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Impact of Winter Cover Crop Usage in Soilborne Disease Suppressiveness in Woody Ornamental Production System

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Abstract: Diseases caused by soilborne pathogens are a major limitation to field grown nursery production. The application of cover crops for soilborne disease management has not been widely investigated in a woody ornamental nursery production system. The objective of this study was to explore the impact of winter cover crops usage on soilborne disease management in that system. Soils from established field plots of red maple (*Acer rubrum* L.) with and without winter cover crops (crimson clover (*Trifolium incarnatum* L.) or triticale (× *Triticosecale* W.)) were sampled following the senescence of the cover crops. Separate bioassays were performed using red maple cuttings on inoculated (with *Phytopythium vexans*, *Phytophthora nicotianae* or *Rhizoctonia solani*) and non-inoculated field soils. The results indicated that winter cover crop usage was helpful for inducing soil disease suppressiveness. There was lower disease severity and pathogen recovery when the cover crops were used compare to the non-cover cropped soil. However, there were no differences in maple plant fresh weight and root weight between the treatments. The rhizosphere pseudomonad microbial population was also greater when the cover crops were used. Similarly, the C:N ratio of the soil was improved with the cover crop usage. Thus, in addition to improving soil structure and reducing erosion, cover crops can provide improved management of soilborne diseases. Therefore, stakeholders can consider cover crop usage as an alternative sustainable management tool against soilborne diseases in field nursery production system.

Keywords: soilborne disease; *Phytopythium vexans*; *Rhizoctonia solani*; *Phytophthora nicotianae*; cover crop; nursery production

1. Introduction

Soilborne diseases are generally considered one of the major constraints to a field grown nursery production system [1]. Soilborne pathogens (*Phytophthora* spp., *Rhizoctonia* spp., *Sclerotinia* spp., *Armillaria* spp., *Fusarium* spp., *Verticillium* spp., and *Pythium* spp.) can cause significant economic losses (50–75%) of the possible yield for diverse crops [2–5]. In the United States, soilborne plant pathogens account for about 90% of the two thousand important diseases of the principle crops [2,6]. Soilborne plant pathogens can generally survive for long periods in host plant debris, soil organic matter, or as free-living organisms. The diversity of the plant species grown in the nursery industry makes the management of soilborne diseases very challenging. Among those soilborne plant pathogens, *Phytophthora nicotianae* (Breda de Haan) is an important oomycete pathogen of concern for the nursery producers that can infect a wide range of plants, causing serious damage in commercial nursery production. *P. nicotianae* can complete multiple disease cycles in woody ornamental nurseries because

of the continuous and repetitive growth of the host plant, making it one of the most common pathogens of woody ornamentals [7–10]. Also known as black shank, *P. nicotianae* can cause root and crown rot, fruit rot and stem infection [11] causing severe loss of other hosts. Another important soilborne plant pathogenic fungi, *Rhizoctonia solani* (J.G. Kühn) can attack the nursery plants, causing pre- and post-emergence damping-off of seedlings, root and stem rot, collar rot, leaf spot and blight, as well as wire stem [4,12,13]. *R. solani* can attack more than 500 species of plants [14], which includes ornamental plants, agronomic crops, fruit and forest trees, as well as turfgrasses [4]. Generally, *R. solani* attacks host plants when they are in an early stage of development. Similarly, *Phytopythium vexans*, a newly reported oomycete in the United States affecting the woody perennials (Baysal-Gurel, unpublished data), may become an important pathogen of interest. Necrosis and defoliation, dark brown to black lesions in the crown area, as well as root rot are the common symptoms on woody ornamentals caused by *P. vexans*.

The introduction of different site-specific fungicides over the period has revolutionized chemical plant protection; however, it has been discovered that the mutations in the plant pathogenic fungi or oomycetes is leading to resistance development [15]. Fungicides of dicarboximide, benzimidazole, azoxystrobine, metalaxyl, and triazole groups have been used to control certain soilborne diseases, however, their effects on microbial growth, productivity, and fungicidal resistance development need to be recognized [5,16]. The haphazard and repetitive use of sole fungicides is leading to the insensitivity of the chemical fungicides against the plant pathogenic fungi or oomycetes. However, rotating or mixing the fungicides, maintaining the recommended dose, and integrating non-chemical treatments such as biorational products are some strategies recommended by Fungicide Resistance Action Committee (FRAC) [17]. In addition to that, a good effort has been made to introduce fungicides with a new mode of action due to regulatory and environmental pressures. These new fungicidal chemistries are environment friendly but not very toxic to fungus/oomycetes isolated from different hosts. Some of these released chemicals, such as acibenzolar-S-methyl, have a suitable mode of action that can increase the natural defense system of the host plants [18]. These fungicides that induce natural host defenses have increased efficacy and residual activity. However, there are always some problems associated with the chemicals, thus alternative, environmentally sound methods should be studied for sustainable soilborne disease management. However, it is also true that the use of biocontrol agents such as *Rhizobium*, *Trichoderma*, *Bacillus*, *Pseudomonas*, *Coniothyrium*, *Gliocladium*, *Serratia*, and *Streptomyces* is promising [19].

Traditionally defined as crops grown to cover the ground, cover crops are an important component of sustainable agricultural systems. Cover crops were previously used as green manure or as animal feed during the drought period [20]; with time, new roles and better management of cover crops in agricultural systems are being developed. However, the role and the importance of cover crops are generally confined to row crops, vegetables, fruits, and flower production systems. Thus, there is little knowledge about the impact or the management needs of cover crops in perennial nursery crops production systems. The United States Department of Agriculture Natural Resources Conservation Service and land-grant university extension agents have recommended cover crops in between rows of woody ornamentals for some time [21,22], but in many areas of the southeast, the adoption of cover crops has been slow or reduced following the economic crisis of 2008. However, the benefits of cover crops such as improving soil structure [23], increasing in soil organic matter, enhancing nutritional status, improving beneficial microbial populations such as *Pseudomonas* spp. in the soil, and increasing soil disease suppressiveness [24,25] are being realized.

Insertion of cover crops in between rows may provide additional successful and sustainable solutions for soilborne disease control in the field nursery production while improving soil. Therefore, we assessed the winter cover crop usage in between rows of woody ornamental crops to be able to determine their effect on major soilborne plant pathogens (*R. solani* and *P. nicotianae*) prevailing in Tennessee. Additionally, the new emerging pathogen *P. vexans* that has potential for serious losses in woody ornamental crops was also included in the bioassays. The effect of individual cover crops

in the soil system with their natural incorporation (after senescence) into the soil was assessed in different sites. Soils from established field plots of red maple (*Acer rubrum* L.) with and without winter cover crops (crimson clover (*Trifolium incarnatum* L.) or triticale (× *Triticosecale* W.)) were collected, and their ability to suppress these three pathogens was evaluated in the greenhouse bioassays using red maple cuttings.

2. Materials and Methods

### 2.1. Field Experimental Design and Layout

Two replicated field experiments were established at a commercial nursery in Rock Island (production site 1) (35.7416393° N, 85.6590996° W) and another commercial nursery in Smartt (production site 2) (35.3816° N, 85.1519° W) (Warren Co.), TN, USA between August 2018 and June 2019. Field plots with red maple (five-year-old) (9.8 × 9.8 m) previously established by the growers were used for the experiment. Winter cover crop (triticale or crimson clover) and control (no cover crop) plots were established in 1.8 × 1.8 m blocks in a randomized complete block design with four replicates per treatment. The control plots were left uncultivated, maintaining natural weed density. The pre-emergent herbicide SureGuard® (flumioxazin 51%, Valent U.S.A. Corp., Walnut Creek, CA, USA) was applied at a rate of 708.8 g product ha^{-1} in August 2018 to prevent weed/plant competition at the base of the trees in all experimental plots.

2.2. Cover Crop Application

Crimson clover (*Trifolium incarnatum* L.) and triticale (× *Triticosecale* W.) (Adams-Briscoe Seed Company, Jackson, GA, USA) were seeded in September 2018 using a Herd GT77 Spreader (Herd Seeder Co., Inc., Logansport, IN, USA). Crimson clover was chosen for its ability to supply nitrogen to the main nursery crop, avoiding the additional nitrogenous fertilizer input. Triticale was selected for its height to block the arthropod oviposition on red maple tree trunk as well as the possible additional biomass it can supply. Similarly, to protect the tree trunks against insect pests, the cover crops were sown as much as close to red maple trees.

2.3. Soil Moisture and Temperature Measurement

Soil moisture and temperature readings were measured monthly from October 2018 to June 2019 (Figures 1 and 2). Soil temperature was measured using an infrared temperature meter (Spectrum Technologies, Inc., East Plainfield, IL, USA) in probe mode, and soil moisture (percentage of volumetric water content (VWC%)) was measured using a FieldScout time domain reflectometer (TDR) soil moisture meter (Spectrum Technologies, Inc., East Plainfield, IL, USA) inserting probe to a depth of ~7.0 cm. Two readings of volumetric water content as well as temperatures were taken within rows and within middles, where the trees were previously established in the plots.
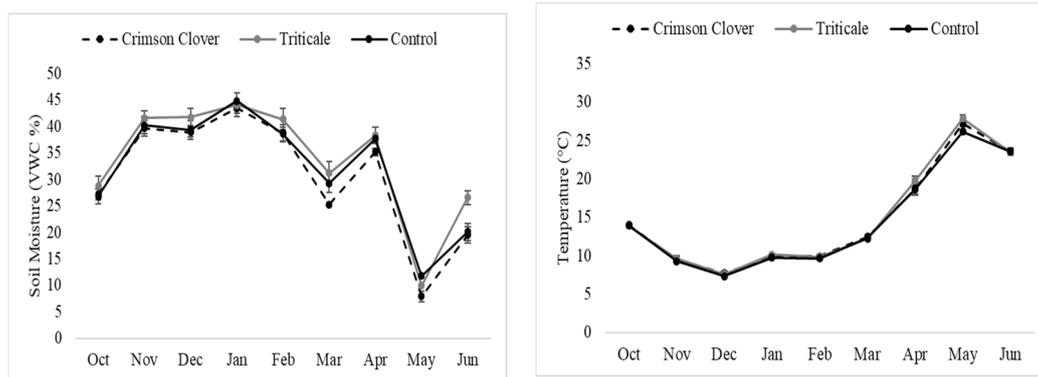
Figure 1. Average volumetric water content (VWC%) of the soil (± SE) starting from October 2018 to June 2019 in cover crop (crimson clover or triticale) and non-cover crop (control) used plots in production site 1, measured with a FieldScout TDR soil moisture meter inserting probe to a depth of ~7.0 cm (**left**). Average soil temperature (∘C) (± SE) starting from October 2018 to June 2019 in cover crop (crimson clover or triticale) and non-cover crop (control) used plots in same production site measured with an infrared temperature meter in probe mode (**right**).
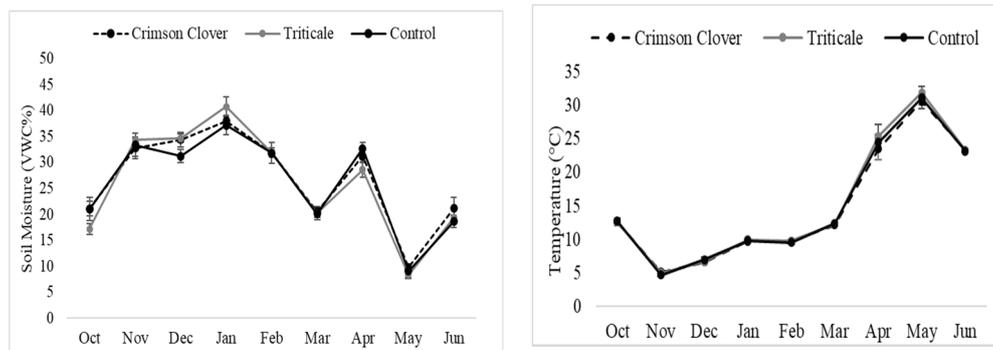
Figure 2. Average volumetric water content (VWC%) of the soil (± SE) starting from October 2018 to June 2019 in cover crop (crimson clover or triticale) and non-cover crop (control) used plots in production site 2, measured with a FieldScout TDR soil moisture meter inserting probe to a depth of ~7.0 cm (**left**). Average soil temperature (∘C) (± SE) starting from October 2018 to June 2019 in cover crop (crimson clover or triticale) and non-cover crop (control) used plots in same production site measured with an infrared temperature meter in probe mode (**right**).

2.4. Soil Sampling

Soil sampling was conducted following the senescence of winter cover crops at both locations (6 June 2019). Four soil samples (30 × 30 cm and 20 cm deep) were taken randomly between tree rows from each treatment plot, mixed in a bucket in situ with a spade, and placed in a clear plastic bag. Soil samples were collected from the region of the tree rows where the soil temperatures and moisture were recorded (about 50 cm radius of the red maple tree to prevent root damage). To prevent contamination between samples, tools such as spade and buckets were cleaned with soap water and then sterilized with 70% ethanol. The collected soil was stored for a week at 22 °C in a greenhouse at the Tennessee State University Otis L. Floyd Nursery Research Center in McMinnville, TN, USA (35.680480°N, 85.774580°W) (TSUNRC) before use in bioassays. The soil sub-samples were sent to Waypoint™ Analytical (Waypoint Analytical Inc., Jackson, TN, USA) for the analysis of soil nutrients and chemical properties.

2.5. Fungal Culture and Pathogen Inoculum Preparation

Isolate FBG201506 of *P. nicotianae* (GenBank accession MK399300) isolated from hydrangea plant, isolate FBG201508 of *R. solani* (GenBank accession MT533254) isolated from red maple plant, and isolate FBG20182 of *P. vexans* isolated from red maple plant (GenBank accession MT076055) were obtained from Dr. Fulya Baysal-Gurel's culture collection at the TSUNRC. The *R. solani* specimen was maintained on potato dextrose agar (PDA: Becton, Dickinson, and Company, Sparks, MD, USA) medium. The *P. nicotianae* and the *P. vexans* specimens were maintained on V8 medium. Preparation of *P. nicotianae* inoculum was done by following rice grain method, modified after Holmes and Benson [26]. Then, 25 g of long grain rice in 20 mL deionized water was autoclaved twice for 30 min. Three plugs of *P. nicotianae* (7 mm) colonized V8-agar (100 ml of clarified V8 juice (Campbell, Camden, NJ, USA), 15 g of agar (Sigma-Aldrich, St. Louis, MO, USA), and 900 ml of deionized water) were placed in the 250 mL flask containing the autoclaved rice and incubated for two weeks at room temperature. The rice inoculum in the flask was mixed thoroughly until final use. Soils in black plastic containers (16 cm diameter×16 cm deep) were artificially inoculated by burying five *P. nicotianae*-colonized rice grains in the soil at 5 cm soil depth. Seven-day old cultures of *R. solani* grown on PDA medium were homogenized in the sterile distilled water, and agar slurry was prepared at the rate of 1 petri plate/L [27] by using a blender (Hamilton Beach hand blender, Model number 59785R). Similarly, for the *P. vexans* inoculum, an agar slurry (2 petri plates of a 7-day-old *P. vexans* culture blended with 1 L of sterile distilled water) (Panth, M. Unpublished data) was prepared. Each container was drenched with 150 mL of agar slurry after red maple transplantation.

2.6. Red Maple Propagation

Healthy parent red maple "October Glory" (*Acer rubrum* L.) was chosen for preparation of softwood stem cuttings. In September 2018, stem cuttings of 0.5–1.0 cm thick and 10–15 cm long were prepared and treated with 2500 ppm of rooting hormone, Indole-3-Butyric acid (4-[3-Indolyl] butanoic acid) (Sigma Chemical Co. St. Louis, MO, USA). The cuttings were dipped very shortly (~2 s) in the hormone solution and inserted into the soilless potting mix (Morton's Nursery Mix: Canadian sphagnum peat [55–65%]) (Morton's Horticultural Products, McMinnville, TN) using $10 \times 10 \times 9$ cm black containers. Average relative humidities of 90.8, 77.0, 79.0, 79.1, 72.4, 76.3, 93.9, and 99.7 were maintained by misting between September 2018 to April 2019, respectively. Eight-month-old rooted cuttings were then used for the bioassays.

2.7. Bioassays for Soilborne Disease Suppressiveness

Bioassays were conducted to determine whether cover crop soil can suppress two common maple tree pathogens (*R. solani* and *P. nicotianae*) and a newly emerging pathogen (*P. vexans*). Soil samples from each treatment were taken and divided into #1 size black nursery containers (16 cm diameter × 16 cm deep) for each soil pathogen (n = 3) and control (n = 1). The control consisted of non-cover crop used soils from the same production fields to assess background pathogen pressure. The bioassays were conducted under 54% shade at the TSUNRC. Rooted maple cuttings were transplanted into the containers filled with the soil collected from both field experiments on 13 June 2019. One week after transplanting, plants were either inoculated with *P. vexans, P. nicotianae,* or *R. solani* or non-inoculated. For each bioassay, ten single-container replications per treatment were laid out in a completely randomized block design. Maple plants were irrigated for 2 min twice a day using an irrigation system with a grey spot-spitter spray stick (90° spray pattern) (Primerus Products, LLC., Encinitas, CA, USA). The average photosynthetic photon flux received by the shade house during the experimental period was 1200 µmol $m^{-2}s^{-1}$ (Basic Quantum Meter, Apogee instruments, Inc., Logan, UT, USA). The experiment was conducted between 13 June–30 September 2019. Average maximum temperatures for June, July, August, and September 2019 were 29.5, 31.9, 32.1, and 33.2 °C; average minimum

temperatures were 17.9, 19.5, 19.4, and 17.6 °C; and total rainfall amounts were 9.62, 21.83, 7.48, and 0.43 cm, respectively.

2.7.1. Crop Health Assessment

At the end of the bioassays, a visual assessment of roots was done to evaluate the root rot severity using a scale of 0–100% of the total maple root system affected. Maple plants were uprooted, and roots were washed with running tap water to remove the soil particles. After the assessment of roots, ten randomly selected root pieces (~1 cm long root tip, ten replicates per treatment) were plated on *Rhizoctonia* selective medium and PARPH-V8 selective medium, respectively. To prepare PARPH-V8 selective medium, 1% $CaCO_3$ (98% Acros Organics, Geel, Belgium) was mixed to V8 juice and centrifuged for 10 min at 10,000 rpm (Sorvall LEGEND X1R Centrifuge, ThermoFisher Scientific, Am Kalkberg, Germany). Then, 50 mL of buffered and clarified V8 juice was added to 450 mL deionized water (10% V8) along with 8 g agar (Sigma-Aldrich, St. Louis, MO, USA) and autoclaved at 121 °C at 15 psi for 15 min. Afterwards, 500 µL of fungicide and antibiotics (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), rifampicin (Sigma-Aldrich, St. Louis, MO, USA) (0.05 g/50 mL ethanol), pimaricin (2.5%) (MP Biomedicals, Santa Ana, CA, USA), and hymexazol (Sigma-Aldrich, St. Louis, MO, USA) (250 mg/50 mL sterile water)) were added to the medium [28,29]. Similarly, for *Rhizoctonia* semi-selective medium, 9 g of agar (Sigma-Aldrich, St. Louis, MO, USA) was added in 500 mL of deionized water and autoclaved at 121 °C at 15 psi for 15 min. Afterwards, 50 mg of each streptomycin sulfate (ACROS organics, Morris Plains, NJ, USA) and penicillin-G Na salt (Alfa Aesar, Ward Hill, MA, USA) and 400 µL of 1M NaoH (AMRESCO Inc., Solon, OH, USA) was added [30]. Plates were then incubated at 25 °C in the dark (VWR incubator, Radnor, PA, USA). The total number of root pieces showing the growth of the respective pathogen was counted after three days, and the pathogen recovery percentage was calculated for each pathogen according to the formula: [(total number of root pieces showing pathogen growth/total number root pieces) × 100%]. Dense cottony mycelium with a light rosette pattern for *P. nicotianae* [31] and a radiate growth pattern without aerial mycelium for *P. vexans* [32] was used as colony morphology to differentiate between the pathogens while counting the plates. Plant height was measured on 13 June 2019 and 30 September 2019. Total plant height increment was calculated by calculating the difference between the height at the beginning and the end of the experiment. Total fresh weight and root weight were also recorded on 30 September 2019.

2.7.2. Fluorescent Pseudomonad Population

Following Gould and his colleague's method [33], a selective medium (S1 medium) was used for enumeration of fluorescent pseudomonad from the soil. One gram of soil sample from each treatment container was transferred into a 15 mL tube (Thermo Fisher Scientific Inc., Waltham, MA, USA) containing 10 mL of sterile distilled water. The tubes containing samples were ultrasonicated (Fisherbrand M-Series 5.7 L Mechanical Ultrasonic Cleaning Bath, Thermo Fisher-Scientific Inc., Watham, MA, USA) for 3 min and then agitated with a shaker (Fisherbrand Incubating Mini-Shaker, Thermo Fisher-Scientific Inc., Watham, MA, USA) at 250 rpm for 30 min at room temperature. The particles were then allowed to settle for 30 min. Dilutions up to 10^{-3} were prepared and spread-plated using glass beads (3-mm solid glass beads, Walter Stern, Inc., Manorhaven, NY, USA) as well as 100 µL of the undiluted sample. The numbers of *Pseudomonas* colonies on plates were recorded after 48–72 h incubation at 25 °C. The number of colonies forming units (CFUs) per gram of soil sample was calculated from the plate counts, the dilution factor, and the plated volume for each sample.

2.8. Statistical Analysis

Plant height increase, total plant fresh weight and root fresh weight, root rot disease severity, and percent recovery of *P. vexans*, *P. nicotianae,* and *R. solani* from root samples, total nutrient content of the soil, and pseudomonad CFUs were compared among the treatments for both production sites.

Analysis of variance of all recorded data sets was performed using the general linear model procedure with SAS statistical software 2016 (SAS Institute Inc., Cary, NC, USA), and the means were separated using Tukey's test for post -hoc analysis. The graphs for the study were plotted using Sigma plot 12.0 (Systat Software, Inc., Santa Clara, CA, USA). Initially, the pseudomonad CFUs were log transformed for analysis, but original mean values are presented in the figures. All analyses were carried out for both artificially infested soil and non-infested soil.

3. Results

3.1. Bioassay with Natural Pathogen Pressure

In a bioassay without the artificial inoculation of pathogen, both cover crops (crimson clover and triticale) reduced the root rot disease severity compared to the non-cover crop used soil collected from both production sites (Site 1: $F = 19.93$, $P < 0.0001$; Site 2: $F = 17.71$, $P < 0.0001$; df $_{MST, MSE} = 2, 27$)) (Figure 3). There were no significant differences between cover crops in root rot disease severity for both production sites.
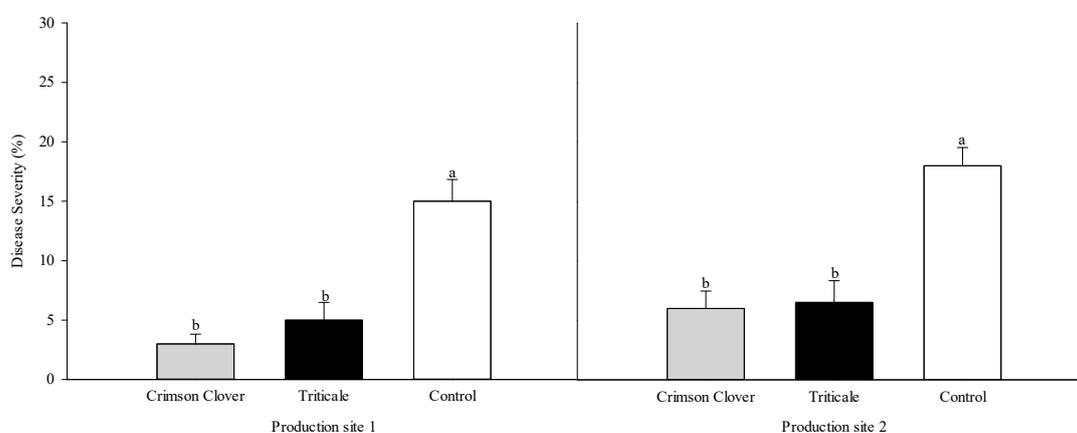
Figure 3. Root rot disease severity (mean ± SE) of red maple seedlings in non-inoculated cover crop used and non-cover crop used (control) soils collected from two experimental sites. For root rot disease severity, each plant was evaluated using a scale of 0–100% of roots affected. Different letters beside bars indicate significantly different root rot disease severity among treatments (Site 1: $F = 19.93$, $P < 0.0001$; Site 2: $F = 17.71$, $P < 0.0001$; df $_{MST, MSE} = 2, 27$; $\alpha = 0.05$, least square means).

The roots of maples grown in the cover crop or non-cover crop used soil collected from both locations were cultured for oomycete as well as *Rhizoctonia solani* recovery. There were significant differences in *R. solani* or oomycete pathogen recovery percentage between the cover crop used and the non-cover crop used (control) soils collected from two production sites (Table 1). There were no significant differences in *R. solani* recovery between crimson clover used soil and triticale used soil collected from production site 1. Oomycete recovery percentage was numerically low in triticale used soil compared to crimson clover used soil. *R. solani* recovery percentage was numerically low in triticale used soil compared to crimson clover used soil collected from production site 2. There were no significant differences in oomycete recovery between crimson clover used soil and triticale used soil collected from production site 2. There were no significant differences in plant height increment, total plant fresh weight, and total fresh root weight between red maples planted in cover crop and non-cover crop used soils collected from both locations.

Table 1. Recovery of oomycetes and *Rhizoctonia* from the root of red maple plants, total plant fresh weight, fresh root weight, and height increment (mean ± SE) of red maple plants planted in non-inoculated cover crop used (crimson clover or triticale) and non-cover crop used (control) soils collected from two experimental locations.

Treatment	Production Site 1					Production Site 2				
	Rhizoctonia solani Recovery (%) [z]	Oomycete Recovery (%) [z]	Total Height Increment (cm) [y]	Total Plant Fresh wt. (g)	Total Root Fresh wt. (g)	*Rhizoctonia solani* Recovery (%) [z]	Oomycete Recovery (%) [z]	Total Height Increment (cm) [y]	Total Plant Fresh wt. (g)	Total Root Fresh wt. (g)
Crimson Clover	12.0 ± 3.3 b [x]	24.0 ± 2.7 ab	9.3 ± 1.8 a	47.3 ± 4.3 a	28.6 ± 2.7 a	16.0 ± 2.7 ab	16.0 ± 2.7 b	9.6 ± 2.9 a	48.9 ± 3.9 a	33.2 ± 2.7 a
Triticale	10.0 ± 4.5 b	18.0 ± 3.6 b	14.3 ± 3.0 a	51.6 ± 6.4 a	34.2 ± 5.1 a	14.0 ± 4.3 b	16.0 ± 4.0 b	4.0 ± 1.3 a	58.7 ± 5.3 a	41.3 ± 3.7 a
Control	26.0 ± 3.1 a	34.0 ± 3.1 a	8.1 ± 2.0 a	64.5 ± 9.5 a	43.5 ± 7.8 a	28.0 ± 3.3 a	36.0 ± 2.7 a	10.8 ± 2.6 a	46.7 ± 4.8 a	32.9 ± 3.9 a
F value	5.70	6.68	1.97	1.62	1.79	4.78	13.24	2.37	1.49	1.83
df	2	2	2	2	2	2	2	2	2	2
P value	0.0086	0.0044	0.1585	0.2169	0.1870	0.0167	<0.001	0.1128	0.2441	0.1796

"[z] For each plant (ten replications per treatment), five randomly selected maple root samples were plated on V8-PARPH oomycete-selective medium or *Rhizoctonia* semi-selective medium to determine the percent recovery of oomycete or *R. solani* from root samples"; "[y] height increment was measured by subtracting the height of plant at the beginning of the experiment from the height of the experiment at the end of the experiment"; "[x] treatment means that do not share the same letter are significantly different (α = 0.05)".

The mean pseudomonad counts were similar in both cover crop (triticale and crimson clover) used soils collected from both locations (Figure 4). The mean pseudomonad population counts were significantly higher in the cover crop used soil compared to the non-cover crop used soil collected from production sites.
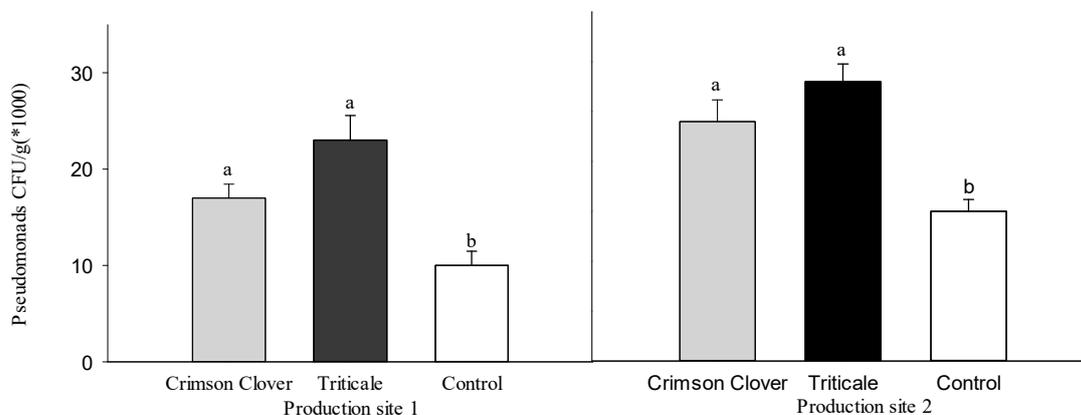
Figure 4. Mean (± SE) pseudomonad colony forming units (CFUs/g of soil) in cover crop used and non-cover crop used (control) soil with natural pathogen pressure. Values were log transformed for analysis purposes, but the original mean values are presented in the figure. Different letters beside bars indicate significantly different mean pseudomonad CFUs/g among treatments (Site 1: $F = 12.89$, $P = 0.0023$; Site 2: $F = 16.83$, $P = 0.0009$; df $_{MST, MSE}$ = 2, 9; $\alpha = 0.05$, least square means).

3.2. Bioassay with Phytopythium vexans

There were significant differences in Phytopythium root rot disease severity between cover crop used soil and non-cover crop used soil collected from both locations when *P. vexans* inoculum was added (Figure 5). The cover crops (crimson clover and triticale) reduced the level of Phytopythium root rot disease severity on red maple plants compared to the control for both locations. There were no significant differences in Phytopythium root rot disease severity between triticale and crimson clover used soils collected from both locations.
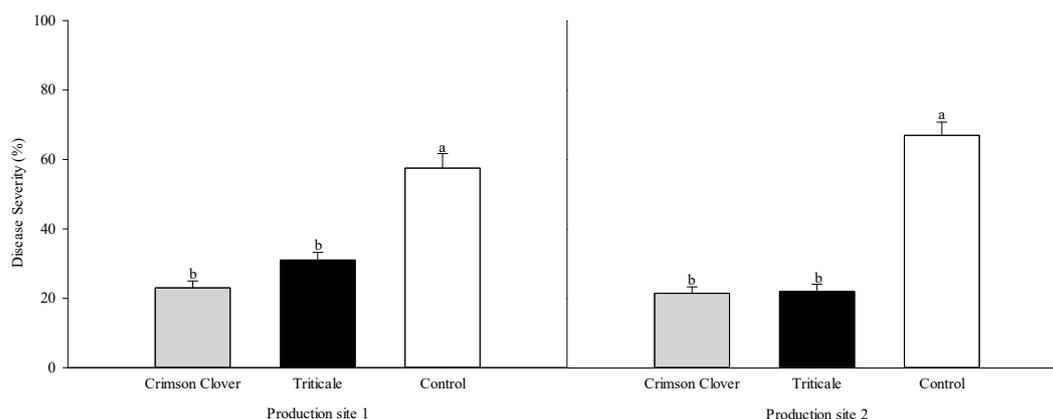

Figure 5. Root rot disease severity (mean ± SE) of red maple plants when *Phytopythium vexans* was inoculated into cover crop used and non-cover crop used (control) soil. Phytopythium root rot disease severity was evaluated using a scale of 0–100% of roots affected. Different letters beside bars indicate significantly different Phytopythium root rot disease severity among treatments (Site 1: $F = 37.27$, $P < 0.0001$; Site 2: $F = 91.12$, $P < 0.0001$; df $_{MST, MSE}$ = 2, 27; $\alpha = 0.05$, least square means).

The pathogen recovery percentage was higher in the non-cover crop used soil (control) when *P. vexans* inoculum was added compared to the cover crop used soil collected from two production sites (Table 2). There were no significant differences in pathogen recovery between crimson clover

used soil and triticale used soil collected from both locations. Red maple plant height increase was significantly or numerically greater in crimson clover used soil compared to non-cover crop used soil collected from production site 1 and site 2, respectively. There were no significant differences in total plant fresh weight and total fresh root weight between red maples planted in cover crop and non-cover crop used soils collected from both locations.

The mean pseudomonad population counts were similar in both cover crop (triticale and crimson clover) used and non-cover crop used soils collected from production site 1 (Figure 6). The mean pseudomonad counts were higher in triticale used soils compared to crimson clover used or non-cover crop used soils collected from production site 2.
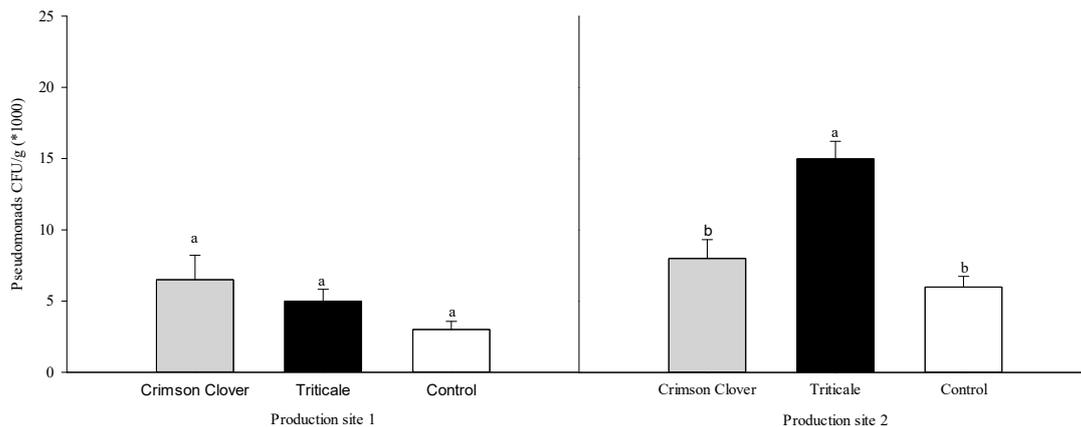
Figure 6. Mean (±SE) pseudomonad colony forming units (CFUs/g of soil) in cover crop used and non-cover crop used (control) soil when *Phytopythium vexans* was added. Values were log transformed for analysis purposes, but the original mean values are presented in the figure. Different letters beside bars indicate significantly different mean pseudomonad CFUs/g among treatments (Site 1: $F = 2.97$, $P = 0.1025$; Site 2: $F = 11.37$, $P = 0.0034$; df $_{MST, MSE}$ = 2, 9; $\alpha = 0.05$, least square means).

Table 2. Recovery of *Phytopythium vexans* from the root of red maple plants, total plant fresh weight, fresh root weight, and height increment (mean ± SE) of red maple plants planted in inoculated (with *P. vexans*) cover crop used (crimson clover or triticale) and non-cover crop used (control) soils collected from two experimental locations.

Treatments	Production Site 1				Production Site 2			
	Pathogen Recovery of *P. vexans* [z]	Total Height Increment (cm) [y]	Total Plant Fresh wt. (g)	Total Root Fresh wt. (g)	Pathogen Recovery of *P. vexans* [z]	Total Height Increment (cm) [y]	Total Plant Fresh wt. (g)	Total Root Fresh wt. (g)
Crimson Clover	38.0 ± 4.7 b [x]	10.5 ± 1.0 a	45.5 ± 4.1 a	29.4 ± 2.7 a	28.0 ± 3.3 b	8.1 ± 1.6 a	59.5 ± 3.6 a	36.4 ± 3.3 a
Triticale	32.0 ± 4.4 b	6.8 ± 1.4 ab	48.6 ± 4.9 a	31.4 ± 3.1 a	30.0 ± 4.5 b	4.7 ± 2.3 a	69.5 ± 4.0 a	49.6 ± 3.7 a
Control	64.0 ± 5.8 a	5.3 ± 1.0 b	51.7 ± 3.7 a	33.6 ± 2.7 a	74.0 ± 4.3 a	5.6 ± 2.0 a	57.2 ± 5.3 a	39.5 ± 4.3 a
F value	11.56	5.55	0.52	0.55	41.48	0.78	2.25	3.34
df	2	2	2	2	2	2	2	2
P value	0.0002	0.0095	0.5992	0.5824	<0.001	0.4688	0.1243	0.0507

"[z] For each plant (ten replications per treatment), five randomly selected maple root samples were plated on V8-PARPH oomycete-selective medium to determine the percent recovery of *P. vexans* from root samples"; "[y] height increment was measured by subtracting the height of plant at the beginning of the experiment from the height of the experiment at the end of the experiment ";" [x] treatment means that do not share the same letter are significantly different (α = 0.05) ".

3.3. Bioassay with Phytophthora nicotianae

There were significant differences in Phytophthora root rot disease severity between cover crop used soil and non-cover crop used soil collected from both locations when *P. nicotianae* inoculum was added (Figure 7). The cover crops (crimson clover and triticale) reduced the level of Phytophthora root rot disease severity on red maple plants compared to the control for both locations. There were significant differences in Phytophthora root rot disease severity between triticale and crimson clover used soils collected from both locations. Triticale significantly reduced Phytophthora root rot disease severity compared to crimson clover used soil collected from production site 1. Crimson clover significantly reduced Phytophthora root rot disease severity compared to triticale used soil collected from production site 2.
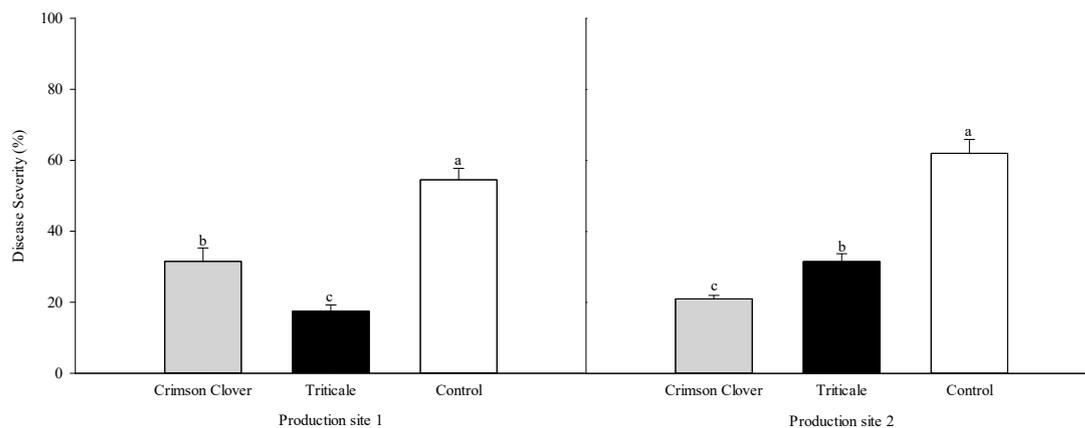
Figure 7. Root rot disease severity (mean ± SE) of red maples when *Phytophthora nicotianae* was inoculated in cover cropped and non-cover crop used (control) soil. For root rot disease severity, each plant was evaluated using a scale of 0–100% of roots affected. Different letters beside bars indicate significantly different *Phytophthora* root rot disease severity among treatments (Site 1: $F = 37.88$, $P < 0.0001$; Site 2: $F = 64.37$, $P < 0.0001$; df $_{MST, MSE}$ = 2, 27; $\alpha = 0.05$, least square means).

The pathogen recovery percentage was higher in the non-cover crop used soil (control) when *P. nicotianae* inoculum was added compared to the cover crop used soil collected from two production sites (Table 3). There were no significant differences in pathogen recovery between crimson clover used soil and triticale used soil collected from both locations. Red maple plant height increase was significantly greater in crimson clover used soil compared to the non-cover crop used soil collected from production site 1. There were no significant differences in plant height increase between red maples planted in cover crop and non-cover crop used soils collected from production site 2. There were also no significant differences in total plant fresh weight and total fresh root weight between red maples planted in cover crop and non-cover crop used soils collected from both locations.

The mean pseudomonad counts were higher in both cover crop (triticale and crimson clover) used soils compared to the non-cover crop used soils collected from both production sites (Figure 8). There was no difference in mean pseudomonad population counts between the cover crop (triticale and crimson clover) used soils from both production sites.

Table 3. Recovery of *Phytophthora nicotianae* from the root of red maple plants, total plant fresh weight, fresh root weight, and height increment (mean ± SE) of red maple plants planted in inoculated (with *P. nicotianae*) cover crop used (crimson clover or triticale) and non-cover crop used (control) soils collected from two experimental locations.

| Treatments	Production Site 1				Production Site 2			
	Pathogen Recovery of *P. nicotianae* [z]	Total Height Increment (cm) [y]	Total Plant Fresh wt. (g)	Total Root Fresh wt. (g)	Pathogen Recovery of *P. nicotianae* [z]	Total Height Increment (cm) [y]	Total Plant Fresh wt. (g)	Total Root Fresh wt. (g)
Crimson Clover	48.0 ± 4.4 b [x]	12.0 ± 1.5 a	51.1 ± 5.0 a	35.2 ± 3.7 a	24.0 ± 4.0 b	5.7 ± 1.8 a	56.6 ± 4.0 a	40.6 ± 3.1 a
Triticale	36.0 ± 2.7 b	8.1 ± 2.4 ab	59.7 ± 3.3 a	38.5 ± 2.3 a	30.0 ± 4.5 b	3.8 ± 1.9 a	46.4 ± 5.2 a	32.4 ± 3.8 a
Control	66.0 ± 5.2 a	4.9 ± 0.9 b	62.2 ± 5.1 a	41.4 ± 3.4 a	70.0 ± 5.4 a	5.9 ± 1.9 a	55.3 ± 5.4 a	38.5 ± 4.1 a
F value	12.72	4.18	1.65	0.96	28.91	0.39	1.30	1.32
df	2	2	2	2	2	2	2	2
P value	0.0001	0.0261	0.2106	0.3944	<0.001	0.681	0.289	0.2851

"[z] For each plant (ten replications per treatment), five randomly selected maple root samples were plated on V8-PARPH oomycete-selective medium to determine the percent recovery of *P. nicotianae* from root samples "; "[y] height increment was measured by subtracting the height of plant at the beginning of the experiment from the height of the experiment at the end of the experiment ";" [x] treatment means that do not share the same letter are significantly different ($\alpha = 0.05$) ".
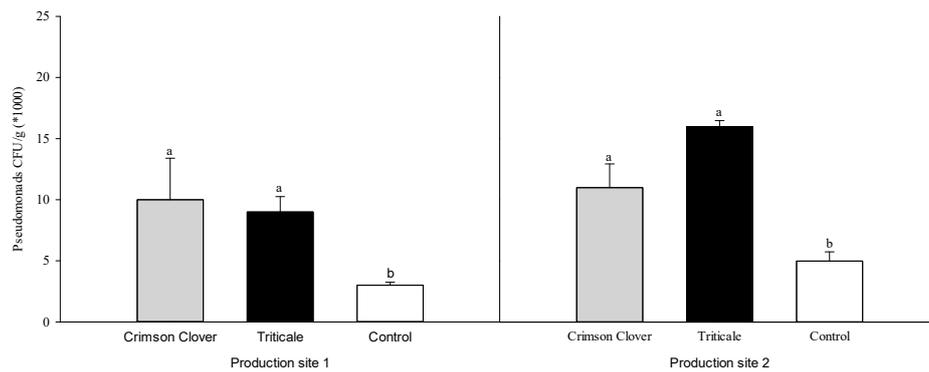
Figure 8. Mean (±SE) pseudomonad colony forming units (CFUs/g of soil) in cover crop used and non-cover crop used (control) soil when *Phytophthora nicotianae* was added. Values were log transformed for analysis purposes, but the original mean values are presented in the figure. Different letters beside bars indicate significantly different mean pseudomonad CFUs/g among treatments (Site 1: $F = 7.70$, $P = 0.0112$; Site 2: $F = 19.03$, $P = 0.0006$; df $_{MST, MSE}$ = 2, 9; $\alpha = 0.05$, least square means).

3.4. Bioassay with Rhizoctonia solani

There were significant differences in Rhizoctonia root rot disease severity between cover crop used soil and non-cover crop used soil collected from both locations when *R. solani* inoculum was added (Figure 9). The cover crops (crimson clover and triticale) reduced the magnitude of Rhizoctonia root rot disease severity on red maple plants compared to the control for both locations. There were no significant differences in Rhizoctonia root rot disease severity between triticale and crimson clover used soils collected from both locations.
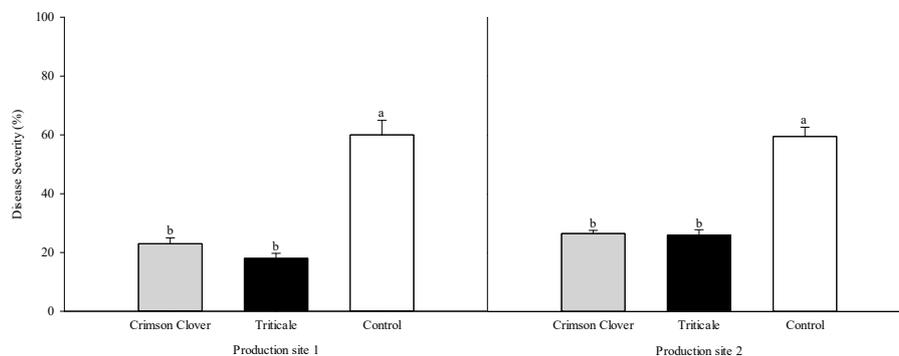
Figure 9. Root rot disease severity (mean ± SE) of red maples when *Rhizoctonia solani* was inoculated in cover crop used and non-cover crop used (control) soil. For root rot disease severity, each plant was evaluated using a scale of 0–100% of roots affected. Different letters beside bars indicate significantly different Rhizoctonia root rot disease severity among treatments (Site 1: $F = 50.39$, $P < 0.0001$; Site 2: $F = 78.67$, $P < 0.0001$; df $_{MST, MSE}$ = 2, 27; $\alpha = 0.05$, least square means).

The pathogen recovery percentage was higher in the non-cover crop used soil (control) when *R. solani* inoculum was added compared to the cover crop used soil collected from two production sites (Table 4). The pathogen recovery percentage was significantly higher in crimson clover used soil compared to triticale used soil collected from production site 1. There were no significant differences in pathogen recovery between crimson clover used soil and triticale used soil collected from production site 2. Red maple plant height increase was significantly greater in crimson clover used soil compared to the non-cover crop used soil collected from production site 1. There were no significant differences in plant height increase between red maples planted in cover crop and non-cover crop used soils collected from production site 2. There were also no significant differences in total plant fresh weight and total fresh root weight between red maples planted in cover crop and non-cover crop used soils collected from both locations.

Table 4. Recovery of *Rhizoctonia solani* from the root of red maple plants, total plant fresh weight, fresh root weight, and height increment (mean ± SE) of red maple plants planted in inoculated (with *R. solani*) cover crop used (crimson clover or triticale) and non-cover crop used (control) soils collected from two experimental locations.

Treatments	Production Site 1				Production Site 2			
	Pathogen Recovery of *R. solani* [z]	Total Height Increment (cm) [y]	Total Plant Fresh wt. (g)	Total Root Fresh wt. (g)	Pathogen Recovery of *R. solani* [z]	Total Height Increment (cm) [y]	Total Plant Fresh wt. (g)	Total Root Fresh wt. (g)
Crimson Clover	52.0 ± 4.4 b[x]	11.4 ± 2.1 a	41.9 ± 4.6 a	29.7 ± 1.9 a	32.0 ± 3.3 b	5.1 ± 1.4 a	62.a ± 6.2 a	44.7 ± 4.6 a
Triticale	28.0 ± 3.3 c	6.6 ± 1.6 ab	51.1 ± 3.5 a	32.6 ± 2.3 a	36.0 ± 4.0 b	2.3 ± 0.5 a	57.8 ± 2.9 a	41.0 ± 2.3 a
Control	76.0 ± 5.8 a	4.0 ± 1.1 b	51.9 ± 3.7 a	34.3 ± 2.8 a	70.0 ± 3.3 a	6.7 ± 2.5 a	59.0 ± 6.3 a	40.9 ± 5.2 a
F value	27.00	5.35	1.98	2.73	34.62	1.73	0.17	0.26
df	2	2	2	2	2	2	2	2
P value	<.0001	0.0110	0.1575	0.0834	<0.001	0.1967	0.8476	0.7705

" [z] For each plant (ten replications per treatment), five randomly selected maple root samples were plated on *Rhizoctonia* semi-selective medium to determine the percent recovery of *R. solani* from root samples "; "[y] height increment was measured by subtracting the height of plant at the beginning of the experiment from the height of the experiment at the end of the experiment ";" [x] treatment means that do not share the same letter are significantly different (α = 0.05)".

The mean pseudomonad population counts were significantly different between both cover crop (triticale and crimson clover) used and non-cover crop used soils collected from both production sites (Figure 10). The mean pseudomonad counts were higher in triticale used soils compared to crimson clover used soils collected from both production sites.
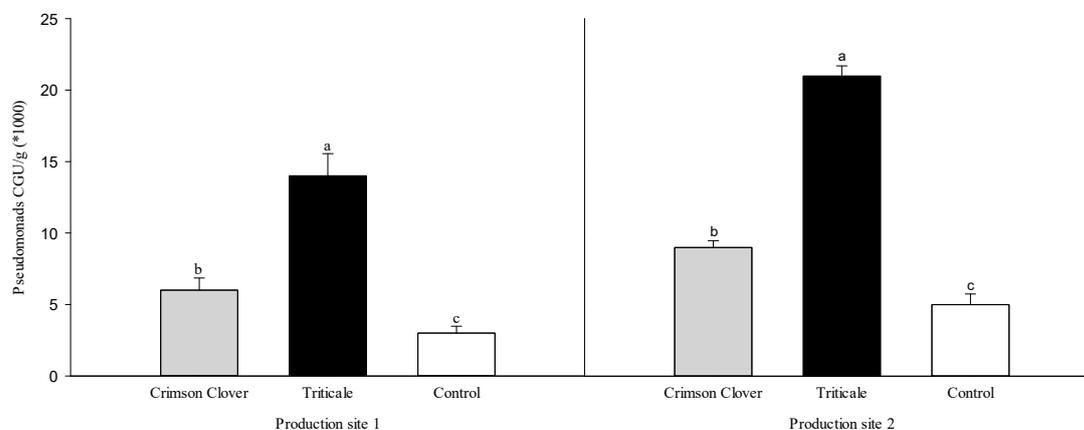
Figure 10. Mean (±SE) pseudomonad colony forming units (CFUs/g of soil) in cover crop used and non-cover crop used (control) soil when *Rhizoctonia solani* was added. Values were log transformed for analysis purposes, but the original mean values are presented in the figure. Different letters beside bars indicate significantly different mean pseudomonad CFUs/g among treatments (Site 1: $F = 31.61$, $P < 0.0001$; Site 2: $F = 53.05$, $P < 0.0001$; df $_{MST, MSE}$ = 2, 9; $\alpha = 0.05$, least square means).

3.5. Soil Moisture and Temperature

Triticale planted soil had comparatively higher soil moisture content in all recorded months in production site 1, except for January and May. Both cover crops maintained higher soil moisture in all recorded months in production site 2, apart from October and May. The lowest moisture differences were 0.42 VWC% in November 2018 and 0.03 VWC% in February 2019 between cover crop and non-cover crop used soils for production site 1 and site 2, respectively. The highest moisture differences were 2.9 VWC% in June 2019 and 3.2 VWC% in December 2018 between cover crop and non-cover crop used soils for production site 1 and site 2, respectively. The temperature differences between cover crop used soil and non-cover crop used soil varied from 0.08 °C in October 2018 to 0.05 °C in June 2019 and from 0.2 °C in October 2018 to 0.1 °C in June 2019 for production sites 1 and 2, respectively.

3.6. Soil Chemistry

Slightly acidic pH was observed in all soil samples collected from both locations (Table 5). C:N ratio was significantly higher in triticale used soils compared to non-cover crop used soils collected from production site 2. However, there were no significant differences in other soil chemical and nutritional properties between cover crop and non-cover crop used soils collected from both locations.

Table 5. Mean value of soil chemical and nutritional properties of the soil sampled from cover crop and non-cover crop used soil from both experimental locations.

Treatments	Production Site 1						Production Site 2					
	OM [y]	CEC [y] (meq/100 g)	C:N Ratio [y]	pH	P (kg/ha)	K (kg/ha)	OM [y]	CEC [y] (meq/100g)	C:N Ratio [y]	pH	P (kg/ha)	K (kg/ha)
Crimson Clover	2.9 a [z]	5.9 a	8.5 a	5.9 a	41.4 a	411.6 a	2.6 a	5.8 a	9.3 ab	6.0 a	27.4 a	242.5 a
Triticale	2.9 a	6.6 a	11.5 a	5.9 a	31.4 a	320.9 a	2.5 a	6.0 a	11.9 a	5.9 a	29.7 a	241.4 a
Control	2.9 a	6.3 a	9.1 a	5.9 a	36.9 a	371.8 a	2.8 a	5.3 a	8.2 b	5.9 a	22.4 a	201.6 a
P value	1.0000	0.7103	0.2391	0.9866	0.5928	0.7132	0.2933	0.3939	0.0401	0.5413	0.5368	0.7016

"[z] Treatment means that do not share the same letter are significantly different ($\alpha = 0.05$) ";" [y] OM = Organic matter, CEC = cation exchange capacity, C:N = carbon nitrogen, P = phosphorus, K = potassium".

4. Discussion

Over a long period of time, soil fumigants have become more common in the field of agriculture for controlling soilborne diseases. Chemicals such as methyl bromide, chloropicrin [34], and metam sodium [35] were commonly used fumigants to control soilborne fungal as well as oomycete pathogens, most likely with the compensation of the environmental health. Panth and his colleagues [5] reviewed and recommended different methods for management of soilborne pathogens such as sanitation, legal methods, soil solarization, biofumigation, anaerobic soil disinfection, cropping systems, and biological control as alternative options for chemical fumigants. However, the use of cover crops to manage soilborne disease in a woody ornamental production system is underexploited.

Cover crops are an integral part of crop production systems with their ability to suppress weeds, host beneficial microbes, reduce erosion, preserve and improve soil quality, provide organic matter, and aid in nutrient cycling [36]. Additional features such as providing suitable habitat for predatory insects [20], improving soil structure, and acting as a non-host crop for pathogens could be important for a woody ornamental production system. Although the mechanisms of disease suppression by cover crops are not yet fully understood, some possible ways that cover crops could be effective against suppressing soilborne plant pathogens are: production of phytochemicals that can be toxic to many soilborne plant pathogens [10]; providing substrate to beneficial microorganisms such as *Pseudomonas*, *Rhizobacteria*, and *Trichoderma* species [5,25,37]; allelopathic effect of cover crops [38]; and reducing pathogen dispersal by rain splash [39]. Not only antagonistic bacteria and fungi, cover crops can host arbuscular mycorrhizal fungi, which can effectively reduce soilborne diseases, supported by the abundancy of mycorrhizal fungi from short term cover crop-maize rotation [40] and use forage oat as fall cover crop [41]. Generally, these mycorrhizal fungi cover and protect the plant roots by forming a mat like structure, produce antagonistic chemicals, compete with the pathogens, and solubilize the nutrients [42]. In this research, we tried to address a few possible ways of soilborne disease management in a woody ornamental production system.

In bioassays with *Phytopythium vexans* and *Phytophthora nicotianae* added, use of cover crops reduced the root rot disease severity. Recovery of the pathogens was also lower when cover crop was used. Similarly, 10–20% reduction in disease severity as well as recovery of *P. nicotianae* were reported with the use of mixed crimson clover and winter wheat by Dawadi and his colleagues [25]. Moreover, Baysal-Gurel and Liyanapathiranage [43] reported the potential use of brassica cover crops to reduce the root rot caused by *P. nicotianae*. Grazieli [44] also suggested the use of winter rye as a cover crop in soybean production to lower the disease pressure caused by *Pythium* species. The incorporation of organic amendments into the soil in different forms has also been associated with the reduction of soilborne plant pathogens such as *Phytophthora* and *Pythium* in different systems [25,45–48]. Furthermore, Ristaino and Johnston [49] found the reduced splash dispersal of *Phytophthora* pathogens by introduction of wheat or winter rye cover crop in bell pepper field. This suggests that the cover crops may hinder the dissemination of *P. nicotianae* spores in soil physically by reducing the splash effect or chemically by producing antimicrobial compounds. Another mechanism of soil suppressiveness might be associated with the relationship between the microbial biodiversity and the frequency of parasitism [50]. As these cover crops increase the host diversity, it can make the hosts difficult to find, leading to "dilution effect" with species richness [51]. Vukicevich and his colleagues [52] reviewed the effect of cover crops in a perennial cropping system and suggested the use of cover crops such as legumes and grasses to minimize the proliferation of pathogens. In our study, growing the winter cover crops up to senescence led to an increase in substrate for beneficial microorganisms, increased nutrient availability, and a higher number of *Pseudomonad* populations, which are likely to have played a role in suppressing *Phytopythium* and *Phytophthora* disease.

A similar pattern of reduction in disease severity as well as recovery from red maple roots was observed with *Rhizoctonia solani*. Aggressiveness of *R. solani* was severe when the cover crops were not used, causing severe root rot of maples. When triticale and crimson clover were used as cover crops, the pathogen was significantly contained. A similar pattern was observed by Wen and

colleagues [53] with short-term cereal rye cropping, which was effective in suppressing *R. solani* compared to fallow. However, they recommended an extended period of cover cropping to observe the sustainable effects. Similarly, Larkin and his colleagues [54] demonstrated the use of winter rye to reduce the root rot disease severity by 18–38% caused by *R. solani* in a potato production system. Populations of *Rhizoctonia* causing root rot of apple [55] and root rot disease incidence in sugerbeet caused by *R. solani* [56] were reduced with the brassica cover crops. Baysal-Gurel and her colleagues [57] reported a successful suppression of *R. solani* by 20–30% using biofumigant cover crop as green manure in a woody ornamental production system. Similarly, Dawadi and colleagues [25] reported a long-term usage of cover crop to increase the soil suppressiveness against *R. solani*.

In this study, we also found that use of cover crops increased C:N ratio, phosphorus, and potassium content of the soil, which might affect the host–pathogen interface. As mineral nutrients are integral components of plants regulating metabolic activities, nutrients are directly correlated with plant resistance and virulence of pathogen [5,58]. Similarly, increased numbers of *Pseudomonas* populations in the cover crop used soil compared to the non-cover crop used soil over the period can contribute to boost the soil suppressiveness against the soilborne pathogens. We did not observe a significant difference in soil temperature and moisture in this experiment. This is in contrast with the reduction in moisture level that was reported by Dawadi and his colleagues [25] when cover crops were used in a woody ornamental production system that can reduce the opportunity of pathogen development [59].

Soils that exhibit suppressiveness to soilborne pathogens have been identified for a long period of time, which are a natural microbe-based plant defense [60]. These suppressive soils can support the microorganisms in a plant root system and develop them as the first line of defense against soilborne plant pathogens [61,62]. In our experiment, we also observed that, even in the non-inoculated cover crop used soil, disease severity as well as recovery of *Rhizoctonia* and oomycetes were low. The increase in pseudomonad counts in the cover crop used soil also aids in the hypothesis of cover crop increasing the disease suppressiveness of the soil. This is also supported by many researchers, as they have reported the increase in the pseudomonad population in the soil system with the use of cover crops [25,53–65]. The pseudomonad population can rapidly colonize in the rhizosphere to produce varieties of growth promoting materials as well as metabolites promoting plant growth [66] and competes aggressively with the other microorganisms [67]. The increase in pseudomonad counts during the short experimental period also suggests possible improvement in soil properties, microbial populations, and better soil suppressiveness in long term use of cover crops.

During our study in two different types of soil systems, we found that the winter cover crops (triticale or crimson clover) effectively reduced the root rot disease severity as well as the recovery of soilborne fungi such as *R. solani* and oomycetes such as *P. vexans* and *P. nicotianae*. Not only able to reduce the soil erosion, cover crops can contain the pathogens by reducing the runoff and blocking further contamination of the nearby fields. The use of these cover crops could also increase the beneficial arthropod population such as ladybugs (cereals), parasitic wasps, minute pirate bugs, and tachinid flies (clover) in the production system [68], which might save the plant from physical injury from harmful insects, thus blocking possible entry of plant pathogens. Similarly, as the effect of the cover crops could be better realized with longer time, perennial woody ornamental production makes it an ideal candidate for the cover crop usage. This suggests that the use of cover crops could be an alternative option for chemical fungicides in a woody ornamental production system to prevent the harmful pests as well as pathogens.

Lastly, a grower's choice to incorporate the cover crops into the production depends on the advantages and the disadvantages the cover crop possesses. We should also keep in mind that the priority of growers is economic survival. Although the cover crops are generally used for its benefits to soil and water quality, a potential threat of the cover crop hosting the plant pathogens cannot be ignored. Bakker and his colleagues [69] suggested a potential elevation of corn seedling pathogens with the use of cereal rye. Similarly, in a greenhouse study, Robertson et al. [70] suggested the potential ability of cover crops to increase the inoculum level in the soil. However, the field trial

showed no such phenomena. Thus, further research on the effect of cover crops on soil microbiology and their substantial benefits as well as the potential threats in different production systems with different crop rotation programs should be studied. We recommend expanding not only the research to different systems with different cover crops and pathogens but also to the comparative economic evaluation of cover crop incorporation in the production system. We evaluated the effect of cover crops against soilborne pathogens in only one woody ornamental crop, thus further investigation is vital for conclusive recommendation.

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