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Increased pepper yields following incorporation of biofumigation cover crops and the effects on soilborne pathogen populations and pepper diseases

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ABSTRACT

The use of brassica cover crops and their associated degradation compounds as biofumigants to manage soilborne pathogens could offer vegetable growers an alternative to the restricted broad-spectrum fumigant methyl bromide. Biofumigation was tested in two experiments to manage *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Pythium* spp., and the diseases they cause on pepper. Field plots were seeded in fall 2010 and spring 2011 to oilseed radish (*Raphanus sativus* L.), 'Pacific Gold' mustard (*Brassica juncea* (L.) Czern), or 'Dwarf Essex' winter rapeseed (*Brassica napus* L.). Cover crops were disked into soil in spring 2011 and immediately covered with virtually impermeable film (VIF) to reduce the escape of volatile pesticidal compounds. Controls included fallow plots with (CVIF) and without (fallow) VIF. Green bell pepper was transplanted into all plots. Concentrations of isothiocyanates (ITCs), the brassica degradation compounds primarily responsible for pesticidal activity, were highest following incorporation of mustard. Rapeseed yielded the second highest ITC concentrations. Radish yielded very low ITC concentrations in experiment 1, and none during experiment 2. ITCs also were detected in low concentrations in CVIF treatments. All treatments that received VIF reduced populations of *R. solani* compared to fallow, with no differences between biofumigation treatments and CVIF. Biofumigation treatments did not reduce populations of *Pythium* spp. or *S. rolfsii* compared to CVIF. Pepper stunting was significantly lower in treatments that received VIF compared to fallow, with no consistently significant differences between biofumigation treatments and CVIF. *Pythium* isolated from roots of stunted peppers was identified as *Pythium aphanidermatum*. Biofumigation treatments did not reduce plant mortality. Pepper yields were highest in biofumigation treatments compared to CVIF, and CVIF yields were higher than fallow yields.

1. Introduction

Biofumigation is the use of isothiocyanates (ITCs) produced during the break-down of *Brassica* green manures to control soilborne pathogens, nematodes, or weeds (Matthiessen and Kirkegaard, 2006). This is accomplished by a reaction between two isolated compounds that occur naturally within the plant: the enzyme myrosinase (thioglucoside glucohydrolase), located in myrosin cells, and the secondary metabolites glucosinolates (GSLs), which are contained in cell vacuoles. Upon tissue disruption the myrosinase catalyzes a hydrolysis reaction that converts GSLs into ITCs, the compounds primarily responsible for the plant's pesticidal properties, as well as a variety of other breakdown products such as nitriles, thiocyanates, oxazolidine-2-thiones, and sulfides (Fenwick et al., 1983; Morra and Kirkegaard, 2002; Wang et al., 2009). Biofumigation, the subject of research since the early 1990s with varying levels of success, remains an active area of research.

Different species and cultivars of brassicas vary in their biofumigation potential. *Brassicaceae* species should be selected based on the amount of GSL that they contain, the type of resulting ITC that will be produced, as well as the amount of biomass they are capable of producing (Matthiessen and Kirkegaard, 2006). *Brassica juncea* (L.) Czern cv. Pacific Gold ('Pacific Gold' mustard) was reported to have the highest above-ground biomass (5.7 kg m^{-2}) and GSL content of seven *B. juncea* species tested (Antonious et al., 2009). The allyl ITC released from macerated *B. juncea* tissue has been shown to be suppressive to in vitro growth of several plant pathogens including *Pythium ultimum* and *R. solani* (Mayton et al., 1996). *Brassica napus* L. PI 169083 (winter rapeseed) had the highest above-ground biomass (7.74 kg m^{-2}) of ten *Brassica* species studied (Antonious et al., 2009). Although its GSL content was relatively low, its high biomass produced high GSL yields per unit area relative to the other species tested. Furthermore, because winter rapeseed requires vernalization to flower, it overcomes the limitation of early flowering that affects many other brassica species, making it an excellent

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candidate for a biofumigant crop (Sarwar and Kirkegaard, 1998). Another member of the *Brassicaceae* that is used as a cover crop, *Raphanus sativus* L. (oilseed radish), has the potential to produce approximately 10 kg m^{-2} biomass (Sundermeier, 2008).

In addition to brassica species and cultivar, time of planting and growth stage at incorporation are important to consider for the success of biofumigation. GSL concentration was highest at the bud-raised growth stage prior to flowering and higher in spring- versus fall-seeded brassicas (Sarwar and Kirkegaard, 1998). The type of plastic used to cover the fumigated area may also be an important consideration. Virtually impermeable film (VIF) composed of polyethylene and ethylene vinyl alcohol greatly reduces the quantity of volatile chemicals allowed to escape from the soil into the atmosphere compared to conventional low density polyethylene mulch (LDPE) (Noling et al., 2010; Hanson et al., 2010). This reduces both the rate at which volatile chemicals must be applied to the soil to obtain adequate fumigation results and the harmful effects that may result from releasing toxic volatile chemicals into the atmosphere.

R. solani, a soilborne fungal plant pathogen within the phylum Basidiomycota, reduces vigor and yield of mature pepper plants (Roberts, 2003a,b). Control of *R. solani* through biofumigation has shown varying success, at least in part due to the conditions discussed above (Motisi et al., 2010). Allyl ITC was suppressive to *R. solani* in a controlled laboratory study (Charron and Sams, 1999). Significant *R. solani* population reductions were observed in greenhouse assays and disease reductions were observed in field tests following incorporation of brassica plant tissue, including *B. juncea*, *B. napus*, and *R. sativus* (Larkin and Griffin, 2006). Essential oil extracted from mustard seeds (*Brassica rapa*) significantly reduced the saprophytic growth of *R. solani* in vitro and in three separate field soils, although significantly higher mustard oil concentrations were required for field soils (Dhingra et al., 2004). In another field experiment, there were no significant differences in *R. solani* population densities between non-amended treatments and treatments amended with *B. juncea* or *B. napus* (Njoroge et al., 2008).

S. rolfsii, another soilborne fungal plant pathogen within the phylum Basidiomycota, causes the economically important disease southern blight in the southern United States. Symptoms on peppers include yellowing, wilting, and eventual plant death (Roberts, 2003a,b). Germination of *S. rolfsii* sclerotia was inhibited by brassica amendments in conjunction with a diurnal heat treatment with a maximum and minimum of 38 °C and 27 °C (Stapleton and Duncan, 1998). The heat treatment alone was not suppressive. *S. rolfsii* was suppressed in vitro by phenyl and allyl ITC released from cabbage and mustard soil amendments (Gamliel and Stapleton, 1993; Harvey et al., 2002).

Pythium spp. are commonly occurring fungus-like soilborne plant pathogens that are classified in the kingdom Straminopila and the phylum Oomycota. Many species of *Pythium*, with varying degrees of pathogenicity, are capable of colonizing pepper roots. *Pythium aphanidermatum* and *Pythium myriotylum* were the most aggressive of 10 *Pythium* species, leading to approximately 50% mortality in inoculated pepper plants (Chellemi et al., 2000). *Pythium periplocum* and *Pythium spinosum*, although capable of colonizing pepper roots, resulted in no adverse effects on root system weight or plant growth.

The diversity in *Pythium* spp. results in differential sensitivity to ITCs. Significant variations in responses to 2-phenylethyl ITC among 13 different isolates across 3 species of *Pythium* (*P. sulcatum*, *P. ultimum*, and *P. violae*) have been reported (Smith and Kirkegaard, 2002). The ITC concentration required to reduce in vitro radial growth by 90% ranged from 0.005 mM to 0.862 mM for the different isolates. The least sensitive was *P. ultimum*. Allyl ITC was suppressive to *P. ultimum* in a controlled laboratory study (Charron and Sams, 1999). Njoroge et al. (2008) and Collins et al. (2006) both found that the incorporation of brassica tissue did not significantly reduce, and in some cases increased, soil population densities of *Pythium* spp. In contrast, Stapleton and Duncan (1998) reported significant reductions in survival of *P. ultimum* following the incorporation of 2% brassica tissue into soil in a controlled experiment.

Bell pepper (*Capsicum annuum* L.) is a good candidate crop for the incorporation of biofumigation into the production system. All pepper cultivars are susceptible to the pathogens *R. solani*, *S. rolfsii*, and *Pythium* spp. Methyl bromide is only allowed in pepper production in the southeastern United States where there is a moderate to severe nutsedge, nematode, or *Pythium* infestation under an EPA critical use exemption (USEPA, 2011). This highlights the need for the development of alternative disease management strategies in pepper, so that pepper growers can transition to more sustainable methods to manage soilborne pathogens. Many growers may also be attracted to the relatively low cost and ease of implementing biofumigation compared to conventional fumigants like methyl bromide or metam sodium. Bell pepper production in the southeastern US was valued at over half of the country's total production value, $239 million, making bell peppers an important crop for Southeastern vegetable growers (USDA ERS, 2011).

The first objective of this study was to determine if biofumigation with three representative brassica cover crops, oilseed radish, 'Pacific Gold' mustard, or 'Dwarf Essex' winter rapeseed, would reduce populations of the soilborne plant pathogens *R. solani*, *S. rolfsii*, and *Pythium* spp. in the field. The second objective was to determine the effect of biofumigation on pepper production, including plant stunting; incidence of damping-off, root rot, and southern blight; and yield. The third objective was to determine the pathogenicity of *Pythium* spp. isolates on pepper in order to better understand which were responsible for the majority of the damping-off and root rot observed in the field.

2. Materials and methods

2.1. Field plot establishment

The experiment was conducted at the Clemson University Coastal Research and Education Center (CREC), Charleston, SC. The soil in the fields used is Yonges loamy fine sand. The experiment was done twice. Prior to planting the experimental cover crops, the fields were planted in a mixture of rye (*Secale cereale*) and crimson clover (*Trifolium incarnatum*) in fall 2009. The experimental design was a randomized complete block design with four replications. Experiment 1 was reduced to three replications due to poor brassica emergence in one of the blocks. Prior to planting brassica cover crops, each experimental field was sectioned into 16 equally sized plots. Each plot measured 9.1 m by 1.8 m, with a 3.0-m fallow space between each plot.

On 18 October 2010, prior to planting brassica cover crops, field plots were infested with *S. rolfsii* at a rate of approximately 21,800 sclerotia per plot (13.0 g sclerotia) by spreading sclerotia over the soil surface. Sclerotia were produced on autoclaved green beans using a technique developed for *R. solani* (van Bruggen and Arneson, 1985). Dried beans were ground in a blender, and sclerotia were separated from larger bean pieces with a sieve with 2-mm openings. Sclerotia were stored in a refrigerator until use.

The three brassica treatments were oilseed radish (*R. sativus*), mustard (*B. juncea* 'Pacific Gold'), and winter rapeseed (*B. napus* 'Dwarf Essex') seeded at 28.0 kg ha^{-1} for radish and 11.2 kg ha^{-1} for mustard and rapeseed. Seeds were purchased from Johnny's Selected Seeds (Winslow, ME), and seeding rates were based on the highest rates recommended by the supplier. All brassica treatments were covered with 1.25-mm white-on-black VIF (Berry Plastics, Washington, GA) after incorporation, with black side up