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Fungal and oomycete pathogen detection in the rhizosphere of organic tomatoes grown in cover crop-treated soils

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ABSTRACT

Soil management practices, including the use of cover crops, affect soil and plant health through varied mechanisms. Impacts on microbial communities are known to be important, but are not well understood. Various techniques are used to measure the effect of treatments on microbial communities, but rarely are the results of more than one technique compared. This field study examined the impacts of a single-season application of cover crops on detection of pathogen species in the tomato crop rhizosphere. The study took place in Maryland, New York and Ohio (MD, NY and OH) in the summers of 2010 and 2011, with a total of 260 plots tested using both macroarray and T-RFLP analyses. The macroarray used in this study was specifically designed to detect thirty-one pathogens of solanaceous crops and had not previously been used for such a field study. The results of T-RFLP analysis, which is a common tool for examining microbial communities, were compared to the macroarray results and the limitations and benefits of each are presented. While not a quantitative measure, the macroarray was able to detect certain fungi with much greater sensitivity than T-RFLP. Our findings suggest that the results of PCR-based techniques used for microbial community studies should be compared to other methods to verify sensitivity.

1. Introduction

Plant type, soil type and management practices all affect the microbial community structure of the soil ecosystem. Plants induce changes in soil microbial communities because the rhizosphere of plants encourages diverse and abundant microbial communities due to chemical exudates, mucilage production, improved aeration and moisture retention (Angers and Caron, 1998). Likewise, microbial populations in the rhizosphere influence plant health, both directly and through interactions with other soil microbes (Kim et al., 2011; Whipps, 2001). Different plant species have been found to encourage distinct microbial species populations in the rhizosphere after only four weeks of plant growth (Grayston et al., 1998).

The interaction of microorganisms with each other and with plants both result in differences in plant growth and in the extent to which disease can be suppressed in the agroecosystem (Garbeva et al., 2004). Suppressive soils are soils that limit the survival, growth or disease causing activity of plant pathogens. Suppression can be general or specific. General suppression reduces fungal, oomycete and nematode damage. Mechanisms of action are unclear and dynamic, however, suppression often appears to be due to total microbial biomass (Weller et al., 2002). Cover crops have been found to increase soil microbial biomass, which could allow for general suppression (Mendes et al., 1999; Schutter and Dick, 2002). Research supports the role of enhanced microbial diversity in the disease suppression exhibited by cover crops (Abawi and Widmer, 2000; Mazzola, 2004; Van Bruggen and Semenov, 1999).

Abbreviations: T-RFLP, terminal restriction fragment length polymorphisms; GLM, generalized linear model; CCT, cover crop treatment; HSD, Honestly Significant Differences; TRF, terminal restriction fragment; Aa, *Alternaria alternata*; Fo, *Fusarium oxysporum*.

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However, while research supports this association, the profile of microbial communities involved and their role in soilborne disease control are not well understood. Benitez et al. (2007) observed that disease suppression of damping-off on tomato and soybean increased following a mixed-species hay cover crop. Furthermore, terminal restriction fragment length polymorphism (T-RFLP) analysis of bacterial communities was used to examine the rhizosphere of crop plants and correlate suppression to members of the genera *Burkholdaria*, *Bacillus*, *Paenibacillus*, and *Streptomyces*, all genera previously found to contain beneficial species. Larkin and Griffin (2007) observed suppression of various soilborne pathogens of potato in the field. The suppression occurred for all species of cover crop tested, which implies a role for microbial communities in the soil. A study of the short-term effects of an oat-vetch mixture on the growth of damping off pathogens, *Pythium aphanidermatum* (Edson) Fitzp. and *Rhizoctonia solani* (J.G. Kühn) suggests that cover crop incorporation leads to a suppressive effect *in vitro* (Grünwald et al., 2000).

In order to examine green manure impacts on soilborne pathogens in the crop rhizosphere, this project implemented macroarray and T-RFLP analyses. The macroarray detects thirty-one different fungal and oomycete pathogens of solanaceous crops common in the Northeastern region of the US, even at extremely low inoculum levels (Zhang et al., 2008). The limit of detection for the macroarray was determined to be 0.04 pg (Zhang et al., 2008). This technique was developed as a diagnostic tool for diseased plant samples and has not previously been used in a field study.

T-RFLP analysis has been used to determine the profile of microbial communities and identify potentially-beneficial bacteria (Benitez et al., 2007). Subsequent application of the technique revealed both beneficial and detrimental populations of fungi (Benitez, 2009). As T-RFLP was previously used successfully in the above field study (Benitez, 2009) to profile diverse species of microorganisms, this study tested the potential for detection of specific pathogens. The limit of detection of specific organisms by T-RFLP was not determined for this study. However, quantitative real-time PCR has been used in conjunction with T-RFLP in other studies to allow the quantification of targeted template in environmental samples (Yu et al., 2005). T-RFLP is relatively less expensive and more time efficient than macroarray. While T-RFLP can be used to identify specific organisms through *in silico* correlation with terminal restriction fragment lengths, the true accuracy of these assignments is not verifiable given the high degree of microbial diversity present in the soil. However, it is unknown if significant interference would occur with such assignments made from assays of root samples which would be expected to harbor less diversity by volume. Because specific species are able to be accurately targeted by macroarray, this experiment provided a unique opportunity to assess the relative detection power of the two techniques.

In this study, the single-season impacts of mixed-species cover crops on organic tomato (*Solanum lycopersici* L.) crop rhizosphere pathogen detection was evaluated in three states with distinct soilborne disease pressure and repeated over two field seasons. Two PCR-based molecular techniques, macroarray and T-RFLP, were used to detect species of fungal and oomycete pathogens. The results of each were used to evaluate and compare the efficacy of each technique. Finally, macroarray analysis was used to assess cover crop treatment effects on pathogen presence.

2. Materials and methods

2.1. Transplant production

Tomato cultivar Celebrity (Johnny's Select Seed, Winslow, ME) was used. This cultivar has disease resistance to Verticillium wilt, Fusarium wilt Races 1 and 2, root-knot nematodes, Alternaria stem canker and tobacco mosaic virus (Rutgers Cooperative Extension, 2013).

Tomato seeds were sown into a locally-produced organic potting mix in 50 cell flats in NY and OH (TO Plastics, Clearwater, MN) and 128 cell flats in MD, then maintained in a greenhouse with 16 h of both natural and supplemental light per day. Seedlings were moved into a cold frame for at least 24 h before transplant.

Table 1
Field setup and timeline.

	MD	NY	OH
Number of fields	1	2	3
Reps per field	6	4	4
Total reps per year	6	8	12
Total plots	30	40	60
Plot size (m)	6.4 × 12.2	2.4 × 7.6	3.1 × 6.1
Rows/plot	2	1	4
Plant distance (m)	0.9	0.6	0.6
Tilled CC 2010	4/16/2010	5/5/2010	4/14/2010
Tilled CC 2011	5/2/2011	5/14/2011	5/10/2011
Transplanted Tomatoes 2010	5/14/2010	5/27/2010	6/3/2010
Transplanted Tomatoes 2011	5/20/2011	6/10/2011	6/15/2011
Rhizosphere Collection 2010	6/14/2010	6/30/2010	7/13/2010
Rhizosphere Collection 2011	6/21/2011	7/11/2011	7/25/2011

2.2. Field design

Research was conducted in 2010 and 2011 at the University of Maryland Lower Eastern Shore Research and Education Center, Salisbury, the New York Agricultural Experimental Station, Phytophthora blight research farm in Geneva and the Ohio Agricultural Research and Development Center, Wooster. The experiment was conducted as a randomized complete-block design with five treatments and six, eight or twelve replicates, for MD, NY and OH respectively (Table 1). The five treatments of single or mixed-species cover crop combinations were different in each state based on local growing conditions and practices. The experiment included the legumes hairy vetch (*Vicia villosa* Roth), crimson clover (*Trifolium incarnatum* L.) and alfalfa (*Medicago sativa* L.). Grasses used were annual rye (*Lolium multiflorum* Lam.), winter rye (*Secale cereal* M. Bieb) and mixed-species hay which included red fescue (*Festuca rubra* L.), orchard grass (*Dactylis glomerata* L.), and timothy (*Phleum pratense* L.), as well as the legumes crimson clover and alfalfa. The brassica species used were forage radish (*Raphanus sativus* var. *longipinnatus* L.) and forage turnip (*Brassica rapa* var. *rapa* L.). MD treatments included vetch (79 kg/ha) + winter rye (79 kg/ha); vetch (25 kg/ha); vetch (42 kg/ha) + radish (42 kg/ha); mixed-species hay (125 kg/ha with composition of equal seed number); and no cover. NY treatments included vetch (34 kg/ha) + winter rye (79 kg/ha); clover (10 kg/ha) + annual rye (18 kg/ha); turnip (15 kg/ha) + winter rye (45 kg/ha); winter rye (135 kg/ha); and no cover. OH treatments included winter rye (150 kg/ha); vetch (50 kg/ha); vetch (25 kg/ha) + winter rye (75 kg/ha); radish (10 kg/ha); and mixed-species hay with 56 kg/ha in 2010 and 112 kg/ha in 2011, also with composition of equal seed number. Cover crop seed was sown in the fall and the cover crop was mowed and tilled in as a green manure the following spring three to five weeks before transplanting the tomatoes (Table 1). Fields in all states had raised beds covered with black plastic and drip irrigation. Tomatoes were grown using standard organic practices including trellising.

2.3. Tomato rhizosphere DNA extraction

DNA extraction was performed on rhizosphere samples collected at four weeks post-transplant (Table 1). Rhizosphere samples were collected from two plants per plot from each plot and each