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Response of Cover Crops to *Phytopythium vexans*, *Phytophthora nicotianae*, and *Rhizoctonia solani*, Major Soilborne Pathogens of Woody Ornamentals

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Abstract: Management of plant diseases is a subject of concern for researchers as well as growers. Different management practices are being developed and used to combat the rising number of plant pathogens, which threaten nursery crop production. Use of cover crops for sustainable management of soilborne diseases is being explored as an alternative strategy to the chemicals. However, the potential threat of these cover crops acting as a secondary host of these devastating soilborne pathogens has not been described. We studied the response of the major cover crops being used by woody ornamental growers in the Southeastern United States to *Phytopythium vexans*, *Phytophthora nicotianae*, and *Rhizoctonia solani* in greenhouse conditions to identify the effective cover crops that can be used in a nursery field production system. Data related to post-emergence damping-off and plant growth parameters (plant height increase and fresh weight) were recorded. Similarly, cover crop roots were assessed for root rot disease severity using a scale of 0–100% roots affected. Among the tested cover crops, the grass cover crops triticale (×*Triticosecale* Wittm. ex A. Camus.), annual ryegrass (*Lolium multiflorum* L.), Japanese millet (*Echinochloa esculenta* (A. Braun) H. Scholz), and the legumes Austrian winter pea (*Pisum sativum* var. *arvense* (L.) Poir) and cowpea 'Iron and Clay' (*Vigna unguiculata* (L.) Walp.), showed lower root rot disease severity and post-emergence damping-off in the soil inoculated with *P. nicotianae*, *R. solani*, or *P. vexans* compared to the other crops. Since these cover crops can act as non-host crops and benefit the main crop in one way or another, they can be used in the production system. Further research is recommended to evaluate their performance in a natural field setting.

Keywords: soilborne diseases; nursery crops; cover crops; secondary host; susceptibility

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1. Introduction

The nursery crop industry is the fastest growing sector of US agriculture, contributing USD 5.1 billion to the economy [1]. Although nursery crops are primarily grown for their aesthetic value, crop production must remain economically viable for the continued promotion and production of these plants. Due to the increased area and production of nursery crops [1], exploration of the constraints of their production is necessary. One of the major limitations of nursery crop production is soilborne diseases caused by *Phytophthora* spp., *Phytopythium* spp., *Rhizoctonia* spp., *Fusarium* spp., *Sclerotinia* spp., *Armillaria* spp., *Pythium* spp., and *Verticillium* spp. [2,3]. Additionally, the diversity of plant species grown in ornamental nursery sectors poses a challenge to the management of soilborne diseases, especially in susceptible woody ornamental crop species.

Among these soilborne pathogens, *Phytophthora nicotianae* (Breda de Haan) is one of the important oomycete pathogens of concern for nursery growers. A wide range of

plants (more than 255 plant genera of 90 families) are susceptible to *P. nicotianae,* which causes significant loss in different crop production systems [4]. Because woody ornamental plants are perennial, this pathogen can complete multiple cycles, making it one of the most common and devastating pathogens in woody ornamental nurseries [5–8]. Root and crown rot and stem infections are the common symptoms associated with *P. nicotianae* [9] in plants, which accelerates chlorosis as well as defoliation of the plants.

Similarly, *Phytopythium vexans* (de Bary) is an oomycete which has been observed to be pathogenic in plants such as kiwi, citrus, avocado, flowering cherry, ginkgo, and maple in Turkey, Tunisia, Canary Islands, and the United States, respectively [10–14]. This pathogen poses a potential threat in woody ornamental production systems, resulting in chlorosis, necrosis, and defoliation, dark brownish to blackish lesions in the crown area, and root rot in plants.

Rhizoctonia solani (J.G. Kühn) is an important and notorious plant pathogenic fungi which is found in both cultivated and non-cultivated soil. This pathogenic fungus can attack over 500 species of plants including cereals, fruit trees, forest trees, ornamental plants, as well as turfgrasses [5,15]. Early developmental stages of plants are at increased risk of *R. solani* causing pre- and post-emergence damping-off of seedlings, which contributes to irreversible loss of plants. Additionally, root and stem rot, collar rot, blight, leaf spot, and wire stem [5,16,17] are common symptoms associated with *R. solani*.

The revolution of chemical plant protection and the development of site-specific chemicals are leading the way in disease management. However, continuous and haphazard use of these chemicals makes them less effective on these pathogens. The effect of those chemicals on environmental and human health, soil microbiology, plant growth, and resistance development need to be addressed. We cannot ignore the fact that new fungicidal chemistries with new modes of action are being developed, which are effective and have low residual activity. However, the production, as well as environmental costs associated with these chemicals, always demands a better, sustainable approach. Some biological control agents such as *Bacillus*, *Trichoderma*, *Rhizobium*, *Streptomyces*, *Serratia*, and *Pseudomonas* are being developed and used effectively [18]. Some other disease management options, such as sanitation, soil solarization, soil biofumigation, anaerobic soil disinfection, and mixed cropping, solely or in combination with each other, can be used [3]. The use of cover crops is promising in woody ornamental production systems; however, there is a long way to go before drawing a core conclusion and making proper recommendations. A few researchers [19–21] screened several cover crops such as winter wheat, crimson clover, triticale, and Brassicaceae crops against soilborne plant pathogens and discovered potential benefits of the cover crops.

Cover crops are an important part of the crop production system. Although they are generally used to cover the field, more often than not they are used as green manure or animal forage. Cover crops slow soil erosion, improve soil physical (structure) [22] and chemical properties (pH balance, increase C:N ratio) [21], smother weeds, conserve moisture, and may attract beneficial insects, which prey on pests. Cover crops are also known to increase beneficial microorganisms in the soil such as *Pseudomonas* spp. as well as increase soil disease suppressiveness [19,21]. Although the use of cover crops is generally confined to agronomic and vegetable crops, their benefits in woody ornamental production systems are also being realized. Research on cover crops generally focuses on their potential beneficial aspects, which ignores the possibility of cover crops acting as a potential host for a specific pathogen. If these cover crops harbor the unnecessary pathogens, it can elevate the risk of plant pathogens invading the main crop. It is therefore critical to screen these cover crops against these major soilborne pathogens before initiating cover crop introduction in the field. In this study, we selected 14 cover crops which are generally used in the Southeastern U.S. and screened them for germination. Those exceeding an 80% germination rate (9 cover crops) were tested against *P. vexans*, *P. nicotianae*, and *R. solani*; which are among the major soilborne pathogens in woody ornamental production systems such as maple, gingko, boxwood, hydrangea etc. [12,19,20].

2. Materials and Methods

2.1. In Vitro Screening of Cover Crop Germination

Germination percentages were determined by placing 10 seeds from 14 cover crop species (Table 1) on 10 cm petri-plates (Fisher Scientific Inc., Hampton, NH, USA) which were lined with #1 Whatman filter paper (VWR International LLC., Radnor, PA, USA) and wetted with 10 mL sterilized distilled water. The cover crops were selected as per the growers use and availability in Tennessee. Four single-plate replications per cover crop were laid out in a completely randomized design and were incubated at 25 °C (VWR incubator, Radnor, PA, USA) in the dark for 12 days. After three days of incubation, germination counts were performed 4 times with two-day intervals. The percentage of germination was calculated by dividing the total number of seeds germinated by the total number of seeds plated [23]. The experiment was repeated twice.

Table 1. List of cover crops used for in vitro screening for germination.

Cover Crop	Scientific Name	Seed Source	Growing Season	Germination Rate (%) *
Crimson clover	*Trifolium incarnatum* L.	Summitville Grain & Feed	Cool	87.5
Triticale	×*Triticosecale* Wittm. ex A. Camus.	Summitville Grain & Feed	Cool	97.5
Austrian winter pea	*Pisum sativum* var. *arvense* (L.) Poir	Summitville Grain & Feed	Cool	100.0
Annual ryegrass	*Lolium multiflorum* L.	Summitville Grain & Feed	Cool	100.0
Red clover 'Kenland'	*T. pratense* L.	Summitville Grain & Feed	Cool	82.5
White clover	*T. repens* L.	Summitville Grain & Feed	Cool	22.5
Buckwheat	*Fagopyrum esculentum* Moench	Summitville Grain & Feed	Warm	100.0
Turnip 'Pointer'	*Brassica rapa subsp. rapa* L.	Summitville Grain & Feed	Cool	67.5
Tillage radish 'Daikon'	*Raphanus sativus* L.	Summitville Grain & Feed	Cool	97.5
Cowpea 'Iron and Clay'	*Vigna unguiculata* (L.) Walp.	Summitville Grain & Feed	Warm	95.0
Japanese millet	*Echinochloa esculenta* (A.Braun) H.Scholz	Summitville Grain & Feed	Warm	97.5
Browntop millet	*Urochloa ramosa* L.	Summitville Grain & Feed	Warm	62.5
Proso millet	*Panicum miliaceum* L.	Summitville Grain & Feed	Warm	25.0
Pearl millet	*Pennisetum glaucum* (L.) R. Br.	Summitville Grain & Feed	Warm	62.5

* Values represent the mean of the two experimental setups.

2.2. Pathogens: Culture and Inoculum Preparation

Isolate FBG201506 of *P. nicotianae* (GenBank accession MK399300), isolate FBG201508 of *R. solani* (GenBank accession MT533254), and isolate FBG20182 of *P. vexans* (GenBank accession MT076055) isolated from hydrangea and red maple plant, respectively, were obtained from Dr. Fulya Baysal-Gurel's culture collection at the Tennessee State University, Otis L. Floyd Nursery Research Center (TSUNRC), McMinnville, TN. *R. solani* specimen was cultured and maintained on potato dextrose agar (PDA: Becton, Dickinson, and Company, Sparks, MD, USA) medium. *P. nicotianae* and *P. vexans* specimens were cultured and maintained on V8 medium (50 mL of clarified V8 juice (Campbell, Camden, NJ, USA), 7.5 g of agar (Sigma-Aldrich, St. Louis, MO, USA), and 450 mL of deionized water). Preparation of *P. nicotianae* inoculum was performed by following Holmes and Benson's [24] rice grain method. Shortly thereafter, 25 g of long grain rice in 18 mL deionized water was autoclaved twice. Three plugs of 7 mm-sized *P. nicotianae*-colonized V8-agar were placed in the 250 mL flask containing the autoclaved rice, followed by incubation at room temperature. To ensure uniform colonization, rice inoculum in the flask was mixed meticulously until final use. For *P. vexans* inoculum, an agar slurry (2 petri plates of a 7-day-old *P. vexans* culture transferred into a sterilized beaker with 1 L of sterilized distilled water and homogenized with a blender (Hamilton Beach hand blender, Model number 59785R, Hamilton Beach Brands, Inc., Glen Allen, VA, USA)) was prepared [12]. For *R. solani* inoculum, an agar slurry of 7-day old cultures of *R. solani* grown on PDA was prepared at the rate of 1 petri plate/L [25].

2.3. Greenhouse Bioassay for Pathogenicity Test

A set of nine different cover crops (crimson clover, triticale, Austrian winter pea, annual ryegrass, red clover, buckwheat, tillage radish, cowpea, and Japanese millet), which surpassed the 80% germination rate (Table 1) according to the in vitro experiments, was sown into 10 cm diameter and 9 cm deep black plastic pots filled with sterilized clay

loam soil. The seed sown were surface sterilized with 1% sodium hypochlorite solution. Sterilization of soil was done by using an electric soil sterilizer (Pro-Grow Supply Corp., Model SS-30 Brookfield, WI, USA) for 100 min at 85 °C.

For each bioassay, ten individual cover crop seeds were sown per pot and a separate set of pots was used for each cover crop species. For each treatment (pathogen and control, n = 4), six single-pot replications were arranged in a randomized block design in a greenhouse at the TSUNRC. For *P. nicotianae* experiment, treated pots received three *P. nicotianae* infested rice grains while the control pots received pathogen free rice grains. Similarly, for *R. solani* and *P. vexans* inoculation, 100 mL of agar slurry was drenched while the control pots received the pathogen free agar-slurry. All the plants for each pathogen were inoculated on the same day of sowing. All pots were hand watered (100 mL/pot) twice a day. The whole set of experiments was repeated twice. Trials were conducted between 15 March and 16 April, and 12 May–13 June, respectively. Average maximum temperatures for March, April, May, and June 2020 were 28.3 °C, 28.9 °C, 27.7 °C, and 30.2 °C; average minimum temperatures were 15.9 °C, 17.1 °C, 18.3 °C, and 18.7 °C; and average relative humidity levels were 95.4%, 91.3%, 68.0%, and 95.3%, respectively.

2.4. Assessment of Cover Crop Health

After complete germination of cover crops, stand data were recorded in the greenhouse bioassays. Those that failed to germinate were counted and were reported as part of the damping-off percentage. Damping-off percentage was calculated as 100 times the ratio of plant that failed to germinate or died after germination to the total seeds sowed. Plant growth parameters such as plant height (from the soil line to the tallest point of foliage), total plant fresh weight and total fresh root weight was recorded at the end of each experiment. Cover crops were uprooted, and the roots were cleaned with running tap water to remove the soil debris. Evaluation of root rot severity was done with visual assessment of roots using a scale of 0–100% of total root system affected. After the assessment of roots, from each replication, ten randomly selected root pieces of ~1 cm long root tip were plated on PARPH-V8 selective medium and *Rhizoctonia* selective medium, respectively. To prepare PARPH-V8 selective medium, 500 µL of PCNB (pentachloronitrobenzene (99%) (GC) Sigma-Aldrich, St. Louis, MO, USA; 0.63 g/50 mL ethanol), ampicillin (Sigma-Aldrich, St. Louis, MO, USA; 1.25 g/50 mL ethanol), pimaricin (2.5%) (MP Biomedicals, Santa Ana, CA, USA), rifampicin (Sigma-Aldrich, St. Louis, MO, USA; 0.05 g/50 mL ethanol), and hymexazol (Sigma-Aldrich, St. Louis, MO, USA; 250 mg/50 mL sterile water) were added to the V8 medium after autoclaving [26,27]. Similarly, for the preparation of *Rhizoctonia* semi-selective medium, 18 g of agar (Sigma-Aldrich, St. Louis, MO, USA) was added in 1000 mL of deionized water and autoclaved (121 °C at 15 psi for 15 min). After autoclaving, 100 mg of each streptomycin sulfate (ACROS organics, Morris Plains, NJ, USA) and penicillin-G Na salt (Alfa Aesar, Ward Hill, MA, USA), and 800 µL of 1 M NaoH (AMRESCO Inc., Solon, OH, USA) was added [28]. The plates were then incubated at 25 °C in an incubator (VWR incubator, Radnor, PA, USA). After three days, the total number of root pieces showing growth of pathogen was counted. Percentage of recovery was calculated as 100 times the proportion of root pieces showing pathogen growth. Randomly selected colonies were used for identification using DNA sequencing. Mycelia was scrapped and total genomic DNA was extracted using the UltraClean Microbial DNA Isolation Kit (MO-BIO Laboratories Inc., Carlsbad, CA, USA). The primer pairs ITS1 and ITS4 were used to amplify ribosomal DNA 'internal transcribed spacer (ITS)' region [29]. Positive PCR products were identified using gel electrophoresis and purified using Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) following the manufacturer's instructions and sent for sequencing to GenHunter Corporation (Nashville, TN, USA).

2.5. Data Analysis

Cover crop seed germination rate, Phytopythium, Phytophthora, and Rhizoctonia root rot disease severity and recovery percentage, and total damping-off were analyzed among the cover crops; and plant growth parameters (height, total plant fresh weight and total fresh root weight) were analyzed among the pathogens using the general linear mixed model procedure (PROC GLIMMIX) with SAS statistical software 2016 (SAS Institute Inc., Cary, NC, USA). The plant growth parameters were compared among pathogens (within cover crops) to remove statistical bias as different plant species may have different natural growth parameters. However, response parameters such as disease severity, pathogen recovery, germination rate, and damping-off were compared among cover crops as to compare the susceptibility among cover crops. The means were separated using Tukey's post-hoc test and Pearson correlation coefficient was calculated. The graphs were created using Sigma plot 12.0 (Systat Software, Inc., San Jose, CA, USA).

3. Results

In the in vitro experiment, 100% germination was recorded for Austrian winter pea, annual ryegrass, and buckwheat (Table 1). Similarly, triticale, tillage radish, and Japanese millet showed 97.5% germination rate. A germination rate higher than 80% was observed with cowpea (95%), crimson clover (87.6%), and red clover (82.5%). Lowest germination was observed with white clover, followed by proso millet, brown top millet, pearl millet, and turnip with the germination rate of 22.5%, 25.0%, 62.5%, 62.5%, and 67.5%, respectively. Since there were only nine cover crops exceeding 80% germination rate, only those cover crops were selected for a pathogenicity test to evaluate their response to major soilborne pathogens.

All the tested soilborne plant pathogens showed similar symptoms such as damping-off, stunted growth, chlorosis, and defoliation, in extreme cases. Damping-off of seedlings was observed within the first ten days of inoculation. Clear symptoms were observed in the root and crown areas of the plant system after one month of pathogen inoculation. Randomly selected water-soaked or necrotic root pieces were cultured on V8-PARPH and *Rhizoctonia*-selective media for the confirmation of presence of *Phytophthora, Phytopythium,* or *Rhizoctonia* for both trials and colonies were confirmed as *P. nicotianae*, *P. vexans,* and *R. solani* using DNA sequencing.

All cover crops exhibited root damage from each pathogen, but that severity as well as recovery varied among cover crops. Cover crops grown on *P. nicotianae*-infested soil showed a range of 3.3% to 28.3% and 6.7% to 27.5% disease severity while pathogen recovery percentages ranged from 15.0% to 28.3% and 18.3 to 38.3% for Trials 1 and 2, respectively (Figure 1). All the cover crop species had lower disease severity compared to buckwheat in Trial 1. Buckwheat, crimson clover, and red clover showed the highest root rot disease severity compared to other cover crops in the second trial, while triticale, tillage radish, Japanese millet, and Austrian winter pea showed the least disease severity. No difference was observed in the pathogen recovery from the roots of cover crops in Trial 1, while Austrian winter pea showed less pathogen recovery (18.3%) followed by triticale (25.0%), annual ryegrass (30.0%), tillage radish (30.0%), and cowpea (33.3%) from their roots in Trial 2.

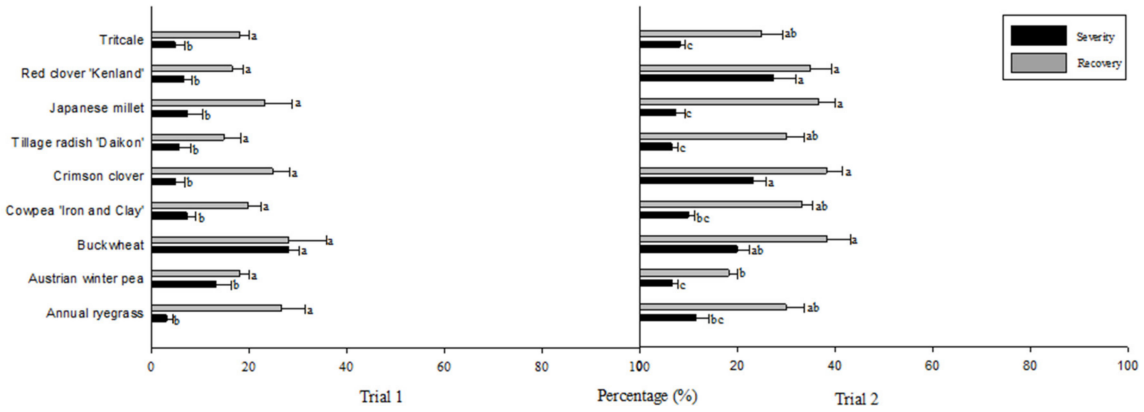

Figure 1. Disease severity and recovery (mean ± SE) of cover crop roots when *Phytophthora nicotianae* was inoculated in the soil. Phytophthora root rot disease severity was evaluated using a scale of 0–100% of roots affected. For each replication, ten randomly selected cover crop root samples were plated V8-PARPH oomycete-selective medium to determine the percent recovery of *P. nicotianae* from root samples. Different letters on the bars indicate significant differences in Phytophthora root rot disease severity or pathogen recovery among cover crops (Trial 1: Severity: $F = 12.57$, $p < 0.0001$; Recovery: $F = 1.31$, $p = 0.2633$; Trial 2: Severity: $F = 12.29$, $p < 0.0001$; Recovery: $F = 3.51$, $p = 0.0031$; df $_{MST,MSE} = 8, 45$; $\alpha = 0.05$, least square means).

Cover crops grown on *P. vexans*-infested soil showed a range of 12.5–19.2% and 9.0–40.8% disease severity for Trials 1 and 2, respectively. A range of 20.0–30.0% and 28.3–51.7% (for Trials 1 and 2, respectively) of *P. vexans* was recovered from the roots of cover crops (Figure 2). In Trial 1, disease severity and pathogen recovery from the roots was similar among all cover crops. However, in Trial 2 lower disease severity was observed for triticale, cowpea, annual ryegrass, and Japanese millet, while pathogen recovery was lowest for Austrian winter pea and annual ryegrass compared to buckwheat and crimson clover.

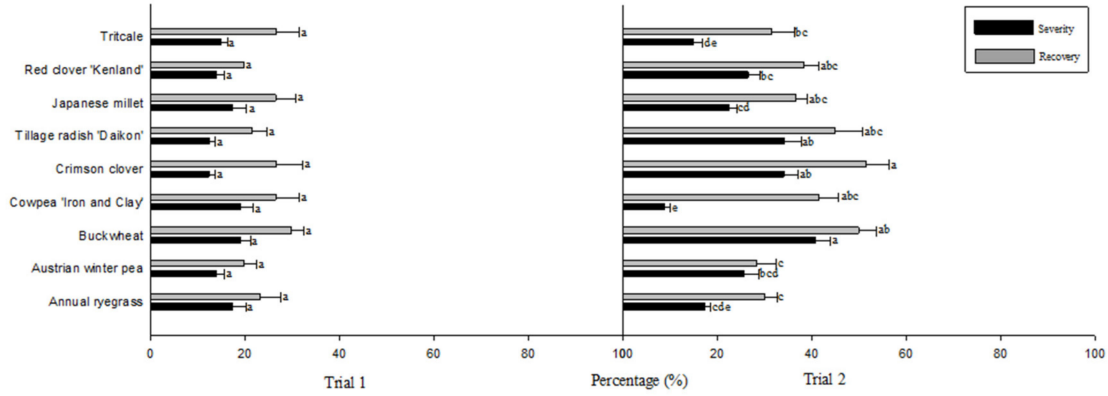

Figure 2. Disease severity and recovery (mean ± SE) of cover crops when *Phytopythium vexans* was inoculated in the soil. Phytopythium root rot disease severity was evaluated using a scale of 0–100% of roots affected. For each replication, ten randomly selected cover crop root samples were plated V8-PARPH oomycete-selective medium to determine the percent recovery of *P. vexans* from root samples. Different letters on the bars indicate significant differences in Phytopythium root rot disease severity and pathogen recovery among cover crops (Trial 1: Severity: $F = 1.75$, $p = 0.1129$; Recovery: $F = 0.8$, $p = 0.0603$; Trial 2: Severity: $F = 16.13$, $p < 0.0001$; Recovery: $F = 4.53$, $p = 0.0004$; df $_{MST,MSE} = 8, 45$; $\alpha = 0.05$, least square means).

In Trial 1, Japanese millet, Austrian winter pea, and annual ryegrass had lower Rhizoctonia root rot disease severity compared to buckwheat, cowpea, and red clover (Figure 3). In Trial 2, disease severity was lower in all cover crops compared to buckwheat, crimson clover, and red clover. Cowpea, tillage radish, Japanese millet, Austrian winter pea, annual ryegrass, and triticale consistently showed lower disease severity in both trials.

Crimson clover and Austrian winter pea showed higher *R. solani* recovery from their roots than buckwheat and triticale in Trial 1. However, in Trial 2, Buckwheat, crimson clover, and red clover showed higher pathogen recovery from the roots compared to triticale.

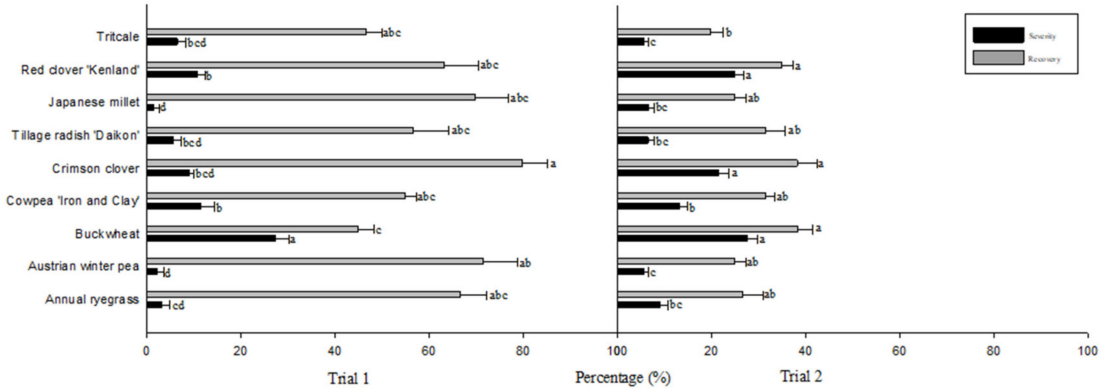

Figure 3. Disease severity and recovery (mean ± SE) of cover crops when *Rhizoctonia solani* was inoculated in the soil. Rhizoctonia root rot disease severity was evaluated using a scale of 0–100% of roots affected. For each replication, ten randomly selected cover crop root samples were plated on Rhizoctonia semi-selective medium to determine the percent recovery of *R. solani* from root samples. Different letters on the bars indicate significant differences in Rhizoctonia root rot disease severity and pathogen recovery among cover crops (Trial 1: Severity: $F = 19.22$, $p < 0.001$; Recovery: $F = 4.28$, $p = 0.0007$; Trial 2: Severity: $F = 33.76$, $p < 0.0001$; Recovery: $F = 4.43$, $p = 0.0005$; df $_{MST,MSE} = 8, 45$; $\alpha = 0.05$, least square means).

Tillage radish, buckwheat, red clover, and Japanese millet exhibited no differences in height with or without inoculation of pathogens among trials (Table 2). In Trial 1, the height of annual ryegrass was not impacted by pathogen inoculation; however, *P. vexans* reduced the height of annual ryegrass compared to control in Trial 2. Significant reduction of height was observed in Austrian winter pea when *R. solani* and *P. nicotianae* was inoculated compared to control in Trial 1, and when *P. nicotianae* was inoculated in Trial 2. In Trial 1, cowpea showed no difference in height with or without the inoculation of the pathogen; however, in Trial 2, *R. solani* reduced the height compared to control and other pathogens. Crimson clover height was greater than the control even with the inoculation in Trial 1 and the control was similar to all others in Trial 2. Similar height was observed among the treatments compared to control for triticale in both trials.

Total fresh weight of annual ryegrass was lower when infected with *P. vexans* in Trial 2; however, a height reduction because of pathogen inoculation was observed in Trial 1 (Table 3). The height of Austrian winter pea was reduced by *R. solani* in Trial 1, and both *P. vexans* and *P. nicotianae* in Trial 2, compared to control. No significant difference in total plant fresh weight was observed among the treatments for buckwheat in Trial 2; however, *R. solani* reduced the total plant fresh weight compared to control in Trial 1. In both trials, *R. solani* was more aggressive in reducing the weight of cowpea. No significant reduction of total fresh weight was observed in both trials for crimson clover. *P. vexans* reduced the fresh weight of tillage radish in Trial 2; however, no difference among treatments was observed in Trial 1. *P. vexans* reduced the total fresh weight of Japanese millet and Austrian winter pea compared to control in Trial 1; and including those of Trial 1, *P. vexans* reduced the total fresh weight of annual ryegrass, crimson clover, tillage radish, and red clover in Trial 2. In Trial 1, red clover showed no weight difference among the treatments; however, in Trial 2, *R. solani* and *P. vexans* reduced the total fresh weight compared to control. Triticale showed similar total fresh weight among treatments in Trial 1; however, *R. solani* reduced its weight in Trial 2.

No reduction in total root weight was observed for annual ryegrass, tillage radish, Austrian winter pea, crimson clover, and triticale when inoculated with pathogens in both trials compared to control (Table 4). Buckwheat and red clover showed no difference in root weight among the treatments in Trial 1; however, in Trial 2, *P. vexans* and *R. solani*

significantly reduced the root weight compared to control. *R. solani* reduced the root weight of cowpea compared to control in both trials. *P. vexans* reduced the root weight of Japanese millet in Trial 1, but root weight was similar among all treatments in Trial 2.

The cover crops exhibited no differences in seed germination with or without inoculation of *P. nicotianae* in Trial 1; however, buckwheat, crimson clover, and red clover showed a reduced germination rate in Trial 2 as compared to Japanese millet and Austrian winter pea (Table 5). The germination of buckwheat, tillage radish, and red clover were reduced by the presence of *P. vexans* in Trial 1, and likewise in Trial 2, including crimson clover. The germination of buckwheat was severely reduced (28.3% and 30.0% in Trials 1 and 2, respectively) due to *R. solani* compared to the control (75.0% and 71.7% in Trials 1 and 2, respectively). Similarly, cowpea and red clover in Trial 1, and cowpea, crimson clover, and triticale in Trial 2 showed a reduction in the germination rate compared to other cover crops when challenged with *R. solani*. Comparing the aggressiveness of pathogens, *R. solani* was more virulent than the oomycete *P. vexans* and *P. nicotianae,* which is shown by the overall reduction of germination rate (67.2%) compared to *P. nicotianae* (92.2%), *P. vexans* (75.5%) and control (87.8%) (data not shown). Overall, cover crops such as annual ryegrass, Austrian winter pea, Japanese millet, and triticale performed better, even with the threat of these pathogens among the trials.

However, not surprisingly, almost all cover crops showed post-emergence damping-off with the inoculation of pathogens. We did not observe significant post-emergence damping-off of seedlings when *P. nicotianae* was inoculated in Trial 1; however, cowpea and triticale showed 3.3% of post emergence damping-off in Trial 2 (Table 6). Although almost all cover crops showed a small degree of damping-off, no significant difference between the cover crops were observed when *P. vexans* was present in the soil in both trials. Cowpea, tillage radish, crimson clover, buckwheat, Austrian winter pea, red clover, and triticale showed significant damping-off against *R. solani* among cover crops in Trial 1; however, no significant difference between the cover crops was observed in Trial 2. Japanese millet and annual ryegrass showed no or minimum damping-off among all pathogens and in both trials.

Cover crops inoculated with *P. vexans* did not have an effect on height, total fresh weight, and fresh root weight in Trial 1; however, they had significant height, total fresh weight, and fresh root weight reductions ($p = 0.0379$, $p = 0.0011$ and $p = 0.0001$, respectively) in Trial 2 (Table 7). Similarly, cover crops inoculated with *P. nicotianae* did not have any significant effect on height in both trials. Additionally, no significant difference on total fresh weight and fresh root weight was observed among cover crops inoculated with *P. nicotianae* in Trial 1; however, a significant total fresh weight and fresh root weight reduction percentages ($p = 0.0045$ and $p = 0.0001$, respectively) was observed in Trial 2 for all cover crops. A negative correlation was observed between plant growth parameters (total height, total plant fresh weight and total fresh root weight) and the root rot disease severity caused by *R. solani* in both trials. A significant reduction of total fresh weight and root weight was observed in both trials and reduction in height was only observed in Trial 2, when the cover crops were infected with *R. solani*. Additionally, no significant positive or negative relation between the disease severity and plant growth parameters in between the trials was observed (data not shown). At the end of the experiment, the pathogens were re-isolated from the infected roots and sequenced. The morphology of the isolates was same. All three pathogens were found to be (100%) similar with the initial isolates used at the beginning of experiment which was confirmed with NCBI database.

Table 2. Total height of different cover crops with the respective of *Phytophthora nicotianae*, *Phytopythium vexans*, *Rhizoctonia solani*, and control.

Treatment	Height of Cover Crops (cm)																	
	Annual Ryegrass		Austrian Winter Pea		Buckwheat		Cowpea 'Iron and Clay'		Crimson Clover		Tillage Radish 'Daikon'		Japanese Millet		Red Clover 'Kenland'		Triticale	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Phytophthora nicotianae	20.9 ± 0.7 b [x]	15.9 ± 0.8 bc	31.4 ± 0.7 bc	24.6 ± 1.9 b	22.2 ± 0.6 a	20.9 ± 0.8 a	18.5 ± 0.9 a	27.6 ± 0.9 a	8.5 ± 0.1 a	6.8 ± 0.2 a	13.9 ± 0.6 a	10.2 ± 0.4 a	25.6 ± 0.7 a	18.7 ± 0.4 a	4.7 ± 0.1 a	4.63 ± 0.1 a	24.9 ± 0.7 b	18.9 ± 0.7 b
Rhizoctonia solani	21.7 ± 0.7 a	19.1 ± 0.8 b	27.0 ± 0.5 c	28.8 ± 1.8 ab	21.5 ± 1.2 a	22.7 ± 2.4 a	20.0 ± 1.4 a	14.5 ± 2.0 b	8.6 ± 0.2 a	5.6 ± 0.6 ab	13.7 ± 0.7 a	10.7 ± 0.5 a	30.7 ± 5.7 a	20.2 ± 1.3 a	5.2 ± 0.4 a	4.76 ± 0.1 a	24.3 ± 0.8 b	24.2 ± 1.9 a
Phytopythium vexans	20.6 ± 0.6 b	14.6 ± 0.2 c	36.9 ± 2.8 ab	26.97 ± 2.5 ab	23.9 ± 1.2 a	20.7 ± 2.4 a	18.4 ± 1.7 a	23.1 ± 1.5 a	7.7 ± 0.2 b	5.1 ± 0.2 b	14.0 ± 0.6 a	8.9 ± 0.5 a	23.2 ± 1.8 a	18.3 ± 0.7 a	4.9 ± 0.1 a	4.78 ± 0.4 a	30.7 ± 1.5 a	18.7 ± 0.7 b
Control	20.6 ± 1.0 b	16.9 ± 0.5 ab	40.8 ± 0.3 a	33.4 ± 1.6 a	21.9 ± 0.1 a	22.7 ± 1.3 a	16.2 ± 0.9 a	24.1 ± 0.8 a	6.9 ± 0.2 c	5.9 ± 0.1 ab	13.9 ± 0.6 a	9.6 ± 0.5 a	26.6 ± 0.9 a	17.8 ± 0.3 a	4.5 ± 0.1 a	4.21 ± 0.2 a	27.8 ± 2.3 ab	20.2 ± 0.6 ab
F value	0.41	10.5	17.55	3.56	1.2	0.32	1.52	16.08	17.98	4.46	0.31	2.71	1.05	1.73	1.63	1.47	4.03	5.37
df	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
p value	0.7479	0.0002	<0.0001	0.0327	0.3351	0.8099	0.2403	<0.001	<0.0001	0.0148	0.8169	0.0726	0.3942	0.1934	0.2135	0.2521	0.0216	0.0071

[x] Means (± SE) with different letters (within columns) are significantly different at $p \leq 0.05$, according to Tukey's post hoc test.

Table 3. Total plant fresh weight of different cover crops with the respective treatments of *Phytophthora nicotianae*, *Phytopythium vexans*, *Rhizoctonia solani,* and control.

Treatment	Total Plant Fresh Weight of Cover Crops (g)																	
	Annual Ryegrass		Austrian Winter Pea		Buckwheat		Cowpea 'Iron and Clay'		Crimson Clover		Tillage Radish 'Daikon'		Japanese Millet		Red Clover 'Kenland'		Triticale	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Phytophthora nicotianae	4.0 ± 0.2 a [x]	3.8 ± 0.6 ab	16.2 ± 1.1 a	14.9 ± 1.6 b	7.2 ± 0.6 ab	4.1 ± 0.9 a	10.2 ± 0.4 a	27.4 ± 2.7 a	2.5 ± 0.1 a	2.0 ± 0.3 a	11.6 ± 1.1 a	9.5 ± 0.6 ab	6.9 ± 0.5 a	3.9 ± 0.4 ab	1.0 ± 0.1 a	1.9 ± 0.2 ab	10.1 ± 0.5 a	11.6 ± 1.9 ab
Rhizoctonia solani	3.9 ± 0.5 ab	4.5 ± 0.4 a	9.6 ± 0.8 c	21.0 ± 1.9 a	3.5 ± 0.4 c	3.4 ± 0.5 a	5.4 ± 1.2 b	10.7 ± 1.6 b	2.9 ± 0.4 a	1.0 ± 0.2 b	7.0 ± 1.3 b	10.3 ± 0.6 a	6.5 ± 0.9 a	5.2 ± 0.2 a	0.7 ± 0.1 a	1.2 ± 0.2 c	8.6 ± 1.3 a	7.3 ± 1.9 b
Phytopythium vexans	2.7 ± 0.4 ab	2.8 ± 0.2 b	12.3 ± 1.1 bc	15.4 ± 1.1 b	8.3 ± 0.5 a	3.4 ± 0.6 a	10.2 ± 0.9 a	25.5 ± 2.9 a	3.8 ± 0.5 a	1.1 ± 0.2 b	7.5 ± 1.5 ab	7.3 ± 0.7 b	3.3 ± 0.6 b	3.7 ± 0.3 b	0.8 ± 0.1 a	1.3 ± 0.3 c	6.8 ± 0.8 a	9.7 ± 0.7 ab
Control	2.4 ± 0.4 b	4.1 ± 0.3 ab	13.4 ± 0.3 ab	21.6 ± 3.1 a	4.9 ± 0.9 bc	7.2 ± 0.5 a	9.9 ± 0.6 a	21.9 ± 2.0 a	2.7 ± 0.3 a	1.3 ± 0.2 ab	8.7 ± 0.5 ab	9.9 ± 0.6 a	6.6 ± 0.4 a	3.2 ± 0.4 b	0.9 ± 0.1 a	2.1 ± 0.3 a	9.2 ± 1.2 a	16.3± 2.6 a
F value	4.35	3.51	10.06	3.12	12.61	7.44	8.18	10.47	2.71	5.01	3.15	4.45	7.97	6.48	2.07	3.72	1.97	4.06
df	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
p value	0.0163	0.0341	0.0003	0.0488	<0.0001	0.0016	0.0009	0.0002	0.0724	0.0094	0.0478	0.015	0.0011	0.003	0.1365	0.0282	0.1507	0.021

[x] Means (± SE) with different letters are significantly different at $p \leq 0.05$, according to Tukey's post hoc test.

Table 4. Total plant root weight of different cover crops with the respective treatments of *Phytophthora nicotianae*, *Phytopythium vexans*, *Rhizoctonia solani*, and control.

Treatment	Total Plant Root Weight of Cover Crops (g)																	
	Annual Ryegrass		Austrian Winter Pea		Buckwheat		Cowpea 'Iron and Clay'		Crimson Clover		Tillage Radish 'Daikon'		Japanese Millet		Red Clover 'Kenland'		Triticale	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Phytophthora nicotianae	2.2 ± 0.1 a [x]	3.1 ± 0.5 a	9.2 ± 0.6 a	11.0 ± 1.1 a	0.6 ± 0.1 a	1.4 ± 0.3 ab	2.8 ± 0.2 a	12.2 ± 1.0 ab	1.5 ± 0.1 a	1.1 ± 0.2 a	1.0 ± 0.2 a	4.8 ± 0.4 a	3.8 ± 0.6 a	3.1 ± 0.4 a	0.5 ± 0.1 a	1.1 ± 0.1 ab	8.2 ± 0.4 a	10.5 ± 1.8 a
Rhizoctonia solani	2.1 ± 0.3 a	3.7 ± 0.4 a	5.8 ± 0.7 b	14.6 ± 1.6 a	0.3 ± 0.0 a	1.0 ± 0.2 b	1.5 ± 0.3 c	4.1 ± 0.7 c	1.4 ± 0.2 a	0.4 ± 0.1 b	0.9 ± 0.4 a	4.1 ± 0.5 a	3.5 ± 0.6 a	2.9 ± 0.2 a	0.4 ± 0.1 a	0.7 ± 0.1 c	6.6 ± 1.2 ab	6.3 ± 1.8 a
Phytopythium vexans	1.4 ± 0.3 ab	2.2 ± 0.2 a	6.4 ± 0.8 ab	11.0 ± 0.7 a	1.0 ± 0.4 a	0.8 ± 0.3 b	1.6 ± 0.3 bc	13.1 ± 1.1 a	2.0 ± 0.3 a	0.6 ± 0.1 b	1.0 ± 0.3 a	3.0 ± 0.5 a	1.1 ± 0.2 b	2.1 ± 0.2 ab	0.4 ± 0.1 a	0.8 ± 0.2 c	4.6 ± 0.6 b	8.4 ± 0.7 a
Control	1.1 ± 0.2 b	3.0 ± 0.3 a	6.9 ± 0.8 ab	13.2 ± 1.9 a	1.0 ± 0.3 a	2.0 ± 0.3 a	2.5 ± 0.3 ab	8.9 ± 0.9 b	1.7 ± 0.2 a	0.8 ± 0.1 ab	1.0 ± 0.1 a	4.1 ± 0.4 a	3.8 ± 0.1 a	1.6 ± 0.3 b	0.5 ± 0.0 a	1.2 ± 0.2 a	5.8 ± 0.9 ab	12.0 ± 1.2 a
F value	4.77	3	4.17	1.66	1.75	4.43	6.68	19.34	1.73	4.97	0.02	2.87	9.53	6.44	0.91	3.19	3.51	3.05
df	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
p value	0.0115	0.055	0.019	0.2085	0.1886	0.0152	0.0026	< 0.001	0.1928	0.0098	0.9953	0.0622	0.0004	0.0031	0.4524	0.0459	0.034	0.0525

[x] Means (± SE) with different letters are significantly different at $p \leq 0.05$, according to Tukey's post hoc test.

Table 5. Germination rate of different cover crops with the respective treatments of *Phytophthora nicotianae*, *Phytopythium vexans*, *Rhizoctonia solani*, and control.

Cover Crop	Germination Rate of Different Cover Crops with the Respective Treatments (%)							
	Phytophthora nicotianae		*Phytopythium vexans*		*Rhizoctonia solani*		Control	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Annual Ryegrass	95.0 ± 2.2 a [x]	73.3 ± 8.8 a–d	85.0 ± 5.0 ab	90.0 ± 2.6 ab	91.7 ± 6.5 a	81.7 ± 6.0 abc	95.0 ± 2.2 a	95.0 ± 3.4 a
Austrian Winter Pea	96.7 ± 3.3 a	98.3 ± 1.7 a	88.3 ± 6.0 a	76.7 ± 6.7 abc	83.3 ± 5.6 ab	91.7 ± 3.1 a	81.7 ± 3.1 ab	95.0 ± 2.2 a
Buckwheat	88.3 ± 7.5 a	53.3 ± 10.2 cd	56.7 ± 8.8 cd	40.0 ± 5.8 de	28.3 ± 3.1 d	30.0 ± 4.5 d	75.0 ± 8.5 ab	71.7 ± 4.8 b
Cowpea 'Iron and Clay'	96.7 ± 2.1 a	86.7 ± 7.6 ab	81.7 ± 3.1 abc	83.3 ± 4.9 abc	45.0 ± 7.6 cd	55.0 ± 7.2 cd	81.7 ± 4.0 ab	93.3 ± 3.3 a
Crimson Clover	86.7 ± 3.3 a	51.7 ± 3.1 d	76.7 ± 4.9 a-d	31.7 ± 4.8 e	75.0 ± 6.7 abc	28.3 ± 4.0 d	91.7 ± 4.8 ab	71.7 ± 4.0 b
Tillage Radish 'Daikon'	96.7 ± 2.1 a	80 ± 5.8 abc	53.3 ± 5.6 d	56.7 ± 10.5 b-e	65.0 ± 9.2 abc	70.0 ± 11.0 abc	81.7 ± 3.1 ab	96.7 ± 2.1 a
Japanese Millet	96.7 ± 3.3 a	98.3 ± 1.7 a	95.0 ± 2.2 a	95.0 ± 3.4 a	95.0 ± 3.4 a	88.3 ± 4.8 ab	86.7 ± 4.9 ab	96.7 ± 2.1 a
Red Clover 'Kenland'	86.7 ± 4.9 a	68.3 ± 6.0 bcd	60.0 ± 6.8 bcd	50.0 ± 14.8 cde	53.3 ± 5.6 bcd	85.0 ± 5.6 abc	71.7 ± 4.8 b	75.0 ± 5.0 b
Triticale	86.7 ± 3.3 a	90.0 ± 3.7 ab	83.3 ± 4.9 ab	75.0 ± 7.6 a-d	68.3 ± 10.5 abc	56.7 ± 12.6 bcd	86.7 ± 4.9 ab	95.0 ± 2.2 a
F value	1.57	8.22	7.32	8.67	10.34	11.35	2.41	10.95
df	8	8	8	8	8	8	8	8
p value	0.1594	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0295	<0.0001

[x] Means (± SE) with different letters are significantly different at $p \leq 0.05$, according to Tukey's post hoc test. Germination percentage was calculated using the formula: (total seed germinated/total seed sowed) * 100%.

Table 6. Post emergence damping-off of different cover crops with the respective treatments of *Phytophthora nicotianae*, *Phytopythium vexans*, *Rhizoctonia solani*.

Cover Crop	Post Emergence Damping-off of Different Cover Crops with the Respective Treatments (%)					
	Phytophthora nicotianae		*Phytopythium vexans*		*Rhizoctonia solani*	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Annual Ryegrass	0 a [x]	0 b	6.7 ± 3.3 a	0 a	1.7 ± 1.7 c	0 a
Austrian Winter Pea	1.7 ± 1.7 a	0 b	6.7 ± 2.1 a	0 a	5.0 ± 2.2 b	0 a
Buckwheat	3.3 ± 2.1 a	0 b	5.0 ± 2.2 a	1.7 ± 1.7 a	5.0 ± 2.2 b	5.0 ± 2.2 a
Cowpea 'Iron and Clay'	5.0 ± 3.4 a	3.3± 2.1 a	8.3 ± 4.0 a	3.3 ± 2.1 a	13.3 ± 5.6 a	0 a
Crimson Clover	8.3 ± 3.1 a	0 b	6.7 ± 3.3 a	0 a	10.0 ± 3.7 b	1.7 ± 1.7 a
Tillage Radish 'Daikon'	6.7 ± 3.3 a	0 b	5.0 ± 2.2 a	0 a	13.3 ± 3.3 a	3.3 ± 3.3 a
Japanese Millet	6.7 ± 2.1 a	0 b	3.3 ± 2.1 a	0 a	1.7 ± 1.7 c	0 a
Red Clover 'Kenland'	10.0 ± 3.7 a	0 b	0 a	1.7 ± 1.7 a	6.7 ± 2.1 b	6.7 ± 2.1 a
Triticale	8.3 ± 4.0 a	3.33 ± 2.1 a	0 a	1.7 ± 1.7 a	6.7 ± 2.1 b	1.7 ± 1.7 a
F value	1.36	2.19	1.4	1.03	2.17	2.1
df	8	8	8	8	8	8
p value	0.2383	0.0465	0.2245	0.4263	0.0482	0.0555

[x] Means (±SE) with different letters are significantly different at $p \leq 0.05$, according to Tukey's post hoc test. Post emergence damping-off percentage was calculated using the formula: (total seedling died/total seed germinated) * 100%.

Table 7. Pearson correlation coefficient between the plant growth parameters and root rot severity caused by *Phytophthora nicotianae*, *Phytopythium vexans* and *Rhizoctonia solani* for all cover crops.

Plant Growth Parameter	Root Rot Disease Severity (%)					
	Phytopythium vexans		*Phytophthora nicotianae*		*Rhizoctonia solani*	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Height	$R^2 = 0.01630$ $p = 0.9069$	$R^2 = -0.28598$ $p = 0.0379$	$R^2 = 0.25443$ $p = 0.0634$	$R^2 = -0.213$ $p = 0.1217$	$R^2 = -0.18175$ $p = 0.1884$	$R^2 = -0.43242$ $p = 0.0011$
Total fresh weight	$R^2 = -0.09847$ $p = 0.4787$	$R^2 = -0.43648$ $p = 0.0011$	$R^2 = 0.18645$ $p = 0.1770$	$R^2 = -0.381$ $p = 0.0045$	$R^2 = -0.37014$ $p = 0.0059$	$R^2 = -0.55140$ $p = <0.0001$
Total root weight	$R^2 = -0.17408$ $p = 0.2080$	$R^2 = -0.50503$ $p = 0.0001$	$R^2 = -0.06612$ $p = 0.6348$	$R^2 = -0.496$ $p = 0.0001$	$R^2 = -0.45395$ $p = 0.0006$	$R^2 = -0.56087$ $p = <0.0001$

[x] The value −1 represent a strong negative correlation and the value +1 represent strong positive relationship between plant growth parameters and root rot disease severity. The negative value represents that as severity increases, growth parameters decrease at $p \leq 0.05$.

4. Discussion

Cover crops are usually grown to cover the soil to avoid soil erosion and nutrient loss between periods of crop production. The term 'cover crop' commonly refers to 'green manure legumes', which are used to enhance nitrogen content of soil, capture soluble excess nutrients, increase the biological activity of the soil via addition of organic matter, and sometimes to achieve biological control of insects and pests [30]. Still, soil fumigants are being used to manage soilborne diseases. The chemical fumigants such as methyl bromide, chloropicrin, [31] as well as meta sodium [32] are commonly used. Although these chemical fumigants are quick and effective for a short run, they negatively impact environmental health, which ultimately impacts human health. Aside from agronomic crops and a vegetable cropping system, the use of cover crops is still in its early phase. Some studies have been conducted [19–21,33,34] which proved the beneficial aspects of cover crops in different crop productions, their benefits, and methods, as well as timing of application.

Although the benefits of cover crops have been recognized for a long period of time, their adoption to crop production is still slow and uncertain. The complex economic, biological, and operational issues attached to the use of cover crops make growers reluctant to incorporate these cover crops in their growing system [30,35,36]. Along with the benefits, occasional yield decrease in corn was observed following winter grass cover crops [33] which may lead farmers to abandon the cover crops. In this experiment, we aimed to find

the potential issues associated with the cover crops which may harm the principal crop production system. One of the major hypotheses was that the cover crops may act as a secondary host for the potential harmful pathogen and benefits the survival of the pathogen. In this experiment, we evaluated nine different cover crops used in the southeastern U.S. for their susceptibility to soilborne plant pathogens *P. nicotianae, R. solani*, and *P. vexans*.

All tested cover crops showed some extent of root rot disease and recovery of pathogen from the roots. Except for buckwheat, crimson clover, and red clover, all the other cover crops exhibited lower extent of root rot disease severity caused by *P. nicotianae,* which is also explained by the higher percentage of pathogen recovery from the soil and lower germination rate. Robertson et al. [34] categorized millet, ryegrass, and triticale as non-hosts for *Fusarium virguliforme*, suggesting those cover crops are unlikely to host or increase the pathogen inoculum. Similarly, lower Phytophthora root rot severity was observed with brassica cover crops [20], crimson clover and winter wheat [19], and crimson clover and triticale [21]. They also reported the increase in disease suppressiveness of soil when the cover crops were used with woody ornamentals. Grazieli [37] used winter rye to suppress the disease pressure caused by *Pythium* species in soybean production systems. Similarly, a reduction in splash dispersal of *Phytophthora* was observed by introduction of rye or wheat as a cover crop in a bell pepper field [38]. This also suggests that the cover crops may hinder the dissemination of *P. nicotianae* spores, either acting as a physical barrier or by producing antimicrobial compounds.

Similarly, with the *R. solani* inoculation, some of the legumes (red clover and crimson clover) and buckwheat showed higher disease severity compared to other crops, which is also exhibited in their germination rate. However, grass cover crops such as Japanese millet, triticale, annual ryegrass, and legumes such as Austrian winter pea and cowpea showed low root rot disease severity. The success of Austrian winter pea and cowpea might be due to their prolific growth habit, higher biomass, and nitrogen addition [39] to the soil, increasing the microbial interaction and competition. This is also supported by their higher germination, total fresh root weight and total fresh plant weight in our experiment. A similar pattern of lower Rhizoctonia root rot disease severity was observed when the cover crops were used in a different production system [19,21]. Pathogen population of *Rhizoctonia* was reduced by using cover crops in apple [40] and beet [41] production systems. Similarly, Baysal-Gurel et al. [42] reported 12–30% of disease suppression when the biofumigant cover crops were used in woody ornamental production systems.

Furthermore, *P. vexans* was more severe on those cover crops compared to the other two. Grass cover crops such as triticale, Japanese millet, and annual ryegrass; and legumes such as cowpea and Austrian winter pea were least susceptible to the pathogen. Panth et al. [21] reported lower root rot disease severity of maple caused by *Phytopythium* when the cover crops were used. Similarly, the use of cover crops increased the Pseudomonads population of soil [19] and C:N ratio of the soil [21], thus increasing the microbial population of the soil and suppressing the disease severity. Additionally, cover crops can host arbuscular mycorrhizal fungi, which effectively reduce soilborne diseases when used in a short-term cover crop-maize rotation [43] or as a forage crop [44]. These mycorrhizal fungi form a mat-like structure on roots, thereby protecting the roots physically by producing antagonistic chemicals, competition with pathogens, and solubilization of nutrients [45]. Grass cover crops are also known to attract ladybugs, which feed on aphids, and legumes can attract parasitic wasps and predatory bugs, which can feed on spider mites and flower thrips [46].

Although tillage radish is supposed to produce glucosinolates and related substances to reduce the disease severity [20], such phenomena was not observed during the extremely short experimental period. This was due to their lower germination and extremely slow growth habit as explained by their low root and plant weight. The fast-growing legumes such as cowpea, Austrian winter pea, and non-host grass cover crops such as triticale, Japanese millet, and annual ryegrass were standing above all in terms of resisting all tested pathogen attacks. A similar suggestion of using cover crops such as legumes and grasses

to minimize the rapid development of inoculum was made by Vukicevich et al. [47] in a perennial cropping system. Additionally, despite their wide use on the strength of their short growth habit and nitrogen fixing ability, both red clover and crimson clover did not perform well in containing pathogen to lower extent in greenhouse set up. However, the favorable environmental set up for a pathogen to attack the plant in controlled setup may not correlate to the natural field setting. This demands further research in field setup.

There are many benefits associated with the cover crops, such as reducing soil erosion, increasing nutrient content of the soil, maintaining soil physical and chemical stability, increasing beneficial microorganisms such as Pseudomonads, suppressing weeds, and attracting beneficial insects. The use of cover crops may also reduce the cost of chemicals and their operational cost. Although some cover crops can be a secondary host for the pathogen, most of the cover crops tested in this experiment performed better and the disease risks associated with them are low. Similarly, the perennial growth habit of woody ornamentals makes them suitable for cover cropping as the beneficial effects of cover crops are often being realized in the long run. We recommend using grass cover crops, such as triticale, Japanese millet, or annual ryegrass, and legumes, such as Austrian winter pea and cowpea, in the cropping system. Additionally, further research on field trials and wider pathogen range is necessary for narrowing down the search for the most effective cover crops for use in woody ornamental crop production.

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