THE EFFECT OF WOOD-FIBER SUBSTRATES ON NATURAL DISEASE SUPPRESSION AND THE EFFICACY OF ROOTSHIELD®WP AGAINST RHIZOCTONIA SOLANI

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THE EFFECT OF WOOD-FIBER SUBSTRATES ON NATURAL DISEASE SUPPRESSION
AND THE EFFICACY OF ROOTSHIELD® WP AGAINST RHIZOCTONIA SOLANI

BY

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Bachelor of Art, Luther College, 2018

THESIS

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# TABLE OF CONTENTS

ACKNOWLEDGEMENTS ........................................................................................................ iv

TABLE OF CONTENTS ........................................................................................................... v

LIST OF TABLES .................................................................................................................... vii

LIST OF FIGURES ................................................................................................................ x

ABSTRACT .............................................................................................................................. xii

CHAPTER 1 INTRODUCTION ................................................................................................. 1

1.1 Overview ......................................................................................................................... 1

1.2 The plant microbiome in the context of CEA and soilless cropping systems .............. 7

1.3 Soilless Substrates ........................................................................................................ 10

1.4 Wood Substrates .......................................................................................................... 17

1.5 Biopesticides as a tool of Sustainable Disease Control ........................................... 20

1.6 Natural disease suppressiveness or biopesticide efficacy in WF substrates is unknown ... 23

1.7 Objectives, Overview, and Aim .................................................................................. 26

1.8 Expected outcomes ...................................................................................................... 27

CHAPTER 2 ROOT DISEASES, VIRULENCE, AND DISEASE SEVERITY CAUSED BY RHIZOCTONIA SOLANI ................................................................................................. 28

2.1 Introduction .................................................................................................................. 28

2.2 Materials and Methods ............................................................................................... 32

   Radish bioassay ................................................................................................................ 37

   Chrysanthemum bioassay ............................................................................................... 38

2.3 Results and Discussion ............................................................................................... 49

2.4 Conclusion ................................................................................................................... 55

CHAPTER 3 THE EFFECT OF WOOD FIBER PROCESSING AND BLEND RATIO ON DAMPING-OFF DISEASE SEVERITY CAUSED BY RHIZOCTONIA SOLANI ......................................................... 56

3.1 Introduction .................................................................................................................. 56

3.2 Material and Methods ............................................................................................... 64

3.3 Results ........................................................................................................................ 73

3.4 Discussion ................................................................................................................... 84

3.5 Conclusions and Future Direction ............................................................................ 87

CHAPTER 4 THE EFFECT OF Wood Fiber Substrate on the EFFICaCY OF THE BIOPESTICIDE ROOTSHIELD®WP ON DISEASE SEVERITY CAUSED BY RHIZOCTONIA SOLANI IN GREENHOUSE CROPS ..................................................................................... 89

4.1 Introduction .................................................................................................................. 89

4.2 Materials and Methods ............................................................................................. 96
4.3 Results................................................................................................................................. 110
4.4 Discussion............................................................................................................................ 130
4.5 Conclusions and Future Direction ...................................................................................... 140
CHAPTER 5 CONCLUSION ........................................................................................................ 141
LIST OF REFERENCES.............................................................................................................. 144
APPENDIX A SUBSTRATE BLENDING ...................................................................................... 157
APPENDIX B RHIZOCTONIA SOLANI PROTOCOLS ............................................................ 163
APPENDIX C SOILLESS SUBSTRATES OVERVIEW ............................................................... 165
LIST OF TABLES
Table 1.1. Substrates vary widely by intrinsic (unchangeable) physical, chemical, and biological characteristics ................................................................. 12
Table 2.1. Experimental Treatment groups (12) to test the efficacy of three isolates of R. solani on radish damping-off ................................................................. 33
Table 2.2. R. solani isolate treatments tested in the root and crown rot assay ................ 35
Table 2.3. pH and EC measurements ........................................................................ 42
Table 2.4. Damping-off disease scale (1-5) on radish developed in collaboration with M. Krause ........................................................................................................ 45
Table 2.5. Crown and root rot disease scale (1-6) on chrysanthemum developed with M. Krause. ........................................................................................................ 47
Table 2.6. Full factorial ANOVA performed to assess main effect of R. solani isolate (BW-R and RS-33) and subplot effect of dose on radish damping-off disease severity in a split-plot RCBD 51
Table 2.7. Average Damping-off Disease severity (1-5) by rice inoculum dose ............... 52
Table 2.8. Factorial ANOVA performed to assess the effect of R. solani isolate and substrate treatment on crown and root rot disease severity in chrysanthemum in a completely randomized design (n=1 per treatment) ........................................ 54
Table 2.9. Average disease±SE by R. solani isolate and substrate ................................ 54
Table 3.1. Treatments evaluated for the effect of WF processing type on damping-off disease severity in radish ................................................................. 66
Table 3.2. Treatments evaluated for the effect of WF blend ratio on disease severity in radish ................................................................. 67
Table 3.3. Mean soil moisture content (SMC), pH, and electrical conductivity (EC)±standard error (SE) (n=3) ........................................................................ 73
Table 3.4. Mean physical properties of substrate treatments±SE (n=3) ......................... 74
Table 3.5. Mean soil moisture content (SMC), pH, and electrical conductivity (EC)±SE (n=3). 75
Table 3.6. Mean physical properties of the substrate treatments ±SE (n=3) ..................... 75
Table 3.7. Natural germination rates of radish ± standard error planted in each WF processing substrate blend ........................................................................ 76
Table 3.8. Natural germination rates±standard error of radish seeds sown in different WF processing type and blend ratio ........................................................................ 76
Table 3.9. Full factorial ANOVA performed to assess main effect of R. solani infestation (infested or non-infested) and subplot effect of WF processing type substrate treatment on radish damping-off disease severity in a split-plot RCBD ........................................................................ 78
Table 3.10. Damping-off disease severity in R. solani infested and non-infested pots 7 days post seeding in the four substrate treatments ........................................ 78
Table 3.11. Full factorial ANOVAs performed to assess main effect of R. solani infestation (infested or non-infested) and subplot effect of percent WF blend substrate treatment on radish damping-off disease severity in a split-plot RCBD ........................................................................ 80
Table 3.12. Damping-off disease severity in *R. solani* infested and non-infested (control) pots. 81

Table 3.13. Mean aboveground fresh weight biomass (g) in *R. solani* infested and non-infested pots across all substrate treatments. 81

Table 3.14. Full factorial ANOVAs performed to assess main effect of *R. solani* infestation (infested or non-infested) and subplot effect of percent WF blend substrate treatment on radish aboveground biomass in a split-plot RCBD. 82

Table 3.15. Aboveground fresh weight biomass (g) in *R. solani* infested and non-infested (control) pots. 83

Table 4.1. Substrates by cellulose content. 93

Table 4.2. Treatment groups for the effect of WF blends on the efficacy of Rootshield WP®’s. 97

Table 4.3. Crown and root rot disease scale (1-6) on young chrysanthemum. 107

Table 4.4. Mean chemical properties of the four substrate treatments used in radish bioassay study±standard errors (n=3). 110

Table 4.5. Mean physical properties of substrate treatments study±standard errors (n=3). 110

Table 4.6. Natural germination rates. 113

Table 4.7. Full factorial ANOVA performed to assess main effect of *R. solani* infestation (infested or non-infested) and subplot effect of WF percent blend and Rootshield treatment (1.93g/L) on radish damping-off disease severity in a split-plot RCBD. 115

Table 4.8. Disease severity 7 days post infestation at high application (1.93g/L) of Rootshield®WP. 115

Table 4.9. Full factorial ANOVA performed to assess main effect of *R. solani* infestation (infested or non-infested) and subplot effect of WF percent blend and Rootshield treatment (1.93g/L) on radish aboveground biomass in a split-plot RCBD. 117

Table 4.10. Aboveground biomass of radish 7 days post infestation at high application (1.93g/L) of Rootshield®WP. 117

Table 4.11. Full factorial ANOVA performed to assess main effect of *R. solani* infestation (infested or non-infested) and subplot effect of WF percent blend and Rootshield treatment (0.4g/L) on radish damping-off disease severity in a split-plot RCBD. 119

Table 4.12. Damping-off disease severity in *R. solani* infested versus non-infested (control) pots under different substrate blend ratios with and without Rootshield WP. 119

Table 4.13. Full factorial ANOVA performed to assess main effect of *R. solani* infestation (infested or non-infested) and subplot effect of WF percent blend and Rootshield treatment (0.4g/L) on radish aboveground biomass in a split-plot RCBD. 121

Table 4.14. Aboveground biomass of radish under *R. solani* and no infestation (control). 121

Table 4.15. Full factorial ANOVA performed to assess main effect of *R. solani* infestation (infested or non-infested) and subplot effect of WF percent blend and Rootshield treatment on crown and root rot in mature chrysanthemum (infested at age 6 weeks) in a split-plot RCBD. 124
Table 4.16. Crown and root rot disease severity of chrysanthemums infested with *R. solani* six weeks after transplant (mature mums) in different substrate treatments with two applications of Rootshield®WP ................................................................. 125

Table 4.17. Full factorial ANOVA performed to assess main effect of *R. solani* infestation (infested or non-infested) and subplot effect of WF percent blend and Rootshield treatment) on crown and root rot disease severity of young chrysanthemums (infested at age one-week) in a simple RCBD ................................................................................................................................. 128

Table 4.18. Crown and root rot disease severity of young chrysanthemums infested with *R. solani* one week after transplant and Rootshield®WP ................................................................. 129
LIST OF FIGURES
Figure 1.2. Sustainable CEA requires addressing several components a) energy consumption, b) the use of growing media, and c) disease and nutrition............................................................... 4
Figure 1.3. The five main plant-microbe mutualisms related to disease suppression .......... 8
Figure 1.4. A generalized diagram noting the four main influences on microbial activity .... 10
Figure 1.5. The Integrated Pest (Pathogen) Pyramid.......................................................... 21
Figure 1.6. The benefits of biopesticide use as part of an IPM disease management program .... 22
Figure 2.1. Sclerotia of isolate BW-R (AG 1-IC) (A) and its diagnostic features.................. 30
Figure 2.2. Radish bioassay set up in growth chamber......................................................... 34
Figure 2.3. Diagram of table of dripline fertigated crops arranged in a CRD ...................... 35
Figure 2.4 A-F. Unrooted chrysanthemum cuttings (URCs) of Chrysanthemum Breeze Yellow 39
Figure 2.5 A-D. Rooted cuttings (A-C) prior to transplant................................................. 40
Figure 2.6. Humidity chamber............................................................................................. 41
Figure 2.7. A total of 1.6 grams of pulverized rice inoculum.................................................. 43
Figure 2.8. Damping-off disease scale developed from Krause et al. (2001)............................. 44
Figure 2.9. Range of “4” or post-emergent damping-off...................................................... 44
Figure 2.10 A-D. Culture morphology of nine isolates of Rhizoctonia solani grown PDA........ 49
Figure 2.11 A-D. Isolates of R. solani under compound microscope....................................... 50
Figure 2.12. (A) R. solani AG 4 HG-I ............................................................... 54
Figure 3.1. The three differently processed Wood Fiber blends............................................. 63
Figure 3.2. Diagram of split-plot design showing randomization for one replicate experiment in the growth chamber................................................................. 66
Figure 3.3. Pots after seeding, watering, and inoculation with R. solani................................. 70
Figure 3.4. Damping-off disease scale developed from Krause et al. (2001)........................... 71
Figure 3.5. Range of “4” or post-emergent damping-off....................................................... 71
Figure 3.6. Visual representation of the effect of R. solani infestation across different blend ratios ................................................................................................................ 83
Figure 4.1. Growth Chamber and Radish: .............................................................................. 97
Figure 4.2. Greenhouse chrysanthemum crown and root rot bioassay .................................. 98
Figure 4.3. Greenhouse and chrysanthemum...................................................................... 98
Figure 4.4. Damping-off disease scale developed from Krause et al. (2001)....................... 105
Figure 4.5. Examples of “4” disease rating: post-emergent damping-off............................. 105
Figure 4.6. Full 1-6 crown and root rot disease scale ........................................................... 106
Figure 4.7. pH (A) and EC (mS/cm) (B) of substrates used in mature chrysanthemum bioassay ................................................................. 111
Figure 4.8. pH (A) and EC (mS/cm) (B) of substrates used in young chrysanthemum bioassay. ........................................................................................................................ .................... 112
Figure 4.9. Aboveground biomass of radish across treatment. Rootshield was applied at 0.4g/L ....................................................................................................................... 122
Figure 4.10. Mature Chrysanthemums crown rot and root mass by substrate treatment, Rootshield application, and R. solani infestation ................................................................................................................................. 126
Figure 4.11. Young Chrysanthemums aboveground growth ................................................................. 129
Figure 4.12. Young Chrysanthemums root mass ......................................................................................... 130
Figure A-1. One-cubic foot box ........................................................................................................ 158
Figure A-2. The addition and incorporation by hand of WF, peat, wetting agent, and limestone ................................................................................................................................. 158
Figure A-3. Final Blended substrates ..................................................................................................... 158
Figure A-4. Substrate pH of peat moss amended .................................................................................... 160
Figure A-5. Porometer method performed at North Carolina State University .................................. 162
Figure B-1. After 6-7 days after PDA bores of R. solani were added .................................................. 163
ABSTRACT

THE EFFECT OF WOOD-FIBER SUBSTRATES ON NATURAL DISEASE SUPPRESSION AND THE EFFICACY OF ROOTSHIELD® WP AGAINST RHIZOCTONIA SOLANI

by

Isobel Michaud

As by-products of the forest industry, wood fibers (WFs) are affordable and sustainable raw materials that have risen in popularity in the last decade to help meet the demand for soilless substrates. Wood fibers are high in hemicellulose and cellulose content yet have lower lignin content than Sphagnum peat, coconut coir, and tree bark-based growing medium components. Researchers have demonstrated that wood is more microbially active than peat and that WFs from pine and poplar have significantly greater fungal diversity and abundance than peat and coir, which can have higher bacterial diversity than WFs. However, the effects of WF-associated microbial communities on disease suppressiveness are unknown. While other organic substrates such as peat, fresh and composted hardwood and pine barks and wood chips, and other composts have been assessed for natural disease suppression and their interactions with biopesticides, WFs have not. Given increasing demand for affordable, sustainable soilless substrates like WF, our study sought to provide growers with information on WFs under disease pressure and in a realistic greenhouse production system, with the ultimate aim to better inform growers on how to incorporate WFs into their own production operations. Two bioassays were developed for Rhizoctonia Damping-Off of Radish (growth chamber) and and Rhizoctonia Crown and Root Rot of Chrysanthemum (greenhouse).

In the first part of this study, WF-Sphagnum peat-based growing media were examined for natural disease suppressiveness or conduciveness to Rhizoctonia damping-off in comparison to a general peat-perlite potting mix. Specifically, I examined whether potting mixes differing in WF processing type (disc refined, extruded or hammer milled) and the percent WF content (v/v) significantly differed from each other in conduciveness or suppressiveness to Rhizoctonia damping-off in radish. No differences in natural disease suppression across WF were found regardless of processing type or percent WF content. Moreover, radishes grown in WF blends had consistently lower damping-off disease severity than radishes grown in peat:perlite controls, suggesting that compared to peat:perlite blends, WFs are naturally suppressive to R. solani.

Using both bioassays, the efficacy of Rootshield® WP, a registered Trichoderma-based biofungicide reference treatment was examined. Previous studies on composted hardwood and pine barks found a correlation between carbon content and the efficacy of specific Trichoderma spp. biocontrol strains, with efficacy of these strains generally increasing with decreasing cellulose content. This suggested that WFs’ high hemicellulose and cellulose content could potentially influence Rootshield’s efficacy. To test this hypothesis, I assessed whether potting mixes differing in the percent WF content (v/v) significantly affected Rootshield® WP efficacy against Rhizoctonia solani in both radish (damping-off) and chrysanthemum (crown and root rot). Plants grown in WFs and infested with R. solani across experiments had significantly lower disease severity than plants grown in peat-lite, despite similar health in control plants. Across experiments, there were no instances of percent WF blend significantly affecting Rootshield
application, suggesting that there is no synergistic effect of WF content and Rootshield efficacy on disease severity. While Rootshield application was non-significant across substrate treatment, there were observable differences in aboveground plant biomass and root growth, highlighting opportunity for future work to more fully capture the effects of Rootshield.

Our results suggest that WF blends offer more protection against the soilborne pathogen *R. solani* than peatlite blends and do so across the plant production cycle. This research provided industry professionals with insight on WF substrate performance in terms of disease management and offered much needed guidance to growers seeking to integrate WF-amended substrates into their operations. Biopesticides and WFs are increasingly integral components of sustainable controlled environmental agriculture (CEA)—with biopesticides as part of IPM and WFs as renewable substrates. While interactions in greenhouse production systems are complex, studies that focus on a few aspects of this complexity will improve our overall understanding of the functional phytobiome and give growers improved working knowledge of the tools that are integral to sustainable crop production, like WFs and registered biopesticides. Given that the composition of WF-associated microbial communities has not been characterized with recent molecular and biochemical methods, future work should examine these unique communities and their interactions with different model plants, biocontrol agents, and soilborne pathogens. However, such studies must be complemented by pilot-scale greenhouse trials under practical conditions to show growers the economic, safety, and environmental merits of incorporating sustainable products like WFs and biopesticides into their production systems.
CHAPTER 1
INTRODUCTION

1.1 Overview

From factories to farms, sustainability is defined as present action with long-term viability. Also known as the triple-bottom line of People, Planet, and Profits, it can be expressed in three-dimensions: social, environmental, and economic (Saunila et al., 2019; University of Wisconsin, 2021). This holistic definition of sustainability is exemplified in sustainable agriculture, which provides a framework for addressing the difficult and inevitable tradeoffs among environmental, economic, and social considerations.

Sustainable agriculture is defined as agriculture that, according to the United Nation’s Food and Agriculture Organization (FAO) meets “the needs of the present and future generations, while ensuring profitability, environmental health, and social and economic equity” (2021). While laudable in its vision, sustainable agriculture must address several problems; namely, the crisis of producing more food using less land and fewer resources (Figure 1.1). For example, with a population of over 9 billion by 2050, food production will need to increase by 60% from 8.4 to 13.5 billion tons annually yet the earth’s finite capacities of land, soil, water, and energy are already strained and further threatened by pollution, resource extraction, the loss of biodiversity, and climate change (FAO, 2014) (Figure 1.1).

Meeting food demands must also be met by simply wasting less. Undoubtedly, reducing food spoilage and waste on the retail and consumer side are essential given 30-40% of food supply is lost at this point in the chain (USDA, 2021), however, we must also focus on disease management to reduce production and supply-side losses. Pests, including invertebrates, pathogens and weeds, cause a staggering 27-42% losses in production for major crops around the world—and without the use of pest control, this would double to 48–83% (Birch et al., 2011).
However, most plant disease management is heavily reliant on chemical pesticide use—a practice increasingly regarded as unsustainable in the long-term (Pimental et al., 2014; Carvalho, 2017; FAO & WHO, 2019) and one that must shift to the more holistic approach of Integrated Pest Management (IPM). IPM includes, among other tools, the use of biological control ("biocontrol") or the use of biological control organisms/agents (BCAs) to reduce the harmful effects of one or more other pest organisms (Pal and Gardener, 2006; Glare et al., 2012; Damalas and Koutroubas, 2018).

![A growing population is increasing strain on our finite world](fig1.jpg)

Figure 1.1. By 2050 rising population, rising food, land, and resource needs will reach unprecedented levels (FAO, 2014). Sustainable agriculture, including sustainable CEA and greenhouse production, will help address these demands.

Current food demands in the US are overwhelmingly met using conventional industrial agriculture practices, with a mere 4% of total food sales in the US in 2012 from organic agriculture (ERS USDA, 2013). While an impressive jump to 7% organic production was achieved in 2019 ($9 billion organic production out of a total $136.1 billion produced; Matlock, 2020; ERS USDA, 2020), meeting rising food demands requires sustainable agricultural
practices that look beyond the narrow definition of organic agriculture. Addressing this crisis requires a multifaceted approach and one essential component is controlled environment agriculture (CEA). Broadly speaking, CEA is intensive agricultural production that integrates horticulture, chemistry, plant physiology and pathology, engineering, entomology, and computer technology to optimize plant growth, yields, and quality in controlled environments, such as greenhouses (Raviv et al., 2019; Mattson, 2021). Along with a growing global population, rising standards of living have contributed to increased demand for high-quality out-of-season produce and ornamentals (Raviv et al., 2019, Davis and Lucier, 2021). In response, CEA has risen (Sparks, 2020) and it will increasingly do so, in order to help address these demands and other agricultural resource needs (Davis and Lucier, 2021, Figure 1.1).

The main benefits of CEA greenhouse crop production are higher yields, water efficiency, and year-round production (Khoshnevisan et al., 2014; Barrett et al., 2016; Savvas and Gruda, 2018; Raviv et al., 2019). In the most complex operations, nearly every environmental condition—irrigation, temperature, humidity and moisture, EC and pH, carbon dioxide, and solar radiation—can be monitored and modified (Kime 2016; Wang and Li 2012). This high level of control mitigates weather risks and allows growers to produce consistent and high quality food and ornamentals year-round (Kime 2016, Raviv et al. 2019). While current greenhouse production only accounts for 1-2% of overall U.S. produce production, several greenhouse-grown crops dominant the market (Davis and Lucier, 2021). For example, greenhouse grown tomatoes account for upwards of 70% of U.S. tomato sales, while greenhouse pepper and cucumber production have dramatically risen; 508% and 92%, respectively, since 2009 (Davis and Lucier, 2021). Due to tremendous investments in CEA technology, Mexico and Canada now dominate the U.S. high-value crop and horticulture (Davis and Lucier, 2021,
LaPlante et al., 2021). However, upfront costs, operating costs, labor shortages, disease management remain some of the largest barriers to greater CEA adoption (Mattson et al., 2015; LaPlante et al., 2021). Nevertheless, rising incomes have increased the demand for premium produce and horticulture products, demonstrating that the cost challenge of CEA is addressable for high-value plants (Halliburton, 2013; Davis and Lucier, 2021; LaPlante et al., 2021). While the U.S. lags behind in CEA investment and technology (Mattson et al., 2015; Davis and Lucier, 2021), a post-pandemic world may encourage more CEA in the U.S., as the COVID-19 pandemic placed acute stress on global food systems, as exemplified in a recent report focused on Canadian greenhouse production (LaPlante et al., 2021). Supply chain stress has also encouraged particularly food insecure, urbanized countries, such as Singapore, to concentrate investments in CEA. For example, the city-state imports 90% of its food and in April 2020, only two days after partial lockdowns, Singapore’s government committed S$30 million to CEA (Tatum, 2020). As CEA-greenhouse production rises (Sparks, 2020; Davis and Lucier, 2021), it is essential to make production more sustainable in terms of energy use, growing media, nutrient inputs, and disease control (Figure 1.2).

Figure 1.2. Sustainable CEA requires addressing several components a) energy consumption, b) the use of growing media, and c) disease and nutrition. This work focused on two of these aspects of sustainability 1) soilless substrates and 2) the use of biopesticides in disease mitigation.
Energy and water use are one component of sustainable greenhouse production (Khoshnevisan et al., 2014; Raviv et al., 2019). High-tech greenhouses can be incredibly energy intensive, specifically in terms of the additional heating and electricity requirements (Canakci and Akinci 2006; Pahlavan et al., 2011; Khoshnevisan et al., 2014). Generally, these requirements inversely vary by location and season; for example, greenhouse imports from Mexico and Canada meet a significant portion of U.S. produce demands (Davis and Lucier, 2021), but Mexico requires cooling compared to Canada, which requires heating and supplemental lighting (Moghaddam et al., 2011; Pahlavan et al., 2011; Khoshnevisan et al., 2014). The energy requirements can be substantial; for example, Moghaddam et al. (2011) found the energy efficiency ratio of greenhouse-grown tomatoes in Iran to be 0.18 compared to 1.42 for field-grown tomatoes, with the total energy requirement for greenhouse-grown tomatoes (2102.68 GJ ha⁻¹) 97.7% higher than for field tomato production (47.65 GJ ha⁻¹) (Moghaddam et al., 2011). However, while energy uses are generally higher in CEA, the gains in water-use efficiency of CEA are substantial, particularly in soilless substrates (and recirculated) systems (Raviv et al., 2019). For example, the water-use efficiency of CEA tomatoes grown in substrate was found to be 15-25L of freshwater per 1 kg of tomato compared to 25-40L for soil-grown greenhouse and 40-60L for outdoor field production (Stanghellini et al., 2003). In general, greenhouse production results in significantly higher yields and improved water use efficiency, but at the expense of significantly higher energy costs.

Substrates are another essential component of sustainable CEA production. Unlike field-grown plants, those grown in CEA are mainly produced using soilless culture systems (SCS) rather than “soils.” Broadly speaking, SCS is any method of growing plants without soil as the rooting medium (Savvas, 2003; Gruda et al., 2016a; Savvas and Gruda, 2018). Hydroponics is
exclusive to plants grown in nutrient solutions, whereas soilless substrates (the focus of this work) is defined by inclusion of solid media (Raviv et al., 2019). Soilless CEA systems have tremendous advantages over field-grown plants: increasing water-use efficiency, mitigating soil-borne diseases, improving fertility, and providing the right balance of saturation and water storage at such a small volume (Barrett et al., 2016; Savvas and Gruda, 2018). In fact, the shift from soils to substrates was originally driven by the high prevalence of soil-borne pathogens in soils (Raviv et al., 2019); this shift demonstrates that a substrate’s disease suppressiveness is a key consideration for growers. While the physical, hydraulic, and chemical properties of soilless substrates are, in general, better for plant growth than soils (Raviv et al., 2019), there is an immense variety of substrates on the market and each has different physiochemical and biological characteristics. Moreover, substrates vary in terms of practical considerations including cost, ease of use, availability, disposal, and sustainability.

Disease management is a third component of sustainable CEA greenhouse production—and soilless substrates are essential to successful disease control. Pests such as insects and pathogens—fungi and fungal-like organisms, viruses, bacteria, and nematodes—are serious threats to crop production; these threats are generally combated with the use of chemical pesticides. However, there are also significant costs to using chemical pesticides to combat disease, including those to public health, pesticide resistance, crop losses, and environmental degradation (USGS, 2007; Pimental et al., 2014; Kumar and Singh, 2015; Carvalho, 2017; Lichtfouse, 2018). Moreover, pest-resistance has rendered many synthetic crop protection products ineffective, requiring the costly development of new chemical active ingredients or increasingly higher doses or combinations of current ones (Owen, 2012; Glare et al., 2012; Seiber et al., 2014). Such arms-race tactics are short-sighted, increasingly expensive, and simply
inefficient. Truly sustainable disease management requires a holistic approach, one which mitigates the risks of traditional synthetic pesticides while ensuring pest populations are maintained below economic thresholds. IPM does just that, using ecology and economics to coordinate multiple tactics of pest control (Krejci, 2014; Figure 1.4). As a “toolbox” for addressing disease, IPM is grounded in good cultural and physical practices—for example the proper sanitation of tools and spacing of plants. In IPM, biocontrols, or organisms that suppress pests and pathogens (Glare et al., 2012), are applied as preventative measures and complement the targeted use of chemical pesticides (Krejci, 2014). IPM is important for disease management in both the field and CEA. However, CEA production by definition offers a more controlled environment to implement IPM—for example, containing applied beneficial insects in greenhouses as well as growing plants in disease-free substrates. In sum, sustainable CEA greenhouse production requires addressing a) energy consumption, b) the use of growing media, and c) disease and nutrition. This thesis focused on two of these three aspects 1) soilless substrates and 2) the use of biocontrols—specifically biopesticides—in disease mitigation (Figure 1.2).

1.2 The plant microbiome in the context of CEA and soilless cropping systems

Plants live in close association with edaphic, endophytic, and root- and foliar-associated epiphytic microbes, known collectively as the plant microbiome or phytomicrobiome. Together, the plant, and these soil and foliar microbes exist in an ecosystem known as the phytobiome (Berendsen et al., 2012; Bulgarelli et al., 2013; Hawkes and Conner, 2017). Much like the human microbiome, which is estimated to consist of over 30 trillion human cells and 38 trillion bacterial cells (Sender et al., 2016; Cani et al., 2018), the plant microbiome consists of trillions of plant cells and trillions of microbial cells—bacteria, fungi, oomycetes, nematodes, and viruses
(Rodriguez et al., 2019). Defined as the volume of soil surrounding plant roots influenced by the living root (Hartmann et al., 2008), the rhizosphere supports a diverse microbiome and is the foundation of plant health and production (Berendsen et al., 2012). While there are a myriad of plant-microbe and microbe-microbe interactions that occur in the rhizosphere, there are five main plant-microbe mutualisms specific to disease suppression (competition, antibiosis, parasitism, growth promotion, and induced resistance) (Hoitink and Boehm, 1999; Whipps, 2001; Rodriguez et al., 2019) (Figure 1.3). With microbial biopesticides, these mutualisms are utilized as modes of action (MOAs) against plant pathogens (Harman, 2000; Woo et al., 2014).

Figure 1.3. The five main plant-microbe mutualisms related to disease suppression: (#1-3) competition with, antibiosis against, and parasitism of pathogens 4) plant-growth promotion, and 5) induced plant resistance Figure from Rodriguez et al. (2019)

Ultimately the rhizosphere is the interface at which the plant, beneficial microbes (including biopesticides), and pathogens interact (Bulgarelli et al., 2013; Parnell et al., 2016).
Since 2010, substantial commercial investments in the research and development of BCAs have been made (Krause, expert testimony 2021). However, in the public sphere where research has examined single strains of BCAs and their interactions with the host plant and pathogens, the effect of the rhizosphere phytobiome remains an understudied factor and may hold some keys to understanding why biopesticides tend to exhibit inconsistent efficacy (Nicot et al., 2011; Grosch et al., 2012; Massart et al., 2015; Parnell et al., 2016; Hawkes and Conner, 2017).

In an agricultural setting, a biopesticide’s activity, as well as the microbial composition and function of the rhizosphere, are influenced by several factors in rhizosphere and bulk soil (Nicot et al., 2011; Ravensberg, 2011; Grosch et al., 2012; Massart et al., 2015; Parnell et al., 2016) (Figure 1.4). First and foremost, the plant and the soil (or soilless substrate) play significant roles in determining the microbial composition (and by extension microbial function) of the rhizosphere; however, there is no consensus on which is the greater determinant (Cardon and Whitbeck, 2007; Berg and Smalla, 2009; Berendsen et al., 2012). Some assert that plant species is the key driver (Garbeva et al., 2004) while others conclude that rhizosphere microbial composition is an “initial substrate-driven community shift” which the plant fine tunes (Bulgarelli et al., 2013). Along these same lines, biopesticide performance is thought to be heavily influenced by the greenhouse production environment (e.g. fertigation, disease management, soilless substrate) (Reviewed in Raviv et al., 2019). Ultimately, the confluence of the plant, the growing substrate, the biopesticide product, and the greenhouse production system can significantly influence biopesticide efficacy (Figure 1.4). (Massart et al., 2015; Parnell et al., 2016). As the physical foundation of the rhizosphere, soilless substrates and their impact in the phytobiome represents a tremendous opportunity to understand the role of substrate in
biopesticide efficacy and consistency (Massart et al., 2015; Parnell et al., 2016) and ultimately help improve CEA greenhouse production.

Figure 1.4. A generalized diagram noting the four main influences on microbial activity in the rhizosphere in an agricultural or greenhouse setting using a biological control program.

1.3 Soilless Substrates

In greenhouse production, soilless substrates replace soils because of their immense advantages: mitigating soil-borne diseases, improving soil fertility, providing the optimal balance of saturation and water storage for plant growth at such a small volume, and producing high quality plants (Agung-Putra and Yuliando, 2015; Barrett et al., 2016; Savvas and Gruda, 2018, Davis and Lucier, 2021). Initially, soils were used to grow plants in containers in greenhouse production and over time growers began to amend their soil with organic materials (i.e. compost) to improve water retention and increase air-pore space (Raviv et al., 2019). Peat has been used as a soil amendment as early as the 19th century. Peat-based growing media became more
intensively studied in the 1950s and increased in popularity with growers, except for greenhouse
tomato, cucumber, and pepper growers who primarily used mineral wool (rockwool) or coconut
coir (Raviv, 2019; Davis and Lucier, 2021). The main driver for the transition to soilless media
was to reduce contamination by soilborne plant pathogens (Baker, 1957) and the loss of methyl
bromide to manage pathogens (Holmes et al., 2020). The shift in CEA away from soils to soilless
substrates was further driven by the desire for control over key physiochemical properties of the
growing media. Soilless substrates have superior physical and hydraulic properties compared to
soil, allowing for optimal water and oxygen and their lower substrate matric forces reduce the
energy needed for plants to uptake water (Raviv et al., 2019).

In soilless culture systems (SCS), plants are grown without soil as the rooting medium
(Savvas, 2003; Gruda et al., 2016a; Savvas and Gruda, 2018). Soilless substrates are a
component of SCS, where plants are grown in various growing media (GM), rather than in the
soilless cultures (solutions) used in hydroponics (Adams, 2002; Savvas and Gruda, 2018). The
term GM can also refer to the solid constituents of the media alone, with additives (e.g. fertilizer,
wetting agents, liming materials and biocontrol) considered separately (Gruda et al., 2013;
Savvas and Gruda, 2018). In this thesis, I will use the term soilless substrates (or only substrates)
as any form of growing plants within a tangible growing media (Savvas, 2003; Agung-Putra and
Yuliando, 2015; Gruda et al., 2016).

A wide variety of substrates are used in horticultural crop production, and each varies in
physical, chemical, and biological characteristics (Table 1.1). Substrates can be classified as
organic (containing carbon) or inorganic (lacking carbon). Organic examples include sphagnum
peat moss, wood fibers, bark, coconut coir, and composts. Inorganic (synthetic, inert) substrates
include stonewool, Oasis™ foam, and nutrient solutions, with stonewool and coconut coir
dominating vegetable production (Abad et al., 2005; Agung-Putra and Yuliando, 2015; Mariotti et al., 2020). Ultimately, substrates provide a growing medium that is able to support the growth of plants under high-intensity agriculture production system (Robbins et al., 2011a; Barrett et al., 2016). Grower selection of a substrate will vary by the crop grown, but in general growers are looking for substrates that are pest and pathogen free and have, chemical stability, a pH from 5-6.5, and balanced water-holding capacity (Table 1.1).

Table 1.1. Substrates vary widely by intrinsic (unchangeable) physical, chemical, and biological characteristics. When choosing media, there are also a host of important economic and emerging considerations that continue to change (Robbins et al., 2011a and b; Schmilewski, 2012; Agung Putra et al., 2015; Barrett et al., 2016; Langenhoven, 2017; Savvas and Gruda et al., 2018).

**Overview of Common Organic Substrates**

Growers have an immense number of substrate products to choose from. To date, grower substrate selection has been driven by performance and cost (Barrett et al., 2016) and less on sustainability or microbial activity. While achieving specific physical and chemical qualities is key, a substrate’s disease suppressiveness is an important quality to consider. In general,
inorganic growing media, such as mineral wool and the standalone use of perlite, pumice, or vermiculite, are free of organic carbon prior to use and thus considered sterile or a “microbial vacuum” (Paulitz and Belanger, 2001; Raviv et al., 2019). However, organic soilless substrates are not a microbial vacuum; rather, they can be characterized by their organic (carbon) constituents, microbial communities, and ultimately by their disease conduciveness or suppressiveness (Bohem et al., 1997; Krause et al., 2001; Borrero et al., 2004).

Organic substrates have vastly different physical and chemical characteristics that not only affect substrate properties and plant growth but also the persistence and activity of both plant-pathogenic and beneficial microbes with implications for CEA IPM (Nelson and Hoitink et al., 1982; Nelson et al., 1983; Kwok et al., 1987; Chen et al., 1988; Hoitink, 1999; Krause et al., 2001; Koohakan et al., 2004; Scheuerell et al., 2005; Lundberg et al., 2012; Parnell et al., 2016; Raviv et al., 2019). The wide variety of organic substrate products available represents both a great opportunity and a challenge for research on the effect of substrate on disease suppression and biopesticide efficacy.

Substrate-choice is in part dictated by practical considerations—the ease and familiarity of a given substrate, available suppliers, and environmental sustainability (Barrett et al., 2016) (Table 1.1). Peat has long been the standard for organic substrate mixes, particularly in greenhouse ornamental production, whereas stonewool (or mineral or rockwool) is more common in greenhouse hydroponic vegetable production (Robbins et al., 2011a and Barrett et al., 2016; Bek et al., 2020). Peat is generally amended with perlite and vermiculite. More recently, US growers have started incorporating wood byproducts and coco coir. Peat has dominated horticulture for decades; in the 1960s, it became a leading component of GM and by the late 1990s it was the predominant constituent of GMs in ornamental and nursery production.
For example, peat was about 94% of GM in UK commercial horticulture in the 90’s and today it still meets the majority of substrate demand in UK commercial horticulture (Bek et al., 2020). Peat is the product of decomposition of aquatic plants (mosses, reeds, and sedges) growing in bogs, or wetlands. In these wet areas, biomass has accumulated in the postglacial period about 11,000-14,000 years ago (Raviv, 2019). Peat bogs vary in location, vegetation, age, and degree of decomposition which leads to differences in physiochemical properties of the harvested peat product. As a result, peats are classified into three categories using the von Post scale (von Post, 1922) — younger, under decomposed H1-H3, partly decomposed (H4-H6) and older highly decomposed (H7-H10). In horticulture, peat is also defined, somewhat subjectively, into three categories “white peat” (H1-H4), “dark peat” (H4-H6), and “black peat” (H7 and above) (Raviv et al., 2019). Peat is widely used because it provides optimal physiochemical conditions for plant growth (Table 1.1) it has low bulk density, high porosity and water-holding capacity, provides good aeration, has high cation exchange capacity (CEC), low microbial activity, is of consistent quality, and has few issues of pathogen, pest, or weed contamination (Barrett et al., 2016; Raviv et al., 2019; Bek et al., 2020). However, due to its inherent low microbial activity, peat is considered “disease conducive” meaning it does not inhibit development of soil-borne plant pathogens, at least compared to composts (Chen et al., 1988; Boehm et al., 1997; Borrero et al., 2004). Although peat has a naturally low pH (raw peat ranges from 3.5 to 4.1) and limited nutrient availability, it can be easily adjusted with lime to raise pH and fertilizer to provide nutrients (Barrett et al., 2016); moreover, for the last four decades, it has been a relatively cheap substrate to extract and transport (Bek et al., 2020). However, there is an environmental cost of peat extraction. As wetlands, peatlands provide many essential ecosystem services including water regulation and filtration, biodiversity, and the largest organic sedimentary store of carbon
(550 Gt of carbon or 42% of ALL soil carbon) (Bek et al., 2020). Given the ecological and climatic importance of peatlands, in the last decade, the UK and Europe have set progressive goals to reduce peat extraction, for example a UK voluntary phase-out of peat for professional growers by 2030 (Defra, 2011; Bek et al., 2020). Increasingly, companies are requiring peat extractions from sites in compliances with the guidelines of responsibly produced peat (RPP), a European certification (Responsibly Produced Peat, 2017; Klasmann-Deilmann, 2021). The worldwide need to reduce and/or responsibly extract peat (Defra, 2011; Responsibly Produced Peat, 2017; Bek et al., 2020) along with the rapid growth in demand (a 240% increase by 2050; Blok, 2021) for soilless substrates in crop production necessitates a dramatic increase in the use of sustainable, affordable alternatives.

Perlite and vermiculite are two common inorganic substrate components that are blended with peat. Formed from minerals that are open-pit mined and heated at extreme temperature to expansion (1600°F, 871°C), perlite (white) and vermiculite (grey) are expensive and non-renewable substrates (Minerals Education Coalition, 2021). In contrast, dozens of sustainable, affordable alternative substrates have re-emerged in the last few decades, including coconut coir, aged pine bark (PB), composted hardwood bark, compost, and wood substrates (Bek et al., 2020) (Appendix C). Sustainable substrates are defined by energy and water use, social compliance, habitat and biodiversity, pollution, renewability, and resource use efficiency (Bek et al., 2020). Many of these organic substrates are, like perlite and vermiculite, blended with peat to achieve the desired pH, porosity, and water retention for optimal plant growth (Langenhoven, 2017; Table 1.1). It is important to note that these substrates alone cannot fully replace peat (Jackson, 2016; Responsibly Produced Peat, 2017, Kiter-Sen et al., 2018)—just as biopesticides can never fully replace chemical pesticides. They are, however, key complements to peat and growers often
experiment, using blends of different substrate components to achieve the desired physical and chemical needs for a specific crop (Table 1.1).

A by-product of the coconut food and fiber industries, coconut coir (the fines and fibers of the husk or mesocarp of the coconut) is a leading renewable substrate (Abad et al., 2005; Robbins et al., 2011a). However, the issue of inconsistent quality across sourcing countries, high EC ($\text{Na}^+$ and $\text{K}^+$), and potential contamination (Abad et al., 2005; Barrett et al., 2016; Raviv et al., 2019) may have reduced grower confidence in the product. Furthermore, there is debate about the true sustainability of coir, particularly in regard to processing energy costs and massive water usage, as well as shipment of the product worldwide (Bek et al., 2020). Nevertheless, improved reliability and consistency in quality control and the supply of coconut coir have bolstered coir’s adoption as an organic substrate (Raviv et al., 2019). Like peat, coconut coir is well-suited to use as a substrate: it has high porosity and water-holding capacity, good aeration, and is generally free of weeds, pests, and pathogens and, unlike peat, it does not require lime for pH adjustment (Abad et al., 2005; Robbins et al., 2011a; Barrett et al., 2016; Raviv et al., 2019).

Other alternative organic substrates include composted hardwood bark, softwood barks such as PB, and composts. Like peat and coconut coir, lignin is a primary component of barks, though the chemical composition of bark varies widely by species, growth conditions, age of trees, and time and method of harvest (Jackson et al., 2009a; Raviv et al., 2019). Both barks and composts rose in popularity from the 1980s, to the early 2000s, however, the cost and availability of many of these substrates has risen since the early 2000s (Nelson and Hoitink, 1982, Nelson et al., 1983; Kwok et al., 1987; Chen et al., 1988; Krause et al., 2001; Scheuerell et al., 2005; Jackson et al., 2009a and b, Altland et al., 2018). Composts in particular have two major drawbacks. First, there is a lack of complete standardization in the compost industry; the definition of “compost” is
vague and “composts” have highest variability of all media materials: physically (e.g. bulk
density, airspace, water retention), chemically (pH and nutrition), and microbiologically (Raviv,
2011; 2013). Secondly, although most pathogens are removed by the composting procedure (21
days at temperature higher than 55°C) (Nobel and Roberts, 2003; 2004), some pathogens, such
as Plasmodiophora brassicae (clubroot of Brassica spp.), Fusarium oxysporum f. sp. lycopersici
(tomato wilt), and Macrophomina phaeolina (dry root rot) have been shown to be resistant to the
composting process, surviving peak temperatures of 62-74°C at 21 days (Nobel and Roberts,
2003; 2004). However, it is important to note that peak temperature is one of several major
factors involved in the composting process. Other factors include bioactive volatiles, release
soluble bioactive materials [e.g phenolics], thermophilic and mesophilic microbes that may feed
on pathogens, enzyme activity, as well as physical and chemical disruption by mechanical
turning (Raviv, 2011; 2013). In sum, some organic substrates, particularly composts, have fallen
in popularity over the last two decades. However, concomitant with a dampening enthusiasm for
composts and barks, wood substrates have emerged as a popular, affordable, and renewable
substrate (Gumy, 2001; Jackson et al., 2009, Owen et al., 2017; Owen et al., 2020).

1.4 Wood Substrates

Made from either the sapwood, heartwood, or both, processed wood substrates are common
soiless substrate components. While they may contain some bark, wood substrates are by
definition mainly made of wood, in contrast to PB substrates, which are a separate category
(Altland et al., 2018). Wood substrate products are made via one of three different processes
(Gumy, 2001; Jackson et al., 2009b; Gaches et al., 2010; Jackson, 2018a):

1) Twin-disc refined

2) Single or double (twin) screw extruded (also called retruders)
3) Hammer-milled

The specific processing procedures are proprietary, however each process involves mechanical break-up and/or thermochemical processing, ultimately leading to differences in fiber size and thickness and the chemical properties of end-products (Jackson, 2018a). Friction used in the processing heats wood materials 80-90°C, resulting in pathogen- and pest-free products (Gumy, 2001; Jackson, 2018a). While all three technologies are used in both Europe and the U.S, disc-refined and screw extruded processes are used extensively in Europe, and hammer-milling is used predominately in the U.S. (Jackson, 2018a). Commercially-available twin-disc refined products include ForestGold (Pinstrup, Denmark) and Hydrafiber® (Hydrafiber, USA) while commercially-available extruded products include GreenFibre® (Klasmann-Deilmann-Americas Inc, USA), HortiFibre® (Fulan, Hong-Kong), and Florafibre®.

Hammer-milling can produce several different products including pine tree substrates (PTS), WholeTree (WT), Clean Chip Residual (CCR), and Pine Wood Chips (PWC). Pine tree substrates are made from harvested pine trees that are de-limbed, chipped or shredded and then further ground in a hammermill and contain a majority of wood (Fain et al., 2006; Laiche A.J., 1986; Wright and Browder, 2005). WholeTree (WT) substrates are created from entire pine trees harvested at the thinning stage of timber harvest and therefore contain a lower percent wood than PTS. Because all aboveground portions of the pine tree (wood, bark, and needles) are chipped and ground, WT substrates consist of approximately 80% wood, 15% bark, and 5% needles (Gaches et al., 2010). Made from the material left over after pine trees are harvested, clean chip residuals (CCR) are processed into clean chips (that can be used to make paper products or boiler fuel) and contain about 50% wood, 40% bark and 10% needles (Gaches et al., 2010).
Wood fibers (WFs) can be made via each of the three main processes, though the term “wood fiber” still lacks a standard definition (Maher et al., 2008; Barrett et al., 2016; Raviv et al., 2019; Durand et al., 2021). WFs are created from wood chips that are processed by one of the three methods in a high pressure and high temperature environment, one which quickly ages the wood to create a stable and sterile material (Gruda and Schnitzler, 2004; Maher et al., 2008; Schmilewski, 2008). WFs were first developed as soilless substrates in the 1980’s in Germany (Schmilewski, 2008) and since the early 2000s have gained significant interest in the United States (Jackson, 2016). Like other non-traditional substrates, WFs are generally used as an amendment to peat rather than a standalone substrate (Gruda and Schnitzler, 2004; Jackson et al., 2016), with the GM generally made up of 20-40% WF (by volume) in most North American floriculture operations (Drotleff, 2018). Similar to peat and coconut coir, WFs have a low bulk density and high total porosity (Gruda and Schnitzler, 2004); however, they also have high air content (and inversely insufficient plant available water and high compressibility) (Gruda and Schnitzler, 2004). This means that water availability decreases with increasing amounts of WF, requiring more frequent irrigation than other peat-based substrates (Bohne, 2004; Raviv et al., 2019). However, total irrigation requirements has been found to be smaller in WF-amended peat, than for peat alone (Bohne, 2004). Furthermore, both ornamental and vegetable crop plants have been shown to grow successfully in WF blends (Bohne, 2004; Jackson et al., 2007; Boyer et al., 2009; Domeno et al., 2010; Harris et al., 2019). For example, Bohne (2004) found that nursery plants grown in WF blended into peat at 25-50% (v/v), were similar to plants grown in other peat blends, while plant growth in 75-100% WF was found to be (statistically) better than peat.

As with other wood components (Jackson et al., 2009a), WFs have the potential to immobilize N due to microbial decomposition of wood, which has a C:N above 30:1 (Nelson,
The effects of WF blended into peat on N immobilization have been examined (Owen et al., 2016; Harris et al., 2019) and ongoing studies are focused on the in-depth characterization and modeling of WF specific physical and hydraulic properties (Jackson, ongoing). WFs have also recently been evaluated in terms of wettability (Durand et al., 2021), lime pH adjustment (Owen et al., 2020) and substrate shrinkage—a known issue associated with the N immobilization-microbial breakdown of the wood products (Domeno et al., 2010; Owen et al., 2016). However, wood substrate components, including WFs, have only minimally been assessed for their natural disease suppressiveness (Montague et al., 2016; Kaderabek et al., 2019; Owen et al., 2019) and never assessed for their interaction with biopesticides. Examining WFs for disease suppression is of particular importance to growers, who depend on pathogen-free substrates, as concerns over potential pathogen contamination (coco coir; Abad et al., 2005), inconsistent quality and maturity, and disease conduciveness (composts; Nobel and Roberts, 2003; 2004) may have weakened demand for these other substrates. Moreover, when implementing sustainable CEA practices, growers looking to incorporate WFs are also likely to incorporate biopesticides into their production systems, however the efficacy of microbial biopesticides in WFs is unknown.

1.5 Biopesticides as a tool of Sustainable Disease Control

As the world population balloons to 9.7 billion by 2050 (UN, 2019) and climate change disrupts normal pest-crop interactions (Birch et al., 2011; Bebber, 2015), the pressure to use more pesticides against pathogens and pests will grow. Yet traditional agro-chemical pesticide use has well-established negative impacts on people and the planet. For example, pesticide-application poses a risk to farm workers and can leave harmful residues in food (Pimental, 2005; Carvalho, 2017; FAO & WHO, 2019). Furthermore, improper use can negatively impact wildlife and the
soil microbiota by reducing beneficial predators, parasites, and pollinators (Pimental et al., 2014) and pathogen resistance, such as observed in *Botrytis cinerea* the casual agent of post-harvest grey mold, has rendered many chemical synthetics ineffective (Tripathi et al., 2008).

![The Integrated Pest (Pathogen) Pyramid](image)

**Figure 1.5. The Integrated Pest (Pathogen) Pyramid** illustrating the basic principles of IPM. Several tactics are combined with an emphasis on prevention, rather than an intervention (Krejci, 2014).

As a major facet of sustainable agriculture, IPM is an alternative to conventional agriculture’s heavy reliance on chemical pesticides and is an effective and sustainable disease management system for CEA (Krejci, 2014). As a holistic approach to disease management, IPM coordinates multiple tactics of pest control—best summarized in the IPM Pyramid (Ehler, 2006; Krejci, 2014; Figure 1.5). Biological control (“biocontrol”) is a broad definition for the use of one or more organisms to reduce the harmful effects of one or more other pest organisms (Damalas and Koutroubas, 2018). The BCAs that control pests range from animal and insect parasites and predators to microbes (fungi, bacteria, viruses, nematodes, etc.) (Pal and Gardener, 2006; Glare et al., 2012). Several beneficial microbes and their metabolites have been studied and formulated as commercial products know as biopesticides. There are three main types of biopesticides: 1) biochemical, 2) plant-incorporated protectants, and 3) microbial (Glare et al., 2012).
Figure 1.6. The benefits of biopesticide use as part of an IPM disease management program.

As a key component of IPM, the advantages of biopesticides are far-reaching (Figure 1.6). For decades, identifying, understanding, and utilizing biological organisms as biopesticides has been an area of active research and this research has led to many commercial products. Moreover, while the costs and time needed to research, develop, and register products were major barriers only two decades ago (Harman, 2000), biopesticides are now economical, effective complements to traditional agro-chemicals (Seiber et al., 2014; Constantine et al., 2020). Biopesticides are becoming a profitable endeavor—as evidenced by the pursuit of the top four major agrochemical companies (Bayer-Monsanto, Dow-DuPont, BASF, and Syngenta) to acquire or partner with biopesticide companies (Olson, 2015) and they are the fastest-growing crop protection market sector (Alexander, 2014).
Representing a mere 5% of the global crop protection market (Damalas and Koutroubas, 2018), biopesticides have yet to reach their full market potential. Growers will not use biopesticides unless they are affordable, reliable, and effective. Efficacy issues—which contribute to a lack of grower confidence in the products—remain substantial barriers to greater biopesticide adoption (Seiber et al., 2014; Kumar and Singh, 2015; Summerfield et al., 2015; Wang et al., 2018; Constantine et al., 2020). More specifically, while the use of biocontrol for insect pests (e.g. thrips, spider mites, whitefly, and aphids) has risen dramatically, biocontrol use for disease management (e.g. soilborne diseases) lags behind (Summerfield et al., 2015). While microbial biopesticides are an essential component of IPM and sustainable greenhouse agriculture, unlike chemical pesticides, they are more directly influenced by the plant and native microbial life in the phytobiome (Massart et al., 2015, Raviv et al. 2019). Under these dynamic conditions, the efficacy of microbial-based biopesticide, particularly those applied to the soil, is largely influenced by interactions within the plant microbiome and the broader CEA greenhouse production environment (Harman, 2000; Berg and Smalla, 2009; Glare et al., 2012; Grosch, et al., 2012; Massart et al., 2015).

1.6 Natural disease suppressiveness or biopesticide efficacy in WF substrates is unknown

Natural disease suppression is related to competition between microbes for space and nutrients, with high microbial activity and biomass reported to keep pathogen populations in check (Chen et al., 1988; Whipps et al.,1991; Hoitink and Boehm 1999; Postma et al., 2000). High microbial activity is particularly effective against pathogens that are considered poor competitors such as *Pythium* spp. (Whipps, 1991; Postma, 2000) as labile carbohydrates, like cellulose and simple sugars, feed high microbial activity—and thus high levels of microbial competition (Bonanomi et al., 2018). However, the same simple carbohydrates that feed general saprotrophs also feed
pathogens that are better soil competitors, such as *Rhizoctonia solani*, whose facultative abilities allow it to eat both dead organic matter and parasitize living plants (Nelson and Hoitink; 1982; Kwok et al., 1987; Scheuerell et al., 2005; Chung et al., 1988; Hoitink and Boehm, 1999). Thus, a substrate’s carbon source influences its natural disease suppressiveness and the degree of suppression may not be equal for all plant pathogens (Chen et al., 1988; Whipps et al., 1991; Hoitink and Boehm 1999; Postma et al., 2000; Krause et al., 2001; Bonanomi et al., 2018).

Organic substrates vary by natural disease suppression. For example, peat is considered disease conducive due to low inherent microbial activity (Chen et al., 1988; Boehm et al., 1997; Borrero et al., 2004) while composts are generally disease suppressive (Raviv et al., 2019) but they range in disease suppression due to high variability physically (e.g. bulk density, airspace, water retention), chemically (pH and nutrition), and microbiologically (Bonanomi et al., 2007; Raviv, 2005; 2011; 2013). Biochar, another substrate amendment, has had similarly inconsistent effects on disease suppression. It has been found to enhance microbial populations (Graber et al., 2010) but also to be neither conducive nor suppressive to disease (Blok et al., 2017); however, Frenkel et al. (2017) found biochar suppressed soil-borne diseases when blended into peat at low concentrations (≤1%), but at higher concentrations (≥3%), biochar actually induced plant disease. Ultimately, organic substrates vary by carbon content and this C influences a substrate’s natural disease suppressiveness; however, in WF the effect of carbon content on natural disease suppressiveness is unknown.

A substrate’s carbon-content also influences the activity and efficacy of biocontrol agents such as *Trichoderma* spp. Some *Trichoderma* spp. suppress fungal plant-pathogens by producing cell-wall degrading enzymes, such as chitinases (Nelson et al., 1983; Chen et al., 1988; De la Cruz et al., 1993; Krause et al., 2001; Borrero et al., 2004; Raviv, 2011; 2013; Francois and
Kronstad, 2017), but as a facultative parasite, *Trichoderma* spp. are also excellent decomposers and able to exist on cellulose and simple sugars (Harman, 2000). Adhering to the biological rule of efficiency, *Trichoderma* spp. produce cellulases and glucanases to consume simple carbohydrates like hemicellulose, cellulose and monosaccharides, rather than more complex carbohydrates like chitin. *Trichoderma* spp. are thus satiated and do not produce chitinases—reducing their effectiveness against pathogens such as *R. solani*. Furthermore, *R. solani* also prospers in the presence of these same simple carbohydrates. Thus, the pathogen thrives, and the biocontrol sits around being “lazy” (Benhamou and Chet, 1997; Pal and Gardener 2006). For several substrates, including dark and light peats and composted pine bark (Krause et al. 2001), the natural disease suppressiveness of carbon-based soilless substrates and their influence on *Trichoderma* spp. biopesticide efficacy have been tested; however, these effects have never been examined in WF substrates.

Wood substrates are high in hemicellulose and cellulose content and have lower lignin content than peat, coir, and bark (Domeno et al., 2010). Based on what is known about carbon source and disease suppression, substrates containing wood may be naturally suppressive to some pathogens, such as *Pythium* and *Phytophthora* spp., but not as suppressive to others, such as *Rhizoctonia solani*. Moreover, while researchers have demonstrated that wood fibers from pine and poplar had significantly greater fungal diversity and dominance compared to peat and coconut coir, with peat and coir having higher bacterial diversity (Montagne et al., 2015; 2016; 2017), microbial activity in WFs remains understudied. As a result, there is little information to support growers looking to use biopesticides in WF substrate blends.
1.7 Objectives, Overview, and Aim

As reviewed here, researchers have demonstrated that WFs differ in microbial activity compared to peat and other organic substrates, however the implications of these microbial differences on natural disease suppressiveness are unknown. Moreover, with the increasing adoption of biopesticides in CEA IPM, it also remains unknown what, if any, influence wood components in substrates will have on biopesticide efficacy. The goal of this study was to elucidate how wood fiber substrate blends affect disease severity of *R. solani* damping-off in seedlings and crown and root rots in mature plants, and to document whether these different blends influence the biocontrol efficacy of Rootshield® WP (*Trichoderma harzianum strain T-22*) against *R. solani*.

The three specific objectives were:

1. To evaluate the effect of differently-processed wood-fibers blended in peat on *Rhizoctonia solani* disease severity

2. To evaluate the effect of different peat wood-fiber blend ratios on *Rhizoctonia solani* disease severity

3. To evaluate the efficacy of the biocontrol fungus *T. harzianum* against *R. solani* disease severity in different peat-wood fiber blend ratios

The overall hypothesis of the project was that WF-amended peat blends would differ in natural disease suppression among blends and influence Rootshield® WP (*T. harzianum T-22*) efficacy. More specifically, we hypothesized that 1) natural disease suppression in WF-amended blends and peatlite controls would differ and 2) that wood fibers added to peat would negatively influence *T. harzianum* efficacy against disease caused by the pathogen *R. solani*. 
1.8 Expected outcomes

This study was the first to document the disease suppressiveness of peat-WF blends and the efficacy of Rootshield® WP in WF blends. Results from this project will provide foundational knowledge on the impact of WFs on disease severity and biopesticide efficacy. Knowledge gained in this work will provide industry professionals with insight on WF substrate performance in terms of disease management, offering much needed guidance to growers seeking to integrate WF-amended substrates into their operations and IPM programs. Biopesticides and WFs are increasingly integral components of sustainable CEA—biopesticides as part of IPM and WFs as affordable, renewable substrates. However, their potential will not be realized unless a better understanding of the variables which influence biopesticide efficacy and disease suppressiveness are first realized. **Thus, this study contributed to the long-term goal of increasing grower confidence in and the use of WF substrates and biopesticides.**
CHAPTER 2
ROOT DISEASES, VIRULENCE, AND DISEASE SEVERITY CAUSED BY
RHIZOCTONIA SOLANI

2.1 Introduction

*Rhizoctonia solani* is a ubiquitous soil-borne basidiomycete fungal plant pathogen (Ajayi and Bradley, 2017; Gondal et al., 2019) that infects over 200 plant species (Adams et al., 1988; Sneh et al., 1991, Prabha et al., 2014). In addition to food and fiber crops, *R. solani* is a significant threat to ornamentals such as poinsettia, geranium, impatiens, and chrysanthemum (Gondal et al., 2019; Lawson, 2021). Moreover, *R. solani* causes a wide range of diseases (e.g. damping-off, hypocotyl, root, stem, and crown rots, stem cankers, blights, even black scurf on potato tubers) (Krause et al., 2001; Ajayi and Bradley, 2017; Gondal et al., 2019; Lawson, 2021), affecting a wide range of plant parts (e.g. roots, stems, tubers, corms, aboveground portions) (Agrios, 2005).

To attack plants, the pathogen produces cell-wall degrading enzymes (CWDE), such as pectin esterases, polygalacturonases, and various cellulolytic enzymes (Sharon et al., 2006). Its diverse ability to induce disease on a wide variety of plants and its strong competitive abilities make it one of the most prominent and devastating soil-borne pathogens (Sneh et al., 1991).

*Methods of Classification*

*R. solani* can be categorized in several ways: by morphotaxonomy, anastomosis group, and various genomic sequencing methods. *R. solani* can be characterized as uninucleate (UNR; teleomorph *Ceratobasidium bicorne*), binucleate (BNR; teleomorphs *Ceratobasidium* spp. and *Tulasnella* spp.), and multinucleate (MNR; teleomorphs *Thanatephorus* and *Waitea*) (Sharon et al., 2006; Sharon et al., 2008). However, it is mainly classified by anastomosis group (AG) or the ability of hyphae to anastomose (fuse) with known isolates of designated anastomosis groups (AGs) (Sneh et al., 1991; Tredway and Burpee, 2001). This technique is considered to be the classical method of identification (Sharon et al., 2006). Numbered AG groups (e.g. AG 1, AG 2)
generally denote multinucleate (MNR) isolates (Sharon et al., 2006) while letters (e.g. AG A, AG E) denote binucleate (BNR) and/or uninucleate (UNR) (Sharon et al., 2008). Currently, there are 13 anastomosis groups, with AG BI recently moved to AG 2 (Woodhall et al., 2021). Strains classified within the same AG have a close relationship to each other and oftentimes have the same host range. For example, the majority of AG 1, 2, and 4 are pathogenic on radish while AG 3 isolates are not (Ichielevich-Auster et al., 1985); however, it is difficult to draw clear patterns of pathogenicity among AGs (Melzer et al., 2016).

Historically, *R. solani* classification by AG is most-common, however alone it is an imperfect way to determine genetic similarity (Sharon et al., 2006 and Sharon et al., 2008), as some isolates of different AGs can anastomose with one another—for example AG-2, 3, 6, and 8 (Sneh et al., 1991; Carling, 1996). Determining AG is also a time-consuming process requiring significant expertise (Sharon et al., 2006). These issues have contributed to considerable inconsistencies over and re-classification of AG groups (Sharon et al., 2006; Sharon et al., 2008; Ajayi and Bradley, 2017; Gondal et al., 2019), with certain isolates in GenBank inaccurately identified (Sharon et al., 2008).

There are several molecular classification methods that can complement the classical anastomosis grouping method. Of these methods, rDNA-ITS sequence analysis is accurate, easy to use, and a strong complement to classical anastomosis group (Sharon et al., 2006; Sharon et al., 2008). This technique connotes genetic relatedness of isolates by the clustering of isolate sequences in a phylogenetic tree along with a detailed percent sequence similarity within and among AGs and subgroups. rDNA-ITS sequence similarity ranges are large within some AGs (e.g. AG 4 88-97%) (Sharon et al., 2006; Sharon et al., 2008), but generally AGs contain distinct
subgroups with narrow percent sequence similarity ranges (e.g. AG 4 HG-I {94-100%}, AG 4 HG-II {98-100%}, AG 4 HG-II {99-100%}) (Sharon et al., 2006).

At the microscopic level, the diagnostic features of \textit{R. solani} include branching of septate hyphae, hyphae at right-angles, constriction at the branching point, dolipore septa that permit unrestricted cell-to-cell movement, no clamp connections, septae near branching points, and (depending on the isolate) monilioid cells (Figure 2.1) (Tredway and Burpee, 2001 and Ajayi and Bradley, 2017). Ultimately, the hyphal fusion method remains the most common mode of \textit{R. solani} identification, and molecular techniques, for the most part, verify/support those classifications (Sharon et al., 2008).

\textbf{Figure 2.1. Sclerotia of isolate BW-R (AG 1-IC) (A) and its diagnostic features under (B) light scope: 1) right-angled mycelium and 2) “pinching” at the septum as well as 3) multinucleate isolates with several nuclei observable}

\textbf{R. solani as a soil inhabitant}

\textit{R. solani} is highly successful soil competitor due to its facultative saprophytic abilities and the formation of long-lived ‘nutrient-independent propagules’ called sclerotia (Hoitink et al., 1991), which can survive in the soil for several years (Sneh et al., 1991). While \textit{R. solani} produces
sclerotia, it cannot produce spores except under special laboratory or rare natural conditions (Agrios, 2005; Ajayi-Oyetund & Bradley, 2017); this lack of airborne transport is key to mitigating the pathogen’s spread. Unlike some soil-borne pathogens that are poor soil competitors, such as the Oomycetes Pythium spp. and Phytophthora spp., high microbial activity is not necessarily sufficient for disease suppression of R. solani (Chung et al., 1988; Hoitink and Boehm, 1999; Krause et al., 2001). However, R. solani’s diverse range and pathogenic abilities makes it difficult to observe clear patterns. For example, despite being a strong soil competitor, disease suppression of R. solani (AG-3) was correlated with high levels of soil microbial activity in grasslands compared to croplands (Garbeva et al., 2006). Overall, specific microbial antagonists are responsible for suppression of R. solani (Nelson and Hoitink; 1982; Kwok et al., 1987; Scheuerell et al., 2005), (Grosch et al., 2012).

**Disease Prevention and Mitigation: An IPM approach**

The best prevention measure against R. solani infestation is to avoid pathogen-infested soils and mitigate the spread of mycelia and sclerotia. For example, R. solani spread can be mitigated by sanitizing tools and equipment, rotating crops, and incorporating of soil amendments such as plant materials, mulch, or green manure to increase antagonistic soil microbial populations and reduce disease caused by R. solani. For field production, increasing aboveground biodiversity (Garbeva et al., 2006) and crop rotation, alongside other best management practices (e.g. proper spacing, aeration, and field drainage) are the best methods of mitigating disease caused by R. solani. For container-crop production of annual monocultures, best practices include proper sanitation of tools, pots, tables, driplines, and equipment, alongside the use of pathogen-free substrates and plant propagation material (Agrios, 2005). Given a lack of Rhizoctonia-specific resistant crop varieties, an IPM approach is the best disease management method.
The overall aim of this study was to establish protocols and assess pathogenicity of isolates of *R. solani* representing approximately seven AG groups. Specific objectives were to:

1) evaluate virulence of three *R. solani* isolates causing damping-off on radish and to determine the optimum rice inoculum dose to induce a medium level of damping-off disease severity

2) evaluate the virulence of nine isolates of *R. solani* on chrysanthemum and to develop a root and crown rot scale

### 2.2 Materials and Methods

**Pathogen isolates**

Nine isolates of *R. solani* were used to develop a radish damping-off assay and a chrysanthemum crown and root rot assay. Isolate RS-33 (isolated from radish) was obtained from Ram Khadka at Ohio State University. Isolates BW-R and BW-P were obtained from Chris Rose at BioWorks. Seven isolates (identified by AG group) were obtained from Dr. James Woodhall at the University of Idaho. All isolates except BW-P were sequenced by Dr. James Woodhall and Lara Brown at the University of Idaho using rDNA-ITS techniques. The Bioworks isolate RS-33 was identified as AG 2-1 clade HK and BW-R is AG 1-IC. The University of Idaho isolates were grouped to AG 1 I-B, AG 2-1, AG 4 HG-I, AG 4 HG-II, AG 5, AG E, and AG F. To document morphologies of the isolates microscopically, hyphal wet mounts were prepared from pure cultures and viewed using a compound microscope at 20X magnification (Olympus CX43RF). To document macroscopic differences, isolates were grown on PDA incubated at room temperature (21-24°C) for 10 weeks and imaged.
2.2.1 TREATMENTS AND EXPERIMENTAL DESIGN

RADISH DAMPING-OFF ASSAY

To test inoculum rates and characterize *R. solani* isolates, an experiment was set up as a split-plot RCBD with each shelf in the growth chamber as a block. The response variable was damping-off on a scale 1-5, with main plot treatment of isolate (3) and subplot treatment rice inoculum concentration (4) for a total of 12 treatments (Table 2.1). The experimental unit was one tray (main plot treatment of isolate) and one pot (subplot treatment of rice inoculum dose) (Figure 2.2). For isolate BW-P, they were four replicates per treatment and only one experiment was performed. For isolates BW-R and RS-33, there were 16 replicates per treatment, with four total experiments (n=4 per treatment per experiment) performed.

Table 2.1. Experimental Treatment groups (12) to test the efficacy of three isolates of *R. solani* on radish damping-off and to determine an inoculum dose to induce a medium level of damping-off disease severity.

<table>
<thead>
<tr>
<th>R. solani Isolate</th>
<th>Dose (g/L)</th>
<th>BW-R</th>
<th>RS-33</th>
<th>BW-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>1</td>
<td>5</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>2</td>
<td>6</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>7</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>4</td>
<td>8</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.2. Radish bioassay set up in growth chamber. Three trays fit half of one block/shelf. For the final replicate experiment, only BW-R and RS-33 were used (two trays per shelf). Numbered pots represent the subplot randomization of the four dosages (0, 0.5 g/L, 1g/L, 1.5g/L) of each isolate applied to pots.

**CHRYSANTHEMUM CROWN AND ROOT ROT BIOASSAY**

Treatments consisted of ten different infestations of *R. solani* (nine isolates and one control) applied as a rice grain inoculum and four substrate treatments (70:30 peatlite, peat:PTS WF 70/30, 80/20, 90/10) for a total of 40 treatments (Table 2.2) arranged in a completely randomized design (CRD) on one table in the greenhouse with a single experimental unit of one pot. WF substrate blends were made as detailed in Chapter 3 (3.2.2 PREPARATION OF SUBSTRATE TREATMENTS).
Table 2.2. *R. solani* isolate treatments tested in the root and crown rot assay and their respective anastomosis group (AG) (rDNA-ITS sequencing by Dr. James Woodhall and Lara Brown, University of Idaho).

<table>
<thead>
<tr>
<th>Treatment By <em>R. solani</em> Isolates</th>
<th>Isolate Name</th>
<th>AG Group</th>
<th>Isolated from</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RS-33</td>
<td>AG 2-1 HK clade</td>
<td>Radish</td>
</tr>
<tr>
<td>2</td>
<td>BW-R</td>
<td>AG 1-IC</td>
<td>Radish</td>
</tr>
<tr>
<td>3</td>
<td>AG 2-1</td>
<td>AG 2-1</td>
<td>Potato</td>
</tr>
<tr>
<td>4</td>
<td>AG1-1B</td>
<td>AG1-1B</td>
<td>Bean</td>
</tr>
<tr>
<td>5</td>
<td>AG 4-HG I</td>
<td>AG 4-HG I</td>
<td>Bean</td>
</tr>
<tr>
<td>6</td>
<td>AG 4-HG II</td>
<td>AG 4-HG II</td>
<td>Bean</td>
</tr>
<tr>
<td>7</td>
<td>AG 5</td>
<td>AG 5</td>
<td>Bean</td>
</tr>
<tr>
<td>8</td>
<td>AG E</td>
<td>AG E</td>
<td>Wheat</td>
</tr>
<tr>
<td>9</td>
<td>AG F</td>
<td>AG F</td>
<td>Bean</td>
</tr>
<tr>
<td>10</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

Figure 2.3. Diagram of table of dripline fertigated crops arranged in a CRD experimental design randomized by *R. solani* treatment (numbers 1-10) (Table 2.2)
2.2.2 PATHOGEN PREPARATION

Sclerotia of isolates were stored in -80°C freezer and grown on 39% Potato Dextrose Agar (PDA) media plates (39 g commercial PDA powder VWR and 1L DO water) (1-2 sclerotia per plate) one to two weeks before preparation of the inoculum. The pathogen was prepared as a rice grain inoculum as described by Owen (2019). 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 once for 60 min at 121°C and 15 psi. Autoclaved rice grains were then inoculated with 10 PDA plugs taken from one to two-week old R. solani cultures using a size 7 mm cork borer. Beakers were covered with aluminum, wrapped with parafilm, and placed in a cabinet at room temperature for 6-7 days. One week later, colonized rice grains were pulverized with a mortar and pestle to produce inoculum of uniform size (~1-3mm). This uniform size was initially determined using a 2mm mesh cheese cloth to establish a standard, and then was visually determined for subsequent inoculum preparations.

To confirm that symptoms observed were caused by R. solani, pathogen identity from infested radish was confirmed by root plating on acidified potato dextrose agar (APDA) media plates (amended with concentrated lactic acid 85%). Blackened roots were removed with sterilized tweezers and rinsed three times: twice in petri dishes of sterile RO water and once in dish containing a 10% bleach solution. Root material in the “transition zone” (between rotted & healthy root) was taken to minimize the risk of accidentally isolating secondary pathogens. Four root pieces (~2-5mm in length) were placed into four different quadrants on a dish of APDA. Dishes were parafilmed and incubated at room temperature and after 2-5 days and if roots showed fungal growth, colonized APDA disc samples were sub-cultured onto new APDA or
PDA. Samples were examined morphologically on agar plates as well as with light microscopy to identify the diagnostic right-angle branching hyphae (Figure 2.1).

2.2.3 PLANT PREPARATION AND EXPERIMENTAL SET-UP

RADISH BIOASSAY

Pots were filled in the following order: 1) substrate, 2) water, 3) radish seed, 4) *R. solani* inoculum was drizzled on top of pots, and then 5) randomized trays placed in growth chamber for one week, three trays randomly assigned to each shelf (block) (Figure 2.2).

1) **Pot Filling:** The prepared substrate treatments were added to fill each 11 cm pot three-quarters full (and 4.5in (~11cm) STD Thermo, The HC Companies, Boise, ID). Three pots per substrate treatment were weighed to get the average dry weight and this average weight was used to fill all other pots for a given treatment. Eight pots were placed on 27.9 x 54.3 cm standard 1020 Flat Black trays (The HC Companies, Boise, ID).

2) **Watering:** All pots were hand-watered once by graduated cylinder at the time of seeding with ~150-200 mL of tap water.

3) **Plant material:** Thirty-two Organic Early Scarlet Globe (*Raphanus sativus*) seeds were sown into each pot by hand using tweezers.

4) **Inoculation of Pots:** Each pot was infested with one of three different isolates (BW-R, BW-P, and RS-33) at four different rice dosage treatments (0, 0.5 g/L, 1g/L, 1.5g/L) (g rice per L of soil) for eight pots n=2 per dose concentration*isolate.

5) **Growth Chamber:** Pots were incubated in a walk-in growth chamber environment set at 22 to 24°C, 67-70% relative humidity under 24-h illumination by T5 120v Florescent lights (fluence rate of 55-65 umol m⁻² s⁻¹) for one week.
**CHRYSANTHEMUM BIOASSAY**

1) **Establishment of unrooted cuttings in propagation house and transplant**

Unrooted cuttings (URCs) of ‘Breeze Yellow’ Chrysanthemum (*Chrysanthemum x morifolium*) (Dummen Orange, Columbus OH) were dipped in powder rooting hormone (indole-3-butyric acid IBA; Hormodin®1, OHP, Inc., Bluffton, SC), sprayed with the organo-silicone foliar spray Capsil® at a rate of 4 mL/gal (Aquatrols, Paulsboro, NJ) to ensure uniform spreading of rooting hormone and water and inserted into 1.0 in³ Oasis Cubes (Smithers Oasis CO, Walterboro, SC) (Figure 2.4). URCs were then placed under natural light for eight days and misted at an increasingly longer misting interval to allow roots to establish. URCs were taken off of mist but kept in the humidity of the propagation house for another seven days and overhead watered twice daily at a rate of 17-4-17 (Pure Water LX, JR Peters Inc., PA.) at 100 ppm N to reduce the risk of transplant shock. The Pure Water LX (JR Peters Inc., PA) contained 3.73% ammoniacal nitrogen and 13.27% nitrate nitrogen. Approximately two weeks after receiving chrysanthemum URCs, roots were established and cuttings were transplanted to into 15 cm tall Euro Black Pots (The HC Companies, Twinsburg, OH) with their respective WF:peat or perlite:peat substrate treatment containing an average 250-270g of substrate. Transplants were overhead fertilized for two days at 17-4-17 (Pure Water LX, JR Peters Inc., PA.) at 300 ppm for to adjust for young leaf chlorosis. Plants were then switched to driplines and fertigated at the same rate for three days. Fertigation was then lowered to 17-4-17 (Pure Water LX, JR Peters Inc., PA.) 150 ppm (balanced nitrate-ammonical fertilizer) at rate of 40 mL/min four times a day.
Figure 2.4 A-F. Unrooted chrysanthemum cuttings (URCs) of Chrysanthemum Breeze Yellow (*Chrysanthemum* spp.) (Dummen Orange, Columbus OH) (A). URCs were dipped in rooting hormone (B) and inserted into Oasis cubes, creating a new hole (C and D). Each tray was then sprayed with Capsil® (4mL/gal) (E) and immediately placed under mist in the propagation house (F).
About a week later, to help raise and then maintain pH, pots were switched to a calcium nitrate fertilizer containing 2.88% ammoniacal nitrogen and 12.12% nitrate nitrogen (Poinsettia FeED, JR Peters Inc, PA, USA, 15-4-15 at 150 ppm) at the same irrigation rate. This rate was then adjusted in accordance with plant development. Approximately one week after transplant, mums were also given either a “hard” pinch or “soft” pinch by cutting off the top growth of each plant to maintain uniform height and promote the vertical growth of plants.

Figure 2.5 A-D. Rooted cuttings (A-C) prior to transplant (D) chrysanthemum cuttings after transplanting into their respective substrate treatments. Plants were transplanted 14 days post-sticking. The following day each plant was raised about an inch and substrate was added to adjust for shrinkage.
2) **Greenhouse Environmental Conditions**

Chrysanthemums were grown in the UNH Macfarlene greenhouse from April 1\textsuperscript{st} through June 25\textsuperscript{th}, 2021 and May 31\textsuperscript{st} through June 14\textsuperscript{th}, 2021. For the mums grown in April, temperature was kept at 70°F (~21°C) during the day, 65°F (18.3°C) at night with the light window 5am-7pm (5:00-19:00), lights turning on when light dropped below 150wm\(^{-2}\) (~315 \(\text{umol m}^{-2}\text{s}^{-1}\)). However, as the days lengthened, these controls were removed. Average greenhouse temperatures were 72.0°F (~22.2°C), average light was 374 \(\text{umol m}^{-2}\text{s}^{-1}\), and average relative humidity 52.7%. Once plants were infested with *R. solani*, a humidity chamber was constructed using growth tunnel plastic (SunMaster, FarmTek) placed over a PVC-pipe cage to increase relative humidity (Figure 2.6). Under plastic relative humidity was an average 72.5% and average temperature 81.5°F (27.5°C). For the ‘young’ mum experiments, humidity chambers were not and average temperature was 76.8°F (~24.9°C), average light was 348 \(\text{umol m}^{-2}\text{s}^{-1}\), and average relative humidity 54.80%.

![Image](image.jpg)

**Figure 2.6. Humidity chamber** constructed of a PVC-pipe cage covered with growth tunnel plastic to increase humidity and temperature.
3) Irrigation, pH & EC monitoring, and Disease Management

Four days after transplant, dripline fertigation of pots began at a rate 17-4-17 (Pure Water LX, JR Peters Inc., PA.) 150 ppm at rate of 40 mL/min on four one-minute cycles a day for a total of 160mL. This rate was progressively tweaked to adjust for plant growth and increasingly hot, humid, and bright conditions. Specifically, plants were fertigated for the majority of their production cycle (April 24-June 2) at a rate of 40 mL/min on 2.5-minute irrigation intervals six times a day (8:00, 12, 15, 18, 20) for a daily total of 500mL water per pot per day.

Optimal substrate pH for chrysanthemum growth is 5.5-6.3 (Whipker et al., 2020). At the time of transplant, substrate pHs were slightly lower than this range and for peatlite substrates, pH dropped significantly when fertilizer was applied (Table 2.3). pH and EC for substrate blends were recorded weekly by soil media extract (SME; Warncke, 1986; University of Minnesota, 2020). One week after transplant, pH and EC of potted chrysanthemums were measured using the pour-thru method (LeBude and Bilderback, 2009). Due to timetable constraints, only one corrective action was taken, with a change from a balanced nitrate-ammoniacal fertilizer (17-4-17 (Pure Water LX, JR Peters Inc., PA.) at 150ppm) to calcium nitrate fertilizer (Poinsettia FeED, JR Peters Inc, PA, USA, 15-4-15 at 150 ppm). However, a flowable lime drench, a quick method to raise pH, was not used and pH remained substantially lower than the optimal range.

Table 2.3. pH and EC measurements of pots immediately prior to R. solani infestation. Pour-thru method used.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>pH</th>
<th>EC (mS/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peatlite 70/30</td>
<td>3.19</td>
<td>1.92</td>
</tr>
<tr>
<td>70/30 WF</td>
<td>5.03</td>
<td>1.81</td>
</tr>
<tr>
<td>80/20 WF</td>
<td>5.28</td>
<td>2.36</td>
</tr>
<tr>
<td>90/10 WF</td>
<td>4.97</td>
<td>1.81</td>
</tr>
</tbody>
</table>
A mix of insect biocontrols were sprinkled weekly on top of all young plants and later on applied as Swirskii-Breeding-System sachets (*Amblyseius swirskii*) (BioBest, Westerlo, Belgium) hung on mature plants. Both sachets and sprinkled biocontrols contained the following: *Amblyseius cucumeris* and *Orius insidiosus* (thrips control), *Phytoseiulus persimilis* (spider mite, whitefly control), *Encarsia Formosa* and *Eretmocerus eremicus* (whitefly control), and *Aphidius colemani* (aphid control). Yellow sticky cards (BASF Corporation, Research Triangle Park, NC) were placed in the greenhouse, one per table at plant level, to monitor pest populations. Additionally, plants were treated every two weeks with preventative soil drench applications of *Steinernema feltiae* (150,000-200,000 nematodes per plant) (BioBest, Westerlo, Belgium) to control fungus gnats.

4) **Pathogen infestation**

Rice inoculum was added at a rate of 1.3g/L to chrysanthemums (age three weeks post-transplant). The *R. solani* rice inoculum was pulverized and buried about one cm at four cardinal pots on the surface of each pot (Figure 2.7). Because each pot is ~1.56 liters in volume, 2.0 of *R. solani* rice grains were added to each pot. This protocol combines the methods of Owen et al. (2019) and Krause et al. (2001) (Appendix B).

![Figure 2.7. A total of 1.6 grams of pulverized rice inoculum (application rate 1g rice/L of soil) were added to the surface of the media about an inch from the stem of the chrysanthemum at the four cardinal directions. Small divots (~1 cm deep) were dug at each point point and rice was covered lightly with media.](image-url)
2.2.4 DATA COLLECTION
FOR RADISH DAMPING-OFF ASSAY

**Disease Assessment:** Damping-off severity was determined 7 days after seeding based on a disease severity scale developed by Krause et al. (2001) (Figure 2.8) 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, and 5 = pre-emergence damping-off. A more detailed description of the rating scale is shown in Table 2.4.

**Figure 2.8.** Damping-off disease scale developed from Krause et al. (2001).

**Figure 2.9.** Range of “4” or post-emergent damping-off. A rating of 4 was characterized by blackened stem and girdling of greater than 50%.

For each pot, the 32 seeds were assessed on the categorical scale (Figure 2.8) and then averaged to calculate the mean disease severity per pot.
Table 2.4. Damping-off disease scale (1-5) on radish developed in collaboration with M. Krause.

<table>
<thead>
<tr>
<th>Scale</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1=healthy roots</td>
<td>• No apparent symptoms of disease</td>
</tr>
</tbody>
</table>
| 2=mild disease severity | • Distinct lesions or “bite” marks, but NO girdling.  
• These are superficial stem/hypocotyl lesions only; surface or hairline stem/hypocotyl lesions that do not cut/sink into the cortex  
• The plant can overcome these lesions |
| 3=moderate disease severity | • Deep or major lesions  
• Girdling but no more than 50%  
• Typically, one large or multiple non-lethal lesions that do not kill the plant after 7 or 8 days after planting. |
| 4=post-emergence damping off | • Blackened wiry stem  
• Lethal stem girdling (>50% stem girdling), hypocotyl (stem) rot or root rot that kills plant before rating or within 1 day of rating  
• Oftentimes blackened or non-existent stem, wrinkled leaves, dead plants  
• Plant germinates as normal, but *R. solani* strikes it quickly and kills it after emergence (progressing through category 2 through 3 very quickly) |
| 5=pre-emergent damping-off | • Seeds do not germinate or germinating plant is killed before emergence = no emergence |

Fives were calculated as the difference between the #seeds sown and the number of 1-4s recorded.
FOR CHRYSANTHEMUM CROWN AND ROOT ROT

**Disease Assessment:** Disease progress was checked weekly and the onset of disease for each isolate was recorded. Five weeks after pathogen infestation, an early disease assessment was made and seven weeks after pathogen infestation, final disease assessment was performed, with crown and root rot disease severity were measured according to the scale adapted from Krause et al. (2001) with 1-6 in which: 1 = symptomless, 2 = mild root rot OR crown rot, 3 = mild root rot AND mild crown rot, 4 = severe crown rot OR severe root rot, 5 = severe crown rot AND severe root rot, and 6 = dead plant
Table 2.5. Crown and root rot disease scale (1-6) on chrysanthemum developed with M. Krause.

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1=</td>
<td><strong>symptomless</strong></td>
</tr>
<tr>
<td></td>
<td>• Healthy plant</td>
</tr>
<tr>
<td></td>
<td>• No observable root or crown rot</td>
</tr>
</tbody>
</table>

| 2=    | **mild crown rot OR mild root rot**                                                              |
|       | • Image depicts mild root rot with no crown rot                                                  |

| 3=    | **mild crown AND mild root rot**                                                                 |

| 4=    | **severe crown rot OR severe root rot**                                                           |

| 5=    | **severe crown rot AND severe root rot**                                                          |

| 6=    | **dead plant**                                                                                 |
### 2.2.5 DATA ANALYSIS

All analyses were performed in R version 3.6.2 (2019-12-12) --"Dark and Stormy Night” ©2019. For the radish bioassay, analysis of variance (ANOVA) was used to determine the effects of *R. solani* isolate and inoculum dose on damping-off severity. Mean disease severity which ranged in discrete categories from 1 to 5, was transformed to $R^* = (R^{1.5} - 1)/1.5$ (Krause et al., 2001) to obtain a linear scale and meet the assumptions of ANOVA: normality of residuals, homogeneity of variances, and block-factor additivity. The mix treatment factors included isolate treatment (RS-33, BW-R, and BW-P) and dose (rice dosage: 0, 0.5 g/L, 1g/L, 1.5g/L). Factorial ANOVA and Tukey HSD were performed to compare differences in transformed mean disease severity and to determine the isolate*dosage treatment that would induce medium to high disease severity or about ‘3 to 4’ on the disease scale (Figure 2.8, Table 2.5).

For the chrysanthemum bioassay, analysis of variance (ANOVA) was also used to determine the effects of *R. solani* isolate and inoculum dose on damping-off severity. Mean disease severity which ranged in discrete categories from 1 to 6, was transformed to $R^* = (R^{1.5} - 1)/1.5$ (Krause et al., 2001) to obtain a linear scale and meet the assumptions of ANOVA: normality of residuals, homogeneity of variances, and block-factor additivity. The mix treatment factors included isolate treatment (10) and substrate (4). Factorial ANOVA and Tukey HSD were performed to compare differences in transformed mean disease severity.
2.3 Results and Discussion

**ISOLATE CHARACTERIZATION**

Figure 2.10 A-D. Culture morphology of nine isolates of *Rhizoctonia solani* grown PDA in cabinet at room temperature (21-24°C) for 10 weeks. (A) BW-R is AG 1-IC. (B) AG 1-IB and (C) RS-33 is AG 2-1 HK clade, and (D) AG 2-1. BW-R and RS-33 were isolated from radish, AG 2-1 from potato, and AG 1-IB from bean. AG-E was isolated from wheat and AG 4 HG II, AG 4 HG I, AG 5, and AG F were isolated from a variety of bean plants (Fabaceae). AG 2-1 HK clade has slightly different culture morphology at different temps (at 30º Celsius mycelia growth is faster than typical AG 2-1, but at 25 and 35 ºC is similar to other AG 2-1. For detailed review of distinctions between AG 2-1 HK clade and general AG 2-1 see Misawa et al. (2018)).
Figure 2.11 A-D. Isolates of R. solani under compound microscope. (A) isolate BW-R (AG 1-IC) and (B) AG 1-IB with characteristic right-angled branching, with hypha constricted at branching point. Both are multinucleate isolates with several nuclei observable in an individual cell. (C) RS-33 (AG 2-1 HK clade) under scope, also exhibiting hypha constricted at branching point, but with less distinct right-angled branching. (D) AG 2-1. Both AG 2-1 isolates are also multinucleate. AG were identified by Dr. James Woodhall and Lara Brown (University of Idaho) using rDNA-ITS techniques. Photos taken by Isobel Michaud using Microscope Digital Camera: Olympus LC30 (Olympus Soft Imaging Solutions, Munster, Germany).
**RADISH DAMPING-OFF BIOASSAY**

Isolate BW-P was the only isolate that was not pathogenic on radish; disease severity of radishes grown in pots infested with BW-P was similar to disease severity of the non-infested control plants (ANOVA p=0.744, F_{2,33}= 0.344) and was not analyzed further. BW-P never formed sclerotia, and so it could not be stored in a long-term -80°C freezer. Therefore, it was the only isolate in which the original culture was used to prepare the rice inoculum and it could have lost its virulence after being stored on PDA for 4 months (due to delays caused by the COVID-19 epidemic).

Another full factorial ANOVA was performed for only isolates BW-R and RS-33 (Table 2.6). There was also no significant effect of blocking by shelf (p≥0.112) thus subsequent growth chamber experiments were not blocked by shelf. For BW-R and RS-33, there was a significant interaction effect of dose and isolate (ANOVA p=5.03x10^{-9}, F_{3, 118}=16.52). Simple effects ANOVAs were performed and a significant dose effect was found for each isolate (BW-R: ANOVA p= 2.2e-16, F_{3, 60}= 730.42 and RS-33 ANOVA p= 2.2e-16, F_{3, 60}= 362.95).

**Table 2.6.** Full factorial ANOVA performed to assess main effect of *R. solani* isolate (BW-R and RS-33) and subplot effect of dose on radish damping-off disease severity in a split-plot RCBD. Given significant interaction effect of dose and isolate (ANOVA p=5.03x10^{-9}, F_{3, 118}=16.52) simple effects ANOVAs were then performed.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block (Shelf)</td>
<td>1</td>
<td>1.144</td>
<td>1.144</td>
<td>31.71</td>
<td>0.1119</td>
</tr>
<tr>
<td><em>R. solani isolate</em></td>
<td>1</td>
<td>26.254</td>
<td>26.254</td>
<td>728.12</td>
<td>0.0236</td>
</tr>
<tr>
<td>Residuals*</td>
<td>1</td>
<td>0.036</td>
<td>0.036</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Split-plot analysis uses Block:Isolate as error term for main effect*
Biopesticides are best used proactively against moderate to intermediate levels of disease as part of an IPM program along with best management practices (e.g. sanitation, aeration and spacing) and in rotation with both other biopesticides and traditional synthetic pesticides (Glare et al. 2012; Seiber et al., 2014). Because biopesticides are ineffective at high levels of disease (Glare et al. 2012), one of the main aims of this study was to determine an appropriate dose of *R. solani* rice inoculum to create medium levels (rating ‘3’ to ‘4’) of damping-off disease severity. While we found that BW-P (AG unknown) was not virulent on radish, BW-R (AG-1 IC) and RS-33 (AG 2-1 HK clade) were virulent. Both isolates applied at 0.5 g/L, resulted in disease severity in the targeted 3-4 range on the 1-5 scale. Additionally, pots infested with 0.5 g/L contained plants with significantly lower disease than at doses of 1 and 1.5 g/L and significantly higher disease than the control. This dose of 0.5g/L was also used by Owen and colleagues (Owen et al., 2019). However, because BW-R caused higher disease severity (4.48) in radish than RS-33 (3.67), Isolate RS-33 was chosen for subsequent radish damping-off studies (Table 2.7).

**Table 2.7. Average Damping-off Disease severity (1-5) by rice inoculum dose for three isolates of *R. solani*.** Letters indicate significant differences for two separate Tukeys performed (Tukey HSDs a=0.05, n=16 MSD\textsubscript{BW-R}= 0.3819228, MSD\textsubscript{RS-33}= 0.4553317).

<table>
<thead>
<tr>
<th>Dose (g/L)</th>
<th>BW-R Average Disease (Group)</th>
<th>RS-33 Average Disease (Group)</th>
<th>BW-P Average Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>1.43±0.05 a</td>
<td>1.30±0.04 a</td>
<td>1.37±0.05</td>
</tr>
<tr>
<td>0.5</td>
<td>4.48±0.07 b</td>
<td>3.67±0.04 b</td>
<td>1.39±0.09</td>
</tr>
<tr>
<td>1</td>
<td>4.77±0.03 c</td>
<td>4.22±0.07 c</td>
<td>1.58±0.11</td>
</tr>
<tr>
<td>1.5</td>
<td>4.63±0.05 bc</td>
<td>4.31±0.08 c</td>
<td>1.49±0.10</td>
</tr>
</tbody>
</table>

**CHrysanthemum Crown and Root Rot BIOASSAY**

Chrysanthemums infested with isolate AG 4 HG-I were the earliest to exhibit signs of disease at three weeks post-infestation (Figure 2.12 A) and for this reason the isolate was chosen for subsequent chrysanthemum studies (Chapter Four). Of the other eight *R. solani* isolates,
chrysanthemums infested with seven of them (RS-33, BW-R, AG 2-1, AG1 IB, AG 4 HG-I, AG 4 HG-II, AG 5 and AG E) also exhibited signs of disease, albeit at five weeks post-infestation. AG F was the only isolate that did not appear to cause disease after five weeks.

Seven weeks post infestation a final disease assessment was performed and isolates AG 4 HG-I, AG 4 HG-II, and AG 5, and BW-R were found to cause significantly higher crown/root rot of chrysanthemum than the control and isolate AG F (Tukey HSD $a=0.05$, $n=4$, MSD=2.757467). AG-4 HG-I caused the highest level of disease (Table 2.9). Furthermore, while disease symptoms were observed for chrysanthemums infected with isolates AG E, RS-33 (AG 2-1 HK clade), AG 1 I-B, AG 2-1, and AG F, disease severity was not greater than the control (Table 2.9).

In terms of substrate treatment, chrysanthemum crown and root rot caused by R. solani was significantly greater in mums grown in peatlite compared to the three WFs (Tukey HSD $a=0.05$, $n=10$, MSD= 1.387482) (Table 2.9). This is most likely attributed to the extremely low pH of peatlite (Table 2.3) as was found in previous work where in vitro R. solani mycelial (AG 2-2) growth was optimal at pH 4.5-5.5 and 6.0-6.5, but declined as pH dropped or rose (Watanbe et al., 2011). However, the significant disease caused by isolates AG-4 HGI, AG-4 HG-II, and AG-5, and BW-R in peatlite pots suggests that R. solani can cause infection despite low pH. Due to low sample size ($n=1$ per isolate*substrate), the interaction between R. solani isolate and substrate treatment could not be determined (Table 2.8).
Table 2.8. Factorial ANOVA performed to assess the effect of R. solani isolate and substrate treatment on crown and root rot disease severity in chrysanthemum in a completely randomized design (n=1 per treatment).

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. solani isolate</td>
<td>9</td>
<td>36.4</td>
<td>4.0444</td>
<td>9.5789</td>
<td>2.173e-06</td>
</tr>
<tr>
<td>Substrate</td>
<td>3</td>
<td>16.1</td>
<td>5.3667</td>
<td>12.7105</td>
<td>2.294e-05</td>
</tr>
<tr>
<td>Residuals</td>
<td>27</td>
<td>11.4</td>
<td>0.4222</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.9. Average disease±SE by R. solani isolate and substrate. Disease assessment was performed seven weeks post R. solani infestation. Letters indicate significant differences for separate analyses (Tukey HSD a=0.05, n=4, MSD_{isolate}=2.757467 and Tukey HSD_{substrate} a=0.05, n=10, MSD= 1.387482).

<table>
<thead>
<tr>
<th>R. solani isolates</th>
<th>Average Disease (1-6)</th>
<th>Group</th>
<th>Substrate</th>
<th>Average Disease (1-6)</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG-4 HG-I</td>
<td>4.75±0.25</td>
<td>d</td>
<td>Peatlite</td>
<td>4.1±0.44</td>
<td>b</td>
</tr>
<tr>
<td>AG-4 HG-II</td>
<td>4.00±0.58</td>
<td>cd</td>
<td>70/30 WF</td>
<td>3.0±0.58</td>
<td>a</td>
</tr>
<tr>
<td>AG-5</td>
<td>4.00±0.41</td>
<td>cd</td>
<td>80/20 WF</td>
<td>2.5±0.68</td>
<td>a</td>
</tr>
<tr>
<td>BW-R</td>
<td>3.25±0.48</td>
<td>bc</td>
<td>90/10 WF</td>
<td>2.6±0.59</td>
<td>a</td>
</tr>
<tr>
<td>AG-E</td>
<td>3.00±0.41</td>
<td>abc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RS-33</td>
<td>2.75±0.48</td>
<td>abc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AG-1 1-B</td>
<td>2.75±0.48</td>
<td>abc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AG 2-1</td>
<td>2.50±0.65</td>
<td>abc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AG-F</td>
<td>2.25±0.63</td>
<td>ab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.25±0.25</td>
<td>a</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2.12. (A) R. solani AG 4 HG-I “bite marks” observed on stems three weeks post R. solani infestation, (B) disease progression one and (C) two weeks later. (D) Healthy control stem at the same age as the infested (A).
2.4 Conclusion

RADISH DAMPING-OFF BIOASSAY

In the first study, three isolates were assessed for 1) their ability to cause damping-off in radish and 2) a concentration of rice inoculum to induce a medium level of disease (‘3’ to ‘4’ on the damping-off scale). Determining this level was essential for determining an appropriate dose of *R. solani* that for subsequent studies (CHAPTERS THREE AND FOUR). We found that BW-P (AG unknown) was not virulent on radish, however BW-R (AG 1-IC and RS-33 (AG 2-1 HK clade)) were virulent. Furthermore, for both BW-R and RS-33, a rice inoculum dose of 0.5 grams of rice per liter of soil was determined to cause about moderate (range of 3-4) level of damping-off severity, a dosage rate used in previous studies (Owen et al. 2019). However, BW-R was a more aggressive isolate and did have higher overall disease than isolate RS-33.

CHRYSANTHEMUM CROWN AND ROOT ROT BIOASSAY

In the second study, the objective was to evaluate the virulence of nine isolates of *R. solani* on chrysanthemum and to develop a root and crown rot scale. Of the nine isolates, only four (AG-4 HG-I, AG-4 HG-II, AG-5, BW-R) caused significantly higher disease than that observed on non-infested control chrysanthemums. AG-4 HG-I caused disease the fastest, at three weeks post-infestation, compared to five weeks for all other isolates. AG-4 HG-I was selected for chrysanthemum studies documented in Chapter Four.
CHAPTER 3
THE EFFECT OF WOOD FIBER PROCESSING AND BLEND RATIO ON DAMPING-OFF DISEASE SEVERITY CAUSED BY *RHIZOCTONIA SOLANI*

3.1 Introduction

Soilless substrates are essential for the greenhouse horticulture industry, providing growers with consistent, high quality growing media for efficient and intensive production (Savvas, 2003; Robbins et al., 2011a; Barrett et al., 2016). Soilless substrates help mitigate soil-borne diseases, improve soil fertility, and provide the right balance of saturation and water holding capacity (Barrett et al., 2016; Savvas and Gruda, 2018). These advantages, along with a global rise in greenhouse production (Sparks, 2020; Davis and Lucier, 2021), have contributed to a projected 240% increase in the demand for soilless substrates by 2050 (Blok, 2021). Historically, the need for organic (carbon-based or non-synthetic) soilless substrates has been filled by sphagnum peat moss (Bek et al., 2020), but that has dramatically changed in the last two decades. For example, grower use of peat in the UK horticulture industry has been cut in half—from over ninety-percent of UK soilless substrates in 2000 to 50% today (Bek et al., 2020). Peat is generally amended with perlite (‘peatlite’) and remains the dominant carbon-containing soilless substrate used by ornamental growers (Owen et al., 2016; Schmilewski, 2017; Bek et al., 2020; Blok, 2021). However, peatlite alone cannot meet the rising demand, particularly considering questions of non-renewable harvest and rising costs for both peat and perlite (Robbins et al.; 2011a; Barrett et al. 2016; Gruda et al., 2016b). A wide variety of soilless substrates have entered the market to help fill the gap: coconut coir, followed by hardwood bark and pine bark, are three peat complements that have dominated in recent decades (Abad et al., 2005; Robbins et al., 2011a; Altland and Krause, 2012; Barrett et al., 2016; Altland et al., 2018). First developed as soilless substrates in the late 1970’s and 80’s in Germany (Schmilewski, 2008), wood fibers (WF), a by-
product of the timber industry, have received renewed interest by growers and the substrate industry in the last decade (Jackson, 2019) due to worldwide availability, a reduced carbon footprint compared to peat and other substrate materials, and low production costs (Gumy, 2001; Jackson et al., 2009a and b; Jackson, 2019; Owen et al., 2017; Owen et al., 2020; Durand et al., 2021). Although the term “wood fiber” still does not have a standard definition (Barrett et al., 2016; Jackson, 2018a and b), wood fibers are usually made from conifers, e.g. loblolly pine (Pinus taeda) in the Eastern US and Scots pine (Pinus sylvestris) in Europe and have lower phytotoxic molecule content compared to hardwood tree species (Jackson, 2018a and 2018b). WF substrates have several advantages including being capable of receiving OMRI-listed certification, locally available in many parts of the country and world to both substrate manufacturers and growers, affordable, recyclable, and disposable (Jackson, 2018b). However, WFs foster unique microbial communities and the effects of these associated microbes on disease suppressiveness are unknown.

Wood fibers (WF) are blended with peat at different percentages, generally ranging from 10 to 40% by volume (Jackson and Bartley, 2017; Drotleff, 2018). The main advantage of WF over hardwood and pine barks (also by-products of the forest industry) is economic. Specifically, WFs can be harvested as the whole tree (WT) and then processed. WT harvest eliminates dependency on the forest products industry where barks are also used as fuel, in landscaping, and for biochemical extractions (Altland and Krause, 2012). For WF, as with other wood components and barks (Jackson et al., 2009a), N immobilization is a concern; however, several studies have provided the industry with best practices for managing plant nutrition when using wood (Owen et al., 2016; Harris et al., 2019). Other recent studies have focused on the characterization and modeling of WF specific physical and hydraulic properties (Fields et al., 2014; Jackson,
ongoing) and the use of lime for pH adjustment (Owen et al., 2020). However, little is known about the effects of WFs on the general (natural) suppressiveness, or the intrinsic ability of a soil or substrate to suppress soilborne plant pathogens and promote beneficial plant microbes.

**Natural disease suppression is driven by microbial competition and varies by substrate**

Every soil or organic substrate has the natural ability to suppress pathogens, known as general or natural disease suppressiveness (Whipps, 2001), but substrates differ in their natural disease suppressiveness (Nelson et al., 1983; Chen et al., 1988; Hoitink and Boehm, 1999; Krause et al., 2001; Raviv, 2011; 2013). In essence, organic substrates are living systems within which microbial activity—with both positive and negative effects—can occur (Bonanomi et al., 2018; Raviv et al., 2019). Microbial activity can result in physical instability (and therefore breakdown) of the substrate and/or N immobilization (e.g. Jackson et al. 2009a; Owen et al., 2016; Harris et al., 2019) and disease conduciveness (Raviv et al., 2019). However, the main positive effects are the release of nutrient ions from slow-release fertilizers and natural disease suppression (Raviv et al., 2019). Microbial activity that contributes to either natural disease suppressiveness or conduciveness is influenced by several interrelated factors: the microbial degradation of a substrate’s organic components, competition between microbes for space and nutrients, specific microbial functions, and total microbial biomass (Hoitink and Boehm 1999; Raviv et al., 2019). Overall, high levels of microbial activity are correlated with lower pathogen populations, particularly for pathogens that are poor soil competitors, such as *Pythium* spp. (Chen et al., 1988; Whipps et al., 1991; Hoitink and Boehm 1999; Postma et al., 2000).

Substrates can be characterized by their natural disease suppression or conduciveness (Raviv et al., 2019). For example, peat has inherent low decomposition and microbial colonization rates and is thus considered biologically stable compared to other substrates. Both
low rates are thought to contribute to peat’s inability to buffer against invading pathogens and is therefore considered in many studies as a “disease conducive control” to the development of soil-borne plant pathogens (Chen et al., 1988; Boehm et al., 1997; Borrero et al., 2004; Blok et al., 2017). Sphagnum peat mosses do not possess lignin, but contain “lignin-like” polymers and “pectin-like” substances both linked to recalcitrance, resulting in the low decomposition rate (Hajek et al., 2011; Rydin and Jeglum, 2013). While peat it is generally considered disease conducive (Chen et al., 1988; Boehm et al., 1997; Borrero et al., 2004), conducive or suppressiveness varies by the type of peat (Boehm and Hoitink, 1992). For example, Boehm and Hoitink (1992) found substrate blends containing dark peat (H4 on von Post scale) were naturally conducive to Pythium root rot in poinsettia while light peat mixes (H2-3 on von Post scale) were naturally suppressive, but in contrast Krause et al. (2001) found damping-off in radish caused by Rhizoctonia solani to be higher in light peats mixes than dark ones. Peat has also been shown to be more disease conducive than other substrates—for example, Fusarium wilt of tomato in peat compared to various composts (Borrero et al., 2004). These differences in peat suppressiveness-conduciveness are linked to carbon availability for microbial degradation, as well as peat’s phenolic antimicrobial and humic substances which are highly resistant to microbial breakdown (Rydin and Jeglum, 2013).

It is difficult to draw clear patterns for specific substrates and their respective natural disease suppression abilities (Parnell et al., 2016; Bonanomi et al., 2007, 2018; Raviv et al., 2019). For example, a meta-analysis by Bonanomi et al. (2007) found that organic (carbon-based) substrate amendments were suppressive in 45% of cases, but had no effect in 35% of cases, and had a significant increase in disease incidence in 20% of the tests, suggesting that the relationship between the organic matter available in a substrate and its impact on natural disease
suppression is complex, fluid, and involves an intricate set of mechanisms (Hoitink and Boehm, 1999; Parnell et al., 2016; Bonanomi et al., 2018; Raviv et al., 2019). This is in part because substrates differ with respect to their C:N ratio and, more importantly, substrates vary in the availability of simple and complex forms of carbon (C), a quantity not accounted for by the C:N alone (Bonanomi et al., 2018). In substrates, the type and amount of carbon present influence the composition and function of soil microbial communities, ultimately affecting a substrate’s natural disease suppressiveness (Chen et al., 1988; Hoitink and Boehm, 1999; Krause et al., 2001; Parnell et al., 2016; Bonanomi et al., 2018).

In addition to differences across substrates in terms of natural disease suppression against a range of pathogens, substrates also vary in natural suppression of specific soil-borne pathogens (Bonanomi et al., 2007, 2018). For example, composts that consistently controlled *Pythium* and *Phytophthora* (oomycetes), did not control *Rhizoctonia solani* (fungi basidiomycete), which is a good soil competitor (Chung et al., 1988; Hoitink and Boehm, 1999; Krause et al., 2001). In essence, a substrate’s natural suppression against specific soil-borne pathogens is not “one-size-fits-all” (Chen et al., 1988; Whipps et al., 1991; Hoitink and Boehm 1999; Postma et al., 2000; Whipps, 2001).

Both general natural disease suppression and natural suppression (or conduciveness) to certain soil-borne pathogens are driven by the forms of carbon available in an organic substrate (reviewed in Hoitink and Boehm 1999 and Bonanomi et al., 2018). Much research in the 70s and 80s, primarily from the Hoitink lab, examined this, evaluating natural disease suppression in different substrates against different pathogens. Hoitink (1982) observed that composted pine bark suppressed *Pythium* and *Phytophthora* root rots but not *Rhizoctonia solani* damping-off. The Hoitink lab also found that hardwood bark (HB) mixed with 60% wood chips did not
suppress *Phytophthora* root rot of *Rhododendron* sp. in nurseries (Hoitink and Boehm, 1999). Similarly, Nelson et al. (1983) reported that aging of composted HB was more suppressive to *Rhizoctonia* damping-off than non-composted HB and slightly more suppressive than Canadian peat substrate. Differences in natural suppression against different soilborne pathogens is strongly driven by the carbon available in an organic substrate, because labile carbon sources, such as cellulose and hemicellulose, are most readily available for decomposition by microbes, and microbial activity influences natural disease suppression (Nelson et al., 1983; De la Cruz et al., 1993; Domeno et al., 2010; Bonanomi et al., 2018).

**Natural disease suppression in Wood Fiber substrates are unknown**

Wood substrates are known to be high in hemicellulose and cellulose content and have lower lignin content than peat, coir, and bark (Domeno et al., 2010). Based on what we know about carbon source and disease suppression, we might expect substrates containing wood to be naturally suppressive to some pathogens, such as *Pythium* and *Phytophthora* spp., but not as suppressive to others, such as *Rhizoctonia solani*, however, this remains understudied. Moreover, little is known about microbial communities in wood products, although researchers have demonstrated that wood is more microbially active than peat in part because of its high carbon: N ratio (Jackson et al., 2009a). For example, Montagne et al. (2015) found that wood fibers from pine and poplar had significantly greater fungal diversity and dominance compared to peat and coconut coir, with peat and coir having higher bacterial diversity. Nevertheless, the effects of these unique microbial communities associated with WFs on disease suppressiveness are unknown.

WF substrate components vary in two main ways: 1) sourcing and processing and 2) the percent (by volume) mixed into peat (generally 10 to 40% WF by volume) (Jackson and Bartley,
WFs have unique physiochemical properties compared to other soilless substrate materials; for example, WFs have lower EC and higher pH than peatlite, hardwood bark, pine bark, and coco coir (Owen et al., 2016; Barrett et al., 2016). WFs also typically have higher total porosity and air space and lower container capacity than both peat and perlite (Smith et al., 2019), and higher wettability than peat and barks (though lower than coco coir (Michel, 2015))—all traits that make WF an excellent substrate component to increase moisture retention (Smith et al., 2019; Durand et al., 2021).

Prior to use, raw WF materials (e.g. sapwood, heartwood, or both) undergo mechanical churning, pressure, and heating processes. There are three main processes of defibration, all involving mechanical break-up and thermochemical processing: 1) Twin-disc refined, 2) Single or double screw extruded (also called retruders), and 3) Hammer-milled (Gumy, 2001; Jackson et al., 2009b; Gaches et al., 2010; Jackson, 2018a). While the specific secondary processing varies by company, processing can quickly age and sterilize the wood (Gruda and Schnitzler, 2004; Maher et al., 2008; Schmilewski, 2008). Importantly, the friction used in the processing heats the wood materials 80-90°C or higher, which results in a pathogen and pest free, stable, consistent WF product (Gumy, 2001; Schmilewski, 2008; Jackson, 2018a). However, differences in WF processing results in WF products with visually distinct products (Figure 3.1) as well as differences in fiber size/thickness and physiochemical properties (Jackson et al., 2018 a and 2018b) which may affect plant growth, nutrient availability, and microbial activity.
Figure 3.1. The three differently processed Wood Fiber blends. (A) disc-refined, ForestGold (FG), (B) extruded, GreenFibre (GF), and (C) Hammer-milled product, Pine Tree Substrate (PTS).

While the characterization of physiochemical and hydraulic properties of WFs (i.e. by processing type and percent blend) is ongoing (Fields et al., 2014; Smith et al., 2019; Jackson, in press), it is well-established that a soilless substrate’s physical, chemical, and biological characteristics ultimately influence its natural disease suppressiveness (Nelson et al., 1983; Chen et al., 1988; Hoitink, 1999; Krause et al., 2001; Koohakan et al., 2004; Lundberg et al., 2012). WFs’ unique physiochemical properties may create conditions that affect plant growth, pest and pathogen dynamics, nutrient access, but such effects have not been well-studied. Examining WFs for disease suppression is of particular importance to growers, who depend on pathogen-free substrates, as issues of disease have slowed the adoption of other substrates. For example, coco coir, another organic substrate made from the by-product of the coconut industry, has been shown to have contamination issues (Abad et al., 2005, Robbins et al., 2011a), while some composts (Nobel and Roberts, 2003; 2004), peats (Bonanomi et al., 2007), and amounts of biochar (Frenkel et al., 2017) have been indicted as conducive to plant pathogens.

The overall objective of this study was to examine whether substrates that contain wood fiber are naturally disease conducive or disease suppressive compared to traditionally used peat moss substrates using a radish damping-off bioassay. We hypothesized that damping-off disease severity on plants grown in WF varying in processing and blend ratio would not differ, however
there would be differences in damping-off disease severity between plants grown in peat:WFs and peat-lite amended blends.

Specifically, we had two objectives:

1) To compare the effect of hammer-milled Pine Tree Substrate (PTS), disc-refined ForestGold (FG), and extruded GreenFibre (GF) WF components blended with peat on damping-off disease severity caused by *Rhizoctonia solani*

2) To evaluate the effect of peat wood-fiber blend ratio on damping-off disease severity caused by *R. solani* and aboveground biomass.

Our study was the first to examine the effects of WF-amended substrates on disease severity and plant growth. Results from this research will help growers select substrates and make informed management decisions. Insights into how wood fibers influence disease severity will also provide information to growing media producers to help them provide much needed guidance to growers seeking to integrate these new substrates into their operation and IPM programs.

### 3.2 Material and Methods

We chose to use *R. solani*-radish as a model (Krause et al., 2001) to study peat-wood fiber-pathogen interactions. This damping-off assay is high-throughput and quick, allowing us to survey several WF treatments. Because general suppression of pathogens can decline over time, short-term assays are best suited to study a substrate’s ability to support or suppress pathogens. Experiments were conducted to investigate the effects of (1) WF processing and (2) WF:peat blend ratios on damping-off disease severity. For the second objective, each of the three WF processing types (disc-refined {ForestGold, FG}, extruded {GreenFibre, GF}, hammer-milled {pine tree substrate, PTS}) were tested separately. The experiments were performed using the same experimental design and methodology as detailed in Chapter 2.
3.2.1 TREATMENTS AND EXPERIMENTAL DESIGN

Experiments for objectives one and two consisted of 4 substrate treatments at 2 levels of infestation (yes or no) of *Rhizoctonia solani* in a 4x2 factorial design for a total of 8 treatments (Tables 3.1 & 3.2). Treatments were arranged in a split-plot randomized complete block design (RCBD) with four blocks for a total of 32 pots per block (8 treatments x 4 pots per treatment) where each replicate experiment was considered a block (Figure 3.2). Blocking by replicate experiment controlled for natural variability associated with substrate blending, *R. solani* inoculum preparation, and other small stochastic differences among replicate experiments.

The experimental unit for the main plot effect of *R. solani* infestation was one tray, while the experimental unit for substrate treatment was a single pot, with 32 seeds planted per pot. Main plot randomization was by *R. solani* infestation, with all pots (8 per tray) in each tray either infested or not infested and two trays placed on each shelf. The subplot randomization was by substrate treatment (WF processing type or %blend ratio) applied to each pot within each tray. This split-plot design increased the precision in detecting differences in disease severity attributable to substrate treatment.

There were four replicate experiments/blocks for each objective, performed sequentially. Within replicate experiment (4), there were four pots (n=4) per treatment for a total of n=16 pots per treatment for objective one (Effect of WF processing type on natural disease suppression). For objective two, four replicate experiments per WF-processing type were performed (4 replicate experiments * 3 WF-processing types=12 total experiments). Thus for objective two there were also n=16 per treatment.
Figure 3.2. Diagram of split-plot design showing randomization for one replicate experiment in the growth chamber. Each tray was randomized for main plot effect of *R. solani* infestation (infested or non-infested) and subplot effect of substrate type (numbers 1-4 in diagram, specific treatments shown in Tables 3.1 & 3.2). A total of four replicate experiments were performed for a split-plot RCBD blocked by replicate experiment. Within replicate experiment, there were four pots (n=4) per treatment for a total of n=16 pots per treatment for each RCBD.

Table 3.1. Treatments evaluated for the effect of WF processing type on damping-off disease severity in radish in objective one.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>R. solani infestation (Main plot effect)</th>
<th>Substrate (Subplot effect)</th>
<th>Substrate by Product Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>1. Peat 70% Perlite 30% (Control)</td>
<td>Peatlite</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>2. Peat 70% WF Hammer-Mill 30%</td>
<td>Pine Tree Substrate (PTS)</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>3. Peat 70% WF Disc-refined Wood 30%</td>
<td>ForestGold (FG)</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>4. Peat 70% WF Extruded Wood 30%</td>
<td>GreenFibre (GF)</td>
</tr>
<tr>
<td>5</td>
<td>No</td>
<td>1. Peat 70% Perlite 30% (Control)</td>
<td>Peatlite</td>
</tr>
<tr>
<td>6</td>
<td>No</td>
<td>2. Peat 70% WF Hammer-Mill 30%</td>
<td>Pine Tree Substrate (PTS)</td>
</tr>
<tr>
<td>7</td>
<td>No</td>
<td>3. Peat 70% WF Disc-refined Wood 30%</td>
<td>ForestGold (FG)</td>
</tr>
<tr>
<td>8</td>
<td>No</td>
<td>4. Peat 70% WF Extruded Wood 30%</td>
<td>GreenFibre (GF)</td>
</tr>
</tbody>
</table>
Table 3.2. Treatments evaluated for the effect of WF blend ratio on disease severity in radish in objective two. **Note:** Each of the three WF processing types (ForestGold (FG), GreenFibre (GF), pine tree substrate (PTS)) were tested separately.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>R. solani infestation (Main plot effect)</th>
<th>Substrate (Subplot effect)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>1. Peat 70% Perlite 30% (Control) (Peatlite)</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>2. Peat 70% WF 30%</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>3. Peat 80% WF 20%</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>4. Peat 90% WF 10%</td>
</tr>
<tr>
<td>5</td>
<td>No</td>
<td>1. Peat 70% Perlite 30% (Control) (Peatlite)</td>
</tr>
<tr>
<td>6</td>
<td>No</td>
<td>2. Peat 70% WF 30%</td>
</tr>
<tr>
<td>7</td>
<td>No</td>
<td>3. Peat 80% WF 20%</td>
</tr>
<tr>
<td>8</td>
<td>No</td>
<td>4. Peat 90% Wood Fiber 10%</td>
</tr>
</tbody>
</table>

For objective one (Table 3.1), the four substrate treatments were (1) hammer-milled PTS, Brian Jackson, North Carolina State University), (2) disc-refined FG (Pinstrup, Denmark), (3) extruded GF (Klasmann-Delimann, Geeste Germany,) (Figure 3.1) and (4) peatlite control (70% sphagnum peat and 30% perlite). All peat-WF treatments were blended at a ratio of 70% sphagnum peat to 30% WF (Table 3.1). For objective two (Table 3.2), the four substrate treatments were (1) 70% sphagnum peat to 30% WF, (2) 80:20 WF, (3) 90:10 WF and (4) peatlite control (70% sphagnum peat and 30% perlite). These peat:WF ratios were chosen to align with industry standards, which can be upwards of 40% WF, but is generally 10-30% WF (Jackson, 2016; Kaderabek et al., 2019; Owen et al., 2019).

### 3.2.2 PREPARATION OF SUBSTRATE TREATMENTS

Substrate blends were prepared by mixing each WF with raw sphagnum peat (H3-H4 Promix, Premier Tech, Canada) as described in Appendix A. Substrate pH was adjusted to achieve a pH of 5.4-6.4 with the target being 5.8 (Nelson, 2012; Jackson, 2016). The pH adjustments were performed by adding carbonate dolomitic limestone (PLANTEX #80 Pulverized 50 lbs, Master Plant-Prod-Inc.) at a rate of 6 lbs/yd³ (~3.56 kg/m³) (100g/ft³). At the time of blending, wetting agent (PsiMatric, Aquatrols; 3mL per ft³) dissolved in tap water at a 1:150 rate (1 mL wetting
agent mixed into 150 mL water) was also incorporated to increase the spreading ability of fertilizer, water, and Rootshield® WP application by lowering surface tension in the substrates. An additional 250-350 mL per 0.5 ft³ of tap water was also added to blend to increase SMC. Substrates were then sealed in 20-30-gallon plastic bins and allowed to equilibrate for 2-7 days for proper lime and moisture distribution. Previous work performed by Brian Jackson (in press) indicates that two days is the minimum time required for pH to equilibrate, and that pH stabilizes within 7-14 days. The pH and EC measurements were then made one-week after blending using the soil media extract method (SME; Warncke, 1986; University of Minnesota, 2020). Samples were randomly collected from each bulk substrate blend (three per treatment) prior to potting. Liquid extracts were then used to measure pH and EC in each sample using a Orion™ Versa Star Pro advanced electrochemistry meter (Thermo Scientific, Waltham, MA). Substrate moisture content (SMC) was also measured one-week post blending using a MB27 moisture analyzer (OHAUS, Parsippany, NJ) and the SMC of all blends fell within the desired range of 50-60±5%. Substrates were used to fill pots one-week post preparation. Total porosity, container capacity, air space, bulk density, and initial moisture content, were measured for each substrate blend by Brian Jackson (NCSU) following the North Carolina State University Porometer method (Fonteno et al., 1995).

3.2.3 PATHOGEN PREPARATION

*R. solani* isolate RS-33 was obtained from Ohio State University and identified as anastomosis group (AG) 2-1 HK clade (sequenced by Dr. James Woodhall at the University of Idaho using rDNA-ITS techniques). Sclerotia of the isolate were stored in -80°C freezer and grown on Potato Dextrose Agar (PDA) media plates (39 g/1L DO water) one to two weeks before preparation of the inoculum. The pathogen was prepared as a rice grain inoculum as described by Owen (2019).
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 once for 60 min at 121°C and 15 psi. Autoclaved rice grains were then inoculated with 10 PDA plugs taken from one to two-week old *R. solani* cultures using a size 7 mm cork borer. Beakers were covered with aluminum, wrapped with parafilm, and placed in a cabinet at room temperature for 6-7 days. One week later, colonized rice grains were pulverized with a mortar and pestle to produce inoculum of uniform size (~1-3mm). This uniform size was initially determined using a 2mm mesh cheese cloth to establish a standard, and then was visually determined for subsequent inoculum preparations.

To confirm that symptoms observed were caused by *R. solani*, pathogen identity from infested radish was confirmed by root plating on acidified potato dextrose agar (APDA) media plates (amended with concentrated lactic acid 85%). Blackened roots were removed with sterilized tweezers and rinsed three times, twice in petri dishes of sterile RO water and once dish containing a 10% bleach solution. Root material in the “transition zone” (between rotted & healthy root) was taken to minimize the risk of accidentally isolating secondary pathogens. Four root pieces (~2-5mm in length) were placed into four different quadrants on a dish of APDA. Dishes were parafilmed and incubated at room temperature and after 2-5 days and if roots showed fungal growth, colonized APDA disc samples were sub-cultured onto new APDA or PDA. Samples were examined morphologically on agar plates as well as with light microscopy to identify the diagnostic right-angle branching hyphae (Chapter Two).

3.2.4 EXPERIMENTAL SET-UP

Pots were filled in the following order: 1) substrate, 2) water, 3) radish seed, 4) *R. solani* inoculum was drizzled on top of pots and then 5) randomized trays placed in growth chamber for
one week (Figure 3.3)

1) **Pot Filling:** The prepared substrate treatments were added to fill each 11 cm pot three-quarters full (and 4.5in (~11cm) STD Thermo, The HC Companies, Boise, ID). Three pots per substrate treatment were weighed to obtain the average dry weight and this average weight was used to fill all other pots for a given treatment. Eight pots were placed on 27.9 x 54.3 cm standard 1020 Flat Black trays (The HC Companies, Boise, ID).

2) **Watering:** All pots were hand-watered once by graduated cylinder at the time of seeding with ~150-200 mL of tap water.

3) **Plant material:** Thirty-two Organic Early Scarlet Globe (*Raphanus sativus*) seeds were sown into each pot by hand using tweezers.

4) **Inoculation of Pots:** Each pot was infested with 0.5 grams of *R. solani* rice/liter of soil with 0.25 grams of rice grain spread across the surface of each 500 mL pot, but not mixed into the media. This concentration was determined during preliminary work designed to achieve a medium disease severity in **CHAPTER TWO**.

5) **Growth Chamber:** Pots were incubated in a walk-in growth chamber set at 22 to 24°C, 67-70% relative humidity under 24-h illumination by T5 120v Florescent lights (fluence rate of 55-65 \( \text{umol m}^{-2} \text{s}^{-1} \)) for one week (Figure 3.2).

Figure 3.3. Pots after seeding, watering, and inoculation with *R. solani* prior to entering the growth chamber for one-week. Afterward, pots were randomized within each tray. From left to right, (A) 70:30 peat perlite control, (B) 70:30 peat:WF, (C) 80:20 peat:WF, and (D) 90:10 peat:WF PTS. This is an example for the second objective: WF by percentage blend.
3.2.5 DATA COLLECTION

**Disease Assessment:** Damping-off severity was measured 7 days after seeding based on a disease severity scale developed by Krause et al. (2001) (Figure 3.4) 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.

![Figure 3.4. Damping-off disease scale developed from Krause et al. (2001).](image)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptomless</td>
<td>Small root/stem lesion</td>
<td>Stem lesions Girdling &lt;50%</td>
<td>Post-emergent damping-off Blackened stem Girdling &gt;50%</td>
<td>Pre-emergent damping-off</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3.5. Range of “4” or post-emergent damping-off. A rating of 4 was characterized by blackened stem and girdling of greater than 50%.
For each pot, the 32 seeds were assessed on the categorical scale (Figure 3.4) and then averaged to calculate the mean disease severity per pot. For each pot, natural germination was calculated as the percent of “1”s and “2”s out of the 32 seed subsamples.

**Total aboveground biomass:** For the second objective, total aboveground 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).

### 3.2.6 DATA ANALYSIS

All analyses were performed in R version 3.6.2 (2019-12-12) --"Dark and Stormy Night” ©2019. Analysis of variance (ANOVA) was used to assess differences in physical and chemical properties among substrate blends and assumptions of ANOVA (normality of residuals and homogeneity of variances) were met. Analysis of variance (ANOVA) was also used to determine the effects of substrate blend and inoculation treatment on damping-off severity. Mean disease severity which ranged in discrete categories from 1 to 5, was transformed to $R^* = \frac{(R^{1.5}-1)}{1.5}$ (Krause et al., 2001) to obtain a linear scale and meet the assumptions of ANOVA: normality of residuals, homogeneity of variances, and block-factor additivity. Data were only transformed once and homogeneity of variance was always met; however, in some instances, residuals were not normally distributed, fortunately ANOVA is robust to deviations from normality. The mix treatment factors included substrate treatment (either WF processing type or percent blend) and *R. solani* infestation (yes/no). Simple effects analyses and Tukey HSDs were performed to determine differences in 1) transformed mean disease severity and 2) aboveground fresh weight biomass (g) in substrate treatments within and without *R. solani* infestation. Natural
germination rates with the non-infested treatment group were also compared using ANOVA, simple effects analyses, and Tukey HSDs.

3.3 Results

3.3.1 SUBSTRATE CHEMICAL AND PHYSICAL PROPERTIES ANALYSES

Average substrate chemical and physical properties were comparable across treatments, with some minor significant differences (Tables 3.3-3.6). For objective one (all 70/30 blends), peatlite control blends had significantly lower SMC at time of seeding (i.e. prior to hand watering) (mean 44.8%) followed by PTS (51.8%). SMCs were highest in FG (59.0%) and GF (57.2%) (Table 3.3). pH was highest in PTS (6.18) but within the desired range (5.4-6.4 with the target 5.8). Peatlite (5.80), FG (5.72), and GF (5.68) also fell within this range (Table 3.3). PTS also had higher EC (0.789 mS/cm) than the other substrate blends, however this was not seen in the same substrate (i.e. peat:PTS at 70/30 ratio) treatment blended for objective two where EC was 0.417±0.027 mS/cm (Table 3.5). The high EC in PTS substrates during objective one may have been due to blending inconsistencies. Overall SMCs and pH for substrate treatments fell within the desired ranges; moreover, pH measurements were similar to those recorded in previous work on PTS wood fiber substrates (Jackson et al., 2009).

Table 3.3. Mean soil moisture content (SMC), pH, and electrical conductivity (EC)+standard error (SE) (n=3) of the substrate treatments in objective one. Measurements were taken one-week after blending, before radishes were sowed. All blends consisted of 70 Peat 30 WF or perlite (% by volume). Substrates in replicate experiment one were amended at a rate of 8 lbs/yd$^3$ (~4.75 kg/m$^3$), but this raised pH above the desired range and in subsequent replicate experiments at a rate of 6 lbs/yd$^3$ (~3.56 kg/m$^3$).

<table>
<thead>
<tr>
<th>Substrate Treatment</th>
<th>SMC (%)</th>
<th>pH</th>
<th>EC (mS/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peatlite</td>
<td>44.82±3.15$^b$</td>
<td>5.80±0.24$^{ab}$</td>
<td>0.354±0.033$^a$</td>
</tr>
<tr>
<td>ForestGold (FG)</td>
<td>59.04±2.06$^a$</td>
<td>5.72±0.20$^b$</td>
<td>0.332±0.041$^a$</td>
</tr>
<tr>
<td>GreenFibre (GF)</td>
<td>57.18±2.63$^a$</td>
<td>5.68±0.24$^b$</td>
<td>0.373±0.039$^a$</td>
</tr>
<tr>
<td>Pine Tree Substrate (PTS)</td>
<td>51.81±1.74$^{ab}$</td>
<td>6.18±0.14$^a$</td>
<td>0.789±0.215$^b$</td>
</tr>
</tbody>
</table>

For each substrate property, letters indicate significant differences among substrates (Tukey HSDs, a=0.05, MSD$_{SMC}$=9.12, MSD$_{pH}$=0.457, MSD$_{EC}$=0.392)
The three different WF processing types, despite their visual differences, had similar physical properties: total porosity, container capacity, and SMC, but were significantly different from the peatlite controls (Table 3.4). Likewise, for objective two, WFs had overall similar physiochemical properties within WF blend ratio (for example PTS 70/30, 80/20, and 90/10) as well as among WF processing types (e.g. 80/20 of FG, GF, PTS) (Table 3.5 and 3.6) and similar to previous work (Smith et al., 2019). Peatlite controls and PTS blends had lower SMC (both mean 51%) than FG (58.4%) and GF (56.07%). Generally speaking, the first set of experiments (PTS) had significantly higher pH and EC, but in subsequent experiments (FG and GF), a lower rate of lime (from 8lbs/yd$^3$ (~4.75 kg/m$^3$) to 6lbs/yd$^3$ (~3.56 kg/m$^3$)) was added to blends to ensure pHs in the target range (5.5-6.0). Overall and across the three sets of experiments, SMCs, pH, and ECs fell within the desired ranges.

Table 3.4. Mean physical properties of substrate treatments±SE (n=3) in objective one. All blends consisted of 70 Peat 30 WF or perlite (% by volume). Physical properties were measured for each substrate blend following the North Carolina State University Porometer method (Fonteno et al., 1995).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Total porosity$^1$</th>
<th>Container capacity$^2$</th>
<th>Air space$^3$</th>
<th>Bulk density (lbs/ ft$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peatlite</td>
<td>81.0±0.83c</td>
<td>56.5±0.15b</td>
<td>24.5±0.84a</td>
<td>5.6±0.07a</td>
</tr>
<tr>
<td>ForestGold (FG)</td>
<td>86.5±1.07a</td>
<td>62.9±0.98a</td>
<td>23.6±0.52a</td>
<td>4.3±0.09c</td>
</tr>
<tr>
<td>GreenFibre (GF)</td>
<td>85.3±0.20ab</td>
<td>63.5±0.55a</td>
<td>21.8±0.67ab</td>
<td>4.9±0.17b</td>
</tr>
<tr>
<td>Pine Tree Substrate (PTS)</td>
<td>82.2±1.04bc</td>
<td>62.8±0.80a</td>
<td>19.5±0.41b</td>
<td>5.5±0.00a</td>
</tr>
</tbody>
</table>

$^1$ Total porosity is equal to container capacity/ air space (%vol)
$^2$ Container capacity is (wet weight - oven dry weight) / volume (%vol)
$^3$ Air space is the volume of water drained from the sample / volume of sample (%vol)

For each substrate property, letters indicate significant differences among substrates (Tukey HSDs, $a=0.05$, MSD$_{Totalporosity} = 3.905323$, MSD$_{Container capacity} = 3.153951$, MSD$_{Airspace} = 2.852311$, MSD$_{Bulkdensity} = 0.2923334$)
Table 3.5. Mean soil moisture content (SMC), pH, and electrical conductivity (EC)±SE (n=3) of the substrate treatments ±standard error for objective two. Measurements were taken one-week after substrate blending, right before radishes were sowed. Substrates were amended at a rate of 6 lbs/yd$^3$ (~3.56 kg/m$^3$). Three ‘peatlite’ controls were made at a 70:30 (peat:perlite % volume) for each set of experiments (ForestGold, GreenFibre, Pine Tree Substrate).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>SMC (%)</th>
<th>pH</th>
<th>EC (mS/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peatlite 70/30 (FG)</td>
<td>51.27±0.98 cwde</td>
<td>5.61±0.11 cdde</td>
<td>0.345±0.016 bcde</td>
</tr>
<tr>
<td>ForestGold (FG) 70/30</td>
<td>57.74±1.23 ab</td>
<td>5.81±0.12 cbed</td>
<td>0.357±0.015 abed</td>
</tr>
<tr>
<td>ForestGold (FG) 80/20</td>
<td>58.01±1.66 cbc</td>
<td>6.01±0.16 cbed</td>
<td>0.363±0.014 abc</td>
</tr>
<tr>
<td>ForestGold (FG) 90/10</td>
<td>59.43±0.84 w</td>
<td>5.47±0.17 cdde</td>
<td>0.350±0.016 bcde</td>
</tr>
<tr>
<td>Peatlite 70/30 (GreenFibre)</td>
<td>50.30±0.61 cd</td>
<td>6.13±0.07 cde</td>
<td>0.320±0.005 de</td>
</tr>
<tr>
<td>GreenFibre (GF) 70/30</td>
<td>55.15±1.18 bcd</td>
<td>5.74±0.05 bcd</td>
<td>0.296±0.002 de</td>
</tr>
<tr>
<td>GreenFibre (GF) 80/20</td>
<td>56.00±1.16 cde</td>
<td>5.58±0.04 cde</td>
<td>0.292±0.002 cde</td>
</tr>
<tr>
<td>GreenFibre (GF) 90/10</td>
<td>57.07±1.69 cde</td>
<td>5.22±0.04 cde</td>
<td>0.295±0.003 de</td>
</tr>
<tr>
<td>Peatlite 70/30 (Pine Tree Substrate)</td>
<td>52.02±2.42 c</td>
<td>6.28±0.07 bc</td>
<td>0.394±0.019 ab</td>
</tr>
<tr>
<td>Pine Tree Substrate (PTS) 70/30</td>
<td>49.49±1.17 a</td>
<td>6.08±0.06 ab</td>
<td>0.417±0.027 a</td>
</tr>
<tr>
<td>Pine Tree Substrate (PTS) 80/20</td>
<td>52.45±1.51 abc</td>
<td>6.01±0.05 abc</td>
<td>0.394±0.018 ab</td>
</tr>
<tr>
<td>Pine Tree Substrate (PTS) 90/10</td>
<td>51.34±1.63 bcde</td>
<td>5.73±0.07 bcde</td>
<td>0.415±0.014 a</td>
</tr>
</tbody>
</table>

Letters indicate significant differences among substrates for each property: (Tukey HSDs, a=0.05, n=12 MSD$_{SMC}$=6.2276, MSD$_{pH}$=0.430 MSD$_{EC}$=0.063)

Table 3.6. Mean physical properties of the substrate treatments ±SE (n=3) for objective two. Total porosity, container capacity, air space, bulk density, and initial moisture content were measured for each substrate blend one to two months after blending by Brian Jackson following the North Carolina State University Porometer method (Fonteno et al., 1995).

<table>
<thead>
<tr>
<th>Substrate Treatment</th>
<th>Total porosity$^1$</th>
<th>Container capacity$^2$</th>
<th>Air space$^3$</th>
<th>Bulk Density (lbs/ft$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peatlite 70/30</td>
<td>78.6±0.86</td>
<td>62.1±0.50</td>
<td>16.5±1.33</td>
<td>6.6±0.07ab</td>
</tr>
<tr>
<td>ForestGold (FG) 70/30</td>
<td>81.3±2.42</td>
<td>63.7±1.52</td>
<td>17.6±1.01</td>
<td>4.4±0.00c</td>
</tr>
<tr>
<td>ForestGold (FG) 80/20</td>
<td>84.7±0.90</td>
<td>65.1±2.54</td>
<td>19.6±1.73</td>
<td>5.3±0.06de</td>
</tr>
<tr>
<td>ForestGold (FG) 90/10</td>
<td>84.7±0.83</td>
<td>67.4±0.72</td>
<td>17.4±0.52</td>
<td>5.7±0.20bcd</td>
</tr>
<tr>
<td>GreenFibre (GF) 70/30</td>
<td>84.2±0.44</td>
<td>61.3±0.09</td>
<td>25.4±0.49</td>
<td>4.7±0.12de</td>
</tr>
<tr>
<td>GreenFibre (GF) 80/20</td>
<td>81.8±1.51</td>
<td>65.2±0.43</td>
<td>16.6±1.10</td>
<td>5.0±0.03de</td>
</tr>
<tr>
<td>GreenFibre (GF) 90/10</td>
<td>81.9±0.54</td>
<td>62.3±0.78</td>
<td>19.6±0.99</td>
<td>8.1±0.30a</td>
</tr>
<tr>
<td>Pine Tree Substrate (PTS) 70/30</td>
<td>79.3±1.72</td>
<td>60.1±2.85</td>
<td>19.2±2.76</td>
<td>5.9±0.10bcd</td>
</tr>
<tr>
<td>Pine Tree Substrate (PTS) 80/20</td>
<td>79.7±3.42</td>
<td>63.8±1.40</td>
<td>15.9±2.05</td>
<td>8.6±0.43a</td>
</tr>
<tr>
<td>Pine Tree Substrate (PTS) 90/10</td>
<td>80.1±1.04</td>
<td>59.6±2.74</td>
<td>20.5±2.08</td>
<td>6.8±0.31b</td>
</tr>
</tbody>
</table>

$^1$Total porosity is equal to container capacity / air space (%vol)

$^2$Container capacity is (wet weight - oven dry weight) / volume (%vol)

$^3$Air space is the volume of water drained from the sample / volume of sample (%vol)

For bulk density, letters indicate significant differences among substrates (Tukey HSDs, a=0.05, MSD=1.049). Total porosity (p=0.137, ANOVA $F_{9,20}$=1.774), container capacity (p=0.0635, ANOVA $F_{9,20}$=2.244), and air space (p=0.36, ANOVA $F_{9,20}$=1.78) were not significantly different among substrates.
3.3.2 NATURAL GERMINATION RATES OF RADISH SEEDS

For objective one (experiments focused on the effect of WF processing type on natural disease suppression), radish seeds sown in the GF blends had significantly lower germination rates compared to radish seeds planted in PTS and FG, however this was not the case during objective two (Tables 3.7 and 3.8). Across all experimental treatments (substrate processing type and blend ratio), the natural germination rates were similar but below the expected rate for seed lot (90%) (var. Early Scarlet Globe).

Table 3.7. Natural germination rates of radish ± standard error planted in each WF processing substrate blend in objective one averaged across replicate experiments (blocks) Tukey-HSD (a=0.05, n=16 MSD= 10.10) test was performed. All blends consisted of 70 Peat: 30 WF or perlite (% by volume). Letters indicate significant group differences.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Commercial Name</th>
<th>Natural Germination (%)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peatlite</td>
<td>Peatlite</td>
<td>81.6±2.28, ab</td>
</tr>
<tr>
<td>Disc-refined</td>
<td>ForestGold (FG)</td>
<td>91.6±1.7, a</td>
</tr>
<tr>
<td>Extruded</td>
<td>GreenFibre (GF)</td>
<td>74.8±5.2, b</td>
</tr>
<tr>
<td>Hammer-milled</td>
<td>Pine Tree Substrate (PTS)</td>
<td>86.7±1.8, a</td>
</tr>
</tbody>
</table>

¹Calculated as fraction of “1”s and “2”s out of 32 seeds per non-infested pot (no R. solani)

Table 3.8. Natural germination rates±standard error of radish seeds sown in different WF processing type and blend ratio in objective two. Germination rates were similar across processing type and percent blend by volume (p=0.0743 ANOVA F_{11,180}=1.71, n=16).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>ForestGold</th>
<th>GreenFibre</th>
<th>Pine Tree Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peatlite</td>
<td>84.0±1.3</td>
<td>88.5±1.8</td>
<td>85.4±2.1</td>
</tr>
<tr>
<td>70/30</td>
<td>87.7±1.9</td>
<td>85.4±1.9</td>
<td>90.0±1.5</td>
</tr>
<tr>
<td>80/20</td>
<td>86.7±1.8</td>
<td>86.5±2.0</td>
<td>89.1±1.9</td>
</tr>
<tr>
<td>90/10</td>
<td>83.2±1.8</td>
<td>85.0±2.1</td>
<td>90.6±1.5</td>
</tr>
</tbody>
</table>
3.3.3. **EFFECT OF WF PROCESSING TYPE ON DISEASE SEVERITY**

Pots infested with *R. solani* had significantly higher mean disease severity (4.32) than non-infested pots (1.66) indicating that the inoculation was successful (p= 0.000482; ANOVA F<sub>1,3</sub>= 273.055)

We observed differences in radish damping-off disease severity in treatments. Radish grown in PTS had significantly lower disease (4.21±0.05) compared to those grown in the peatlite control (4.43±0.07) (p<0.05). Among WF processing types, disease severity tended to be slightly lower on radish grown in PTS compared to those grown in FG (4.23±0.06) (p>0.05) and trending toward significantly lower in GF (4.23±0.06) (p<0.05) blends (Table 3.9). Under infestation, plants grown in both FG (4.23) and PTS (4.21) had significantly lower disease severity than the peatlite control (4.43) (p<0.05 Dunnett’s multiple comparisons; Quantile=2.4093, 95% confidence interval. However, it is important to acknowledge that under the Tukey-HSD (which has a lower level of precision than Dunnett’s), only radish grown in PTS substrate had significantly lower disease severity than peatlite (a=0.05, n=16 MSD=0.428895; Table 3.9). Differences in radish disease severity were also found under control conditions (no pathogen present). Specifically, radishes in blends of FG and PTS had significantly lower disease severity than plants grown in the GF blend (Table 3.9), a pattern reflective in the significantly lower (p<0.05) natural germination rates of GF (74.8±5.2%) compared to FG (91.6±1.7) and PTS (86.7±1.8) (Table 3.7).
Table 3.9. Full factorial ANOVA performed to assess main effect of *R. solani* infestation (infested or non-infested) and subplot effect of WF processing type substrate treatment on radish damping-off disease severity in a split-plot RCBD. Although there was no interaction effect of infestation and substrate (p=0.0901, ANOVA $F_{3,114}=2.216$) simple effects ANOVAs were performed to assess disease severity across substrates under *R. solani* infestation and no infestation.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block (Replicate Experiment)</td>
<td>3</td>
<td>1.6</td>
<td>0.5</td>
<td>0.227</td>
<td>0.872971</td>
</tr>
<tr>
<td><em>R. solani</em> infestation</td>
<td>1</td>
<td>653.0</td>
<td>653.0</td>
<td>273.055</td>
<td>0.000482</td>
</tr>
<tr>
<td>Residuals*</td>
<td>3</td>
<td>7.2</td>
<td>2.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Split-plot analysis uses Block:Isolate as error term for main effect

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peatlite</td>
<td>3</td>
<td>8.645</td>
<td>2.8817</td>
<td>12.318</td>
<td>4.88e-07</td>
</tr>
<tr>
<td>ForestGold</td>
<td>3</td>
<td>1.555</td>
<td>0.5183</td>
<td>2.216</td>
<td>0.0901</td>
</tr>
<tr>
<td>GreenFibre</td>
<td>3</td>
<td>2.02</td>
<td>0.6667</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pine Tree Substrate</td>
<td>114</td>
<td>26.670</td>
<td>0.2339</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.3.4. **EFFECT OF WF BLEND RATIO ON DISEASE SEVERITY**

Across blend ratio, radishes grown in WF blends infested with *R. solani* had lower mean disease severity than the peatlite controls (Table 3.12). However, the differences in disease severity between WF blend ratio treatments varied by processing method. For example, within GF WF blends, plants grown in 70/30 and 80/20 had significantly lower disease severity than the peatlite control and the 90/10 GF blend (Table 3.12 p<0.05). However, within PTS and FG WF blends,
plants grown in 80/20 and 90/10 had significantly lower disease than plants in the peatlite control but not compared to the 70/30 blend (Table 3.12).

Overall, there were some significant differences in disease severity within WF blend ratio, with radishes grown in 80/20 blends from each of the three WF processing-type having significantly lower damping-off disease severity than the peatlite control and, in some cases, compared to other WF blend ratios (Table 3.12; p<0.05). Under control conditions, disease severity was similar across WF type and blend ratio and there was no significant difference between WFs and peatlite. In contrast, under *R. solani* infestation, radishes grown in WFs had significantly lower disease than the peatlite control (Tukey *HSDs* a=0.05, n=16 MSD_{disc}= 0.3174008, MSD_{extruded}= 0.3174008, MSD_{hammer}= 0.3939673) (Table 3.12).
Table 3.11. Full factorial ANOVAs performed to assess main effect of *R. solani* infestation (infested or non-infested) and subplot effect of percent WF blend substrate treatment on radish damping-off disease severity in a split-plot RCBD. Three separate ANOVAs were performed, one for each WF processing type (ForestGold, GreenFibre, Pine Tree Substrate). Given significant interaction effect of infestation and substrate, simple effects ANOVAs were then performed to assess disease severity across substrates (for each set of WF processing experiments at each %blend) under *R. solani* infestation and no infestation.

<table>
<thead>
<tr>
<th>ForestGold (FG)</th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block (Replicate Experiment)</td>
<td>3</td>
<td>4.6</td>
<td>1.5</td>
<td>2.242</td>
<td>0.262</td>
</tr>
<tr>
<td><em>R. solani</em> infestation</td>
<td>1</td>
<td>768.8</td>
<td>768.8</td>
<td>1119.509</td>
<td>5.87e-05</td>
</tr>
<tr>
<td>Residuals*</td>
<td>3</td>
<td>2.1</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Split-plot analysis uses Block:Isolate as error term for main effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substrate</td>
<td>3</td>
<td>2.021</td>
<td>0.6736</td>
<td>6.189</td>
<td>0.000621</td>
</tr>
<tr>
<td>Infestation x Substrate</td>
<td>3</td>
<td>2.345</td>
<td>0.7817</td>
<td>7.181</td>
<td>0.000185</td>
</tr>
<tr>
<td>Residuals</td>
<td>114</td>
<td>12.409</td>
<td>0.1089</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GreenFibre (GF)</th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block (Replicate Experiment)</td>
<td>3</td>
<td>0.6</td>
<td>0.2</td>
<td>0.604</td>
<td>0.655</td>
</tr>
<tr>
<td><em>R. solani</em> infestation</td>
<td>1</td>
<td>828.2</td>
<td>828.2</td>
<td>2404.060</td>
<td>1.87e-05</td>
</tr>
<tr>
<td>Residuals*</td>
<td>3</td>
<td>1.0</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Split-plot analysis uses Block:Isolate as error term for main effect</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Substrate</td>
<td>3</td>
<td>2.304</td>
<td>0.7681</td>
<td>5.97</td>
<td>0.000812</td>
</tr>
<tr>
<td>Infestation x Substrate</td>
<td>3</td>
<td>4.017</td>
<td>1.3391</td>
<td>10.41</td>
<td>4.17e-06</td>
</tr>
<tr>
<td>Residuals</td>
<td>114</td>
<td>14.667</td>
<td>0.1287</td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Pine Tree Substrate (PTS)</th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block (Replicate Experiment)</td>
<td>3</td>
<td>2.7</td>
<td>0.9</td>
<td>2.389</td>
<td>0.247</td>
</tr>
<tr>
<td><em>R. solani</em> infestation</td>
<td>1</td>
<td>892.4</td>
<td>892.4</td>
<td>2344.477</td>
<td>1.94e-05</td>
</tr>
<tr>
<td>Residuals*</td>
<td>3</td>
<td>1.1</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Split-plot analysis uses Block:Isolate as error term for main effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substrate</td>
<td>3</td>
<td>3.772</td>
<td>1.2573</td>
<td>10.376</td>
<td>4.323-06</td>
</tr>
<tr>
<td>Infestation x Substrate</td>
<td>3</td>
<td>0.805</td>
<td>0.2683</td>
<td>2.214</td>
<td>0.0903</td>
</tr>
<tr>
<td>Residuals</td>
<td>114</td>
<td>13.813</td>
<td>0.1212</td>
<td></td>
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</tr>
</tbody>
</table>
Table 3. Damping-off disease severity in *R. solani* infested and non-infested (control) pots 7 days post seeding in each WF type blended with peat at different ratios. For each set of experiments, a separate 70/30 peatlite control was blended. Mean original disease severity (M) (categorical #1-5) was transformed to a linear scale (M\(^1.5\))/1.5 (Krause et al., 2001). Mean separation was conducted using the Tukey HSD.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>ForestGold (FG) Infested</th>
<th>ForestGold (FG) Non-infested</th>
<th>GreenFibre (GF) Infested</th>
<th>GreenFibre (GF) Non-infested</th>
<th>Pine Tree Substrate Infested</th>
<th>Pine Tree Substrate Non-infested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peatlite</td>
<td>4.69±0.05, b</td>
<td>1.73±0.06</td>
<td>4.75±0.02, b</td>
<td>1.56±0.07</td>
<td>4.73±0.04, b</td>
<td>1.6±0.08</td>
</tr>
<tr>
<td>70/30</td>
<td>4.51±0.04, ab</td>
<td>1.59±0.08</td>
<td>4.39±0.06, a</td>
<td>1.71±0.07</td>
<td>4.56±0.05, ab</td>
<td>1.45±0.06</td>
</tr>
<tr>
<td>80/20</td>
<td>4.44±0.06, a</td>
<td>1.65±0.07</td>
<td>4.49±0.04, a</td>
<td>1.63±0.08</td>
<td>4.48±0.05, a</td>
<td>1.41±0.07</td>
</tr>
<tr>
<td>90/10</td>
<td>4.39±0.05, a</td>
<td>1.81±0.08</td>
<td>4.66±0.04, b</td>
<td>1.72±0.08</td>
<td>4.42±0.06, a</td>
<td>1.38±0.06</td>
</tr>
</tbody>
</table>

Simple effects analyses were performed within *R. solani* infestation (infested or control) and letters within a column (FG, GF, PTS) indicate significant differences in disease severity (Tukey HSDs \(a=0.05\), \(n=16\) MSD\(_{FG}\)= 0.3939429, MSD\(_{GF}\)= 0.3174008, MSD\(_{PTS}\)= 0.3939673) (controls: Tukey HSDs \(a=0.05\), \(n=16\) MSD\(_{FG}\)=0.3500114, MSD\(_{GF}\)=0.3697918, MSD\(_{PTS}\)=0.319432).

### 3.3.5. EFFECT OF WF BLEND RATIO ON ABOVEGROUND RADISH BIOMASS

As expected, aboveground biomass was significantly lower in *R. solani* infested pots compared to non-infested (Table 3.15). Under control conditions (no *R. solani*), aboveground biomass was similar across WF type and blend ratio treatments and there was no significant difference between WFs and peatlite (Table 3.15). In contrast, under *R. solani* infestation, pots with WF had significantly higher aboveground radish biomass compared to peatlite controls (Tukey HSDs \(a=0.05\), \(n=16\) MSD\(_{FG}\)= 0.3660805, MSD\(_{GF}\)= 0.2156516, MSD\(_{PTS}\)= 0.3693299) (Figure 3.6).

There were no significant differences among WF blend ratio or processing type (Table 3.15).

Table 3.13. Mean aboveground fresh weight biomass (g) in *R. solani* infested and non-infested pots across all substrate treatments (perlite control, 70/30, 80/20, 90/10). Three separate ANOVAs (Table 3.14) were performed for each set of experiments (FG, GF, PTS) as each WF processing type was used in separate experiments.

<table>
<thead>
<tr>
<th></th>
<th>ForestGold (FG)</th>
<th>GreenFibre (GF)</th>
<th>Pine Tree Substrate (PTS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infested</td>
<td>0.538g</td>
<td>0.42g</td>
<td>0.578 g</td>
</tr>
<tr>
<td>Control</td>
<td>1.718 g</td>
<td>1.95 g</td>
<td>2.059 g</td>
</tr>
<tr>
<td>(F_{1,3}=)</td>
<td>488.21</td>
<td>694.421</td>
<td>216.473</td>
</tr>
<tr>
<td>p-value</td>
<td>0.00020</td>
<td>0.00012</td>
<td>0.00068</td>
</tr>
</tbody>
</table>
Table 3.14. Full factorial ANOVAs performed to assess main effect of *R. solani* infestation (infested or non-infested) and subplot effect of percent WF blend substrate treatment on radish aboveground biomass in a split-plot RCBD. Three separate ANOVAs were performed, one for each WF processing type (ForestGold, GreenFibre, Pine Tree Substrate). Given significant interaction effect of infestation and substrate, simple effects ANOVAs were then performed to assess disease severity across substrates (for each set of WF processing experiments at each %blend) under *R. solani* infestation and no infestation.

<table>
<thead>
<tr>
<th>ForestGold (FG)</th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block (Replicate Experiment)</td>
<td>3</td>
<td>11.62</td>
<td>3.87</td>
<td>42.46</td>
<td>0.005883</td>
</tr>
<tr>
<td><em>R. solani</em> infestation</td>
<td>1</td>
<td>44.53</td>
<td>44.53</td>
<td>488.21</td>
<td>0.000203</td>
</tr>
<tr>
<td>Residuals*</td>
<td>3</td>
<td>0.27</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Split-plot analysis uses Block:Isolate as error term for main effect

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ForestGold (FG)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infestation x Substrate</td>
<td>3</td>
<td>0.546</td>
<td>0.1820</td>
<td>2.329</td>
<td>0.0782</td>
</tr>
<tr>
<td>Residuals</td>
<td>114</td>
<td>8.909</td>
<td>0.0782</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GreenFibre (GF)</th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block (Replicate Experiment)</td>
<td>3</td>
<td>0.71</td>
<td>0.24</td>
<td>2.205</td>
<td>0.26637</td>
</tr>
<tr>
<td><em>R. solani</em> infestation</td>
<td>1</td>
<td>74.44</td>
<td>74.44</td>
<td>694.421</td>
<td>0.00012</td>
</tr>
<tr>
<td>Residuals*</td>
<td>3</td>
<td>0.32</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Split-plot analysis uses Block:Isolate as error term for main effect

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GreenFibre (GF)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infestation x Substrate</td>
<td>3</td>
<td>2.615</td>
<td>0.8717</td>
<td>8.088</td>
<td>6.24e-05</td>
</tr>
<tr>
<td>Residuals</td>
<td>114</td>
<td>12.287</td>
<td>0.1078</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Pine Tree Substrate (PTS)</th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block (Replicate Experiment)</td>
<td>3</td>
<td>5.65</td>
<td>1.88</td>
<td>5.804</td>
<td>0.091315</td>
</tr>
<tr>
<td><em>R. solani</em> infestation</td>
<td>1</td>
<td>70.20</td>
<td>70.20</td>
<td>216.473</td>
<td>0.000681</td>
</tr>
<tr>
<td>Residuals*</td>
<td>3</td>
<td>0.97</td>
<td>0.32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Split-plot analysis uses Block:Isolate as error term for main effect

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pine Tree Substrate (PTS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infestation x Substrate</td>
<td>3</td>
<td>0.452</td>
<td>0.1506</td>
<td>1.327</td>
<td>0.269</td>
</tr>
<tr>
<td>Residuals</td>
<td>114</td>
<td>12.939</td>
<td>0.1135</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3.1. Aboveground fresh weight biomass (g) in *R. solani* infested and non-infested (control) pots for each WF type blended with peat at four ratios. For each set of experiment, a separate 70/30 peatlite control was blended.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>ForestGold (FG) Infested</th>
<th>ForestGold (FG) Non-infested</th>
<th>GreenFibre (GF) Infested</th>
<th>GreenFibre (GF) Non-infested</th>
<th>Pine Tree Substrate (PTS) Infested</th>
<th>Pine Tree Substrate (PTS) Non-infested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peatlite</td>
<td>0.16±0.07 b</td>
<td>1.54±0.11</td>
<td>0.16±0.03 b</td>
<td>2.12±0.10</td>
<td>0.25±0.07 b</td>
<td>1.86±0.11</td>
</tr>
<tr>
<td>70/30</td>
<td>0.65±0.09 a</td>
<td>1.84±0.08</td>
<td>0.68±0.08 a</td>
<td>1.86±0.09</td>
<td>0.66±0.09 a</td>
<td>2.24±0.09</td>
</tr>
<tr>
<td>80/20</td>
<td>0.67±0.11 a</td>
<td>1.82±0.09</td>
<td>0.54±0.05 a</td>
<td>1.97±0.12</td>
<td>0.68±0.11 a</td>
<td>2.11±0.11</td>
</tr>
<tr>
<td>90/10</td>
<td>0.67±0.12 a</td>
<td>1.68±0.14</td>
<td>0.31±0.06 b</td>
<td>1.85±0.11</td>
<td>0.72±0.12 a</td>
<td>2.03±0.10</td>
</tr>
</tbody>
</table>

Simple effects analyses were performed within *R. solani* infestation (infested or control) and letters indicate significant differences in disease severity within set of experiments of infestation (Tukey HSDs a=0.05, n=16 MSD$_{FG}$= 0.3660805, MSD$_{GF}$= 0.2156516, MSD$_{PTS}$= 0.3693299). There were no significant differences in disease severity of radish in non-infested pots.

Figure 3.6. Visual representation of the effect of *R. solani* infestation across different blend ratios of GreenFibre (GF) WF compared to the 70:30 peatlite control. WF blends had significantly lower disease severity and higher biomass than the peatlite control.
3.4 Discussion

*REGARDLESS OF PROCESSING METHOD, WF BLENDS WERE LESS CONDUCIVE TO DAMPING-OFF COMPARED TO PEATLITE*

Across substrate treatments, natural germination rates were similar but also lower than the variety’s expected rate (90%), a difference we attributed to the age of the seed. Minor differences in germination rates for radishes planted in different substrate treatments could have contributed to disease severity observed in infested pots, however this did not appear to be the case. For example, natural germination rates were significantly lower in GF compared to FG, but under *R. solani* infestation, there was no significant difference in disease severity across the three WF treatments. These results suggest that the poor natural germination rates under GF treatments did not artificially skew average disease severity of GF pots under *R. solani* infestation.

Since natural germination rates were not significantly different among plants grown in peatlite, PTS, and FG, we can conclude that the infested 70:30 peat:PTS and peat:FG blends were less conducive to damping-off caused by *R. solani* than the peatlite control. While we found statistical significance, the difference in damping-off severity was small. These results suggest that the inclusion of the wood components tested, when incorporated into peat, may not significantly negatively or positively affect damping-off.

*OVERALL, THERE IS NO EFFECT OF WF BLEND RATE OR PROCESSING METHOD ON DAMPING-OFF DISEASE SEVERITY OR ABOVEGROUND BIOMASS*

WFs are commonly blended into peat at 10 to 40% by volume but little is known about how blend ratio affects severity of soilborne plant diseases. While we observed small effects of WF:peat blend ratio and processing method, overall there were no significant differences in damping-off disease severity or aboveground biomass among WF treatments suggesting that the processing method and percent of WF mixed with peat has no influence on natural damping-off disease suppressiveness/conduciveness. Furthermore, under *R. solani* infestation, WF blends
across WF processing type had significantly higher aboveground biomass than the peatlite control, suggesting that plants grown in WFs exhibit better growth than the peatlite control in the presence of disease pressure. While radish plants grown in WFs were broadly found to have significantly lower disease severity and higher aboveground biomass than the peatlite control, the differences were small and may not have economic significance for growers.

**DAMPING-OFF BIOASSAY AND SUBSTRATE BLENDING METHODS ARE A FOUNDATION FOR FUTURE WORK**

The one-week bioassay of damping-off in radish provided a high degree of replication at both the sample size and at the experimental level. However, the bioassay’s brevity (one week) also limits analyses of the effect of WF to damping-off at the earliest stage of plant growth (germination). The effect of WFs on disease severity and aboveground plant growth was similar or better than the peatlite standard at the stage of germination and emergence of cotyledons. While this is a pivotal stage—and arguably the most vulnerable—of plant growth, examining the effect of WF on later stages of plant development will provide further evidence of the effect of WF blends at other horticulturally-important stages.

Another concomitant limit and strength of this study was the control blend used, which was made by hand and included the same source of sphagnum peat moss, limestone (for pH adjustments), and wetting agent as the WF substrate blends. Performing blends by hand ensured consistency across substrate treatments and is a strength of our study. However, the majority of growers using peat:perlite blends as purchased products, and often with additional amendments. For example, Pro-Mix BX Mycorrhizae, contains sphagnum peat moss (75-85%), perlite, vermiculite, limestone, wetting agent, and mycorrhizae-PTB297 Technology (ProMix, 2021). By extending the plant’s root system, the addition of mycorrhizae to peatlite blends is known to significantly improves crop yield and resistance to root diseases (Berruti et al., 2016; Parnell et
al., 2016; ProMix, 2021). While the positive benefits of arbuscular mycorrhizal fungi (AMF) additions to soils are well-established and include improved nutrient and water acquisition, increased resistance to drought, salinity, and disease (reviewed in Berruti et al., 2016), the effects of this beneficial fungi addition have not been well-studied in WF blends and represent a future direction for WF research.

WOOD FIBERS BLENDED INTO PEAT PERFORM SIMILAR OR BETTER THAN PEATLITE

WFs vary in many ways, for example in terms of the processing type (e.g. hammer-milled, disc-refined, extruded) and by the percent blend (by volume) used by growers when amended into peat. While the effects of WF blended into peat on other microbe-mediated activities—namely N immobilization (Harris et al., 2019) and the effects of pine wood chips (PWC) on disease severity (Owen et al., 2017; Kaderabek et al., 2019)—have been examined, our results represent the first examination of the effect of WFs on natural disease suppression and disease severity. Specifically, this study examined whether differences within WF (processing type and blend ratio) contribute to differences in natural disease severity and plant biomass. We found that across processing type and blend ratio, WFs did not negatively affect disease or plant growth compared to the peatlite control. As a rule, every soil has the natural ability to suppress pathogens. This natural disease suppressiveness varies by organic substrate due to differences in physical, chemical, and biological characteristics (Nelson et al., 1983; Chen et al., 1988; Hoitink and Boehm 1999; Whipps, 2001; Krause et al., 2001; Bonanomi et al., 2007 and 2018).

Substrates vary in overall disease suppressiveness, as well as suppression against specific pathogens; for example, peat is considered a “disease conducive control” (Chen et al., 1988; Boehm et al., 1997; Borrero et al., 2004) with composts generally considered suppressive (Raviv et al., 2019). Given their high level of readily-degradable hemicellulose and cellulose (Domeno
et al., 2010) and their smaller particles with a large surface area favorable to microbial colonization (Raviv et al., 2019), WFs may be particularly good at promoting the high levels of microbial activity associated with natural disease suppressiveness. However, more work is needed to characterize the microbial profiles of WF components, especially considering supplementary N is essential to avoid microbial N drawdown detrimental to plant growth (Gruda et al., 2004; Jackson et al., 2009a; Harris et al., 2019).

Our findings show that WF blends had better or similar disease suppression to the peatlite standard. Taken in sum with the results of Harris et al. (2020), who found that peat amended with 30% hammer-milled PTS could be used for petunia production with minimal effects on plant growth, fertilizer adjustment needs, or the risk of N immobilization, our results show that WF blends are worthwhile for growers looking to switch from the tried and true to more sustainable options.

3.5 Conclusions and Future Direction

Our research is among the first to evaluate the effect of WF components on disease in container production under controlled conditions. Results suggest that WFs, regardless of blend ratio or type, do not have a negative effect on damping-off disease severity and plant growth. Our findings provide direction for further research as we examined only three types of pine wood materials which represents a small percentage of wood materials growers have access to. There is still very little known about the effects wood component type and inclusion rate on soilborne diseases and disease management. We evaluated wood components for 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 diseases such as root and crown rot. R. solani causes a wide range of diseases (e.g. damping-off, hypocotyl, root, stem, and crown rots, stem cankers) on a wide a range plants
(estimates of over 200 species), infecting a range of plant parts (e.g. roots, stems, tubers, corms, aboveground portions) (Adams et al., 1988; Sneh et al., 1991, Krause et al., 2001; Agrios, 2005; Prabha et al., 2014; Ajayi and Bradley, 2017; Gondal et al., 2019; Lawson, 2021). Moreover, \textit{R. solani} has been shown to cause different levels of disease in both different substrates and on different plants (Nelson and Hoitink, 1982; Krause et al., 2001; Scheuerell et al., 2005). For example, Krause et al. (2001) found that \textit{R. solani} induced damping-off in radish was higher than \textit{R. solani} induced crown and root rot of poinsettia across three substrate mixes (dark peat, light peat, and composted pine bark). \textit{R. solani}'s versatility makes it difficult to predict how the pathogen would cause disease under different conditions, particularly given the unique physiochemical properties of substrates used. Additionally, the unique physiochemical properties of substrates that influence natural disease suppression could also affect specific disease suppression, such as the use of biopesticides. Biopesticides, like WFs, are a sustainable alternative/complement growing in importance in the horticulture industry and the use of both rests on improving grower confidence in their usage. However, the impacts of biopesticides in WF blends remain unexplored and represent an opportunity for future work.

Our study sought to assess if differences in WFs posed any plant production challenges for growers. Our results indicate that in terms of natural disease suppression, WFs blended into peat are similar or better than perlite blended into peat and that growers should feel confident using WF-peat mixes.
CHAPTER 4
THE EFFECT OF WOOD FIBER SUBSTRATE ON THE EFFICACY OF THE BIOPESTICIDE ROOTSHIELD® WP ON DISEASE SEVERITY CAUSED BY RHIZOCTONIA SOLANI IN GREENHOUSE CROPS

4.1 Introduction

Controlled environmental agriculture (CEA) is essential in addressing the dual demands of food production and high-value crop production (Raviv et al., 2019, Davis and Lucier, 2021). However, for CEA to meet its promise of sustainability, it is essential to make production more sustainable in terms of energy and water use, growing media, nutrient inputs, and disease management. Specifically, the use of sustainable soilless substrates—such as wood fibers (WF)—and biopesticides offer concomitant avenues of improving CEA. While other organic (carbon-based) substrates such as hardwood (Nelson et al., 1983; Chung et al., 1988) and pine barks (Krause et al. 2001) have been examined for their interaction with microbial biocontrol agents such as Trichoderma spp., little is known about the interactions between WFs and biocontrol agents. Evaluating biopesticides in WFs and the impact, if any, of WF content on efficacy will help growers make informed decisions and ultimately promote more sustainable CEA.

Wood fibers are an emerging sustainable soilless substrate

Increasing annual greenhouse crop and horticulture production, coupled with grower demand for economical and sustainable soilless substrates, have contributed to a projected 240% increase in demand of soilless substrates by 2050 (Blok, 2021). Traditionally, the demand for soilless substrates has been filled by sphagnum peat moss, but that has changed dramatically in the last two decades (Bek et al., 2020). Substrate manufacturers have identified wood fibers (WFs), a by-product of the forest industry, as an affordable and sustainable raw material that will help meet the demand for soilless substrates and growers have started to incorporate these products into
their production systems (Gumy, 2001; Barrett et al. 2016; Owen et al. 2017 and 2020; Altland et al. 2018, Jackson, 2019).

Generally, WFs are used as an amendment in sphagnum peat moss-based substrates in which peat is amended with WFs from 10 to 40% WF by volume in order to achieve certain physical and chemical properties needed for optimal plant growth (Jackson and Bartley, 2017). While WFs have the potential to meet the growing demand for soilless substrates in a cost-effective and sustainable manner, their use in combination with other sustainable practices such as biopesticides, must be evaluated to provide growers with better information and best practices.

**Wood fibers’ unique physiochemical content may influence biopesticide efficacy**

Beneficial microbes, formulated commercially as biopesticides, are important tools in integrated pest management (IPM) for combating plant pathogens (Glare, 2012; Seiber et al., 2014; Damalas and Koutroubas, 2018; Krause, 2019). While biopesticides have been studied for decades, it remains unknown what, if any, effect wood components have on their efficacy.

Wood fibers and other wood substrates are known to be high in hemicellulose and cellulose content and have lower lignin content than peat, coir, and bark (Domeno et al., 2010). WFs have most recently been examined for natural disease suppression (CHAPTER THREE) because natural suppression against soilborne pathogens is strongly driven by the carbon available in an organic substrate; specifically, labile carbon sources, such as cellulose and hemicellulose found in WF, are most readily available for decomposition by microbes, and their microbial activity influences natural disease suppression (Nelson et al., 1983; De la Cruz et al., 1993; Domeno et al., 2010; Bonanomi et al., 2018). Researchers have demonstrated that wood is more microbially active than peat in part because of its high carbon: N ratio (Jackson et al., 2009) and that wood fibers from pine and poplar have significantly greater fungal diversity and
dominance compared to peat and coconut coir, with peat and coir having higher bacterial diversity (Montagne et al., 2015, 2017). Yet overall, little is known about microbial communities in WFs and the effect of WFs on biopesticide efficacy has never been examined.

**Biopesticide Efficacy is influenced by a substrate and its carbon content**

Biopesticides play an important role in CEA disease management and while the use of biocontrol for pests (e.g. thrips, spider mites, whitefly, and aphids) has risen dramatically, biocontrol use for disease management (e.g. soilborne diseases) against root pathogens lags behind (Summerfield et al., 2015). Microbial biopesticides are an essential component of IPM and sustainable greenhouse agriculture, but unlike chemical pesticides, their biocontrol agents (BCA) interact with the plant and native microbial life in the phytobiome (Massart et al., 2015, Raviv et al. 2019). Therefore, the efficacy of microbial-based biopesticides applied to the soil is largely influenced by interactions within the plant microbiome and the broader CEA greenhouse production environment (Harman, 2000; Berg and Smalla, 2009; Glare et al., 2012; Grosch, et al., 2012; Massart et al., 2015).

A substrate’s carbon source can affect a biocontrol agent efficacy in two main ways: 1) affecting rhizosphere competence/establishment of biocontrol agents (Nelson et al., 1983; Schlaeppi and Bulgarelli, 2014) and 2) influencing a biopesticide’s modes of action (MOAs) (de la Cruz et al., 1993; Hoitink lab’s work, reviewed in Chung et al., 1988; Krause et al., 2001; Swiontek et al., 2014). While rhizosphere competence and an effective MOA are both necessary to a biopesticide’s overall efficacy, the influence of carbon-source may inversely affect each. For example, Nelson et al. (1983) found that although there was greater colonization of *T. harzianum* with addition of fresh hardwood bark compared to composted bark, the greater colonization did not correlate with improved disease suppression of *Rhizoctonia solani*. In other words, the
amount of *T. harzianum* present was higher in the high cellulose substrate (i.e. better rhizosphere competence), but this larger population did not contribute to higher disease suppression. Yet as hardwood bark was aged and its cellulose content decreased, disease suppression of *R. solani* increased. Supporting this finding, De la Cruz et al. (1993) found that chitinase (a cell wall-degrading enzyme) was produced by *T. harzianum* in presence of chitin while chitinase production was suppressed in the presence of glucose. These findings indicate that the production of chitin-degrading enzymes by BCAs may be repressed in the presence of a more-easily broken down carbohydrates like glucose and (hemi)cellulose present in organic substrate.

Another member of the Hoitink lab, Krause et al. (2001) found that substrate’s carbon source was a key driver of biocontrol efficacy. Krause found that *T. hamatum* strain T382 reduced severity of *Rhizoctonia* damping-off of radish in the infested composted pine bark mixes, but was less effective in the light peat mixes and not effective in dark peat mixes. While hardwood bark is composted mainly to reduce toxic compounds (Robbins et al., 2011a), pine bark (PB) has somewhat fewer leachable organic compounds (Robbins et al., 2011a) and PB is also composted to strip waxes and improve water holding capacity, increase surface area and promote microbial activity (Table 4-1). In contrast to both types of bark, light peat mixes (Von post scale H1-H3) have high amounts of cellulose as they largely consist of undecomposed organic material (Robbins et al., 2011a; Premier Horticulture Ltd., 2020). Dark peat mixes (H4 and up) are more decomposed and have a medium level of cellulose content (Table 4-1). Interestingly, sphagnum peat mosses do not possess lignin like that found in vascular plants (Rydin and Jeglum, 2013) but their cell walls contain “lignin-like” polymers and “pectin-like” substances both linked to peat’s low decomposition rate (Hajek et al., 2011).
Table 4.1. Substrates by cellulose content. In the horticulture industry, peats are also labeled as “white peat” (H1-H3), “dark peat” (H4-H6), and “black peat” (H7 and above) (Raviv et al., 2019).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Cellulose Content</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardwood bark</td>
<td>Low; mainly lignin, composted to remove toxic organic acids</td>
<td>Robbins et al. (2011a)</td>
</tr>
<tr>
<td>Canadian peat moss (H1-H3; von Post Scale) “light or white peat”</td>
<td>High: (H1 is totally undecomposed) Younger, undecomposed of low humification; composted to lower cellulose content</td>
<td>Kaila (1956); Premier Horticulture Ltd. (2020); Robbins et al. (2011); Zaccone et al. (2018); Raviv et al. (2018)</td>
</tr>
<tr>
<td>Canadian peat moss (H4-H6) “dark or black peat”</td>
<td>Medium: partly decomposed and older than H1-H3, with lower cellulose and higher lignin/humic content</td>
<td>Kaila (1956); Premier Horticulture Ltd. (2020); Robbins et al. (2011); Zaccone et al. (2018); Raviv et al. (2018)</td>
</tr>
<tr>
<td>Canadian peat moss (H7-H10)</td>
<td>Low: highly decomposed and older than H1-H6</td>
<td>Raviv et al. (2018)</td>
</tr>
<tr>
<td>Pine bark mulch</td>
<td>Low: mainly composed of lignin, composted/age to strip waxes</td>
<td>Altland et al. (2018)</td>
</tr>
<tr>
<td>Wood products</td>
<td>High in cellulose and hemicellulose</td>
<td>Domeno et al. (2010); Jackson (2016)</td>
</tr>
</tbody>
</table>

Overall, results from Krause et al. (2001) supported Nelson et al. (1983) and de la Cruz (1993), showing that a lower cellulose content improved *T. hamatum*’s biocontrol efficacy. However, it is also important to note that in Krause’s same study, *T. hamatum* similarly suppressed *R. solani* crown and root rot in poinsettia regardless of substrate type; substrate and cellulose type significantly influenced *T. hamatum*’s suppression against one type of disease caused by *R. solani* on one specific crop, but not against *R. solani* on another crop. However, these results may be attributed to the difference between short-term assays (e.g. damping-off) and longer term assays (e.g. crown rot). In general, these studies showed that a substrate’s high cellulose concomitantly stimulated the pathogen *R. solani* and repressed parasitism by the biocontrol *Trichoderma* spp. (Nelson et al., 1983; Chen et al., 1988; De la Cruz et al., 1993; Krause et al., 2001).
Given their diverse abilities, *Trichoderma* spp. dominate the microbial biopesticide market

*Trichoderma* spp. are one of the most commonly used agents in microbial biopesticides in agriculture accounting for over 60% of all registered biopesticides (Mukherjee et al., 2013; Woo et al., 2014). As mycoparasites, they are effective against a range of taxonomically diverse pathogens, from soilborne fungal pathogens such as *Rhizoctonia*, *Pythium* and *Sclerotinia* to foliar pathogens such as *Botrytis* and *Alternaria* (Woo et al., 2014). *Trichoderma* spp. suppress plant pathogens via several modes of action (MOA): 1) Direct parasitism or mycoparasitism by physical penetration and the secretion of lytic enzymes such as chitinase (de la Cruz et al., 1993; Francois and Kronstad, 2017), 2) Antibiosis, 3) Competition for nutrients or ecological niches (Harman, 2000; Woo et al., 2014), 4) Activating induce systemic acquired resistance (ISR) (Woo et al., 2014) and 5) Improving the plant's resistance to abiotic stresses such as drought, salinity, pH and nutrient fluctuations (Mukherjee et al., 2013; Woo et al., 2014).

As endophytes, *Trichoderma* spp. colonize plant organs and grow between living cells (Mukherjee et al., 2013) in a mutualism where *Trichoderma* is protected by the plant, while also protecting the plant (Raviv et al., 2019). However, as facultative parasites, *Trichoderma* spp. are also excellent decomposers and able to exist in the rhizosphere without a pathogen present and is rhizosphere competent (Harman, 2000). *Trichoderma* spp. survive in the soil by producing cellulase and other enzymes to break down dead plant material (Mukherjee et al., 2013; Woo et al, 2014). As a biopesticide, *Trichoderma* spp. produce chitinases to hydrolyze (breakdown) chitin and attack the structural integrity of fungal plant pathogens, whose cell walls are mainly composed of chitin (Francois and Kronstad, 2017). Several different types of chitinases are often produced, for example, *T. harzianum* produces two *N*-acetylglucosaminidases, four endochitinases, and one chitobiosidase (Swiontek Brzezinska et al., 2014). *Trichoderma* spp. can
also produce β-glucanases and proteinases (De la Cruz et al., 1993) depending on the carbon type available. *Trichoderma* spp.’s generalist characteristics make it an ideal biopesticide, but also suggest that the presence of the more labile cellulose in a substrate may repress the biopesticide’s chitin-degrading enzyme production (Nelson et al., 1983; De la Cruz et al., 1993; Krause et al., 2001; Francois and Kronstad, 2017).

**Overview**

A substrate’s cellulose-content can influence the activity of the *Trichoderma* spp., ultimately affecting *Trichoderma*’s ability to suppress plant pathogens. This is important for growers because substrates differ with respect to forms of carbon. As a result, we cannot assume that biopesticides will “behave” the same in wood-containing substrates as they do in more traditional substrate blends. Based on what we know about the effects of carbon source on *Trichoderma* spp. efficacy as a biocontrol, the high cellulose content of WFs could reduce the efficacy of fungal biocontrol agents.

The overall goal of this study was to explore the effect of WF on the efficacy of *T. harzianum* T-22 the active ingredient in Rootshield®WP to suppress damping-off and crown and root rot caused by the common soilborne pathogen *R. solani*. We expected that peat wood-fiber blend ratio would affect the efficacy of Rootshield®WP. Specifically, we hypothesized that blends with higher ratios of WF would result in higher damping-off and crown/root rot disease severity because Rootshield®WP would be less effective in media with higher amounts of WF. To examine the effect of WFs on biopesticide efficacy under different disease scenarios at different stages of plant growth, two model plants were used: radish (Organic Early Scarlet Globe, *Raphanus sativus*) and chrysanthemum (*Chrysanthemum x morifolium* ‘Breeze Yellow’).
The specific objectives were to evaluate the effect of peat-wood fiber blend ratio on the efficacy of *T. harzianum* T-22 against:

1) Damping-off disease caused by *R. solani* on radish

2) Crown and root rot disease caused by *R. solani* on ‘mature’ chrysanthemum

3) Crown and root rot disease caused by *R. solani* on ‘young’ chrysanthemum

### 4.2 Materials and Methods

#### 4.2.1 TREATMENTS AND EXPERIMENTAL DESIGN

Experiments in each objective included 4 substrates and 2 Rootshield® WP applications (yes or no) at 2 levels of infestation (yes or no) of *R. solani* in a 4x2x2 factorial design for a total of 16 treatments (Table 4.2). For objectives one and two, treatments were arranged in a split-plot randomized complete block design (RCBD) with four blocks. The block was one replicate experiment (Figure 4.1) or table (Figure 4.2 B). Within replicate experiment or table, there were four pots (n=4) per treatment for a total of n=16 pots per treatment. The experimental unit for the main plot effect of *R. solani* infestation was one tray, while the experimental unit for subplot (substrate blend ratio*Rootshield) treatment combination was a single pot. A single pot was either 32 radish seeds planted per pot or one chrysanthemum plant. This split-plot design increased the precision in detecting differences in disease severity attributable to substrate treatment and Rootshield application (Figures 4.1 and 4.2). For objective three, a simple RCBD 4x2x2 factorial design was used, blocked by table. One of sixteen treatments (*R. solani*Substrate*Rootshield) was applied to each pot and 64 pots (n=4 per treatment) randomized by table. There were two blocks (tables) (Figure 4.3).
Table 4.2. Treatment groups for the effect of WF blends on the efficacy of Rootshield WP®’s

<table>
<thead>
<tr>
<th>Treatment</th>
<th>R. solani infestation (Main plot)</th>
<th>Subplot Treatment</th>
<th>Substrate Blend (Subplot effect)</th>
<th>Rootshield® WP Application (Subplot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>1</td>
<td>Peatlite 70:30 (Control)</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>2</td>
<td>Peat 70% WF 30%</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>3</td>
<td>Peat 80% WF 20%</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>4</td>
<td>Peat 90% WF 10%</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>Yes</td>
<td>5</td>
<td>Peatlite 70:30 (Control)</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>Yes</td>
<td>6</td>
<td>Peat 70% WF 30%</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>Yes</td>
<td>7</td>
<td>Peat 80% WF 20%</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>Yes</td>
<td>8</td>
<td>Peat 90% WF 10%</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>No</td>
<td>1</td>
<td>Peatlite 70:30 (Control)</td>
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</tr>
<tr>
<td>10</td>
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<td>2</td>
<td>Peat 70% WF 30%</td>
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</tr>
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<tr>
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<td>6</td>
<td>Peat 70% WF 30%</td>
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<td>15</td>
<td>No</td>
<td>7</td>
<td>Peat 80% WF 20%</td>
<td>No</td>
</tr>
<tr>
<td>16</td>
<td>No</td>
<td>8</td>
<td>Peat 90% WF 10%</td>
<td>No</td>
</tr>
</tbody>
</table>

Figure 4.1. Growth Chamber and Radish: Radish bioassay set up in growth chamber (B). Numbered pots (A) 1-8 on pots represent the subplot randomization of the eight substrate*Rootshield application treatments. Within replicate experiment, there were four pots (n=4) per treatment for a total of n=16 pots per treatment.
Figure 4.2. Greenhouse chrysanthemum crown and root rot bioassay: (A) Full RCBD assessing crown and root rot disease in mature chrysanthemums. Blocking was by table (4), with the main effect of infestation (*R. solani* infested or none) applied to each half-table section. Numbered pots (1-8) represent subplot randomization (B). Within each table, there were four pots (n=4) per treatment for a total of n=16 per treatment. Visual representation of A and B, with the full RCBD (C) and one block (D) respectively.

Figure 4.3. Greenhouse and chrysanthemum: Chrysanthemum crown and root rot bioassay in “young” chrysanthemum. Blocking was by table (2), with 64 pots per table. Numbered pots represent one of 16 treatments. n=4 per treatment per table, 8 total per treatment (*R. solani*+Substrate+RootShield) applied to each pot and
4.2.2 PREPARATION OF SUBSTRATE TREATMENTS

Substrates blends were prepared by mixing each WF with raw sphagnum peat (H3-H4 Promix, Premier Tech, Canada) as described in Chapter Two and detailed in Appendix A. Substrate pH was adjusted to achieve a pH of 5.4-6.4 with the target being 5.8 (Nelson, 2012; Jackson, 2016) and SMC of all blends fell within the desired range of 50-60±5%. Substrates were used to fill pots one-week post preparation. Total porosity, container capacity, air space, bulk density, and initial moisture content, were measured for each substrate blend following the North Carolina State University Porometer method (Fonteno et al., 1995).

For chrysanthemum experiments, additional corrective actions were taken to raise substrate pH, which dropped dramatically after transplant. These actions included a switch from a balanced nitrate-ammoniacal fertilizer (17-4-17 (Pure Water LX, JR Peters Inc., PA.) at 150ppm) to calcium nitrate fertilizer (Poinsettia FeED, JR Peters Inc, PA, USA, 15-4-15 at 150 ppm) and an eight ounce (~227mL) flowable lime drench (LimestoneF, Clearly, NJ) applied at a 1:100 rate (3.785L/ 378.5L). A second flowable lime drench was applied to only pots containing the peatlite treatment at a 1.5:100 rate (5.878L/378.5L) (CalFlow, Burnett Company Inc, Campobello, SC). Once mums were transplanted into pots, pH and EC were measured weekly, with samples collected using the pour-through method (LeBude and Bilderback, 2009) rather than SME.

4.2.3 PATHOGEN PREPARATION

For the radish damping-off bioassay R. solani isolate RS-33 (isolated from radish) was obtained from Ohio State University and identified as anastomosis group (AG) 2-1 HK clade (sequenced by Dr. James Woodhall at the University of Idaho using rDNA-ITS techniques). For the chrysanthemum crown and root rot bioassay, R. solani isolate AG 4 HG-I (isolated from bean)
was also received and sequenced by Dr. James Woodhall at the University of Idaho using rDNA-ITS techniques. Sclerotia of isolates were stored in -80°C freezer and grown on Potato Dextrose Agar (PDA) media plates (39 g/1L DO water) one to two weeks before preparation of the inoculum and the pathogen was prepared as a rice grain inoculum as described in Chapter Two. To confirm that symptoms observed were caused by *R. solani*, pathogen identity was confirmed by root plating and light microscopy to identify the diagnostic right-angle branching hyphae as described in Chapter Two.

4.2.4 PLANT PREPARATION AND EXPERIMENTAL SET-UP

**RADISH BIOASSAY**

Pots were filled in the following order: 1) substrate, 2) Rootshield or water, then two days later 3) radish seed, 4) *R. solani* inoculum was drizzled on top of pots and then 5) randomized trays placed in growth chamber for one week as described in Chapter Three. Approximately two days before seeding and *R. solani* infestation, Rootshield®WP (Bioworks, Victor, NY) (or tap water) was applied as a soil drench at 1.93 g/L of water in one replicate experiment and at 0.4g /L in other replicates. One hundred mL of the Rootshield suspension was applied to each pot and 100 mL of tap water was applied to control pots. In addition to the Rootshield drench or tap water control, all pots were hand-watered at the time of seeding (but prior to *R. solani* infestation) with ~150 mL of tap water.
CHRYSANTHEM BIOASSAY

1) Establishment of unrooted cuttings and transplant

Two sets of chrysanthemum experiments were conducted. The first used unrooted cuttings (URCS) received from Dummen Orange and the second used cuttings taken from plants grown from original URCS.

Unrooted cuttings of ‘Breeze Yellow’ Chrysanthemum (Chrysanthemum x morifolium) (Dummen Orange, Columbus OH) were rooted in Oasis Cubes (Smithers Oasis CO, Walterboro, SC) as detailed in Chapter Two. For the second set of experiments, URCS cut from the original mother chrysanthemum plants were similarly rooted into one of four respective substrate treatments (peat:perlite 70:30, peat:WF 70:30, 80:20, or 90:10). URCS were placed under natural light for eight days (Oasis Cubes) or two weeks (peatlite or peat:WF) and misted at an increasingly longer misting interval or light threshold to allow roots to establish. URCS were taken off of mist but kept in the humidity of the propagation house for another seven to ten days and overhead watered twice daily at a rate of 17-4-17 (Pure Water LX, JR Peters Inc., PA) at 100 ppm N to reduce the risk of transplant shock (Figure 2.4).

Approximately two weeks after receiving chrysanthemum URCS, roots were established and cuttings were transplanted to into 15 cm tall Euro Black Pots (The HC Companies, Twinsburg, OH) (mature mums) three-quarters full with their respective WF:peat or perlite:peat substrate treatment or into 11 cm pot (4.5in (~11cm) STD Thermo, The HC Companies, Boise, ID)(young mums). Transplants were overhead fertilized for two days at 17-4-17 (Pure Water LX, JR Peters Inc., PA) at 300 ppm N for to adjust for young leaf chlorosis. The Pure Water LX (JR Peters Inc., PA) contained 3.73% ammoniacal nitrogen and 13.27% nitrate nitrogen. Plants were then switched to driplines and fertigated at the same rate for three days. Fertigation was then
lowered to 17-4-17 (Pure Water LX, JR Peters Inc., PA,) at 150 ppm N (balanced nitrate-ammonical fertilizer) at rate of 40 mL/min four times a day for a total of 160 mL. One week later, to help raise and then maintain pH, pots were switched to a calcium nitrate fertilizer (Poinsettia FeED, JR Peters Inc, PA, USA, 15-4-15 at 150 ppm N) at the same irrigation rate. This rate was then adjusted in accordance with plant development. For the first set of experiments, approximately one week after transplant, mums were also given either a “hard” pinch (B) or “soft” pinch (C) by cutting off the top growth of each plant to maintain uniform height and promote vertical growth of plants.

2) **Greenhouse environmental conditions**

Chrysanthemums were grown in the University of New Hampshire Macfarlane greenhouse from April 1st through June 25th, 2021 and May 31st through June 14th, 2021. For the mums grown in April, temperature was kept at 70°F (~21°C) during the day, 65°F (18.3°C) at night with the light window 5am-7pm (5:00-19:00); lights turned on when light dropped below 150wm⁻² (~315 μmol m⁻² s⁻¹). However, as the days lengthened, these controls were removed. Average greenhouse temperatures were 72.0°F (~22.2°C), average light was 374 μmol m⁻² s⁻¹, and average relative humidity 52.7%. Once plants were infested with *R. solani*, a humidity chamber was constructed using growth tunnel plastic (SunMaster, FarmTek) placed over a PVC-pipe cage to increase relative humidity (Figure 2.6). Under plastic relative humidity was an average 72.5% and average temperature 81.5°F (27.5°C). For the ‘young’ mum experiments, humidity chambers were not used and average temperature was 76.8°F (~24.9°C), average light was 348 μmol m⁻² s⁻¹, and average relative humidity 54.80%.
3) Rootshield® WP application

For chrysanthemums used for objective 2, Rootshield® WP (Bioworks, Victor, NY) was applied four days post-transplant as a soil drench at the recommended rate of 0.4 g/L. A drench of either 250 mL (water mixed with Rootshield® WP) or control water was added to every pot and irrigation was paused for the next 12 hours to allow Rootshield® WP to establish. A second application of Rootshield® WP was applied at the same rate six weeks later, which was one week prior to *R. solani* infestation. For the second set of experiments, Rootshield was applied only once, at the same rate and at the time of transplant.

4) Irrigation, pH & EC monitoring, and Disease Management

For mature chrysanthemum experiments, four days after transplant and overhead irrigation, dripline fertigation of pots began at a rate 17-4-17 (Pure Water LX, JR Peters Inc., PA.) 150 ppm N at rate of 40 mL/min four times a day for a total of 160 mL. This rate was progressively increased to adjust for plant growth and increasingly hot, humid, and bright conditions. Specifically, plants were watered for the majority of their production cycle (April 24th-June 25th) at a rate of 40 mL/min on 2.5 minute irrigation intervals six times a day (8:00h, 12:00, 15:00, 18:00, 20:00) for a daily total of 12.5 minutes or 500mL water per pot per day. For young mums, transplants were similarly overhead irrigated and then switched to dripline fertigation of pots began at a rate 17-4-17 (Pure Water LX, JR Peters Inc., PA.) 150 ppm N at a rate of two one minute intervals of 40 mL/min for a total of 80mL of water per pot per day.

Optimal substrate pH for chrysanthemum growth is 5.5-6.3 (Whipker et al., 2020), however for mature chrysanthemum work, peatlite pH was lower than this optimal range at time of transplant and dropped significantly when fertilizer was applied. pH and EC were recorded weekly: first by soil media extract (SME; Warncke, 1986; University of Minnesota, 2020) taken
from substrates after blending and then later on (once mums were transplanted) using the pour-thru method (LeBude and Bilderback, 2009). Corrective actions noted previously were taken to correct low substrate pH. These included a switch to a calcium nitrate fertilizer (Poinsettia FeED, JR Peters Inc, PA, USA, 15-4-15 at 150 ppm N) and an 8oz (~227mL) flowable lime drench (LimestoneF, Clearly, NJ) applied at a rate of 1:100 rate. A second flowable lime drench was applied two weeks later only to the peatlite pots at a 1.5:100 rate (CalFlow, Burnett Company Inc, Campobello, SC).

As detailed in Chapter Two, insect biocontrols were sprinkled weekly on top of young plants and later on applied as Swirskii-Breeding-System sachets (*Amblyseius swirskii*) (BioBest, Westerlo, Belgium) hung on mature plants. Yellow sticky cards (BASF Corporation, Research Triangle Park, NC) were placed in the greenhouse and plants were treated every two weeks with preventative soil drench applications of *Steinernema feltiae* (150,000-200,000 nematodes per plant) (BioBest, Westerlo, Belgium) to control fungus gnats.

5) **Pathogen infestation**

Rice inoculum (isolate AG-4 HG-I; received from Dr. Woodhall at the University of Idaho) was added at a rate of 1.0g/L to chrysanthemums. Chrysanthemums were infested at 5-6 weeks post-transplant (mature plants) or one-week post-transplant (young plants). The inoculum was pulverized applied as detailed in Chapter Two. For each 15cm ~1.56L pot, 1.6 of *R. solani* rice grains were added and for 11 cm pots (~0.5L), 0.5 grams were added per pot.
4.2.5 *DATA COLLECTION:*

**RADISH BIOASSAY**

**Disease Assessment:** Damping-off severity was determined 7 days after seeding based on a disease severity scale developed by Krause et al. (2001) (Figure 4.4; Chapter Two) 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, and 5 = pre-emergence damping-off. For each pot, the 32 seeds were assessed on the categorical scale (Figure 4.4) and then averaged to calculate the mean disease severity per pot. For each pot, natural germination was calculated as the percent of “1”s and “2”s out of the 32 seed subsamples.

**Figure 4.4. Damping-off disease scale developed from Krause et al. (2001).**

**Figure 4.5. Examples of “4” disease rating: post-emergent damping-off.** A rating of 4 was characterized by blackened stem and girdling of greater than 50%.
**Total aboveground biomass:** Damping-off rates are not directly correlated with aboveground biomass. For example, a “4” or post-emergent damping-off is characterized by blackened stem and girdling greater than 50% regardless of aboveground stem health (Figure 4.5). To account for this discrepancy, total aboveground biomass was recorded 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).

**CHRYSTANTHEM BIOASSAY**

**Disease Assessment:** Disease progress was checked weekly. For objective two with “mature mums” (infested with *R. solani* at age six weeks post-transplant), disease assessment was made six-seven weeks after infestation. For objective three, chrysanthemums were infested with *R. solani* one week after transplant and Rootshield application (i.e. ‘young mums’) and disease assessment was made two weeks after infestation. Crown and root rot disease severity were measured according to the scale adapted from Krause et al. (2001). The scale, developed while testing *R. solani* isolates for pathogenicity (Chapter Two), was 1-6 in which: 1 = symptomless, 2 = mild root rot OR crown rot, 3 = mild root rot AND mild crown rot, 4 = severe crown rot OR severe root rot, 5 = severe crown rot AND severe root rot, 6 = dead plant (Figure 4.6)

![Figure 4.6. Full 1-6 crown and root rot disease scale](image)

*Figure 4.6. Full 1-6 crown and root rot disease scale* for work performed on chrysanthemums infested with *R. solani* one week after transplant ‘young mums.’
Table 4.3. Crown and root rot disease scale (1-6) on young chrysanthemum developed in collaboration with M. Krause. Radish damping off and mature chrysanthemum scales detailed in Chapter Two.

<table>
<thead>
<tr>
<th>Scale</th>
<th>Description</th>
</tr>
</thead>
</table>
| 1     | **symptomless**  
|       | - Healthy plant  
|       | - No observable root or crown rot |
| 2     | mild crown rot OR mild root rot  
|       | - Image depicts mild crown rot with no root rot |
| 3     | mild crown AND mild root rot |
| 4     | severe crown rot OR severe root rot  
|       | - Image depicts severe crown rot |
| 5     | severe crown rot AND severe root rot |
| 6     | dead plant |
4.2.6 DATA ANALYSIS

All analyses were performed in R version 3.6.2 (2019-12-12) -- "Dark and Stormy Night"
Copyright (C) 2019. Analysis of variance (ANOVA) was used to assess differences in physical
and chemical properties among substrate blends and assumptions of ANOVA (normality of
residuals and homogeneity of variances) were met. Analysis of variance (ANOVA) was also
used to determine the effects of substrate blend and inoculation treatment on damping-off
severity. Mean disease severity which ranged in discrete categories from 1 to 5, was transformed
to \( R^* = (R^{1.5} - 1)/1.5 \) (Krause et al., 2001) to obtain a linear scale and meet the assumptions of
ANOVA: normality of residuals, homogeneity of variances, and block-factor additivity. Data
were only transformed once and homogeneity of variance was always met; however, in some
instances, residuals were not normally distributed, but ANOVA is robust to deviations from
normality. The mix treatment factors included substrate treatment (either WF processing type or
percent blend) and \( R. solani \) infestation (yes/no). Simple effects analyses and Tukey HSDs were
performed to determine differences in 1) transformed mean disease severity and 2) aboveground
fresh weight biomass (g) in substrate treatments within and without \( R. solani \) infestation. Natural
germination rates with the non-infested treatment group were also compared using ANOVA,
simple effects analyses, and Tukey HSDs. Given significant block:factor interactions
(\( p \leq 0.000369 \)), including block:biopesticide application (\( p = 0.000369 \)), replicate experiment one
was analyzed separately from replicate experiments two-four. The significant interactions are
attributed to the rate of Rootshield applied, which was higher (1.93 g/L) in replicate one than in
the following three (0.4 g/L).

For the chrysanthemum bioassay, analysis of variance (ANOVA) was also used to
determine the effects of \( R. solani \) isolate and inoculum dose on damping-off severity. Mean
disease severity which ranged in discrete categories from 1 to 6, was transformed to $R^* = (R^{1.5} - 1)/1.5$ (Krause et al., 2001) to obtain a linear scale and meet the assumptions of ANOVA: normality of residuals, homogeneity of variances, and block-factor additivity. The mix treatment factors included Rootshield application (2) and substrate (4). Factorial ANOVA and Tukey HSD were performed to compare differences in transformed mean disease severity.
4.3 Results

4.3.1 SUBSTRATE PHYSICAL AND CHEMICAL PROPERTIES ANALYSES

Substrate blends were created for each replicate experiment performed with goal soil moisture content of 50-60±±5 and pH between 5.5-6.0 with the target being 5.8. However, there was inherent variability across substrate blends. Substrate chemical and physical properties are reported in Tables 4.4 and 4.5 and Figures 4.7 and 4.8.

Table 4.4. Mean chemical properties of the four substrate treatments used in radish bioassay study±standard errors (n=3). Substrates were amended at a rate of 6 lbs/yd³ (~3.56 kg/m³). pH generally fell within the desired range and those recorded in previous work on PTS wood fiber substrates (Jackson et al., 2009).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>SMC (%)</th>
<th>pH</th>
<th>EC (mS/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peatlite (70/30)</td>
<td>53.44±1.25a</td>
<td>5.35±0.16b</td>
<td>0.215±0.039a</td>
</tr>
<tr>
<td>Peat:PTS WF (70/30)</td>
<td>54.96±1.04a</td>
<td>5.82±0.12a</td>
<td>0.221±0.035a</td>
</tr>
<tr>
<td>Peat:PTS WF (80/20)</td>
<td>57.23±1.35a</td>
<td>5.46±0.08b</td>
<td>0.210±0.038a</td>
</tr>
<tr>
<td>Peat:PTS WF (90/10)</td>
<td>57.86±1.13a</td>
<td>5.57±0.05ab</td>
<td>0.224±0.215a</td>
</tr>
</tbody>
</table>

Letters indicate significant differences among substrates for each property (Tukey HSDs, α=0.05, MSD	}_{SMC}= 4.484, MSD_{pH}= 0.3455, MSD_{EC}=0.0244)

Table 4.5. Mean physical properties of substrate treatments study±standard errors (n=3) used across objectives. All blends consisted of 70 Peat 30 WF or perlite (% by volume). Total porosity, container capacity, air space, bulk density, and initial moisture content were measured for each substrate blend one to two months after blending by Brian Jackson following the North Carolina State University Porometer method (Fonteno et al., 1995).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Total porosity¹</th>
<th>Container capacity²</th>
<th>Air space³</th>
<th>Bulk Density (lbs/ft³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peatlite 70/30</td>
<td>78.7±0.84</td>
<td>61.6±0.58ab</td>
<td>17.2±0.93</td>
<td>5.9±0.06⁶</td>
</tr>
<tr>
<td>Pine Tree Substrate (PTS) 70/30</td>
<td>83.5±1.70</td>
<td>67.7±1.04a</td>
<td>15.8±0.72</td>
<td>6.0±0.06b</td>
</tr>
<tr>
<td>Pine Tree Substrate (PTS) 80/20</td>
<td>79.7±3.42</td>
<td>63.8±1.40ab</td>
<td>15.9±2.05</td>
<td>8.6±0.43a</td>
</tr>
<tr>
<td>Pine Tree Substrate (PTS) 90/10</td>
<td>80.1±1.04</td>
<td>59.6±2.74b</td>
<td>20.5±2.08</td>
<td>6.8±0.31b</td>
</tr>
</tbody>
</table>

¹ Total porosity is equal to container capacity/ air space (%vol)
² Container capacity is (wet weight - oven dry weight) / volume (%vol)
³ Air space is the volume of water drained from the sample / volume of sample (%vol)

For container capacity and bulk density, letters indicate significant differences among substrates (Tukey HSDs, α=0.05, MSD_{container capacity}= 7.465289, MSD_{bulk density}= 1.200585). Total porosity (p= 0.411 ANOVA F₃,₈=1.079) and Air space (p= 0.189, ANOVA F₃,₈= 2.026) were not significantly different among substrates
Figure 4.7. pH (A) and EC (mS/cm) (B) of substrates used in mature chrysanthemum bioassay amended with dolomitic lime at a rate of 6 lbs/yd3 (~3.56 kg/m3) and tracked over the course of the experiment on “mature” chrysanthemums. pH was within the desired pH range at transplant, but dropped after transplant, when fertigation began (Week 2). Corrective actions included switching to nitrate-based fertilizer and flowable lime drenches. EC dramatically increased with fertigation, then leveled out.
Figure 4.8. pH (A) and EC (mS/cm) (B) of substrates used in young chrysanthemum bioassay. WF substrates were amended with dolomitic lime at a rate of 8lbs/yd$^3$ (~4.75 kg/m$^3$) and peatlite peatlite amended 9lbs/yd$^3$ (~5.34 kg/m$^3$). pH fell within range at time of transplant (Week 2) and remained level throughout the plant production cycle with calcium nitrate fertilizer applications (Poinsettia FeED, JR Peters Inc, PA, USA, 15-4-15 at 150 ppm N).
OBJECTIVE ONE: RADISH BIOASSAY

Analysis of disease data for radish bioassay showed a significant interaction effect of block*biosticide application (p=1.702x10^-5 ANOVA F(3,192)=8.80)(Table. This was due to differences in application rate (1.93g/L) of Rootshield® WP in the first replicate experiment. Therefore, replicate one was analyzed separately from replicates 2-4 (rate 0.4g/L) to account for this difference in application.

4.3.2 SUBSTRATE DOES NOT AFFECT NATURAL GERMINATION RATE

Natural germination rates of radish were calculated as a percent of plants scored as “1”s and “2”s on the damping-off scale out of 32 seeds per pot under control (no R. solani) conditions. Germination was similar across treatments (p=0.324 ANOVA F(7,119)=1.1662) although lower than the 90% germination rate for the variety. This lower germination is attributed to age of seeds (Table 4.6).

Table 4.6. Natural germination rates were similar across treatments (p=0.324 ANOVA F(7,119)=1.1662) although lower than the 90% germination rate for the variety. This lower germination is attributed to age of seeds.

<table>
<thead>
<tr>
<th>Rootshield Application</th>
<th>Peatlite</th>
<th>70/30</th>
<th>80/20</th>
<th>90/10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>86.9±2.6</td>
<td>86.9±2.1</td>
<td>81.4±2.7</td>
<td>83.4±3.1</td>
</tr>
<tr>
<td>Control</td>
<td>85.4±2.0</td>
<td>78.7±3.9</td>
<td>83.6±2.7</td>
<td>80.5±4.3</td>
</tr>
</tbody>
</table>

4.3.3 DAMPING-OFF DISEASE SEVERITY AT HIGH ROOTSHIELD® WP APPLICATION RATE

R. solani infestation (p=1.18e-5), substrate treatment (p=0.00933), and Rootshield application (p=1.2e-5) had a significant effect on damping-off disease severity; however, due to a significant interaction effect between infestation and substrate (p=0.01539, ANOVA
simple effects ANOVAs were performed to assess disease severity across substrates under *R. solani* infestation and no infestation (Table 4.7).

Under *R. solani* infestation, there was a significant effect of biopesticide (*p*=8.912e-05 ANOVA $F_{1,24}$=22.0868), but not substrate (*p*=0.9521 ANOVA $F_{3,24}$=0.1122) or substrate x biopesticide treatment (*p*= 0.3593, ANOVA $F_{3,24}$= 1.1233) on disease severity. However, under no infestation, there were significant effects of both substrate (*p*= 0.01104 ANOVA $F_{3,24}$=4.6069) and biopesticide (*p*= 0.02415 ANOVA $F_{1,24}$=5.7921) separately on disease severity. As with *R. solani* infestation, under no infestation there was no interaction effect of substrate*biopesticide treatment (*p*= 0.48711, ANOVA $F_{3,24}$= 0.8365) on damping disease severity. Examining differences further, plants treated with Rootshield and grown in the 80/20 peat:WF blend exhibited significantly lower damping-off disease severity (3.66±0.12, *p*<0.05) compared to plants in the non-Rootshield 80/20 peat:WF control (4.25±0.16). Plants in all other substrate treatments had similar damping-off disease ratings regardless of Rootshield application (Table 4.8). For experiment one, total disease severity was lower in pots under *R. solani* infested pots and higher in non-infested (control) pots than for experiments two-four (Tables 4.8 and 4.10).
Table 4.7. Full factorial ANOVA performed to assess main effect of *R. solani* infestation (infested or non-infested) and subplot effect of WF percent blend and Rootshield treatment (1.93g/L) on radish damping-off disease severity in a split-plot RCBD. Because there was a significant interaction effect of infestation and substrate (*p*=0.01539, ANOVA $F_{3,42}=3.887$), simple effects ANOVAs were performed to assess disease severity across substrates under *R. solani* infestation and no infestation ($n=4$ per treatment).

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. solani</em> infestation</td>
<td>1</td>
<td>157.80</td>
<td>157.80</td>
<td>173.4</td>
<td>1.18e-05</td>
</tr>
<tr>
<td>Residuals*</td>
<td>6</td>
<td>5.46</td>
<td>0.91</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Split-plot analysis uses Block:Isolate as error term for main effect

Table 4.8. Disease severity 7 days post infestation at high application (1.93g/L) of Rootshield®WP. Damping-off disease severity in *R. solani* infested versus non-infested (control) pots under different ratio blends and Rootshield®WP application ($n=4$). Mean original disease severity (M) (categorical #1-5) was transformed to a linear scale ($M^{1.5}-1)/1.5$ (Krause et al. 2001).

<table>
<thead>
<tr>
<th>R. solani infestation</th>
<th>Non-infested (No R. solani)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rootshield</td>
<td>Control</td>
</tr>
<tr>
<td>Peatlite</td>
<td>3.91±0.07, ab</td>
</tr>
<tr>
<td>70/30</td>
<td>3.73±0.09, ab</td>
</tr>
<tr>
<td>80/20</td>
<td>3.66±0.12, a</td>
</tr>
<tr>
<td>90/10</td>
<td>3.80±0.12, ab</td>
</tr>
</tbody>
</table>

Simple effects analyses were performed within *R. solani* infestation (infested or control) and letters indicate significant differences in disease severity within infested and non-infested groups (Tukey HSDs $a=0.05$, $n=4$ MSD$_{infested}=1.098814$, MSD$_{noninfested}=1.800972$).
4.3.4 ABOVEGROUND FRESHWEIGHT BIOMASS AT HIGH ROOTSHIELD® WP APPLICATION

Due to a significant interaction effect between infestation and substrate (p=0.040542, ANOVA F₃,₄₂=3.013) on radish aboveground biomass, simple effects ANOVAs were performed to assess disease severity across substrates under *R. solani* infestation and no infestation (Table 4.9).

Under both *R. solani* (p=0.595398, ANOVA F₃,₂₄=0.6421) and non-infested pots (p=0.71104, ANOVA F₃,₂₄=0.4626), there was not a significant Rootshield application*substrate treatment interaction effect on aboveground biomass. However, Rootshield application alone had a significant effect on aboveground biomass under both *R. solani* infestation (p=0.001742, ANOVA F₁,₂₄=12.409) and for non-infested pots (p=0.02355, ANOVA F₁,₂₄=5.8476). Interestingly, there was no significant effect of substrate alone on aboveground biomass under *R. solani* infestation (p=0.710867, ANOVA F₃,₂₄=0.4628) (Table 4.10), but under no *R. solani* (control), there were significant (p=9.715e-05 ANOVA F₃,₂₄=11.0107) differences in aboveground biomass among substrate treatments. Specifically, radish biomass was lower in both 80/20 (1.18±0.12) and 90/10 (1.33±0.19) without Rootshield than in both peatlite controls (Table 4.10). Regardless of infestation, aboveground biomass of radishes was higher in pots with Rootshield compared to pots with the same substrate treatment and no Rootshield applied, but not significantly so (p>0.05 Tukey HSDs MSDinfested=0.924477; MSDnoninfested=1.179925; Table 4.10).
Table 4.9. Full factorial ANOVA performed to assess main effect of *R. solani* infestation (infested or non-infested) and subplot effect of WF percent blend and Rootshield treatment (1.93g/L) on radish fresh aboveground biomass in a split-plot RCBD. Because there was a significant interaction effect of infestation and substrate (p=0.040542, ANOVA F<sub>3,42</sub>=3.013), simple effects ANOVAs were performed to assess disease severity across substrates under *R. solani* infestation and no infestation (n=4 per treatment).

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. solani</em> infestation</td>
<td>1</td>
<td>0.0011</td>
<td>0.001</td>
<td>0.003</td>
<td>0.956</td>
</tr>
<tr>
<td>Residuals*</td>
<td>6</td>
<td>1.9488</td>
<td>0.3248</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Split-plot analysis uses Block:Isolate as error term for main effect*

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td>3</td>
<td>3.803</td>
<td>1.268</td>
<td>6.754</td>
<td>0.000806</td>
</tr>
<tr>
<td>Rootshield</td>
<td>1</td>
<td>3.725</td>
<td>3.725</td>
<td>19.844</td>
<td>6.12e-05</td>
</tr>
<tr>
<td>Infestation x Substrate</td>
<td>3</td>
<td>1.697</td>
<td>0.566</td>
<td>3.013</td>
<td>0.040542</td>
</tr>
<tr>
<td>Infestation x Rootshield</td>
<td>1</td>
<td>0.336</td>
<td>0.336</td>
<td>1.792</td>
<td>0.187863</td>
</tr>
<tr>
<td>Substrate x Rootshield</td>
<td>3</td>
<td>0.301</td>
<td>0.100</td>
<td>0.535</td>
<td>0.661141</td>
</tr>
<tr>
<td>Infestation x Rootshield x Substrate</td>
<td>3</td>
<td>0.404</td>
<td>0.135</td>
<td>0.718</td>
<td>0.546879</td>
</tr>
<tr>
<td>Residuals</td>
<td>42</td>
<td>7.884</td>
<td>0.188</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.10 Aboveground biomass of radish 7 days post infestation at high application (1.93g/L) of Rootshield® WP. Aboveground biomass was not significantly different under *R. solani* infestation (p=0.710867, ANOVA F<sub>3,24</sub>= 0.4628) (n=4 per treatment).

<table>
<thead>
<tr>
<th></th>
<th>R. solani infestation</th>
<th>Non-infested (No R. solani)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rootshield</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Rootshield</td>
<td>Control</td>
</tr>
<tr>
<td>Peatlite</td>
<td>2.05±0.22</td>
<td>1.7±0.28</td>
</tr>
<tr>
<td>70/30</td>
<td>2.04±0.24</td>
<td>1.28±0.24</td>
</tr>
<tr>
<td>80/20</td>
<td>2.18±0.24</td>
<td>1.21±0.35</td>
</tr>
<tr>
<td>90/10</td>
<td>1.81±0.26</td>
<td>1.38±0.14</td>
</tr>
</tbody>
</table>

Letters indicate significant group differences (Tukey HSD MSD<sub>noninfested</sub>=1.179925) under control conditions and error bars are ±standard error.

4.3.5 DAMPING-OFF DISEASE SEVERITY AT RECOMMENDED (0.4G/L) ROOTSHIELD® WP APPLICATION

Due to a significant interaction effect between infestation and substrate (p=0.00108, ANOVA F<sub>3,172</sub>=5.612) on damping-off disease severity, simple effects ANOVAs were performed to assess
disease severity across substrates under \( R.\ solani \) infestation and no infestation (Table 4.11). Under \( R.\ solani \) infestation, there was a significant effect of Rootshield (\( p= 0.003344, \) ANOVA \( F_{1,86} = 9.1101 \)) and substrate (\( p= 5.645e-16, \) ANOVA \( F_{3,86} = 38.8772 \)) on disease severity but no interaction effect (\( p= 0.819932, \) ANOVA \( F_{3,86} = 0.8226 \)), which does not support our hypothesis that there is a synergistic effect of WF\% and Rootshield, as \( T.\ harzianum \) was less effective at higher WF\%.

While there were no significant differences in disease severity under control conditions (Tukey HSD \( a=0.05, n=12, MSD_{\text{noninfested}} = 0.610 \); Table 4.12), radish grown in all WF blends, regardless of Rootshield application, had significantly lower disease severity than both the peatlite and peatlite+Rootshield controls (\( p<0.05 \) Tukey-HSD \( n=12, MSD_{\text{infested}} = 0.382 \); Figure 4.6). Within WF blends, there was no difference in disease severity between WF+Rootshield blends and their control counterparts (70/30, 80/20, 90/10) without Rootshield (\( p<0.05 \) Tukey-HSD \( n=12, MSD_{\text{infested}} = 0.382 \); Figure 4.6). Moreover, while disease severity under \( R.\ solani \) infestation was lowest in 90/10 WF+Rootshield (4.24±0.05, a), there was no significant difference in disease severity among WFs with Rootshield applied (4.39±0.05 ab for 70/30 and 4.38±0.06 80/20 ab).
Table 4.11. Full factorial ANOVA performed to assess main effect of *R. solani* infestation (infested or non-infested) and subplot effect of WF percent blend and Rootshield treatment (0.4g/L) on radish damping-off disease severity in a split-plot RCBD. Because there was a significant interaction effect of infestation and substrate (p=0.00108, ANOVA F<sub>3,172</sub>=5.612), simple effects ANOVAs were performed to assess disease severity across substrates under *R. solani* infestation and no infestation (n=12 per treatment).

<table>
<thead>
<tr>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. solani infestation</td>
<td>1</td>
<td>1117.4</td>
<td>1117.4</td>
<td>1652.346</td>
</tr>
<tr>
<td>Block (Replicate Experiment)</td>
<td>2</td>
<td>2.3</td>
<td>1.1</td>
<td>1.666</td>
</tr>
<tr>
<td>Residuals*</td>
<td>2</td>
<td>1.4</td>
<td>0.7</td>
<td></td>
</tr>
</tbody>
</table>

*Split-plot analysis uses Block:Isolate as error term for main effect

Table 4.12. Damping-off disease severity in *R. solani* infested versus non-infested (control) pots under different substrate blend ratios with and without Rootshield WP. For each set of experiment, a separate 70/30 peatlite control was blended. Mean original disease severity (M) (categorical #1-5) was transformed to a linear scale (M<sup>-1</sup>/1.5) (Krause et al., 2001).

<table>
<thead>
<tr>
<th>R. solani infestation</th>
<th>Non-infested (No R. solani)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rootshield Control</td>
<td>Rootshield Control</td>
</tr>
<tr>
<td><strong>Peatlite</strong></td>
<td></td>
</tr>
<tr>
<td>4.66±0.04, c</td>
<td>4.78±0.03, c</td>
</tr>
<tr>
<td>4.39±0.05, ab</td>
<td>4.45±0.04, b</td>
</tr>
<tr>
<td>4.38±0.06, ab</td>
<td>4.42±0.05, ab</td>
</tr>
<tr>
<td>4.24±0.05, a</td>
<td>4.36±0.03, ab</td>
</tr>
</tbody>
</table>

Letters indicate significant differences in disease severity within infestation (Tukey HSDs α=0.05, n=12 MSD<sub>infested</sub>= 0.382), with no significant differences in disease severity found under control conditions (p= 0.4338, ANOVA F<sub>7,36</sub>= 1.0046) (n=12 per treatment).
4.3.6 ABOVEGROUND FRESHWEIGHT BIOMASS AT RECOMMENDED (0.4G/L) ROOTSHIELD® WP APPLICATION

Although there was not a significant interaction effect of infestation and substrate (p=0.530, ANOVA F$_{3,42}$=0.738), simple effects ANOVAs were performed to assess disease severity across substrates under *R. solani* infestation and no infestation (Table 4.13).

Under *R. solani* infestation, there was a significant effect of substrate (p<2.2e-16 ANOVA F$_{3,86}$=46.8434), but not of Rootshield application (p=0.3263 ANOVA F$_{1,86}$=0.9746) on aboveground biomass and there was also no significant interaction effect between Rootshield application and substrate treatment (p=0.8054 ANOVA F$_{3,86}$=0.3276). Similarly, for non-infested pots, there was a significant effect of substrate (p=1.397e-06 ANOVA F$_{3,86}$=11.8748), but not of Rootshield application (p=0.6197372 ANOVA F$_{1,86}$=0.2480) on aboveground biomass and there was also no significant interaction effect between Rootshield application and substrate treatment (p=0.7547997 ANOVA F$_{3,86}$=0.3981).

In examining individual substrate treatments under *R. solani* infestation, radish aboveground biomass of radish was significantly lower in both peatlite (Rootshield® WP and none) compared to all WF blends regardless of Rootshield® WP application (p<0.05; Table 4.14). However, aboveground biomass was also significantly lower in both peatlite treatments under control conditions (no *R. solani* infestation). Under both *R. solani* infestation and control conditions, radishes grown in WFs had similar aboveground biomass regardless of Rootshield application (Table 4.14).
Table 4.13. Full factorial ANOVA performed to assess main effect of *R. solani* infestation (infested or non-infested) and subplot effect of WF percent blend and Rootshield treatment (0.4g/L) on radish aboveground biomass in a split-plot RCBD. Although there was no interaction effect of infestation and substrate (*p*=0.530, ANOVA $F_{3,172}=0.738$), simple effects ANOVAs were performed to assess disease severity across substrates under *R. solani* infestation and no infestation (*n*=12 per treatment).

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. solani</em> infestation</td>
<td>1</td>
<td>79.53</td>
<td>79.53</td>
<td>80.423</td>
<td>0.0122</td>
</tr>
<tr>
<td>Block (Replicate Experiment)</td>
<td>2</td>
<td>3.80</td>
<td>1.90</td>
<td>1.919</td>
<td>0.3426</td>
</tr>
<tr>
<td>Residuals*</td>
<td>2</td>
<td>1.98</td>
<td>0.99</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Split-plot analysis uses Block:Isolate as error term for main effect

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td>3</td>
<td>14.038</td>
<td>4.679</td>
<td>37.512</td>
<td>&lt;2e-16</td>
</tr>
<tr>
<td>Rootshield</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.995</td>
</tr>
<tr>
<td>Infestation x Substrate</td>
<td>3</td>
<td>0.276</td>
<td>0.092</td>
<td>0.738</td>
<td>0.530</td>
</tr>
<tr>
<td>Infestation x Rootshield</td>
<td>1</td>
<td>0.099</td>
<td>0.099</td>
<td>0.797</td>
<td>0.373</td>
</tr>
<tr>
<td>Substrate x Rootshield</td>
<td>3</td>
<td>0.211</td>
<td>0.070</td>
<td>0.563</td>
<td>0.640</td>
</tr>
<tr>
<td>Infestation x Rootshield x Substrate</td>
<td>3</td>
<td>0.076</td>
<td>0.025</td>
<td>0.104</td>
<td>0.894</td>
</tr>
<tr>
<td>Residuals</td>
<td>172</td>
<td>21.456</td>
<td>0.125</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.14. Aboveground biomass of radish under *R. solani* and no infestation (control) when Rootshield or tap water (control) is applied to four different substrates (Peatlite, WF 70:30, 80/20, 90/10).

<table>
<thead>
<tr>
<th>Aboveground biomass</th>
<th><em>R. solani</em> infestation</th>
<th>Non-infested (No <em>R. solani</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rootshield</td>
<td>Control</td>
</tr>
<tr>
<td>Peatlite</td>
<td>0.20±0.04, b</td>
<td>0.11±0.03, b</td>
</tr>
<tr>
<td>70/30</td>
<td>0.78±0.09, a</td>
<td>0.72±0.08, a</td>
</tr>
<tr>
<td>80/20</td>
<td>0.75±0.07, a</td>
<td>0.68±0.09, a</td>
</tr>
<tr>
<td>90/10</td>
<td>0.86±0.09, a</td>
<td>0.89±0.10, a</td>
</tr>
</tbody>
</table>

Letters indicate significant group differences within infested and non-infested pots (Tukey HSD $a=0.05$, $n=12$ MSD$_{infested}=0.288$; MSD$_{noninfested}=0.564$)±standard error.
Figure 4.9. Aboveground biomass of radish across treatment. Rootshield was applied at 0.4g/L.
OBJECTIVE TWO: CROWN AND ROOT ROT DISEASE ON ‘MATURE’ CHRYSANTHEMUM

4.3.7 THERE WAS NO SYNERGISTIC EFFECT OF WF AND ROOTSHIELD EFFICACY ON DISEASE SEVERITY

Due to significant interaction effect of *R. solani* infestation and substrate on crown and root rot severity (p=0.000266, ANOVA F$_{3,234}$=6.604) (Table 4.15), simple effects analyses were performed to assess *R. solani* infested and non-infested pots separately. There was also a trending toward significant effect of blocking (p= 0.0583, ANOVA F$_{3,3}$=8.251) and no significant interaction between substrate and Rootshield (p= 0.312514, ANOVA F$_{3,234}$= 1.6836).

Under *R. solani* infestation, there was a significant effect of substrate (p= 6.164e-09, ANOVA F$_{3,117}$= 16.3444), and trending toward significant effect of Rootshield application (p=0.1763, ANOVA F$_{1,117}$= 1.8510), but a strongly non-significant interaction effect between substrate and Rootshield application (p= 0.5981, ANOVA F$_{3,117}$= 0.6283). Chrysanthemums potted in peatlite without Rootshield application had the highest disease severity (4.19±0.14) with peatlite+Rootshield (3.94±0.19) the next highest (p>0.05 Tukey HSD MSD= 1.685515). Infested pots of 70/30 and 80/20 without Rootshield and 90/10 with Rootshield had significantly lower disease severity (p<0.05) (Table 4.16) compared to chrysanthemums potted in peatlite and peatlite+Rootshield. However, there were no significant differences in disease severity among WFs or for each WF+Rootshield compared to WF+controls. There was also no significant effect of blocking by table (p= 0.7608, ANOVA F$_{3,117}$= 0.3895).

Under control conditions, there was also a significant effect of substrate (p= 0.0008689, ANOVA F$_{3,117}$= 5.9045), and trending toward significant effect of Rootshield application (p=0.0647917, ANOVA F$_{1,117}$= 3.4755) but no significant interaction effect between substrate and Rootshield application (p= 0.2258979, ANOVA F$_{3,117}$= 1.4715). There was also no
significant effect of blocking by table (p=0.4375306, ANOVA $F_{3,117} = 0.9120$). Specifically, chrysanthemums potted in peatlite without Rootshield had significantly higher disease than chrysanthemums planted in all WFs treatments and peatlite+Rootshield (Table 4.16).

Across treatments, there were visual differences in root mass and vigor observed, with observably healthier roots seen in pots with Rootshield applied, regardless of substrate treatment and infestation (Figure 4.10). However, roots were also observably healthy across WF substrates regardless of Rootshield application, in contrast to chrysanthemums potted in peatlite, where differences in root healthy between Rootshield+peatlite and peatlite control were more distinct (Figure 4.10).

Table 4.15. Full factorial ANOVA performed to assess main effect of *R. solani* infestation (infested or non-infested) and subplot effect of WF percent blend and Rootshield treatment on crown and root rot in mature chrysanthemum (infested at age 6 weeks) in a split-plot RCBD. Although there was not a significant interaction effect of infestation and substrate (p=0.000266, ANOVA $F_{3,234}=6.604$), simple effects ANOVAs were performed to assess disease severity across substrates under *R. solani* infestation and no infestation.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. solani infestation</em></td>
<td>1</td>
<td>549.7</td>
<td>549.7</td>
<td>3469.594</td>
<td>1.08e-05</td>
</tr>
<tr>
<td>Block (Table)</td>
<td>3</td>
<td>3.9</td>
<td>1.3</td>
<td>8.251</td>
<td>0.0583</td>
</tr>
<tr>
<td>Residuals*</td>
<td>3</td>
<td>0.5</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Split-plot analysis uses Block:Isolate as error term for main effect

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td>3</td>
<td>97.9</td>
<td>32.65</td>
<td>21.954</td>
<td>1.46e-12</td>
</tr>
<tr>
<td>Rootshield</td>
<td>1</td>
<td>6.2</td>
<td>6.24</td>
<td>4.193</td>
<td>0.041704</td>
</tr>
<tr>
<td>Infestation x Substrate</td>
<td>3</td>
<td>29.5</td>
<td>9.82</td>
<td>6.604</td>
<td>0.000266</td>
</tr>
<tr>
<td>Infestation x Rootshield</td>
<td>1</td>
<td>0.2</td>
<td>0.23</td>
<td>0.152</td>
<td>0.697416</td>
</tr>
<tr>
<td>Substrate x Rootshield</td>
<td>3</td>
<td>5.3</td>
<td>1.78</td>
<td>1.195</td>
<td>0.312514</td>
</tr>
<tr>
<td>Infestation x Rootshield x Substrate</td>
<td>3</td>
<td>1.8</td>
<td>0.59</td>
<td>0.396</td>
<td>0.756252</td>
</tr>
<tr>
<td>Residuals</td>
<td>234</td>
<td>348.0</td>
<td>1.49</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.16. Crown and root rot disease severity of chrysanthemums infested with *R. solani* six weeks after transplant (mature mums) in different substrate treatments with two applications of *Rootshield® WP*: at transplant and eight weeks later. Mean original disease severity (M) (categorical #1-6) was transformed to a linear scale (M^{1.5}-1)/1.5 (Krause et al., 2001).

<table>
<thead>
<tr>
<th>R. solani infestation</th>
<th>Non-infested (No R. solani)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rootshield</strong></td>
<td><strong>Control</strong></td>
</tr>
<tr>
<td>Peatlite</td>
<td>3.94±0.19 bc</td>
</tr>
<tr>
<td>70/30</td>
<td>2.56±0.22 a</td>
</tr>
<tr>
<td>80/20</td>
<td>3.06±0.27 ab</td>
</tr>
<tr>
<td>90/10</td>
<td>2.63±0.22 a</td>
</tr>
</tbody>
</table>

Simple effects analyses were performed within *R. solani* infestation (infested or control) and letters indicate significant differences in disease severity (two Tukey HSDs a=0.05, n=16 MSD\textsubscript{infest}=1.685515 MSD\textsubscript{control}=0.8370737) while error bars are ±standard error.
Figure 4.10. Mature Chrysanthemums crown rot and root mass by substrate treatment, Rootshield application, and *R. solani* infestation.
OBJECTIVE THREE: CROWN AND ROOT ROT DISEASE ON ‘YOUNG’ CHRYSANTHEMUM

4.3.8 THERE WAS NO SYNERGISTIC EFFECT OF WF AND ROOTSHIELD EFFICACY ON DISEASE SEVERITY

Due to significant interaction effect of R. solani infestation and substrate on crown and root rot severity (p=2.026e-05, ANOVA F₃,₁₁₄=9.0411) (Table 4.17), simple effects analyses were performed to assess R. solani infested and non-infested pots separately. There was no effect of blocking by table (p= 0.8906, ANOVA F₁,₁₁₄= 0.0190), Rootshield application (p= 0.1971, ANOVA F₁,₁₁₄= 1.6836), or interaction between substrate and Rootshield (p= 0.1971, ANOVA F₁,₁₁₄= 1.6836) (Table 4.17). Young chrysanthemums (infested with R. solani one week after transplant) showed symptoms of disease within seven days. In contrast, in chrysanthemums infested at a mature age (six weeks old), symptoms of disease were observed four weeks after R. solani infestation. Within one week of infestation, some plants transplanted into peatlite were dead while plants in WFs showed only minor symptoms of crown disease. Disease assessment was performed two weeks post-infestation. Across substrate treatment and Rootshield application, control chrysanthemums were healthy, with all 64 plants rated as “1” on the severity scale (Table 4.18).

4.3.9 THERE WAS NO SYNERGISTIC EFFECT OF WF AND ROOTSHIELD EFFICACY ON DISEASE SEVERITY

Under simple effects analysis for plants infested with R. solani, there was a significant effect of substrate (p= 6.079e-05, ANOVA F₃,₅₅= 8.9947), but not Rootshield application (p= 0.2010, ANOVA F₁,₅₅= 1.6750), and there was no significant interaction between substrate and Rootshield application (p= 0.3441, ANOVA F₃,₅₅= 1.1323), indicating there was not a synergistic effect of substrate content and Rootshield application. When infested with R. solani, chrysanthemums grown in peatlite had the highest disease severity, with all chrysanthemums
exhibiting severe crown and root rot and several dead plants observed (Table 4.18 and Figure 4.11). WFs 70/30 (3.00±0.38) and 80/20 (3.00±0.46) applied with Rootshield had significantly lower (p<0.05) disease severity than peatlite with (5.00±0.33) and without Rootshield (5.25±0.31) (Tukey HSD n=8 MSD_{infested}= 3.49). While the effect of Rootshield application on disease severity was non-significant effect (p= 0.2010, ANOVA F_{1,55}= 1.6750), chrysanthemums grown in pots with Rootshield tended to have lower disease severity than their corresponding non-Rootshield pots. For example, in 80/20 WF, disease severity was 3.00±0.46 compared to 3.63±0.32 (Table 4.18; Figures 4.11 and 4.12).

Table 4.17. Full factorial ANOVA performed to assess main effect of *R. solani* infestation (infested or non-infested) and subplot effect of WF percent blend and Rootshield treatment) on crown and root rot disease severity of young chrysanthemums (infested at age one-week) in a simple RCBD. Because there was a significant interaction effect of infestation and substrate (p=2.026e-05, ANOVA F_{3,114}=283.41), simple effects ANOVAs were performed to assess disease severity across substrates under *R. solani* infestation and no infestation.

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>p(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block (Table)</td>
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<td>0.05</td>
<td>0.05</td>
<td>0.0190</td>
<td>0.8906</td>
</tr>
<tr>
<td><em>R. solani</em> infestation</td>
<td>1</td>
<td>693.94</td>
<td>693.94</td>
<td>279.1316</td>
<td>&lt;2.2e-16</td>
</tr>
<tr>
<td>Substrate</td>
<td>3</td>
<td>67.43</td>
<td>22.48</td>
<td>9.0411</td>
<td>2.026e-05</td>
</tr>
<tr>
<td>Rootshield</td>
<td>1</td>
<td>4.19</td>
<td>4.19</td>
<td>1.6836</td>
<td>0.1971</td>
</tr>
<tr>
<td>Infestation x Substrate</td>
<td>3</td>
<td>67.43</td>
<td>22.48</td>
<td>9.0411</td>
<td>2.026e-05</td>
</tr>
<tr>
<td>Infestation x Rootshield</td>
<td>1</td>
<td>4.19</td>
<td>4.19</td>
<td>1.6836</td>
<td>0.1971</td>
</tr>
<tr>
<td>Substrate x Rootshield</td>
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<td>8.49</td>
<td>2.83</td>
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<td>0.3368</td>
</tr>
<tr>
<td>Residuals</td>
<td>114</td>
<td>283.41</td>
<td>2.49</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.1. Crown and root rot disease severity of young chrysanthemums infested with *R. solani* one week after transplant and Rootshield® WP. Mean original disease severity (M) (categorical #1-6) was transformed to a linear scale (M^{1.5-1}/1.5 (Krause et al., 2001).

<table>
<thead>
<tr>
<th>R. solani infestation</th>
<th>Non-infested (No R. solani)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rootshield</td>
<td>Control</td>
</tr>
<tr>
<td>Peatlite</td>
<td>5.00±0.33 bc</td>
</tr>
<tr>
<td>70/30</td>
<td>3.00±0.38 a</td>
</tr>
<tr>
<td>80/20</td>
<td>3.00±0.46 a</td>
</tr>
<tr>
<td>90/10</td>
<td>3.75±0.45 abc</td>
</tr>
</tbody>
</table>

Simple effects analyses were performed within *R. solani* infestation. Letters across within infestation column indicate significant differences in disease severity (Tukey HSD a=0.05, n=8 MSD_{infested}= 3.49) while error bars are ±standard error. with no significant differences in disease severity found under control conditions where all plants were healthy and rated as 1.

Figure 4.11. Young Chrysanthemums aboveground growth (three-weeks post-transplant) by substrate treatment, Rootshield application, and *R. solani* infestation.
4.4 Discussion

**RADISH BIOASSAY**

*SMALL DIFFERENCES IN SUBSTRATES AND NATURAL GERMINATION RATES INDICATE NO “BACKGROUND” NOISE CONTRIBUTING TO DISEASE SEVERITY*

There were only minor differences in the physical and chemical properties of substrates (Tables 4.4 and 4.5), for example differences in container capacity. This suggests that physical and chemical variation in substrates did not substantially influence radish damping-off or aboveground biomass. While natural germination rates were lower than the expected for the seed lot (90% var. *Early Scarlet Globe*), there was no significant difference in germination rates among the substrate treatments, implying that the lower germination rates were due to aging seed rather than the substrates in which the radishes were planted. Most importantly, the similar
germination rates indicate that there was no “background noise” among substrates that would contribute to higher disease severity or lower aboveground biomass under *R. solani* infestation.

**ROOTSHIELD® WP EFFICACY WAS MOST OBSERVABLE AT HIGH APPLICATION RATE**

When applied at a rate of 1.93 g/L, Rootshield® WP significantly lowered damping-off disease severity compared to controls (p=1.2e-05), but this was not seen when Rootshield® WP was applied at the commercially-recommended rate (0.4g/L) (p= 0.11660). However, when applied at the recommended rate, there was a trend toward significant interaction effect between Rootshield® WP and *R. solani* infestation (p= 0.10680) suggesting that there is an effect of biopesticide on *R. solani* disease severity, but it was masked by a lack of effect under control conditions (no *R. solani*). This was confirmed in simple effects analyses performed for radish bioassay experiments 2-4 (Rootshield applied at 0.4g/L). Specifically, in *R. solani* infested radish planted in the four substrates, there was a significant effect of Rootshield on disease severity (p= 0.003344), but there was no effect of Rootshield under control conditions (no *R. solani*) (p=0.9793).

When applied at a high (1.93g/L) rate, Rootshield contributed to lower disease severity across substrate treatments under *R. solani* infestation, compared to the recommended rate (0.4g/L). However, for this high application rate in experiment 1, radish damping-off disease severity was also lower overall compared to the experiment in which Rootshield was applied at the recommended rate. While the difference in disease severity among experiments could have been due to *R. solani* rice inoculum, this is unlikely as rice inoculum was consistent in other studies (Chapter Three). Rather, the lower disease severity in the experiment 1 (1.93g/L rate Rootshield) may have been due Rootshield® WP spreading to control pots in the same tray. During this experiment, pots were overwatered and Rootshield-containing water may have been
uptaken by non-Rootshield pots—an effect visually observed on disease severity and aboveground biomass in control (non-Rootshield) pots. This mistake was remedied for the experiments 2-4 when Rootshield was also applied at the commercial rate (0.4g/L).

The high application of Rootshield® WP, coupled with the Rootshield® WP spread among pots in the same tray suggest several things about the higher than recommended application of Rootshield® WP. First, that it was efficacious in mitigating damping-off disease severity of R. solani despite the high level of disease. Second, that the high rate of Rootshield® WP had a negative, and potentially phytotoxic, effect on radish biomass in the absence of R. solani in WFs, but a positive effect on radishes grown in peatlite.

Specifically, under high Rootshield application (1.93g/L) with no R. solani infestation (control pots), the aboveground fresh weight of radishes grown in WFs was lower compared to where Rootshield was applied at the recommended rate (0.4g/L). In contrast, a reduction in biomass was not observed for radishes grown in peatlite. This suggests that at a high rate, Rootshield application could have phytotoxic effects and contribute to lower biomass for plants grown in peat:WF blends. However, this phytotoxicity may not extend to peat:perlite mixes, as the positive effect of high Rootshield on plants grown in peatlite has been found in other studies. For example, when Rootshield® PLUS WP (T. harzianum strain T-22 AND T. virens strain G-41) was applied at a rate of 0.6g/L to viburnum planted in R. solani infested peatlite, viburnum with Rootshield had aboveground biomass similar to plants treated with other biopesticides and/or fungicides; in other words, no substantial phytotoxic effects due to a higher rate of Rootshield application were observed (Gurel and Kabir, 2018). Moreover, the application of Rootshield® PLUS WP significantly reduced root rot disease (26-33% disease severity) despite the moderate to high level (60-66%) of disease severity observed in non-treated, R. solani
infested media (Morton’s #2 Growing Mix) (Gurel and Kabir, 2018). Despite this promising finding, most studies seem to indicate that *Trichoderma* spp. should be applied at a moderate rate, as a high level of *T. harzianum* (the active ingredient of Rootshield®WP) can have phytotoxic effects, though what defines a “high” rate is uncertain (MacKenzie et al., 2000; Zhang et al., 2020).

**CHRYSANTHEMUM BIOASSAY**

*PH MAY HAVE INFLUENCED CHRYSANTHEMUM HEALTH, R. SOLANI AND ROOTSHIELD*

pH adjustments are notoriously difficult to do with precision and recent work by Owen et al. (2020) determined that higher rates of dolomitic liming (9-12lbs/yd$^3$ (~5.34-7.11 kg/m$^3$)) were needed to adjust pH to optimal chrysanthemum growth. However, in the first experiment (“mature” chrysanthemums), substrates were blended at a rate of 6lbs/yd$^3$ (~3.56 kg/m$^3$). Therefore, extremely low pH likely contributed to the higher disease severity and poorer root health observed; low pH could have made plants more susceptible to soil-borne pathogens due to root tip burn and generally reduced plant health. In this study, corrective actions were taken to raise substrate pH to recommended range (5.5-6.3; Whipker et al., 2020) and control chrysanthemums grown in peatlite (not infested with *R. solani*) were observably healthy plants, indicating that low pH was an influencer, rather than a driver, of chrysanthemum health and disease severity.

Furthermore, low pH was not an issue in the “young” mum bioassay where substrates were blended at 8lbs/yd$^3$ (~4.75 kg/m$^3$) (peat:WFs) and 9lbs/yd$^3$ (~5.34 kg/m$^3$) (peatlite) and substrate pH fell within or above the optimal range for chrysanthemums (Figure 4.8). Importantly, as with both the radish and mature mum bioassays, young chrysanthemums planted in WF had lower disease severity than chrysanthemums planted in peatlite. For example, under
R. solani infestation, chrysanthemums planted in both 70/30 and 80/20 WF with Rootshield applied had significantly lower disease severity than both peatlite+Rootshield and peatlite control. Moreover, under control conditions, young chrysanthemums were healthy regardless of substrate treatment. Taken in sum, these results strongly suggest that the lower disease severity observed in WFs compared to peatlite was not a fluke side effect created by confounding variables such as substrate blending, irrigation rates, or low pH, but evidence that peat:WFs blends offered a better substrate environment against R. solani infestation than peatlite.

The pH could have also affected R. solani and T. harzianum. For example, in vitro R. solani mycelial (AG 2-2) growth was found to be optimal at pH 4.5-5.5 and 6.0-6.5, but declined as pH dropped or rose (Watanbe et al., 2011), although our previous work (Chapter Two) suggests that R. solani can cause significant infection despite low pH. Low pH, among other environmental conditions such as lighting and pathogen presence, has also been implicated in determining Trichoderma spp. growth through conidiation for asexual reproduction (Mukherjee et al., 2013). It has been suggested that these conditions could influence gene expression of Trichoderma’s mycoparasitic enzymes and its stimulation of the plant’s defenses, though these effects are unknown (Mukherjee et al., 2013). While Rootshield application did not have a significant effect on crown and root rot disease severity, it was visual efficacious and improved root health across substrate treatment for chrysanthemums infested with R. solani (Figures 4.11 & 12).

**NO CLEAR INTERACTION BETWEEN ROOTSHIELD AND WF OBSERVED**

While we hypothesized that the percent of WF in a blend would have an inverse effect on Rootshield efficacy, we found that there were no significant interaction effects between
Rootshield and substrate treatment and no clear differences in disease severity were observed at different WF amendment rates with Rootshield applied.

Overall, chrysanthemums with Rootshield applied had lower crown and root rot disease severity than their control counterparts, though in most cases the differences in disease severity were non-significant. For example, in young chrysanthemums infested with *R. solani* one-week post transplant, the effect of Rootshield application on crown and root rot severity was non-significant (*p* = 0.2010), however chrysanthemums grown in pots with Rootshield all had lower disease severity than their corresponding non-Rootshield pots regardless of WF blend (Table 4.12; Figures 4.11 and 4.12). These results suggest that the percent of WF blended into peat does not adversely affect Rootshield® WP efficacy.

**THE EFFICACY OF ROOTSHIELD® WP ON DISEASE SEVERITY LOWER THAN EXPECTED**

The efficacy of a biocontrol product often involves a trade-off between immediate short-lived impact and persistence in the substrate environment (Barea, 2015). Moreover, the substrate environment influences biopesticide’s MOAs (e.g. de la Cruz et al., 1993; Krause et al., 2001; Swiontek et al., 2014) and most likely this influence increases with time. We examined Rootshield® WP’s efficacy in three separate models that address three parts of plant development: a radish damping off bioassay, chrysanthemums infected at a young age and at a mature age. Despite differences in bioassays, we found similar efficacy of Rootshield® WP in WFs compared to peat-lite across experiments.

Microbial biopesticides are influenced by a given substrate’s unique physiochemical properties (Chen, 1988; Hoitink, 1999; Koohakan et al., 2004; Lundberg et al., 2012) and while substrates high in cellulose have been shown to reduce the biocontrol efficacy of *Trichoderma* spp. in hardwood and pine barks (Nelson et al., 1983; Chung et al., 1988; Krause, 2001), this was
not found to be the case in wood fiber blends, though Rootshield’s reduced efficacy could have also been affected by other factors such as older product or simply due to an off-batch of product. High disease severity could also have reduced Rootshield’s effectiveness against damping-off in radish, though high disease was not observed in the chrysanthemum bioassays.

*Trichoderma* spp. are some of the most extensively researched and most commonly used biopesticides (Woo et al., 2014; Nelson et al., 1983; Harman, 2000). To our knowledge, only one other study has examined the effect of *Trichoderma* (*T. atroviridae* strain MUCL45632) in WF and found that cucumbers inoculated with this antagonist *Trichoderma* and infested with the pathogen *Fusarium oxysporum* f. sp. *radicis-cucumerinum* had significantly higher disease than cucumbers grown in WF and only infested with the pathogen (Montagne et al., 2016). While this suggests that WF’s unique physiochemical properties adversely influenced *T. atroviridae* against *F. oxysporum*, our study suggests the opposite for *T. harzianium* (strain T-22) against *R. solani*; overall, plants grown WF-blends with Rootshield®WP applied had lower disease severity than those grown in peatlite.

The suppression of fungal pathogens by *Trichoderma* spp.’s is often attributed to its production of cell-wall degrading enzymes and research has shown that *Trichoderma* spp.’s enzyme production is influenced by the type of carbon available in the substrate (de la Cruz et al., 1993; Francois and Kronstad, 2017). While we did not examine the specific effects of carbon content on *T. harzianum*’s production of chitinase, our findings suggest that a) there is no interaction effect of WF carbon content and Rootshield efficacy and/or b) Rootshield®WP was not effective at the commercial rate or c) *Trichoderma* sp. in Rootshield®WP may have been using a different MOA, such as induced systemic resistance.
As with results from the radish bioassay, results from the chrysanthemum bioassays indicate that the disease scale offered only a glimpse at the effects of Rootshield. Root development was oftentimes observably better in chrysanthemums where Rootshield was applied, particularly in peatlite pots. For example, for mature chrysanthemums, there was little to no root development in the bottom of pots of peatlite without Rootshield, regardless of *R. solani* infestation, most likely due to low pH. However, when Rootshield was applied, peatlite pots had visually better root biomass and while the disease scale addressed root rot, it did not capture the full effect of Rootshield on root growth. Quantifying root surface area in pot, as well as weighing dry root biomass would better address the effect of Rootshield’s on the chrysanthemum’s belowground health. Moreover, tracking aboveground growth would be useful, although for mature chrysanthemums, differences in height and aboveground biomass of chrysanthemums were not distinctly different in Rootshield versus non-Rootshield pots. However, for mature mums, it is worth noting that a few chrysanthemums infected with *R. solani* were rated as ‘1’ and had healthy roots and crown, but were shorter in height than chrysanthemums rated as ‘1’ but not infested with *R. solani*.

Another issue found during disease assessment was how to account for stems that broke off and then either a) died or b) re-rooted. In both cases, chrysanthemums generally callused at the break point, suggesting that plant defenses activated in response to the pathogen. Anecdotally, callusing was better in plants with Rootshield pots vs control and in plants in WFs compared to peatlite, with most falling in the ‘3’ or ‘4’ categories of disease severity; however, this gap is an opportunity to improve the disease scale to address callusing.
The chrysanthemum scale was designed to capture different stages of disease progression in a manner representative of disease levels where growers can take corrective actions. Specifically, the difference between “3” (mild both) and “4” (severe crown or root rot) represents the cut-off point for grower intervention. Importantly, across WFs, chrysanthemums infested with *R. solani* infestation tended to be rated as “3’s,” particularly when Rootshield was applied, while chrysanthemums in peatlite were generally rated as 4s and 5s. This suggests that preventative use of Rootshield®WP in WF blends works well as part of an IPM program.

**HIGH HUMIDITY DID NOT SIGNIFICANTLY INCREASE DISEASE SEVERITY CAUSED BY R. SOLANI**

In mature chrysanthemums infested with *R. solani*, there was no significant effect of blocking by table (p= 0.7608, ANOVA F3,117= 0.3895), indicating that the sole table where a humidity chamber was not added did not significantly influence disease severity. Moreover, significant disease was observed in young chrysanthemum experiments, where no humidity chambers were used. Taken in sum, these findings indicate that extremely high relative humidity (~70%) was not needed for *R. solani* to induce significant disease. However, the higher humidity and temperature could have increased stress in chrysanthemums, as flowering was observed only in *R. solani* infested mature chrysanthemums under humidity chambers, despite chrysanthemums being a short-day plant experiencing summer sunlight conditions.

Additionally, while irrigation rates were consistent across substrate treatment, differences in the water holding capacity and porosity of peatlite compared to the WF blends could have influenced plant health, root growth, and the severity of disease caused *R. solani*. While pH probably had little effect on *R. solani* pathogenicity, peatlite pots noticeably held more water than those of WF, perhaps a combination of slower root growth due to pH and lower container
capacity of peatlite compared to 70/30 and 80/20 WF blends (Table 4.5). Regardless of the cause, wet peatlite pots may have concomitantly encouraged poor root health and *R. solani*.

While substrate moisture has not been shown to affect the severity of root rot caused by *R. solani* in other cases (Paula and Hau, 2007), *Pythium* spp. are known to thrive in wet conditions (Whipps et al., 1991; Postma et al., 2000). Previous work by the Poleatewich lab in the Macfarlene greenhouses has found evidence of *Pythium* spp. in the water supply and in this study sloughed-off roots were observed, although only in a few (~5) young chrysanthemum pots out of nearly 200.

*THE MICROBIAL COMMUNITY IN WOOD FIBERS IS STILL UNKNOWN*

The main objective of this study was to examine the effect of WF blends on the efficacy of Rootshield® WP on disease severity caused by the soilborne pathogen *R. solani* in two different model plants (radish and chrysanthemum). While examining the microbial communities was not one of our aims, we did observe fungal growth on the roots of chrysanthemums grown in WF blends regardless of Rootshield application. The fungi grew on top of roots, its white mycelia spanning across large swaths of substrate and also produced large white, nodules. Little is known at the molecular level (Montagne et al., 2015, 2016, 2017) about these unique microbial environments, and even less is known about the interactions of biocontrols like *T. harzianum* and soil-borne pathogens *R. solani* within these unique WF communities. For example, *Trichoderma* spp.’s mycoparasitic interactions are driven by several different factors. One factor is approximately 200 genes for elicitor-like proteins that are involved in mycoparasitism, and *Trichoderma*’s interactions with both the plant and other fungi, yet these genes have rarely been
examined (Mukherjee et al., 2013). Ultimately, the unique microbe communities associated with WFs offer a rich opportunity for examination.

4.5 Conclusions and Future Direction

A by-product of the forest industry, wood fibers are emerging as promising raw materials for use in soilless substrate blends in horticultural production systems. To encourage their greater adoption, WFs must be examined for compatibility with other aspects of sustainable CEA, specifically microbial biopesticides such as Rootshield® WP. Our study was the first to evaluate the effect of WF components on biopesticide efficacy under both growth chamber and commercial horticulture systems. Results indicate that the efficacy of Rootshield® WP against diseases caused by *R. solani* did not differ significantly among percent WF blends and that, in regard to Rootshield® WP efficacy, WFs blended into peat performed better than peatlite mixes. Moreover, our study showed that both radishes and chrysanthemums grown in WF blends infested with *R. solani* generally had significantly lower disease severity than both the peatlite and peatlite+Rootshield controls, while there were no serious differences in disease severity among non-infested controls.

Growers should feel confident using Rootshield as a preventative measure in different peat:WF blends. Future studies examining the interaction of WFs and Rootshield should collect data on root surface area and mass, aboveground plant biomass, and microbial composition of rhizosphere and bulk soil to better capture the interactions between WF content and the biopesticide.
CHAPTER 5
CONCLUSION

The overarching goal of this research was to examine how wood fiber substrate blends affect soilborne disease severity and biopesticide efficacy using *R. solani*, a common greenhouse pathogen, and Rootshield® WP, a widely used biocontrol, as models. This study was the first to examine the disease suppressiveness of peat-WFs blends and the efficacy of Rootshield® WP in WF blends to provide foundational knowledge to growers on the impact of wood fibers on disease severity and biopesticide efficacy.

Wood substrates are generally high in hemicellulose and cellulose content and have lower lignin content than peat, coir, and bark (Domeno et al., 2010). Researchers have demonstrated that wood is more microbially active than peat (Jackson et al., 2009a) and that WFs from pine and poplar have significantly greater fungal diversity and dominance compared to peat and coconut coir, with peat and coir having higher bacterial diversity (Montagne et al., 2015). However, the effects of these unique microbial communities associated with WFs on disease suppressiveness are largely unknown. While other organic substrates have been assessed for natural disease suppression and interaction with biopesticides, WFs had not.

Natural disease suppression was examined using the radish damping-off bioassay. No differences in natural disease suppression across WF processing type or percentage in peat blend were found, suggesting that WFs are naturally suppressive to *R. solani*. In contrast, other organic substrates, such as dark peat (Krause et al., 2001; Bonanomi et al., 2007), some composts (Nobel and Roberts, 2003; 2004), and large amounts of biochar (Frenkel et al., 2017) have been implicated in being disease conducive to various soil-borne pathogens. Additionally, against *R. solani*, radishes grown in WF blends consistently performed better than radishes grown in peatlite controls.
Both radish and chrysanthemum bioassays were used to examine the efficacy of the microbial biopesticide Rootshield® WP. Previous studies on hardwood and pine barks found a correlation between carbon content and the efficacy of *Trichoderma* spp. biocontrols, with *Trichoderma* spp. efficacy generally increasing with decreased cellulose content (Nelson et al., 1983; De la Cruz et al., 1993; Krause et al., 2001). This suggested that WFs’ high hemicellulose and cellulose content (Domeno et al., 2010) could influence Rootshield’s efficacy, however this was not found to be the case in our studies. Across bioassays and experiments, WF percentage blend into peat did not impact Rootshield efficacy, suggesting that there is no synergistic effect of WF content and Rootshield efficacy on disease severity. However, there was only one instance where WF+Rootshield had significantly lower disease severity than the non-Rootshield counterpart. Specifically, infested WFs 80/20+Rootshield blend had significantly lower damping-off in radish than infested WF 80/20 control (no Rootshield) but only when Rootshield was applied at a rate (1.93g/L)—nearly five times the recommended rate (0.4g/L). This result reveals that an interaction between WF content and Rootshield application may simply be unobservable at the commercial rate. While Rootshield application was non-significant across substrate treatment, there were observable differences in aboveground plant biomass and root growth, particularly in plants grown in peatlite and in the chrysanthemum bioassays, suggesting opportunity for future studies to more fully capture Rootshield’s effects.

Perhaps most importantly, plants grown in WFs and infested with *R. solani* across experiments had significantly lower disease severity than plants grown in peatlite, despite the fact that control plants grown in WFs had similar health and root growth across substrate treatment. Our results suggest that WF blends offer more protection against the soilborne
pathogen *R. solani* than peatlite blends and that this is true across the plant production cycle (damping-off, transplant age, and maturity).

**Future Direction**

This research provides industry professionals with insight on WF substrate performance in terms of disease management and offers much needed guidance to growers seeking to integrate WF-amended substrates into their operations and IPM programs. Biopesticides and WFs are becoming increasingly integral components of sustainable CEA (controlled environmental agriculture)—biopesticides as part of IPM and WFs as affordable, renewable substrates. The interactions among the plant, pathogen(s), biocontrol agent(s), native substrate microbe community, and greenhouse production system at large are complex. Studies that focus on a few aspects of this complexity will provide essential pieces of information to improve our overall understanding of the phytobiome puzzle and provide growers with improved working knowledge of the tools, like WFs and biopesticides, integral to sustainable disease management and CEA production.

In recent decades, an explosion of genomic sequencing techniques has driven studies on the composition and structure of plant microbiomes (e.g. Sharon et al., 2008; Mukherjee et al., 2013, Montagne et al. 2015, 2016, 2017; Rodriguez et al., 2019). Given that the makeup of WFs microbial communities is still relatively unknown (Montagne et al., 2016, 2017), future work should examine these unique communities in different model plants and in concert with biocontrol agents and soilborne pathogens. However, these should be complemented by greenhouse-based studies to bridge the gap between genomic research and the proof-of-concepts necessary to show growers that adopting WFs and biopesticides, will provide them with products of similar or better caliber than those currently used.
LIST OF REFERENCES


APPENDIX A

SUBSTRATE BLENDING

A cubic-foot box (1ft³) was created by a previous member of the lab, Crysta Harris (Figure A1-A) and this box was labeled with percentage marks (10, 20, 30, 40, 50, 60, and 70%) along the inside as reference points (Figure A1-B). For more precise measurements, 12-inch marks were measured out using a ruler for height, length, and width (Figure A1-C). Note: the inside of the box measured 12’’x12’’x12’’

MIXING SUBSTRATE BLENDS

1. Measuring out each blend material by volume: The cubic foot box was used to measure out each component separately according to desired blend percentage. Measuring each component separately ensures components do not compress each other and alter measurements.
   a. Example Calculation: For a 70% peat, 30% perlite blend:
      i. 0.70*12 inches=8.4 inches of peat
      ii. 0.30*12 inches=3.6 inches of perlite
      iii. To measure, fill box 8.4 inches tall with peat.
   b. Put another way, we have a one-cubic ft box (12*12*12) or 1728 inches³
      i. If 70% peat is wanted, that’s 70% of 1728 in³ or 1209.6 in³. Dividing 1209.6 in³ by the area of the box (144 in²) is 8.4 inches. Therefore, to achieve a 70% peat blend for one-cubic foot, peat was filled to the 8.4-inch high marking on the blend box (Figure A1-C).

2. Peat, perlite, and WF materials were added together in one large plastic bin
3. Carbonate dolomitic limestone (PLANTEX #80 Pulverized 50 lbs, Master Plant-Prod-Inc.) was added at a rate of 6 lbs/yd³ (~3.56 kg/m³)
4. Wetting agent (PsiMatric, Aquatrols; 3mL per ft³) was added at the recommended “Initial Substrate Hydration: Media Treatment” rate of 2-4 fl oz of wetting agent in 2 gallons of water per yd³ of substrate mix. This translated into about 1 mL wetting agent per cubic foot of substrate made. To ensure effective spread of wetting agent while also adjusting for soil moisture content, 500 mL of water was mixed with each 1mL of wetting agent.
5. All components were added to a large plastic tub
6. Substrates were blended by hand to ensure thoroughly incorporation of all components
Figure A-1. **One-cubic foot box** used for measuring out raw peat, wood fiber, and perlite for blending.

Figure A-2. The addition and incorporation by hand of WF, peat, wetting agent, and limestone.

Figure A-3. **Final Blended substrates**: A) 70/30 peat:lime, B) 70/30 peat:hammer WF, C) 80/20, D) 90/10
ADJUSTING pH VIA LIMING BATCH TESTING METHODS

1. Generally, nutrients are most accessible to crops at pH from 5.4-6.4 (Nelson, 2012). For these studies, the goal pH of substrates was 5.5-6, with 5.8 as ideal, however adjusting pH in WF blends is not an absolute science. I tested substrate blends at two different rates: 8lbs/yd$^3$ and 6lbs/yd$^3$ and determined that a rate of 6lbs/yd$^3$ generally met the range of 5.5-6 and did so within the one-week turnaround time that substrate blends were generally used. 6lbs/yd$^3$ is approximately ~3.56 kg/m$^3$ or 100g/ft$^3$.

2. After blending and liming, substrates were incubated in sealed plastic bins for at least 2-3 days prior to experiment to give pH time to equilibrate. pH and EC were determined by using saturated media extract method to collect leachate (see below) and measured with an Orion™ Versa Star Pro advanced electrochemistry meter (Thermo Scientific, Waltham, MA) and Thermo Scientific™ Orion™ ROSS Ultra™ Refillable pH/ATC Triode™ probe, either 8157BNUMD or 8107BNUMD.

3. Overall, pH tends to equilibrate one week after blending, confirming previous lime adjustment work (Figure A-5 and A-6, supplement to Jackson’s Chasing pH paper, in press). Note: Adjusting pH in WF blends is difficult and not much data exists on variations in WF that affect adjustments (Figure A-4). Thus, this work represents experimental practice, rather than the use of a refined and well-established method.

![20% Amendment Rate](image)
Liming Requirements for Chrysanthemum planted in pine wood chips (PWC)

Other recent work by Brian Jackson (Owen et al., 2020) tested five different dolomitic liming rates (0, 3, 6, 9, 12 lbs/yard$^3$) on eleven different substrates of peat moss amended with either perlite or pine wood chips (PWC), another type of wood substrate. They determined that to meet the pH optimal for chrysanthemum growth (5.5-6.3 Whipker et al., 2000), substrates formulated to contain ≤40% perlite (by volume) needed to be amended with higher dolomitic limestone rates (9 and 12 lbs/yard$^3$). More specifically, they found that high rates of dolomitic limestone (9 and 12 lbs/yard$^3$) were required for peat:PWC 90/10 however for all substrates with ≤30% PWC, 6-12 lbs/yard$^3$ to meet the optimal chrysanthemum pH. For my own work with chrysanthemum, I added dolomitic limestone at a rate of 6lbs/yard$^3$ (~3.56 kg/m$^3$) across substrate blend (70/30 peat:lite, 70/30, 80/20, 90/10 peat:WF (PTS)) which may have been too low, particularly for the peatlite blend. In sum, higher dolomitic liming rates may have been needed for my substrate blends. However, Owen et al. (2020) did make an important qualification, noting that different pH adjustments may be needed for wood processed into fibers (which increases surface area) compared to PWCs, particularly given that physical substrate characteristics will be different.

TESTING PH: SATURATED MEDIA EXTRACT (SME)

SME is the industry standard and most accurate way to test pH and EC

1. Two to five days after substrate blending, three representative samples of each substrate blend were collected, approximately 25 mL of substrate per 50 mL conical centrifuge Falcon Tube.
2. Samples of the media were wetted with deionized water until water just barely stands on the surface (“glistening” on surface) and the mixture was allowed to stand for 90 minutes; It was then filtered under suction.

3. Sample-pH and EC measured for each replicate using an Orion™ Versa Star Pro advanced electrochemistry meter (Thermo Scientific, Waltham, MA) and Thermo Scientific™ Orion™ ROSS Ultra™ Refillable pH/ATC Triode™ probe, either 8157BNUMD or 8107BNUMD.

4. pH average of 3 samples per treatment.
   a. For reporting purposes, most precise average of pH, first convert to \([H^+]\) by taking \(10^{-\text{pH}}\) and taking the average \([H^+]\) and then converting back to pH via \(-\log_{10}[H^+]\). I simply averaged pH values, as the range of pH (5.5-6) does not need this level of precision.

### ASSESSING AND ADJUSTING SOIL MOISTURE CONTENT

The goal is 50-60%±5% moisture content. WFs and peat have an initial level of moisture, but perlite does not. Additional water was added alongside wetting agent, about 500 mL of tap water per cubic foot of substrate made.

A MB27 Soil Moisture Analyzer (OHAUS, Parsippany, NJ) was used to measure SMC for 3 samples (0.5-1.5 grams each) per treatment blend. All blended substrates met the goal 50-60%±5% moisture content. However, to change SMC, substrates can be left exposed to air (to lower SMC) or additional water can be added (to increase SMC). SMC can also be determined by over-drying; weighing initial samples, drying them for 6-12 hours, and then reweighing samples, calculating moisture content by: (wet weight-dry weight)/wet weight *100. Using the soil moisture analyzer drastically reduces the time, energy, and equipment needed to assess SMC.

### FILLING POTS

Substrate blend was added to three pots to approximate similar heights, then weighed and recorded to calculate an average weight. This average weight was then used to approximately fill all pots of that treatment. **Note:** Given the similarities of WF by processing type and blend ratio, the weights were similar across treatments; however, this method was key to ensure uniformity of pot fills within each treatment.
ASSESSING PHYSICAL PROPERTIES

For each experiment and treatment, two gallons of each mix were sent to Brian Jackson at North Carolina State University to determine the physical properties of a blend. These properties included porosity (air, water, solid by volume), bulk density, and water-holding capacity and were measured for each substrate type using the Porometer method (Figure A-5).

Figure A-5. Porometer method performed at North Carolina State University. All 70/30 mixes B: peatlite, C: peat WF hammer-milled, D: peat WF disc-refined, E
B.1 Rice Inoculum Preparation

The rice inoculum (Owen et al., 2017) was used to grow *R. solani*. Twenty-five grams of white long grain rice were weighed, placed into a beaker containing 18 mL of deionized water, covered with aluminum foil, and autoclaved once on a 60 min liquid cycle (121°C 15 psi). Autoclaved rice grains were then inoculated with colonized 10 PDA disc samples of *R. solani* using a size 7 mm cork borer and beakers were covered with aluminum, wrapped with parafilm, and placed in a cabinet at room temperature to allow 6-7 days of fungal colonization. One week later, colonized rice grains were pulverized with mortar and pestle to produce inoculum of uniform size (~1-3mm). This uniform size was initially determined using a 2mm mesh cheese cloth to establish a standard, and then was visually determined for subsequent inoculums. For radish experiments, rice inoculum was evenly spread across the surface of pot media but was not mixed into the media.

Figure B-1. After 6-7 days after PDA bores of *R. solani* were added (A), rice grain inoculum was grounded with mortar and pestle and then added at concentrations of grams of rice per liter of soil (B). For example, for a 500 L pot at a concentration of 0.5g/L, 0.25 grams of grounded rice would be added to each pot. Note: Rice inoculum is pathogenic for several weeks after preparation. Note: According M Krause, a drawback of the rice inoculum (in contrast to the potato soil inoculum) is that there is still some rice grain leftover as a food resource for *R. solani* and could therefore contribute to poor disease efficacy. However, I. Michaud’s work with radish has had high levels of disease in both growth chamber and greenhouse settings. Moreover, the advantages of the rice inoculum are that rice is cheap, easy to purchase/obtain, and grains are of uniform quality. The set-up itself is also quick, easy, and nearly 100% efficacious. Moreover, unlike the potato-soil inoculum, there is minimal risk of soil-borne pathogens hitchhiking.

For chrysanthemum experiments, rice inoculum was also placed on the surface of the media, but at the four cardinal directions of the pot (N, S, E, W) approximately one inch from the stem of the plant.
B.2 Short-term Storage

Growth on PDA or APDA plates (APDA for root plating isolations in particular) is best for short-term storage of *R. solani* isolates. As with most plant pathogens, too much time on agar plates will reduce the isolate’s virulence, however according to Dr. James Woodhall (University of Idaho), *R. solani* on PDA is virulent for up to a year and, due to its formation of sclerotia, has been shown to cause disease several years after growth on PDA.

B.3 Long-term Storage (adapted from Chris Rose at Bioworks March 2020)

Microcentrifuge tubes (0.5mL) were filled with 50% glycerin and 50% water solution and autoclaved on a standard 20 min liquid cycle at 121°C, 15 psi. Sclerotia from the edge of actively growing plates of *R. solani* on PDA were removed and added to the sterile (autoclaved) 50% glycerin and 50% water solutions. One to several sclerotia were added per microcentrifuge tube. Tubes were then stored in a -80°C freezer. *Note from Bioworks: they have had great recovery results taking them out years later and placing them on PDA plates: This method is easy and effective but requires ultra-cool storage access. Long-term storage can also be done at room temperature using the cereal grain method (Sneh et al. 1991).*

B.4 Pathogen Identity

To confirm that symptoms observed were caused by *R. solani*, pathogen identity from infested radish was confirmed by root plating on acidified potato dextrose agar (APDA) media plates (amended with concentrated lactic acid 85%). Blackened roots were removed with sterilized tweezers and rinsed three times, in petri dishes of sterile RO water and one dish containing a 10% bleach solution. Root material in the “transition zone” (between rotted & healthy root) was taken to minimize the risk of accidentally isolating secondary pathogens. Four root pieces (~2-5mm in length) were placed into four different quadrants on a dish of APDA. Dishes were sealed with parafilm and incubated at room temperature and after 2-5 days and if roots showed fungal growth, colonized APDA disc samples were sub-cultured onto new APDA or PDA. Samples were examined morphologically on agar plates as well as with light microscopy to identify the diagnostic right-angle branching hyphae (Figure 2.1)
## APPENDIX C
### SOILLESS SUBSTRATES OVERVIEW

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Type &amp; Material</th>
<th>Benefits</th>
<th>Drawbacks</th>
<th>References</th>
</tr>
</thead>
</table>
| Sphagnum Peat Moss  | Organic; Major component of most medias (75-85%) then amended with perlite, vermiculite, coir, wood, etc. Dominants the nursery and pot flower industries, but has more limited use in vegetable and cut-flower production | • “Gold Standard”  
• High water-holding capacity  
• High porosity  
• Reliable, consistent, and inexpensive  
• Minimal amendments necessary  
• Low bulk density lowers shipping costs | • Rising costs  
• Non-renewable | Barrett et al. (2016); Robbins et al. (2011a); Gruda et al. (2016b) |
| Perlite             | Inorganic; Volcanic rock; Standard blend with peat mixes to improve drainage and/or increase the percent aeration | • Standard alone or amendment to peat  
• Lightweight  
• Neutral (pH 7-7.5) and chemically inert  
• Sterile and odorless | • Expensive addition to soil mixes  
• Non-renewable  
• Adds no nutrients or buffering capacity | Robbins et al. (2011a); Savvas and Gruda (2018); Kaderabek et al. (2019) |
| Vermiculite         | Inorganic; originates from same volcanic rock as perlite, but heated differently | • Holds large-quantities of water  
• Positively-charged nutrients (K, Mg, Ca)  
• Sterile and light weight  
• pH varies, neutral to alkaline | • Respiratory concerns related to asbestos  
• Expensive addition to mixes  
• Non-renewable | Robbins et al. (2011a) |
| Coco coir           | Organic; Waste product of coconut industry. The mesocarp or “husk” of coconuts. After peat (2/3 of market) and mineral substrates (vermiculite, perlite, rockwool), it’s the third most common growing media used by nurseries in horticulture | • Porosity and water holding capacity comparable to peat and higher re-wetting capacity  
• Higher pH than peat  
• Inexpensive, abundant, and renewable | • High EC  
• Lower CEC than peat  
• pH range 5.5-6.8  
• Secondary processing costs (toxic levels of Na and K from coastal marine areas)  
• Inconsistent quality  
• Contamination | Barrett et al., (2016); For an extensive review Abad et al. (2005); Robbins et al. (2011a); Mariotti et al. (2020) |
| Pine bark (mulch, wood chips) | Organic; major substrate used in Eastern U.S. | • Renewable  
• Favorable physical properties  
• Used for decades (since 1970s)  
• Greater disease suppressiveness compared to perlite  
• Preferred over hardwood bark (resists decomposition and contains fewer leachable materials) | • Physical and hydraulic properties variability due to the supplier/source (e.g., lumber mill type) and methods of additional processing or aging  
• Issue of Nitrogen drawdown if not amended with N  
• Secondary processing costs | Altland et al. (2018); Barrett et al. (2016); Owen et al. (2019); Robbins et al. (2011a) |
<table>
<thead>
<tr>
<th>Substrate</th>
<th>Type</th>
<th>Characteristics</th>
<th>Costs and Limitations</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardwood Bark</td>
<td>Organic</td>
<td>• pH 5.5-5.5, less acidic than peat moss or pine bark</td>
<td>Toxic compounds, compost first, requires significant amendments of N, P, S, secondary processing costs</td>
<td>Robbins et al. (2011a)</td>
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<td></td>
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<td>• Composted bark may be alkaline (pH 7-8.5)</td>
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<tr>
<td>Wood Substrates</td>
<td>Organic;</td>
<td>• Renewable alternative to pine bark</td>
<td>Huge variability by source, processing, type, etc., initially high level of investment to obtain the machinery required for manufacture, rarely used as stand-alone substrate due to insufficient water holding capacity and issue of compaction</td>
<td>Barrett et al. (2016), Robbins et al. (2011a)</td>
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<tr>
<td>(Chips and Fiber)</td>
<td>includes fibers, chips</td>
<td>• High total porosity and air holding capacity</td>
<td></td>
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<td></td>
<td>Also a major substrate used in Eastern U.S. Used as amendment alternative to perlite</td>
<td>• Comparable production practices (fertility and irrigation) to other blends, specifically as replacement to perlite in peat-perlite blends</td>
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<tr>
<td></td>
<td>Loblolly pine is best species</td>
<td>• Best as an amendment to peat moss in range of 10-40%</td>
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<tr>
<td>Rockwool (mineral wool)</td>
<td>Dominant Inorganic, mineral aluminum silicates, heated to form fibers that are blocks as a final product</td>
<td>• Alkaline pH</td>
<td></td>
<td>Robbins et al. (2011a), Agung Putra and Yuliando (2015), Gruda et al. (2016b)</td>
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<td></td>
<td></td>
<td>• Sterile and chemically inert</td>
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<td></td>
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<td>• Provide optimum air: water ratio</td>
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