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ORIGINAL PAPER

Root, shoot tissues of *Brassica juncea* and *Cereal secale* promote potato health

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Abstract *Brassica* species are increasingly being used as cover crops to suppress soil-borne diseases in potato cropping systems. Experiments were conducted in controlled environments and in the field to evaluate the effects of cover crop root or shoot or a combination of root and shoot tissues on potato root and tuber health. In a lab assay we examined the extent to which volatile compounds released from tissues of two cover crop species, rye (*Cereale secale* L.) and oriental mustard (*Brassica juncea* L.), could inhibit mycelium growth of two important potato diseases, *Rhizoctonia solani* and *Pythium ultimum.* Twenty-four hours into the lab assay, volatile compounds from all residues suppressed fungal growth. After 48 h, marked suppression of hyphal growth continued in the presence of mustard residues but not in the presence of rye tissues or the control without tissues. A 75 L volume container experiment evaluated the effect of incorporating different quantities of mustard shoot and root tissues (none, comparable to field level and fourfold field level) into *R. solani* and *P. ultimum* infested soil on potato growth, root health and tuber disease. In the container study, incorporating mustard shoots at the highest dose increased potato yield by 54% and reduced disease rating to 2.3 compared to a severe rating of 4.4 in the control. In the field trial, potato growth, root health and tuber disease levels were evaluated in plots where disease management involved either incorporation of mustard or rye cover crop roots, shoots and whole plants (roots plus shoots) or standard farmer practice of a fumigated fallow as a control. White root tissue was used as a health indicator, and averaged 58 and 78% in the fumigated control and mustard cover crop treatments, respectively. The highest healthy root tissue status (91%) was recorded where whole plants of mustard were incorporated. In contrast to the visual assessment of root and tuber health, tuber yield in the field was not influenced by cover crop treatment. Across experiments, the incorporation of or exposure to whole mustard plants was consistently effective at suppressing soil-borne fungi and promoting healthy roots and tubers, especially at higher rates of biomass. Mustard should be managed so as to maximize incorporated biomass for effective biofumigation. Multipurpose management requiring removal of mustard shoots is incompatible with promoting potato rhizosphere health.

Keywords Cover crop · Bio-suppression · Soil-borne disease · Brassicaceae

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Abbreviations

PDA Potato dextrose agar
N Nitrogen

Introduction

The incorporation of *Brassica* cover crop residues initiates a series of complex biological and chemical interactions that have short- and long-term consequences for soil and plant health. For example, *Brassica* residues have been shown to alter the soil nematode community structure, which in turn profoundly affects the soil microbial food web, as indicated by studies of biosuppressive effects on plant parasitic nematodes such as *Pratylenchus penetrans* (Porter et al. 1998). Though the level of nitrogen (N) immobilization or release from residues depends on residue quality and other environmental factors, N cycling by *Brassicas* can influence soil microbial activity and main crop growth (Snapp and Borden 2005). Because of their potential as both a cover and oilseed crop, farmers are particularly interested to know the potential effects of incorporating different rates and types (roots versus shoots) of *Brassica* tissues on disease suppression and root health within typical field conditions.

Numerous studies have identified an association of *Brassica* residue incorporation with soil-borne disease and pest suppression, through mechanisms that affect soil organisms directly as well as indirectly (Brown and Morra 1997). For example, *Brassica* cover crop tissue incorporation yields energy and nutrient sources that support soil biota and can increase their activity (Kirkegaard et al. 1999), an effect that may lead indirectly to suppression of soil-borne disease organisms (Workneh and van Bruggen 1994). The decomposition of *Brassica* tissues also releases glucosinolates, and hydrolysis of these volatile compounds leads to the formation of isothiocyanates (ITCs), which have fumigant properties similar to metham-sodium (Sarwar et al. 1998). Isothiocyanates and other secondary compounds of glucosinolates can act as biocides as well as suppress the growth of a wide range of soil-borne disease organisms (Kirkegaard and Sarwar 1998). Glucosinolate-containing tissues from the Brassicaceae family have been reported to suppress activity of *Pythium ultimum*, *Fusarium oxysporum* f. sp *cumini* and *Rhizoctonia solani* (Charron and Sams 1999; Marwar and Lodha 2002). Usually, the quantity and effect of biotoxic compounds released from *Brassica* tissues is a small fraction of the potential and is greatly influenced by the edaphic environment (Morra and Kirkegaard 2002), tissue qualities (Sang et al. 1984) and extent to which *Brassica* plant cells are ruptured by maceration, freezing, or drying, which brings glucosinolates and hydrolyzing enzymes into contact (Watt et al. 2006).

Identifying the potential for using green manures to decrease root rot incidence and promote healthy crop plants is of growing importance, since few disease management practices are consistently successful, economically feasible and environmentally sound (Stone et al. 2001; Williams-Woodward et al. 1997). Soil quality problems in North America are increasing with land values as short rotation sequences with few soil building 'break crops' become the norm (Snapp et al. 2005). More growers are becoming interested in testing the soil amelioration characteristics of alternative cover crops and alternative cash crops as potential additions within conventional crop rotations (Abdallahi and N′Dayegamiye 2000).

Cereal rye (*Cereale secale* L.) has been used for decades in irrigated cropping systems in North America as a remarkably cold-tolerant, inexpensive and soil building cover crop. While allelopathic weed suppression involving rye plants has been widely studied, including the production of biocidal compounds from rye tissues (Burgos and Talbert 2000; Reberg-Horton et al. 2005; Virtanen and Hietala 1955), rye grown as a cover crop has not been systematically investigated with regard to its capacity for fungal suppression. Seeking to manage disease levels in their fields, some innovative potato farmers have modified their crop rotations, substituting a species with fumigation properties, oriental mustard (*Brassica juncea* L.), for a rye cover crop or fallow (McGuire 2002). Managed as a green manure, mustard is planted in late fall or early spring and

incorporated in the vegetative stage, prior to planting a potato crop (Snapp et al. 2006). Because oriental mustard is also grown as an oilseed crop, it is important to identify the biosuppressive capacity of root tissues, the main material remaining following grain removal. Comparisons of disease potential, root dynamics and soil biology have not been studied extensively following the incorporation of roots versus shoots of *Brassica* cover crop tissues.

The objective of this study was to evaluate the of potential of oriental mustard root and shoot tissues to promote potato root and tuber health through suppression of the fungus *R. solani* and other soil-borne diseases under controlled and field conditions. Effects of tissue incorporation rate and tissue type were tested in a laboratory assay and a glasshouse experiment. In addition, a field experiment compared pathogen biosuppression and potato growth following either incorporation of an oriental mustard cover crop, a rye winter cover crop or a fumigated fallow rotation. The latter practices are typical of North American potato farmers with irrigated production systems (Snapp et al. 2005).

Materials and methods

Experiment 1—laboratory assay

The effect of volatile compounds released from two plant species (oriental mustard, cv. 'Pacific Gold,' and cereal rye, cv. 'Wheeler') and different tissue types (shoots, roots and shoots plus roots) was investigated using a Petri plate-container bioassay modified from Charron and Sams (1999). Suppression of hyphal growth by tissue volatiles was quantified for important potato disease organisms, *R. solani* and *P. ultimum.* The experimental design was completely randomized with four replications.

The cultivars of rye and mustard were chosen based on earlier reports of allelopathic activity (Charron and Sams 1999; Reberg-Horton et al. 2005). Rye and mustard cover crop tissues for the bioassay were obtained from plants grown in 17 L containers at a Michigan State University glasshouse in East Lansing, MI. The containers were filled with sandy, autoclaved soil (121°C for 120 min) to which slow-release 14N–6.2P–12K fertilizer (Osmocote, The Scotts Co., Marysville, OH, USA), was incorporated at a rate of 22 g kg^{-1} to provide nutrients. Plants were grown under natural light supplemented with 400 W lamps (photon flux of 200 µmol m^{-2} s^{-1}) to provide 16 h illumination day^{-1}. Air temperature was maintained at 26°C (standard deviation 4°C). Plants received consistent watering, using an automatic irrigation system.

For the bioassay, *R. solani* cultures were grown in the dark at 22°C for 10 days on autoclaved potato dextrose agar (PDA) plus 25 g L^{-1} ampicillin (Sigma-Aldrich chemicals, used to eliminate bacterial contamination). *P. ultimum* was cultured in the light under the same conditions, in the absence of ampicillin. *R. solani* was originally isolated from potato tubers at six sites across Michigan in 2000. The anastomosis group of all isolates was AG–3 as determined by vegetative compatibility with a known AG-3 isolate. The isolates were grown together to produce a composite isolate, used in this experiment. Under aseptic conditions, an agar disc (5 mm diameter) was transferred from the edge of actively growing colonis to 100 mm diameter. Petri dishes containing PDA. Meanwhile, plant roots and shoots (near flowering stage) were harvested from the 17 L containers, washed with distilled water, blotted dry on filter paper, and cut into 2 cm pieces. Tissue pieces were macerated completely using a mortar and pestle. Immediately following maceration, 10 g fresh weight (fwt) of residue (90% moisture), corresponding to each treatment (5 g each of roots and shoots for the whole plant treatments) were placed inside 400 mL glass containers. The Petri dishes inoculated with *R. solani* or *P. ultimum* were inverted over the mouths of the 400 mL containers holding macerated tissues and sealed using Parafilm (Fig. 1). The control treatment used the same container-Petri dish set-up without the addition of macerated plant tissues. Container-Petri dish assays were maintained at constant light and temperature (24°C) for 2 days to observe fungal growth.

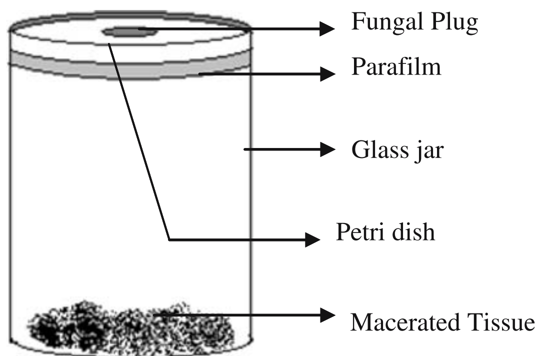

Fig. 1 Laboratory assay of the effect of volatile compounds from macerated cover crop tissues on fungal growth in agar on an inverted Petri dish

The radial mycelial growth of *R. solani* or *P. ultimum* was recorded at 24 and 48 h as the mean of two perpendicular diameters (mycelial coverage was measured using digital imaging for a randomly chosen 20 sub-sample; the correlation was $R^2 = 0.95$ between the two diameter measurements and the area covered by mycelial growth, diameter monitoring was used for the remainder of the experiment). Measurements stopped at 48 h since release of ITCs from macerated tissues occurs rapidly, usually by 24 h (Morra and Kirkegaard 2002), and because of the likelihood of rapid colony growth in the control treatment where mycelial growth completely cover Petri dishes after 2 days (=100% in Fig. 2). The experiment was repeated once.

Experiment 2—glasshouse container study

A glasshouse experiment compared potato health following soil amendment with three types of oriental mustard residues (root, shoot and whole plant) and two residue loading rates at Michigan State University, in E. Lansing, MI (USA). The experiment included a control treatment of non-amended soil. The experiment had a randomized complete block design (RCBD) with three replications.

The soil (a Metea loamy sand) used to fill 75 L containers (58 cm height and 48 cm diameter) was collected from the Ap horizon (0–20 cm) of an area located adjacent to the field site described in experiment 3. Fresh soil was steamed for 1 h at 82°C to suppress pathogens, sieved through a 10-mm mesh sieve, then mixed with perlite (3:2 ratio of soil:perlite, by volume) to prevent compaction.

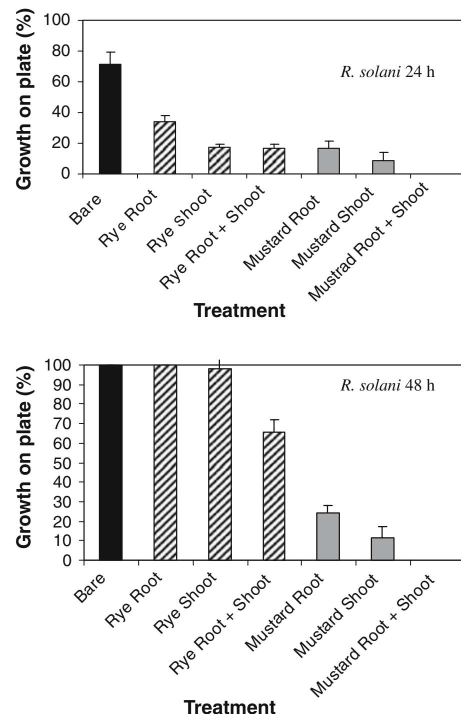

Fig. 2 Area of *Rhizoctonia solani* growth after 24 and 48 h exposure to macerated cover crop residues, comparing mustard and rye root and shoot tissues. Columns present average values with standard deviation bars from two experimental runs

Twenty days prior to planting potato tubers, each container was inoculated with *R. solani* and *P. ultimum* fungal isolates. The isolates were first cultured on potato dextrose media as described earlier, then inoculated on millet seed that was spread onto soil in containers at a rate of 6 g seed m^{-1}, and incorporated to 20 cm. To quantify inoculation rates, 1 g of millet seed was placed in a beaker and mixed with 5 mL of distilled water for 5 min. After filtering through cheesecloth, 40 μL of filtrate was loaded on a hemacytometer grid. Spores were counted on the grid and diluted to obtain a final spore count of 2,000 oospores mL^{-1} for *P. ultimum.* Because *R. solani* does not produce asexual spores or conidia, the inoculum rate was determined based on earlier field-based experimentation involving the establishment of *R. solani* in similar soil (W. Kirk, personal communication).

Ten days before planting potato tubers, oriental mustard residues were obtained from glasshouse-grown plants as described for experiment 1. Fresh residues were added to soil at one of two application rates. The first (base) application rate was equivalent to the cover crop biomass incorporated in the field study (experiment 3, described below): 17 and 40 g fwt container^{-1} for the root and shoot treatments, respectively. This is equivalent to the field root treatment of 780 kg ha^{-1} and shoot treatment of 1,820 kg ha^{-1} (Fig. 3). The second application rate was a fourfold increase of the base rate. Residues were incorporated evenly into the soil within the containers to a depth of 20 cm. Following tissue incorporation, the top of each container was sealed with plastic wrap to reduce the loss of volatile compounds (e.g., glucosinolates and their degradation products) from the system over a 10-day period.

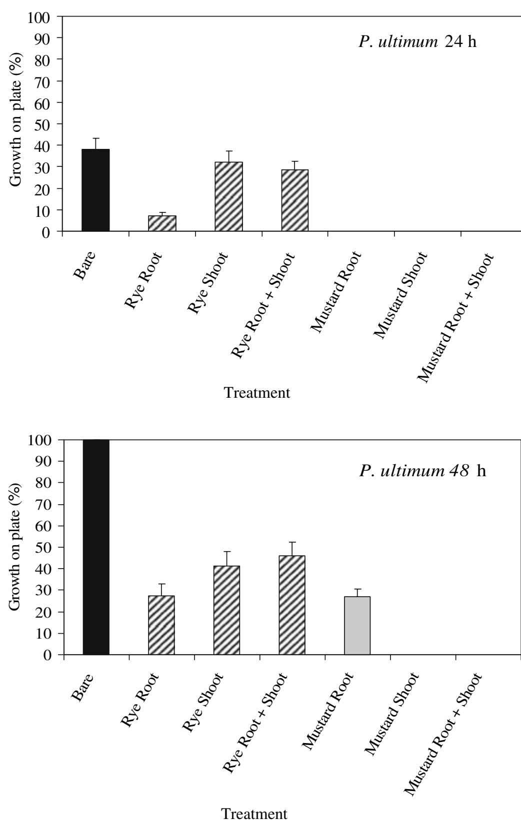

Fig. 3 Area of *Pythium ultimum* growth after 24 and 48 h exposure to macerated cover crop residues, comparing mustard and rye root and shoot tissues. Columns present average values with standard deviation bars from two experimental runs

Immediately prior to planting, potato seed tuber pieces (cv. 'Onaway') were surface sterilized with 5% bleach solution, rinsed thoroughly with distilled water and blotted dry. Two seed pieces were planted per container at a depth of 8 cm. Seedlings emerged after 1 week, and where there were two seedlings, the less vigorous one was removed. Daytime and nighttime temperatures were controlled during potato growth at 25 and 20 ± 2°C, respectively. Containers were irrigated to maintain soil at approximately 20–30% of soil water depletion from field capacity, Soil water potential was monitored using tensiometers inserted to a depth of 15 cm. Twenty grams of K m^{-2} as K_2O and 3.5 g P m^{-2} as P_2O_5 were applied as pre-plant fertilizer, respectively. Nitrogen as NH_4NO_3 was incorporated shallowly in three split applications—at planting, early tuber formation and tuber expansion—at a rate of 20 g m^{-2}.

After 80 days of growth, when potato plants were starting to senesce, they were cut at the shoot–root interface. Soil was carefully washed from the potato roots on a 10-mm screen to facilitate recovery of root material, which was patted dry with paper towels. Root and shoot fresh weights were recorded. Dry weights of shoots and of a randomly selected sub-sample of the roots were recorded after drying at 70°C for 4 days. A fresh weight:dry weight ratio of root sub-samples was used to estimate the total dry weight of roots collected from the containers. Remaining fresh roots were stored at 10°C for no longer than a week after harvest. During this time, dark brown (diseased) and white (healthy) root tissue was quantified using WinRhizo^{TM} (Regents Instruments, Quebec), a scanner-based image analysis system program. Ten sub-samples per plot of root pieces were plated on selective media to identify the presence of specific root rot organisms. The selective media contained K_2HPO_4 (1.0 g), KCl (0.5 g), $NaNO_2$ (0.2 g), Agar 20 g, $MgSO_4{\cdot}7H_2O$ (0.5 g), $FeSO_4{\cdot}7H_2O$ (10 mg) and Gallic acid 0.4 g mixed in 1 L distilled water. After autoclaving, fenaminosulf (90 mg), chloramphenicol (50 mg) and streptomycin (50 mg) were added.

At tuber harvest, detailed score ratings were generated by a single experienced observer who visually compared harvested tubers with pictures of tubers infected to different degrees with *R. solani*. Detailed ratings were conducted by an experienced observer, using the scoring system of 1–6: 1 = white tissue, no observable disease symptoms, 2 = 1–20% brown, diseased tissue on tuber surface; 3 = 21–40% diseased; 4 = 41–60% diseased, 5 = 61–80% diseased, 6 = 80–100% diseased.

Experiment 3—field study

A field experiment was conducted at the Sandhill research station located at Michigan State University in East Lansing, Michigan, USA (42°67′N; -84°49′W). To prepare the field site for this experiment, potatoes (cv. 'Onaway') were grown continuously from 2001 through 2003 to enhance the presence of pest organisms and reduce soil quality. The experimental design was a randomized complete block with four blocks and seven treatments. Treatments were two cover crops (oriental mustard and rye) applied as root, shoot and whole plant (root plus shoot) and a fall fumigated, winter-fallowed control, that followed common farmer practice. Each plot measured 7.6 by 10.7 m.

The experiment was situated on a soil classified as a Metea loamy sand (loamy, mixed, active, mesic Arenic Hapludalfs; USDA 2006). Composite samples (eight cores per plot, 0–20 cm depth) were taken using a core auger (1 cm diameter) on 31 March 2004 to determine topsoil characteristics. Soil was air-dried and ground to pass a 2-mm sieve. Organic carbon (C) content was determined by modified Walkley–Black method (Allison et al. 1965). Soil pH was determined using a 1:2.5 soil:water (vol:vol) solution. Phosphorus, potassium and calcium concentrations were determined by Mehlich III extraction (Mehlich 1984).

The field site was inoculated with *P. ultimum* and *R. solani* using the fungal isolates described above, cultured on PDA media then inoculated on millet seed. The field was inoculated on 7 June 2004 by spreading the infected millet seed at rates of 3 and 6 g millet seed m^{-2}, for *R. solani*, and *P. ultimum*, respectively. The fallow treatment was fumigated following farmer practice on 22 October 2003 with metham (*N*-sodium methyl dithiocarbamate) applied at 350 L ha^{-1}, injected to a soil depth of 20 cm in three parts water to one part metham.

Field management

Rye was planted at a rate of 100 kg ha^{-1} on 10 October 2003, and oriental mustard was planted at a rate of 23 kg ha^{-1} on 5 May 2004. Cover crop shoots were cut using a flail mower on 22 June 2004, and the three treatments per cover crop species were established by removing shoots (raking onto a plastic sheet) for the root treatment, incorporating shoots from the root plots for the shoot treatment, and incorporation in situ after mowing for the whole plant treatment. Residues and the control fallow plots were incorporated with a disk to a depth of approximately 20 cm on 24 June 2004.

Potassium as K_2O and phosphorous as P_2O_5 were applied at recommended rates of 200 and 35 kg ha^{-1} respectively, 7 days before potato planting on 30 June 2004. The variety of potato used (cv. 'Onaway') is widely grown in the Upper Midwest for fresh market sales. Between and within row spacing of potato seed pieces (average weight 52 g) was 0.86 and 0.30 m, respectively. Nitrogen fertilizer (as NH_4NO_3) was applied to the potato crop at the following rates: 100 kg N ha^{-1} at planting, 50 N kg ha^{-1} at hilling and 50 N kg ha^{-1} at tuberization. A N credit of 25 kg N ha^{-1} was deducted from the hilling application for the cover crop treatments that included both shoots and root tissues. The size of this credit was determined by estimating that 30% of the approximate 75 kg N ha^{-1} added with rye and mustard biomass would be available over the season (Snapp et al. 2005). Irrigation totaling 0.27 m was applied to the field site using a traveler irrigation system to supplement precipitation of 0.21 m during the growing season from potato planting to harvest. Irrigation was conducted five times over the growing season, when precipitation, for a total of 0.48 m.

Soil respiration and mineral N analysis

Soil microbial respiration (glucose-induced) was measured to estimate potential biological activity early in the growing season (Kumar and Goh 2000). One composite soil sample was collected per plot from the top 0–20 cm on 15 July 2004 (eight sub-samples per composite, 1 cm diameter soil probe). Soil sub-samples of 100 g fwt (three replicates per sample) were brought to 85% of field capacity by adding a glucose solution (1:10 glucose-deionized water), to measured substrate inducible respiration (SIR). These sub-samples were incubated in 500 mL plastic containers for 20 days at 25°C in a temperature-controlled chamber. Vials containing 10 mL of 1 N NaOH were placed in each container to trap CO_2 evolution over two back-to-back 10-day incubation periods. Trapped carbonates in the vials were precipitated with $BaCl_2$, remaining NaOH in the vials was neutralized, and accumulated CO_2 was determined using an automatic titration meter containing 0.25 N HCl (Anderson 1982). Using the same composite soil samples, soil inorganic N concentration was assessed at the beginning and end of the incubation periods. A 2 M KCl solution (1:4 soil–solution ratio) was used to extract mineral N (Bremner and Mulvaney 1982), which was quantified using a Technicon Autoanalyzer and used to calculate N mineralized over the 20 days incubation (Technicon Industrial Systems; Tarrytown, NY, USA).

Plant monitoring

Cover crop biomass was destructively harvested on 14 June 2004. The amount of shoot biomass incorporated into the soil—4,500 kg ha^{-1} dwt for rye root and shoot residues combined and less than 3,000 kg ha^{-1} dwt for mustard root and shoot residues combined (Fig. 4)—was calculated using a 0.5×0.5 m quadrat randomly thrown twice in the plot, within which shoots were cut and the fresh weight measured. To quantify the root biomass, six soil cores per quadrat at 0–8 inches depth were taken and composited. The composite samples were wet sieved using 8-mm mesh and roots were collected with tweezers. The roots and shoots were placed in a 70°C oven, after which their dry weights were measured. Sub-sample of cover crop shoot tissues, ground to pass a 1-mm mesh sieve were used to determine sample N concentration using the total Kjeldahl N digestion procedure (Bremner and Mulvaney 1982).

Six potato plants per plots were randomly sampled on 30 July 2004 and again on 30 August 2004. Following removal of shoot materials, a volume of soil 0.3 m deep by 0.2 m^2 was excavated to recover roots. After being wet sieved (10-mm mesh) to remove soil, the fresh weight of

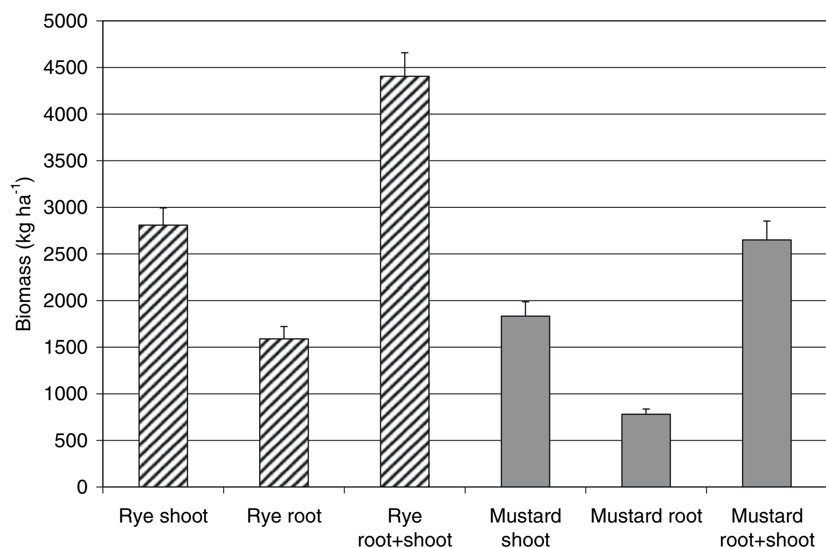

Fig. 4 Means and error bars (standard error, $n = 4$) for cover crop shoot and root biomass on 14 June 2004 for root, shoot and combined root + shoot tissue treatments in biofumigant field experiment at Sandhill MSU research farm, East Lansing, MI, USA

root materials was determined. A sub-sample of root materials was rinsed with distilled water. The percentage (by area) of root tissues that were white (healthy) versus brown (diseased) was clearly observable and was quantified using Win-Rhizo image analysis system, as in experiment 2. An evaluation of disease organism presence in both white and brown root pieces was conducted using a random selection of root pieces that were cut out using a scalpel, sterilized with repeated washing in sterile water and ethanol, then transferred onto Petri plates containing PDA and selective media (name what the media was) for *P. ultimum* and *R. solani*. Petri plates were incubated for 4–5 days before visual evaluation for disease organism presence.

A one-row tractor was used to harvest potatoes on 11 October 2004, 10 days after plants were desiccated using Matrix^{TM} (Rimsulfuron) at 0.98 L ha^{-1} (0.25 L ha^{-1} a.i.) and Poast^{TM} (Sethoxydim) at 1.23 L ha^{-1} (0.55 L ha^{-1} a.i.) with 0.9 L ha^{-1} crop oil concentrates. Potatoes harvested from two 3 m middle sections of row were used to determine tuber yield. Tubers were sorted by diameter size into USDA market classes: oversize ≥8.3 cm; US No. 1 ≥ 5.1 cm; B < 5.1 cm; tubers with external physiological deformities were placed in a defect category. The fresh weight of potatoes in each category was determined. Rates of disease in potato tubers were scored by an experienced plant pathology technician on a scale of 0–4, where 0 indicated tubers with no signs of disease, 1 indicated (1–25% disease), 2 indicated (26–50% disease) and 3 indicated severe disease presence.

Soil disease and nematode monitoring

Disease potential bioassays were carried out, where the surface of tubers was monitored for disease symptoms during a glasshouse incubation of soil collected from the field experiment. Soil for the assay was sampled on 10 June 2004 (before cover crop incorporation), 22 July 2004 (during early potato growth) and on 2 September 2004 (during tuber-filling). On each date, six soil sub-samples were randomly collected from each plot (0–10 cm depth using a trowel) and composited by treatment. Additional topsoil was collected from a location adjacent to the field experiment site on the same dates and autoclaved at 121°C for two consecutive 60-min time periods, with 24 h between autoclaving operations. Soil was not expected to remain completely free of microorganisms over the 2- to 4-day time period between autoclaving and the initiation of the disease potential assay.

Soil was collected fresh for each assay, and moist sieved through a 8-mm sieve. Some of the autoclaved soil was reserved for a control treatment; the rest was inoculated with either *R. solani* or *P. ultimum* cultured as previously described. The fungal inoculation rate was 12 g m^{-2}, double the rate applied to the field, to ensure that infection would occur in these positive controls. Ten treatments were tested in all with five bioassay replicates of each per time point: field experiment soil from mustard root, mustard shoot or whole mustard plots, soil from rye root, rye shoot or whole rye plots, soil from the fumigated fallow control plots, autoclaved control soil, and autoclaved soil inoculated with either *R. solani* or *P. ultimum*. Bioassay soil was placed into 39 cm^3 plastic containers (5 replicates × 10 treatments = 50 containers).

For the 10-day bioassay, 'Onaway' tubers were surface sterilized by washing surface dirt off with distilled water, dipping tuber for 60 s in 5% Clorox® bleach and washing with two rinses of distilled water. Using a knife, each tuber was halved into similarly sized seed pieces (approximately 52 g), and four halves were placed, cut surface downward, on top of the soil in each container. The soil was compacted around each half tuber to provide maximum contact. The containers were watered every day to maintain uniform moisture as judged by moisture condensation on top of lid and appearance of the soil. Pictures taken at day 10 were used to rate tubers according to their degree of infection. The ratings were scored on a range of 0–4: 0 = white tissue, no observable disease symptoms, 1 = 1–24% brown, diseased tissue on tuber surface; 2 = 25–49% diseased; 3 = 50–74% diseased, and 4 = 75–100% diseased.

Soil was sampled on 30 May 2004 and 24 September 2004 to evaluate early- and late-season incidence of *P. penetrans* and *Verticillium*

dahliae, two important soil-borne potato pests. A modified centrifugation–flotation method for counting *P. penetrans* was used Jenkins (1964), where a 100 cm^3 sample was sieved through nested 16-mesh (openings = 1 mm) and 400-mesh sieves (openings = 38 μm), then *P. penetrans* individuals were enumerated. To assay for *V. dahliae*, a modified wet-sieving technique was used (Ashworth et al. 1972). Ten grams of air-dried soil were poured over nested 100-mesh and 400-mesh sieves with the resulting soil trapped on the 400-mesh sieve spread onto four Petri plates (100 × 15 mm) half-filled with a *V. dahliae* selective alcohol agar medium (Nada-Kavukaren and Horner 1959). Colonies were enumerated 14 days after plating.

Statistical analysis

SAS (Cary, NC, USA) was used to perform data analyses, using MIXED PROC (SAS, 2002). Mean differences were considered to be significantly different at $p < 0.05$ in the laboratory and glasshouse experiments (1 and 2), and $p < 0.10$ for the field experiment (3) due to the greater variability expected in the latter. For the laboratory assay, three-way analysis of variance (ANOVA) was used to evaluate fungal growth in response to volatile compounds. Main factors were trial (two trial runs), cover crop (none, mustard or rye) and tissue type (root, shoot or root plus shoot).

For the container glasshouse experiment, a two-way ANOVA compared tissue type (root or shoot) and two residue application rates (base rate and base rate × 4) to evaluate the effect of incorporating mustard cover crop residues on healthy potato root percentage, potato root weight, tuber disease and tuber yield. Pre-planned contrasts of means (root versus shoot and rate 1 versus rate 2) were carried out where a main factor was significant. For the field experiment, a two-way ANOVA tested the main effects of cover crop treatment (fallow, mustard or rye) and tissue type (roots, shoots or roots plus shoots) on root and tuber disease level, soil pest incidence, biomass of potato roots and shoots and tuber yield. Data normality was tested using residuals, and where needed (nematode population density) data was log transformed. Pre-planned mean contrasts were used to test differences where a main factor was significant. Data for soil-borne disease organisms, soil respiration and N mineralization potential was evaluated using the same model.

Results

Laboratory assay

Fungal suppression from released volatiles was observed with all tissues studied in the laboratory, including root and shoot residues of cereal rye as well as oriental mustard. In both runs of the experiment, volatile compounds released from macerated mustard tissues completely or almost completely inhibited *R. solani* hyphal growth after 24 and 48 h (Fig. 2). By comparison, fungal suppression by volatiles released from macerated rye tissues was relatively moderate at 24 h and did not persist at 48 h, except in the root plus shoot treatment (Fig. 2). In the control treatment, with no residues present, *R. solani* growth completely covered the Petri dish by 48 h.

A similar suppression pattern was observed for *P. ultimum* mycelium in the presence of macerated rye and mustard residues (Fig. 3). Rye tissues had moderately suppressive effects, whereas mustard tissues from roots and shoots were markedly effective at complete suppression of *P. ultimum* hyphal growth.

Glasshouse study

The results from a large volume (75 L) container experiment are presented in Table 1. Mustard residues added to field soil led to substantial disease suppression in potato. Mustard tissue type (roots, shoots, or roots plus shoots) and tissue loading rate were both highly significant factors that affected potato root health (white surface area), and tuber disease rating. Tuber disease rating was lower in treatments with higher tissue loading rates, and lowest overall in the highest tissue loading rate of root plus shoot treatment. Container-grown tubers to which oriental mustard shoots were added had low disease ratings (average 3.1), and white ‘healthy’ root tissue of

Table 1 Effects of mustard root and shoot tissues applied at different rates on root health (% of white area), tuber disease rating and potato tuber yield from a large-container experiment conducted in the glasshouse at East Lansing, MI campus

	Healthy root (% white surface area)	Disease rating tuber (score: 1–6[a])	Root weight (dwt) g plant^{-1}	Tuber yield (fwt) g plant^{-1}
Rate 0 (control)[b]	17.8	4.4	1.34	432
Root rate 1	24.5	4.3	1.70	568
Root rate 2	23.4	2.5	1.47	557
Shoot rate 1	71.1	3.6	1.25	399
Shoot rate 2	80.6	2.5	2.27	666
Root + shoot rate 1	57.4	2.2	1.49	531
Root + shoot rate 2	68.3	1.8	1.51	661
Factorial main effects				
Rate (*R*)	<0.69 NS	<0.02*	<0.43 NS	<0.03*
Tissue (*T*)[c]	<0.0003***	<0.05*	<0.30 NS	0.23 NS
$R \times T$	<0.45 NS	<0.38	<0.61 NS	0.56 NS
Planned contrasts				
Rate 1 vs. rate 2	0.33 NS	0.05*	NA	0.12 NS
Shoots vs. roots	0.07 NS	0.44 NS	NA	NA

Rate 0 = no cover crop residues added; *rate 1* = 40 g of cover crop tissue (dwt) per container, the rate being similar to that applied in the field experiment; *rate 2* = 160 g of cover crop tissue (dwt) per container

NS non-significant, *NA* not applicable as ANOVA was not significant so contrasts not conducted for means

*, **, *** Significant at 0.01, 0.05 and 0.001 probability levels, respectively

[a] Visual disease rating based on a score of 1 = no disease and 6 = entire tuber diseased

[b] No residues incorporated

[c] Tissue type contrast compares shoot, root and root + shoot

71% or greater. Following additions of *B. juncea* roots, the average disease rating of tubers and healthy root tissue percentage was 3.4 and 24%, respectively (Table 1). By comparison, where no mustard residue was added, average tuber disease rating was 4.4 and the percentage of healthy white root tissue was 18% (control, Table 1).

Tuber yield responses were variable, and significant differences were observed only for the factor of tissue addition rate. Significantly higher yields (about 600 g fwt plant^{-1}) were associated with the elevated dose of shoots or root plus shoot tissues. Residue dose did not affect yield (average of 563 g fwt plant^{-1}) in containers to which only *B. juncea* roots were added. Average tuber yield in the control treatment was 432 g fwt plant^{-1} (Table 1).

Field study

Soil organic C content at the field site was 0.59 mg g^{-1}, pH was 7.7 and P, K and Ca concentrations were 30, 35 and 2,525 mg kg^{-1}, respectively. The amount of cover crop biomass incorporated in the whole plant treatments was 4,500 kg ha^{-1} for rye root and shoot residues combined, and about 3,000 kg ha^{-1} dwt for mustard root and shoot residues (Fig. 4). Root biomass was about 30% of shoot biomass in mustard treatments, and 55% of rye shoot biomass in rye treatments (Fig. 4).

Potato characteristics

Potato tuber fresh weight ranged from 350 to 488 g plant^{-1}, equivalent to 24–32 Mg ha^{-1}, and was not significantly influenced by cover crop treatment (Table 2). Root biomass was influenced by cover crop treatment, at the first destructive harvest, when potato root weight was greater following incorporation of a rye cover crop than following other treatments (Table 2). The extent of root and tuber disease incidence is presented numerically in Table 2. Ratings were based on percent of brown tissue observed, a tissue reaction consistent with

Table 2 Influence of cover crop residues on average potato root biomass, average disease severity of roots and tubers and average tuber yield in the field experiment, East Lansing, MI

	Healthy root 31 July 2004 (% white surface area)	Healthy root 30 August 2004 (% white surface area)	Root weight. 31 July 2004 [g plant^{-1} (dwt)]	Root weight. 30 August 2004 [g plant^{-1} (dwt)]	Tuber disease rating[c] (0–4)	Tuber yield (fwt) (g plant^{-1})
Bare[a]	67.3	57.7	5.95	5.70	1.00	354
Rye shoot	55.2	60.6	6.02	5.52	2.62	443
Rye root	56.0	57.0	7.21	5.63	2.37	400
Rye root + shoot	88.1	87.9	7.81	5.43	1.37	350
Mustard shoot	76.1	69.3	4.39	4.88	1.87	370
Mustard root	74.2	71.2	7.11	5.39	1.25	488
Mustard root + shoot	90.6	90.7	4.33	6.43	0.75	429
Factorial main effects						
Cover crop (CC)	<0.0001***	<0.003***	<0.019*	0.89 NS	<0.0001	0.54 NS
Tissue type (*T*)[b]	<0.0001***	<0.0001***	0.095 NS	0.17 NS	NS	0.69 NS
CC × *T* interaction	<0.048*	0.24 NS	0.17 NS	0.19 NS	0.44 NS	0.37 NS
Planned contrasts						
Bare versus cover crop	0.45 NS	NS	0.19 NS	NA	0.003	NA
Mustard versus rye	0.003**	0.29 NS	0.03*	NA	0.05*	NA
Shoot versus root	0.07****	0.32 NS	0.24 NS	NA	NA	NA

NS non-significant, *NA* not applicable as ANOVA was not significant so contrasts not conducted for means

*, **, ***, **** Significant at0.05, 0.01, 0.001 and 0.10 probability levels, respectively

[a] Winter bare fallow, fumigant treated in the fall

[b] Tissue type main effect examines shoot, root and root + shoot

[c] Tubers were rated using the scale *0* = white tissue, no observable disease symptoms; *1* = 1–24% brown, diseased tissue on tuber surface; *2* = 25–49%, diseased; *3* = 50–74%, diseased; and *4* = 75–100% diseased

R. solani symptoms in potato (W. Kirk, personal communication). Overall, the lowest levels of disease were observed in mustard cover crop treatments (Table 2). The whole plant mustard treatments were associated with the healthiest potato root tissues (based on digitized percentage of white root tissue, 91% in this treatment), while roots in the bare control treatment were 63%. Tuber disease incidence was significantly ($p < 0.0001$) related to cover crop species but not to tissue type. Tuber disease severity was greatest in rye residue treatment plots, and smallest in the mustard root plus shoot and fallow-fumigated plots (Table 2).

Soil biology

Three years of growing continuous potatoes prior to conducting this experiment did not successfully build up *P. penetrans* or *V. dahliae* populations at the field site. While *P. penetrans* was not detected in May following whole plant inputs (root plus shoot) of rye and mustard cover crops, populations rose to an average of 9.2 nematodes 100 cm^{-3} soil in September (Table 3). *P. penetrans* populations were low in the root or shoot alone treatments, and in the fallow fumigated treatments at both dates (Table 3). Variability within *V. dahliae* data was high. In May, average presence of *V. dahliae* in soil taken from cover crop plots was 0.1 colonies gm $soil^{-1}$. This was significantly less than the average of 0.2 colonies gm $soil^{-1}$ observed in soil taken from the fallow fumigated plots, but no treatment had levels of *V. dahliae* above 1 colony gm $soil^{-1}$ the threshold for plant injury (Michigan State University *V. dahliae* recommendations, G. Bird, personal communication). Later in the growing season (September), there were no significant treatment effects on *V. dahliae* or *P. penetrans* population density.

Soil disease potential results from a tuber assay are shown in Table 3. The assay conducted in June, before cover crops were incorporated,

Table 3 Influence of cover crop treatment on soil microbial biomass (substrate induced respiration, SIR), N mineralization potential (NMP 10-day assay, 15 July 2004), tuber assay of disease potential (12 July 2004 and 2 September 2004) and nematode (*Pratylenchus penetrans*) population numbers in May and September in a potato field experiment in East Lansing, MI

	SIR 0 to 10-day assay (CO_2 mg^{-1} kg h^{-1})	NMP (NH_4 + NO_3 mg^{-1} kg day^{-1})	Disease potential July rating (0–4)	Disease potential September rating (0–4)	Prat. May (no. 100 cm^{-3} soil)	Prat. September (no. 100 cm^{-3} soil)
Bare[a]	9.8	0.54	1.00	1.10	0.5	0.2
Rye shoot	9.2	0.60	3.00	2.37	2.5	1
Rye root	9.3	0.79	2.37	2.62	0.25	10.3
Rye root + shoot	8.4	0.75	1.12	1.37	0	7.7
Mustard shoot	9.9	1.22	0.87	1.25	4.25	1.5
Mustard root	9.8	1.19	1.62	1.87	2.5	3.5
Mustard root + shoot	8.9	1.32	0.62	0.75	0	10.7
Factorial main effects						
Cover crop (CC)	0.09****	0.02*	<0.001***	<0.001***	0.31 NS	0.76 NS
Tissue type (*T*)[b]	0.18 NS	0.10****	<0.001***	<0.001***	0.10****	0.18 NS
CC × *T*	0.25 NS	0.29 NS	<0.001***	0.43 NS	0.57 NS	0.49 NS
Planned contrasts						
Bare versus cover crop	NA	0.01**	<0.001***	<0.001***	NA	NA
Mustard versus rye	0.07****	0.03*	0.08****	0.78 NS	NA	NA
Shoot versus root	NA	NA	0.22 NS	0.39 NS	0.27 NS	NA

NS non-significant, *NA* not applicable as ANOVA was not significant so contrasts not conducted for means, *Prat. Pratylenchus penetrans*

*, **, ***, **** Significant at 0.05, 0.01, 0.001 and 0.10 probability levels, respectively

[a] Winter bare fallow, fumigant treated in the fall

[b] Tissue type main effect examines shoot, root and root + shoot

resulted in an average disease potential score of 2.8 (±1.1) with no treatment effects observed. July and September results from the field bioassay were similar to the container study; mustard shoot and shoot plus root treatments were associated in both cases with the lowest level of disease, 0.7–1.2 (Tables 1, 3). Rye tissue treatments were included in the field experiment and found to be associated with higher levels of disease than mustard treatments, particularly the rye single tissue treatments (root or shoot), which were associated with disease levels ≥2.4. Similarly, the tuber assay of disease potential in field soil indicated moderate to high (2.4–3.0) disease scores in rye single tissue treatments compared to all other treatments (1.0–1.9; Table 3).

Glucose-inducible respiration rates were monitored as an indicator of microbial activity (Table 3). Overall, the soil microbial activity as measured by SIR did not vary much across treatments for the initial 10-day assay, other than a modest response to the rye whole plant treatment (Table 3). There was no treatment effect for the 11 to 20-day assay (mean response 1.1, standard deviation 0.4). Nitrogen mineralization potential was determined using a standard incubation process, an aerobic 30-day assay where release of inorganic N was calculated on a per day basis (after subtracting initial soil inorganic N). Interestingly, there was no difference in initial mineral N (data not shown), yet there was a significant cover crop species effect on N mineralization potential (Table 3). This dynamic inorganic N pool was increased by approximately twofold in the mustard residue treatments compared to the other treatments (Table 3). No tissue type effects were observed.

Discussion

Suppressive effects of *Brassica* species on fungal organisms have been clearly shown, in container studies (Lazzeri and Manici 2001) and in vitro assays (Sarwar et al. 1998). *B. juncea*, the species selected for our research, was reported as being the most effective fungal growth suppressor in a laboratory assay that compared the effects of six *Brassica* species on mycelium radial growth of *P. ultimum* and *R. solani* (Charron and Sams 1999). Our laboratory bioassay confirmed the effectiveness of the newly released mustard cultivar 'Pacific Gold' as a source of fungal-suppressive volatile compounds.

A comprehensive study on tissue biochemical compounds and biosuppression found that both root and shoot tissues of oriental mustard and related genotypes contained relatively high levels of glucosinolates (Kirkegaard and Sarwar 1998). Our controlled environment studies documented a similar pattern, as both root and shoot tissues of mustards had biosuppressive properties against soil-borne diseases (Figs. 2, 3 and Table 1). Mustard shoots and roots by themselves suppressed *R. solani* growth in the laboratory assay, but provided significantly less ($p < 0.001$) suppression compared to the root plus shoot treatment (Fig. 2). This result is not surprising because the dose was higher in the combined treatment, and aromatic compounds found in *B. juncea* roots are expected to be biotoxic in a soil-free environment.

The differences observed among treatments in their effects on fungal suppression within the 2-day time span of the bioassay illustrate the rapid rate of release and dissipation of the effects of volatile compounds released from macerated tissues. The expected persistence time of biotoxic ITC compounds released from *Brassicas* is brief, less than a week within the soil environment (Rumberger and Marschner 2003). To use *Brassicas* effectively for biofumigation purposes, plant cells must be extensively damaged, for example by mowing or freezing and incorporated at a vulnerable point in the life-cycle of the targeted pest (Matthiessen et al. 2004; Morra and Kirkegaard 2002; Matthiessen and Kirkegaard 2006). The extent of volatile compound release and subsequent biotoxic effects is complex, as it is influenced by plant age and species, and extent of plant incorporation in the soil, soil type, soil moisture level and other factors (Watt et al. 2006; Matthiessen and Kirkegaard 2006), thus posing significant challenges for farmers searching for successful biological alternatives in pest management.

There have been a limited number of studies in the field environment, where biosuppressive

activity is challenging to predict. In our field study, root tissues were not always associated with healthy potato roots or biosuppression; however, the combined treatment of mustard roots and shoots was consistently associated with the healthiest potato roots and tubers (Tables 2, 3). Understanding the biosuppression activity of root tissues is complex; the amount of glucosinolate compounds incorporated with root residues vary with root system growth and biochemistry, and activity of compounds released is influenced markedly by residue management and environment (Morra and Kirkegaard 2002). The total amount of root tissues relative to shoots tends to be less, thus the glucosinolate dose from *Brassica* roots is often less than from shoots (Matthiessen and Kirkegaard 2006). However, root plasticity is tremendous, and root growth is apt to be enhanced in low fertility soils compared to shoot growth. In our field experiment, the amount of cover crop root tissues present was 35–55% of shoot tissues (Fig. 4). Our earlier research indicated that the proportion of cover crop root to shoot residues varies from 120% in highly infertile, sandy soil to 25% in fertile, sandy loam soil (Snapp et al. 2005).

Our field results indicate that mustard root tissues can have biosuppression activity in relationship to soils inoculated with *P. ultimum* and *R. solani*, however using whole plants (root plus shoot) tissue appeared to be the most effective at improving root health (Table 3). *Brassica* roots have been shown to effectively suppress growth of take-all fungus (Angus et al. 1994). Understanding root tissue biosuppressive effects is complicated by recent research findings on the short-chain aliphatic and long-chain aromatic structures of ITC compounds in *B. juncea.* A study by Matthiessen and Shackleton (2005) found that the former, primarily found in shoot tissues, have greater biotoxic potential than the latter, which are primarily found in root tissues and whose toxicity was readily disabled within the soil environment. This may explain, in large part, the contrasting results we observed between the laboratory assay, where any amount of root or shoot residues tested had biosuppressive activity, and the container and field experiments where shoot residues were consistently superior to root residues in biosuppressive activity (Fig. 2, Tables 1, 2).

To disaggregate the effect of quantity of residues versus quality of residues (root versus shoot tissue), the glasshouse container experiment was carried out with different doses of root, shoot and root plus shoot residues. The treatments with shoot residues, at the higher doses, had marked positive effects on both tuber health and yield (Table 1). A dose of tissue equivalent to 1,600 kg ha^{-1} or higher was associated with disease suppression as tuber ratings were reduced to ≤2.4 regardless of tissue type (Table 1), on a scale of 1.0 (no observable disease) to 6 (80–100% disease). This compares to disease ratings above 3.0 associated with doses of residue <1,600 kg ha^{-1}. Biomass production of *Brassica* species varies widely, from less than 500 kg ha^{-1} to more than 7,000, and the minimal amount of biomass associated with biofumigation requires more study (Kirkegaard and Sarwar 1998; Snapp et al. 2005). Climatic constraints often preclude production of large amounts of cover crop biomass in a crop rotation sequence. Detailed study of how to effectively manage moderate doses of cover crop residues to optimize biosuppressive effects would provide useful information for biologically based pest management.

Rye versus mustard

An early study by Virtanen and Hietala (1955) indicated that alleopathy involving rye plants could include fungal suppression, and this cold-tolerant species is widely used as a winter cover crop by Northern hemisphere potato producers (Snapp et al. 2005). The nematicidal potential of rye residues has also been shown, in a glasshouse study by Guertal et al. (1998), where *Rotylenchulus reniformis* populations were reduced following a rye cover crop. Yet to our knowledge there has been no systematic investigation of rye for disease suppression activity, nor has the biofumigation potential of rye been evaluated in relationship to *Brassica* species. Confirming Virtanen and Hietala's research, our experimentation under controlled, laboratory conditions showed that macerated rye tissues produced volatiles suppressive of fungal growth (Figs. 2, 3). Oriental

mustard tissues showed more long-lasting and substantive effects in the same assays, as suppression of *P. ultimum* and *R. solani* growth was 80–100% after 48 h exposure to mustard residues (shoot plus root) versus 30–55% after 48 h exposure to rye residues (shoot plus root).

Mustard cover crop residues were substantially more disease suppressive than rye residues in the field environment, where complex interactions of soil, climate and management are expected. While mustard roots were somewhat effective at reducing tuber disease ratings in the field, the most effective disease suppression was consistently achieved with the incorporation of whole mustard plants (roots and shoots). The mustard whole plant treatment was associated with the highest level of white root and tuber tissue (indicative of a healthy potato crop), and with disease suppression as indicated by the tuber assay (Table 3). This is consistent with high biosuppressive activity of mustard tissues compared to rye tissues, as mustard growth was moderate and 2,000 kg ha^{-1} less residues were incorporated compared to rye residues (Fig. 4). The container experiment indicated that quantity of residues was important, yet even relatively large amounts of rye residues had no detectable biosuppressive activity in the field, as indicated by tissue ratings and by tuber assay (Tables 2, 3). The biofumigant effect that was observed with rye residues in the laboratory assay was apparently short-lived, and did not hold up in the presence of the biologically and chemically complex soil environment (Fig. 2).

The field and large-volume container experimentation reported on here provide insights into performance of a potato cash crop subsequent to a biosuppressive cover crop. In the field, potato tuber yield was 26.2 Mg ha^{-1} (± standard deviation 2.7). This is in the expected yield range of 20–36 Mg ha^{-1} for fresh market cultivars in North Central USA (Long et al. 2004). A moderate yield potential was expected at our site given the short-season cultivar 'Onaway' used, and the late June planting time. Yield and root biomass in the field were not significantly influenced by cover crop treatments, except at the first destructive harvest, when potato root weight was greater following incorporation of a rye cover crop than following other treatments (Table 3). There is not a clear explanation for this observation. It is possible that the high biomass rye cover crop reduced soil moisture and this enhanced potato root system growth. However, recommended potato production practice was followed including supplemental irrigation, to ameliorate soil moisture differences across treatments (0–20 cm gravimetric moisture measurement on 15 July 2004 indicated no treatment effect, soil moisture content 52 ± 6%).

Soil microbial activity was monitored using glucose inducible respiration and limited effects of cover crop residues were observed. The greater amount of biomass incorporated, and a low tissue N concentration in the biomass (1.5% N in rye shoot tissue versus 2.7% N in mustard shoot tissue) could have led to N-limitations in decomposition of rye compared to mustard, indicated by the low microbial SIR activity observed with the rye root + shoot treatment (Table 3). The soil N mineralization potential data was not consistent with this explanation as N mineralization in a short-term assay was low in all of the rye treatments and in the fallow, not just in the root plus shoot treatment (Table 3). Incorporation of a nutrient-enriched substrate such as cover crop residues is expected to enhance soil microbial activity (Hoitink and Boehm 1999), however the effect may be short-lived (Abdallahi and N'Dayegamiye 2000). Management practices had a rapid and transitory impact on bacterial and fungal community growth and activity in California irrigated agro-ecosystems, as shown by evaluating community composition and activity through phospholipid ester-linked fatty acid and microbial biomass assays (Lundquist et al. 1999). The temporally dynamic and spatially variable nature of microorganism response (Grunwald and van Bruggen 2000), in addition to the functional redundancy of soil communities in agro-ecosystems (Cavagnaro et al. 2006), poses challenges to understanding the relationship of microbial activity and disease suppression.

Nitrogen mineralization potential was found to unresponsive to a mustard cover crop in a similar potato systems trial carried out on coarse soils of the Pacific Northwest, USA (Collins et al. 2006). However, in our study, mustard residues were

associated with significantly higher NMP than rye residues, 1.2 mg^{-1} N kg day^{-1} compared to 0.7 mg^{-1} N kg day^{-1}. Although the bulk composition of the soil samples tested and dynamic nature of microbial activity pose methodological challenges, cover crop treatment influence was observed for gross N mineralization potential trends. Despite the moderate quantities of mustard residues incorporated, N mineralization potential pool responded; presumably due to the high N content of mustard tissues relative to rye tissues (Table 3). An earlier study of cover crop management supported these findings, as residue quality was the driving factor in N mineralization response of large cores from a related potato systems trial (Snapp and Borden 2005).

The relatively low density of *V. dahliae* and *P. penetrans* populations observed at the field site was incompatible with testing the impact of cover crop species on this important plant parasitic nematode and disease complex (Table 3). The coarse textured soil environment present may support pathogens less well than in heavier textured soils, which explains in part the possible advantage of producing potatoes on sandy soils (Knudsen et al. 2002).

Conclusion

Where mustard tissues were incorporated, disease suppression was consistently observed across all of the experimental approaches tested here, under controlled and field conditions. Different mechanisms may have been in operation in the lab assays versus the experiments with field soil present. Volatiles from rye tissues were effective, in the short-term, at suppressing *R solani* and *P. ultimum* hyphal growth under controlled laboratory conditions. In contrast, field experimentation indicated that disease suppression in a potato crop was not observed after incorporation of rye residues. Healthy potato roots and tubers were consistently associated with high doses of mustard residues, particularly for root plus shoot tissues (Fig. 4, Tables 1, 3). Soil media provides a complex environment that supports diverse biosuppression processes (Wiggins and Kinkel 2005). In our study, quantification of disease potential (tuber bioassay) and plant response (root and tuber tissue observations, biomass accumulation and plant development) indicated the following: (1) incorporation of large doses of *Brassica* tissues was markedly effective at supporting healthy plant growth and (2) laboratory assays conducted in the absence of soil appear to overestimate biofumigation potential. Predicting longer term (the duration of a growing season, or multiple year) effects of incorporating cover crop tissues containing biosuppressive chemicals requires additional *in situ* field studies of controlling factors and modeling of fungi, residue, root and soil environmental dynamics.

Combined, the lab and field study results presented here have implications for farmers who use cereal rye as a spring grazing crop or who want to include mustard as an oilseed crop within their field crop rotations. Soil-borne disease levels must be considered when weighing the costs of removing cover crop shoots either for feed or as part of a seed production system against the decreased potential of the cover crop to suppress disease. Identifying seeding rates and planting windows that maximize cover crop biomass prior to incorporation should lead to increased disease suppression in potato and other cash crops. Although the root health promoting effects of a rye cover crop were modest to nil relative to those provided by a mustard cover crop, seed costs of rye are relatively inexpensive and rye has greater cold tolerance. Additional study on the biosuppressive effects of rye used in combination with mustard is warranted.

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