Crop Protection 96 (2017) 59–67

Contents lists available at ScienceDirect

Crop Protection

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

Consistency of control of damping-off of cucumber is improved by combining ethanol extract of *Serratia marcescens* with other biologically based technologies

Daniel P. Roberts*, Laurie F. McKenna, Jeffrey S. Buyer

Sustainable Agricultural Systems Laboratory, USDA-ARS, Beltsville Agricultural Research Center, Beltsville, MD 20705, United States

ARTICLE INFO

Article history:
Received 13 September 2016
Received in revised form
22 December 2016
Accepted 14 January 2017

Keywords:
Cover crops
Natural products
Pythium ultimum
Seed treatments
Trichoderma virens

ABSTRACT

Disease control tactics that rely less on synthetic pesticides are needed that are consistently effective in soils that vary with regard to their biotic and abiotic components. An ethanol extract of *Serratia marcescens* N4-5, when applied as a cucumber seed treatment, effectively suppressed damping-off of cucumber caused by *Pythium ultimum* in a natural sandy loam soil and a natural sand soil but not in a natural loam soil. A combination treatment containing seed treatment with this N4-5 ethanol extract and a drench containing *Trichoderma virens* GL21 improved disease control performance relative to individual application of both of the treatment components. Plant stand associated with this combination treatment was significantly greater than the no-treatment control in all three natural soils at all levels of pathogen inoculum used. In some cases plant stand associated with this combination treatment was significantly greater than that resulting from individual application of N4-5 ethanol extract or *T. virens* GL21. Data presented here indicate that strategic combinations of biologically based disease control tactics can increase consistency of performance in multiple soils that vary in biotic and abiotic characteristics.

Published by Elsevier Ltd.

1. Introduction

Pythium ultimum Trow is an important soil-borne oomycete that causes disease under favorable environmental conditions on greater than 300 diverse plant species (Kamoun et al., 2015; Okubara et al., 2014). Seed treatment with pesticides, especially mefenoxam when available, can be very effective in managing damping-off caused by this pathogen (Garzón et al., 2011). However, there are concerns regarding the development of resistance to pesticides in pathogen populations (Lamour and Hausbeck, 2000; Moorman and Kim, 2004; Okubara et al., 2014; Taylor et al., 2002). Alternate control measures such as crop rotation can be ineffective due to the large host range of *P. ultimum* and the ability of this pathogen to persist in soil for extensive periods of time. Resistant cultivars can be a powerful tactic for disease control, however, cultivars resistant to this pathogen are limited in effectiveness (Louws et al., 2010; Okubara et al., 2014).

In prior work, we demonstrated the ability of seed treatment with cell-free ethanol extract of *Serratia marcescens* Bizio N4-5 to provide suppression of damping-off of cucumber caused by *P. ultimum* (Roberts et al., 2007, 2014; 2016). Seed treatment with N4-5 ethanol extract has potential for use in cucumber production systems as it resulted in suppression of damping-off caused by *P. ultimum* that was equivalent to that provided by the seed treatment pesticide Thiram in certain planting media and had a shelf-life of at least 14 weeks (Roberts et al., 2014). This work was focused on seed treatment due to the fact that some crops, such as cucumber, have a very short window of vulnerability to this disease and hence an infection court limited in time and space; allowing inundation of the infection court with N4-5 ethanol extract at the time of seed treatment (Fukui et al., 1994; Hadar et al., 1983; Nelson, 1988; Roberts et al., 1997, 2007; Windstam and Nelson, 2008).

Biologically based control tactics, such as N4-5 ethanol extract, and other tactics directed at control of soil-borne pathogens must be tested in a number of soils as biotic (e.g. genetic structure of target and non-target pathogen populations and soil microbial community) and abiotic (e.g. pH, mineral content, oxygen tensions) factors in soil can influence disease control efficacy and consistency

* Corresponding author. Building 001, Rm. 245B, 10300 Baltimore Avenue, Beltsville, MD 20705 United States.
E-mail address: dan.roberts@ars.usda.gov (D.P. Roberts).

http://dx.doi.org/10.1016/j.cropro.2017.01.007
0261-2194/Published by Elsevier Ltd.

(Roberts and Kobayashi, 2011). Indeed, N4-5 ethanol extract provided inconsistent control of damping-off of cucumber caused by *P. ultimum* between two different natural soils in past experiments (Roberts et al., 2016). Control associated with seed treatment with N4-5 ethanol extract was equivalent to that provided by seed treatment with Thiram in a sandy loam soil but ineffective and inconsistent in a loam soil.

Here we tested combinations of N4-5 ethanol extract with other biologically based control measures for increased efficacy and consistency of control of damping-off of cucumber caused by *P. ultimum* in several natural soils. It is thought that combinations of biologically based disease control tactics can improve consistency of performance (Lemanceau and Alabouvette, 1991; Pierson and Weller, 1994; Raupach and Kloepper, 1998). Specifically, we *i*) screened cover crops for suppression of damping-off of cucumber caused by *P. ultimum*. Chemical compounds released from cover crops affect plant pathogens and pests, and other soil microbes (Akhtar and Malik, 2000; Bailey and Lazarovits, 2003; McSorley, 2011; Zhou and Everts, 2004). We also *ii*) tested combinations of seed treatment with N4-5 ethanol extract, cover crops identified in this screen, and a biological control isolate of *Trichoderma virens* that was shown to be compatible with N4-5 ethanol extract (Roberts et al., 2005, 2016) for increased consistency of disease control over soils that differed in abiotic and biotic characteristics.

2. Materials and methods

2.1. Preparation of cover crop biomass

BMR sorghum-sudangrass (*Sorghum bicolor* × *S. bicolor* var. *Sudanese*), sunn hemp (*Crotalaria juncea* cv. Tillage Sunn), velvet bean (*Mucuna pruriens*), jack bean (*Canavalia ensiformis*), an Indian mustard (*Brassica juncea*) + white mustard (*Sinapis alba*) mixture, Martigena (*Brassica hirta* cv. Martigena), oilseed radish (*Raphanus sativus*), and Dwarf Essex rape (*Brassica napus*) were grown in 14 × 20 inch flats containing commercial potting mix (Pro-Mix PGX, Premier Horticulture, Inc. Quakertown, PA) for 16 weeks (cover crops were starting to flower) in the greenhouse, the shoots harvested, dried for 10 days at 160 °C, ground with a Wiley mill (#20 mesh screen), and stored in the dark at room temperature until used. All seeds were from Johnny's Select Seeds, Winslow, ME with the exception of velvet bean and jack bean which were the gift of Dr. Waldy Klassen (retired), Univ. FL Tropical Research and Education Center, Homestead, FL.

2.2. Microbial strains, seed treatments, and formulations

S. marcescens N4-5, *Enterobacter cloacae* 501R3, *Pseudomonas protegens* Pf-5, and *Trichoderma virens* GL21 have all been shown to control *P. ultimum* on cucumber (Loper et al., 2007; Roberts et al., 1997, 2005; 2007). Strains N4-5, 501R3, and Pf-5 were routinely grown on nutrient broth (NB) or nutrient agar (NA) while isolate GL21 was grown on potato dextrose agar. *P. ultimum* Puzc is pathogenic on cucumber (Roberts et al., 1997, 2005; 2007) and was maintained on corn meal agar at room temperature. All microbial isolates were from the USDA-ARS, Sustainable Agricultural Systems culture collection.

Dried ethanol extract was prepared from *S. marcescens* N4-5 grown on peptone glycerol (PG) agar plates at 28 °C as described (Matsuyama et al., 1985; Roberts et al., 2007). For treatment of cucumber seed (*Cucumis sativum* cv. Marketmore 76; no pesticide), dried N4-5 ethanol extract was resuspended in ethanol, incubated with seed for 30 s (4 PG agar plates produced 2 mL ethanol extract which treated 3.5 g seed), and dried under a laminar flow hood. The control was seed incubated in ethanol but no N4-5 ethanol extract. *E. cloacae* 501R3 and *P. protegens* Pf-5 were grown overnight in NB, washed and resuspended in sterile distilled water (SDW), and applied to seed in a gelatin formulation essentially as described (Roberts et al., 2005). Seed coated with gelatin plus SDW, but no microbes, was the control. Formulated *T. virens* GL21 was prepared on Biodac (Kadant Gran Tek, Inc. Green Bay, WI) in mycobags (Unicorm Imp. and Mfg. Corp. Commerce, TX) as described by Roberts et al. (2010). For preparation of drenches for in-furrow application of *T. virens*, 50 g Biodac formulation was suspended in 200 mL SDW and ground with a tissue homogenizer (Ultra-Turrax T 25 basic, IKA-Werke, Staufen, Germany) prior to application. The control was sterile Biodac in SDW.

2.3. Abiotic and biotic characterization of natural soils

Physical-chemical characteristics of natural soils #2 (Ultisol, loam, 106 ppm available P, 48 ppm K, 50 ppm Mg, 250 ppm Ca, 3.1 CEC, 3.0% organic matter, pH 4.5), #11 (Ultisol, sandy loam, 108 ppm available P, 141 ppm K, 60 ppm Mg, 620 ppm Ca, 6.3 CEC, 4.8% organic matter, pH 5.1), #13 (Ultisol, sand, 12 ppm available P, 40 ppm K, 65 ppm Mg, 240 ppm Ca, 4.0 CEC, 2.3% organic matter, pH 4.7), #15 (Inceptisol, loam, 41 ppm available P, 134 ppm K, 105 ppm Mg, 480 ppm Ca, 5.8 CEC, 1.4% organic matter, pH 5.1), and #18 (Ultisol, sandy loam, 32 ppm available P, 31 ppm K, 45 ppm Mg, 170 ppm Ca, 3.1 CEC, 2.7% organic matter, pH 4.6) were determined by A&L Eastern Laboratories, Inc. (Richmond, VA). All soils were collected from the Beltsville Agricultural Research Center.

For determination of differences in the soil microbial community between treatments, sunn hemp and sorghum-sudangrass cover crops, prepared as above, were mixed with natural soils #2, #11, #13, #15, and #18 at a rate of 0.5% (*w/w*), or no cover crop was added, the soils incubated for three days at 22 °C, and applied on top of potting mix in cups as indicated below. Cucumber seed was layered on top of the soil and a layer of soil treatment applied on top of the seed. Cups containing these treatments were incubated in the growth chamber at 22 °C for 14 days as for the disease assay described below. Soil microbial biomass and community composition were determined by phospholipid fatty acid (PLFA) analysis according to a previously published protocol (Buyer and Sasser, 2012). The experiment was performed twice with treatments replicated three times. Experiments were analyzed independently.

2.4. Suppression of damping-off of cucumber caused by P. ultimum

Experiments to determine suppression of damping-off of cucumber caused by *P. ultimum* were performed essentially as described (Roberts et al., 2005, 2007; 2016) using potting mix and natural soils #2, #13, #15, and #18 as the planting medium. Differences in abiotic and biotic characteristics of these soils are listed above and in Fig. 1. Formulations and cucumber seed treatments were prepared as described above. Treatments applied are stated in Tables 1 through 6. Treatments containing cover crops had cover crops mixed homogeneously into the planting medium at 0.5% (*w/w*) three days prior to infestation with *P. ultimum.* The Biodac granular formulation used in these experiments contained approximately 7.0 $\log_{10}$ CFU *T. virens* per g, with 1 g being applied to the seed region as a drench. Seed treated with live bacteria contained approximately 7.0 $\log_{10}$ CFU per seed.

To produce sporangia, *P. ultimum* was grown at 25 °C for 3 days, flooded with sterile soil extract (Ayers and Lumsden, 1975), and incubated at 25 °C for 7–28 days. Sporangia from these plates were washed and incorporated into potting mix. Potting mix, potting mix incorporated with cover crop (where appropriate) amended with sporangia of *P. ultimum* or with SDW, treated seeds or non-treated seeds, and potting mix amended with sporangia of