

Moths that Vector a Plant Pathogen also Transport Endophytic Fungi and Mycoparasitic Antagonists

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Abstract *Claviceps paspali*, a common fungal pathogen of *Paspalum* grasses, attracts moth vectors by producing sugary exudates in the grass florets it infects. These exudates also support mycoparasitic *Fusarium* species that may negatively influence *C. paspali* fitness. We examined the potential for moths on which *C. paspali* depends to also transmit mycoparasitic *Fusarium* and fungal endophytes, which inhabit asymptomatic plant tissue and may influence host susceptibility to pathogens. We quantified infections by *C. paspali*, *Fusarium* spp., and endophytic fungi associated with *Paspalum* spp. at focal sites in the southeastern USA and used data from the nuclear internal transcribed spacer (ITS rDNA) to compare communities of plant-associated and moth-borne fungi. ITS sequences of moth-borne fungi were identical to reference sequences of mycoparasitic *Fusarium heterosporum* and to three distinct endophytic fungi isolated from *Paspalum* species. Our results demonstrate an unexpected overlap of fungal communities between disparate locations and among plant

species and plant tissues, and suggest an unexpected role of moths, which vector a plant pathogen, to transmit other guilds of fungi. In turn, the potential for insects to transmit plant pathogens as well as mycoparasites and endophytic fungi suggests complex interactions underlying a commonly observed grass–pathogen system.

Introduction

Interactions among plants, pathogenic fungi, and insect vectors occur in the context of other microbial species that may alter the nature and outcomes of plant–pathogen interactions [8, 19, 22, 64]. For example, mycoparasitic fungi occurring on plant surfaces may attack pathogens directly (e.g. [22]), and infection of above-ground tissues by asymptomatic fungi (endophytes, *sensu* [9]) may directly or indirectly inhibit colonization of plant tissues by pathogens [8, 19, 64]. Together, these organisms have the potential to affect the ecological and evolutionary trajectories of host plants, vectors, and pathogens, as well as their multipartite interactions. However, mycoparasites and endophytes are rarely studied in the context of plant–pathogen–vector systems.

The fungal pathogen *Claviceps paspali* (Clavicipitaceae, Hypocreales, Ascomycota) frequently infects grasses in the genus *Paspalum* in the southern United States [3]. At the beginning of the infection cycle, sexual spores (ascospores) of *C. paspali* are spread by wind [1, 45, 46] and possibly insects [14] to uninfected hosts. The fungus infects ovaries after colonizing styles and subsequently prevents seed production in infected florets [14, 45]. During the growing season, asexual spores (conidia) of *C. paspali* are borne in sugar-rich exudates (honeydew) of infected florets, which attract and feed diverse insects [11, 33, 42, 43]. Moths

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(Lepidoptera) attracted by these exudates transfer conidia of *Claviceps* from infected to uninfected grass stalks while foraging [51, 60]. Infection rates peak in August or September in the southeastern USA (Feldman, personal observation), due to movement of conidia by wind, water, and insects [10, 51, 60]. At the end of the growing season, *C. paspali* produces sclerotia or ergots [45], which are toxic or sickening to livestock [20] and remain dormant on or in soil until the following summer.

The honeydew resulting from *C. paspali* infections is often colonized by the mycoparasite *Fusarium heterosporum* (Hypocreales, Ascomycota; [18]), which reproduces asexually on infected inflorescences of *Paspalum notatum* and *Paspalum dilatatum* [21]. Although Raybould et al. [63] found no significant effects of *F. heterosporum* on survival and sclerotia production in the ergot fungus *Claviceps purpurea*, Cunfer [21] determined that *F. heterosporum* can inhibit sclerotia formation in *C. paspali*. Moths such as *Spodoptera ornithogali* (Noctuidae) and *Utetheisa bella* (Arctiidae; Fig. 1) visit inflorescences of *Paspalum* spp. infected by both *C. paspali* and *F. heterosporum* in pastures in the southeastern USA (Feldman, personal observation). Although insects transmit several other species of *Fusarium* among plants (e.g., [32, 37, 49]), the mechanism by which *F.*



Figure 1 *Utetheisa bella* (Lepidoptera, Arctiidae) feeding on exudates from a *Claviceps paspali* infection of a *Paspalum notatum* floret (the moth's proboscis is yellow). Exudates are in turn infected by *Fusarium heterosporum*. The white arrow points to the orange sporodochium of *F. heterosporum* in the infected floret

heterosporum is transmitted among *Paspalum* inflorescences has not been identified. Similarly, folivorous insects often increase the incidence of endophytic fungi by damaging leaf tissue, which facilitates colonization [23–25, 30], but the role of non-herbivorous insects in transmitting endophytes has only rarely been investigated (see [15]).

Here, we characterize fungal communities associated with *Paspalum* spp. in four sites in the southeastern USA, focusing on fungi within *C. paspali*-infected florets, endophytic fungi from healthy foliage and stems of *Paspalum* spp., and fungi borne on the bodies of moths that visit infected *Paspalum* inflorescences. In addition to providing the first characterization of the floret, foliage, and stem fungal associates of economically and ecologically important *Paspalum* grasses, our study shows that moths visiting *C. paspali*-infected florets of *Paspalum* spp. also carry viable propagules of fungi with nuclear ribosomal internal transcribed spacer (ITS) sequences identical to mycoparasitic *F. heterosporum* and several endophytic fungi. The potential for insects to transmit mycoparasites and endophytes that may affect plant–pathogen–vector interactions suggests that complex species interactions underlie this common grass–pathogen system.

Materials and Methods

Study Species and Field Sites

The pantropical and warm-temperate genus *Paspalum* (Poaceae) comprises at least 400 species of annual and perennial grasses [59]. Eighty-three species are known from North America, including the invasive tropical perennials *P. dilatatum* (dallisgrass) and *P. notatum* (bahiagrass), and the native perennials *Paspalum floridanum* and *Paspalum laeve* [72]. *P. dilatatum* and *P. notatum* were introduced from South America in the late 1800s for forage and are now widely established in the southern United States (21 and 16 states, respectively; [48, 72]).

Claviceps (Clavicipitaceae, Ascomycota) contains 36 species that associate with Poaceae and, less frequently, Cyperaceae [39]. *C. paspali* was originally described in association with *P. dilatatum* and *P. laeve* in the southeastern United States [71], but its status as a native or introduced pathogen is unclear [2]. Infection of *Paspalum* florets by *C. paspali* leads to destruction of ovaries and replacement with sclerotia that contain potent mycotoxins responsible for ergotism in livestock [20, 69].

Fusarium refers to the asexual states of several genera in the Hypocreales (Ascomycota), many of which are plant pathogens and endophytes. *Fusarium* contains 70 recognized species [44], although molecular evidence suggests many currently recognized species harbor numerous cryptic

phylogenetic species [55, 56]. The cosmopolitan species *F. heterosporum* was described by Nees in 1832 [12], and has been found in soils and in association with diverse plants from tropical to subantarctic sites [35, 65]. *F. heterosporum* also has been recorded in association with several *Claviceps* species, and with head blight of grasses [5, 44]. A second *Claviceps*-associated species, *Fusarium gramineum*, is recognized as distinct by some authors (e.g. [29]), though most taxonomists consider these species to be synonymous (see [16, 44]). *F. heterosporum* has the potential to decrease sclerotia production in *C. paspali* [21], and has been explored as a biocontrol agent of this plant pathogen.

We examined fungal communities associated with *Paspalum* spp. at four open pastures and wood edges in the southeastern USA. Stowe Farm, near Gainesville, FL (SF-FL; 29°37'45" N, 82°17'35" W) consists of a hammock forest edge with abundant *P. notatum*. Couch Farm, near Durham, NC (CF-NC; 36°00'50" N, 79°00'10" W) consists of small pastures at the edge of deciduous forest with abundant *P. dilatatum* and a small population of *P. laeve*. Pickett Road, near Durham, NC (PR-NC; 35°58'33" N, 78°59'00" W) consists of forest-edge pastures dominated by *P. dilatatum* and contains small populations of *P. notatum* and *P. floridanum*. Biology Lawn, in Durham, NC (BL-NC; 36°00'12" N, 78°56'33" W) is an unmowed lawn with both *P. dilatatum* and *P. notatum* on the campus of Duke University.

Infection Frequencies of *Claviceps* and *Fusarium*

We quantified symptomatic infections by *C. paspali* and *F. heterosporum* in three sites when symptoms of *C. paspali* infection were most visible (August 2004, SF-FL; September

2004, CF-NC and PR-NC). At each of 20 points along a 10–20 m transect, we examined the ten nearest inflorescences of the most common *Paspalum* species (*P. notatum* at SF-FL and *P. dilatatum* at PR-NC and CF-NC) for symptomatic infections by *C. paspali* and *F. heterosporum* and calculated the proportion of inflorescences infected at each site by averaging across the 20 points. Data were arcsine square-root transformed and ANOVA was used to compare infection frequencies among sites.

Fungal Community of Infected *Paspalum* Florets

We extracted total genomic DNA from symptomatic *Paspalum* florets and directly amplified the nuclear ribosomal internal transcribed spacer (ITS rDNA) region using fungus-specific primers (see below). We examined florets of *P. notatum*, *P. dilatatum*, *P. laeve*, and *P. floridanum* that were symptomatic of infection by *C. paspali* alone ($N=13$ florets; Table 1), and florets of *P. notatum*, *P. dilatatum*, and *P. laeve* that were symptomatic of infection by both *C. paspali* and *F. heterosporum* ($N=14$ florets; Table 1). To more carefully assess richness of fungi associated with florets, amplicons from *P. notatum* were cloned and sequenced (see below). To determine the degree to which culture-based methods alone would capture diversity, we examined two florets of *P. notatum* from SF-FL. One floret was streaked directly onto 2% malt extract agar (MEA). The second was agitated in 10 ml of sterile water and the resulting suspension streaked on 2% MEA at both full concentration and following a 10× dilution.

The 103 cultures obtained in this study were vouchered as mycelial samples in sterile water and have been deposited at

Table 1 Number of florets, tissue segments, and moths sampled for fungi from the four study sites

Site	Grass species	Infection status	Florets sampled	Tissue segments plated (stems, leaves)	Moths sampled (individuals, species)
SF-FL	<i>P. notatum</i>	Uninfected	–	–	–
		<i>C. paspali</i>	4	12, 12	4, 4
		<i>F. heterosporum</i>	4	–	–
CF-NC	<i>P. dilatatum</i>	Uninfected	–	–	–
		<i>C. paspali</i>	3	6, 6	4, 3
		<i>F. heterosporum</i>	5	6, 6	2, 2
	<i>P. laeve</i>	Uninfected	–	15, 15	–
		<i>C. paspali</i>	1	15, 15	–
		<i>F. heterosporum</i>	5	–	–
PR-NC	<i>Digitaria</i> sp.	Uninfected	–	–	1, 1
		Uninfected	–	6, 6	–
	<i>P. notatum</i>	Uninfected	–	6, 6	–
		<i>C. paspali</i>	4	6, 6	–
<i>P. floridanum</i>	Possible <i>C. paspali</i> symptoms	1	12, 12	–	
	Uninfected	–	–	–	
BL-NC	<i>P. notatum</i>	Uninfected	–	6, 6	–
	<i>P. dilatatum</i>	Uninfected	–	6, 6	–

Sites are Stowe Farm, Gainesville, FL (SF-FL), collected on 18 August 2004; and two sites near and within Durham, NC: Pickett Road (PR-NC), Couch Farm (CF-NC), and Biology Lawn (BL-NC), collected on 19 September 2004

the Robert L. Gilbertson Mycological Herbarium, University of Arizona (ARIZ).

Fungal Endophytes

We examined two to three inflorescence stalks (hereafter, stems) and associated foliage per species per site for fungal endophytes (Table 1). Plant material was surface-sterilized using sequential immersion in 95% ethanol (10 s), 0.5% NaOCl (2 min), and 70% ethanol (2 min) following Arnold et al. [7]. Six small sections (ca. 2 mm²) of each leaf or stem were placed onto 2% MEA (12–18 segments per tissue per plant; 192 total; Table 1) and incubated at room temperature for up to 2 weeks. Emergent fungi were isolated into axenic culture on 2% MEA.

Fungal Isolates from Moths

We captured representative nocturnal moths feeding on exudates from *C. paspali* on *Paspalum* spp. in small fields and wood edges at SF-FL and CF-NC, where *F. heterosporum* infections were common (Table 1). Moths were frozen for ≤48 h at –20°C within 1 h of capture. To sample viable fungi from moth bodies, we pressed moth legs and proboscises into 2% MEA in sterile Petri dishes (two replicates per moth), incubated plates at room temperature for up to 96 h, and transferred all unique fungi into axenic culture on 2% MEA.

DNA Extraction, PCR, and Sequencing

We extracted total genomic DNA from fungal isolates and grass florets using a SDS-phenol-chloroform extraction protocol [75]. The nuclear ribosomal internal transcribed spacer region and 5.8S gene (ITS rDNA) was amplified using the universal primer ITS4 [74] and the fungal-specific primer ITS1F [28] with the following PCR protocol: initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, 50°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. Each 25 µl PCR reaction consisted of 25 µg BSA, 0.625 U *Taq* DNA polymerase (ABgene, Rochester, NY), 1.5 mM MgCl₂, dNTPs (0.2 mM each), primers (0.5 µM each), and PCR buffer.

Amplicons obtained directly from a representative floret of *P. notatum* were cloned using the Topo-TA cloning kit (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The maximum recommended incubation times were used for each step with 4 µl of PCR product. Colonies were screened for inserts by adding whole cells directly to PCR reactions and amplifying the inserts with PCR using M13 F and R primers (Invitrogen) and a “colony PCR” protocol: initial denaturation of 94°C

for 10 min, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, with a final extension of 72°C for 10 min.

PCR products were purified by affinity chromatography using Qiaquick spin columns (Qiagen, Valencia, CA, USA). Cycle sequencing was performed with Big Dye chemistry version 3.1 (Applied Biosystems, Foster City, CA, USA) and sequences were determined with an ABI3700 automated sequencer (Applied Biosystems). All sequence data obtained in the present study have been submitted to the National Center for Biotechnology Information's GenBank database under accession numbers EU680480–EU680567.

Sequence Analyses

Sequences were assembled using Sequencher 4.0 (Gene Codes, Ann Arbor, MI, USA) and were identified at higher taxonomic levels using BLAST searches of GenBank [6]. Because five of 13 clone amplicons were identical, and seven others differed from these by three or fewer bases, these 12 sequences were combined into a consensus sequence to control for potential insertion of PCR products with errors into cloning vectors. Sequence comparisons were made using MacClade [47]. Fungal community overlap between plant- and moth-associated fungi and between plant tissue types, grass species, and locations, was assessed by clustering identical ITS sequences and treating them as operational taxonomic units (rather than as species). Results did not differ substantially when lower similarity thresholds were used (see supplementary information; Figure S1a,b).

Results

At the height of the infection season, 50.5–81.5% of *Paspalum* inflorescences were infected with *C. paspali* (Table 2). Symptomatic *F. heterosporum* infections were relatively uncommon, occurring in fewer than 20% of inflorescences at SF-FL and CF-NC, and in no inflorescences at PR-NC (Table 2). Infection frequency varied across sites for *C. paspali* ($F=13.0$, $df=57$, $P<0.001$) and *F. heterosporum*

Table 2 Percent of *Paspalum* inflorescences with symptoms of infection by *Claviceps paspali* and *Fusarium heterosporum* in three sites

Site	Dominant <i>Paspalum</i> species	% <i>C. paspali</i> infection (95% confidence interval)	% <i>Fusarium heterosporum</i> infection (95% confidence interval)
SF-FL	<i>P. notatum</i>	81.5 (75.7–86.5)	16 (11.4–21.5)
CF-NC	<i>P. dilatatum</i>	58 (51.1–64.7)	19.5 (14.4–25.4)
PR-NC	<i>P. dilatatum</i>	50.5 (43.5–57.4)	–

($F=19.8$, $df=57$, $P<0.001$). Although *Fusarium* was only detected on *C. paspali* infected florets, the frequency of *F. heterosporum* symptoms was not significantly correlated with the frequency of symptomatic *C. paspali* infections at the three sites (data not shown).

Fungal sequences confirmed the presence of *Fusarium* on florets infected by *C. paspali*. We obtained 12 ITS sequences from *Paspalum* florets (10 from direct PCR and two unique clone sequences). Four identical sequences showed highest BLAST affinity for *Fusarium lateritium* AF310979 (Table S1; supplemental material). However, when these sequences were compared to the partial ITS sequence from *F. heterosporum* in GenBank (AF111064), similarities were consistently higher (100%) than with the homologous portion of *F. lateritium* (98.5%). These sequences were obtained from florets visibly infected with fungi morphologically consistent with *Fusarium* infection.

Additional fungi were present in florets, including *Cladosporium* sp. (Mycosphaerellaceae) and *Didymella* (Pleosporales; Table S1). Although visible *Fusarium* infections were common among *P. dilatatum* at CF-NC, we did not recover *Fusarium* strains from *P. laeve* at that site; representative fungi included *Ampelomyces* (Leptosphaeriaceae, Pleosporales) and *Cephalosporium* (Hypocreales; Table S1).

We recovered 45 endophytic isolates from 192 leaf and stem segments and sequenced the ITS of 30 representative isolates (Table S2; supplemental material). Endophytes were frequently recovered from plants with and without visible infection by *C. paspali* (Table 3). All endophytes were members of the Ascomycota except two stem endophytes from *P. notatum* (highest BLAST affinity for *Stereum*, Basidiomycota). The majority of endophytes represented the Pleosporales: *Ampelomyces* (stems only; *P. laeve* at CF-NC and *P. notatum* at SF-FL), *Curvularia*

(leaves only; *P. dilatatum* and *P. notatum* at PR-NC and BL-NC), and *Bipolaris* (leaves only; *P. notatum* at PR-NC) were the most commonly recovered genera.

We recovered culturable fungi from the bodies of eight moth species representing four individuals captured on *P. notatum* at SF-FL, six on *P. dilatatum* at CF-NC, and one resting on *Digitaria* sp. within the *P. dilatatum* population at CF-NC. Thirty-five isolates from moth bodies were sequenced for ITS (Table S3; supplemental material). With the exception of one isolate matching *Umbelopsis* (Zygomycota), all are Ascomycota. Most fungi recovered from moths are Pleosporales, including *Ampelomyces*, *Curvularia*, *Phaeosphaeria* (CF-NC), *Coniothyrium* (CF-NC and SF-FL), and *Paraphaeosphaeria* (SF-FL). Eight isolates from the Hypocreales were also recovered, including three *Fusarium* isolates (CF-NC) and five isolates of *Trichoderma* or *Hypocrea* (SF-FL). Five isolates from the Mycosphaerellaceae matched *Cladosporium* (SF-FL and CF-NC).

Fungal Community Overlap

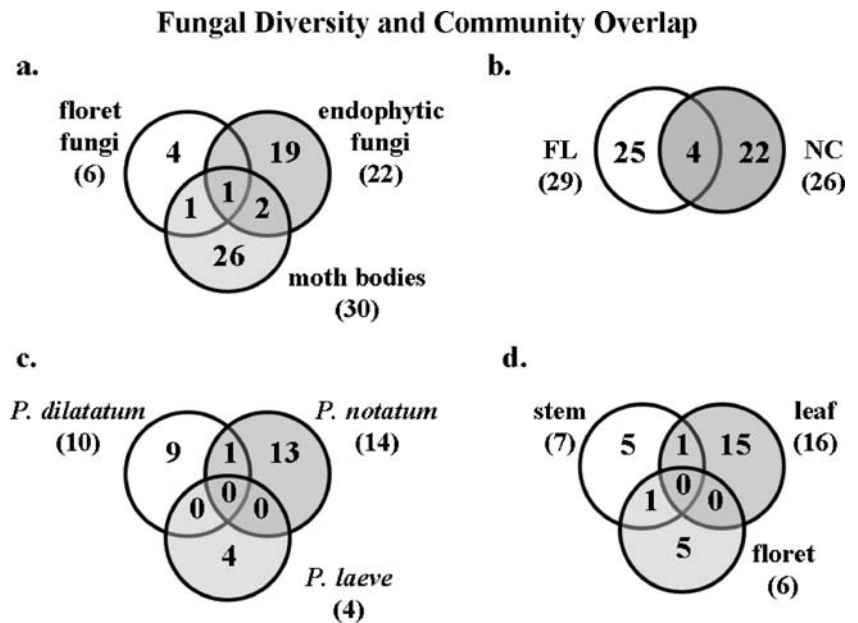
Over the entire dataset (88 sequences), we found 53 different sequence types, 42 of which occurred only once in the data set (singletons). Of the 11 non-singleton groups, four were represented by both plant-associated and moth-borne fungi (Fig. 2a). These included *F. heterosporum*, represented by nine sequences (group 1); an unidentified hypocrealean taxon, represented by two sequences (group 2); a group of pleosporealean fungi, represented by four sequences (group 3); and a *Cladosporium* species, represented by three isolates (group 4) (Table 4). The *F. heterosporum* group (group 1) included the consensus sequence from floret clones, three cultures from *P. notatum* florets (SF-FL), and one fungus cultured from a moth captured near infected *P. dilatatum* (CF-NC). No endophyte sequences matched *F. heterosporum*. The Hypocreales group (group 2) included a leaf endophyte from *P. notatum* (SF-FL) and a moth-borne fungus (CF-NC). Members in the Pleosporales group (group 3) included one endophyte from *P. laeve* and two moth-borne fungi (CF-NC). Representatives of the *Cladosporium* group (group 4) included one floret isolate and one stem endophyte from *P. notatum* and one moth-borne fungus (SF-FL) (Table 4).

In addition to overlap between moth-borne and plant-associated fungi, our results indicated some overlap of fungal communities among study sites, *Paspalum* species, and host tissue types (Fig. 2b–d). Groups 1 and 2 were recovered from *Paspalum* plants in FL and from moths in NC. Group 5 (*Curvularia* sp. A) was recovered from different *Paspalum* species in FL and NC (*P. notatum* at SF-FL and PR-NC; *P. dilatatum* at PR-NC). Group 6 (*Cladosporium* sp. B) was recovered from moths in FL and

Table 3 Isolation frequency for endophytes from stems and leaves of *Paspalum* spp. as a function of site, tissue type, and co-infection by *C. paspali*

Grass species	<i>C. paspali</i> infection status	Site	Percentage of tissue segments with cultivable endophytic fungi	
			Stems	Leaves
<i>P. notatum</i>	Uninfected	PR-NC	0	33.3
	Uninfected	BL-NC	0	16.7
	Infected	SF-FL	83.3	58.3
<i>P. dilatatum</i>	Uninfected	PR-NC	0	16.7
	Uninfected	BL-NC	0	66.7
	Infected	PR-NC	50	16.7
<i>P. laeve</i>	Uninfected	CF-NC	0	0
	Infected	CF-NC	13.3	0
<i>P. floridanum</i>	Uninfected	CF-NC	33.3	0
	Uninfected	PR-NC	0	8.3

Figure 2 Venn diagrams depicting overlap between fungal communities, based on 100% ITS sequence similarity. **a** Overlap between plant-associated fungi (floret fungi and endophytes) and fungi isolated from moth bodies. **b** Overlap between fungi from FL and fungi from NC. **c** Overlap between fungi in different *Paspalum* species. **d** Overlap between fungi in different plant tissues. In all cases, the total number of fungal groups for each category is in parentheses



NC. Two groups were recovered from different tissues of the same *Paspalum* species: group 4 from both stem and floret tissues of *P. notatum* (SF-FL; see above), as well as from a moth at the same site, and group 7 (*Stereum*) from both stem and leaf tissues of *P. notatum* (SF-FL) (Table 4).

Three other groups were restricted to one tissue type of a single *Paspalum* species at one site: group 8 (Pleosporales sp. B) from stems of *P. notatum* (SF-FL), group 9 (*Curvularia* sp. B) from *P. notatum* leaves (SF-FL), and group 10 (*Cephalosporium* sp.) from florets of *P. laeve* (CF-NC) (Table 4). The final group included three Hypocreales isolates from a moth at SF-FL (group 11).

Discussion

Many plant pathogens are vectored by herbivorous and nectar-feeding insects (e.g., [4, 17, 26, 32, 40, 41, 60, 66, 68]). Some plant-pathogenic fungi produce chemical attractants (volatiles) and food (sugars) that lead to spore transmission by insects to previously uninfected hosts (e.g., [27, 52–54, 62, 67]). Because plant pathogens can influence the quantity and quality of nutrients available in plant tissues and nectar, insect vectors with herbivorous or nectarivorous life stages are likely to be directly or indirectly affected by the fungi they transmit [50, 57].

Table 4 Groups based upon 100% ITS sequence similarity

Group number ^a	Top match in GenBank	Floret fungi	Endophytes		Moth-associated fungi	Smallest overlapping region ^b
			Leaf	Stem		
1	Fusarium heterosporum	4 ^c			1	481
2	Hypocreales sp. A		1		1	527
3	Pleosporales sp. A			1	3	461
4	Cladosporium sp. A	1		1	1	453
5	<i>Curvularia</i> sp. A		4			407
6	<i>Cladosporium</i> sp. B				2	543
7	<i>Stereum</i> sp.		1	1		605
8	Pleosporales sp. B			4		545
9	<i>Curvularia</i> sp. B		2			498
10	<i>Cephalosporium</i> sp.	4				462
11	Hypocreales sp. B				3	546

Entries in **bold** denote groups with both plant-associated and moth-associated fungi. Groups are ranked according to the order in which they are discussed in the text

^a Pleosporales sp. A and B and *Cladosporium* sp. A and B are >99% similar to each other. All other groups in this table are <98% similar to any others

^b Refers to the number of bases in the smallest overlapping region between any pair of sequences within a group

^c Includes three fungal cultures and the consensus sequence of 12 of 13 sequences from a single cloning reaction

Moths that visit *Paspalum* florets infected by *C. paspali* carry viable propagules of fungi with ITS sequences identical to mycoparasitic and endophytic fungi isolated from *Paspalum*. Although ITS data are of limited use in designating species boundaries in some groups of *Fusarium* (see [55, 56]), this marker provided a solid link between evident *F. heterosporum* infections on *C. paspali* florets and sterile cultures of that fungus obtained from spores on moth bodies. Overall, our data suggest that moths have the potential to act as dispersal vectors for several different guilds of fungi associated with these ecologically and economically important grasses, including the mycoparasite *F. heterosporum* and endophytic fungi representing two orders of Ascomycota. Overlap between groups from moth bodies in one location and plants in another suggests either that moths carry viable propagules over long distances (~900 km), or that these plant-associated fungi have broad geographic ranges. Although several moth species that visit *C. paspali*-infected grass florets are capable of traveling long distances [34, 38] comparable to the distance between sites in North Carolina and Florida, we cannot yet distinguish between these interpretations. For similar reasons, it is difficult to determine whether the limited overlap between endophytes and floret fungi indicates that these fungi represent distinct guilds. Greater community overlap may be detectable with additional sampling, as the diverse community of fungi recovered here included 42 sequence types that were found only once.

While conclusive evidence of moth dispersal of mycoparasites and endophytes requires tracking of individual fungal spores, our data indicate that successful transmission of these fungi by moths is plausible. Moths frequently visit more than one infected grass inflorescence per night (Feldman, personal observation), and the moth species sampled have been collected frequently on infected inflorescences of *Paspalum* spp., as well as at bait traps, in Florida [42]. In addition to carrying spores externally, moths may transmit spores that pass through their digestive tracts after having been ingested. For example, Prom and Lopez [61] showed that *Claviceps* spores ingested by *Helicoverpa zea* remain viable for up to 72 h after consumption, indicating the potential for long-distance dispersal.

The recovery of *Cladosporium* from *C. paspali*-infected florets and moth bodies raises the possibility that some endophytes may colonize tissues via *Paspalum* florets, and are transmitted by an insect vector. Under this scenario, moth attraction by *C. paspali* could positively impact endophyte transmission. This case differs from the well-described *Neotyphodium/Epichloë* system, in which insect vectors are attracted to and visit the fungus they transmit, but no additional fungi are known to act as facilitators [15, 19]. Our data suggest that several endophyte species associate with the same set of host plant species and insect

vectors. Endophytes may affect interactions between *C. paspali* and its insect vectors by competing with *C. paspali* for resources within the grass hosts or promoting host defense against the pathogen (e.g., see [8]). Moreover, some endophytes reduce plant seed set [58] thereby reducing the substrate available for seed pathogens like *C. paspali*.

Placing the *C. paspali*–*Paspalum* interaction into the ecologically realistic context of the fungal community [31], which includes mycoparasites and endophytes, highlights a potentially complex interplay of species underlying this plant–pathogen association. The *C. paspali*–moth interaction may be similar to pollination mutualisms, in that food (nectar) is traded for transportation of reproductive propagules (*C. paspali* spores). Moth transport of *Fusarium* spores to previously uninfected *C. paspali*, however, may reduce benefits received by *C. paspali*—both directly, by spreading a mycoparasite that is implicated in decreased sclerotia production [21] and indirectly because exudates provided by *C. paspali* also are used by *Fusarium* [11, 18]. Reduced benefits, combined with increased costs to both mutualist partners in the presence of *Fusarium*, could shift the relationship between moths and *C. paspali* into an antagonism ([13], also see [70] for a similar graphical model of a simpler system). Such shifts between parasitism and mutualism occur in all categories of mutualisms (reviewed in [13, 36], e.g. [73]), but this phenomenon has been studied relatively rarely. The potential for competition and antagonism among fungal symbionts raises questions about whether mycoparasite and endophyte infections influence insect visitation rates, insect fitness, or spore transmission patterns in this system and in other plant–pathogen–vector systems. Our study highlights the importance of considering interspecific interactions, specifically plant–pathogen associations, within their broader ecological context and sets the stage for finer-scale molecular analyses and experimental trials to assess the fitness consequences of these distinctive fungal guilds.

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