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Mycorrhizal composition can predict foliar pathogen colonization in soybean

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HIGHLIGHTS

- Foliar pathogen colonization is dependent on AM-fungal identity.
- Nitrogen application also limits pathogen colonization.
- Bioprotection can be voided by inter-specific competition among AM-fungi.

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ABSTRACT

Arbuscular mycorrhizal (AM) fungi may contribute to plant protection against pathogens. However, AM-fungal bioprotection may depend upon AM-fungal species identity and plant-pathosystem. Here, the aim is to determine if AM-fungal composition can alter *Pseudomonas syringae* pv. *glycinia's* (*Psg*) effect on soybean (*Glycine max*). Two experiments were performed simultaneously. The first experiment assessed the effect of soil treatment on pathogen (*Psg*) growth. While the second experiment assessed the interactive effects of *Psg* and soil treatment on soybean growth. In the first experiment, mycorrhizal composition and soil nutrients (nitrogen and phosphorous) were manipulated for *Glycine max* under growth chamber conditions. Mycorrhizal treatments included four single species of AM-fungi (*Entrophospora infrequens*, *Funneliformis mosseae*, *Claroideoglomus claroideum*, and *Racocetra fulgida*) and a mix (Fungal Community) of all four species. Three nutrient addition treatments included nitrogen (N), phosphorous (P), and nitrogen with phosphorous (NP). *Psg* colonization was assessed at 40 and 120 h post infection (HPI). In the second experiment, also under growth chamber conditions, soybean biomass in response to the interactive effect of *Psg* and soil environments (AM-fungal community, N, P, NP, and control) was assessed after a four month growing season. AM-fungal species *Entrophospora infrequens* reduced *Psg* colonization, while three other fungal species did not (*F. mosseae*, *C. claroideum*, and *R. fulgida*). Addition of supplemental nitrogen inhibited *Psg* colonization, suggesting a resource provisioning mechanism of AM-fungal bioprotection. Assessment of plant growth revealed that an AM-fungal inoculum mix increases soybean leaf mass over a four month growing period. Meanwhile, *Psg* markedly increased stem mass. An interaction between AM-fungi and *Psg* on plant growth was not detected. In mixed communities, AM-fungal sporulation was only detected for a single species (*F. mosseae*). These findings provide insight onto the role of AM-fungal identity in bioprotection against a foliar pathogen. Although additional work is needed to fully determine ecological processes that provide selective advantages to host plant, these findings indicate that such ecological processes include nutrient provisioning and competition among AM-fungi. Together, these processes may have an underlying role in bioprotection.

1. Introduction

Although ecologists have focused on how species interactions and resource abundance affect trophic levels (Hairston et al., 1960; Leibold, 1989; Moore et al., 2004; Reynolds et al., 2003; Wall and Moore, 1999), the role of mycorrhizal symbionts may be just as important. AM-fungi are plant root symbionts that supplement their host with phosphorous (P) and nitrogen (N) and may alter plant-enemy interactions (Bennett et al., 2006; Gianinazzi-Pearson, 1996). While increasing resource quantity may not have a direct effect on plant enemies, its enhancements may lead to greater exploitation of the host plant by the plant enemy (Bennett et al., 2006). Alternatively, the propensity of AM-fungi

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to increase plant vigor may provide bioprotection against insects, pests, and pathogens (Harrier and Watson, 2004; Pozo et al., 1999; Ryan et al., 1994; Zhang et al., 2008)

Plants that associate with AM-fungi have been shown to have increased resistance or tolerance toward enemies (Bennett and Bever, 2007; Jung et al., 2012; Li et al., 2013). Induced systemic resistance is the alteration of plant hormonal balance by beneficial microbes which impacts pathogen performance at distal tissues (der Ent et al., 2009; Pieterse and Zamioudis, 2014; Pozo and Azcón-Aguilar, 2007). While tolerance includes the ability to grow vegetatively despite enemy damage (Strauss and Agrawal, 1999), Gruntman and Novoplansky (2011) quantified tolerance as an index scoring the physical difference between damaged and non-damaged plants. In the context of fungal mediated bioprotection, tolerance or resistance can result from either direct or indirect protection provided by AM-fungi. Direct protection involves the ability of AM-fungi to compete with pathogens for colonization, space, and photosynthates (Harrier and Watson, 2004). This may explain the observation that root lesion nematode (*Pratylenchus penetrans*) and root knot nematode (*Meloidogyne exigua*) abundance and colonization were inversely proportional to AM-fungal abundance (Peña and Echeverría, 2006; Schwob et al., 1999). AM-fungi can also provide indirect protection against intracellular root pathogens by increasing lignification of root mass, thickening host cell wall with pectin, inducing chitinase activity and enabling localization of *PATHOGENESIS-RELATED-1A* to the site of the intracellular pathogen, *Phytopthora parasitca* (Gianinazzi-Pearson, 1996). Similarly, AM-fungi may have an indirect effect on above ground herbivores. In milkweed, mycorrhizal abundance has been observed to increase host-plant phosphorous levels and caterpillar growth rate (Vannette and Hunter, 2013). However, AM-fungi can provide additional protection against herbivores by facilitating the recruitment of herbivore-enemy by altering plant volatile composition (Schausberger et al., 2012). While at the same token, species of AM-fungi may vary in their effect on host tolerance and host chemical defense (Bennett and Bever, 2007; Bennett et al., 2009).

AM-fungi also have the ability to modify plant-pathogen interactions by affecting defense signaling. In rice, AM-fungi induce defense genes of the salicylic acid (SA) pathway, including *pathogenesis related-1* (*PR1*) and *non-expresser of PR-1* (*NPR1*), as well as transcription factors and calcium (Ca^{2+}) -mediated signaling genes (Campos-Soriano et al., 2012). In tomato, AM-fungi elicit systemic induced resistance by enabling a threefold increase of jasmonic acid (JA) pathway defense genes that code for lipoxygenases (*LOX*) and phenylalanine ammonia lyase (*PAL*) (Nair et al., 2014). Observations of AM-fungal effects on disease have been variable. For instance, AM-fungal species *Rhizophagus intraradices* enhances disease severity of *tobacco mosaic virus* infection (*TMV*), and disease severity of *Boltrytis cinera* infection (Shaul et al., 1999). In contrast, other case studies have shown the exact opposite trend where *Rhizophagus intraradices* reduced disease severity of blast fungus in rice and *Phytopthora sojae* in soybean (Campos-Soriano et al., 2012; Yuanjing et al., 2013). Mycorrhizal composition and the particulars of the plant-pathosystem is likely to influence the role of AM-fungi in bioprotection.

To date, research on AM-fungal modulation of induced systemic resistance has narrowly focused on a few species of AM-fungi, such as *Rhizophagus intraradices* and *Funneliformis mosseae* (Cordier et al., 1998; Elsen et al., 2008; Elsharkawy et al., 2012; Khaosaad et al., 2007; Pozo et al., 2002; Saldajeno and Hyakumachi, 2011; Slezack et al., 2000). There is a need to study additional species of AM-fungi and to test the potential for synergistic effects of an AM-fungal community on plant-enemy outcomes. This is the first study to investigate the effect of multiple AM-fungal species on leaf pathogen colonization. Here, the role of AM-fungi in moderating infection of the crop plant *Glycine max* (soybean) by the bacterial pathogen *Pseudomonas syringae* pv. *glycinea* (*Psg*) is investigated. The following questions are addressed: (1) Do select species of AM-fungi differ in their ability to inhibit *Psg* colonization? (2) What are the effects of AM-fungi and *Psg* on soybean growth? (3) What effect does *Psg* have on AM-fungal sporulation? Given the potential of AM-fungi to facilitate resource acquisition, it is hypothesized that AM-fungi will reduce *Psg* colonization through a nutrient provisioning mechanism.

2. Methods

2.1. Study system

Glycine max (soybean) is a global source of vegetable oil (Yuanjing et al., 2013) and animal feed (Barrett, 2006). A proportion of soybean yield loss is due to *Pseudomonas syringae* pv. *glycinea* (*Psg*), the causal agent of bacterial blight (Williams and Nyvall, 1980). *Psg* can infect young and mature soybean plants through stomatal openings on the underside of leaflets. Disease symptoms include lesions and small reddish-brown spots that can be observed on leaves, stems, petioles, and pods, as well as deteriorating leaf mass. *Psg* can passage via precipitation while disseminating into irrigation systems and agricultural fields (Morris et al., 2008). Optimal conditions for this pathogen are moist soil surfaces at temperatures ranging from −12 °C to 4 °C (Park and Lim, 1985). Due to overwintering of *Psg*, bacterial blight is most prevalent in the early growing season and can be transmitted from soil to seed after winter subsides (Park and Lim, 1985). The ability of *Psg* to persist between seasons makes *Psg* a threat to soybean (Park and Lim, 1985).

2.2. Experimental design

Two experiments were performed at the same time under growth chamber conditions (Table 1). In one experiment, the growth of *Psg* was assessed in response to soil treatment. Soybean plants were grown under varying mycorrhizal and nutrient treatments in a randomized block design. Plants were assigned nine different soil treatments. These treatments included four single species of AM-fungi, all four species of AM-fungi (fungal community), nutrient treatments (N, P, NP), as well as a control (soil treated with neither nutrients nor AM-fungi). Each treatment

Table 1
Experimental Design of Experiment I & II.

Soil Treatment		Traits/Response Variables
Exp. 1: Psg Colonization in response to Treatment		
Control (n = 6)	+Psg	Psg Colony Forming Units (CFU) and Chlorophyll Content
Claroideoglomus claroideum (n = 7)	+Psg	
Entrophospora infrequens (n = 7)	+Psg	
Funneliformis mosseae (n = 6)	+Psg	
Fungal Community (n = 7)	+Psg	
Racocetra fulgida (5)	+Psg	
Nitrogen (N) (n = 6)	+Psg	
Phosphorous (P) (n = 5)	+Psg	
Nitrogen + Phosphorous (NP) (n = 5)	+Psg	
Exp. 2: Interactive effect of Psg and Treatment		
AM – Fungal Community (n = 8)	+/−Psg	Stem Mass, Leaf Mass, Pod Mass, and AM-fungal Sporulation
Nitrogen (N) (n = 8)	+/−Psg	
Phosphorous (P) (n = 8)	+/−Psg	
Nitrogen + Phosphorous (NP) (n = 8)	+/−Psg	
Control (n = 8)	+/−Psg	

Experiment 1 assesses bacterial leaf pathogen (Psg) in response to soil treatment. *Experiment 2* assesses plant phenological traits in response to soil treatment and pathogen, as well as AM-fungal sporulation in response to pathogen. Number of replicates are in ().