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A mutualistic interaction between a fungivorous nematode and a fungus within the endophytic community of *Bromus tectorum*

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ABSTRACT

In its invaded range in western North America, *Bromus tectorum* (cheatgrass) can host more than 100 sequence-based, operational taxonomic units of endophytic fungi, of which an individual plant hosts a subset. Research suggests that the specific subset is determined by plant genotype, environment, dispersal of locally available endophytes, and mycorrhizal associates. But, interactions among members of the endophyte community could also be important. In a sampling of 63 sites throughout the invaded range of *B. tectorum*, a fungivorous nematode, *Paraphelenchus acontioides*, and an endophyte, *Fusarium* cf. *torulosum*, were found together in two sites. This positive co-occurrence in the field led to an experimental investigation of their interaction and its effects on relative abundances within the endophyte community. In greenhouse and laboratory experiments, we determined first that *P. acontioides* preferred *F.* cf. *torulosum* to other endophytes, and secondly that the relative abundance of *F.* cf. *torulosum* within the endophyte community was increased by the nematode in experimental plants. Taken together our results suggest that the fungivorous *P. acontioides* uses living plants to cultivate or increase the relative abundance of its preferred fungus. Surprisingly, host plant growth was unaffected by this endophytic, cultivation-based mutualism between a nematode and a fungus.

Introduction

Endophytic fungi are ubiquitous in nature (Petrini 1986; Schulz & Boyle 2006). Although infection is typically asymptomatic (Wilson 1995), symbioses with a plant host can range from mutualistic to antagonistic (Clay 1996; Kuldau & Bacon 2008; Saikkonen *et al.* 2010). A few endophytic species, sometimes known only as sequence-based, operational taxonomic units (OTUs), often dominate within a host (Ahlholm *et al.* 2002; Shipunov *et al.* 2008). For instance, research investigating the endophytic community of western white pine (*Pinus monticola*) from multiple populations throughout the Rocky Mountains revealed that *Lophodermium* endophytes were dominant (Ganley & Newcombe 2006). Although a few species are often

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dominant, endophytic fungi still form diverse community assemblages (Arnold & Lutzoni 2007; Shipunov *et al.* 2008). For instance, Vega *et al.* (2010) found 257 fungal endophytes in a single plant species and 17 fungal species have been found within a single leaf (Lodge *et al.* 1996; Gamboa & Bayman 2001). Even at a small spatial scale, singletons often comprise a significant portion of the endophyte community (Arnold *et al.* 2000; Arnold & Lutzoni 2007).

The factors affecting endophyte community structure are starting to be explored. For example, Arnold & Lutzoni (2007) found biogeography to be an important factor for the incidence and diversity of endophytes in leaves. Their research demonstrated that the diversity of endophytes at both the individual and plant community levels increased with decreasing latitude (i.e., from poles to equator). Furthermore, they also found that endophytes isolated within a specific biogeographic zone (i.e., arctic, temperate or tropical) were often absent from other zones.

At the local level, other factors are operative. Water availability, temperature, agricultural chemicals, and plant metabolites affect the endophyte community in maize (*Zea mays*) (Marin *et al.* 1998; Seghers *et al.* 2004; Saunders & Kohn 2009). Marin *et al.* (1998) demonstrated that inter- and intraspecific endophytic interactions resulted in different fungi dominating at different temperatures and water availabilities. Saunders & Kohn (2009) demonstrated that production of plant defense compounds influenced the endophyte community within maize, and variable leaf chemistry generally explained differences in endophyte communities among host species (Arnold & Herre 2003).

A living plant can serve as a significant filter for diversity since it controls entry of fungi into its tissues. Thus, it is not surprising that host genotype affects the structure of mycorrhizal communities (Mummey & Rillig 2006; Korkama *et al.* 2006), as well as richness, diversity and composition of endophytes within plants (Todd 1988; Bailey *et al.* 2005; Pan *et al.* 2008). In western North America, for example, the endophyte community of *Bromus tectorum* (Baynes *et al.* 2012) differs substantially from that of *Centaurea stoebe* (Shipunov *et al.* 2008), another common plant invader of the region. Although both species are native to Eurasia and both were sampled within similar habitat types in their invaded range, little overlap was observed between their endophyte communities.

In addition to these community-structuring factors, members of endophyte communities could also directly affect the relative abundance of one another. Some endophytes reduce colonization by other endophytes. Schulthess & Faeth (1998) found that, when *Neotyphodium* was present in Arizona fescue (*Festuca arizonica*), the frequency of other endophytes declined. Specific endophytes may be competitively superior because of mycotoxin production or stimulation of host plant defenses (e.g., premature leaf abscission and chemical toxin production) that limit colonization and growth of other endophytes (Saikkonen *et al.* 1998; Schulthess & Faeth 1998). Therefore, the presence of one dominant or beneficial endophyte may influence the presence and diversity of other potential endophytes within a host. Endophyte–endophyte interactions may be similar to microbial interactions within soil communities. Some microarthropods are selective feeders (Maraun *et al.* 1998) with a preference for conidial fungi over arbuscular mycorrhizal fungi (Klironomos & Kendrick 1996). Likewise, nematodes, which are common in soil communities (Bongers & Bongers 1998; Newsham *et al.* 2004), can also influence growth of fungi (Shafer *et al.* 1981; Ingham 1988; Giannakis & Sanders 1989) and species composition (Newsham *et al.* 2004). Interactions between endophytic nematodes and fungi can have consequences for host plant health (Nordmeyer & Sikora 1983a,b; Sikora & Carter 1987), contributing to diseases like vascular wilt and root-rot in banana (Sikora & Schlösser 1973; Sikora & Carter 1987; Gowen *et al.* 2005). Conversely, Stewart *et al.* (1993) found that endophytic fungi could inhibit gall-forming nematodes, improving plant health.

Fungivorous nematodes are sometimes isolated as endophytes along with fungi (Christie & Arndt 1936; Wasilewska 1967; Sosamma 2001). Since fungivorous nematodes can alter fungal community diversity (Barnes *et al.* 1981), these nematodes could change the relative abundance of endophytic fungi that they selectively or preferentially consume within plant tissue. An *in planta* interaction between a fungivorous *Botanophila* fly species and endophytic *Epichloe festucae* in *Festuca* spp. has been demonstrated (Rao & Baumann 2004; Rao *et al.* 2005). However, to our knowledge, no other research has been conducted on fungivore–endophyte associations that could affect endophyte community structure. From *B. tectorum*, we isolated a fungivorous nematode with an endophytic *Fusarium*. We investigated their *in planta* association and the effect of that interaction on relative abundances within the endophyte community of *B. tectorum*. We hypothesized that the nematode was using living *B. tectorum* plants to 'cultivate', or increase the relative abundance of, the endophytic *Fusarium* that it preferred to consume.

The objectives of our research were to test this cultivation hypothesis *via* preference and suitability assays directed at the nematode, and secondarily *via* inoculations of *B. tectorum* with the nematode and/or its putative fungal cultivar. Finally, we determined whether this nematode–fungus interaction affected host plant fitness (i.e., height and biomass).

Materials and methods

Sampling of endophyte communities in Bromus tectorum

Bromus tectorum was collected from 63 sites throughout the United States and Canada (i.e., British Columbia, Colorado, Idaho, Illinois, Iowa, Nevada, New Mexico and Washington – Table 1) during 2009 and 2010. Collections were made from a variety of habitats, including coniferous forest, sagebrush-grassland, desert scrub, agricultural fields and disturbed roadside. At each site 20 green stems were collected (Seabloom *et al.* 2009). Sampling was conducted twice at one site; Piney River, CO, USA was sampled in 2009 (Piney River) and again in 2010 (Piney River '10).

A 2-cm segment centered on the lowest culm node was removed from each plant. Culm segments were surface-sterilized in 50 % ethanol (EtOH) for 5 min and rinsed with sterile, deionized (DI) water for 1 min (Schulz *et al.* 1993). For each population, imprint plates were made to ensure efficacy of sterilization. Culm segments were placed on potato dextrose agar (PDA) in Petri dishes and sealed with parafilm.

Table 1 – Richness (R), evenness (E) and diversity (D) of operational taxonomic units of fungal isolates for 63 *B. tectorum* populations sampled

Collection site	State/Province[a]	GPS Coordinates		R	E	D
Aztec	NM	36°47′57.42″N	107°53′02.67″W	11	0.913	0.861
Bandelier	NM	35°47′36.37″N	106°16′12.43″W	4	0.876	0.667
Benewah	ID	47°20′15.00″N	116°40′12.00″W	3	0.999	0.667
Berthoud Falls	CO	39°46′10.72″N	105°48′45.75″W	10	0.959	0.880
Big Meadow	ID	46°47′14.14″N	116°48′10.45″W	5	0.885	0.722
Bruno Gulch	CO	39°31′42.09″N	105°44′53.21″W	14	0.952	0.910
Camp Grizzly	ID	46°56′36.68″N	116°39′19.86″W	8	0.971	0.860
Colorado Springs	CO	38°55′11.23″N	104°51′56.63″W	5	0.847	0.698
Coyote Run	WA	46°07′03.16″N	117°10′58.01″W	10	0.978	0.891
Crow Hill	CO	39°24′07.61″N	105°28′10.79″W	8	0.918	0.831
Dillon Lake	CO	39°36′47.33″N	106°03′54.56″W	3	0.625	0.379
Dworshak	ID	46°32′55.31″N	116°15′22.69″W	6	0.915	0.782
East Suzie	NV	40°47′21.09″N	115°55′51.38″W	4	0.874	0.654
Elk Meadow	CO	39°40′18.12″N	105°21′37.68″W	6	0.808	0.717
Fall Creek	ID	44°38′04.39″N	116°21′05.84″W	18	0.918	0.913
Freeman	ID	46°34′20.95″N	116°16′31.47″W	5	0.894	0.734
Georgetown	CO	39°42′07.76″N	105°41′50.87″W	5	0.881	0.720
Granite Point	ID	46°48′49.31″N	116°52′49.61″W	3	0.960	0.640
Guanella Pass	CO	39°42′07.76″N	105°41′50.87″W	5	0.935	0.760
Hagenah	WA	46°18′30.53″N	117°07′36.89″W	10	0.912	0.858
Heyburn Lake	ID	47°21′16.13″N	116°45′48.48″W	9	0.973	0.875
Heyburn State Park	ID	47°20′50.02″N	116°41′06,11″W	7	0.941	0.815
Highway 95	ID	46°17′52.26″N	116°35′38.99″W	9	0.931	0.848
Highway 99	ID	46°37′3.27″N	116°40′27.40″W	11	0.961	0.889
Hubert Gulch	WA	46°14′6.24″N	117°12′14.69″W	6	0.993	0.828
Johnson	WA	46°14′19.23″N	117°12′30.35″W	10	0.959	0.881
Kendrick	ID	46°37′03.00″N	116°38′43.98″W	5	0.916	0.750
Kootenay	BC	49°38′58.01″N	115°38′55.06″W	6	0.885	0.765
Little Boulder	ID	46°46′20.07″N	116°27′23.45″W	2	1.000	0.500
Little Salmon River	ID	45°20′33.59″N	116°21′00.53″W	7	0.969	0.840
Lower Deadman	WA	46°40′10.60″N	117°26′51.32″W	5	0.946	0.765
Lower Moscow Mtn.	ID	46°47′56.08″N	116°53′56.36″W	6	0.931	0.781
Mississippi	IL	41°24′58.78″N	91°01′50.12″W	4	0.409	0.259
Moose Creek	ID	46°52′19.43″N	116°24′56.36″W	5	0.963	0.776
Moscow Mtn. So.	ID	46°53′32.80″N	116°53′32.80″W	4	0.959	0.722
Nelson	**BC**	49°29′09.43″N	117°18′07.21″W	**6**	**0.601**	**0.497**
Nisqually John	WA	46°30′42.08″N	117°13′51.59″W	1	n/a	0.000
Parker Farm	ID	46°43′28.57″N	116°57′13.55″W	6	0.999	0.833
Pearl Button	IA	41°25′11.61″N	91°02′44.00″W	3	0.790	0.500
Piney River '09	**CO**	39°50′24.99″N	106°38′26.85″W	**4**	**0.548**	**0.394**
Piney River '10	CO	39°50′24.99″N	106°38′26.85″W	8	0.956	0.854
Potlatch	ID	46°54′55.99″N	116°53′45.16″W	7	0.935	0.819
Puffer Butte	WA	46°04′14.01″N	117°10′15.50″W	14	0.895	0.878
Rest Area	WA	46°47′24.53″N	118°44′38.36″W	21	0.947	0.934
Rock Creek	ID	46°51′57.33″N	116°53′51.44″W	5	0.928	0.750
Ryegrass	WA	47°00′27.36″N	120°27′29.88″W	3	0.870	0.571
San Ysidro	NM	35°33′56.23″N	106°46′08.37″W	7	0.250	0.815
Sandia Mountain	NM	35°12′49.26″N	106°24′30.19″W	12	0.827	0.800
Santa Fe	NM	35°37′02.83″N	106°00′14.64″W	8	0.901	0.821
St. Joe Nat'l Forest	ID	47°00′29.76″N	116°12′43.30″W	8	0.974	0.860
Sky City	NM	35°04′29.52″N	107°33′22.39″W	8	0.801	0.734
Skyline	ID	47°03′41.84″N	116°56′37.62″W	4	0.882	0.680
Sperry Grade	ID	46°36′33.23″N	116°39′15.12″W	11	0.962	0.891
St. Maries	ID	47°19′52.09″N	116°38′37.78″W	0	0.000	1.000
Steptoe Canyon	WA	46°26′57.77″N	117°12′17.52″W	8	0.834	0.771
Strychnine Creek	ID	46°56′54.16″N	116°38′18.45″W	6	0.936	0.790
Tent Rock	NM	35°39′32.09″N	106°24′47.40″W	4	0.726	0.533
UI Exp. Station	ID	46°50′17.27″N	116°52′45.55″W	8	0.885	0.793
Wawawai	WA	46°38′17.16″N	117°22′33.91″W	7	0.866	0.776
Weiser	ID	44°38′42.74″N	116°22′50.53″W	20	0.937	0.926
WERC	ID	46°13′40.08″N	116°38′12.50″W	7	0.946	0.827
White Bird	ID	45°51′55.05″N	116°14′12.00″W	11	0.954	0.886
Winchester	ID	46°15′07.34″N	116°36′16.89″W	6	0.796	0.671

Bolded text indicates two sites from which *P. acontioides* nematodes were isolated.
a BC = British Columbia, CO = Colorado, ID = Idaho, IL = Illinois, IA = Iowa, NV = Nevada, NM = New Mexico and WA = Washington.

Endophytic fungi and nematodes emerging from segments were isolated and cultured. Fungal isolates were subcultured bi-monthly onto PDA and nematodes were reared on cultures of the *Fusarium* sp. with which they were isolated. Cultures were stored in the laboratory at ambient conditions (20 °C with a 10:14 hr photoperiod, light:dark).

Identification of endophytic fungi and nematodes

Endophytes isolated from all 63 *B. tectorum* populations were morphotyped based on culture and microscopic characteristics. A subset of these isolates (221 of 1064) was sent to the USDA-ARS Systematic Mycology and Microbiology Laboratory for sequence-based identification. These included two morphologically similar *Fusarium* cultures from Piney River (CID 018) and Nelson (CID 273), a *Curvularia* culture (CID 031) and a *Penicillium* culture (CID 098), both from Piney River.

Two additional cultures of *Fusarium* isolated from Piney River (CID 314 and CID 383), morphologically identical to CID 018, were also identified using morphological and molecular approaches. For the morphological identification, cultures were grown on PDA for 2 weeks to measure colony diameter and allow for the production of sporulating structures. In addition to the sequence data, the identification was confirmed by comparing the macroconidia, microconidia, chlamydospores, and colony morphology with the description and illustrations of *Fusarium torulosum* in Leslie & Summerell (2006).

For sequencing and phylogenetic analysis, isolates of *Fusarium* were grown in 5 ml of potato dextrose broth in 15 × 60 mm Petri dishes incubated at 25 °C for 3 d. Mycelium from the cultures was separated from the media and pressed between paper towels to remove excess media and used for DNA extraction. DNA was extracted using ArchivePure DNA cell/tissue kit from 5 PRIME, Inc. (Gaithersburg, MD) following the protocol provided by the manufacturer. The DNA was used as a template in polymerase chain reactions. A section of translation elongation factor (EF1-α) was amplified using primers EF-700f (Samuels & Ismaiel 2011) and EF2 (O'Donnell *et al.* 2000). Internal transcribed spacer (ITS) was amplified using primers ITS5 and ITS4 (White *et al.* 1990). The PCR mixture and the thermalcycler program for amplification of both loci were the same as described previously (Samuels & Ismaiel 2009). Approximately 0.5 kb and 0.6 kb products of EF1-α and ITS were amplified, respectively. The amplicons were cleaned enzymatically using Exosap-IT (USB Corporation, Cleveland, OH). The purified products were directly sequenced using BigDye Terminator v3.1 chemistry on an automated 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Both strands of each amplicon were sequenced using the primers used in generating them.

The sequences were assembled and edited to construct a consensus sequence using Sequencher 4.9 (Gene Codes, Madison, WI). The sequences of the two isolates were 100 % identical. One of the two sequences was subjected to basic local alignment search tool (BLAST) using BLASTN program available at http://www.ncbi.nlm.nih.gov. The search indicated that several species of *Fusarium* in the study of Kristensen *et al.* (2005) and a few isolates in the study of O'Donnell *et al.* (2009) had high sequence similarity to the two isolates under study. The nexus alignment file from Kristensen *et al.* (2005) was retrieved from Treebase home page (http://www.treebase.org). The sequences of our isolates plus the few isolates of O'Donnell *et al.* (2009) were added to the alignment file. We also reduced the number of taxa in each clade in the tree. The additional sequences were realigned manually.

A phylogenetic tree was obtained under parsimony criterion using PAUP 4.0b10 (Swofford 2002) with a heuristic search, 1000 random stepwise addition, tree bisection reconnection (TBR) as branch swapping algorithm and MULTREES on. All characters were equally weighted and gaps were treated as missing. The tree was rooted using *Fusarium equiseti* as the outgroup based on the study of Kristensen *et al.* (2005). Support for the branches was performed with bootstrap using 1000 pseudoreplicates of the data, 100 random additions per replicate and TBR branch swapping. Bootstrap values greater or equal to 70 % were considered significant (Hillis & Bull 1993).

The two *Fusarium* isolates (CID 314 and CID 383) were deposited in CBS as CBS **130337** and CBS **130338**, respectively. The sequences were deposited in GenBank as CID 314 (ITS **JN133579**, TEF **JN133577**) and CID 383 (ITS **JN133580**, TEF **JN133578**). *Curvularia inaequalis* and *Penicillium olsonii* sequences were deposited in GenBank (ITS **HQ829066** and ITS **JQ663620**, respectively).

The USDA-ARS Nematology Laboratory identified endophytic nematodes that were isolated, and subsequently co-cultured with, two *Fusarium* isolates (CID 314 and CID 383). Nematodes were rinsed from the Petri plates, placed in 4 % formalin for 24 hr and then rinsed in sterile DI water, or rinsed from the plates and placed in 70 % alcohol before identification (Carta *et al.* 2011).

Effects of a fungivorous nematode and a putative fungal cultivar on the endophyte community

Field surveys

Prior to endophyte and nematode isolation, individual *B. tectorum* plant weight (aboveground fresh weight) and height (from base to first inflorescence branch) were recorded; endophyte isolation and identification results were also compiled into the database. These data allowed for analyses of plant height and weight as well as endophyte frequency, richness, evenness and diversity within and by population (refer to "Statistical Methods", below, for details).

Endophyte isolation assay

Endophytes were isolated from *B. tectorum* seed to ascertain the frequency of infection in field-collected seed. Two hundred seeds were surface-sterilized in 50 % EtOH for 5 min and rinsed with sterile, deionized (DI) water for 1 min (Schulz *et al.* 1993). Sterilized seeds were placed on PDA in Petri dishes and sealed with parafilm; imprint plates were made to ensure sterilization efficacy. Petri dishes were stored in the laboratory at ambient conditions (20 °C with a 10:14 hr photoperiod, light:dark). Observations were made daily; all emerging endophytes were recorded, isolated and cultured.

Experiment 1

In an *in planta* greenhouse experiment, we investigated the effect that the isolated nematodes and *Fusarium* sp. had on the

endophytic community in *B. tectorum*. Experimental design included two treatments: *F.* cf. *torulosum* inoculum with (N+) or without (N−) nematodes. One local population of *B. tectorum* was employed with 15 replicates (i.e., plants) per treatment. The two fungal inoculant solutions were prepared by removing a 12 cm^2 section of mycelium from a *F.* cf. *torulosum* culture with nematodes (N+) and thoroughly mixing into 150 ml of sterile DI water. The same procedure was employed for the second inoculation solution from a *F.* cf. *torulosum* culture without nematodes (N−).

Seeds were harvested from a *B. tectorum* population on Hog Island along the Clearwater River near Lewiston, ID, in 2009 [46°26′52.77″N 116°51′42.42″W]. Seeds were surface-sterilized in 50 % EtOH for 5 min and rinsed with sterile DI water for 1 min (Schulz *et al.* 1993). The seeds were placed in UV-sterilized, covered Petri plates and allowed to germinate at ambient room temperature and light.

Seedlings of *B. tectorum* were transplanted into autoclaved potting soil (Sunshine Mix #2) and UV-sterilized trays (20 × 25 × 8 cm). For each treatment, three seedlings were planted into five trays. Seedlings were planted at an equal distance from each other and the inoculant was immediately pipetted into shallow holes in the soil, equidistant to each plant (3 ml of inoculant per hole for a total of 9 ml per tray). Roots, fungi and nematodes were allowed to freely interact within the soil environment.

Plants of *B. tectorum* were harvested after 4 weeks. Excess soil was rinsed from each plant, and aboveground and belowground fresh weights were recorded. Three random 3 cm sections were clipped from both the root and leaf tissue of the harvested plants. Fresh weight of the clippings and remaining plants (aboveground and belowground biomass) were recorded. After weighing, the plants were placed into separate paper bags and dried for 72 hr at 60 °C. Following drying, plant dry weight biomass was recorded for each plant. These results along with the fresh weight results were used to calculate total dry weight biomass for each individual plant.

Root and leaf tissue was surface-sterilized using the same procedure used to sterilize seed. Sterilized plant tissue was plated onto PDA; Petri plates were sealed with parafilm and stored in the laboratory at ambient conditions. Cultures were observed daily and fungal isolates were identified morphologically to genus based on macroscopic and microscopic morphology.

Experiment 2

A repeat greenhouse study was conducted to validate the effect that the nematodes and *Fusarium* sp. had on the endophytic community in *B. tectorum*. Using the same seed source, the experimental design from Experiment 1 was repeated but with additional replication for each treatment ($n = 50$). Five seedlings were planted into each tray, equidistant from one another. The inoculant was pipetted into shallow holes in the soil at an equal distance from each plant (3 ml of inoculant per hole for a total of 15 ml per tray). The solution, plant ratios, and proportions were equivalent to those in Experiment 1. Trays containing plants and fungi were covered in Experiment 2 to minimize contamination. Plants of *B. tectorum* were harvested after 4 weeks and the same procedures were followed as in Experiment 1.

Experiment 3

We conducted a third *in planta* greenhouse experiment to test the effect of the nematodes and *Fusarium* sp. on the endophytic community in the presence of competition. Experimental design was similar to the first two experiments, but inoculum comprised all four of the endophytes isolated from the Piney River *B. tectorum* population rather than just *F.* cf. *torulosum*. Specifically, *F.* cf. *torulosum*, *C. inaequalis*, *P. olsonii* and an unidentified endophyte (B115) were used to make the inoculum, both with (N+) and without (N−) nematodes. A 3 cm^2 section of mycelium from each fungal culture was removed and mixed together thoroughly into 150 ml of sterile DI water. Inoculum:plant ratios were equivalent to those in the first experiments; each treatment was replicated ($n = 50$) and the same seed source was utilized. Plants of *B. tectorum* were harvested after 4 weeks and the same procedures were followed as in Experiments 1 and 2.

Fungal preference and suitability assays

Preference assay

To determine if nematodes had a preference for particular fungal endophytes, nematodes were offered a choice of two fungal endophytes in a preference assay. Three endophytes (i.e., *F.* cf. *torulosum*, *P. olsonii* and *C. inaequalis*) isolated from Piney River *B. tectorum* in two different combinations (*F.* cf. *torulosum*—*P. olsonii* and *F.* cf. *torulosum*—*C. inaequalis*) were tested. Fungi were cultured on PDA in Petri dishes (8.5 cm diam.). Small plugs (0.5 cm^2) of two inoculants, *F.* cf. *torulosum* and *P. olsonii* or *F.* cf. *torulosum* and *C. inaequalis*, were placed on opposite sides of each plate. The plates were sealed with parafilm and the fungi were allowed to grow for 3 d. On the third day, a diameter line was drawn on the back of the plate, halfway between the mycelium of each fungus. Approximately 50 nematodes (*Paraphelenchus acontioides*) were pipetted along the line onto the agar. The plates were resealed and left for 3 d under ambient laboratory conditions. Under a dissecting microscope, nematodes were counted, in each of the sectors delineated by the diameter line. Counts were repeated three times and averaged. Each assay was repeated four times.

Suitability assay

Nematodes (~75) were placed into Petri dishes containing only *C. inaequalis*, *F.* cf. *torulosum* or *P. olsonii* to test whether *P. acontioides* would graze, survive and reproduce on fungi other than *F.* cf. *torulosum*. For each fungus, four plates were prepared (two N+ and two N−), sealed with parafilm and left undisturbed for 2 weeks under ambient laboratory conditions. Six plugs (0.5 cm^2) were randomly removed from each culture and observed. Under a dissecting microscope nematodes (alive, eggs and dead) were counted. Counts for each plug were repeated three times and averaged.

Because nematodes were often concealed within the mycelium and agar, plug data were supplemented by a secondary method for determining density (number of individuals per 0.5 cm^2). Once plug counts were completed, six plugs of each endophyte type were placed into a small glass bottle with 6 ml of sterile DI water and vigorously shaken for 1 min. From the solution, 1 ml was pipetted into a 0.5 cm^2

gridded Petri plate. Nematodes (alive, eggs and dead) were counted three times and averaged. This process was repeated for all 6 ml of solution for each of the cultures.

Additional observations related to grazing suitability were made using *Agaricus bisporus* to assess the diversity of fungi suitable as food and whether *P. acontioides* could have any economic effect on mushroom cultivation. *Agaricus bisporus* was grown in culture but was not isolated as an endophyte from cheatgrass. Four plates (two N+, two N−) were observed over the same time period as the other fungi. Observational data rather than density data were recorded for *A. bisporus*.

Statistical methods

Data were analyzed with SysStat 12.02.00 (SysStat Software, Inc. 2007) and online computer software (Preacher 2001). For field-collected samples, chi-square analyses were used to compare the frequency of the putative fungal cultivar with the presence or absence of nematodes (Preacher 2001). Richness, diversity (Simpson's) and evenness (Shannon's) analyses were conducted for each of the 63 populations. For the three greenhouse experiments, chi-square analyses were conducted to compare the re-isolation frequency of the putative fungal cultivar when nematodes were included (N+) *versus* excluded (N−) in the inoculum (Preacher 2001). To determine endophyte preference, chi-square analyses were conducted (Preacher 2001) and density data from the suitability assays was analyzed using ANOVA with Bonferroni pairwise comparisons (SysStat Software, Inc. 2007). Field and greenhouse biomass data were analyzed using Student's two-sample *t*-test with separate variances (SysStat Software, Inc. 2007).

Results

Sampling and isolation of endophyte communities in Bromus tectorum

From the 63 populations sampled, 1 064 fungal endophytes were isolated, comprising more than 100 sequence-based identifications. Results are unpublished with the exception of the thermotolerant fungal isolates, which were presented in Baynes *et al.* 2012. Of the 63 sites sampled in 2009, only two yielded co-occurring endophytic nematodes and fungi (i.e., Nelson, BC and Piney River, CO).

Identification of endophytic fungi and nematodes

Sequence-based identifications were made for three of the *Fusarium* isolates (CID 018, CID 314 and CID 383) from Piney River. Isolates CID 314 and CID 383 were identified as *F.* cf. *torulosum*; CID 018 was initially identified as a *Fusarium* sp. Isolate CID 018 as well as all other *Fusarium* cultures from Piney River were morphologically identical to CID 314 and CID 383 and thus were morphotyped as *F.* cf. *torulosum*. A sequence-based identification was also made for the morphologically similar isolate CID 273 from Nelson. Results from a BLAST search identified this isolate as *Fusarium* sp.

With sequences from GenBank added to the tree of Kristensen *et al.* (2005), the final sequence data had 27 taxa and 723 characters of which 545 were constant, 58 parsimony-uninformative, and 120 (17 %) were parsimony-informative characters. The two isolates under study, along with an isolate deposited as *Fusarium* sp. (GenBank accession number GQ505419), formed a highly supported subclade (Fig 1). This subclade had a strong sister-relationship with *F. torulosum*. In Kristensen *et al.* (2005), all the species in Fig 1 were included in the monophyletic group M that included all the species that produced moniliformin but not trichothecene. Even though moniliformin production has not been reported for *F. torulosum*, inclusion of the species within the group suggests the potential for such activity. The internal transcribed spacer region (ITS) of the two isolates was identical. When an ITS sequence of one of the two isolates was used in a BLAST search, many identical or highly homologous hits deposited under different species names, or as *Fusarium* sp., were available suggesting inability of this locus to distinguish between closely related species of *Fusarium*; therefore, we did not use the ITS in any phylogenetic analysis.

Endophytic nematodes were only isolated with the *F.* cf. *torulosum*. Nematodes were absent from all other endophyte cultures isolated from the Piney River and Nelson populations, and from the other 61 populations. Two species of nematodes were co-isolated with *F.* cf. *torulosum*, and identified as the polyphagous *Panagrolaimus artyukhovskii* (Blinova & Mishina 1975) and the fungivorous *Paraphelenchus acontioides* (Taylor & Pillai 1967; Carta *et al.* 2011). All greenhouse and laboratory experiments were conducted using *P. acontioides* isolated with one culture of *F.* cf. *torulosum* from Piney River.

Effects of a fungivorous nematode and a fungal cultivar on the endophyte community

Field surveys

Host plant fitness in the Piney River and Nelson sites was unaffected by *F.* cf. *torulosum* and the nematodes. Plant height did not differ significantly between *B. tectorum* with *F.* cf. *torulosum* and nematodes (N+) and those without nematodes (N−) (Piney River, $t = 1.467$, df $= 2.486$, $p = 0.256$; Nelson, $t = -1.253$, df $= 7.724$, $p = 0.247$). Likewise, for both sites, there was no significant difference in fresh weight between N+ and N− plants (Piney River, $t = 2.050$, df $= 11.875$, $p = 0.063$; Nelson, $t = -0.490$, df $= 2.541$, $p = 0.663$).

Relative abundance (i.e., isolation frequency) of *F.* cf. *torulosum* at Piney River and Nelson sites was high: 73 % and 69 %, respectively. Nematode isolation frequency was high at both sites; nematodes were observed in 84 % and 89 % of the *F.* cf. *torulosum* isolates at Piney River and Nelson, respectively. In these two sites, the isolation frequency of *Fusarium* spp. was much higher (a near 3:1 ratio) than the 1:9 ratio of the other 61 sites. Not surprisingly, a chi-square analysis of the 63 sites demonstrated that the isolation frequency of *Fusarium* spp. was significantly higher when the latter was associated with nematodes (chi-square = 159.427, df = 1, $p < 0.001$) (Table 2). Resampling at Piney River in 2010 yielded a low isolation frequency of *Fusarium* sp. (20 %) relative to 2009 efforts; nematodes were absent from all Piney River 2010 isolates.

Endophytic *F.* cf. *torulosum* and its co-occurring nematodes influenced indices of richness, diversity and evenness of the

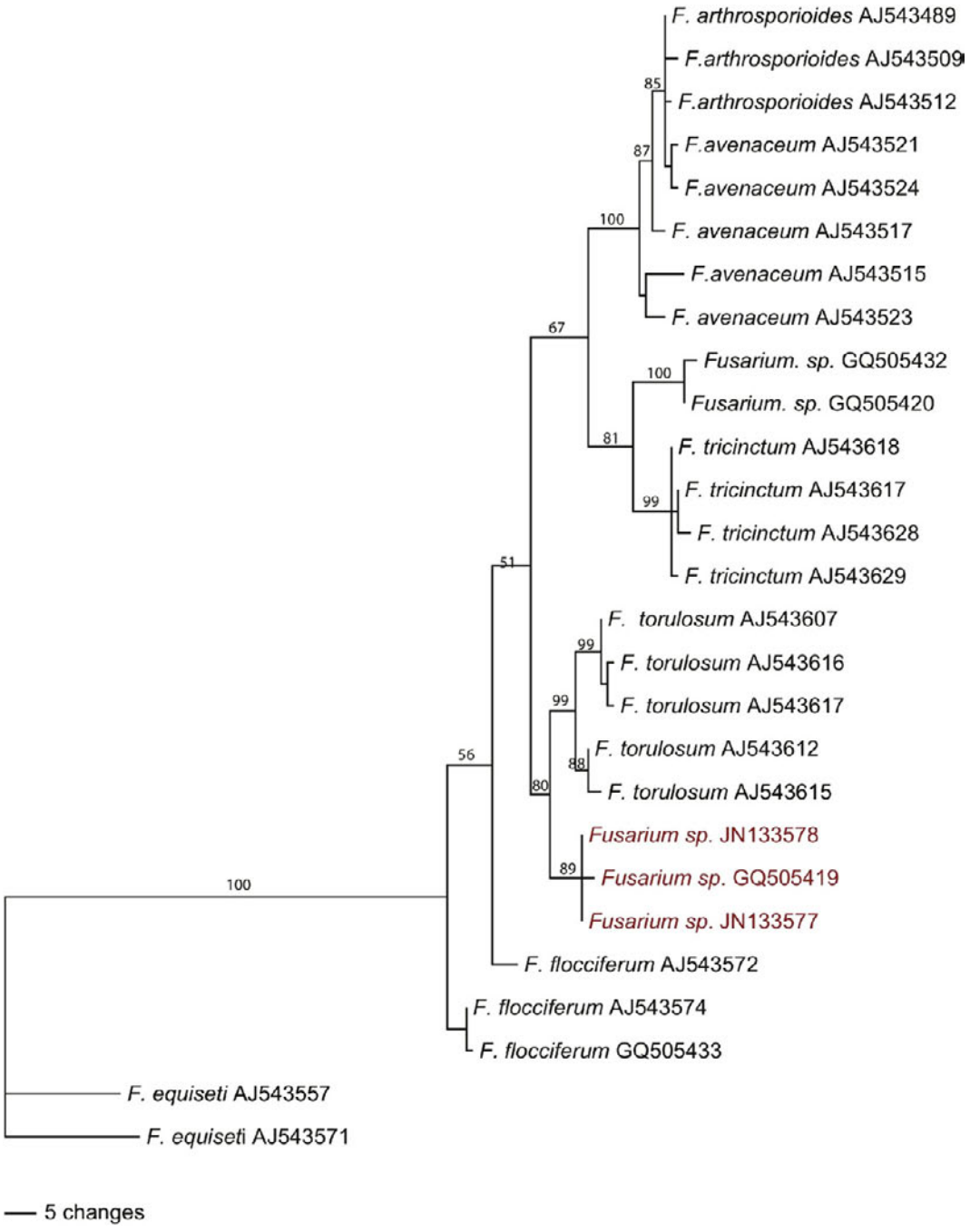

Fig 1 – Parsimonious tree showing position of *Fusarium* cf. *torulosum* (JN133578 and JN133577) within the phylogeny of related *Fusarium* species. The tree was based on translation elongation factor 1 alpha sequence data. Tree had 220 steps, consistency index 0.87, Homoplasy index 0.13. Numbers on the branches represent bootstrap values greater than 50 % obtained *via* 1 000 replicates. Two isolates of *F. equiseti* were used as outgroup taxa.

endophytic communities at these two sites (Table 1). For the 63 populations sampled, the richness of fungal OTUs varied from 0 to 21 with a mean of 7.18. Both Piney River and Nelson sites were below the mean with values of 4 and 6, respectively. With respect to evenness, values ranged from 0.000 to 1.000 among the 63 populations sampled; Piney River and Nelson values were 0.548 and 0.601, respectively. Only three populations had lower values; one of these, St. Maries, produced no endophytes and in another, Mississippi, *Aspergillus niger* was the dominant endophyte. Endophytic diversity values ranged from 0.000 to 1.000 among all 63 populations. Diversity at Piney River and Nelson was low compared to the other populations (0.394 and 0.497, respectively); only three populations had lower values. One population (Nisqually John) had a very low isolation rate (one endophyte) and the two other populations had a high isolation rate of a single

Table 2 – In field-collected *B. tectorum*, relative isolation frequency of *Fusarium* spp. was significantly higher when *P. acontioides* was present (N+): $n = 63$, chi-square = 159.427, df = 1, $p \leq 0.001$

Field-collected *B. tectorum*	*Fusarium* spp.	Other endophytes	Total	Relative frequency
N+ plants	37	14	51	0.73
N– plants	107	906	1013	0.11
Total	144	920	1064	0.14

endophyte (*A. niger* at Mississippi and *Fusarium* sp. at Dillon Lake) that reduced their respective diversities.

Endophyte isolation assay

Endophyte isolation frequency from field-collected *B. tectorum* seed was relatively low. Endophytes were isolated from 30 of 200 seed (15 %). Endophytes isolated included *Alternaria* sp., *Aureobasidium* sp., *Cladosporium* sp., *Trichoderma* sp. and three unknown species. No bacterial endophytes were observed.

Experiment 1

The re-isolation frequency of *F.* cf. *torulosum* was significantly higher in N+ plants than in N– plants, 38 % and 14 %, respectively (chi-square = 4.406, df = 1, $p = 0.036$) (Table 3). *Alternaria* sp., *Penicillium* sp., *Fusarium oxysporum* (N+ only), *Rhizopus* sp. and several unidentified bacterial endophytes were also isolated, but they are common to greenhouse materials and experiments (Ganley & Newcombe 2006). *Fusarium oxysporum* was distinguished from *F.* cf. *torulosum* by comparing both culture morphology and micromorphological features (Nelson *et al.* 1983). Nematodes did not affect host plant biomass ($t = -1.401$, df = 20.410, $p = 0.176$) and were only isolated with *F.* cf. *torulosum*.

Table 3 – In greenhouse experimental *B. tectorum*, relative re-isolation frequency of *F.* cf. *torulosum* was significantly higher when *P. acontioides* was present (N+)

Greenhouse experimental *B. tectorum*	*F.* cf. *torulosum*	Other endophytes	Total	Relative frequency
Experiment 1				
F. cf. *torulosum* (N+)	11	18	29	0.38
F. cf. *torulosum* (N–)	4	25	29	0.14
Total	15	43	58	0.26
Experiment 2				
F. cf. *torulosum* (N+)	35	93	128	0.27
F. cf. *torulosum* (N–)	21	107	128	0.20
Total	56	200	256	0.22
Experiment 3				
F. cf. *torulosum* (N+)	23	109	132	0.17
F. cf. *torulosum* (N–)	7	112	119	0.06
Total	30	221	251	0.12

Experiment 1: chi-square = 4.406, df = 1, $p = 0.036$, Experiment 2: chi-square = 4.480, df = 1, $p = 0.034$, and Experiment 3: chi-square = 7.922, df = 1, $p = 0.005$.

Experiment 2

Fusarium cf. *torulosum* re-isolation frequency was significantly higher in N+ plants than in N– plants, 27 % and 20 %, respectively (chi-square = 4.480, df = 1, $p = 0.034$) (Table 3). *Aspergillus* sp., *Alternaria* sp., *F. oxysporum*, *Penicillium* sp., *Rhizopus* sp., *Trichoderma* sp., and *Ulocladium* sp. were isolated as greenhouse contaminants. Again, nematodes were re-isolated from plants inoculated with *F.* cf. *torulosum* and always in association with this fungus and no other. Nematode presence again did not affect host plant biomass ($t = 0.145$, df = 82.918, $p = 0.885$).

Experiment 3

Once again, re-isolation frequency of *F.* cf. *torulosum* was significantly higher in N+ *versus* N– plants, 17 % and 6 %, respectively (chi-square = 7.922, df = 1, $p = 0.005$) (Table 3). Other inoculants (i.e., *C. inaequalis* and *P. olsonii*) were also re-isolated from both treatments although the unidentified endophyte (B115) was not. The greenhouse contaminants in this experiment were *Acremonium* sp., *Aspergillus* sp., *Alternaria* sp., *Chaetomium* sp., *F. oxysporum*, *Rhizopus* sp., *Trichoderma* sp., and a second species of *Penicillium*. Nematodes were re-isolated exclusively in association with *F.* cf. *torulosum*. Plant biomass was not analyzed.

Fungal preference and suitability assays

Preference assays

Three endophytes from the Piney River site (i.e., *F.* cf. *torulosum*, *P. olsonii* and *C. inaequalis*) were employed in assays to determine whether the nematode, *P. acontioides*, preferred *F.* cf. *torulosum*. In all four plates of the *F.* cf. *torulosum*–*P. olsonii* preference assay, more nematodes were observed within the mycelial sector of *F.* cf. *torulosum* (chi-square = 12.875, df = 3, $p = 0.005$) than in the sector of *P. olsonii* (Table 4). Likewise in the *F.* cf. *torulosum*–*C. inaequalis* preference assay, the nematodes preferred *F.* cf. *torulosum* to *C. inaequalis* (chi-square = 7.883, df = 3, $p = 0.049$) (Table 4).

Suitability assays

Paraphelenchus acontioides grazed and reproduced upon the *F.* cf. *torulosum* cultures but also upon the *A. bisporus* and *C. inaequalis* cultures (Fig 2A, B, D). In contrast, nematode survival and

Table 4 – In preference assays, 3 d post-inoculation with ~50 living *P. acontioides* in each plate, *P. acontioides* abundance was significantly greater in *F.* cf. *torulosum* relative to *P. olsonii* (chi-square = 12.875, df = 3, $p = 0.005$) and *C. inaequalis* (chi-square = 7.883, df = 3, $p = 0.049$) cultures

	Nematodes				Total
	Plate 1	Plate 2	Plate 3	Plate 4	
F. cf. *torulosum*	42	61	56	103	262
P. olsonii	6	10	1	3	20
Total	48	71	57	106	282
F. cf. *torulosum*	46	51	60	44	201
C. inaequalis	7	10	1	7	25
Total	53	61	61	51	226

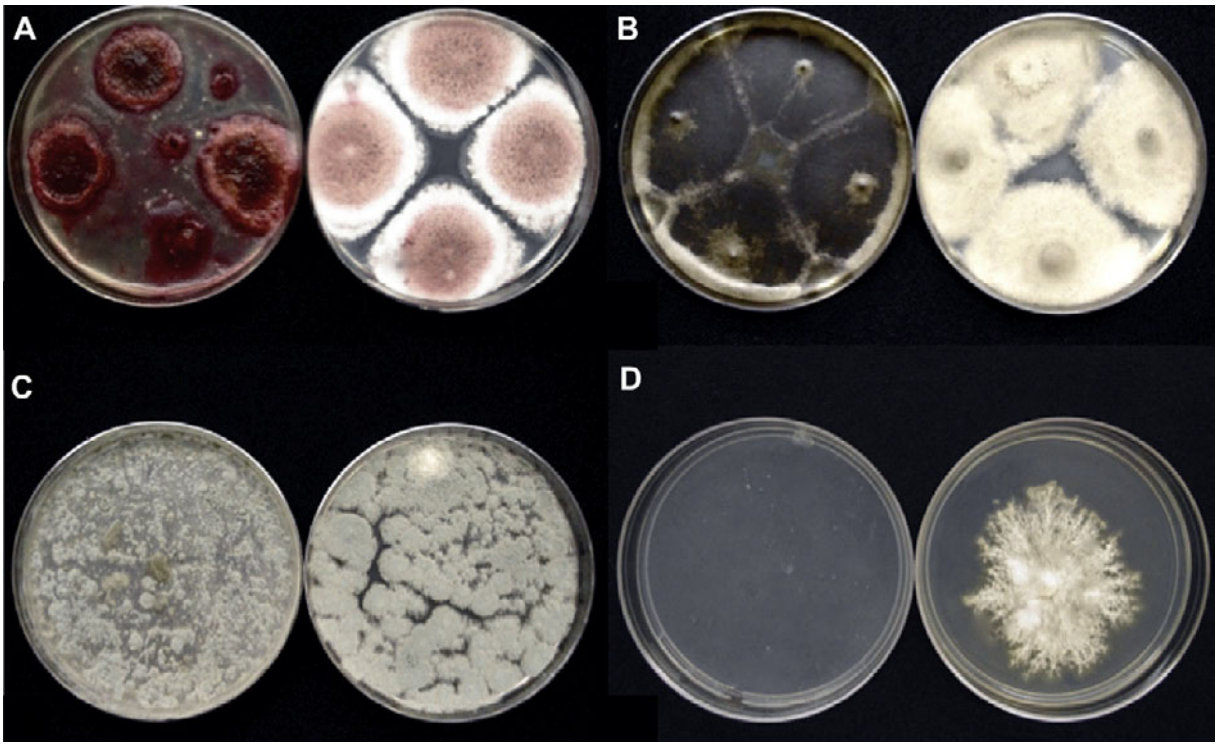

Fig 2 – Suppression by *P. acontioides* of growth of (A) *F.* cf. *torulosum*, (B) *C. inaequalis*, (C) *P. olsonii*, and (D) *A. bisporus* cultures 2 weeks post-inoculation with ~75 living nematodes. For each set, left image (N+) and right image (N−). *Paraphelenchus acontioides* least affected culture morphology of *P. olsonii*.

reproduction was limited in the *P. olsonii* cultures (Fig 2C). Aerial and radial mycelial growth of *F.* cf. *torulosum* was significantly impacted by nematode presence (Fig 2A). Nematode density within *F.* cf. *torulosum* averaged 54 (alive), 5 (eggs) and 0 (dead) from the plug counts and 133 (alive), 17 (eggs) and 1 (dead) from the solution counts.

Nematode grazing also reduced aerial and radial growth of the *Curvularia* species. In the N− plates, *C. inaequalis* filled the plate within the 2-weeks but the fungus was completely grazed in the N+ plates (Fig 2B). From the plug counts, nematode density averaged 66 (alive), 5 (eggs) and 0 (dead). Nematode counts from solution averaged 159 (alive), 5 (eggs) and <1 (dead). Living (alive + eggs) nematode counts from N+ *F.* cf. *torulosum* and N+ *C. inaequalis* cultures did not differ significantly in either the plug ($p = 0.289$) or solution counts ($p = 0.138$) (Fig 3). Nematode reproduction (i.e., eggs) in cultures of these two fungi did not differ significantly with plug counts ($p = 1.000$). But, for solution counts, reproduction was greater in *F.* cf. *torulosum* cultures ($p = 0.010$).

Nematode grazing was limited at the end of the 2-weeks in the N+ *P. olsonii* cultures. Hyphae appeared to be partially grazed although radial growth was not suppressed; the fungus grew rapidly and filled the entire plate (Fig 2C). While nematodes survived initially within the *P. olsonii* cultures, their activity and mobility were diminished compared to the *C. inaequalis* and *F.* cf. *torulosum* cultures. Nematodes did not reproduce within the *P. olsonii* cultures. From both the plug and solution counts, nematode density averaged 0 (alive), 0 (eggs) and <1 (dead). Compared to the N+ *F.* cf. *torulosum* and *C. inaequalis* cultures, living (alive + egg) nematode counts from *P. olsonii* cultures were significantly lower for both the plug ($p \leq 0.001$) and solution counts ($p \leq 0.001$) (Fig 3). This was also true for nematode reproduction, i.e., significantly greater reproduction in the *F.* cf. *torulosum* cultures with both plug ($p \leq 0.001$) and solution ($p \leq 0.001$) counts.

Aerial and radial growth was completely suppressed in both sets of *A. bisporus* N+ plates; the fungus was entirely grazed within the 2-weeks (Fig 2D). In the control plates, the mycelium grew and filled approximately half of the plate in the 2-weeks. Reproduction and consumption by *P. acontioides* of *A. bisporus* established the nematode as a polyphagous fungivore and potential mushroom pathogen.

Discussion

This research provides evidence that a fungivorous nematode can become endophytic by colonizing the tissues of a living host plant. In doing so, the nematode can increase the relative abundance of its preferred endophyte, or fungal cultivar, thereby shifting relative abundances of other members of the endophyte community. Because both the nematode and its fungal cultivar benefit from their interaction the association is mutualistic. However, in relation to the host plant this cultivation-based mutualism appears commensalistic. Both the nematode and its cultivar benefit from the endophytic niche provided by the host plant that is itself unaffected (i.e., no effect on plant biomass in the field or the greenhouse). However, *F.* cf. *torulosum* could in theory indirectly affect the

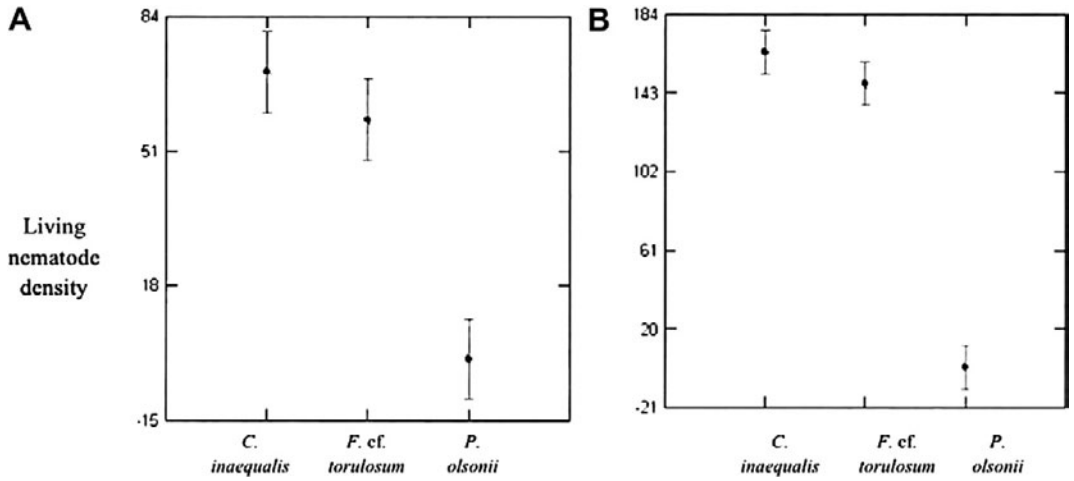

Fig 3 – Suitability assays (plug (A) and solution (B) densities for living *P. acontioides* in *C. inaequalis*, *F.* cf. *torulosum* and *P. olsonii* cultures) 2 weeks post-inoculation with ~75 living *P. acontioides*. Because plug densities were relatively low, supplemental solution densities were analyzed. Analyses for plug and solution counts were conducted using ANOVA ($F = 65.754$, $p \leq 0.001$ and $F = 296.257$, $p \leq 0.001$, respectively). Results from a pairwise comparison (using Bonferroni test) indicated that *C. inaequalis* and *F.* cf. *torulosum* were significantly ($p \leq 0.001$) more suitable for *P. acontioides* survival and reproduction than *P. olsonii*. No significant differences were detected between *F.* cf. *torulosum* and *C. inaequalis* plug ($p = 0.289$) and solution ($p = 0.138$) densities.

host plant negatively or positively if it displaced a mutualist or a parasite, respectively.

Horizontally transmitted endophytes must first infect living plants; host-mediated, differential infection may thus be the most important factor in endophyte community structure (Todd 1988; Bailey *et al.* 2005; Pan *et al.* 2008). However, our results indicate that interactions among members of the community can be significant. When nematodes were present (i.e., the Piney River and Nelson field sites, and the experiments), the relative abundance of *F.* cf. *torulosum* increased. This relationship held regardless of *B. tectorum* population; the greenhouse experiments utilized seedlings from a *B. tectorum* population from the Clearwater River, ID, USA distinct from the field populations. Our field data demonstrated that an increase in *P. acontioides* and *F.* cf. *torulosum* reduced endophyte richness, evenness and diversity. In some ecological systems, an increase in relative abundance of one species will not reduce diversity. However, when resources are limited, a community may become saturated with a few dominant species through competitive exclusion (Mouquet *et al.* 2003). The endophyte community within *B. tectorum* is seemingly such a system as evidenced by the reduction in diversity when a specific endophyte (i.e., *F.* cf. *torulosum*) became relatively abundant.

Fungivorous nematodes feed on a variety of fungi (Giannakis & Sanders 1989; Ruess & Dighton 1996; Hasna *et al.* 2007); *P. acontioides* is known to consume several different fungal species (Pillai & Taylor 1967). However, many fungivorous nematodes show a preference for particular fungi. An example is better survival of *Aphelenchoides* spp. on mycorrhizal fungi than on saprotrophic fungi (Ruess *et al.* 2000). Similarly, *Aphelenchoides bicaudatus* and *Aphelenchus avenae* perform better on *Fusarium chlamydosporum* and *Cladosporium herbarum* than on two species of *Penicillium* or the non-host, *Cladosporium cladosporioides* (Ikonen 2001). Results from our experiments provide evidence that despite the preference of *P. acontioides* for *F.* cf. *torulosum*, other fungi (i.e., *C. inaequalis*) were suitable for its consumption.

Preference may be based on avoidance of fungi toxic to the nematode (e.g., *P. olsonii*). Although grazing was initially evident in the *P. olsonii* cultures, nematode activity diminished over the 2-weeks with no surviving nematodes remaining at the end of the suitability assay. Likewise, in the preference assay (*P. olsonii*–*F.* cf. *torulosum*), nematode activity was initially observed within the *P. olsonii* colony, although it was quite limited relative to that within the *F.* cf. *torulosum* colony. Previous research has demonstrated that nematodes may find a fungus initially favorable but once toxic compounds are produced by the colony, the nematode is negatively affected (Mankau 1969; Ciancio 1995; Hasna *et al.* 2007).

The cultivation mechanism by which nematodes increase the relative abundance of *F.* cf. *torulosum* in *B. tectorum* was not definitively determined here. Previous research with plant parasitic nematodes has shown that nematodes graze more efficiently when a chemical attractant is detected (Perry 1996), and *F.* cf. *torulosum* may produce a signal that attracts *P. acontioides* and stimulates the nematode to cultivate it. Nematodes can promote fungal growth through hyphal grazing (Ingham *et al.* 1985), and fungi can compensate for this grazing pressure (Mikola & Setälä 1998). Positive correlations between fungivorous nematodes and fungal biomass have been documented (Ekschmitt & Griffiths 1998). Nematodes may carry bacteria or hyphal fragments and spores on their surfaces and within their digestive systems, dispersing the microbes as they migrate (Bird & McKay 1987; Fu *et al.* 2005). Fungus-dispersing nematodes can migrate through plant tissue (Neher 2010), and this alone may have allowed *F.* cf. *torulosum* to dominate the endophyte community of *B. tectorum*.

Bromus tectorum is an aggressive invader in western North America and it has the capacity to dominate landscapes upon introduction into new habitats (Stewart & Hull 1949; Mack 1981).

Invasive species are more abundant in their invaded than native ranges (Broennimann et al. 2007), and interaction with novel endophytes may be one of the contributing factors to an invader's success (Baynes et al. 2012). It is unclear, however, whether *P. acontioides* and *F.* cf. *torulosum* are novel symbionts for *B. tectorum*. The native range of *P. acontioides* is unknown, although many species of *Paraphelenchus* described to-date are from Asia or Europe (Carta et al. 2011). There is only one record of *P. acontioides* in the United States (Illinois) prior to the collection made from *B. tectorum* in Piney River. This sole discovery was from the rhizosphere of Kentucky bluegrass (*Agrostis stolonifera*) (Taylor & Pillai 1967; Carta et al. 2011), another grass that was introduced to North America (USDA-ARS 2012).

The systematics of *Fusarium* has reached new levels with the application of the genealogical concordance phylogenetic species recognition (GCPSR) as an approach for defining fungal species based on congruent gene trees (Taylor et al. 2000). O'Donnell et al. (2009, 2010) used this concept to define species in *Fusarium* including *F. torulosum*. In addition, the circumscription of the genus *Fusarium* has been narrowed to include only species that are related to the type species, *Fusarium sambucinum*, including those that have a *Gibberella* sexual state (Gräfenhan et al. 2011). With the transition to one scientific name for one species of fungus, all species outside of *Fusarium* in the strictest sense will be placed in other genera.

The native range of *F.* cf. *torulosum* may be like that of the fungus that it most closely resembles, namely *F. torulosum*, a synonym of *F. sambucinum* var. *coeruleum* among others (Nirenberg 1995), confirmed by Logrieco et al. (1995). *Fusarium torulosum* is reported primarily from post-harvest studies of cereals including *Avena, Hordeum* and *Triticum* but has been reported from *Betula, Buxus, Humulus, Juniperus, Quercus*, soil, *Solanum* and roots of various plants in temperate regions (Benyon et al. 2000; Desjardins et al. 2000; Kristensen et al. 2005), along with a human isolate in GenBank, and as an endophyte of *Pennisetum clandestinum* in Australia (Ryley et al. 2007). This latter report suggests that *F. torulosum* is the cause of kikuyu poisoning of livestock due to the production of mycotoxins. Kristensen et al. (2005) state that *F. torulosum*, and the related species *F. flocciferum* and *F. tricinctum*, are not known to produce trichothecenes but they predict that both *F. flocciferum* and *F. torulosum* may possess the ability to produce moniliformin. They also cite Langseth et al. (1999) who found that "a single strain of *F. torulosum* has produced moniliformin in one out of two experiments". Ryley et al. (2007) cite literature in which a number of mycotoxins are produced by *F. torulosum*. The presence/absence of toxins produced by this fungus could certainly have an influence on the nematode, host plant, herbivory and competition with other endophytes. At present, we do not know whether the interaction of *P. acontioides* and *F.* cf. *torulosum* is restricted to *B. tectorum*.

Although associated with a number of plant hosts, the literature is unclear about whether *F. torulosum* causes plant diseases. Reasons for the absence of disease in *B. tectorum* in this study may include the following: (1) *B. tectorum* is resistant to this fungus–nematode association; (2) *F.* cf. *torulosum* is functionally distinct from *F. torulosum*; and (3) *P. acontioides* reduces the pathogenicity of *F.* cf. *torulosum*.

An early monograph on grass endophytes did not list *Fusarium* as an endophyte (Bacon & Fahey 1994). However, various species of *Fusarium* and nematodes have been isolated as endophytes in more recent literature. Endophytic *F. oxysporum* suppressed the plant parasitic nematodes *Radopholus similis* (Vu et al. 2004), *Meloidogyne incognita* (Dabatat & Sikora 2007), and *Pratylenchus goodeyi* (Mwaura et al. 2010). An endophytic non-pathogenic *Fusarium solani* suppressed plant parasitic root-knot nematode in tomato. This stylet-bearing, plant-feeding nematode promoted inner root colonization by a fungus (Siddiqui et al. 2002), so there is precedent for the ability of the distantly related fungal-feeding, stylet-bearing *P. acontioides* to promote *Fusarium* colonization.

Earlier studies have shown that competitive exclusion can influence relative abundance within the endophyte community (Saikkonen et al. 1998; Schulthess & Faeth 1998). Our results indicate that mutualistic interactions need to be considered as well. We hypothesize that mutualisms are as influential as competitive exclusion in determining the structure of the endophyte community. Since endophytes contribute to larger community processes (Leuchtmann & Clay 1997; Saikkonen et al. 1998; Rudgers & Clay 2007), interactions among endophytes may have unexpectedly significant consequences. We are unaware of any other research demonstrating cultivation by a nematode of one preferred member of a fungal endophyte community. Future studies investigating the role of microfauna in cultivating specific endophytes *in planta* would be valuable to enhance our understanding of how endophyte communities are assembled and how these "bottom-up" processes may affect plant communities.

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