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Impacts of biocontrol products on Rhizoctonia disease of potato and soil microbial communities, and their persistence in soil☆

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ABSTRACT

Four commercial biocontrol formulations (*Bacillus subtilis* GB03, *Burkholderia ambifaria* type Wisconsin isolate J82, *Trichoderma virens* GI-21, and *Trichoderma harzianum* strain T-22), a chemical seed treatment (thiophanate-methyl, mancozeb, and cymoxanil mixture, TMC), and a combination chemical/biological treatment, were compared with no-pathogen and pathogen-treated controls, and monitored in two field seasons in Maine for their effects on the development of Rhizoctonia disease of potato and soil microbial community characteristics. All treatments reduced the incidence and severity of stem canker (37–75% reduction) relative to the pathogen control over both years, with the best control provided by *B. subtilis* and the combination chemical/biological treatment (TMC/Bamb). Both bacterial treatments (*B. subtilis* and *Bu. ambifaria*) reduced severity of black scurf in both years, and *T. virens* reduced scurf in one year, with reductions of 11–20% relative to the pathogen control. Over both years, the *B. subtilis, T. virens*, and TMC/Bamb treatments increased total and marketable yield, and *Bu. ambifaria* increased marketable yield, by 11–15% relative to the pathogen control. Substantial populations of the added fungal agents, but not the bacteria, were detected in bulk soil at the end of the growing season. Biocontrol treatments also significantly ($P < 0.05$) affected soil microbial community characteristics, as assessed by single carbon source substrate utilization (SU) and whole soil fatty acid methyl ester (FAME) profiles. Bacterial biocontrol treatments generally resulted in higher microbial activity and substrate utilization. Some effects on soil microbial communities were also observed the following spring (1 yr after application). This research indicates that biocontrol treatments can assist in the control of Rhizoctonia disease of potato, persist in soil to some degree, and have significant effects on soil microbial communities long after application.

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1. Introduction

The fungal plant pathogen *Rhizoctonia solani* Kühn causes stem and stolon canker on developing potato (*Solanum tuberosum* L.) plants, and also produces black scurf (sclerotia) on potato tubers, resulting in seedling losses and reduced tuber yield, quality, and marketability (Stevenson et al., 2001). Current control measures focus on cultural practices, such as crop rotation, promoting rapid emergence of sprouts, and early harvest of tubers, as well as chemical seed treatments and host resistance (Olanya et al., 2009; Powelson et al., 1993; Secor and Gudmestad, 1999; Stevenson et al., 2001). Although these practices provide some control, they are not always practical or effective, and Rhizoctonia disease remains a persistent problem wherever potatoes are grown. Both tuberborne and soilborne inoculum are important in disease development, with tuberborne inoculum most closely associated with emergence problems and stem canker, and soilborne inoculum more associated with stolon damage and black scurf (Frank and Leach, 1980). In general, seed treatments have been somewhat effective in controlling disease due to tuberborne inoculum, but less effective in controlling disease from soilborne inoculum.

Biological control is an additional strategy that may help provide effective and sustainable management of this disease. Biological control of diseases caused by *Rhizoctonia* on other crops using a variety of bacteria and fungi are well-documented (Asaka and Shoda, 1996; Lewis et al., 1998; Mao et al., 1998; Sczech and Shoda, 2004; Thrane et al., 2001), but only limited studies with biological control on Rhizoctonia disease of potato in the field have

☆ Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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been conducted (Larkin, 2008; Larkin and Tavantzis, 2013; Mrabet et al., 2013). *Trichoderma* spp., including *T. virens* and *T. harzianum*, have been observed to reduce Rhizoctonia disease of potato under field conditions, including the reduction of stem canker and black scurf (Grosch et al., 2006; Tsror et al., 2001). Other biocontrol agents, including *Verticillium bigutatum*, *Laetisaria arvalis*, and binucleate *Rhizoctonia* spp., have all shown some success in reducing Rhizoctonia disease of potato under field conditions (Harris and Adkins, 1999; Jager and Velvis, 1985 Murdoch and Leach, 1993; Sneh, 1991). Brewer and Larkin (2005) screened a wide variety of these and other potential biocontrol organisms for control of Rhizoctonia disease of potato in greenhouse trials and found *B. subtilis* GB03 and *T. virens* GL-21 to be among the most effective agents for control of Rhizoctonia disease, reducing both stem canker and black scurf severity by about 40% over multiple trials.

Burkholderia ambifaria, and other members of the *Burkholderia cepacia* complex, which consists of 10 closely related species (or genomovars) of highly versatile soil bacteria, have also been shown to be effective biocontrol agents against a number of different soilborne plant diseases (Hebbar et al., 1998; Mao et al., 1998; Roberts et al., 2005). However, isolates from the *Bu. cepacia* complex have also been shown to be opportunistic human pathogens of patients with cystic fibrosis (CF) and some other individuals with compromised immunity (Coenye et al., 2001; Holmes et al., 1998; Parke and Gurian-Sherman, 2001). Unfortunately, effective biocontrol isolates may also pose a risk for CF patients, which has resulted in these organisms being discontinued from commercial biocontrol formulations and agricultural use severely limited or discouraged (Coenye et al., 2001; Parke, 2000; Speert, 2001). However, these organisms are widespread and abundant in the natural environment, and have continued to generate interest for their biocontrol activity (Quan et al., 2006; Ren et al., 2011; Zhan et al., 2011). Thus, although no longer commercially available as a biocontrol agent, we still included *B. ambifaria* in this study because it is important to understand its activity, as well as its fate and persistence in the soil environment.

An aspect of biocontrol applications that may be critical for sustained disease control, but is poorly understood, is the persistence of biocontrol agents in the soil and rhizosphere and the interaction of introduced microorganisms with soil microbial communities (Paultiz, 2000). Biocontrol is sometimes promoted as being potentially long-lasting due to the survival and increase of microbial antagonists in the soil (Larkin et al., 1998), but it has also been noted that added organisms generally cannot compete with the native microflora in most soils and do not persist, or cannot sustain themselves at the populations necessary for adequate disease control (Cook, 1993). However, there is little information available on the fate and effects of most biocontrol organisms after application (Longo et al., 2009; Paultiz, 2000). Although it is accepted that biocontrol agents are greatly influenced by the inherent soil microbial communities and, in turn, affect those communities, not much is known regarding these interactions and their relationship to effective biological control. Addition of biocontrol agents may affect microbial communities by displacing, suppressing, or inhibiting particular microorganisms, or by stimulating others, or through changing the microbial environment in some way that affects other organisms (Handelsman and Stabb, 1996).

The use of community-level approaches to characterize soil microbial communities, such as sole carbon source substrate utilization (SU) profiles (Garland and Mills, 1991; Grayston et al., 1998; Zak et al., 1994) and soil fatty acid profiles based on fatty acid methyl esters (FAME) (Cavigelli et al., 1995; Ibekwe and Kennedy, 1999; Larkin, 2003, 2008) enable detailed characterizations of functional and structural aspects of soil microbial communities. These techniques have been used to detect and characterize differences in soil microbial communities based on varying soils and land use (Ibekwe and Kennedy, 1999; Yao et al., 2000), crop rotations (Larkin, 2003; Larkin and Honeycutt, 2006; Larkin et al., 2010, 2011; Lupwayi et al., 1998), and plant species present (Fang et al., 2001; Miethling et al., 2000; Siciliano et al., 1998), although these approaches do not specifically identify the microbial taxa involved. This information combined with traditional approaches, such as microorganism population data from dilution platings, can enable a more comprehensive assessment of changes in multiple aspects of soil microbial community characteristics (Larkin, 2003).

The objectives of this research were to 1) evaluate the efficacy of several readily-available biocontrol formulations in controlling Rhizoctonia disease of potato and improving tuber yield in the field, 2) evaluate persistence of the biocontrol organisms in the soil, and 3) evaluate any effects of the biocontrol applications on soil microbial community characteristics.

2. Materials and methods

2.1. Treatments

Two bacterial and two fungal biocontrol formulations were evaluated in field trials along with a chemical seed treatment and a combination chemical/biological treatment, in relation to pathogen-only and nontreated (no pathogen added) controls. The bacterial biocontrol agents were *Bacillus subtilis* GB03 (Kodiak; Bayer CropScience, Research Triangle Park, NC) and *Burkholderia ambifaria* type Wisconsin isolate J82 (previously available as Deny; Market VI LLC, Shawnee, KS). The fungal biocontrol agents were *Trichoderma virens* Gl-21 (SoilGard 12G; CertisUSA, Inc., Columbia, MD) and *Trichoderma harzianum* strain T-22 (RootShield; BioWorks, Inc., Geneva, NY). These organisms were chosen because they were reported to control *Rhizoctonia*, although not all are currently labeled for use on potatoes. A standard chemical seed treatment, a combination of thiophanate-methyl, mancozeb, and cymoxanil (TMC) (Evolve; Bayer CropScience), was included for comparison with the biocontrol agents and also used in combination with *Bu. ambifaria* as a combined chemical/biological treatment (TMC/Bamb). All treatments, with the exception of the chemical seed treatment, were applied in-furrow at the time of planting. The bacterial products were applied as a liquid, 500 ml/plot of a 5% solution from Deny liquid concentrate, or 1.5 g/L suspension of Kodiak (two 6.1-m rows/plot). Dilution plating of the bacterial suspensions on 0.1 Tryptic Soy Agar determined cell concentrations to be ~1.2×10^8 and 1.3×10^6 Colony Forming Units (CFU)/ml suspension for *B. subtilis* and *Bu. ambifaria*, respectively. The fungal treatments were granular formulations applied at the rate of 100 g/plot. Colony counts for the fungal formulations (plated on potato dextrose agar) were $6.5–7 \times 10^6$ CFU/g formulation for both *T. virens* and *T. harzianum*. The chemical treatment was applied to seed prior to planting at the rate of 35 g/40 seed pieces/plot. Based on preliminary experiments, natural pathogen inoculum levels of *Rhizoctonia solani*, and thus Rhizoctonia disease levels, were low in the soils of these fields, thus pathogen inoculum was added at the beginning of the experiment. Inoculum of *Rhizoctonia solani* AG-3 isolate RS31B was applied in the form of 100 g of cracked wheat colonized by *R. solani* (4×10^6 CFU/g) added to the furrow of each plot at the time of planting for all treatments except the no-pathogen control. Biocontrol treatments were evaluated in two consecutive field seasons.

2.2. Field design

Field plot experiments were established at the USDA Research