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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.