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SHORT COMMUNICATION

Tapping Into the Cotton Fungal Phytobiome for Novel Nematode Biological Control Tools

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ABSTRACT

A number of fungi have been shown to have negative effects on plant-parasitic nematodes. Most of these fungi have been isolated from soil, plant roots, or nematodes themselves. Fungi associated with crops can provide a diverse pool of candidates to test for antagonistic effects against plant parasites and other stressors. We used a hierarchical two-tiered approach to evaluate the efficacy and repeatability of 55 strains of fungi originally isolated as foliar facultative endophytes from upland cotton (*Gossypium hirsutum*) along with one commercial isolate of *Beauveria bassiana* for in planta antagonistic effects on root-knot nematodes (*Meloidogyne incognita*). All fungi were inoculated to cotton using a seed treatment. The number of root galls was quantified 3 weeks after egg inoculation of cotton seedlings. The majority of the fungi tested reduced the number of root galls relative to those on untreated control plants. To assess repeatability, 22 strains that exhibited the strongest reductions in gall numbers were further tested in replicate follow-up assays. Ninety-five percent (21/22) of these retested strains significantly reduced galling in the follow-up assay. Strains that reduced galling the most belonged to the genera *Alternaria*, *Chaetomium*, *Cladosporium*, *Diaporthe*, *Epicoccum*, *Gibellulopsis*, and *Purpureocillium*. On the contrary, three strains in the genera *Alternaria* and *Curvularia* significantly increased gall numbers. Our results indicate that a large proportion of the fungal strains originally isolated from cotton as naturally occurring foliar facultative endophytes are capable of reducing root-knot nematode infection when applied back to the plant as a seed treatment. These findings help establish a rich pool of candidate fungi for further evaluation as novel biological control tools against root-knot nematodes in cotton and other plants.

Keywords: agriculture, crop, nematology, rhizosphere and phyllosphere, soil ecology

Endophytic fungi are key components of the phytobiome that can affect plant–herbivore interactions through a number of non-mutually exclusive mechanisms. These include production of defense-related compounds (Faeth and Fagan 2002; Gurulingappa et al. 2011; Hartley and Gange 2009; McGee 2002; Van der Putten et al. 2001), regulating synthesis of phytohormones (Bilal et al. 2017; Duca et al. 2014) and potentially altering plant quality as a nutritional resource (Bernays 1994; Jallow et al. 2004). To date, most studies conducted on plant–fungus–herbivore systems have focused on the effects of mycorrhizal fungi belowground (Gange and West 1994; Koricheva et al. 2009) or foliar-colonizing fungi aboveground, with particular emphasis in the latter case on a small number of obligate grass endophytes (Cheplick and Faeth 2009). Alternatively, the identity and ecological role (if any) of the vast majority of facultative fungal endophytes that transiently associate with plants is largely uncharacterized (Porras-Alfaro and Bayman 2011; Wani et al. 2015). Importantly, accumulating evidence suggests that facultative fungal endophytes can play important protective roles against invertebrate herbivores and promote plant health (Jaber and Enkerli 2017; Jaber and Ownley 2018; Gange et al. 2019).

A better understanding of plant-fungus-nematode complexes could benefit the development of ecologically based management tools for important crop pests such as the root-knot nematodes, *Meloidogyne* spp. (Perry et al. 2009). The use of beneficial fungi associated with plants that may confer increased resistance or tolerance to nematodes could provide an alternative to chemical applications for their control (Cabanillas et al. 1988; Hallmann and Sikora 1996; Latch 1993; Martinez-Beringola et al. 2013; Mendoza and Sikora 2009; Tian et al. 2014; Waweru et al. 2013). Certain beneficial strains of *Fusarium* spp., *Pochonia chlamydosporia*, *Phialemonium inflatum*, *Piriformospora indica*, and *Chaetomium globosum*, have been reported to have antagonistic effects on nematodes while being present in plants as endophytes (Bajaj et al. 2015; Larriba et al. 2015; Martinuz et al. 2015; Yan et al. 2011; Zhou et al. 2016; Zhou et al. 2018).

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A recent study of facultative fungal endophytes occurring in commercial upland cotton (*Gossypium hirsutum*) recovered thousands of isolates from surface-sterilized leaves or squares (developing flowers). These isolates were grouped into a total of 69 taxa based on morphology and ribosomal internal transcribed spacer sequences (Ek-Ramos et al. 2013). Two of the recovered strains, *Chaetomium globosum* TAMU 520 and *Phialemonium inflatum* TAMU 490, have already been shown to have negative effects in planta on root-knot nematodes when inoculated back to cotton using a simple seed treatment (Zhou et al. 2016; Zhou et al. 2018). Here we report the testing of an additional 56 facultative fungal endophyte strains for potential antagonistic effects against root-knot nematodes in cotton under greenhouse conditions

MATERIALS AND METHODS

Of the 56 strains tested, 55 were originally cultured as endophytes from surface-sterilized cotton foliage on potato dextrose agar and V8 media as described in Ek-Ramos et al. (2013). The other was a commercial isolate of the endophytic fungal entomopathogen, *Beauveria bassiana* (BotaniGard 22WP, ARBICO Organics, Tucson, AZ), an insect pathogen previously shown to have negative effects on insects when inoculated to cotton (Lopez et al. 2014; Lopez and Sword 2015). All fungi were liquid cultured in 1-liter TriForest DuoCap Polycarbonate Erlenmeyer shaker flasks (TriForest Enterprises, Inc., Irvine, CA) containing 400 ml of potato dextrose broth (PDB, HiMedia M403, Mumbai, India). The PDB was sterilized at 121°C for 20 min and cooled down to room temperature. Four milliliters of Penicillin/Streptomycin (penicillin at 10,000 U ml^{-1} and streptomycin at 10 mg ml^{-1}, P4333 Sigma-Aldrich, St. Louis, MO) was added to each flask and mixed well. A 5 × 5 mm plug of each fungal isolate cultured on solid potato dextrose agar was transferred to each flask containing the liquid culture media and placed in an incubator shaker (Southwest Science Inc., Roebling, NJ) at 28°C and 150 rpm for 2 to 3 weeks. Fungal biomass was filtered using sterilized coffee filters and collected into 50-ml Falcon tubes. The wet biomasses were freeze-dried (FreeZone 6 Plus, Labconco, Kansas City, MO) and ground gently into fine powder in a mortar and pestle. Ground dry biomasses were kept refrigerated at 4°C.

A nematode susceptible cotton cultivar PhytoGen PHY499WRF (Dow AgroSciences, Indianapolis, IN) (McPherson 2014; Reid et al. 2012) was used for this study. Methyl cellulose (Sigma-Aldrich, M7140-250G, 15cP viscosity) was used as a sticker to bind fungal biomass to the seeds (Gurulingappa et al. 2010) by mixing 50 mg of ground dry-biomass with 1 ml of 2% methyl cellulose solution, which was then finalized to a concentration of 10^5 CFUs ml^{-1}. Approximately 200 seeds (acid delinted black seed without fungicides or insecticides) were coated using 1 ml of either the sticker solution alone (control) or the fungus-containing sticker solution, and then dried at room temperature and finished with talc powder (Sigma-Aldrich, Product Number 18654) to prevent sticking. Seeds were planted and germinated in pasteurized sand (steamed for 8 h at 72°C) in seed starter trays (each cell pot measured 4 cm top diameter × 6 cm deep) in a plant growth facility at 24°C (12 h light/12 h day photoperiod) until first true-leaf stage.

Root-knot nematode, *Meloidogyne incognita*, eggs were extracted from infected tomato plants maintained on a monthly basis in the greenhouse at Texas A&M University (provided by J. L. Starr) by agitating the roots in 0.6% NaOCl for 4 min and collected on a sieve with a pore size of 25 µm (Hussey and Barker 1973). Egg concentration in the extraction solution was quantified under a microscope using a Neubauer hemocytometer (a modified method of Gordon and Whitlock (1939)). Cotton seedlings at the first true-leaf stage were inoculated by pipetting 2 ml of egg suspension containing approximately 2,000 eggs directly to the soil at the base of the plant. Plants were maintained in the greenhouse for 3 weeks after nematode inoculation, and then carefully removed from pots and washed free of soil from the roots. Root fresh weight was measured and the total number of galls per root system was quantified for each plant. Each treatment group contained a total of 15 replicate plants.

We used a hierarchical two-tiered approach to evaluate the efficacy and repeatability of observed negative effects on nematode galling. We first performed a series of initial assays as described above on all 56 fungal strains. In the second step, we conducted a series of replicate follow-up assays on a reduced set of fungi consisting only of those strains that exhibited the strongest reductions in nematode galls in the first assays based on P values below 0.05 in pairwise statistical comparisons between fungal treatment and control plants (statistical tests described below).

Because of the large number of fungal strains involved in our study, we could not test them all simultaneously. As such, the bioassays were conducted across a total of eight different rounds (six initial and two follow-up rounds), each with a corresponding control treatment group grown for each round. All comparisons between treatment and control plants were made only among plants grown within the same bioassay round. The strains tested in each round are listed in Table 1.

All statistical analyses were performed using JMP Pro, Version 12.0.1 (SAS Institute Inc., Cary, NC). All data were tested for normality and equality of variances. The observed frequency of isolates with a mean number of galls either less than or greater than that of the control in the initial assays (rounds 1 to 6) was compared with the expected frequency of equal numbers under the null hypothesis of no effect of fungal treatment using Fisher's exact test. For each of the eight independent rounds of assays, a one-way analysis of variance (ANOVA) was performed to test for an overall effect of fungal treatment on gall numbers per gram of root tissue ($\alpha = 0.05$). If a significant overall treatment effect was detected, posthoc Dunnett's tests were used to compare the mean of the control against all the fungal treatments in pairwise comparisons ($\alpha = 0.05$). Values below a threshold of $P = 0.05$ in pairwise comparisons from the initial assays were used to select isolates with the strongest negative effects on root galling to be assessed for repeatability in replicate follow-up bioassays.

RESULTS

Of the 56 fungal strains initially assayed in rounds 1 to 6, the number of strains observed to reduce nematode galling relative to the control treatment (77%) was significantly higher than would have been expected by chance under the null hypothesis of no effect of the fungal treatments (50%) (Fisher's exact test, $P = 0.0029$) (Fig. 1; Table 1). This nonrandom negative effect is evident in the strong skew of negative versus positive values in Figure 1, illustrating that the majority of the fungal treatments reduced root galling relative to the controls. Significant overall effects of fungal treatments on nematode gall numbers were found in all six independent rounds of initial bioassays (ANOVA round 1: $F_{4,\ 70} = 7.63$, $P < 0.0001$; round 2: $F_{5,\ 84} = 7.10$, $P < 0.0001$; round 3: $F_{12,\ 182} = 4.84$, $P < 0.0001$; round 4: $F_{10,\ 154} = 10.38$, $P < 0.0001$; round 5: $F_{10,\ 154} = 8.93$, $P < 0.0001$; and round 6: $F_{15,\ 224} = 4.05$, $P < 0.0001$). Results of pairwise comparisons between the individual fungal isolates and their respective control groups are provided in Table 1. In contrast to the general pattern, a minority of the tested strains increased the number of galls in comparison with the controls. The increase in gall number was statistically significant for three of the isolates (Fig. 1; Table 1).

TABLE 1
Number of galls produced by root-knot nematodes per gram of root tissue (mean ± SE) in each fungal seed treatment group across eight bioassay rounds[a]

Bioassay	Fungal seed treatment	Mean ± SE	*P* value
Round 1	Control	28.02 ± 2.81	–
	Curvularia spicifera TAMU189	51.19 ± 6.03	0.0002
	Acremonium alternatum TAMU505	36.97 ± 3.51	0.29
	Cladosporium oxysporum TAMU534	29.01 ± 2.24	1.00
	Curvularia protuberata TAMU105	25.23 ± 3.29	0.96
Round 2	Control	81.17 ± 14.90	–
	Epicoccum layuense TAMU46	69.96 ± 23.48	0.94
	Cladosporium antropophilum TAMU249	39.01 ± 3.64	0.047
	Cladosporium sp. TAMU463	30.27 ± 2.37	0.011
	Epicoccum nigrum TAMU194	8.47 ± 1.28	0.0001
	Chaetomium globosum TAMU554	8.00 ± 1.24	0.0001
Round 3	Control	54.76 ± 5.31	–
	Epicoccum nigrum TAMU89	48.02 ± 4.16	0.74
	Epicoccum nigrum TAMU103	46.57 ± 3.42	0.51
	Alternaria eichorniae TAMU53	40.77 ± 8.09	0.042
	Epicoccum nigrum TAMU125	39.81 ± 1.96	0.024
	Purpureocillium lavendulum TAMU239	39.71 ± 2.81	0.022
	Chaetomium coarctatum TAMU333	38.48 ± 4.59	0.010
	Alternaria eichorniae TAMU87	37.32 ± 3.44	0.0047
	Epicoccum nigrum TAMU131	36.74 ± 2.41	0.0031
	Diaporthe sp. TAMU137	31.76 ± 3.61	<0.0001
	Epicoccum nigrum TAMU497	31.54 ± 3.71	<0.0001
	Alternaria eichorniae TAMU452	29.85 ± 2.37	<0.0001
	Chaetomium globosum TAMU560	28.94 ± 3.24	<0.0001
Round 4	Control	42.18 ± 4.32	–
	Chaetomium globosum TAMU117	52.32 ± 4.64	0.20
	Chaetomium piluliferum TAMU251	48.23 ± 3.30	0.76
	Beauveria bassiana	39.49 ± 3.08	1.00
	Epicoccum nigrum TAMU58	38.41 ± 2.95	0.98
	Alternaria eichorniae TAMU129	35.54 ± 4.51	0.67
	Chaetomium coarctatum TAMU356	31.50 ± 3.36	0.16
	Chaetomium globosum TAMU559	28.57 ± 1.98	0.035
	Gibellulopsis piscis TAMU488	24.30 ± 1.58	0.0020
	Epicoccum nigrum TAMU100	21.19 ± 2.88	0.0002
	Epicoccum nigrum TAMU128	19.43 ± 2.66	<0.0001
Round 5	Control	53.08 ± 4.27	–
	Alternaria eichorniae TAMU179	74.84 ± 6.00	0.018
	Alternaria eichorniae TAMU416	74.42 ± 5.62	0.021
	Cladosporium tenuissimum TAMU494	70.22 ± 5.09	0.10
	Epicoccum nigrum TAMU536	58.21 ± 5.61	0.99
	Alternaria eichorniae TAMU529	57.99 ± 5.38	1.00
	Filobasidiella sp. TAMU514	51.61 ± 5.89	1.00
	Cladosporium sp. TAMU244	50.35 ± 3.57	1.00
	Epicoccum nigrum TAMU32	45.99 ± 4.80	0.92
	Chaetomium sp. TAMU110	32.61 ± 3.56	0.030
	Cladosporium cladosporioides TAMU474	31.86 ± 3.56	0.022

(*Continued on next page*)

[a] Rounds 1 to 6 are the initial tests of all 56 isolates. Rounds 7 and 8 are the follow-up replicate tests of only the best performing isolates in the initial assays. Each bioassay had its own corresponding untreated control for comparison. Pairwise statistical differences between treatments and the control group were compared using Dunnett's test ($\alpha = 0.05$).

The reductions in nematode galling observed in the initial series of assays were highly repeatable. A total of 22 isolates with the strongest negative effects based on P values of less than 0.05 in pairwise comparisons in the initial bioassays were retested in replicate follow-up bioassays in rounds 7 and 8 (Table 1). All of the retested strains reduced root galling in both the initial and follow-up assays (Fig. 2). Significant overall effects of fungal treatments on nematode gall numbers were found in both of the follow-up retesting rounds (round 7: $F_{11,\ 168} = 16.75$, $P < 0.0001$; and round 8: $F_{11,\ 168} = 17.38$, $P < 0.0001$). In pairwise comparisons, 21 of the 22 (95%) retested strains significantly reduced root-knot nematode galling across both replicate trials (Fig. 2; Table 1). Although not strictly statistically significant at the $\alpha = 0.05$ level, the negative effect of *Chaetomium globosum* strain 559 when it was retested was nearly significant at $P = 0.056$.

A taxonomic summary of the observed negative and positive effects on nematode galling grouped by genera of fungi tested is provided in Table 2.

TABLE 1 (*Continued from previous page*)

Bioassay	Fungal seed treatment	Mean ± SE	*P* value
Round 6	Control	57.47 ± 3.25	–
	Cladosporium antropophilum TAMU201	76.87 ± 8.38	0.13
	Cladosporium sp. TAMU190	63.82 ± 7.13	1.00
	Davidiella tassiana TAMU169	58.46 ± 6.34	1.00
	Cladosporium cladosporioides TAMU193	54.84 ± 8.24	1.00
	Chaetomium globosum TAMU355	48.91 ± 4.72	0.95
	Chaetomium sp. TAMU317	46.16 ± 3.98	0.75
	Cladosporium sp. TAMU415	45.99 ± 2.96	0.75
	Cladosporium herbarum TAMU565	44.70 ± 6.76	0.60
	Cladosporium cladosporioides TAMU517	44.65 ± 4.36	0.60
	Gibellulopsis sp. TAMU508	44.60 ± 4.17	0.60
	Fusicoccum sp. TAMU340	43.40 ± 5.62	0.48
	Chaetomium sp. TAMU353	42.75 ± 4.36	0.42
	Penicillium citrinum TAMU413	40.24 ± 5.52	0.24
	Cladosporium sp. TAMU501	34.34 ± 3.46	0.038
	Purpureocillium lavendulum TAMU424	33.60 ± 5.41	0.029
Repeats round 7	Control	44.49 ± 3.88	–
	Chaetomium globosum TAMU559	34.86 ± 2.08	0.056
	Gibellulopsis piscis TAMU488	33.86 ± 1.93	0.026
	Epicoccum nigrum TAMU497	30.26 ± 2.28	0.0008
	Cladosporium antropophilum TAMU249	28.69 ± 2.54	0.0001
	Epicoccum nigrum TAMU100	22.49 ± 2.59	<0.0001
	Epicoccum nigrum TAMU194	22.35 ± 2.63	<0.0001
	Diaporthe sp. TAMU137	18.82 ± 2.70	<0.0001
	Cladosporium sp. TAMU463	16.27 ± 2.05	<0.0001
	Epicoccum nigrum TAMU128	13.85 ± 1.95	<0.0001
	Chaetomium globosum TAMU560	12.79 ± 2.89	<0.0001
	Alternaria eichorniae TAMU452	12.55 ± 1.62	<0.0001
Repeats round 8	Control	47.03 ± 3.57	–
	Cladosporium sp. TAMU501	31.37 ± 2.70	<0.0001
	Epicoccum nigrum TAMU125	27.00 ± 2.16	<0.0001
	Epicoccum nigrum TAMU131	22.25 ± 2.43	<0.0001
	Purpureocillium lavendulum TAMU424	22.03 ± 3.14	<0.0001
	Cladosporium cladosporioides TAMU474	19.41 ± 1.83	<0.0001
	Alternaria eichorniae TAMU53	18.66 ± 2.39	<0.0001
	Alternaria eichorniae TAMU87	17.60 ± 1.94	<0.0001
	Purpureocillium lavendulum TAMU239	15.45 ± 1.64	<0.0001
	Chaetomium sp. TAMU110	14.44 ± 1.31	<0.0001
	Chaetomium coarctatum TAMU333	14.40 ± 1.55	<0.0001
	Chaetomium globosum TAMU554	14.14 ± 1.57	<0.0001

DISCUSSION

Our results indicate that a large proportion of the fungi found to occur naturally in commercial cotton as foliar endophytes are capable of reducing root-knot nematode root gall formation when inoculated back to the plant as a seed treatment. Importantly, this effect was highly repeatable, with 95% of the isolates that were selected for retesting based on their performance in the first assay exhibiting a significant reduction in galling in a follow-up replicate assay.

Although all but one of the fungi evaluated here were originally isolated from cotton as foliar endophytes, endophytic colonization following reinoculation as a seed treatment was not assessed in this

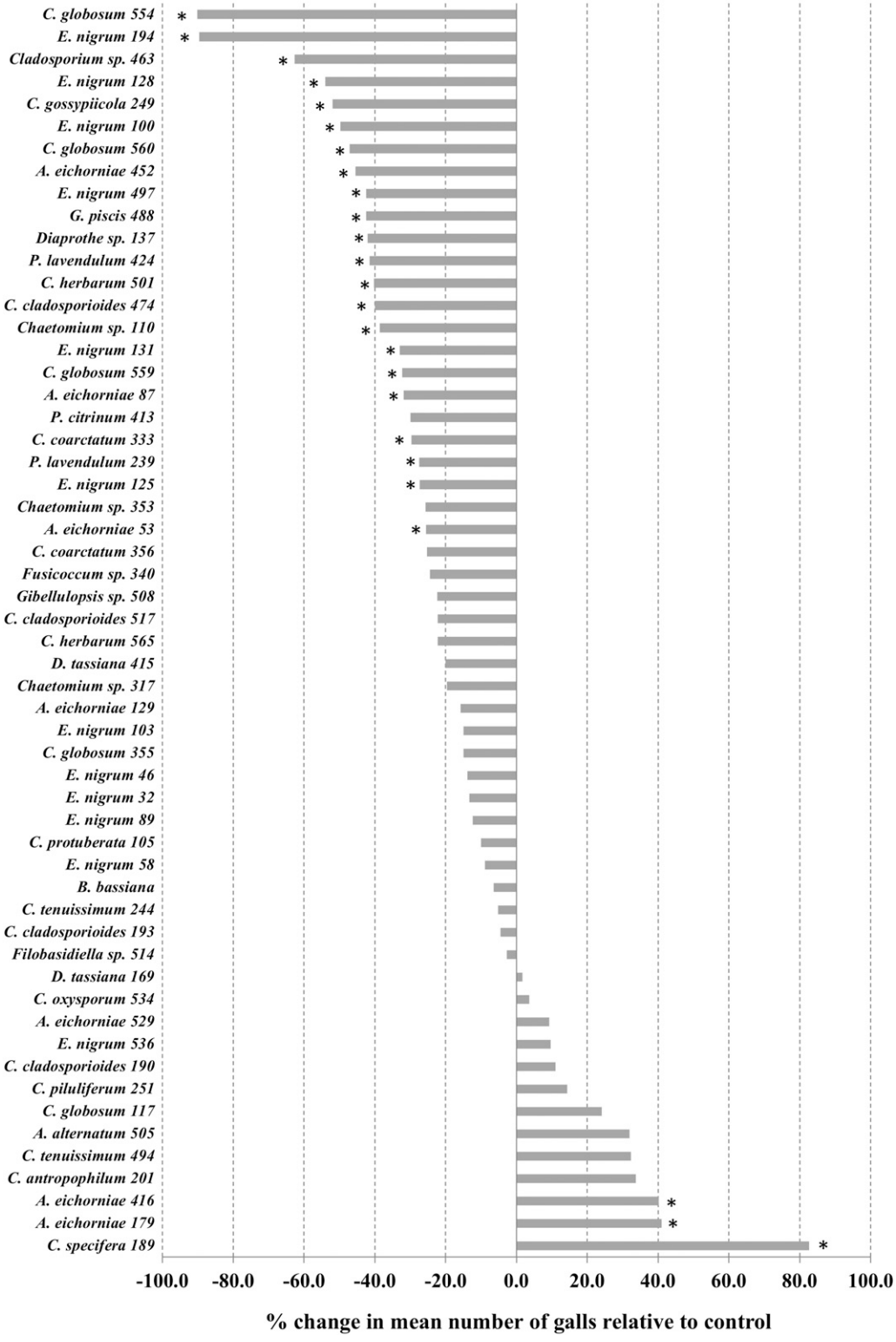

Fig. 1. Treatment of cotton seeds with fungi originally isolated as foliar facultative endophytes can negatively affect root-knot nematode galling of seedlings. Bars represent the percentage of change in mean number of galls relative to the untreated control treatment in the initial bioassays (rounds 1 to 6). Symbol on each bar indicates a significant difference in number of root galls from the control treatment, $^{*}P < 0.05$.

study. As such, we cannot distinguish at this time between the non-mutually exclusive possibilities of endophytic, epiphytic, or rhizospheric effects as causal mechanisms underlying the observed reductions in nematode galling. Using similar seed treatment inoculation protocols and experimental design to distinguish between endophytic, epiphytic, and rhizospheric effects, Zhou et al. (2016) and Zhou et al. (2018) concluded that the negative effects on root-knot nematodes of two other cotton-derived fungal endophytes, *Chaetomium globosum* TAMU520 and *Phialemonium inflatum* TAMU490, were due to their effects as endophytes within the plant. Further study is required to determine whether the activity of the fungi tested here is definitively associated with endophytism and will prove insightful in guiding follow-up hypothesis tests about the mechanisms underlying their observed negative effects on nematodes.

Importantly, taxonomic group was not a reliable predictor of the effects of the fungi on nematode galling. Among the strains from 14 fungal genera that we evaluated, 21 isolates from seven genera including *Alternaria*, *Chaetomium*, *Cladosporium*, *Diaporthe*, *Epicoccum*, *Gibellulopsis*, and *Purpureocillium*, consistently reduced root-knot nematode gall formation by root-knot nematodes across replicated assays (Figs. 1 and 2). In contrast, there were three isolates from the genera *Alternaria* and *Curvularia* that significantly increased root-knot nematode galling in treated plants (Fig. 1; Table 2). While some isolates of *Alternaria eichorniae* were among those that consistently reduced nematode galling, two other *A. eichorniae* isolates had the opposite effect of significantly increasing the number of galls. This example clearly illustrates the importance of strain specificity in affecting the outcome of fungus–plant–nematode interactions.

Our results provide multiple examples of previously unrecognized plant–fungal–nematode interactions. Here we highlight several strains that exhibited robust negative effects on root-knot nematode galling (Fig. 2). Although the nematicidal activity of secondary compounds from *Alternaria* species has been explored (Lou et al. 2016), our study is the first to illustrate the potential ecological significance of specific *Alternaria* strains on nematodes in planta using live plant assays, with both positive and negative effects on root-knot nematode galling. Similarly, some *Cladosporium* strains have been shown to produce secondary metabolites with nematicidal or insecticidal properties (Qureshi et al. 2012; Singh et al. 2016), but our results provide the first examples of negative in planta effects of multiple strains on nematodes. The genus *Diaporthe* (asexual state *Phomopsis*) includes endophytic species (Udayanga et al. 2011) that can produce metabolites and have in planta effects that are deterrent to insect herbivory (Claydon et al. 1985; McGee 2002), but we could find no prior examples of in planta effects on nematodes. The same is true for *Epicoccum* fungi whose filtrates have been shown to have antinematode activity (Meyer et al. 2004), but had not previously been tested in planta. *Gibellulopsis* fungi are largely considered plant pathogens (Zare et al. 2007) and have been reported as asymptomatic

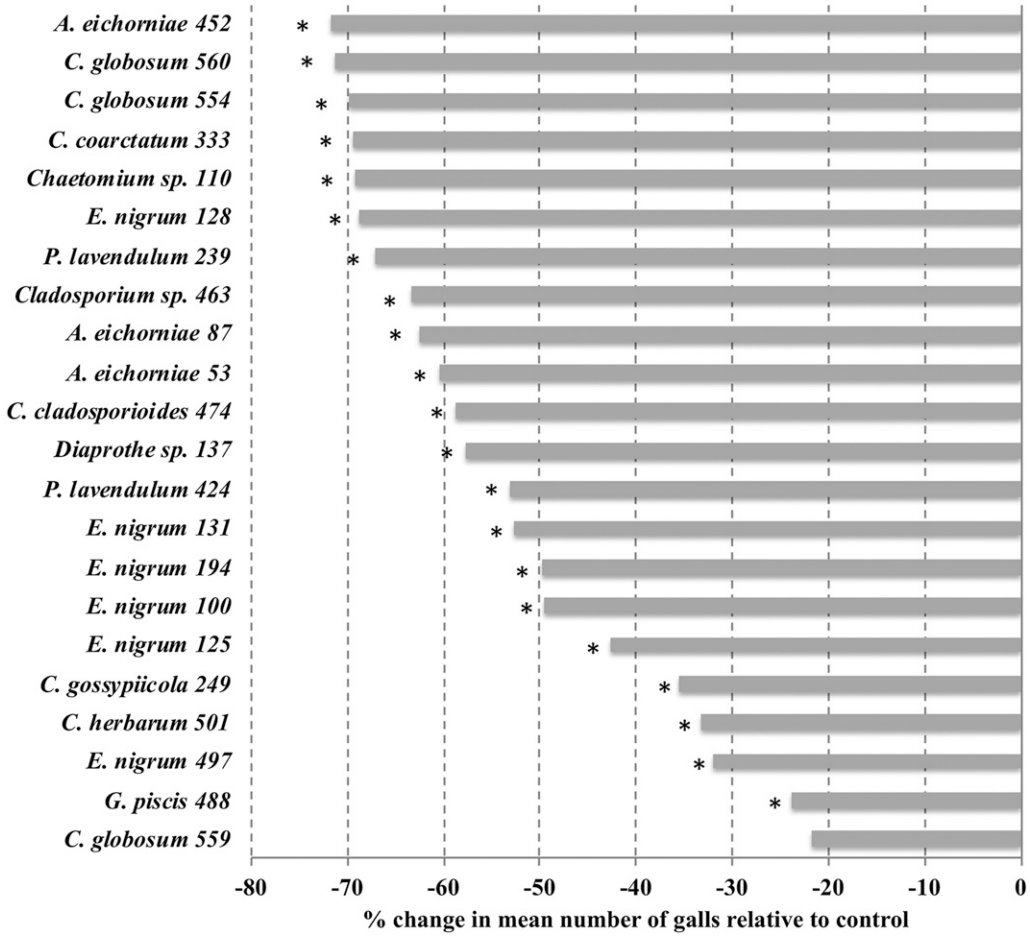

Fig. 2. Negative effects of cotton seed treatment with fungi originally isolated as foliar facultative endophytes on root-knot nematode galling was highly repeatable across independent assays. Bars represent the percentage of change in mean number of galls relative to the untreated control treatment in the follow-up replicate bioassays (rounds 7 and 8). Symbol on each bar indicates a significant difference in number of root galls from the control treatment, $^*P < 0.05$.

endophytes (Khalmuratova et al. 2015), but previous reports of effects on nematodes are lacking.

Our finding of several *Chaetomium* and one *Purpureocillium* isolate with repeatable negative effects on nematodes is consistent with previous studies that have also demonstrated similar effects using whole plant assays. *Chaetomium* strains have been demonstrated to colonize plant tissues endophytically, with some exhibiting antibiosis against nematodes or insects (Gange et al. 2012; Yan et al. 2011; Zhou et al. 2018). The species *Chaetomium globosum* in particular has been frequently assessed for its antagonistic effects against plant-parasitic nematodes (Hu et al. 2012; Meyer et al. 2004; Nitao et al. 2002). Although *Purpureocillium* fungi have been found as endophytes in plants other than cotton (Gong et al. 2017), it is important to note that *Purpureocillium lilacinum* (formerly *Paecilomyces lilacinus*) is a well-known nematode egg pathogen that has been commercialized as a biological control agent for root-knot nematode management and is assumed to act against nematodes in the rhizosphere rather than as an endophyte (Brand et al. 2004; Holland et al. 2003; Kalele et al. 2007).

In conclusion, we have shown that the naturally occurring cotton fungal phytobiome harbors a diverse array of fungi with the potential to negatively affect the performance of root-knot nematodes. These findings help establish a rich pool of candidate fungi for further evaluation as novel biological control agents against root-knot nematodes in cotton and other plants. Several key questions about the mechanistic basis of these interactions will require continued research to elucidate the roles of endophytism versus rhizospheric effects (Zhou et al. 2016; 2018), and the effects of fungal secondary metabolites versus elicitation of plant induced systemic responses (Kusari et al. 2012; Martinuz et al. 2013; Sikora et al. 2008). Plant–fungal interactions can also be affected by variation in specific genotype–genotype combinations of the plant and fungus (Saikkonen et al. 2004). The importance of variation in fungal strains was clearly apparent in our study, but we did not test for the effects of variation in plant genotype. The idea that variation in fungal genotypes, plant genotypes and local environments all interact to affect ecological interactions is well known in studies of plant-associated fungi and often referred to as context-dependency (Davitt et al. 2011; Hartley and Gange 2009). Future studies to better understand fungi–plant–nematode interactions, environmental effects, and the resulting consequences on plant performance will provide insights that can be used to inform the further development of novel ecologically based tools for nematode management.

TABLE 2
A summary of all tested genera of fungi originally isolated from cotton as foliar facultative endophytes and their effects on root-knot nematode gall production[a]

Fungal genera	↓Galling	No effect	↑Galling	Subtotal
Acremonium	0	1	0	1
Alternaria	3	2	2	7
Beauveria	0	1	0	1
Chaetomium	5[b]	6	0	11
Cladosporium	4	9	0	13
Curvularia	0	1	1	2
Davidiella	0	1	0	1
Diaporthe	1	0	0	1
Epicoccum	6	6	0	12
Gibellulopsis	1	1	0	2
Filobasidiella	0	1	0	1
Fusicoccum	0	1	0	1
Penicillium	0	1	0	1
Purpureocillium	2	0	0	2
Total	22[b]	31	3	56

[a] Numbers in each column indicate the number of isolates of each genus tested that either significantly decreased (↓) root galling, had no significant effect, or significantly increased (↑) root galling of seedlings to root-knot nematodes when inoculated back to cotton as a seed treatment.

[b] Effect of *Chaetomium globosum* TAMU559 isolate on root galling was negative in two replicate trials, but the reduction was statistically significant in only one trial. A total of 21 fungal isolates resulted in significant reductions in nematode gall production across both replicate greenhouse trials (Fig. 2).

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