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The Effect of Peat Moss Amended with Three Engineered Wood Substrate Components on Suppression of Damping-Off Caused by *Rhizoctonia solani*

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Abstract: The use of wood-derived materials in soilless substrates for horticultural crop production is increasing; however, there is little information about the effects of wood on the incidence and severity of soilborne diseases of container-grown plants. The objectives of this research were to compare three differently processed wood substrate components blended with sphagnum peat and to investigate the effect of the peat:wood blend ratio on damping-off disease caused by *Rhizoctonia solani* using radish as a model system. In objective one, raw sphagnum peat was blended with three types of processed pine wood, screw-extruded, twin disc-refined, and hammer-milled, at a volumetric ratio of 70:30 and compared to a 70:30 peat:perlite mix. Radish plants grown in the hammer-milled wood and disc-refined wood had significantly lower damping-off disease severity compared to plants grown in the peat–perlite control. In objective two, sphagnum peat was blended with the three types of processed wood at a volumetric ratio of 90:10, 80:20, and 70:30 and compared to a 70:30 peat–perlite mix. The effect of the blend ratio varied by wood processing type. Higher percentages of Forest Gold and pine tree substrate resulted in lower disease severity. In both objectives, radish plants grown in any of the substrate treatments containing wood infested with *R. solani* tended to have lower disease severity compared to plants in the control. Results of this study indicate that the blending of processed pine wood-derived components into peat may enhance the natural suppression of damping-off disease of radish. Further research is needed to elucidate the mode of action of wood-derived materials on disease suppression in container-grown crops and to study the effects for other plant pathogens and crop species.

Keywords: soilless substrate; growing media; wood fiber; damping-off; disease suppression

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1. Introduction

For decades, peat moss has been the primary substrate for container-grown ornamentals and some greenhouse vegetable crops. Increasing annual greenhouse crop production has contributed to a projected four-fold increase (from ~67 million m^3 to almost 300 million m^3 annually) in demand of soilless substrates by 2050 [1]. The global growing media industry, which supplies growers with soilless substrates, has faced unprecedented demand in recent years, which has led to peat and substrate shortages and an inability of substrate suppliers to fulfill orders [2]. As a result, the global growing media industry has needed to identify and develop alternative substrate components to supplement peat usage, extend annual peat supplies, and in some instances reduce dependance on the use of peat moss altogether. In addition to demand, there is also increasing pressure for more economical and sustainable substrates [3,4]. Substrate manufacturers have identified wood byproducts to be some of the most promising sources of raw materials for use in substrate formulations, and growers have started incorporating these products into their production systems [5–8].

The challenge, however, is that several aspects of the production system—ranging from water management to pest control—can be affected by a change in substrate, which has substantial implications for growers [9–12].

Since first offered commercially in the late 1970s, dozens of wood substrate components have been developed, studied, and utilized on a global scale. Wood components can differ greatly in sourcing (tree species and harvest location), processing (machinery type), and pre-treatment (aged, dried, etc.). All current commercially available wood components are made from conifers such as pine (*Pinus* spp.) and spruce (*Picea* spp.) due to their lower concentration of phytotoxic molecules compared to hardwood species [6,8]. Loblolly pine, *Pinus taeda*, is most commonly used in the eastern U.S.; the radiata pine, *Pinus radiata*, is most common in Australia and New Zealand. Scots pine, *Pinus sylvestris*, and Norway spruce, *Picea abies,* are most common across Europe.

Research has demonstrated that wood-derived (engineered wood fiber (WF), and hammer-milled wood) materials have unique properties compared to other organic soilless media components (peat moss, coconut coir, aged bark, etc.) [13–15]. Wood components are processed in multiple ways, with the three most common being those that are hammer-milled, twin-disc-refined, and single- or twin-screw-extruded [16]. These different processing techniques can result in components with unique physical, chemical, hydrological, and biological properties [13,16]. Growers are faced with an increasing number of wood component options with limited science-based published knowledge to support their purchasing or management decisions. Some issues—such as nitrogen immobilization—have been investigated, and researchers have provided the industry with valuable information on how to manage plant nutrition when using wood substrates [13,14,17]. Additionally, hydrophysical behavior and properties of engineered wood components and best practices for adjusting substrate pH have been investigated [15,18]. One of the most poorly understood areas in need of investigation is the microbiological influences of wood substrate components. As researchers investigate and characterize wood materials, it is becoming clear that new engineered wood products differ from their first-generation counterparts and that processed wood components vary significantly in their chemical and physical properties due to material sourcing and differing manufacturing processes [19]. These differences not only affect substrate properties and plant growth but may also affect the persistence and activity of both plant-pathogenic and beneficial microbes, with implications for greenhouse integrated pest management (IPM). Some research suggests that substrates containing wood components are disease-suppressive [20,21]. More research, however, is needed to characterize wood substrates and document if they are naturally disease-suppressive or disease-conducive compared to traditionally used sphagnum peat–perlite substrates.

Naturally disease-suppressive soils are soils that limit growth and infection of soil-borne pathogens despite presence of a susceptible host [22]. This phenomenon is the result of a combination of "general" and "specific" suppression by soil microbes. General suppression is broad spectrum and is the result of pre-existing competitive and antagonistic microbiomes. Specific suppression results from the activity of a single species or a select group of microbes and is pathogen-specific [23]. Suppressive soils have been recognized in soil for over a century [24] and have more recently been documented in soilless substrates such as those containing sphagnum peat and various composted and aged tree barks [25–27]. Substrates can vary in disease-suppressiveness [26], partly because soil- and root-associated microbial communities are highly influenced by the physicochemical properties of the substrate [28]. Food source in a substrate (specifically the form of carbon) is very important [29], as substrates differ with respect to carbon composition. The presence of "simple" carbon sources, such as cellulose, can result in increased microbial activity and competition for resources, such as nitrogen, leading to soils that are suppressive to some plant pathogens [29] but conducive to others [30]. Natural disease suppression tends to be most effective against pathogens that are poor soil competitors such as *Pythium* and *Phytophthora* spp. and less effective against good soil competitors such as *R. solani* [31]. Wood is known to be high in cellulose content [32]. Based on what is known about carbon sources and disease suppression, we might expect substrates containing wood to be naturally suppressive to some soilborne plant pathogens, yet this remains understudied. Researchers have demonstrated that wood is more microbially active than peat in part because of its high carbon: nitrogen ratio [33]. Montagne et al. [34] found that wood fibers from pine and poplar had significantly greater fungal diversity and dominance compared to peat and coconut coir, which had higher bacterial diversity. The implications of these microbial differences on disease are not well studied.

The overall goal of this study was to investigate if peat–wood substrate blends are disease-conducive or disease-suppressive using *Rhizoctonia solani* Kühn as a model soilborne pathogen. *R. solani* is an important pathogen in greenhouse crop production, causing aerial blights, root rot, and stem rot diseases on almost all herbaceous and woody greenhouse crops, leading to significant plant loss [35,36]. The wood product inclusion rates investigated could be considered both as a perlite substitute as well as a product to reduce peat volume in mixes. Our goal in these studies was to mimic the wood inclusion rates currently being used by many horticultural plant producers. Specific objectives were to (1) compare three differently processed wood substrate components blended with peat and (2) investigate the effect of the peat:wood blend ratio on damping-off disease caused by *R. solani*. We hypothesized that damping-off severity would differ in substrates formulated with wood components compared to peat-based substrate blends.

2. Materials and Methods

2.1. Study System

We chose to use the *Rhizoctonia solani*-radish pathosystem [37] to study peat–wood–pathogen interactions as a model soilborne disease in container production. This damping-off assay is high-throughput and quick, allowing us to survey several wood substrate treatments. Because microbes naturally present in soilless substrates can deplete available organic matter over time, short-term bioassays are best suited to study a substrate's ability to support or suppress soilborne pathogen growth and activity [26].

2.2. Analysis and Preparation of Substrates

Hammer-milled pine tree substrate (PTS) was manufactured at the Substrate Processing and Research Center (SPARC) at North Carolina State University by the lab of Dr. Brian Jackson. To create the PTS, 12-year-old loblolly pine trees were harvested at ground-level, delimbed, debarked, and chipped (Salsco Model 8 gas-powered mobile tree chipper, Salsco Inc. Cheshire, CT). Wood feedstock (chips) were then processed in a 50 hp hammer mill (Meadow's Mills, Inc., North Wilkesboro, NC, USA) at 3000 rpms through a 6.35 mm screen. A disc-refined wood product (*Pinus slyvestris*), Forest Gold (FG), was obtained from Pindstrup (Pindstrup Mosebrug A/S, Ryomgård, Denmark), and a screw-extruded wood product (*Pinus sylvestris*), GreenFibre (GF), was obtained from Klasmann-Delimann (Geeste, Germany). Substrate blends were prepared by hand-mixing each wood product or horticultural-grade super coarse perlite (Whittemore Company Inc., Lawrence, MA, USA) with raw sphagnum peat (Promix, Premier Tech, Rivière-du-loup, QC, Canada) classified as H_3–H_4 on the von Post decomposition scale [38]. Substrate pH was adjusted in all substrate treatments to achieve a target pH of 5.8 [39,40] by adding carbonate dolomitic limestone (PLANTEX #80 Pulverized 50 lbs, Master Plant-Prod-Inc., Brampton, ON, Canada) at a rate of 3.56 kg m^{-3}. Additionally, a wetting agent (PsiMatric, Aquatrols; Paulsboro, NJ, USA) was added at 100 mL m^{-3}. Substrate blends were mixed and sealed in 114 L plastic bins and allowed to equilibrate for 7 days.

Several physical and chemical properties were measured to ensure replicability for future experimentation. Initial substrate pH and electrical conductivity (EC) was measured one week after blending using the soil media extract method [41,42]. Three samples per treatment were randomly collected prior to potting. Liquid extracts were then used to measure pH and EC in each sample using an Orion™ Versa Star Pro advanced electrochemistry meter (Thermo Scientific, Waltham, MA, USA). Prior to potting, substrate moisture content was determined (MB27 moisture analyzer; OHAUS, Parsippany, NJ, USA), and clear water was added to increase moisture to achieve a 60% gravimetric substrate moisture content for all treatments. Adjusting/increasing substrate moisture content ensures better uniformity in pot filling and is commonly practiced by growers prior to pot filling and planting. Total porosity, container capacity, air space, and dry bulk density were measured on three replicates of each substrate treatment following the North Carolina State University Porometer method [43].

For each experiment, 11 cm pots (STD Thermo, The HC Companies, Boise, ID) were filled uniformly by weight [14] and placed on 28 × 54 cm standard 1020 flat black trays (The HC Companies, Boise, ID). Each container was weighed to ensure the same mass of substrate was filled in each pot replicate for each substrate treatment. Thirty-two organic radish seeds (*Raphanus sativus* L. "Early Scarlet Globe") were sown into each pot by hand. All pots were hand-watered at seeding with 200 mL of tap water.

2.3. Pathogen Preparation and Inoculation

R. solani isolate RS-33 (anastomosis group 2-1 HK clade, obtained from James Woodhall at The Ohio State University) was prepared as a rice grain inoculum [20]. Twenty-five grams of white long-grain rice were placed into a beaker containing 18 mL of deionized water, covered with aluminum foil, and autoclaved for 60 min at 121 °C and 15 psi. Autoclaved rice grains were then inoculated with ten 7 mm plugs taken from two-week-old *R. solani* RS-33 cultures. Beakers were covered with aluminum foil and incubated at room temperature for 7 days to ensure rice grains were fully colonized. After the incubation period, colonized rice grains were pulverized with a mortar and pestle and sieved through 2 mm mesh to achieve particles of uniform size. Each pot was infested with 0.5 g of inoculum per liter of substrate (0.25 g per pot) by spreading the inoculum across the surface of the substrate. Control pots were not infested. After inoculation, pots were incubated in a walk-in growth room set at 22 to 24 °C, 70% relative humidity, and 24 h illumination by T5 120v florescent lights (fluence rate of 55–65 μmol m^{-2} s^{-1}) for one week.

2.4. Objective 1: Effects of Differently Processed Wood Products on Damping-Off Disease

This experiment consisted of four substrate treatments (3 wood products plus a peat–perlite control) infested or non-infested with *R. solani*. Wood treatments included a commercial wood product produced with a disc-refiner (disc-refined FG), a product produced with a screw extruder (extruded GF), and a manufactured hammer-milled material (Hammer-milled PTS). Wood treatments were blended with sphagnum peat at a volumetric ratio of 70:30 (peat:wood). A control blend was made up of 70% peat and 30% horticultural perlite (peatlite). Treatments were arranged in a split-plot randomized complete block design (RCBD) with four pots (planted with 32 radish seeds) per treatments and four blocks, where each replicate experiment represented a block. Main plot randomization was by *R. solani* infestation. The subplot randomization was by substrate treatment applied to each pot within a tray.

2.5. Objective 2: Effects of Peat–Wood Blend Ratio on Damping-Off Disease

Wood components are commonly blended into peat at 10% to 50% by volume [5], but little is known about how blend ratio affects severity of soilborne plant diseases. To answer this question, we tested each wood treatment (disc-refined FG, extruded GF, and hammer-milled PTS) blended into peat at three ratios (10%, 20%, and 30%) infested or non-infested with *R. solani*. The wood treatments were compared to a peatlite control blend made up of 70% peat and 30% horticultural perlite. Each of the three wood treatments were tested in separate experiments. Treatments were arranged in a split-plot RCBD with four pots per treatment and four blocks, as described for objective one.

2.6. Data Collection

Damping-off severity was measured seven days after seeding based on a disease severity scale developed by Krause et al. [37] in which 1 = symptomless, 2 = small root or stem lesion but not damped-off, 3 = large root or stem lesion but not damped-off, 4 = post-emergence damping-off, 5 = pre-emergence damping-off. For each pot, the 32 seeds were assessed on the categorical scale and then averaged to calculate the mean disease severity per pot. For each pot, germination was calculated as the percent of seedlings scored as "1" or "2" out of the 32 seed sub-samples. Aboveground fresh biomass was measured for each pot by snipping plants at the root–stem interface and measuring total fresh weight (stem and leaves) to the tenth of a gram using AE163 digital laboratory scale (Mettler Toledo, Toledo, OH, USA).

2.7. Data Analysis

All analyses were performed in RStudio version 2021.09.0 "Ghost Orchard" (RStudio Team 2020). Analysis of variance (ANOVA) was used to assess differences in physical and chemical properties among substrate blends. ANOVA was also used to determine the effects of the substrate blend and inoculation treatment on damping-off severity. Mean disease severity ratings (R), which ranged from 1 to 5, were transformed to $R^* = (R^{1.5} - 1)/1.5$ [37] to obtain a linear scale and meet the assumptions of ANOVA: normality of residuals, homogeneity of variances, and block-factor additivity. The model included substrate treatment and *R. solani* infestation. Simple effects analyses and post hoc Tukey means separation tests were performed to determine differences in (1) mean disease severity and (2) aboveground fresh weight in substrate treatments with and without *R. solani* infestation. Natural germination rates with the non-infested treatment group were also compared using ANOVA, simple effects analyses, and Tukey test. To investigate the relationship between substrate blend ratio and disease severity, a regression analysis was performed in the "emmeans" package using the "poly" method. Graphs were created in Rstudio using the package "ggplot2" [44].

3. Results

3.1. Substrate Physiochemical Properties

Physiochemical properties were measured to ensure replicability of future experiments. For both objectives, substrate pH measurements fell within the target range of 5.4 to 6.4 and were similar to pH reported in previous work on wood substrates [45]. The pH was highest in the hammer-milled PTS compared to the other wood types, and similar to peatlite in objective one (Table 1) and objective two experiments (Table 2). Electrical conductivity (EC) was similar across most all treatments, except for hammer-milled PTS, which had higher EC concentrations. The total porosities of the substrates were generally higher in wood products compared to peat, which is expected based on the densities and physical structure of the wood fibers in comparison to the peat. The hammer-milled PTS, disc-refined FG, and extruded GF had similar container capacities, but were significantly higher than those of the peatlite control in objective one (Table 1) yet all mostly similar in objective two across treatments (Table 3). Similarly, air space properties were the same in peat and FG and GF wood fibers, but even for the significant difference between peat and FG to PTS, the extent of the difference was within 4% (by vol), which is quite low. In objective two, air space percentages were consistently higher in most all wood blends compared to peat, which is expected when peat and wood are blended together. These physicochemical findings in this study were similar to previous reports in the literature [46].

3.2. Objective 1: Effect of Differently Processed Wood Products on Damping-Off Disease Severity

We observed a significant effect of the substrate on damping-off disease severity ($p < 0.001$, $F_{3,57} = 6.57$). Radish plants grown in the infested hammer-milled PTS and disc-refined FG had significantly lower damping-off disease severity ($p \leq 0.013$) compared to radish grown in the infested peatlite control (Table 4). Additionally, radish plants grown in the extruded GF had higher damping-off disease severity ($p \leq 0.049$) compared to the other two wood treatments (Table 4).

Table 1. Mean chemical properties (substrate moisture content (SMC), pH, and electrical conductivity (EC)) and physical properties ± standard error (n = 3) at the time of seeding for each substrate treatment in objective one. All blends consisted of 70% peat 30% wood or perlite (by volume).

Treatment	SMC (% vol)	pH ^{z}	EC (mS cm^{-1})	Total Porosity ^{y} (%)	Container Capacity (% vol) ^{x}	Air Space (% vol) ^{w}	Bulk Density (lbs ft^{-3})
Peat	44.82 ± 3.15 b ^{v}	5.80 ± 0.24 ab	0.354 ± 0.03 a	81.0 ± 0.83 c	56.5 ± 0.15 b	24.5 ± 0.84 a	5.6 ± 0.07 a
Disc-refined FG	59.04 ± 2.06 a	5.72 ± 0.20 b	0.332 ± 0.04 a	86.5 ± 1.07 a	62.9 ± 0.98 a	23.6 ± 0.52 a	4.3 ± 0.09 c
Extruded GF	57.18 ± 2.63 a	5.68 ± 0.24 b	0.373 ± 0.04 a	85.3 ± 0.20 ab	63.5 ± 0.55 a	21.8 ± 0.67 ab	4.9 ± 0.17 b
Hammer-milled PTS	51.81 ± 1.74 ab	6.18 ± 0.14 a	0.789 ± 0.23 b	82.2 ± 1.04 bc	62.8 ± 0.80 a	19.5 ± 0.41 b	5.5 ± 0.00 a

^{z} pH and EC of substrate solution determined saturated media extract procedure. ^{y} Total porosity = container capacity plus air space ^{x} Container capacity = wet weight—oven dry weight. ^{w} Air space = volume of water drained from the sample following saturation. ^{v} Within a column, letters indicate significant differences among substrates (α = 0.05, MSD_{SMC} = 9.12, MSD_{pH} = 0.457 MSD_{EC} = 0.392).

Table 2. Mean substrate moisture content (SMC), pH, and electrical conductivity (EC) of the substrate treatments ± standard error for objective two.

Substrate	Blend Ratio ^{z}	SMC (% vol)	pH ^{y}	EC (ms cm^{-1})
Disc-refined FG	Peatlite 70:30	51.27 ± 0.98 cde ^{x}	5.61 ± 0.11 cde	0.345 ± 0.02 bcde
	70:30	57.74 ± 1.23 ab	5.81 ± 0.12 bcd	0.357 ± 0.02 abcd
	80:20	58.01 ± 1.66 ab	6.01 ± 0.16 abc	0.363 ± 0.01 abc
	90:10	59.43 ± 0.84 a	5.47 ± 0.17 de	0.350 ± 0.02 bcde
Extruded GF	Peatlite 70:30	50.30 ± 0.61 de	6.13 ± 0.07 ab	0.320 ± 0.01 cde
	70:30	55.15 ± 1.18 abcde	5.74 ± 0.05 bcd	0.296 ± 0.002 de
	80:20	56.00 ± 1.16 abcd	5.58 ± 0.04 cde	0.292 ± 0.002 e
	90:10	57.07 ± 1.69 abc	5.22 ± 0.04 e	0.295 ± 0.003 de
Hammer-milled PTS	Peatlite 70:30	52.02 ± 1.72 bcde	6.28 ± 0.07 a	0.394 ± 0.02 ab
	70:30	49.49 ± 1.77 e	6.08 ± 0.06 ab	0.417 ± 0.03 a
	80:20	52.45 ± 1.51 bcde	6.01 ± 0.05 abc	0.394 ± 0.02 ab
	90:10	51.34 ± 1.63 cde	5.73 ± 0.07 bcd	0.415 ± 0.01 a

Note: Measurements were taken at seeding stage. The three substrate treatments, disc-refined Forest Gold (FG), extruded GreenFibre (GF), and hammer-milled pine tree substrate (PTS), were tested in separate experiments, each with a peatlite control blend consisting of 70:30 (peat:perlite % volume). ^{z} Blend ratio is a volumetric ratio of peat:wood or peat:perlite (peatlite). ^{y} pH and EC of substrate solution determined on saturated media extract method. ^{x} For each property, letters indicate significant differences: (Tukey HSDs, α = 0.05, n = 12 MSDSMC = 6.2276, MSDpH = 0.430 MSDEC = 0.063).

Table 3. Mean physical properties ± standard error (n = 3) at seeding for each substrate treatment in objective two.

Substrate	Blend Ratio ^{z}	Total Porosity ^{y} (%)	Container Capacity ^{x} (%)	Air Space ^{w} (%)	Bulk Density (lbs·ft^{-3})
Peatlite	70:30	78.6 ± 0.86 ^{v}	62.1 ± 0.50	16.5 ± 1.33	6.6 ± 0.07 bc
Disc-refined FG	70:30	81.3 ± 2.42	63.7 ± 1.52	17.6 ± 1.01	4.4 ± 0.00 e
	80:20	84.7 ± 0.90	65.1 ± 2.54	19.6 ± 1.73	5.3 ± 0.06 de
	90:10	84.7 ± 0.83	67.4 ± 0.72	17.4 ± 0.52	5.7 ± 0.20 cd
Extruded GF	70:30	84.2 ± 0.44	61.3 ± 0.09	25.4 ± 0.49	4.7 ± 0.12 de
	80:20	81.8 ± 1.51	65.2 ± 0.43	16.6 ± 1.10	5.0 ± 0.03 de
	90:10	81.9 ± 0.54	62.3 ± 0.78	19.6 ± 0.99	8.1 ± 0.30 a
Hammer-milled PTS	70:30	79.3 ± 1.72	60.1 ± 2.85	19.2 ± 2.76	5.9 ± 0.10 bcd
	80:20	79.7 ± 3.42	63.8 ± 1.40	15.9 ± 2.05	8.6 ± 0.43 a
	90:10	80.1 ± 1.04	59.6 ± 2.74	20.5 ± 2.08	6.8 ± 0.31 b

Note: The three substrate types (disc-refined FG, extruded GF, and hammer-milled pine tree substrate PTS) were tested in separate experiments, each with a peatlite control blend. ^{Z} Blend ratio is a volumetric ratio of peat:wood or peat:perlite (peatlite). ^{y} Total porosity = container capacity plus air space. ^{x} Container capacity = wet weight—oven dry weight/. ^{w} Air space = volume of water drained from the sample following saturation. ^{v} For bulk density, letters indicate significant differences (Tukey HSDs, α = 0.05, MSD = 1.049). There were no differences among substrates for total porosity, container capacity, or air space ($p \geq 0.064$).

Table 4. Mean damping-off disease severity rates ± standard error in *R. solani*-infested and non-infested pots and natural germination rates in non-infested pots seven days post-seeding in the four substrate treatments.

Treatment [x]	Infested		Non-Infested		Germination (%) [z]	
Peatlite	4.43 [y] ± 0.0	b	1.74 ± 0.09	bc	81.6	bc
Disc-refined FG	4.23 ± 0.06	a	1.35 ± 0.07	a	91.6	a
Extruded GF	4.40 ± 0.04	b	2.02 ± 0.16	c	74.8	c
Hammer-milled PTS	4.21 ± 0.05	a	1.54 ± 0.07	ab	86.7	ab

[z] Calculated as fraction of "1"s and "2"s out of 32 seeds per non-infested pot. [y] Disease severity was measured on a 1–5 scale [37]. Within a column (infested, non-infested), means followed by the same letter are not significantly different (α = 0.05) as determined by the Tukey HSD post hoc test. [x] The three wood treatments were mixed with peat at a volumetric ratio of 70:30 (peat:wood). The peatlite control blend was prepared at 70:30 (peat: perlite). The three substrate treatments were disc-refined Forest Gold (FG), extruded GreenFibre (GF), and hammer-milled pine tree substrate (PTS).

In the non-infested pots, the disc-refined FG had a greater germination rate of radishes ($p = 0.025$) compared to the germination rate in the peatlite control (Table 4). Additionally, the disc-refined FG and hammer-milled PTS had greater germination rates of radishes ($p \leq 0.005$) compared to the germination rate in the extruded GF (Table 4). Disease severity in the non-infested posts was highest in the extruded GF and lowest in the disc-refined FG ($p < 0.001$, $F_{3,57} = 9.74$) (Table 4).

3.3. Objective 2: Effect of Peat:Wood Blend Ratio on Damping-Off Disease Severity

Radish plants grown in any of the wood blends infested with *R. solani* tended to have lower disease severity compared to the infested peatlite control (Table 5). However, differences varied by wood product (tested in separate experiments). There was a significant effect of substrate treatment on disease severity for the disc-refined FG ($p < 0.001$, $F_{3,57} = 15.76$), extruded GF ($p < 0.001$, $F_{3,57} = 16.36$), and hammer-milled PTS ($p < 0.001$, $F_{3,57} = 10.20$). For the disc-refined FG and hammer-milled PTS, plants grown in the 70:30, 80:20, and 90:10 blends had significantly lower disease severity compared to plants grown in the 70:30 peatlite control ($p \leq 0.029$). For the extruded GF, plants grown in the 70:30 and 80:20 blends had significantly lower disease severity compared to plants grown in the 90:10 blend ($p \leq 0.022$) and the 70:30 peatlite control ($p \leq 0.001$). Regression analysis showed that there is a significant negative linear ($p \leq 0.001$) relationship between disease severity and percent wood in the blend for disc-refined FG and hammer-milled PTS in which higher percentages of wood resulted in higher disease severity (Figure 1). For extruded GF, the regression analysis showed that there is a quadratic ($p \leq 0.001$) relationship between disease severity and percent wood in the blend. In the non-infested pots, radish germination rates were similar across all substrate treatments ($p > 0.1619$, $F_{3,57} > 1.89$).

Table 5. Mean damping-off disease severity ± standard error in *R. solani*-infested and non-infested pots seven days post-seeding in wood treatment blended with peat at three ratios compared to a peat:perlite control.

Blend Ratio	Disc-Refined FG [z]		Extruded GF		Hammer-Milled PTS	
	Infested	Non-Infested	Infested	Non-Infested	Infested	Non-Infested
Peatlite 70:30	4.69 ± 0.04 b	1.73 ± 0.06	4.75 ± 0.02 b	1.56 ± 0.07	4.73 ± 0.04 b	1.6 ± 0.08
70:30	4.51 ± 0.05 a	1.59 ± 0.08	4.39 ± 0.06 a	1.71 ± 0.07	4.56 ± 0.05 a	1.41 ± 0.06
80:20	4.44 ± 0.06 a	1.65 ± 0.07	4.49 ± 0.04 a	1.63 ± 0.08	4.48 ± 0.05 a	1.45 ± 0.07
90:10	4.39 ± 0.05 a	1.81 ± 0.08	4.66 ± 0.04 b	1.72 ± 0.08	4.42 ± 0.06 a	1.38 ± 0.06

Note: The three wood treatments were mixed with peat at a volumetric ratio of 70:30 (peat:wood). Wood treatments were tested in separate experiments, each with a volumetric ratio of 70:30 peat:perlite (peatlite) control. Disease severity was measured on a 1–5 scale [37]. Within each wood treatment, means followed by the same letter are not significantly different (α = 0.05) as determined by the Tukey HSD post hoc test. There were no significant differences among treatments in the non-infested pots. [Z] The three wood treatments were disc-refined Forest Gold (FG), Extruded GreenFibre (GF) and hammer-milled pine tree substrate (PTS).

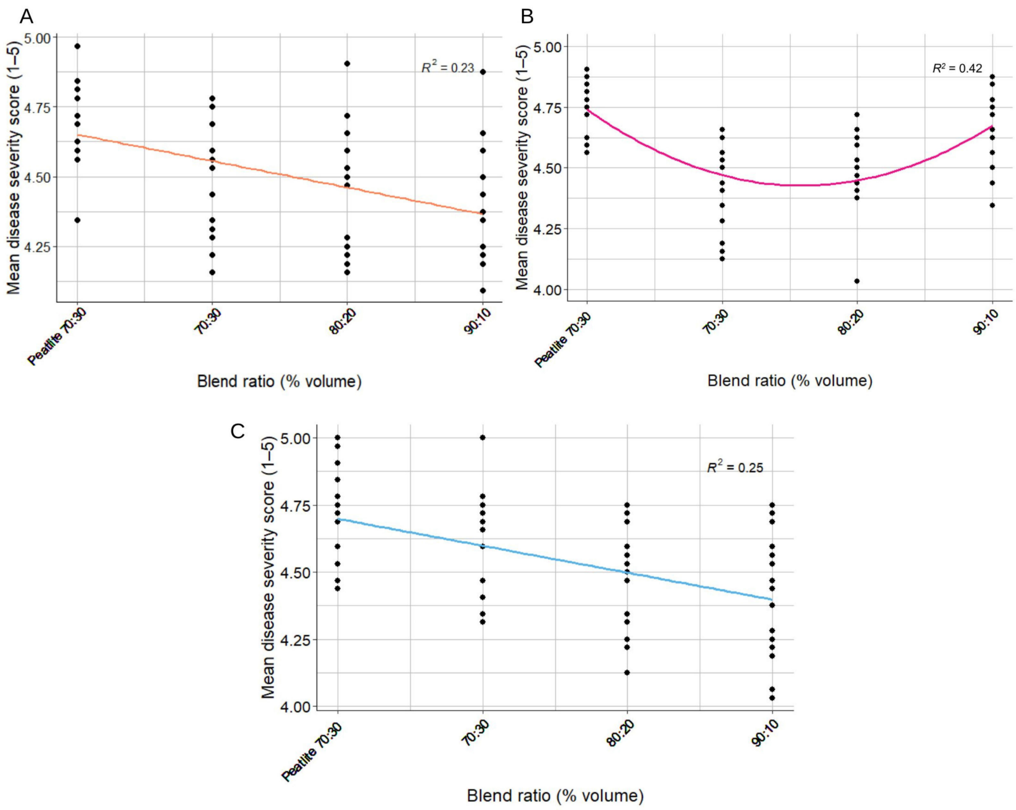

Figure 1. Scatter plots representing the effect of peat:wood blend ratio on mean disease severity of *R. solani* seven days post-infestation for (**A**) disc-refined Forest Gold (FG), (**B**) extruded GreenFibre (GF), and (**C**) hammer-milled pine tree substrate (PTS). Wood treatments were tested in separate experiments, each with a 70:30 peat:perlite control. For disc-refined FG and hammer-milled PTS, a linear regression line was the best fit (R^2 = 0.23; R^2 = 0.25 respectively). For extruded GF, a quadratic regression line was the best fit (R^2 = 0.42).

As expected, radish aboveground fresh weight was lower in *R. solani*-infested pots compared to non-infested pots across all substrate treatments ($p < 0.001$) (Figure 2). Under non-infested conditions, aboveground biomass was similar across all wood and blend ratio treatments. However, plants grown in 70:30 disc-refined FG and 70:30 hammer-milled PTS had significantly higher aboveground fresh weight biomass compared to the plants grown in peatlite ($p \leq 0.0473$) (Figures 2 and 3). Under *R. solani* infestation, there was a significant effect of substrate treatment on aboveground biomass for the disc-refined FG ($p < 0.001$, $F_{3,57} = 17.89$), extruded GF ($p < 0.001$, $F_{3,57} = 16.00$), and hammer-milled PTS ($p < 0.001$, $F_{3,57} = 1095$). Plants grown in wood blends tended to have significantly higher aboveground fresh weight compared to plants grown in peatlite ($p \leq 0.001$) except for plants grown in the 90:10 extruded GF blend ($p = 0.266$) (Figure 2).

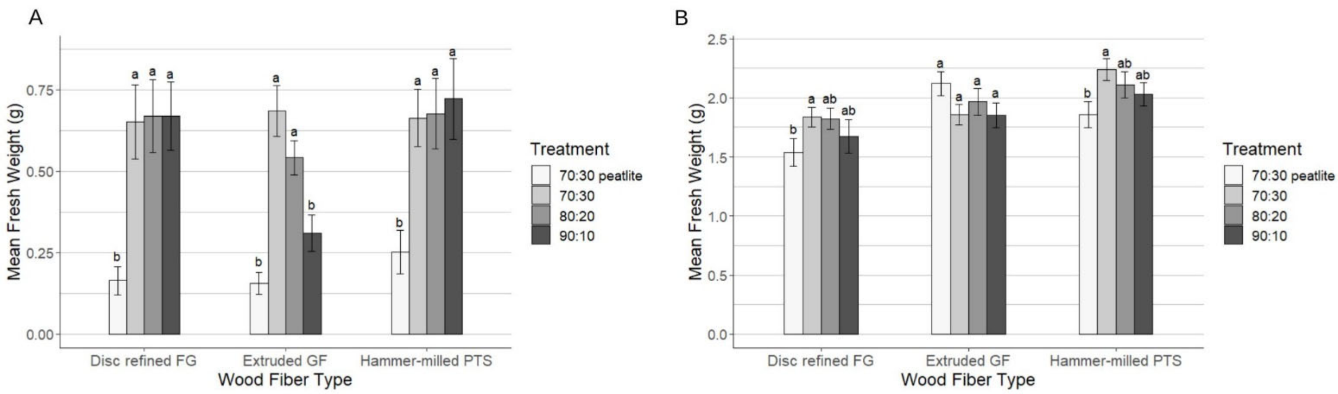

Figure 2. Mean aboveground fresh weight (g) of radish plants in (**A**) *R. solani*-infested and (**B**) non-infested pots 7 days post-infestation. Each wood treatment, disc-refined Forest Gold (FG), extruded GreenFibre (GF), and hammer-milled pine tree substrate (PTS), was blended with peat at four ratios. Wood treatments were tested in separate experiments, each with a 70:30 peat:perlite (peatlite) control. Within wood types, treatment means followed by the same letter are not significantly different ($\alpha = 0.05$) as determined by the Tukey HSD post hoc test ($n = 16$). Errors bars represent standard error.

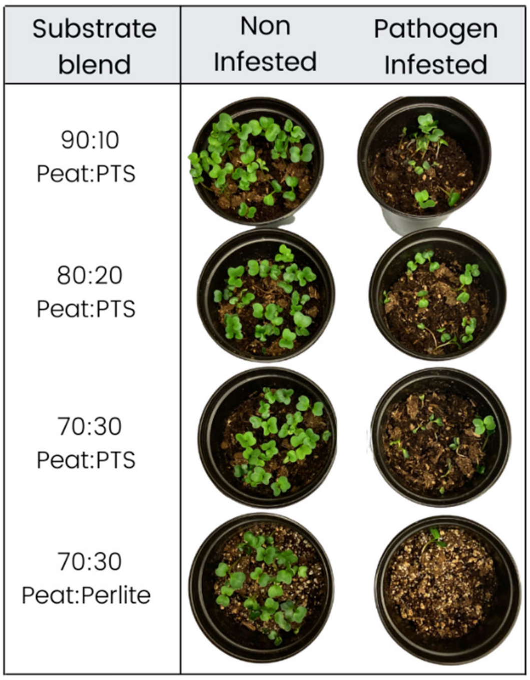

Figure 3. Radish plants grown in non-infested or *R. solani*-infested peat blended with hammer-milled PTS compared to peat blended with perlite. Images were taken 7 days post-infestation.

4. Discussion

Processed wood substrate components hold promise for use in soilless plant production systems. However, there is little information on the effects of wood components on the activity of plant pathogens and development of disease. Our research is among the first to evaluate the effect of processed wood components on damping-off disease. To our knowledge our study is the first to evaluate wood components, manufactured in different ways and blended with peat at different ratios, on damping-off disease in container production.

Sphagnum peat classified as H_3 and H_4 on the von Post decomposition scale [38] is considered to be disease-conducive, particularly to *Pythium* spp. [26]. Our research, and the work of others, suggests that inclusion of processed wood components may reduce this effect and promote disease suppression in peat substrates. In this study, plants grown in peat amended with wood tended to have greater fresh weight and lower disease severity compared to plants grown in the peat–perlite control blend. A similar result was reported by Owen et al. [20] in which substrates amended with pine wood chips exhibited potential suppressiveness to damping-off caused by *Pythium ultimum* and *R. solani*. Similarly, Fuchs et al. reported that wood fibers, blended with peat, were suppressive against *P. ultimum* [21]. In contrast, Montagne found no suppressive effect of screw-refined pine wood fiber on *Fusarium oxysporum* f.sp. *radicis-cucumerinum* [47].

Differences in germination rates and natural incidence of disease (as measured in non-infested pots) could have contributed to differences in disease severity observed in the infested pots. In objective 1, germination rates were highest in the disc-refined FG and lowest in the extruded GF. Furthermore, natural disease severity was highest in the peat–perlite and the extruded GF. In general, the two substrate treatments (disc-refined FG and hammer-milled PTS) with the lowest disease in non-infested conditions also had the lowest disease severity under infested conditions. In objective 2, there were no differences in germination rates or disease severity in the non-infested pots, indicating that baseline germination and disease was not a contributing factor to differences observed in the infested pots. Taken together, these observations suggest that wood component type may affect germination and natural levels of disease, but blend ratio does not.

The mechanisms driving the effects of processed wood components on plant disease are unknown, as this topic is relatively understudied. Much of what is known about disease suppression in substrates derived from forest products/residuals is from studies of pine bark materials [26,30,48], and very little is known about substrates derived from actual wood. The goal of this study was to document if incorporation of processed wood components influences damping-off disease severity to address immediate grower needs. This study provides a foundation for future evaluation of mechanisms contributing to disease suppression. It is possible that suppression observed in this study is due to (1) indirect effects on the pathogen (via shifts in microbial communities) or (2) direct effects of substrate physiochemical properties on pathogen growth. Research has shown that inclusion of wood in soilless substrates alters the physiochemical properties [8,49] and microbial community composition [34] of the substrate and thus is expected to affect plant-microbe interactions [50]. In general, wood is thought to be more microbially active than peat, bark, or composted materials [7,34,49]. While microbial activity was not measured in this study, a possible explanation of the observed reduction in disease severity is that substrates amended with wood supported growth of antagonistic microbes that directly reduced *R. solani* activity [23,51]. This phenomenon has been documented in field soils for suppression of *R. solani* on sugar beet [52]. Future research to investigate correlations between microbial activity and disease suppression is needed. Furthermore, it remains to be determined how physiochemical properties of substrates containing wood influence bulk activity of soil and rhizosphere microbial communities. The addition of wood materials, rich in cellulose, can alter microbial activity in the soil with implications for plant disease. It is possible that addition of cellulose amendments may result in either increased microbial activity leading to enhanced competitive and antagonistic interactions (disease-suppressive) or stimulation of pathogenic fungi and increased disease severity (disease-conducive). Clocchiatti et al. [53] reported that cellulose-rich soil amendments have the potential to suppress *R. solani* and reduce disease. Incorporation of paper pulp initially increased *R. solani* but was followed by a transition from disease-conducive to disease-suppressive effects on damping-off. Wood sawdust was also suppressive, but the effect varied by the type of tree species [53]. Results from this cellulose study support our observation that some wood components, mixed with peat, reduce *R. solani* damping-off severity.

It is also possible that certain physiochemical properties of the wood interfere with the growth of *R. solani* or stimulate plant defenses [54]. Wood fiber and wood chip substrates tend to have a higher pH compared to other substrate components (peat, bark) but are still classified as acidic (4.0–6.0) [8,44]. Research has shown that *R. solani* growth is optimal at pH 4.0–5.5, and growth is reduced as pH increases [55]. In this study, we did not find significant differences in pH between the peatlite control and peat–wood blends, although blends with lower wood amendment rates (i.e., 10%) tended to have lower pH compared to the peatlite control. These experiments also did not test peat or the wood components at their natural inherent pH levels. Understanding more about material pH, independent of lime additions and increases, may help to better understand the natural properties of these materials and their effect on disease suppression or sensitivity.

We observed differences in disease severity in peat amended with wood components manufactured in different ways, suggesting that processing method may influence incidence and severity of disease caused by *R. solani*. Pine tree substrates are produced by chipping and grinding whole pine trees and then further processing in a hammer mill. In contrast, wood fiber materials (FG and GF) are produced by secondary processing of fresh wood chips utilizing mechanical and thermal extraction processes, creating an aged product that is stable and relatively sterile [56,57]. The friction and heating involved in the creation of wood fiber materials is thought to reduce concentration of volatile toxins, such as phenolics, found in fresh wood [16,58]. The various mechanical and thermal extraction techniques create products that vary widely in particle size, volatile organic compounds, and secondary plant metabolites (flavonoids, sterols, pectin, tannins, phenols, resin, etc.) [59]. While research has elucidated the effects of wood secondary metabolites on plant growth [58,60], very little is known about how processed wood chemistry influences activity of plant pathogenic microbes. Additionally, it is not currently understood how aging of processed wood products affects their chemical composition and other properties. Commercially (or experimentally) processed wood components may be stored under various conditions for unknown amounts of time before being sold, used, or tested in trials. The storing conditions and age of processed wood deserves further attention and consideration.

In addition to chemical differences, wood substrate types differ in physical properties, such as particle size distribution and container capacity, which can influence incidence of root rot disease [61]. Dickson and colleagues [13] reported that peat and disc-refined FG had higher percentage (49.0% and 43.7%, respectively) of coarse-sized particles (>2 mm) which was significantly higher than hammer-milled PTS and extruded GF, which contained a higher percentage of particles in the medium-size fraction (0.3–2.0 mm). Dickson also reported that peat blended with disc-refined FG, extruded GF, or hammer-milled PTS at 30% wood had higher container capacity than peat–perlite blended at the same ratio, although extruded GF tended to have the highest CC [13]. When blended, peat and wood interlock to form a matrix and create an environment that is high in porosity, airspace, and water retention that is very different from peat–perlite blends [40]. This matrix effect may vary depending on the ratio of peat to wood and, in turn, could explain differences in disease severity observed in this study. Although we found that disease severity tended to be less in peat amended with wood compared to peat–perlite, a decrease in volumetric disc-refined FG and hammer-milled PTS was significantly correlated with decreased disease severity. Interestingly, the opposite was observed for extruded GF. Statistical significance ($p \leq 0.001$) in our analyses explains the relationship between blend ratio and disease even though the r-squared values were low, indicating high variability around the regression lines. Follow-up studies with increased sample sizes are needed to make predictions about disease based on blend ratio. Overall, differences in disease among the peat:wood blend ratios were small compared differences between peat:wood (at any ratio) and peat:perlite. In general, studies have shown that wood components increase air porosity of peat and improve drainage, which coincidentally creates less favorable conditions for soilborne plant pathogens [18,45]. Data from these studies also showed an increase in air space as the wood percentages increased. It is well established that low oxygen in the root zone increases plant susceptibility to root disease [62,63]. Thus, increases in air space and oxygen in substrates amended with wood may help explain disease-suppressive effects observed in this study. Further work evaluating peat and wood substrates engineered and formulated with differing porosities (air space and container capacities) to test the effect on disease could also be insightful to better understand any potential linkages between the physical environment of the rootzone and plant-disease interactions.

Evaluating substrates (and soils) for disease-suppressiveness is challenging because of the entangled webs of interactions among microorganisms and the environment. The short duration of our damping-off experiments allowed us to reduce any differential effects of water-holding capacity, pH, and nutrient availability on plant growth and germination. Even still, it is possible that differences in disease severity among treatments can be explained by differences in biological and physiochemical properties of the substrates. Because there were little differences in germination among substrate treatments in non-infested pots, we hypothesize that differences in pre-emergent and post-emergent damping-off were due to direct or indirect effects on the pathogen and not an effect of substrate on plant growth. While we found statistical significance in disease severity, the difference was small. This is good news for growers, in that these results suggest that the incorporation of wood components may enhance peat with respect to management of soilborne plant pathogens.

5. Conclusions

Results from this study provide evidence that processed wood components, blended with peat, do not have a negative effect on damping-off caused by *R. solani* and may be disease-suppressive. Our findings provide direction for further research to examine other wood materials and to investigate mechanisms responsible for differences in disease. There is still very little known about the effects of wood component type and inclusion rate on soilborne diseases and disease management. We evaluated wood components for the effects on *R. solani* damping-off on radish, but we do not know if the trends we observed hold for other plant species or other symptomologies such as root and crown rots. Work is needed to examine longer-term effects of WF component type and inclusion rate on disease severity at later stages in plant growth. These questions need to be addressed to provide much needed guidance to growers seeking to integrate these new substrates into their operation and IPM programs.

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