Fungal Ecology 20 (2016) 40–48

Contents lists available at ScienceDirect

Fungal Ecology

journal homepage: www.elsevier.com/locate/funeco

Disease suppression in winter wheat from novel symbiosis with forest fungi

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ARTICLE INFO

Article history:
Received 5 September 2015
Received in revised form
20 October 2015
Accepted 29 October 2015
Available online xxx

Corresponding editor: Luke Barrett

Keywords:
Suppressive soils
Forest microbes
Pine litter microbiome
Winter wheat
Fusarium culmorum
Penicillium
DSE

ABSTRACT

In the Pacific Northwest, USA, pathogenic *Fusarium* spp. are common in agricultural soils but rare in forest soils. We hypothesized that regional forest soils possess indigenous *Fusarium*-suppressive microbes, which may enter into novel associations with introduced crop plants, potentially contributing to suppressive soils. To test this hypothesis, we inoculated winter wheat with regional forest litter microbial communities and rhizosphere fungi and challenged plants with pathogenic *Fusarium culmorum* from regional agricultural fields. We also challenged plants with drought and low-temperature stress, which may augment disease severity. A forest litter microbiome improved survival of *Fusarium*-infected wheat three-fold and increased growth by 67%. Seedlings inoculated with individual forest fungi developed larger root systems during vernalization. *Penicillium* sp. doubled yield of *Fusarium*-infected winter wheat exposed to drought conditions. Indigenous microbes from native plant communities of the interior Northwest mitigate abiotic stresses and may contribute to suppression of a soil-borne wheat disease via novel symbioses.

1. Introduction

Disease-suppressive soils have been defined as soils in which the incidence, severity, or virulence of a soil-borne pathogen is reduced compared to adjacent soils or is lower than expected for environmental or soil conditions (Weller et al., 2002). Disease-suppressive soils have long been of interest to plant pathologists (Burke, 1954; Shipton et al., 1973; Kinkel et al., 2011), and it is now widely recognized that members of soil microbial communities are the primary mediators of disease suppression (Weller et al., 2002; Kinkel et al., 2011).

Symbioses occur when two or more highly differing organisms form a close physical association by which one or more of the organisms benefits (de Bary, 1879). Novel symbioses between indigenous microbes and introduced plants can be mutualistic (Richardson et al., 2000; Baynes et al., 2012), but they have never been shown to contribute to disease suppression in soils. Suppression of plant diseases in soils has been attributed to activities of both single microbes and microbial communities (Kim et al., 1997; Mazzola, 1999), but much of the literature has focused on disease suppression by bacterial communities (Kim et al., 1997; Mazzola, 1999) or single isolates of bacterial and fungal genera previously associated with biocontrol of pathogens, including *Pseudomonas* (Kloepper et al., 1980: Weller and Cook, 1983), non-pathogenic *Fusarium* (Larkin et al., 1996), and *Trichoderma* (Liu and Baker, 1980). Moreover, most active suppressors have been reported from agricultural fields (Liu and Baker, 1980; Scher and Baker, 1980; Weller, 1983) where crop species, tillage practices, and cropping history play a significant role in the formation and dissolution of disease-suppressive microbial communities (Cook, 1981; Mazzola, 1999; Peters et al., 2003). Disease-suppressive soils have been well documented across dryland agricultural fields in the arid interior Pacific Northwest (PNW), USA (Shipton et al., 1973; Weller and Cook, 1983; Weller et al., 2002), with their mechanism most frequently ascribed to *Pseudomonas* bacteria (Weller, 1983; Raaijmakers et al., 1997; Weller et al., 2002). More rare are studies examining the suppressive potential of microbes from undisturbed soils (Smith, 1967; Schisler and Linderman, 1984).

In the arid intermountain region of the PNW, most agricultural fields were developed from shrubsteppe grassland prairie, pine woodlands, and the margins of dry intermountain forests (Black et al., 1998). Conversion of natural communities, followed by continual annual cropping, has led to the build-up of undesirable pathogens (Cook, 1980) and a loss of indigenous diversity in the soil

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http://dx.doi.org/10.1016/j.funeco.2015.10.005
1754-5048/

microbiome (Smiley and Patterson, 1996; Fierer et al., 2013). In these converted fields, the dominant dryland crop of this region is wheat (*Triticum aestivum*), a domesticate of western Asian origin. In dryland wheat production, *Fusarium culmorum* is a significant root and crown rot pathogen (Cook, 2007). *F. culmorum* severity may be mitigated with rotational cropping, but dryland farming in the PNW, where wheat is often grown in short, 2-year rotations to maintain soil moisture, limits rotational effectiveness against outbreaks (Cook, 1980: Paulitz, 2006). Moreover, increased use of conservation tillage to reduce wind and water erosion leaves pathogen-carrying residues on the soil surface, increasing the severity and incidence of the disease (Cook, 2007). Not only are *Fusarium* crown and root rots costly in terms of plant survival and yield, but they are often most severe during periods of drought when plants are already stressed (Cook and Papendick, 1972; Papendick and Cook, 1974). Even among these intensively cultivated soils, *Fusarium*-suppressive soils have been documented, as they have throughout North America and around the world (Burke, 1954; Alabouvette et al., 1979; Lin and Cook, 1979; Sher and Baker, 1980; Larkin et al., 1996; Weller et al., 2002).

Microbial communities of forest litter and forest soils from western North America have been known to suppress pathogenicity and reduce the fecundity of *Fusarium* spp. (Schisler and Linderman, 1984), and may eventually reduce the incidence of the pathogen in the soil. For example, Smith (1967) found forest soils gradually reduced presence and viability of pathogenic *Fusarium oxysporum* in the root zone of infected *Pinus lambertiana*. In pilot surveys of coarse-fine roots of 17 native shrubs and trees in undisturbed forests and shrublands across the arid intermountain PNW, we found only eight isolates of *Fusarium* out of nearly 7000 fungal isolates (Ridout and Newcombe, unpublished data). Interestingly, 65% of all culturable root-associated microbes (both fungal and bacterial) isolated in these surveys belonged to fungal genera associated with the ability to survive xeric conditions and extreme temperatures: *Penicillium* and *Phialocephala* (Bollen and Wright, 1961; Pitt, 1973; Trowbridge and Jumpponen, 2004; Frisvad et al., 2006; Vyas et al., 2007; Houbraken et al., 2012; Knapp et al., 2012). By mediating tolerance to abiotic stresses in plant hosts (Ait Barka et al., 2006; Baynes et al., 2012; Hubbard et al., 2014), plant-associated microbiomes may also reduce the severity of soilborne pathogens, such as *F. culmorum,* that are most damaging under stressful conditions (Cook, 1973; Papendick and Cook, 1974; Gaudet and Chen, 1987).

Novel symbioses that form between indigenous microbes and exotic or non-indigenous plants can be highly beneficial to the host, forming the so-called 'enhanced mutualisms' (Richardson et al., 2000; Baynes et al., 2012). Many forest and woodland soils across the arid interior PNW have never been cultivated or exploited for field crop production (Kershaw et al., 1998; Turner and Kuhlmann, 2014). Therefore, many of the microbes found in the rhizophere soils of these indigenous forests and woodlands may well represent part of indigenous microbial communities once found in soils where dryland wheat is now cultivated. Just as indigenous microbes form enhanced mutualisms with invasive, exotic winter annuals (Baynes et al., 2012), microbes from these indigenous soil and root communities may form beneficial, novel symbioses with wheat. Given the relative absence of *Fusarium* among root-associated microbes of forests and woodlands of the arid interior PNW, we hypothesized that fungi indigenous to these forest microbial communities would form novel symbioses capable of suppressing the severity of Fusarium crown rot if inoculated into wheat under conditions favoring disease due to *F. culmorum*.

To test this hypothesis, we conducted a series of experiments with wheat, testing the *Fusarium*-suppressive effects of a litter microbial community from a PNW Ponderosa pine (*Pinus ponderosa*) woodland and individual fungi including the litter fungus *Morchella snyderi* and species of *Penicillium* and *Phialocephala* from the root microbiome of Douglas fir (*Pseudotsuga menziesii* var. *glauca*). *Penicillium* and *Phialocephala* represented the most common genera found in microbial communities indigenous to these arid forests and woodlands, and *M. snyderi* represented a genus previously associated with increased heat tolerance, growth, and fecundity in an exotic winter annual (Baynes et al., 2012). Since drought-stress augments severity of *F. culmorum* infection, a moisture deficit was added as an abiotic stress in the experiment testing the ability of these single fungi to mediate tolerance to drought stress and suppress *F. culmorum* in winter wheat. We also tested individual microbes for their ability to mediate tolerance of low-temperature stress associated with vernalization—a stress which may reduce plant fitness and ability to resist other abiotic and biotic stresses.

2. Materials and methods

2.1. *F. culmorum, drought, and wheat – effects of single species of fungi*

To determine whether xerophilic and drought-adapted fungi could promote drought tolerance in winter wheat either by directly facilitating drought resistance or by suppressing crown rot pathogens, we designed a three-factor factorial experiment with five fungal treatments, two pathogen treatments, and two levels of moisture. Seeds of a single line of hard red winter wheat (University of Idaho line 306 UI-SRG) were sown in 2 cm^3 200-cell trays nested in seed flats. Seedlings were sprouted in a growth chamber at a 23/16 °C diurnal temperature cycle with a 16 h photoperiod. Seven days after the seed was sown, fully emerged seedlings were inoculated at the crown with 1 ml of a single inoculum per seedling. Inocula selected represented major genera from litter and root microbial communities of arid, drought-adapted intermountain conifer forests of the PNW: *M. snyderi* (recovered from an ascocarp growing in litter of an intermountain conifer forest) and three fungi recovered from the root microbiome of *P. menziesii* var. *glauca*: *Phialocephala* sp. 'BHIAR', a novel xerophilic isolate of *Penicillium* (*Penicillium* sp. *nova* 'WPT111A3'), and the xerophilic *Penicillium goetzii*.

Inocula were generated from pure, 4-week-old cultures cultivated on potato dextrose agar (PDA). Plates containing the non-sporulating, mature cultures of *M. snyderi* and *Phialocephala* were flooded with sterile distilled water (SDW) and scraped with a sterile scalpel to remove the mycelium. Mycelia were then processed in a blender until finely fragmented before suspending in SDW. Final solutions of *M. snyderi* and *Phialocephala* BH1AR inocula were brought to volume at 6.3×10^4 and 2.3×10^6 fragments per ml, respectively. Plates of the sporulating *Penicillium* species *P. goetzii* and WPT111A3 were flooded with SDW and spores were loosened by passing a sterile bent glass rod over the mycelium. Spore solutions were suspended in SDW and final inocula of *P. goetzii* and WPT111A3 were brought to volume at 9.8×10^6 and 2.1×10^7 cells per ml, respectively. The fifth treatment or control consisted of SDW only.

Seedlings were returned to the growth chamber following inoculation, and the temperature was reduced to a 19/16 °C diurnal cycle. Four days after inoculation, growth chamber temperatures were reduced to a 5/5 °C diurnal cycle, and photoperiod was reduced to a 10 h daylength. Plants were vernalized at this temperature and daylength for 8 weeks, at which time seedlings were removed from the growth chamber. During vernalization, seedlings were fertilized with 24-8-16 N–P–K at 300 mg l^{-1} nitrogen every 2 weeks. Observations were taken before 85 seedlings were transplanted.

Following vernalization, seedlings were potted into 3 l pots containing 323 g of soilless potting mix per liter. Plants were placed in a greenhouse at 21/15 °C with a 16 h photoperiod. The plants