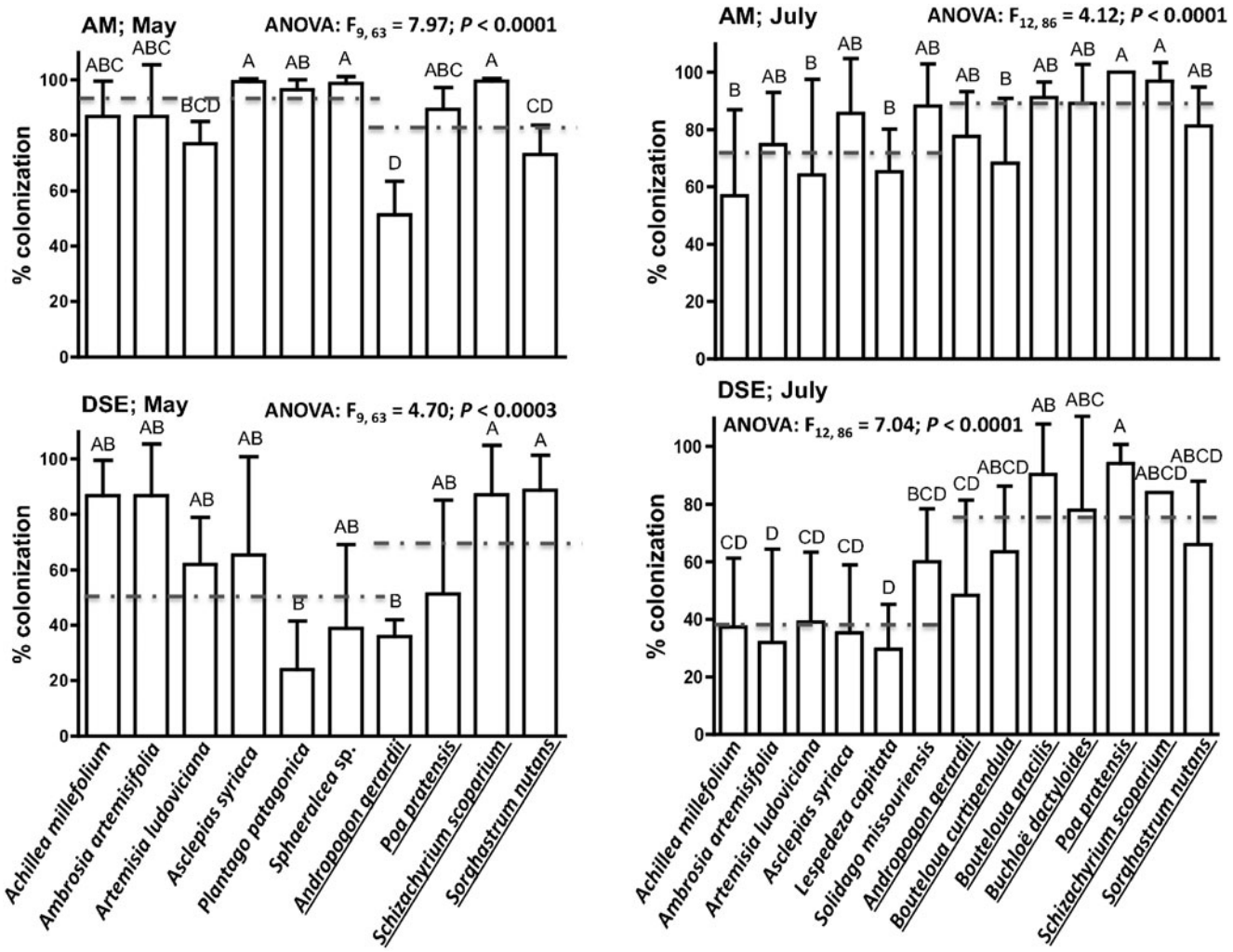

Fig. 3 Colonization (% root length; mean±1 standard deviation) of native forbs and grasses (*underlined*) by arbuscular mycorrhizal (*AM*) and dark septate endophytic (*DSE*) fungi in field-collected roots from Konza Prairie Biological Station. ANOVA table for species effect is provided in the inset. The *dashed lines* identify the grand mean across all forbs or grasses sampled for each of the two (May, July) sampling occasions. Mean AM colonization of forbs exceeded that of grasses in May; in all other comparisons, grass colonization exceeded that of the forbs. *Letters on top* identify significant differences of values transformed by arcsine of the square root based on Tukey's honestly significant difference with α=0.05

colonized than one grass (*B. curtipendula*) and three forbs (*A. millefolium*, *A. ludoviciana*, and *L. capitata*). In contrast to AM, DSE colonization seemed more distinctly higher in grasses. However, although the grass–forb differences among the species were more frequent, these patterns are perhaps best characterized by high variability within and between the species.

We also aimed to compare forb and grass colonization by AM and DSE. These analyses indicated that forbs were colonized by AM to a greater degree than by DSE in both May ($F_{1,\ 86}$=89.32; P<0.0001) and July ($F_{1,\ 84}$=37.03; P<0.0001). The differences in AM and DSE colonization in grasses were less drastic. Grass colonization by AM and DSE did not differ in May ($F_{1,\ 40}$=1.73; P=0.1966), and AM colonization was only marginally greater in July ($F_{1,\ 90}$=4.36; P=0.0397). Overall, these field data from early and peak growing season corroborate the results of our resynthesis study: Grasses have a greater DSE colonization than the forbs and grasses may show a greater compatibility with the DSE fungi than forbs do.

Our broad selection of native forbs also included a combination of annual (*A. artemisiifolia* and *P. patagonica*) and perennial (*A. millefolium*, *A. ludoviciana*, *A. syriaca*, *L. capitata*, *S. missouriensis*) forbs. We omitted *Sphaeralcea* sp. from these analyses because of the uncertainty whether it should be considered annual or perennial. We observed no differences in AM colonization between the annual and perennial forbs in May ($F_{1,\ 44}$=0.24; P=0.6253) or July ($F_{1,\ 41}$=0.001; P=0.9807). In contrast, compared to annual forbs, perennial forbs were more heavily colonized by DSE

in May ($F_{1,\ 33}$=8.14; P=0.0076), but these differences disappeared in the peak season sampling in July ($F_{1,\ 22}$= 1.23; P=0.2951). As the DSE colonization seemed to decline overall, these data suggest that the DSE may primarily inhabit aging tissues in forbs and colonization of the annual tissues may coincide with aging of the root systems.

Discussion

Using combinations of host plants and DSE fungi native to tallgrass prairie in resynthesis studies along with DSE colonization data in the field, this study confirms the "broad host range" of DSE fungi. Interestingly, the data suggest that grasses may be more heavily colonized by DSE and more responsive to DSE colonization than forbs. These observations are congruent with Newsham's (2011) recent meta-analysis: Although there were no differences in the effect sizes between monocots and dicots in that study, the highest effect sizes in biomass responses were observed for monocots. Additionally, regardless of the broad plant groupings, our data confirm the outcomes of plant–DSE symbioses to fall within a range along the mutualism–parasitism continuum and seem to include no pathogenic interactions.

An interesting but yet unanswered question is whether our results represent an in vitro bias or artifact, even though the overall greater grass compatibility was observed both under the field and in vitro conditions. Newsham (2011) concluded that hosts respond more positively if no inorganic nitrogen is made available in the experiments and when nitrogen is supplied in organic forms. At this point, further experiments are necessary to empirically confirm the conclusions of those meta-analyses. However, our choice to use MS medium with exclusively inorganic nitrogen sources may have affected the host responses. If this were true and if inorganic nitrogen supply lead to lesser or fewer positive host responses, then we would have underestimated the proportion of the positive host responses. This underestimation may have been further exaggerated by our use of conservative Bonferroni corrections in the analyses of host responsiveness (Fig. 2).

DSE host range

Broad host range of DSE fungi has been hypothesized, mostly based on surveys of DSE fungal colonization of different plant species (Jumpponen and Trappe 1998) but also based on the limited empirical data from resynthesis studies (Wilcox and Wang 1987; O'Dell et al. 1993; Fernando and Currah 1996; Schadt et al. 2001). Similar DSE colonization in different hosts such as by *P. fortinii* in *Lupinus latifolius* Agardh. and *Pinus contorta* Dougl. (O'Dell et al. 1993) and by an unknown DSE fungus in *R. adoeus* and corn (Schadt et al. 2001) have suggested the broad host range of DSE. In this study, we provide compelling evidence for a broad DSE host range using both resynthesis and field assays of native tallgrass prairie plants. Our data show that native grasses and forbs are colonized by DSE fungi, albeit to varying degrees. *Microdochium* sp. colonized all hosts, whereas the colonization patterns of *Periconia* isolates were more variable: Two strains (KS0045 and KS0100) colonized all plant species producing intracellular microsclerotia and intercellular hyphae, while one strain (KS0019) colonized hosts more sparsely. The inclusion of representative DSE isolates suggests that screening a sizeable number of host-DSE combinations may be necessary to draw meaningful conclusions about the ability of DSE fungi to colonize various hosts.

Of the six native grasses, two responded positively to DSE inoculation, whereas the forbs seemed less likely to benefit from DSE colonization. Based on these results and on our anecdotal observations of more sparse colonization of forbs in the resynthesis experiments, we propose that native tallgrass grasses are more compatible with DSE fungi than co-occurring native forbs. To provide further evidence for the greater grass colonization by DSE fungi, we sampled native hosts from the tallgrass prairie ecosystem where the hosts and fungi naturally co-occur. The results of this field survey were similar to our observations in the resynthesis experiments: DSE fungi colonized all native hosts to some degree. More importantly, as predicted from our observations in the resynthesis experiment, grasses on average hosted greater DSE colonization than forbs. Weishampel and Bedford (2006), similarly, observed that the DSE colonization in monocots was significantly greater than that in dicots. Furthermore, Khidir et al. (2010) evaluated the root-associated fungal communities of three co-occurring species—two grasses (*B. gracilis* and *Sporobolus cryptandrus*) and *Yucca glauca*—and found that the grasses shared a core group of root-associated fungi distinct from that in *Y. glauca*.

In addition to broad monocot vs. dicot differences on AM and/or DSE colonization, we also observed some seasonal patterns. While the AM colonization in forbs exceeded that in grasses in early season, grass AM colonization surpassed that of forbs in the second sampling. In contrast, the DSE colonization was consistently higher in grasses than in forbs, although the effect of including additional species is unclear. Clearly, the plants are colonized to varying degrees by the DSE fungi as indicated by the lower colonization of annual forbs in the early sampling. The decline in the overall forb colonization

suggests root senescence related seasonal dynamics in these systems and a potential DSE function in root nutrient turnover.

DSE symbiosis: mutualism–parasitism continuum

In this study, host biomass was used to screen responses to DSE fungi under resynthesis conditions. Host responses to DSE colonization were highly variable: inoculation either increased, decreased, or had no effect on the biomass or root/shoot ratio, indicating a range of potential and variable interactions. It is notable that many observed responses were neutral, whereas few were mutualistic and fewer yet were parasitic (Fig. 2). While it has been hypothesized that mutualistic interactions are more frequently developed between microbes and roots, only a fraction of root-associated fungi may interact positively with their hosts (Schulz and Boyle 2005; Kageyama et al. 2008). None of our DSE isolates was pathogenic as all the tested plants appeared to be visibly healthy and without any colonization of the root vascular cylinder. These observations suggest that disease and tissue re-organization are exceptions in these endophyte interactions, perhaps an imbalance in symbiosis (Schulz et al. 1999; Kogel et al. 2006). Redman et al. (2001) suggested that a fungal isolate may be pathogenic in one host, mutualistic in another, or colonize some plants as a commensal. Even in mycorrhizal symbioses, neutral and negative responses are commonly encountered (Johnson et al. 1997; Karst et al. 2008). Whether interactions between the plant and its fungal endophytes are balanced (mutualism or commensalism) or imbalanced (parasitism or pathogenicity) depends on the fungal and plant genotypes, plant physiology, developmental stages of the partners, and nutrient availability or other environmental factors (Schulz et al. 1999; Redman et al. 2001; Schulz and Boyle 2005; Kogel et al. 2006; Tanaka et al. 2006; Newsham 2011).

According to Schulz and Boyle (2005), the plant–endophyte interactions fall within the symbiotic continuum, precluding the assignment of a particular life-history strategy to a given endophyte. The outcomes of plant–endophyte interactions depend on a "balance of antagonisms," and the phenotypic plasticity can stem from various factors affecting the continuum (see above) as indicated by the DSE isolates that exhibited negative or neutral effects on some hosts but conferring positive responses in others (e.g., *Periconia* strain KS00045 in *E. canadensis* and in *B. gracilis*). Interestingly, Rodriguez and Redman (2008) suggest that changing life-history strategies in endophytes may signify evolutionary transitions or that the fungi have achieved a greater ecological flexibility ensuring optimal growth and reproduction in different hosts.

Conclusions

To our knowledge, this is the first study to broadly characterize interactions between DSE isolates, grasses, and forbs native to a tallgrass prairie ecosystem. The results support our initial hypotheses that the native DSE fungi possess a broad host range. However, the combination of laboratory resyntheses and microscopic analyses of field-collected materials indicates that the DSE fungi colonize forbs to a lesser degree than they colonize native grasses. However, high temporal, intra-, and interspecific variability preclude explicit statements indicating that DSE colonization in any grass would exceed that in any forb. DSE effects on host growth were variable and represented responses along the mutualism–parasitism continuum. Based on our results and other published reports, the outcome of plant–endophyte symbiosis seems to depend on the host species, endophyte taxa or strains, their genetic makeup, extent of fungal colonization, and experimental conditions.

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