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Impact of brassicaceous seed meals on the composition of the soil fungal community and the incidence of *Fusarium* wilt on chili pepper

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ABSTRACT

Although brassicaceous seed meals (BSMs) have been evaluated for their ability to control various soil-borne diseases, establishing the relationship between disease severity and the soil fungal taxa specifically inhibited or enriched by the seed meal amendment has not been carefully assessed. The purpose of the present work was to elucidate the relationship between the glucosinolate concentration and composition in BSMs applied as soil amendments, the diversity and composition of the resulting soil fungal community, and the incidence of *Fusarium* wilt disease. These relationships were examined using chili pepper (*Capsicum annuum* 'Ben Villalon') as the bioassay plant grown in pots amended with different BSMs in the presence of the *Fusarium* wilt pathogen. We evaluated three BSMs differing in the species from which the seed meal was prepared and hence the glucosinolate content. These included *Camelina sativa* 'Crantz' (CAME), *Brassica juncea* 'Pacific Gold' (PG), and a mixture of PG and *Sinapis alba* cv. 'IdaGold' (IG) (PG + IG, 1:1 ratio). The effect of the BSMs on the fungal community was evaluated after the soil had been incubated for 25 days separately with each seed meal (incubated soil) and again after growing with chili pepper in pots for 35 days (rhizosphere soil). A soil treatment (no BSM amendment, comparable N, P, K added) was used as control. The composition of the soil fungal community in the incubated soil and the rhizosphere soil were examined using DNA based methods including quantitative real-time PCR and high throughput pyrosequencing. Our results indicated that the fungal abundances in the incubated soils were significantly increased by all BSM amendments, however the proliferation became less pronounced during the period of chili pepper growth. Of significance, the BSMs differed in their effect on the fungal community composition and on disease severity. For example, PG and PG + IG specifically enriched *Chaetomium* species, the abundance of which was negatively correlated with disease severity, and simultaneously specifically inhibited *Hypocreales* including the genus of *Fusarium*, the abundance of which was positively correlated with disease severity. PG and PG + IG exhibited good disease control efficacies in the pot experiment suggesting that 2-propenyl (allyl) glucosinolate, the primary glucosinolate component in the PG seed meal, played an important role in the changes in abundance of specific fungi during the 25-day incubation. CAME specifically enriched members of the *Mortierellales*, including the genus *Mortierella*, the abundance of which was not correlated disease severity. Moreover, our data indicated that BSM type was a more important determinant of fungal community composition than the plant host, given that the composition of the fungal communities after 35 days growth in the rhizosphere of chili pepper were not substantially different from the incubated soil communities before planting. Our results suggested that some species in the genus of *Chaetomium* may be important for disease management and deserved further evaluation as potential biocontrol agents.

1. Introduction

Fusarium wilt, caused by the fungal species *Fusarium oxysporum*, is a worldwide disease with a broad host range, including solanaceous crops (tomato, potato, pepper, tobacco and eggplant). Because of the economic significance of these crops, great efforts

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have been made to develop effective measures to control *Fusarium* wilt. Although soil fumigation with bromomethane is an effective control method, due to environmental concerns, it is scheduled to be banned worldwide in 2015 (Shen, 2011). A recent focus has been the development of efficacious and environmentally friendly methods using field trials at multiple scales (Bailey and Lazarovits, 2003; Ślusarski and Pietr, 2009; Thuerig et al., 2009). Based on both effectiveness and environmental safety, one of the most promising control measures to replace bromomethane is the use of brassicaceous seed meal (BSM) as a soil amendment.

Many members of the plant family Brassicaceae contain secondary metabolites known as glucosinolates, which are most concentrated within seeds. By enzymatic hydrolysis, glucosinolates are converted to isothiocyanates, some of which have been shown to be highly active in controlling weeds (Rice et al., 2007; Vaughn et al., 2006), insect pests (Borek et al., 1998; Elberson et al., 1997), nematodes (Walker, 1996), and fungal pathogens (Chung et al., 2002; Mazzola et al., 2001; Smolinska et al., 1997). However, the relationship between the glucosinolate content and the biological control efficacy of the BSMs is not always consistent. For instance, Reardon et al. (2013) demonstrated that BSM with a relatively low glucosinolate content (25.4 μmol g^{-1}) did not significantly reduce the number of nematodes that caused apple disease, whereas a BSM with a higher glucosinolate content (303 μmol g^{-1}) was effective. Other studies (Cohen and Mazzola, 2006; Mazzola et al., 2007) reported that the BSMs differing in glucosinolate contents were equally effective in controlling apple root rot caused by *Rhizoctonia solani*, suggesting the type of glucosinolate present may be more important than the concentration. Relatedly, Handiseni et al. (2013) reported that effectiveness in controlling wheat disease caused by *R solani* was not associated with the glucosinolate concentration of the BSMs used, but with the glucosinolate type. In their report, BSMs containing 4-hydroxybenzyl glucosinolate produced the best results. In vitro assays Mithen et al. (1986) demonstrated that among the hydrolysis products tested, allyl-isothiocyanate, the hydrolysis product of 2-propenyl (allyl) glucosinolate was the most toxic to plant pathogenic fungi. However, the cumulative effects of BSM amendments on the entire soil microbial communities are not sufficiently understood. Several previous studies (Cohen et al., 2005; Omirou et al., 2011; Reardon et al., 2013; Rumberger and Marschner, 2003) addressed this issue using culture-based or other methods, which often have lower resolution than sequencing-based approaches. In a recent study, Hollister et al. (2013) analyzed the effects of *Brassica juncea* seed meal (releasing allyl isothiocyanate) on soil bacterial and fungal communities using a high throughput pyrosequencing approach. Their study identified differential impacts of brassicaceous and non-brassicaceous oilseed meals on the abundance of soil microbial taxa known for contributing to the suppression of fungal diseases. However, that study did not show a direct correlation between the abundance of microbial taxa and disease severity. Additional research is therefore needed to address the possible relationship.

The purpose of the present work is to elucidate the relationship between the glucosinolate concentration and composition in BSMs applied as soil amendments, the diversity and composition of the resulting soil fungal community, and the incidence of *Fusarium* wilt disease. These relationships were studied using chili pepper (*Capsicum annuum* 'Ben Villalon') as the bioassay plant grown in pots amended with different BSMs in the presence of the *Fusarium* wilt pathogen. The effects of the different BSM treatments on the abundance of all soilborne fungal taxa were characterized using quantitative real-time polymerase chain reaction (qPCR) and on the composition of the soil fungal community using high-throughput pyrosequencing. The disease severity of *Fusarium* wilt of chili pepper growing in seed meal-amended soils was quantified. This work was intended to test the hypothesis that seed meals with different glucosinolate compositions inhibited or enriched different specific fungal taxa that were directly related to disease severity.

2. Materials and methods

2.1. Brassicaceous seed meals, soil and chili pepper plants

The three defatted BSMs used in this study were selected based on the concentration and composition of the glucosinolates they contained (Table 1). The nutrient content of CAME was 5.9% N, 1.01% P, and 1.45% K. The nutrient content of PG was 6.1% N, 1.2% P, and 1.5% K. The nutrient content of IG were 6.2% N, 1.18% P, and 1.37% K. Seed meals were sieved through a 1-mm metal mesh and stored at 4 °C before being used. Soil used in this study was collected from Weslaco, Texas, USA, and had a sandy loam texture (sand 69%, silt 14% and clay 17%) with pH 8.2. The nutrient content of soil was 0.72% organic matter, 10 μg/g nitrate-N, 125 μg/g P, and 277 μg/g K. This soil was collected from a commercial chili pepper field where *Fusarium* wilt was a consistent and serious problem and where no seed meal amendments were used previously. The fresh soil with 9.4% of water content was sieved through a 5-mm metal mesh. Chili pepper seeds for pot experiment were provided by Dr. Kevin Crosby, Texas A&M University.

2.2. Incubated soil treatments

Four treatments designated as CK (no seed meal, fertilizer amendment), CAME (amended with CAME), PG (amended with PG) and PG + IG (amended with a mixture of PG and IG in 1:1 ratio by weight) were included. This resulted in the establishment of three levels of 2-propenyl (allyl) glucosinolate content in the amendment materials: 0 μmol g^{-1} in CK, 0 μmol g^{-1} in CAME, 164 μmol g^{-1} in PG and 82 μmol g^{-1} in PG + PI. The application rate

Table 1
Description of Brassicaceous seed meals used for soil amendments

Material code	Source	Glucosinolates content (μmol g^{-1} defatted seed meal)	Glucosinolate composition	Reference
CAME	Seed meal of *Camelina sativa* cv. 'Crantz', provided by Dr. Terry Gentry (Texas A&M University, USA)	23.5	51.9% being 10-methyl-sulfinyl-decyl-glucosinolate; 30.2% being 11-methyl-sulfinyl-decyl; 17.9% being 9-methyl-sulfinyl-decyl glucosinolate	(Hu et al., 2011)
PG	Seed meal of *Brassica juncea* cv. 'Pacific Gold', provided by Farm Fuel Inc., Freedom, CA, USA.	164	99% being 2-propenyl (allyl) glucosinolate	(Handiseni, 2013)
IG	Seed meal of *Sinapis alba* cv. 'IdaGold', provided by Farm Fuel Inc., Freedom, CA, USA	195	97% being *p*-hydroxylbenzyl glucosinolate	(Handiseni, 2013)