Managing Emerging Diseases of Organic Greenhouse Vegetables: Interactions between vermicompost and biological control agents

by

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Abstract

Biological control agents and composted materials, including vermicomposts and their water extracts, are used to suppress plant diseases in organic production systems, where fungicide use is limited. The past decade has seen a doubling in organic horticulture and a dramatic increase in vermicompost research. As disease suppression by vermicomposts has been inconsistent, research in this area requires standardization of methods, and compatibility with current biocontrol agents has not been assessed. I tested the disease suppressive abilities and microbial communities of five vermicomposts with differing characteristics, and developed Petri dish and growth chamber assays to examine compatibility with biocontrol agents. *In vitro* suppression of the pathogens *Fusarium oxysporum* f. sp. *radicis-cucumerinum* D.J. Vakalounakis (Forc), and *Rhizoctonia solani* J.G. Kühn, as well as disease suppression on cucumber and radish plants, respectively, was assessed using vermicomposts incorporated into sterilized substrate and using aerated vermicompost water extract. All vermicomposts provided significant pathogen suppression *in vitro* as well as plant disease suppression. The mechanism for pathogen suppression was negated by autoclaving. A range of responses between the biocontrol agents *Bacillus subtilis* (Ehrenberg) Cohn strain QST 713 (Rhapsody®) and *Clonostachys rosea* f. *catenulata* Samuels, Seifert, and Gams (syn, *Gliocladium catenulatum*) strain J1446 (Prestop®), and vermicomposts, was observed *in vitro*. I tested for interactions between these biocontrol agents and vermicomposts as an example of application of a biological control agent to a microbially competitive growth medium using a mixed effects model approach. Consistent antagonistic to neutral interactions *in vitro*, and a range of interactions from antagonistic to additive *in planta,* suggest that the interaction between a biocontrol agent and a competitive microbial milieu is not additive. The testing strategies investigated provide an efficient screen of vermicomposts for compatibility with existing biocontrol agents, and of biocontrol agent efficacy in a competitive environment. With improved and consistent testing methods, vermicompost can be a reliable approach for plant disease management in organic agriculture.

Keywords: Biocontrol, bioassay, disease suppression, organic greenhouse production, vermicompost

Lisa,

For Love and Patience

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List of Acronyms and Abbreviations

A	Agent: a biological control agent
ACT	Aerated compost tea
AUDPC	Area under the disease progress curve
BC	Biological control agent
BS	*Bacillus subtilis* strain QST 713 (Rhapsody®)
CFU	Colony forming unit
cm	centimetre
°C	Degree Celsius
DGGE	Denaturing gradient gel electrophoresis
DS	Disease severity
EC	Electrical conductivity (commonly used to measure plant growth nutrient solution concentration)
FDA	Fluorescein diacetate hydrolysis assay
Forc, F	*Fusarium oxysporum* f.sp. *radicis-cucumerinum*
h	Hour
ha	Hectare
GC, C, C.r.	*Clonostachys rosea* f. *catenulata* (*Gliocladium catenulatum)* strain J1446 (Prestop®)
HSD	Tukey's honestly significant difference test
ISR	Induced systemic resistance
IPM	Integrated pest management
Lme4	Linear mixed effects package in R
LSD	Fisher's least significant difference test
M	Microbes: a microbial consortium from vermicompost
μm	Micrometres
μL	Microlitres
μmol	Micromols
NCT	Non-aerated compost tea
PDA	Potato dextrose agar
½ PDA	Half-strength potato dextrose agar
PGPR	Plant growth promoting rhizobacteria
PR	Pathogenesis-related

REI	Restricted entry interval
RISR	Rhizobacteria induced systemic resistance
RS, R	*Rhizoctonia solani*
SAR	Systemic-acquired resistance
SNP	Single nucleotide polymorphism
SSCP	Single-strand conformation polymorphism
VC	Vermicompost

Glossary

Hydroponics	Conventional (non-organic) production using liquid fed fertilizer salt solution in media other than soil or soil-like substrates (rockwool, sawdust, peat, etc)
Hydro-organic	Organic production fed with liquid organic fertilizers, may imply inert or containerized substrates. Currently permitted in USA, not in Canada or Europe for certified organic production.
Organic	Production deemed acceptable for certified organic production, especially according to Canadian regulations which include intensive organic greenhouse vegetable production in semi-containerized/demarcated soil-like media.

Chapter 1.

Introduction: Organic greenhouse vegetable production, biological control of plant diseases, and composts

1.1. Global food production outlook

1.1.1. Global food production is slowing

After tripling in production between 1960 and 2015, future increases in agricultural production are expected to come largely from yield increases, but these have stagnated (FAO 2018). Current agricultural intensification strategies are not sustainable, an effect masked by the great progress made in reducing global hunger: Global Hunger Index scores decreased 27% between 2000 and 2017 (von Grebmer et al. 2017). Sustainable strategies for intensification of agriculture are a priority for maintaining food production: with a global population predicted to reach 9.8 billion by 2050 (DESA 2017), and food production in developing countries projected to need to almost double between 2005 and 2050 (FAO 2009), maintaining global food production is a difficult objective requiring yield increases greater than those currently occurring (Ray et al. 2013).

1.1.2. The yield gap between organic and conventional production is narrowing

To support an increasing human population, it is imperative that agriculture uses less arable land per person to maintain food levels. This is a pillar of sustainable intensification, but one that has traditionally been linked to land degradation. using chemical fertilizers is not sustainable without a change in methods and efficiencies (FAO 2018). Modern agricultural practices have led to the reduction of organic matter in soils and potting mixes, leading to several problems including disease epidemics (Hoitink & Boehm 1999). Organic agriculture could contribute to sustainable intensification, although the relationship between organic agriculture and broader sustainability is not clearly defined (Fess 2018), nor is sustainable intensification (Petersen & Snapp 2015). Organic horticulture doubled between 2003 and 2013 and research on organic

horticulture tripled between 1994 and 2013 (Dorais & Alsanius 2015). The intensification of agriculture can be antithetical to some philosophies of organic food production: intensification is sometimes considered allied with environmental harm (Pretty et al. 2018). Organic crops have been found to be 20% lower than conventional crops by meta-analysis (de Ponti et al. 2012), and in long term study (Mäder et al., 2002). In an analysis of 36 studies that compared organic and conventional yields, Dorais and Alsanius (2015) found that although organic yields in field crops can be lower than those in conventional production (90%), organic greenhouses and high-tunnels can approach those of conventional, at 94% and 102% of conventional yields, respectively. Hundreds of studies have investigated organic system performance, reviewed in (Reganold & Wachter 2016). In Canada, public opinion favours biological control over chemical pesticides (McNeil et al. 2010). Organic agriculture can be a sustainable production system, particularly if specific breeding programs close existing production gaps between conventional and organic production (Fess 2018), and some investigators believe that thie gap will close over time simply due to better soil health (Schrama et al. 2018). One way to restore and maintain soil health is by the addition of organic amendments such as composts and vermicomposts. Our knowledge of how organic amendments shape the soil microbiome remains limited, recently reviewed by Bonanomi et al. (2018).

1.2. Greenhouse production increases agricultural intensification

1.2.1. The greenhouse environment: hydroponic and soil based systems

The shift from soil-based, drip-irrigated greenhouse systems to soilless hydroponic greenhouse systems was a successful strategy to reduce the requirement for soil disinfestation using methyl bromide and other chemical fumigants (Postma et al. 2000; 2010) or steaming, which was cost-ineffective after the oil crisis of the 1970s (Raaijmakers et al. 2009). Disinfestation was necessitated by the proliferation of soil-borne plant pathogens (Raviv & Leith 2008). It proved impossible to exclude pathogens even from soilless hydroponic systems, as they are re-introduced into the growing system by many routes (Postma 2010; Al-Sa'di et al. 2008), and hydroponic systems are inherently susceptible to pathogens including aquatic, zoospore–producing oomycetes

such as *Pythium* and *Phytophthora* (Vallance et al. 2011). As such they have failed to reach the theoretical potential to eliminate plant disease (Stanghellini & Rasmussen 1994). Most high-tech greenhouses use hydroponics, and the initially sterile hydroponic plant growth substrates such as rockwool are considered to be at risk of rapid pathogen spread leading to epidemics, because if pathogens can gain access they can take advantage of the organismal vacuum (Stanghellini & Rasmussen 1994): the relatively sterile conditions in hydroponics mean that between crop cycles the fertilization system including the rockwool plant substrate is theoretically free from competing microbes such as the antagonistic microbes naturally present in crop soils (Agrios 2005) and thus pathogens have sole access to the plant roots once they grow into the system and begin to exude nutrients (Postma et al. 2000). The problem of pathogen infestation is compounded with the use of recirculating water systems that can spread plant disease (Stanghellini et al. 1996, Rattink 1996) but in some cases this can lead to disease suppression by unknown organisms (Calvo-Bado et al. 2006). Such suppressiveness to plant diseases has been found in soilless hydroponic systems using rockwool (Postma et al. 2000; Minuto et al. 2007), and perlite and perlite-peat (Clematis et al. 2009), but generally these systems are treated as sterile and pathogen outbreaks are dealt with using fungicides. Recirculation of nutrient solution for water conservation remains one of the primary reasons to use either soil-based or hydroponic greenhouse systems, and methods of recirculating water disinfestation have been attempted including filtration, heat treatment, oxidation, irradiation, activated carbon adsorption, and copper ionization (Postma 2010). All of these methods are variously successful at removal of both beneficial and pathogenic microorganisms, with the possible exception of slow sand filtration (Postma et al. 2007), which may preferentially remove pathogens.

1.2.2. Current greenhouse production

The area covered by greenhouses worldwide is estimated to be between 300 000 hectares and 2 400 000 ha, with a consensus of about 50 000 ha under glass and the remainder under plastic (van Lenteren 2000; van Lenteren 2006; Pilkington et al. 2010). In Canada, there were 2610 ha of total greenhouse area in 2018, mainly in Ontario (61.3%), British Columbia (20.1%), and Quebec (10.3%) (Statistics Canada, 2019 chart 32-10-0018-01). Greenhouse fruit and vegetables from these facilities produced 1.5 billion CDN in 17.4 million m^2 of area (Statistics Canada, 2019 chart 32-10-

0456-01). Greenhouse vegetable production continues to increase rapidly in Canada, and is the largest segment of Canadian horticulture (Agriculture Canada Statistical overview 2017). Greenhouses can produce as much as 40 times more compared with field crops grown in the same area (Paulitz & Bélanger 2001): Van Lenteren (2000) gives the example of production in The Netherlands, where 20% of the total value of agricultural production occurred in 0.5% of the total agricultural area, illustrating the potential of greenhouse agriculture to contribute to intensification of horticultural cropping worldwide.

1.2.3. Organic greenhouses: organic management practices, sustainability, and adoption

Organic management practices can contribute to the four categories of sustainability: production, environmental, economic, and social wellbeing, reviewed in Reganold & Wachter (2016). Certification has narrowed the scope of what is considered organic, and created tension with broader concepts of sustainability, but organic management continues to contribute methods that could make agricultural intensification more sustainable, even if these methods are only partially adopted. For example, biological pest control has been adopted by practitioners of both conventional and organic agriculture: greenhouses are particularly well suited to the use of biological control agents in integrated pest management (IPM), and uptake of IPM has been rapid, particularly in European greenhouse production (Pilkington et al. 2010). Microbial biocontrols offer advantages to both organic and conventional growers: restricted entry interval (REI) for biological controls is favourable compared with chemical fungicides, this is helpful in the greenhouse as the application of the agents does not disrupt operations (Harman, 2000) and is a driver for the uptake of the use of biological controls (Pilkington et al., 2010). Furthermore, a theoretical advantage to using biological control in greenhouse culture is that chemical fungicides often cause plant toxicity because they are not adsorbed and dispersed as they are in the soil (van Lenteren, 2000) but biological controls do not cause this type of toxicity. Organic greenhouse technologies are broadly applicable, and could be transferred to other production systems within the loosely defined area of sustainable intensification of agriculture (Petersen & Snapp 2015) that are not bound to organic certification such as regenerative or conservation agriculture. A partial adoption strategy for organic methods is argued be important to rapidly reduce agricultural greenhouse gas emissions (Quarles 2018).

Organic production is increasing: although worldwide, only about 1 percent of agricultural land under production is occupied by organic agriculture (Reganold & Wachter 2016), in 2016, there were 4289 Canadian farms that reported certified or transitional organic status (Statistics Canada, CANSIM Table 004-0211), and 16.7% of greenhouse vegetable farms (138 farms) reported organic products, an increase from 13.4% of farms in 2011 (Statistics Canada Census of Agriculture 3438). The United States had 596 ha of organic greenhouse area in 2008, Israel had over 500 ha in the same year, and in the European Union, 7.3% of the total greenhouse area was devoted to organic production in 2011, totaling approximately 3,448 ha (Dorais & Alsanius 2015), illustrating that organic greenhouse production is becoming an important agricultural strategy.

1.2.4. Organic greenhouse systems and regulations

Intensive organic greenhouse production occupies a unique niche in agriculture: there is tension between “industrial philosophy” and “agrarian philosophies” of agriculture (National Research Council 2010), and organic greenhouses, especially high-tech glasshouses, fall at the crux of this dichotomy. Intensive organic greenhouse production systems often use retrofitted conventional hydroponic intensive systems including chemigation (chemicals in irrigation) equipment, and also borrow from older greenhouse production systems that would have used fertilized natural soils for plant growth with only irrigation supplied. The established regulations for organic greenhouse production vary by country (van der Lans et al. 2011). Hydro-organic production, similar to hydroponics but with organically certified nutrient feed and substrate, is permitted in the USA, but in most countries this does not meet organic standards as plants must be grown in the soil. Rather than growing plants directly in the soil, Sweden, Finland, the USA, and Canada allow greenhouse plants to be grown in demarcated soil beds or containers, provided that the biological activity of the growing medium generates the majority of the plant nutrition (Dorais & Alsanius 2015). Manufactured greenhouse substrates are often peat-based mixtures, though peat is frowned upon in the European Union because it is a non-renewable resource (Gamliel & van Bruggen 2016), so alternatives such as bark, coco coir and composts are of interest. In Canada, at least 10% of organic growing media must be compost, and vermicompost is permitted with some limitations on feedstocks, for example sewage sludge is prohibited (Canadian

General Standards Board, 2018). An ideal organic greenhouse substrate for demarcated beds would be a soil-like media providing excellent nutrient cycling, and exhibiting a high level of general biological suppression of plant disease, discussed below.

1.3. Organic production uses composts and diverse microbial assemblages

1.3.1. Organic greenhouse production

Organic greenhouse production shares aspects of field horticulture and of hydroponic greenhouse agriculture. Organic greenhouse media contains composts which provide a diverse and active microbiota and have been shown to increase resistance to disease (Hoitink & Boehm 1999). Disease suppression varies between composts (Bonanomi et al. 2007; Larkin 2015; Noble & Coventry 2005; Termorshuizen et al. 2006; Yogev et al. 2006). In industrial organic media production, compost additions can vary between crop cycles, affecting media disease suppressiveness. This is complicated by the disease suppressive properties of other components of the media mixture such as perlite and peat: (Clematis et al. 2009). Because there is a risk of the introduction of pathogenic organisms from composts (Al-Mazroui & Al-Sadi 2015; Noble 2011), it would be useful to be able to add biological control organisms to growth media to reduce disease. It is not known whether biocontrol products are reliably compatible with compost-containing systems because the inherent disease suppressiveness of the media could also suppress the growth and activity of additional biological control organisms.

Figure 1.1. Biologically active organic greenhouse media before planting

1.3.2. Studying the organisms found in hydroponic and organic media

The hydroponic environment contrasts strongly with soil-based agricultural systems, which generally contain 10^7-10^9 colony-forming units (CFU) of bacteria and 10^4-10^6 culturable fungal propagules per gram of soil, compared with 10^5-10^7 CFU of bacteria per mL in hydroponic nutrient solution, and as low as 10^3 CFU per mL before planting (Postma 2010). Another critical difference in the soilless environment is the rate of flow of nutrient solution and the rate of diffusion of root exudates away from the root surface. This is a major difference in the rhizosphere that a microbe finds in a hydroponic system versus one that it would find in a soil- based system. It has been hypothesized that root surface colonization capability is an important attribute for microorganism establishment in soilless systems (Postma 2010). Because of media components such as thermocompost and vermicompost, the levels of microorganisms in organic greenhouse media should be more similar to field soil-based horticultural systems than to hydroponics. Vermicompost in particular has a very high microbial

activity, the properties, production, and disease suppressive abilities of which are discussed extensively in Chapter 2. Compost inputs contribute dense microbial communities that perform nutrient cycling as well as directly supplying plant available nutrients. These are also used to produce compost teas used as a drench for disease suppression and nutrient delivery. High populations of nutrient-cycling microbes are required in this system to render the larger molecules of organic fertilizers available for plants compared with a chemigated system providing nutrient salts. Mäder et al., (2002) found that in addition to greater microbial activity and biomass, organic production methods greatly increased the diversity of microbes, earthworms, carabids, staphylinids, and spiders over conventional production in field crops. Organically managed soils have been shown to have lower levels of nitrate and soluble nitrogen, increased diversity of bacteria and nematodes, as well as more resilience to drying (Van Diepeningen et al. 2006). Organic vegetable greenhouses can develop a prodigious abundance of spiders, earthworms, Collembolans and other organisms such as *Oxidus gracilis* and *Musca domestica* (unpublished observations). Organic production media is necessarily complex, and understanding the communities that deliver its functions requires a combination of methods. The same is true for vermicompost: vermicomposting changes the community structure of composted substrates. The resulting communities and the effect of starting materials, composting conditions, and worm species have been studied, using techniques ranging from chemical and cultural analyses to high-throughput sequencing. These are reviewed in Chapter 2.

1.3.3. Biological control agents versus composts and the holistic approach of organic agriculture

The complex interactions between biocontrol agents and composts are not well understood. For example, a biocontrol agent such as *Bacillus subtilis* (Ehrenberg) Cohn that produces a strong antibiotic (Stein et al. 2005) could eliminate a bacterial species crucial to a compost's ecology, resulting in a collapse of the compost population and its ability to suppress disease. Similarly, an agent such as *Clonostachys rosea*, an excellent fungal hyperparasite producing antifungal enzymes (Chatterton & Punja 2009), could harm the compost's fungal population. Although specific biological suppression has been shown to be the mode of action for some composts (Bonanomi et al. 2007; Suárez-Estrella et al. 2013), in other cases the suppressive effect cannot be narrowed to one agent or mechanism (Hadar & Papadopoulou 2012; Pane et al. 2011) and can be

considered an emergent property of the microbial community (Jack 2011). Furthermore, one of the tenets of organic agriculture is to treat the production system as a holistic ecological system: composts are used to promote soil biology, and it can be argued that seeking a single agent or mode of action violates this holistic philosophy (Doyle 2017). Thus, if we consider disease suppression by composts to be attributable to a diverse assemblage of microbes, rather than one particular member (Hadar 2011), it is necessary to know the effect of perturbing these populations by adding a biocontrol agent. This is in addition to knowing the efficacy of the biocontrol agent when combined with compost.

1.4. Do biological control agents suppress vermicomposts, do vermicomposts suppress biological control agents?

1.4.1. Biological control mechanisms

Antagonistic microbes can use several mechanisms to exact biological control such as hyperparasitism, production of antibiotic compounds or other inhibitory substances, competition for nutrients or space, or induced resistance (Whipps, 2001). For clarity, these mechanisms can be divided into three categories of antagonism: direct (hyperparasitism/predation), mixed-path (antibiotics, lytic enzymes, unregulated waste products, physical/chemical interference), and indirect (resource competition, induction of host resistance) (Pal & Mc Spadden Gardener 2006). Suppression of disease is assumed to result from a combination of these mechanisms: each is discussed in more detail below.

1.4.2. Hyperparasitism and predation

Parasitism and predation can contribute to biocontrol where one fungus directly harms another, and images of *Trichoderma virens* and *Trichoderma harzianum* attacking *Rhizoctonia solani* are commonly found in formal and informal plant pathology literature. Descriptions of mycoparasitic activity are featured in the marketing of biocontrol agents such as Prestop® (*Clonostachys rosea* syn *Gliocladium catenulatum* strain J1446) and Rootshield® (*Trichoderma harzianum* Rifai strain KRL-AG2). Many fungi, bacteria, and even the amoeba *Vampirella* have been found to attack plant pathogenic fungi (Agrios 2005), but there has been a longstanding question as to whether mycoparasitism is a

major mechanism contributing to biocontrol. When this was investigated by testing the biocontrol efficacy of non-mycoparasitic mutants of strongly mycoparasitic *Gliocladium virens* (syn *Trichoderma virens*), no difference was found in biocontrol efficacy between mycoparasitic and non-mycoparasitic mutants (Howell 1987).

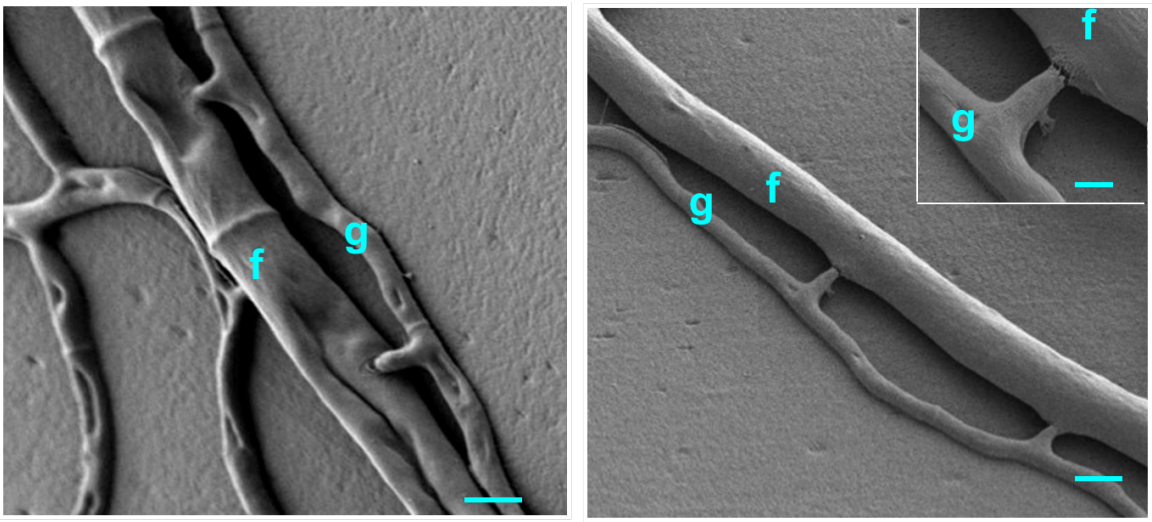

Figure 1.2 Hyphae of *Clonostachys rosea* (g) penetrating hyphae of *Fusarium oxysporum* (f). Scale bar = 30um.
Photo: Syama Chatterton. Reproduced with permission.

As to whether mycoparasitism plays an important ecological role in natural systems, it is present in natural phylloplane populations of powdery mildews (Kiss 1998), but investigating mycoparasitism in the soil is difficult. Mycoparasitism requires four sequential steps: chemotropism, recognition, attachment and coiling, and cell wall penetration and digestion (Chatterton 2010). Mycoparasitic ability can thus be partly inferred from the ability of biocontrol fungi to achieve this crucial final step, which requires the production of lytic enzymes such as chitinases and β-1,3-glucanases to degrade fungal (chitin and β-1,3-glucan) and oomycete cell wall (glucans only) under certain conditions (Viterbo et al. 2002). Agents such as *Clonostachys rosea* f. *catenulata* (syn *Gliocladium catenulatum*) have been shown to exhibit mycoparasitic behaviors (coiling, enzyme production, and penetration) against the cucumber pathogens mentioned above under certain conditions (Figure 1.2)(Chatterton and Punja 2008), in addition to (or perhaps as part of) providing measurable biocontrol (Rose et al. 2003; Punja & Yip 2003).

1.4.3. Antibiosis and other mixed-path antagonism

Many fungi and bacteria produce antibiotics, but not all are suitable for development as biocontrol products. The biocontrol fungi *Trichoderma* and *Gliocladium* have been studied extensively for antibiotic production (Whipps 2001). Fungi of these genera produce two main antibiotics: gliovirin, primarily effective against oomycetes (Howell 2006), and the broad spectrum gliotoxin, effective against bacteria, actinobacteria, and fungi (Chatterton 2010). As well, *T. harzianum* can produce peptaibols, antibiotics that inhibit β-glucan production and are antimicrobial against bacteria and fungi (Lorito et al. 1996). Not all antibiotic-producing fungi use this capability equally: a *T. virens* non-antibiotic mutant was found to be just as effective in biocontrol as antibiotic producing mutants (Howell 2006), but *T. harzianum* has been shown to use volatile pyrone-like antibiotics against *Gaemannomyces graminis* var *tritici* to suppress the pathogen (Vinale et al. 2008). Fluorescent *Pseudomonas* spp. are the agents responsible for some suppressive soils. They possess the ph1D gene and can produce the antibiotic 2,4-diacetylphloroglucinol. For example, *P. fluorescens* strain CHA0 suppresses pythium damping off of cucumber, among many other diseases. Among the disease suppressive phlD+ fluorescent *Pseudomonas* spp. antibiotic production, rhizosphere competence, and disease suppression vary between subspecies: some are more effective against pythium damping off of cucumber and fusarium crown and root rot of tomato than others (Weller et al. 2002). Although this antibiosis mechanism appears simple, it is affected by factors such as the ability of *P. ultimum* to reduce the ecological fitness of *P. fluorescens* F113 (Fedi et al. 1997). *Bacillus cereus* UW85 was shown to protect tobacco roots from *Pythium torulosum* infection through the action of the antibiotics Zwittermicin A and kanosamine, although the effect was stronger with the *B. cereus* cells present than with purified antibiotics, and a mutant that did not produce antibiotics also led to fewer zoospores around the root, fewer encysted zoospores, delayed cyst germination, slower germ tube growth, and lower disease than antibiotics alone (Shang et al. 1999). This suggested that the interaction between *P. torulosum*, *B. cereus*, and tobacco roots is more complicated than antibiosis alone. A more recent example of ecological interactions complicating antibiosis is found in the case of *Pseudozyma flocculosa* (prev. *Sporothrix*). This organism was developed as a biocontrol for powdery mildew because of its production of the antibiotic flocculosin, but recent work demonstrates that its mode of action is more

likely the exploitation of a nutrient that the phytopathogen harvests from the plant (Bélanger et al., 2012). Simon et al. (2001) found that the genotype of tomato can influence the growth of *Bacillus cereus* UW85 and this, in turn affects the suppression of damping off caused by *Pythium torulosum*. The role of antibiosis in biocontrol is complicated, and depends heavily on conditions or organisms involved and is discussed with respect to naturally disease-suppressive soils in section 1.7.

1.4.4. Indirect competition: Resource competition

Competition in biocontrol is thought to occur through two pathways: competition for nutrients and competition for infection sites on the root surface (Alabouvette et al. 2007). Opportunistic pathogens are very susceptible to competition for limited nutrients (Paulitz & Bélanger 2001), so if a biocontrol agent is able to colonize the plant rhizosphere it should be competitive against these pathogens (Alabouvette et al. 2007). Much of the work on competition has been done with non-pathogenic strains of *Fusarium oxysporum* that can suppress pathogenic strains of the same species. The nonpathogenic strains were isolated from individual plants that did not show disease symptoms when other surrounding plants were diseased, not from suppressive soils (Agrios 2005). These strains show better efficacy at low nutrient concentrations (Larkin & Fravel 1999; Lemanceau et al. 1993). Infection sites on the plant root are considered to be finite (Alabouvette et al. 2007), and studies have demonstrated that the total fungal biomass remains the same when roots are inoculated with both pathogenic and nonpathogenic strains of *F. oxysporum* as when they are inoculated with either strain alone (Eparvier & Alabouvette 1994). These results are also consistent with competition for nutrients (Olivain et al. 2006). An important consideration is that the competition mechanism is not useful when the pathogen and the biocontrol occupy different ecological niches, such as root cell surface tissue versus vascular tissue (Bao and Lazarovits 2001).

1.4.5. Indirect competition: Induced Resistance

Plants can be induced to be resistant to disease through several pathways: systemic acquired resistance (SAR) or salicylic acid mediated pathway usually activated

in response to pathogen attack, the wound-induced jasmonic acid pathway (ISR), and the rhizobacteria induced systemic resistance (RISR) pathway. The terminology is confusing because different researchers use the same terms for different effects: many researchers use the term ISR to refer to the rhizobacteria-influenced pathway (Van Loon 1997) that does not involve the production of pathogenesis-related (PR) protein cascades so that both of these different pathways have been termed ISR in different parts of the literature (Harman et al. 2004). All three of these pathways can prime a plant against a pathogen that it is normally susceptible to. In soils and organic production media, plant growth promoting rhizobacteria (PGPR) can act through RISR to prime plants to induce this pathway by colonizing roots. It is likely that field crop plants exist in a state of induced resistance (Walters 2009), organic greenhouse crops grown in soil or organic production media are probably similar to field crops in this regard because of their interaction with soil microbes, and as discussed above, hydroponic media usually host a microbiome as well, and these crops could also be primed through their interactions with biofilm organisms. Systemic-acquired resistance (SAR) is the induction of resistance of a plant to a pathogen that it is normally susceptible to: this can be done by inoculation of the plant with a pathogen, a component of a pathogen, a nonpathogenic organism, or by the application of chemical compounds such as salicylic acid and dichloroisonicotinic acid, and some benzothiazoles (Agrios 2005). This resistance can be epigenetially transferred to a plant's offspring (Luna et al. 2012; Slaughter et al. 2012). In the context of biocontrol, SAR is induced by exposing a plant to a biological agent that induces the response but does not cause disease.

1.5. Biological control and its use in greenhouses

1.5.1. Biological control for soilborne phytopathogens in greenhouse media

Many microbes have been demonstrated to be antagonistic to plant pathogenic organisms in the laboratory and in greenhouse and field trials, but full-scale field applications remain inconsistently effective (Jack 2011). Biocontrol of plant pathogens has yet to reach the level of success realized by the more established field of insect biocontrol despite decades of research (Spadaro & Gullino 2005). Phytopathogen

biological control is necessitated by both public opposition to pesticides (McNeil et al. 2010), and resistance of phytopathogens to existing pesticides: the 1960s saw the emergence of fungicide-resistant fungal phytopathogens, as well as the consolidation of public concern around the environmental effects of pesticide use (Agrios 2005). In the US between the mid-1980s and the early 2000s, 85-90% of historically available pesticides or permitted pesticide uses were banned or discontinued (Agrios 2005), and even fewer remain available to the Canadian market. Public opinion has also limited the success of crops that are genetically modified to be resistant to disease. The compatibility of biocontrol microbes with greenhouse systems makes them a good choice for, phytopathogen biocontrol in these systems.

1.5.2. Biological control agents persist in greenhouses

There are concerns that because many biocontrol agents were isolated from soils, or from crops other than the target crop, that these organisms may not be adapted to soilless systems (Postma 2010). The agents may not be adapted to organic greenhouse media if they were isolated from field soils, as they may not be competitive at the high nutrient concentrations used in these systems, despite the media containing more soil-like organic components than hydroponic media. This view that biocontrols cannot easily proliferate and maintain their populations is strongly refuted by Harman (2000). Inoculation of hydroponic systems with individual biocontrol bacteria has been demonstrated to suppress disease in many studies (Clematis et al. 2009). Examples include plant growth promoting rhizobacteria (PGPR) (McCullagh et al. 1996) and *Pseudomonas* spp. for suppression of pythium root rot on rockwool-grown cucumber (Paulitz 1997; Zheng et al. 2000), lettuce (Utkhede et al. 2000), and sweet pepper (Khan et al. 2003). Inoculation of hydroponic systems with biocontrol fungi has also been successful, this work has mostly focused on *Trichoderma* and *Gliocladium* spp. For example, the biocontrol fungus *Clonostachys rosea* (syn *Gliocladium catenulatum*) has been shown to reduce the pathogens Forc (Rose et al. 2003) and *Pythium aphanidermatum* (Punja & Yip 2003) on hydroponic cucumbers, and *C. rosea* is a better colonizer of cucumber plants in nutrient solution than those in potting mix or field soil (Chatterton & Punja 2010), despite having been originally isolated from field soil.

1.5.3. Adoption of biological controls for disease management in greenhouses

As in all types of biological control, pathogen and antagonist must be synchronized in time and space (Postma 2010). This is theoretically simple for microorganisms in hydroponic systems, but in organic greenhouse systems it may be more difficult for the antagonist to meet the pathogen in more complex media. Even in hydroponic greenhouse systems where biocontrol agents can theoretically be added to the nutrient solution to ensure uniform distribution (Paulitz 1997), it may be difficult to apply microbial controls in this manner due to complications from in-line filtration systems, venturi effects in pumping systems, and concerns over biofilm creation in the irrigation systems. Formulated biological controls must stand up to the rigors of being treated as a pesticide product. Shelf life is less of a problem for sporulating organisms, but non-sporulating organisms present a challenge (Postma 2010). This translates to difficulties in supply chain management for products with a short shelf life. Once formulated, biological control products need to demonstrate consumer safety, environmental safety, and efficacy to be registered. This process is expensive, especially for products that have relatively small markets (Postma 2010). Favourable restricted entry intervals for biological controls should help outweigh the extra complications involved in applying biological fungicides however occupational exposure of greenhouse workers to aerosols from biocontrol and other fungi may be an emerging public health issue (Li and LaMondia 2010).

1.6. Current assays of biocontrol efficacy are limited, biased or cumbersome

Whereas greenhouse and field trials are cumbersome and expensive, existing *in vitro* bioassays for biocontrol efficacy such as dual culture/inhibition zone or spore count studies are too rudimentary (Verma et al. 2007). Although *in vitro* assays are an important tool for screening biological control agents before time-consuming and expensive plant trials, they do not always predict biocontrol efficacy on plants in pots or

in the field (Knudsen et al. 1997). For example, *B. bacillus* B068150 is known for its ability to suppress fusarium wilt of cucumber, but shows no obvious antagonistic activity to *F. oxysporum* f. sp. *cucumerinum* in dual-culture assays on PDA (Li et al. 2012). Of the full range of biocontrol modes of action, *in vitro* dual culture assays are heavily biased towards antagonists using toxin production as a single mode of action and there is a need for an *in vitro* method that is less biased (Köhl et al. 2011). Early work by Davet et al. (1986) used biocontrol-inoculated soil mixed with agar to screen *Trichoderma* isolates for saprophytic competitiveness. The technique was validated by correlating the *Trichoderma* isolates' performance in the agar test to parasitism of *Sclerotium rolfsii* in non-sterile soils (Davet & Roure 1986), and the technique was improved by Naar & Kecskes (1998). Compared with studies of enzyme production and antibiotic production *in vitro*, this type of assay has received little attention. Previous *in vitro* work has focused on testing the compatibility between different biocontrol agents such as different strains of *Pseudomonas* (De Boer et al. 2003; De Boer et al. 1999), and combining biocontrols with chitosan (Khan et al. 2005). The effect of compost tea on plant pathogens *in vitro* has also been studied (Marín et al. 2015). To our knowledge no study has addressed the compatibility of biocontrols and vermicompost tea *in vitro*.

1.7. Suppression of diseases can be through specific or general biological suppression

1.7.1. Complex communities: suppressive soils and other diverse assemblages

The development of biological control began with suppressive soils, when it was noted that certain soils did not allow disease to develop in crops. These suppressive soils operate using two classical types of suppressiveness to soilborne plant pathogens: general suppression uses the total biomass of the soil to produce antagonism, and is not transferable between soils, whereas specific suppression comes from specific microorganisms or groups of microorganisms and is thus transferrable to other soils (Weller et al. 2002). The demonstration that microbial agents are responsible for disease suppression led to the isolation of the organisms suspected to be the cause of suppressiveness, and the development of these organisms as individual biological

control agents for inundative application (Vilich & Sikora 1998). In parallel to this single-agent approach, complex assemblages of microbes such as composts, vermicomposts and compost teas are used for disease suppression in organic greenhouse systems. Alternatively, various organic amendments are incorporated into soils to steer the soil microbiome towards suppressiveness, reviewed in Bonanomi (2018).

1.7.2. Specific suppression may be too simple to explain biocontrol by composts

The disease suppressive ability of complex mixtures of unidentified microbes as found in some suppressive soils, composted substrates, compost teas, and organic amendments is likely to be driven by community-level processes, not just the activity of one easily cultured and mass- produced species, as well as the abiotic factors that this community has developed in, including the availability of substrates required for the production of secondary metabolites such as antibiotics (Vilich & Sikora 1998). Indeed, even the elegant single-agent specific suppression mechanism for the take-all of wheat pathosystem, emblematic of specific suppression, has been questioned as to its simplicity. In this system, fluorescent *Pseudomonas* spp. suppress the pathogen *Gaeumannomyces graminis* var *tritici* (*Ggt*) with repeated cropping in the same soils, and take-all decline results from the building up of these *Pseudomonas* spp. producing the antifungal metabolite 2,4-diacetylphloroglucinol. Take-all decline has, however, been correlated with changes in the community composition of rhizobactera, not just pseudomonads (Sanguin et al. 2009). Fluorescent pseudomonads appear less important for disease suppression in organically managed soils than in conventional management: higher levels of microbial activity and at least some specific bacterial genera are involved (Hiddink et al. 2005).

1.7.3. General suppression and specific suppression in greenhouse media

A biological control agent may not always be able to colonize a medium with an established microbial community: the biologically complex planting medium could buffer

the effect of an added biological control agent. It had been determined that the biological control organism *Clonostachys rosea* was a better colonizer of cucumber plants in nutrient solution than plants in potting mix or field soil (Chatterton & Punja 2010), despite having been originally isolated from soil, which supports this hypothesis. The low biodiversity and organismal vacuum of soilless substrates at the start of production is seen as conferring an advantage to the biocontrol organisms, as they are allowed to establish and proliferate (Fravel, 2005; Postma, 2010) and because of the continuous and even distribution of the agents (and the pathogens) (Grosch et al., 1999). This is where the knowledge gap currently exists: theoretically, a sterile hydroponic system conducive to the survival of biocontrol agents is predictable but not robust, conversely a microbially complex media should be robust but less predictable. In practice, although hydroponic media are not as microbially-rich as composts, they do contain their own native microflora that can suppress disease, or can be inoculated with disease suppressive agents (Hultberg et al. 2000; Khan et al. 2003; McCullagh et al. 1996; Paulitz & Bélanger 2001; Punja & Yip 2003; Utkhede et al. 2000; Vallance et al. 2011). The intrinsic suppression of disease in hydroponic rockwool, nutrient solution, and peat media appear to be correlated with increased bacterial diversity and actinobacterial (Streptomycete) populations in rockwool, unknown organisms in nutrient solution, and *Rhizobium-Agrobacterium* group, Acidobacteria, and Basidiomycetous yeasts in peat (Postma 2010). The bacteria responsible for these effects in rockwool appear to be unculturable (Calvo-Bado et al. 2006). The effects of biofilms on biocontrol agents is not well studied, and there are conflicting *a priori* views as to whether their effect is positive (Scheuerell 2002) or negative (Calvo-Bado et al. 2006) (See Chapter 2). The creation of soilless systems that are microbiologically as well buffered as natural soils rather than attempting to keep these systems sterile is argued to combine the advantages of natural soil with those of soilless systems (Postma 2010). A generally disease suppressive organic media for greenhouse use in lieu of rockwool or similar should provide a robust system, and is only limited by the current understanding of complex soil-like systems. Containerized organic media greenhouse systems mitigate some operational complications of organic greenhouses over conventional hydroponic management. Infection by soil-based phytopathogens has been demonstrated to remain a threat in hydroponic systems. Nutrient delivery from organic sources has been demonstrated to deliver reliable results in practice (unpublished observations), and robust management

of resistance to plant disease in soil and soil-like media based greenhouse systems is the next challenge.

Composts have been investigated for their disease-suppressive activities, and they have been theorized to act using general and specific suppression, including antagonism, nutrient sequestration and release, induced resistance, and inhibition through antibiosis (Hoitink & Boehm 1999; Hoitink et al. 1997). As discussed above, attributing biocontrol effects of composts to specific suppression may be an oversimplification, and in this case the application of a biocontrol product to a compost-containing medium could be viewed as an interplay between general biological suppression and specific biological suppression. Disease suppression by composts is complicated: Composts suppress disease by both abiotic and biotic factors (Krause et al. 2001). Complicating field and pot trials, beyond disease reduction, vermicompost can have beneficial effects on plant growth through nutrition and hormones, (reviewed in Lazcano & Domínguez (2011)). Of the above disease suppression mechanisms, all but induced resistance would be expected to not only antagonize an introduced pathogen but also an introduced biocontrol agent. Conversely, single biocontrol agents use the same mechanisms to provide disease suppression, and could adversely affect a suppressive compost microbial community, reducing its ability to provide disease suppression, as it has been demonstrated that removing the biological components of composts leads to partial or complete loss of disease suppression (See Chapter 2). For example, a biocontrol agent that produces a strong antibiotic such as *Bacillus subtilis* (Stein 2005) could eliminate a bacterial species that is crucial to the compost ecology, resulting in a collapse of the compost population and its ability to suppress disease. Similarly, an agent such as *Clonostachys rosea*, an excellent fungal hyperparasite producing antifungal enzymes (Chatterton & Punja 2009), could harm the compost's fungal population. This idea is not without precedent: AM fungi have been shown to inhibit the growth of other soil microbes (Welc et al. 2010). Because the biological control of plant disease by suppressive composts can be attributed to the function of a diverse assemblage of microbes, rather than one particular member (Hadar 2011) as is the case for specific biological suppression, it is necessary to know the effect of perturbing these populations by adding a biocontrol agent. This is in addition to knowing the efficacy of the biocontrol agent when combined with compost.

1.8. Are biocontrol agents compatible with composts/greenhouse media?

1.8.1. Can biological control agents persist in organic greenhouse media?

Soil health is an important pillar of organic agriculture, and greenhouse organic production reflects this to a large degree. As discussed above, even in jurisdictions where demarcated soil beds are permitted (Northern Europe and Canada) rather than *in situ* soils, plant nutrition must come from nutrient cycling in the media, and media must contain composts. Addition of compost has profound and rapid effects on soil microbial communities (Alabouvette et al. 2006; Pane et al. 2013), and this would be expected to also be the case in plant growth formulations with respect to affecting existing populations of disease suppressive organisms.

On their own, composts can vary widely in their ability to reduce disease. It would be useful to be able to add biocontrol agents to composts to reinforce or diversify their disease suppressive abilities but compatibility studies with existing commercial biocontrol agents are limited. Biological controls can behave unpredictably at the farm scale (Mathre et al. 1999), are not as fast acting as chemical fungicides, nor can they be used successfully as inundative treatments once disease has become established. They may need to be used in conjunction with other farm practices and products as part of a broader disease management program (Spadaro & Gullino 2005), rather than as inundative pesticide replacements. This represents a cultural shift in industrial greenhouse agriculture, but a necessary one because consumer demand for an industry that does not use pesticides is growing (Van Lenteren 2000; McNeil et al. 2010).

Biological control using inundative single agents has been more successful in greenhouse systems than in field soils (Mazzola & Freilich 2016): in field soils there is a significant decline in biocontrol populations after inoculation into soils (Nihorimbere et al.

2011). Suppression of biocontrol agents has been found in soils with higher biomass: introduced *T. harzianum* was suppressed in soils with increased biomass due to supplemented carbon (Bae & Knudsen 2005), and introduced *P. fluorescens* had greater efficacy in conventionally managed soil with lower microbial activity than similar but organically managed soil (Hiddink et al. 2005). These effects could theoretically apply to organic greenhouse media. If the strength of the general suppression exhibited by composts and soil microbes is its microbial diversity, any agent that reduces this diversity could be expected to have a negative effect on general biological suppression, even if that agent were itself exhibiting specific suppression.

1.8.2. Mixing multiple biological control agents: simple antagonist mixtures

Intermediate between individual biocontrol agents and complex assemblages such as suppressive soils or composts are combinations of small numbers of biocontrol organisms whose interactions are simple enough to be understood using current techniques. Some of these are recommended for improving the efficacy of existing biocontrols (Spadaro & Gullino 2005). These include the combination of a fluorescent *Pseudomonas* sp. with a nonpathogenic *F. oxysporum* to suppress pathogenic *F. oxysporum* so that the *Fusarium* species compete for carbon and the bacterium produces an iron chelating siderophore (Alabouvette et al. 1996), and a combination of *Trichoderma* and *Pythium nuun* to suppress *Pythium ultimum* so that the *Trichoderma* protects the plant root, and the *P. nuun* reduces the propagule density of the *P. ultimum* (Paulitz et al. 1990). In both of these cases, care is taken to choose biological controls that have different modes of action, and occupy different ecological niches. Successful combinations of antagonists were also reported by Dunne et al. (1998), who used proteolytic and phloroglucinol producing bacteria to suppress *Pythium* on sugar beet, Raupach & Kloepper (1998) and Liu et al. (2017), who used combinations of PGPRs to suppress several cucumber and tomato diseases, El-Tarabily (2006), who used actinobacteria combinations to suppress *P. aphanidermatum* on cucumber, Roberts et al. (2005) who used combinations of *Trichoderma*, *Burkholderia* and *Serratia* against *R. solani*, *P. ultimum*, and *Meloidogyne incognita* on cucumber, with mixed results, and Kim et al. (2008) who used bacterial combinations to suppress *Phytophthora* on pepper.

Another approach is the use of a biocontrol in conjunction with chitin or chitosan (Sid Ahmed et al. 2003; Benhamou et al. 1998) to improve its efficacy, a technique used successfully for *Pythium aphanidermatum* biological control on cucumber using the chitin-degrading and antifungal producing bacterium *Lysobacter enzymogenes* strain 3.1T8 and chitosan (Postma et al. 2009). The synergistic effect of chitosan and *Lysobacter* has not been elucidated, but could be antifungal (although this was controlled for in the Postma (2009) study), plant resistance inducing, and/or stimulation of the biocontrol: chitosan is a nutritious supplement for microbes that are able to exploit it. Exending the interactions illustrated here to a mechanistic understanding of the full range of possible interactions between a biocontrol agent and a compost or soil's microbiome is more complex and will require a combination of traditional biocontrol research and high throughput community analyses.

1.8.3. Combining biocontrol agents with composts

Composts have been combined with biocontrols in field studies previously with mixed results. Larkin & Tavantzis (2013) performed a study that used *Bacillus subtilis* or hypovirulent *Rhizoctonia solani* alone or in combination, and compared them with composts to reduce soilborne diseases of potato in the field*. B. subtilis* had previously been shown to suppress diseases caused by *Rhizoctonia* (Brewer & Larkin 2005). It would be reasonable to speculate that *B. subtilis* would also suppress a hypovirulent *Rhizoctonia* biocontrol. The authors found that the combination of these two biocontrols was not significantly better than each biocontrol alone. Also importantly, the composts increased yields, without much disease reduction, an effect the authors attributed to greater carbon input. This effect must be mitigated to make fair comparisons between treatments using the strategy of collecting plant dry weights, as well as disease severity or area under the disease progress curve (AUDPC) values. The third objective was to determine if there was a benefit of adding compost and biocontrol agents together, a common practice in the field of fortified composts. The authors used hypovirulent *Rhizoctonia* combined with composts and found some limited synergistic effects. Inconsistent and occasionally marginally additive effects of combining composts and the biocontrol agents *Trichoderma virens*, *Bacillus subtilis*, and *Rhizoctonia solani* hypovirulent isolate Rhs1A1 for disease suppression on potato were reported in a later

study by the same group (Bernard et al. 2014). Interestingly, a green manure crop rotation with *Brassica napus* was more consistently effective than compost or biocontrol agents.

Studies have examined the compatibility of biological control agents with composts in plant media: in particular, Pereira et al. (1998) found incompatibility between *B. subtilis* and *T. harzianum* in vermicompost. Larkin & Tavantzis (2013), Bernard et al. (2014), and Pugliese et al. (2011) tested combinations of *Bacillus subtilis*, hypovirulent *Rhizoctonia solani*, *Trichoderma virens*, and composts to reduce soilborne diseases of potato in the field and on greenhouse cucumber, tomato, bean and basil, finding limited synergistic effects. In the Pugliese et al. (2011) study, when *Trichoderma* and non-pathogenic *Fusarium* were added to composts to suppress diseases of greenhouse cucumber, tomato, bean and basil, *Trichoderma* enriched compost was found to be effective against *R. solani*, but not *P. ultimum* and *P. nicotianae*. The lack of additive effects in these experiments could be explained if the composts are suppressing the biological control agents.

Organic greenhouse production media could serve as a simplified model to investigate biocontrol in soil systems: In industrial organic production the media formulation changes between cropping cycles making controlled studies *in situ* difficult. This contrasts with field soils which have a relatively unchanging microbial population over successive cropping cycles, which allows meaningful repeated experiments. Thus, a model system for this type of production media is needed.

1.9. Pathosystems in the current study

1.9.1. Cucumber/Forc

Cucumbers (*Cucumis sativus* L.) are a well-studied crop because they are widely grown in commercial greenhouses, some of which use intensive organic production

methods. They are susceptible to fungal diseases, and their short cropping cycle lends itself to research use. One common pathogen of greenhouse cucumbers in British Columbia is fusarium root and stem rot caused by *F. oxysporum* Schlechtend:Fr. f.sp. *radicis-cucumerinum* D.J. Vakalounakis (Forc) a Hypocrealean ascomycete (Punja & Parker, 2000; Vakalounakis, 1996), which causes brown crown discolouration and vertical stem lesions, and later white mycelium and masses of orange conidia on the infected plant which wilts and dies (Vakalounakis 1996; Punja & Parker 2000), as depicted in Figure 1.3. Plants are usually infected at the seedling stage, but remain asymptomatic until stressed by environmental factors such as high temperatures or imbalanced nutrient feed, or by fruiting (Punja & Parker 2000). Ridomil Gold 480 EC and 480 SL (both metalaxyl–m and –s isomers, Syngenta) and Previcur (propamocarb, Bayer) are labelled for use as a post-transplant drench in greenhouse cucumbers in British Columbia but are not permitted in organic production. Some cultivars show resistance to *F. oxysporum* (Rose & Punja, 2004). This pathosystem was chosen for our study because Forc is an important disease of greenhouse cucumbers, and because this pathogen has been shown to be susceptible to biocontrol by the fungus *Clonostachys rosea* (Chatterton & Punja, 2010), including by direct parasitism (Figure 1.2).

Figure 1.3 . Growth and sporulation of *Fusarium oxysporum* f. sp. *radicis-cucumerinum*, showing extensive colonization of cucumber stem and abundant production of orange spore masses.
From Punja & Parker (2000), adapted by Syama Chatterton. Reproduced with permission.

1.9.2. Radish/*Rhizoctonia solani*

Radish (*Raphanus sativus* L.) is a widely grown vegetable susceptible to infection by *Rhizoctonia solani* J.G. Kühn, a soilborne plant pathogen with worldwide distribution that also affects a wide variety of other crops. The radish/*Rhizoctonia* pathosystem has been well studied. Of particular interest for our study, rhizoctonia damping off disease is not consistently suppressed by composts alone: the mode of action is thought to be through specific suppression by one or more individual biocontrol agents (Hoitink & Boehm 1999; Santos et al. 2007; Scheuerell et al. 2005; Simsek Ersahin et al. 2009).

F. oxysporum (on flax) and *R. solani* (on cauliflower) were categorized by Termorshuizen et al. (2006) as pathogens that are susceptible to competition. Despite the variability of *Rhizoctonia* suppression by composts, both *F. oxysporum* and *R. solani* were consistently suppressed by composts in greenhouse and growth chamber experiments, reviewed by Noble and Coventry (2005). This suggests that in a study such as ours these organisms could be affected by biocontrol effects from vermicompost microbes.

1.10. Biocontrols and vermicompost in the current study

1.10.1. *Clonostachys rosea*

Clonostachys rosea f. *catenulata* Schroers, Samuels, Seifert, and Gams strain J1446 [syn. *Gliocladium catenulatum* Gilman and Abbott; teleomorph *Bionectria ochroleuca* (Schw.) Schroers and Samuels (Schroers 2001; Schroers et al. 1999)]. commercially formulated as the biocontrol Prestop® (Verdera Oy, Finland) has demonstrated broad-spectrum activity against foliar and root-infecting plant pathogens. Application of Prestop® was shown to reduce root disease caused by Forc on greenhouse cucumber (Rose et al. 2003). *Gliocladium* species have long been shown to suppress rhizoctonia diseases (Lumsden & Locke 1989), and the J1446 strain can be as effective as some chemical fungicides at suppressing rhizoctonia damping off on salvia and allysum (McQuilken et al. 2001). Prestop® is labelled for the suppression of damping off caused by *Rhizoctonia* on a wide variety of vegetable, herbs, and bedding plants in the USA and Canada, and for use on radish in the USA (Plant Products, 2017).

1.10.2. *Bacillus subtilis*

Bacillus subtilis (Ehrenberg) Cohn QST 713 (Rhapsody®) is used to suppress a broad range of foliar and root diseases (Fravel 2005), and *Bacillus subtilis* strains have demonstrated activity against *F. oxysporum* causing diseases of cucumber: *F oxysporum*. f.sp. *cucumerinum* alone and in conjunction with composted materials (Chung et al. 2008; Cao et al. 2011; Yang et al. 2014; N. Huang et al. 2017), and *F. oxysporum* f.sp. *radicis-cucumerinum* (Al-Tuwaijri 2009). *R. solani* is suppressed by commercial strains of *B. subtilis* in many different crop systems, reviewed by Pérez-García et al. (2011), crops on which *B. subtilis* has been studied for suppression of *R.*

solani include potato (Brewer & Larkin 2005), tomato (Asaka & Shoda 1996), and pepper (Y. Huang et al. 2017) and radish (Khabbaz et al. 2015). Biocontrol of *Fusarium oxysporum* has largely focused on fungal agents, but *B. subtilis* has also been studied for its suppression (Lecomte et al. 2016).

1.10.3. Vermicompost as a model system inoculum

Vermicompost is a good candidate for producing a microbial background in our assays: vermicompost provides an active suite of microbes that are selected by the worm gut (Gómez-Brandón et al. 2011). Vermicompost contains a greater diversity of microbes than thermocompost (Scheuerell et al. 2005; Vivas et al. 2009; Neher et al. 2013; Lv et al. 2015; López-González et al. 2015), and aqueous vermicompost extracts have been shown to suppress a wide range of phytopathogens and other fungal species *in vitro* (Arancon et al. 2004; Marín et al. 2013). As such vermicompost should provide a rich suite of organisms as a substitute for generally suppressive soils in disease reduction assays. Preparing compost tea from vermicompost exerts a selective step on the population, and reduces the amount of extra carbon that is added to treatments. Thus using a single vermicompost tea to inoculate into the organismal vacuum of an autoclaved media with relatively high carrying capacity, a consistently generally suppressive experimental model media should be generated. Despite differences between compost biocontrol efficacies, it is not expedient to test biocontrols in a wide variety of composts, a model system that is easily reproducible would allow for comparison between biocontrols. In chapter 3, vermicomposts are evaluated with respect to their suitability to provide general suppression of disease for the *in vitro* and *in planta* trials of chapter 4.

The proposed assay could use a wide variety of pathosystems and associated biocontrols: I chose to use the bacterial biocontrol *Bacillus subtilis*, the fungal biocontrol *Clonostachys rosea*, and the pathosystems of *Fusarium oxysporum* f.sp. *radicis-cucumerinum* on cucumber, an important greenhouse crop and *Rhizoctonia solani* on radish, a well-studied damping-off system as discussed above.

The impact of a microbially-rich substrate on the efficacy of an inundatively applied biocontrol agent, and the converse impact of biocontrols on existing communities, has not been previously studied using a controlled microbial population, and it is not known whether the interaction is additive, synergistic, or antagonistic with respect to disease suppression. To our knowledge, this is the first research that attempts to quantify the relationship between general and specific biological suppression of plant disease in a model system using a reproducible microbial background to allow comparison between experiments.

1.11. General Objectives of the thesis research

The microbial communities in organic media could be cooperative, neutral, or antagonistic towards microbial biological control agents; in the latter case this would reduce a biocontrol agent's ability to suppress disease. More abstractly, general biological suppression of plant disease could be reducing the efficacy of an agent performing specific biological suppression of plant disease, or vice-versa. Our general objective is to develop an experimental model in which to test examples of this interaction. An understanding of the microorganisms that inhabit vermicompost is a first step towards developing a reproducible experimental system in which organic greenhouse media biocontrol can be investigated. A literature review of the suppression of plant disease by vermicomposts is the objective of Chapter 2. Chapter 3 examines the consistency, activity and diversity of microbial communities in five vermicomposts using fluorescein diacetate (FDA) hydrolysis ability, denaturing gradient gel electrophoresis (DGGE) and cultural analyses to determine the suitability of this material as an inoculum for the study of biological control in organic plant growth media. A practical objective of this study is the development of an expedient, quantitative, reproducible assay for the initial screening of biocontrol efficacy for use in biologically active substrates, and this chapter also compares the disease-suppressive activity of these five vermicomposts using the cucumber/Forc pathosystem. General biological suppression of plant disease is represented by a community of microbes derived from vermicompost, and specific suppression of plant disease is represented by the addition of a commercial biocontrol agent, quantified by reduction in pathogen growth *in vitro* and by reduction in disease severity *in planta* in Chapter 4. This chapter develops and implements an *in vitro* method

for testing the theoretical framework using vermicompost and two commercial agents: the fungal biocontrol *C. rosea* and the bacterial agent *B. subtilis*, and further develops and implements an *in planta* growth chamber method for testing the theoretical framework using vermicompost, the aforementioned biocontrols and the two pathosystems cucumber/Forc and radish/*Rhizoctonia*. Chapter 5 will explain the conceptual framework underlying this research and develops a mixed-effects statistical model to test for an interaction between biocontrol agents and vermicompost using the data produced by the *in vitro* and *in planta* experimental systems in Chapter 4.

Chapter 2.

Plant disease suppression by vermicomposts

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2.1. Abstract

Biological control agents and composted materials including vermicomposts (VC) and their water extracts (vermicompost teas) are used to suppress plant diseases in organic and conventional crop production systems. During the past decade, expanding organic horticulture production has prompted an increased interest in utilizing VC. VC production and properties including microbial activity, variability and biological activity have been the subject of several studies. Questions remain regarding the consistency and mechanisms of disease suppression by VC, and their compatibility with biological control agents. Research studies have addressed VC production and their inherent properties, including microbial activity, biological activity, and disease suppression. In this review, we discuss the factors affecting disease suppression by VC. These include the importance of starting materials, the worm species utilized, approaches to assess suppressiveness, and compatibility with biological control agents. VC have potential for disease suppression and provide a basis for studying the interactions between general and specific suppression of diseases.

2.2. Vermicompost production and properties

Vermicomposting is a widely used mesothermic method for converting organic residues into finer particles with higher levels of available nutrients, microbial populations, and porosity, aeration, and water holding capacity desirable for plant growth media (Edwards 1995). The organic matter is ingested and ground in the earthworm gizzard, which increases surface area for microbial breakdown (Dominguez 2011). The worm gut is a relatively anoxic environment which contains a subset of ingested microorganisms (Drake & Horn 2007) that differ from the microbiome of the initial

substrate (Procházková et al. 2018). Worm castings are then excreted along with mucus from the worm's hindgut. Worm castings continue to be digested outside the worms and can also be re-ingested for further digestion. After the worms are removed to make the final vermicompost (VC) product, it remains biologically active because of the secreted mucus, which amplifies the biomass and diversity of bacteria, actinobacteria and fungi (Szczech & Smolinska 2001; Szczech 1999).

Several worm species are used for vermicomposting (Table 2.1): Depending on the area of origin, these worms have different temperature optimuma and ranges (Arancon & Edwards 2004). The most common species used in Canada are the two similar temperate worms *Eisenia andrei* (Bouché) (red tiger worm, striped) and *Eisenia fetida* (Savigny)(red wiggler, not striped) and the tropical *Eudrilus eugeniae* (Kinberg)(African night crawler). *Eisenia andrei* and *Eisenia fetida* are sometimes referred to interchangeably in the literature, but they are different species (Dominguez & Edwards, 2011). Vermicomposting can be used to process a large variety of waste materials, such as crop residues, municipal yard waste, forestry and paper wastes, thermocomposts, industrial, agricultural sludges, municipal sewage sludge and manures (reviewed in Lim et al. (2016)), as well as human waste in vermicomposting toilets (Hill & Baldwin 2012).

Table 2.1. Several worm species commonly used during the production of vermicomposts

Species	Common name	Native climate	Optimal temperature (and limit range)	Comments	References
Eisenia fetida (Savigny)	Red wiggler, brandling worm, tiger worm	temperate	25°C (0°C-35°C)	Most commonly used species worldwide	Edwards & Arancon 2004
Eisenia andrei (Bouché)	Red tiger worm	temperate	25°C (0°C-35°C)	Very similar to *E. fetida*	Edwards & Arancon 2004, Reinecke & Viljeon 1990
Eudrilus eugeniae (Kinberg)	African night crawler	tropical	25°C (16°C - 30°C)	Large and grows rapidly, requires high temperature	Edwards & Arancon 2004, Dominguez & Edwards 2011
Lumbricus rubellus (Hoffmeister)	Red earthworm	temperate	18°C	Earthworms sold as *L. rubellus* were usually found to be *E. fetida or E. andrei*	Edwards & Arancon 2004, Dominguez & Edwards 2011
Dendrobaena veneta (Rosa) aka *Eisenia hortensis* (Michaelsen)	European nightcrawler	temperate	25°C (9°C - 30°C)	Survives in soil	Edwards & Arancon 2004, Dominguez & Edwards 2011
Perionyx excavatus (Perrier)	Blueworm	tropical	25°C (9°C - 37°C)	Very high reproductive rate, requires high temperature	Edwards & Arancon 2004, Dominguez & Edwards 2011
Pheretima hawayana (Rosa)	Alabama jumper	tropical	-	Unable to withstand cold temperatures	Edwards & Arancon 2004, Dominguez & Edwards 2011

2.2.1. Microbial activity of vermicomposts

Vermicomposting changes the community structure of the composted substrates (Gómez-Brandón et al. 2013; Huang et al. 2014; Procházková et al. 2018; Sen & Chandra 2009; Vivas et al. 2009). Certain genera and phyla are amplified including *Bacillus*, *Pseudomonas* and *Microbacterium* (Pathma and Sakthivel 2013), *Bacteroidetes* (Héry et al. 2008; Bernard et al. 2011; Huang et al. 2013), Proteobacteria and γ-Proteobacteria (Koubová et al. 2015; Procházková et al. 2018; Vivas et al. 2009), and Actinobacteria (Huang et al., 2013; Yasir et al., 2009). Other populations are reduced, including the β-Proteobacteria (Procházková et al. 2018; Vivas et al. 2009). Bacterial communities found in VC have attracted interest because of their broad uses in agriculture, and for potential applications in biotechnology (Fernández-Gómez et al. 2012). Many species of bacteria originating from VC have been found to be potentially antagonistic to plant pathogenic fungi (Pathma & Sakthivel 2013). Comparisons between bacterial and fungal communities in VC produced from different starting materials and between vermi- and thermocomposts have also been made. Fracchia et al. (2006) found using single-strand conformation polymorphism (SSCP) that VC contained mainly relatives of Chloroflexi, Acidobacteria, *Bacteroidetes* and Gemmatimonadetes, whereas thermocompost yielded mainly Actinobacteria and Firmicutes. Neher et al. (2013) found using high throughput sequencing that the dominant fungi in VC included members of the class Agaricomycetes, the family Microascaceae, and the genera *Arthrobotrys*, *Zopfiella* and *Mortierella*. In contrast, thermocomposts in the same study were dominated by members of order Pezizales in an aerated static pile, while members of class Sordariomycetes, an unidentified Basidiomycete and *Acremonium* were present in windrows. Neher et al. (2013) also found Bacteroidetes, γ-Proteobacteria, and Verrucomicrobia bacteria to dominate in VC, whereas Chloroflexi and Chlorobi were highest in thermocomposts. VC and thermocomposts have therefore been consistently found to contain different microbial populations and cannot be generalized as “composts”.

2.2.2. Variability in VC microbial communities: starting materials and worm species

The effects of VC starting materials (substrates) and worm species on the bacterial communities of worm castings or VC have been described (Fernández-Gómez

et al. 2012; Grantina-Ievina et al. 2013; Knapp et al. 2009; Koubová et al. 2015; Yakushev et al. 2011). Knapp et al. (2009) simulated *Lumbricus rubellus* habitat and found that the worm substrate influenced the worm gut and casting microbiota. A set of studies looking specifically at *Eisenia andrei* (Aira et al. 2016) determined by pyrosequencing and metagenomics analysis that different manure substrates shaped bacterial communities. This followed from earlier work (Gómez-Brandón et al. 2011) that used phospholipid fatty acid (PLFA) and fluorescein diacetate (FDA) to examine this effect and found that the earthworm gut shaped the microbial populations by reducing them but leaving them more active. Different starting materials processed by *E. andrei* produced similar microbial communities (Lores et al. 2006). These authors were able to fingerprint using fatty acid methyl ester (FAME) methods finished materials according to both the starting material and the worm species used (*Eisenia andrei*, *Eudrilus eugeniae*, or *Lumbricus rubellus*), and found that protozoan markers increased with vermicomposting, as did a fungal biomarker potentially. Another group used PLFA and denaturing gradient gel electrophoresis (DGGE) as well as cultural methods to examine the effect of worms and substrate on communities of bacteria and archaea, and found that processing of substrate by worms resulted in greater viable microbial biomass, and reduced the microbial community richness (Koubová et al. 2015). Cultural analyses of VC found many genera of culturable fungi, and multifactorial analysis showed compost microbial activity and composition was related to manufacturer, substrate, and storage conditions: manufacturer-associated differences were assumed to relate to worm species among other factors (Grantina-Ievina et al. 2013). DGGE analyses have shown that the initial substrate has the greatest effect on the bacterial community in VC, and the worm species had only a secondary effect (Yakushev et al. 2011). DGGE analyses also showed that the concentration of dissolved organic materials in the substrate has the greatest effect on VC microbial communities (Ishii and Takii 2003).

Conversely, Fernández-Gómez et al. (2012) found that earthworm species were able to produce analogous bacterial communities from different wastes processed under different conditions, according to the similarity of DGGE banding patterns among the VC tested. Several other studies used DGGE to investigate VC (Knapp et al. 2009; Vivas et al. 2009; Yakushev et al. 2011) sometimes incorporating quantitative PCR (Huang et al. 2013; Huang et al. 2014), the COMPOCHIP microarray (Fernández-Gómez et al. 2012; Fritz et al. 2012). Other approaches for characterizing VC microbial communities have

ranged from chemical and cultural analyses (Anastasi et al. 2005; Gopal et al. 2009; Hénault-Ethier 2007; Hénault-Ethier et al. 2016; Marín et al. 2013), to high throughput sequencing (Aira et al. 2016; Lv et al. 2015; Neher et al. 2013). Because of contrasting results, more work is needed to determine how starting materials may impact the final outcome of diversity in microbial communities in VC.

2.2.3. Advantages and disadvantages of VC and thermocompost

Both VC and thermocomposts have been shown to suppress soil-borne plant pathogens and the diseases they cause, and there are advantages and disadvantages of each. One main advantages of VC is the relatively short production time: VC can be produced in under 70 days compared to about 6 months for thermophilic compost (Jack 2011). There are some exceptions where thermocomposts can be produced quickly, but these are not suitable for agricultural applications where a high-quality, cured compost is required (Dominguez & Edwards 2011).

Many potentially beneficial microorganisms are lost during the high temperature phase of thermocomposting, and the recolonization by airborne biocontrol agents is random (Hoitink et al. 1997) unless the compost is inoculated with beneficial organisms at the start of the mesophilic curing phase. López-González et al. (2015) conducted extensive analysis of fungi present in composts from agricultural residues. They found that thermocomposting had a strongly selective effect on the fungal populations: only members of the Eurotiomycota were detectable at the late stages of composting. In contrast, even though microbial community diversity can be reduced during vermicomposting (Koubová et al. 2015), VC harbour a wider diversity of bacteria (Neher et al. 2013; Vivas et al. 2009) and fungi (Lv et al. 2015) than thermocomposts. Although Neher et al. (2013) found that fungal diversity was equally abundant in both types of composts, Scheuerell et al. (2005) reported that VC had higher levels of fungi than several thermocomposts, but not more than bark compost, and did not have higher population levels of bacteria, actinobacteria or yeasts than thermocomposts. They also found that microbial populations were not related to disease suppression.

Both thermo- and vermicomposting can produce large volumes of greenhouse gases (reviewed by Swati & Hait 2018): both processes produce large volumes of CO_2 from biomass, vermicomposting produces lower CH_4 emission levels than

thermocomposting, but higher N_2O emission levels (Hobson et al. 2005) although these ratios are variable depending on the feedstocks, amendments and methods used (Lim et al. 2016). Vermicomposting does not require equipment for mechanical turning (Barthod et al. 2018), another source of greenhouse gases. Finally, VC produces a valuable secondary product: worms can be harvested as a protein source for animal feed (Edwards & Bohlen 1996), although care must be taken because worms are bio-accumulators of heavy metals from VC feedstocks (Swati & Hait 2017). Thus, there are aspects of vermicomposting that make it desirable from a sustainability perspective.

2.2.4. Disease suppressive effects

VC are often considered to offer superior disease suppression and plant nutrition than thermocomposts (Edwards et al. 2006; Pant et al. 2013), although this generalization is opposed by some (Tognetti et al. 2005). VC have been found to contain higher nutrient levels than thermocomposts (Doan et al. 2007), but that does not always equate to yields that are comparable to plants receiving synthetic fertilizers (Doan et al. 2013). VC can outperform thermocompost in growth trials (Edwards 1995). Water extracts (tea) of VC have been shown to be more suppressive to diseases than thermocompost tea in some studies (Manandhar & Yami 2008), but less broadly suppressive than thermocompost tea in others (Tian & Zheng 2013). The suppressive effects of organic amendments and thermophilic composts on plant pathogens has been well studied (Noble & Coventry 2005) but general suppression of plant diseases by VC and specific suppression of plant diseases by VC has received less attention (Arancon & Edwards 2004) until recently.

Some starting materials were observed to suppress plant diseases when thermophilically composted but not when vermicomposted (Noble and Coventry 2005). For example *Fusarium oxysporum* f.sp. *radicis cucumerinum* (Forc) (Kannangara et al. 2000) was suppressed by windrow composted dairy solids but not by a vermicomposted set of the same substrate, although in this study, the 20% VC control treatment showed considerable reduction in plant growth which made it difficult to separate the effects due to the pathogen. Neher et al. (2017) found that two stage thermophilic vermicomposting and anaerobic digestion produced composts more suppressive to *Rhizoctonia solani* than windrow or aerated static pile cured composts. VC can have lower electrical conductivity (EC) than thermocomposts, which can increase the basal respiration rate

(BRR) of potting media (Ebrahami 2018). Low EC and higher BRR's can be positively correlated with disease suppression (Ghini et al. 2016; Vekeen et al. 2005), or negatively (Cotxarrera et al. 2002), or neutral (Chitarra et al. 2014), but the low EC of VC allows it to be amended at higher rates, providing increased disease suppression. Direct comparisons of efficacy of VC and thermocomposts is not always possible as they are not always studied side-by-side. Vermicomposting has several favourable attributes, in terms of producing a product with a rich microbiota, and for use in the suppression of plant diseases.

2.2.5. Biological control activities of vermicomposts

VC have been shown to suppress both insect pests (Arancon et al. 2005, Arancon et al. 2007, Edwards et al. 2011) and plant diseases. Beyond disease reduction, VC can have beneficial effects on plant growth through better nutrition and release of hormones: reviewed in Lazcano & Domínguez (2011). These results can vary: (Roberts et al. 2007) found positive and negative VC effects to differ between the plant species sunflower (*Helianthus annuus*), cosmos (*Cosmos bipinnatus*) and California poppy (*Eschscholzia californica*). VC has been shown to have disease suppressive properties in many studies, but it is not universally so. One limitation is that most studies with VC have involved a single pathogen, or have been part of a larger field trial (Jack, 2011) and it is difficult to compare results across studies. VC from different starting materials have been compared for disease suppressive abilities. Szczech & Smolinska (2001) found that VC produced from animal manures were suppressive to the oomycete *Phytophthora nicotianae* Breda de Haan and reduced its infection of tomato, whereas those made from sewage sludge were not suppressive. Reddy (2015) compared the effect of starting materials on suppression *in vitro* of the plant pathogenic bacterium *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye. Among the plants *Azadirachta indica* A. Juss (neem), *Lantana camera* L., and *Parthenium hysterophorous* L., vermicomposted *Azadirachta* produced the most suppressive aqueous extract *in vitro*. This extract also provided excellent suppression of bacterial spot of tomato when applied as a seed treatment and a soil amendment. Further work is needed in this area (Jack 2011). Many techniques have been employed to attempt to determine the main factors that affect VC microbial communities and suppressiveness, with mixed results potentially

because of the wide variety of VC used. With the increasing availability of high-throughput community profiling methods, the differences between the microbiome of disease suppressive and disease conducive VC can be elucidated, helping to reveal what makes VC differ with respect to suppressiveness, and hence produce more reliably suppressive VC.

2.2.6. Earthworms suppress plant disease, vermicomposting reduces plant pathogen propagule density

Removal of plant pathogens is aided by the high-temperature phase of thermocomposting among other mechanisms, reviewed in Wichuk et al. (2011). Earthworms have been shown to reduce plant pathogens and disease despite being limited to mesothermic processes, and passage through the earthworm gut can destroy the ability of some fungal species to germinate, while not harming or even enhancing the germination of others (Moody et al. 1996). Elmer (2009) performed greenhouse trials with the addition of the earthworm *Lumbricus terrestris* L., and showed a reduction in disease caused by *F. oxysporum* f. sp. *asparagi* Cohen, and *Fusarium proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg in asparagus, *Verticillium dahliae* Kleb. in eggplant, *Fusarium oxysporum* f. sp. *lycopersici* Snyder & Hansen race 1 in tomato. In this study tomato and asparagus growth were increased by earthworm addition, and populations of bacilli and *Trichoderma* were not affected by the presence of earthworms whereas populations of fluorescent pseudomonads and actinobacteria increased. *Aporrectodea trapezoides* Dugès and *Aporrectodea rosea* Savigny have also been shown to be capable of spreading *Pseudomonas corrugata* 2140R, which can suppress *Ggt* take-all, a fungal disease of wheat roots (Doube et al. 1994).

Field studies vary on whether disease severity and yield are both affected by earthworm density and species. (Clapperton et al. 2001) found that *Aporrectodea tuberculata* Eisen at conservative densities (70-113 m^{-2}) increased yields without affecting *Ggt* take-all disease severity. At a higher density of earthworms (~300 m^{-2}) *Aporrectodea trapezoides* and *Aporrectodea rosea* generally promoted plant growth and reduced *Ggt* take-all disease, and *R. solani* lesions on subterranean clover and ryegrass, but also led to disease reduction without plant growth promotion (Stephens et

al. 1994) and plant growth promotion without disease reduction (Stephens & Davoren 1997). Bertrand et al. (2015) found that *Lumbricus terrestris* at approximately the same high density as the Stephens et al. (1994) studies reduced severity of eye spot of winter wheat in greenhouse experiments, without increasing plant biomass. Where removal of pathogenic organisms is of paramount importance, thermocomposting and vermicomposting processes can be coupled, with an initial thermocomposting stage to eliminate pathogens, followed by the addition of worms to combine the advantages of both. This adds operational complication because the worms cannot survive the high temperature stage. More work is required in this area: worms can amplify actinobacteria (Elmer 2009), many of which are beneficial to plants, and worms can even inoculate plants with the actinobacterium *Frankia* (Reddell & Spain 1991), but it is not clear if worms control or spread plant pathogenic actinobacteria such as *Clavibacter michiganensis* (Smith) Davis et al. or *Streptomyces scabiei* Lambert & Loria.

2.3. Vermicompost and its aqueous extracts suppress plant disease *in vitro* and *in planta*

2.3.1. Disease suppression by aqueous extracts (teas) of composts

Water extracts of compost and VC (compost teas) are prepared using several methods, mainly divided between those that are aerated during extraction (Aerated Compost Tea- ACT) and those that are not (Nonaerated Compost Tea - NCT). Studies of disease suppression by compost teas are reviewed in St. Martin (2014), including differences between ACT, NCT and the brewing method's effect on disease suppression. Aqueous VC extracts have been reported to suppress a wide range of phytopathogens *in vitro* and in the field such as *F. oxysporum*, *R. solani*, *Botrytis cinerea* Pers., *Sclerotinia sclerotiorum* (Lib.) de Bary, *Verticillium albo-atrum* Reinke & Berthold, *Plasmodiophora brassicae* Woronin, *Phomopsis viticola* Sacc., *Phytophthora cryptogea* Pethybr. & Laff., *P. nicotianae*, *Phytophthora drechsleri* Tucker, *Pythium* sp., *Sphaerotheca fulginea* (Arancon & Edwards 2004), *Athelia rolfsii* (Curtzi) Tu & Kimbr. (anamorph *Sclerotium rolfsii* Sacc.) (Nakasone et al. 1999) and *Fusarium oxysporum* f.sp. *melonis* Snyder & Hansen (Fom.), *Didymella bryoniae* (Fuckel) Rehm., *Pythium aphanidermatum* (Edson) Fitzp., *V. dahliae*, *Lecanicillium fungicola* (Preuss) Zare & W

Gams (Marín et al. 2013). Scheuerell & Mahaffee (2004) showed that VC tea suppressed damping off of cucumber caused by *Pythium ultimum* Trow, particularly when brewed aerobically with molasses-based bacterial nutrients, and with seaweed, humic acid, and glacial rock dust to encourage fungal growth. In these trials, incorporating 25% v/v VC into potting media was not effective at suppressing *P. ultimum*. VC tea suppressed foot rot disease of rice caused by *Fusarium verticillioides* (Sacc.) Nirenberg (syn. *Fusarium moniliforme* J. Sheld) (Manandhar & Yami 2008), and the aerated extract was the most effective treatment. Tian & Zheng (2013) found that a tea made from commercial VC was variable in its ability to inhibit pathogens *in vitro*: *Sclerotinia sclerotiorum* and *Fusarium foetens* Schroers, O'Donnell, Baayen & Hooftman were effectively inhibited, *Pythium ultimum*, *Phytophthora cryptogea* and *Rhizoctonia solani* were inhibited to a lesser degree, and *Pythium intermedium* de Bary was not inhibited at all. Thermocompost tea isolates that suppressed tomato diseases *in vitro* and on tomato fruit caused by *Alternaria solani* Sorauer and *Botrytis cinerea* were shown to have antifungal compounds, suggesting that antibiosis was the most important mode of action (On et al. 2015). Compost teas have been shown to suppress many phytopathogens but not in a consistent manner. They thus could show inconsistent antifungal and antibacterial effects on introduced biocontrol agents.

2.3.2. Pathogen suppression *in vitro* by vermicomposts

Several studies have demonstrated pathogen suppression by VC *in vitro*, (Table 2.2) some of these were part of studies that also included a pot or field trial (Table 2.3). Szczech (1999) isolated organisms from VC that overgrew *Fusarium oxysporum* f.sp. *lycopersici in vitro*, whereas sterilized extracts did not suppress the pathogen. A wide variety of pathogens were screened by Marín et al. (2013): *Botrytis cinerea* Pers, *V. dahliae* Kleb., *Sclerotinia sclerotiorum* (Lib.) de Bary, *Pythium aphanidermatum* (Edson) Fitzp, *Phytophthora parasitica* Dastur var. *nicotianae* (Breda de Haan) Tucker, *Fusarium oxysporum* Schlecht f.sp. *melonis* Snyder and Hansen. (Fom), *Didymella bryoniae* (Fuckel) Rehm., *Verticillium dahliae* Kleb., and *Lecanicillium fungicola* (Preuss) Zare and Gams [synonym: *Verticillium fungicola* (Preuss) Hassebrauk] were all suppressed *in vitro* by VC extracts. Many bacteria have been isolated from VC and tested against a variety of plant and human pathogens and many of them have antagonistic activity *in vitro*.

(Gopalakrishnan et al. 2011; Pathma & Sakthivel 2013; Yasir et al. 2009). Ebrahimi (2018) tested VC *in vitro* against *Pythium ultimum*, finding that although all compost extracts tested showed inhibition, two thermocomposts were more suppressive than the VC tested. Most studies of VC disease suppression *in vitro* have used dual-culture assays, and although results do not universally match those of field or pot trials (Knudsen et al., 1997), these are an important initial step in testing biocontrol efficacy. Several of the studies in Table 2.2 used a sterilized control treatment which is important for determining whether the pathogen suppression is due to the VC organisms, rather than an abiotic factor.

Table 2.2. Pathogen suppression studies using vermicomposts (VC) *in vitro*.

Substrate for VC and worm species used	VC application method	Assay	Pathogen(s), , Significant pathogen suppression (Y/N) or result, Reference
Cattle manure, *Eisenia fetida*	0.5g/centre of plate	Pathogen growth inhibition assay on PDA	*Fusarium oxysporum f. sp. lycopersici* **Y** (Szczech 1999)
Dairy manure separated solids, *Eisenia fetida*	10% water extract	Pathogen growth inhibition assay on acid PDA	*Fusarium oxysporum f. sp. radicis cucumerinum* **N** (Kannangara et al. 2000)
Cattle manure, tree bark, potato culls, apples *Eisenia fetida*	1:2(v/v) water extract	Pathogen growth inhibition assay on PDA, TSA, YPGA, MEA	*Rhizoctonia solani AG-4* **Y** (Simsek-Ersahin et al. 2009)
Paper mill and dairy sludge *Eisenia fetida*	500 μL of 50%(w/v) aqueous extract	Spore germination assay in 0.5% glucose	*Fusarium moniliforme* **Y** (Yasir et al. 2009)
Crop residues N/A	1:3 and 1:4(w/v) compost teas at 15% v/v in agar	Pathogen growth inhibition assay on PDA	*Botrytis cinerea, Verticillium dahliae, Sclerotinia sclerotiorum, Pythium aphanidermatum, Phytophthora parasitica var. nicotianae, Fusarium oxysporum* f.sp. *melonis, Didymella bryoniae, Verticillium dahliae,* and *Lecanicillium fungicola* **Y** (Marín 2013)
Goat manure, straw *Eisenia fetida*	6 mm plug of bacterial isolates	Pathogen growth inhibition assay on PDA	*Sarocladium oryzae, Fusarium oxysporum, Pestalotia theae, Macrophomina phaseolna, Curvularia lunata, Colletotrichum gloeosporioides, Cylindrocladium floridanum, Cy. Scoparium, Bipolaris oryzae* **Y** – at least one isolate inhibited a given pathogen (Pathma 2013)

Substrate for VC and worm species used	VC application method	Assay	Pathogen(s), , Significant pathogen suppression (Y/N) or result, Reference
N/A	400 μL of 24h compost tea	Pathogen growth inhibition assay on PDA	*Sclerotinia sclerotiorum, Fusarium foetens* **Y** *Pythium ultimum, Phytophthora cryptogea, Rhizoctonia solani* **Y/N** *Pythium intermedium* **N** (Tian & Zheng 2013)
Cow manure, bed leachate *Eisenia fetida*	200 μL	Pathogen growth inhibition assay on PDA	*Colletotrichum gloeosporioides* **Y** (Contreras-Blancas et al. 2014)
Leaf litter, *Azardirachta indica*, *Lantana camera*, and *Parthenium hysterophorous* *Eudrilus eugeniae*	1 mL of 1:10 (w/v) water extract filter sterilized at 0.4 μm	Pathogen growth inhibition assay on nutrient agar	*Xanthamonas campetris* pv *vesicatoria* Neem extract and leaf litter VC performed better than *Lantana* and *Parthenium* (Reddy 2015)
Thermocompost *Eisenia fetida*	½ g compost per 100 ml media	Plate competition assay on water agar	*Rhizoctonia solani* **Y** (Neher et al. 2017)
N/A	0.1 mL compost extract at 10%(v/v) and 20% (v/v)	Pathogen growth inhibition assay on PDA	*Pythium ultimum* **Y** (Ebrahami 2018)
Dairy manure solids mixed 7:1:1 with spoiled corn and hay silage, and cured hot compost *Eisenia fetida* *Dendrobaena venata*	Seeds pre-germinated in VC or VC-amended sand	Zoospore microscopy assays on agarose	*Pythium aphanidermatum* Modified seed exudates affected zoospore behaviour (Jack & Nelson 2018)

2.3.3. Disease suppression *in planta* by vermicompost

Suppression of plant diseases by VC has been the subject of several studies (Table 2.3). Early work includes two widely cited studies: addition of VC to tomato seedlings significantly reduced infection caused by *Fusarium oxysporum* f.sp. *lycopersici* (Szczech 1999), and vermicomposted animal manures suppressed *Phytophthora nicotianae* (Szczech & Smolinska 2001). Recent work illustrates the complication of adding an amendment for biocontrol that promotes plant growth: although amendment of VC to field soil increased yield of potato, it also significantly increased the incidence of *Phytophthora infestans* causing late blight (Grantina-Ievina et al. 2015), attributed to the

corresponding increase in foliage. Ebrahimi (2018) found two thermocomposts to be more suppressive than VC *in vitro*, but *in planta* VC was more effective at reducing damping off and promoting plant growth than spent mushroom compost and household waste compost. The two thermocomposts had much higher electrical conductivity (EC) levels than the VC: adding the thermocomposts had a stronger deleterious effect on plant growth than the pathogen had, illustrating the peril of using *in vitro* dual-culture assay data alone.

Table 2.3 Disease suppression studies using vermicompost (VC) *in planta*

Feedstock for VC	Worm species	Substrate	Crop	Pathogen(s), Significant pathogen/disease suppression (Y/N) or other, Reference
Cattle manure	N/A	Peat	Tomato *(Lycopersicon esculentum*), Cabbage (*Brassica oleracea* 'Ditmarska')	*Phytophthora nicotianae var nicotianae* **Y** *Fusarium oxysporum f. sp. lycopersici* **N** *Plasmodiophora brassicae* **Y** (Szczech et al 1993)
Worm addition	N/A	Field soil	Cereals, wheat	*Rhizoctonia, Gaeumannomyces graminis* var. *tritici* **Y** (Doube et al. 1994)
Worm addition	N/A	Calcareous sandy loam Red brown earth field Red brown earth pots	*Triticum aestivum* 'Spear'	*Gaeumannomyces graminis* var. *tritici* **Y** (Stephens et al. 1994)
Cattle manure	*Eisenia fetida*	Peat 7:2:1 pine bark compost, pine sawdust and brown coal powder 7:3 bark compost, brown coal powder	Tomato (*Lycopersicon esculentum* 'Remiz')	*Fusarium oxysporum* f. sp. *lycopersici* **Y** (composts)/ **N** (peat) (Szczech 1999)
N/A	N/A	Naturally infested peat	*Gerbera*, ivy (*Hedera helix*), carnation (*Dianthus*), *Cyclamen*	*Fusarium oxysporum* f. sp. *dianthi*, others **Y** (Orlikowski 1999)
N/A	N/A	Soil	Autumn Squash (*Cucurbita maxima*)	*Rhizoctonia solani* **Y** (Wright et al. 1999)

Feedstock for VC	**Worm species**	**Substrate**	**Crop**	**Pathogen(s), Significant pathogen/disease suppression (Y/N) or other, Reference**
Dairy manure separated solids	N/A	Yellow cedar sawdust	Cucumber (*Cucumis sativa* L. 'Corona')	*Fusarium oxysporum* f. sp. *radicis-cucumerinum* **N** (Kannangara et al. 2000)
Worm addition	*Aporrectodea trapezoides*, *tuberculata* & *rosea*	Dark Brown Chernozem Lethbridge Loam	Soft white spring wheat (*Triticum aestivum* 'Fielder')	*Gaeumannomyces graminis* var. *tritici* **N** (Clapperton et al. 2001)
N/A	N/A	Soil	Eggplant (*Solanum melongena* 'Florida market')	*Rhizoctonia solani* **Y** (Rivera et al. 2001)
Sheep manure; Cattle manure; Horse manure; Municipal sewage w/ 30% coniferous sawdust	*Eisenia fetida*	Peat	Tomato (*Lycopersicon esculentum* 'Remiz')	*Phytophthora nicotianae* var. *nicotianae* **Y** (except cattle manure) (Szczech & Smolinska 2001)
Food waste Paper waste	N/A	Metro mix 360 soil	Cucumber (*Cucumis sativa*), Radish (*Raphanus sativus*) Strawberry (*Fragaria* x *ananassa* 'Chandler')	*Pythium ultimum* **Y** *Rhizoctonia solani* **Y** *Rhizoctonia solani* **Y** (Chaoui et al. 2002)
N/A	N/A	Soil	Rice (*Oryza sativa* 'Pusa basmati')	*Rhizoctonia solani* **Y** (Bhadoria et al. 2003)
Mixed vegetation	N/A	Peat-perlite	Cucumber (*Cucumus sativus* 'Marketmore 76')	*Pythium ultimum* **Y** (drench) **N** (25% v/v) (Scheurell & Mahaffee 2004)
N/A	N/A	Foliar spray	Tomato (*Lycopersicon esculentum*)	*Clavibacter michiganensis* subsp. *michiganensis* **Y** (Utkhede & Koch 2004)

Feedstock for VC	**Worm species**	**Substrate**	**Crop**	**Pathogen(s), Significant pathogen/disease suppression (Y/N) or other, Reference**
Cattle manure, food waste and paper; Dairy manure; Dairy manure, straw and eggshells	N/A	Sunshine mix #1 (Sun Gro) peat perlite medium Sphagnum peat, vermiculite	Cucumber (*Cucumis sativus* 'Marketmore 76') Cabbage (*Brassica oleracea* 'Cheers') Cabbage (*Brassica oleracea* 'Cheers')	*Pythium irregulare* **Y** (except dairy manure) *Pythium ultimum* **Y** *Rhizoctonia solani* **N** (Scheurell et al. 2005)
"Biogreen"	N/A	Unspecified commercial mix	Bedding ornamental (*Impatiens wallerana*)	*Rhizoctonia solani* **N** (Asciutto et al 2006)
Culled produce wastes, coffee grounds, composted horse manure, paper and straw with additives containing clay, blue-green algae, sugar yeast and kelp	N/A	Bangor silt loam	Potato (*Solanum tuberosum* L.) with barley/ryegrass rotation Continuously cropped potato	Stem canker, black scurf (*Rhizoctonia solani*) and common scab (*Streptomyces scabiei*) on tubers **Y** Stem canker, black scurf and common scab on tubers **N** (Larkin 2008)
Vegetable waste and cattle manure	N/A	Soil	Strawberry (*Fragaria* x *ananassa* 'Chandler')	*Botrytis cinerea* **Y** (Singh et al. 2008)
N/A	N/A	Soil	Tomato (*Lycopersicon esculentum* Mill.)	*Nacobbus aberrans* **Y** (Villa-Briones et al. 2008)
Worm addition to soil, plus cow manure added	N/A	1:1 soil and peat amended with dolomitic limestone GH soil	Asparagus, (*Asparagus officinalis*), Eggplant (*Solanum melongena*), Tomato (*Solanum lycopersicum*).	*F. oxysporum* f.sp. *asparagi, F. proliferatum* in asparagus*, V. dahliae* in eggplant*, F. oxysporum* f. sp. *lycopersici* race 1 in tomato **Y** (Elmer 2009)
N/A	N/A	Soil	French bean (*Phaseolus vulgaris* L.)	*Rhizoctonia solani* **Y** *Phaeoisariopsis griseola* **Y** (Joshi et al. 2009)

Feedstock for VC	**Worm species**	**Substrate**	**Crop**	**Pathogen(s), Significant pathogen/disease suppression (Y/N) or other, Reference**
Crop residues	N/A	Peat	Melon (*Cucumis melo*, L. 'Pinonet')	*Didymella bryoniae* **slowed disese development** *Podosphaera fusca* **Y** (Marín 2013)
Worm addition	*Lumbricus terrestris*	Greenhouse pots with maize field soil	Winter wheat (*Triticum aestivum* 'Soissons', 'Aubusson')	*Oculimacula yallundae* **Y** (Bertrand et al. 2015)
7:1:1 dairy manure solids, spoiled corn and hay silage and cured hot compost	*Eisenia fetida* and *Dendrobeaena venata*	Sterile sand	Cucumber (*Cucumis sativus* 'Marketmore 76')	*Pythium aphanidermatum* **Y** (Jack & Nelson 2018)

2.3.4. Vermicompost percent addition to soils

The concentration of VC used varies between investigations and may explain some inconsistency in disease suppression and phytotoxicity. Some studies have tested very high percent VC in their media: 5, 10, 15, 50, even 100%. These concentrations are not economically feasible amounts of VC to add to production-level plant growth media, even in greenhouse horticulture. Consistent disease suppression has been found with 2% (v/v) vermicompost addition to potting media, and with compost tea addition, in cucumber/Forc and radish/*R. solani* pathosystems (Chapters 3 and 4). High levels of VC can lead to phytotoxicity, several aspects of this phenomenon are reviewed in Lim et al. (2015). Szczech & Smolinska (2001) examined suppression of *Phytophthora nicotianae* var. *nicotianae* on tomato seedlings using concentrations of 50% and 100% VC in peat. They found that VC, particularly those derived from sewage sludge, reduced plant growth. The authors attributed plant growth inhibition to high levels of zinc (up to 1458 mg/kg). The poorest faring VC also had the highest salinity (up to 7.29 g/L NaCl for cattle manure VC, and 10.08 g/L NaCl for sewage sludge). Arancon et al. (2004) found that 40% VC substitution in potting mix was the optimum for increasing biomass of greenhouse pepper whereas higher concentrations reduced biomass. A similar result (50% optimum) was found by Amooaghaie & Korrani (2018) and Amooaghaie & Golmohammadi (2017) at 50% substitution. These studies, as well as Asciutto et al. (2006), found that although damaging to plants on their own, higher concentrations of VC reduced disease severity in the presence of pathogens. Lazcano & Dominguez (2010) found a significant reduction in biomass of pansies (*Viola* × *wittrockiana* subsp. Delta) and primulas (*Primula acaulis* subsp. Oriental) with the addition of high doses (15% and 25%) of two different VC to peat based greenhouse media which they attributed to EC, pH, and media air space effects. A 5% addition did not harm plants, highlighting the importance of using VC at the correct levels to prevent plant injury.

2.3.5. The relationship between pathogen density and disease

Measurements of the relationship between pathogen levels and plant disease in disease suppression studies involving VC show inconsistent findings (Jack 2011). Whereas Szczech & Smolinska (2001) and Asciutto et al. (2006) showed significant

disease suppression without significant differences in pathogen propagule density (*Phytophthora nicotianae* and *R. solani*, respectively), another study using *Fusarium oxysporum* f. sp. *radicis cucumerinum* found that Forc propagule density could be significantly reduced without affecting disease levels (Kannangara et al. 2000). Measurement of more detailed factors, such as interactions between VC and pathogens, may provide more meaningful results, including effects on the life stage of the pathogen (Jack 2011), modifications to seed or root exudates (Windstam & Nelson 2008), or rapidly occurring effects in the spermosphere (Chen & Nelson 2008).

2.3.6. Mechanism of pathogen and disease suppression by VC

Autoclaved and filter-sterilized VCs have been observed in several studies to lose their efficacy when autoclaved, suggesting that the mechanism is biological (Asciutto et al. 2006; Simsek Ersahin et al. 2009; Szczech 1999). This has also been observed in thermocompost extracts when autoclaved and filter-sterilized: some filter-sterilized composts actually encouraged pathogen growth (Dionne et al. 2012). Pugliese et al. (2011) showed this effect for fortified thermocomposts, and partially restored suppressiveness by re-inoculating with *Trichoderma viride* Pers. This effect was also observed by Contreras-Blancas et al. (2014) using vermicomposted cattle manure leachate, which was sterilized by sonication, centrifugation, and filtration without autoclaving, which resulted in the loss of ability to inhibit *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc. *in vitro*. Interestingly (Yasir et al. 2009) found that autoclaving VC extract led to the loss of inhibition of spore germination of *Fusarium verticillioides* compared with a filter sterilized extract suggesting that at least part of the suppressiveness of VC is non-cellular, and not heat-stable. Sterilized compost extracts including those from crop residue VC are sometimes partially inhibitory *in vitro* according to Marín et al. (2013), who found this effect for aerated extracts of composts, but not for non-aerated extracts from the same composts. Alfano et al. (2011) found that filter-sterilized compost extracts provided some growth inhibition of *R. solani*, *S. Sclerotiorum*, and *V. dahliae*. Gopalakrishnan et al. (2010) found some filter sterilized fractions of VC to differ in suppression *in vitro* depending on the VC feedstock and pathogen tested, but most were suppressive. Loss of inhibition ability has also been seen *in vitro* with the treatment of compost water extract with activated charcoal (Kannangara et al. 2000).

Removing the biological components of composts including vermicomposts leads to partial or complete loss of suppression.

2.4. Vermicompost plant disease suppression mechanisms

The model for disease suppression by composts derives from that of suppressive soils with two categories: general suppression uses the total biomass of the soil or other media to produce antagonism, and is not transferable between soils, whereas specific suppression comes from specific microorganisms or groups of microorganisms and is thus transferrable to other soils (Weller et al. 2002). The mechanisms by which composts exert general and specific suppression of disease such as competition, antibiosis, hyperparasitism, acquired and induced host resistance, (Hoitink et al. 1997; Lockwood 1988) as well as preventing pathogen proliferation, and compost's physiochemical properties are reviewed in Mehta et al. (2014). The prevailing view of specific vs. general disease suppression from composts is that general biological suppression of plant disease is effective against organisms that are nutrient-limited, such as oomycetes producing small zoospores that will not germinate in a competitive environment. Oomycetes can be suppressed solely by the activities of VC microbes, e.g., interrupting the homing ability of motile zoospores, a mechanism studied in depth by Jack & Nelson (2018), as well as carbon competition (Chen & Nelson 2012). Competition is also one of the mechanisms by which VC suppress *Fusarium*, reviewed in Simsek-Ersahin (2015). Pathogens that can form enduring resting structures such as sclerotia, however, are subject to by specific suppression because they must be parasitized and eradicated by an agent such as *Trichoderma* spp. If these organisms are not present in composts they can be introduced by inoculation.

Specific biological suppression of disease has been shown to be the mode of action for some composts (Bonanomi et al. 2007; Suárez-Estrella et al. 2013); however, in other cases the disease-suppressive effect cannot be narrowed to one agent or mechanism (Hadar & Papadopoulou 2012; Pane et al. 2011) and can be considered an emergent property of the microbial community (Jack 2011). Many microorganisms in compost cannot be cultured (Ivors et al. 2017). Nevertheless, much work has been successful at isolating and testing members of compost consortia for disease

suppressiveness, and the development of these organisms as individual biological control agents for inundative application (Pathma & Sakthivel 2013; Vilich & Sikora 1998). McKellar & Nelson (2003), found 40% of isolates from a disease suppressive compost to be suppressive towards *Pythium ultimum*, whereas 87% of isolates from a disease-conducive compost suppressed the pathogen. When each compost's isolates were mixed and applied as a seed treatment, the treatment containing fewer suppressive members suppressed pythium damping off of cotton, whereas the treatment with a greater amount of disease-suppressive isolates was not. Pathma & Sakthivel (2013) isolated 193 bacteria from VC, and no single isolate inhibited all fifteen plant and human pathogens tested illustrating that the complete consortia is required for broad-spectrum suppression. The disease suppressive ability of complex mixtures of unidentified microbes as found in suppressive soils, composted substrates, compost teas, and organic amendments is likely to be driven by community-level processes, not just the activity of one easily cultured and mass-produced species, as well as the abiotic factors that that community has developed in, including the availability of substrates required for the production of secondary metabolites such as antibiotics (Vilich and Sikora, 1998). Furthermore, in organic agriculture, composts are used to promote soil biology and seeking a single agent or mode of action violates the holistic tenets of organic agriculture according to Doyle (2017).

Other mechanisms suggested for plant disease suppression by VC are induced systemic response (ISR), and plant nutrition. An ISR to preventative foliar application of VC is suggested by the reduction of bacterial canker of greenhouse tomato caused by *C. michiganensis* subsp. *michiganensis* (Utkhede & Koch 2004). Using compost steepages to induce systemic acquired resistance (SAR) in plants were described by Weltzien (1992), and Zhang et al. (1998) found that like other mechanisms, autoclaved compost lost this effect but it could be restored by re-inoculating with compost or with the biocontrol agent *Pantoea agglomerans* 278A. It can be difficult to separate the effect of nutritional plant growth promotion from disease suppression (Berg 2009). Because VC provide excellent plant nutrition (Doan et al. 2007), *in vitro* tests are important for detailed understanding of disease suppression by VC, despite limits to the applicability of these tests to the field. Jack & Nelson (2018) similarly report a technique of soaking VC for 5 minutes in sterile water to reduce the levels of soluble nutrients prior to

experimental use. It is likely that more than one assay will be required to screen VC prior to use, given the complexity of these materials.

2.5. Vermicompost and biocontrol agents in organic and conventional agriculture

2.5.1. Organic and conventional greenhouse media

Organic greenhouse management is beginning to deliver comparable yields to conventional hydroponics, and the reliable management of resistance to plant disease in greenhouse media is the next piece of this puzzle. Organic greenhouse growth media is primarily formulated based on nutrient and physical characteristics: microbial makeup is a secondary consideration, primarily aimed at ensuring the exclusion of pathogenic organisms. This contrasts with, for example, *Agaricus* mushroom production media, wherein a series of composting steps are used to produce a disease suppressive media specifically conducive to *Agaricus*. Compost additions in industrial plant growth systems vary between crop cycles. Because addition of compost has profound and rapid effects on soil microbial communities (Alabouvette et al. 2006; Pane et al. 2013), varying compost inputs should have a profound effect on the disease suppressiveness of the resulting media, and potentially its ability to support additional biocontrol agents.

Understanding the microbial composition of VC is necessary to develop plant growth media inputs that can be produced containing a reproducible suite of organisms with reliable patterns of nutrient mobilization, disease suppressiveness and known compatibilities with biocontrol agents. Because organic production limits fungicides to control pathogenic fungi and oomycetes, free-swimming zoospore-producing pathogens such as the oomycetes *Pythium* and *Phytophthora* can infest irrigation systems. Microbial biocontrol products are an important line of defence against pathogen infestation in organic agriculture, where they are often used in conjunction with compost products. It is not known how existing biological controls work within the context of a competitive media containing composts. They are inherently well suited to greenhouses (Van Lenteren 2000), and restricted entry intervals for biological controls are negligible compared with chemical fungicides: the application of the agents does not disrupt

operations (Harman 2000), a driver for the uptake of the use of biological controls (Pilkington et al. 2010). This advantage offsets the extra complications involved in applying biological fungicides. Occupational exposure of greenhouse workers to aerosols from biocontrol and other fungi may be an emerging public health issue (Li & LaMondia 2010), which would require the use of safety equipment such as respirators.

The microbial communities in organic plant growth media could be cooperative, neutral, or antagonistic towards these microbial biological control agents, in the latter case this would reduce the biocontrol's efficacy. In field soils for example, there is a significant decline in biocontrol populations after inoculation into soils (Nihorimbere et al. 2011; Mazzola & Freilich 2016), more so in organically managed ones (Hiddink et al. 2005). Wood, peat, and coir host different microbial communities, which could affect biocontrol agents differently (Montagne et al. 2017). (Calvo-Bado et al., 2006) suggested that even the communities found in hydroponic systems could interfere with the disease suppressive abilities of introduced biological controls. Initially sterile plant growth substrates such as rockwool are theoretically at even greater risk than organic substrates containing composts, because if pathogens gain access to the organismal vacuum, they can spread rapidly and cause epidemics (Stanghellini & Rasmussen 1994). Although hydroponic media are not as microbially-rich as compost, they do host their own native microflora that can suppress disease, or can be inoculated with disease suppressive agents (Khan et al. 2003; Hultberg et al. 2000; McCullagh et al. 1996; Paulitz & Bélanger 2001; Punja & Yip 2003; Utkhede et al. 2000; Vallance et al. 2011), However unpredictable the results may be in some cases (Rankin & Paulitz 1994), inoculation of hydroponic systems with individual biocontrol bacteria has been demonstrated to suppress disease by many studies (Clematis et al. 2009). For example, plant growth promoting rhizobacteria (PGPR) (McCullagh et al. 1996) and *Pseudomonas* spp reduce pythium root rot on rockwool-grown cucumber (Paulitz 1997; Zheng et al. 2000), lettuce (Utkhede et al. 2000), and sweet pepper (Khan et al. 2003). Inoculation of hydroponic systems with biocontrol fungi has also been successful, this work has mostly focused on *Trichoderma* and *Gliocladium* spp. for example, the biocontrol fungus *C. rosea* (syn *Gliocladium catenulatum*) discussed above has been shown to reduce the pathogens Forc (Rose et al. 2003) and *Pythium aphanidermatum* (Punja & Yip 2003) on hydroponic cucumbers, and *C. rosea* colonizes cucumber plants readily under hydroponic conditions (Chatterton & Punja 2010). Suppressiveness has been found to

develop in soilless hydroponic systems using rockwool (Postma et al. 2000; Minuto et al. 2007), and similarly, perlite and perlite-peat mixtures suppressed *Fusarium oxysporum* f.sp. *radicis*-l*ycopersici* (Forl) (Clematis et al. 2009), but generally these systems are treated as sterile and pathogen outbreaks are dealt with using fungicides. The communities that persist in these systems are dominated by unculturable bacteria (Calvo-Bado et al. 2006) and the mechanism of suppression has not been elucidated (Clematis et al. 2009) but appear to be correlated with increased bacterial diversity and actinobacterial (Streptomycete) populations in rockwool, unknown organisms in nutrient solution, and Rhizobium-Agrobacterium group, Acidobacteria, and Basidiomycetous yeasts in peat (Postma, 2010). The inherent disease suppressiveness of peat mixes colonized by indigenous organisms is unpredictable. Suppression of *Pythium ultimum* was demonstrated with light peat mixes which have high carrying capacity, but not with darker ones containing less available cellulose (Boehm & Hoitink 1992). A following study found that indigenous organisms in both light and dark peat mixes failed to suppress diseases caused by *Rhizoctonia solani* (Krause et al. 2001). Hoitink & Boehm (1999) wrote that dark sphagnum peat mixes were not inherently disease suppressive because the carrying capacity of these reduced substrates was deemed too low to support biocontrol activities. There are some concerns that because many biocontrol agents were isolated from soils, or from crops other than the target crop, that these organisms may not be adapted to soilless systems (Postma 2010). As illustrated above, biocontrols have been shown to colonize well under these conditions. It has been suggested that the suppressive microbes found in hydroponic media could interfere with biocontrol (Calvo-Bado et al. 2006), and conversely Scheurell (2002) theorized that compost tea combined with commercial biocontrol agents could increase the consistency of both treatments on plant surfaces if the tea formed a biofilm to assist the colonization and survival of the biocontrol organism. These are opposing *a priori* views as to whether existing biofilms would help or hinder biocontrol organism colonization: an assay would be useful for screening biocontrols for this effect. Scheuerell & Mahaffee (2004) were successful in using a compost tea brewed to favour fungi as a drench to suppress *P. ultimum*, however, experiments performed by Cummings et al. (2009) saw increased damping off from this pathogen using the same tea, and using other drenched biocontrol products suggesting that the increased moisture from the use of drenches can itself favour *P. ultimum*. Organic greenhouse media may be an intermediate between field soils and hydroponics. The creation of soilless systems that are microbiologically as

well buffered as natural soils rather than attempting to keep these systems sterile is argued to combine the advantages of natural soil with those of soilless systems (Postma, 2010). This is an area where incorporation of composts could be of great benefit and it is important to determine biocontrol agent compatibility with these production methods.

2.6. Combining vermicomposts with biocontrol agents: "Fortified" composts

2.6.1. Biocontrols vs. generally suppressive compost

If the strength of the general suppression exhibited by composts and soil microbes is its microbial diversity, any agent that reduces this diversity could be expected to have a negative effect on general biological suppression, even if that agent were itself exhibiting specific suppression. Furthermore, a biological control agent would not be able to easily colonize a medium with a steady, established microbial community: the biologically complex planting medium would buffer the effect of an added biological control agent. The biological control organism *Clonostachys rosea* is a better colonizer of cucumber plants in nutrient solution than plants in potting mix or field soil (Chatterton & Punja, 2010), which supports this hypothesis. Pauliz and Bélanger (2001) argued that soilless systems have advantages for the introduction of biological control organisms because of a low volume of the matrix around the plant roots, good interaction between host, pathogen, and antagonist, regulated temperature and environmental conditions, and an unbalanced microflora rather than a competitive and microbially buffered environment such as soil. The low biodiversity of soilless substrates at the start of production is thought to confer an advantage to biocontrol organisms, as they are allowed to establish and proliferate (Fravel 2005). The converse to this hypothesis has not been tested: are high-biodiversity organic greenhouse media suppressive to biological control organisms?

2.6.2. Some examples of binary biocontrol combinations

Combinations of two complementary biocontrol agents whose interactions are simple enough to characterize using current techniques is the first step in engineering suppressive microbial consortia. Some simple combinations are recommended for improving the efficacy of existing biocontrols (Spadaro & Gullino 2005). For example, a fluorescent *Pseudomonas* sp. can be combined with a nonpathogenic *F. oxysporum* to suppress pathogenic *F. oxysporum* so that the *Fusarium* spp. compete for carbon and the bacterium produces an iron chelating siderophore (Alabouvette et al. 1996). *Trichoderma* and *Pythium nuun* can be combined to suppress *Pythium ultimum* so that the *Trichoderma* protects the plant root, and the *P. nuun* reduces the propagule density of the *P. ultimum* (Paulitz et al. 1990). Successful combinations of antagonists were also reported by Dunne et al. (1998) who used proteolytic and phloroglucinol producing bacteria to suppress *Pythium* on sugar beet, Raupach and Kloepper (1998), who used combinations of PGPRs to reduce several cucumber diseases, El-Tarabily (2006), who used actinobacteria combinations against *P. aphanidermatum* on cucumber, Roberts et al. (2005) who used combinations of *Trichoderma*, *Burkholderia* and *Serratia* against *Pythium ultimum* with mixed results including potential antagonism between agents, and Kim et al. (2008) who used bacterial combinations to suppress *Phytophthora* on pepper. Another approach is the use of a biocontrol in conjunction with chitin or chitosan (Sid Ahmed et al. 2003; Benhamou et al. 1998) to improve its efficacy, a technique used successfully for *Pythium aphanidermatum* biological control on cucumber using the chitin-degrading and antifungal producing bacterium *Lysobacter enzymogenes* strain 3.1T8 and chitosan (Postma et al. 2009). The synergistic effect of chitosan and *Lysobacter* has not been elucidated but could be antifungal (although this was controlled for in the Postma et al. (2009) study), plant resistance inducing, and/or stimulation of the biocontrol. A review of the history and prospects for combinations of biocontrol agents is found in Szczech (2008).

2.6.3. Combining biocontrols with vermicomposts

The practice of deliberately adding biocontrol agents to composts to produce fortified composts is relatively new (Noble & Coventry 2005; Pugliese et al. 2011). Based on earlier work (Grebus et al. 1993), Hoitink & Boehm (1999) suggested adding composts and biocontrols to potting media to provide a food base for the biocontrol

agents, without stimulating pathogens. DeCeuster & Hoitink (1999) wrote about the importance of striking a balance between immature composts that inhibit biocontrol organisms from producing enzymes necessary for parasitism, and very humified materials that do not support biocontrol organisms. Early success in rendering a conducive compost suppressive was achieved by inoculating disease conducive spent mushroom compost with the biocontrol agent *Trichoderma viride* S17A (Coventry et al. 2006). *Trichoderma* spp. are excellent colonizers of mushroom composts: they are problematic for *Agaricus* mushroom producers (Seaby 1996), illustrating the importance of choosing a compatible biocontrol/compost combination for synergistic effects. Introduced biocontrol agents can provide disease suppression by the same mechanisms as composts, but could adversely affect a suppressive compost microbial community, reducing its ability to provide disease suppression. Alternatively, it would be reasonable to predict that the ability of composts to suppress pathogens and impact soil microbial communities could also translate into the ability of compost to suppress a biocontrol agent. Composts have profound and rapid impacts on microbial populations when they are added to field soils (Pane et al. 2013) but beyond this knowledge the complex interactions between biocontrol agents and composts are unknown. Disease suppression by composts is complicated: Composts reduce disease by both abiotic and biotic factors (Krause et al. 2001) using several mechanisms discussed above: antibiosis, parasitism, nutrient competition, induced resistance, as well as by providing better plant nutrition (Hoitink et al. 1997). Of these five factors, the former three would be expected to not only antagonize an introduced pathogen but also an introduced biocontrol agent. Conversely, single biocontrol agents use the same mechanisms to provide disease reduction, and could adversely affect a suppressive compost microbial community, reducing its ability to provide disease suppression. For example, a biocontrol agent such as *Bacillus subtilis* that produces a strong antibiotic (Stein 2005) could eliminate a bacterial species crucial to a compost's ecology, resulting in a collapse of the compost population and its ability to reduce disease. Similarly, an agent such as *Clonostachys rosea*, an excellent fungal hyperparasite producing antifungal enzymes (Chatterton & Punja 2009), could harm the compost's fungal population. Because the biological control of plant disease by suppressive composts is attributed to the function of a diverse assemblage of microbes, rather than one particular member (Hadar 2011) as is the case for specific biological suppression, it is necessary to know the effect of perturbing these populations by adding a biocontrol agent. This is in addition to knowing

the efficacy of the biocontrol agent when combined with compost to make "fortified" or "biofortified" composts.

Fortification of composts began with work on thermocomposts, and the following work demonstrates effects that are also likely to be seen with VC. Larkin & Tavantzis (2013) performed a study that used *Bacillus subtilis* or hypovirulent *Rhizoctonia solani* alone or in combination, and compared them with composts to reduce soilborne diseases of potato in the field. *B. subtilis* had previously been shown to reduce *Rhizoctonia* diseases (Brewer & Larkin 2005). It would be reasonable to speculate that *B. subtilis* would also suppress a hypovirulent *Rhizoctonia* biocontrol. The authors found that the combination of these two biocontrols was not significantly better than each biocontrol alone. The composts increased yields, without much disease reduction, an effect the authors attributed to greater carbon input. This effect must be mitigated to make fair comparisons between treatments, which could involve considering together both plant dry weights and disease severity or area under the disease progress curve (AUDPC) values. As discussed above, optimum amounts of VC % incorporation for disease reduction are often much greater than those that encourage optimum plant growth. Hypovirulent *Rhizoctonia* combined with composts yielded some limited synergistic effects (Larkin & Tavantzis 2013). Marginally additive effects of combining composts and the biocontrol agents *Trichoderma virens*, *Bacillus subtilis*, and *Rhizoctonia solani* hypovirulent isolate Rhs1A1 for disease reduction on potato were reported in a later study by the same group (Bernard et al. 2014), interestingly a green manure crop rotation with *Brassica napus* was more consistently effective than compost or biocontrols. Limited synergistic effects were also found in a study using *Trichoderma* and non-pathogenic *Fusarium* added to thermocompost to reduce disease of greenhouse cucumber, tomato, bean and basil. In this study the *Trichoderma*-enriched compost was effective against *R. solani*, but not *P. ultimum* or *P. nicotianae* (Pugliese et al. 2011). Ros et al. (2017) found different levels of suppression depending on the biocontrol with which their thermocompost was fortified: *Trichoderma asperellum* Samuels, Lieckf & Nirenberg was more effective than *T. harzianum*.

Similar results to those described for thermocomposts have been demonstrated using VC. Basco et al. (2017) tested the combination of VC with three biocontrol agents: *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis*, finding that the fortified VC treatment provided better suppression than VC alone, although control treatments for the biocontrols were not included in the study so it is not clear what the interaction between the biocontrols and VC may have been. Amooaghaie & Korrani (2018) found that combinations of VC and *B. subtilis* were more effective at lowering disease incidence caused by *F. oxysporum* on psyllium than either *B. subtilis* or VC substitutions alone. Rao et al. (2017) found that VC provided significant suppression of the root knot nematode (*Meloidogyne incognita* Kofoid & White) + *Pectobacterium carotovorum* subsp. *carotovorum* (Jones) Hauben et al. disease complex, and that fortifying VC with a *B. subtilis* isolate provided significantly better suppression than both the VC alone and seed treatment with the *B. subtilis* alone, and even better than Carbofuran/Streptocycline when the bacterium was added to VC at a high rate. Pereira et al. (1998) found incompatibility between *B. subtilis* and *T. harzianum* in VC. Simsek Ersahin et al. (2009) found no beneficial cucumber growth or rhizoctonia damping-off disease control effects when adding *T. harzianum* to VC: in this study they argue that the compost is employing specific suppression on *Rhizoctonia solani* because of the actions of a single antagonistic bacterium. Thilagavathi et al. (2012) used a VC–based formulation for experiments that found a combination of *Pseudomonas fluorescens* strain Pf1 with either *Trichoderma asperellum* strain TTH1 or *Bacillus subtilis* strain EPCO-16 to provide good suppression of sugar beet root rot caused by *Sclerotium rolfsii*. This formulation strategy uses autoclaved VC enriched with each biocontrol so the interaction between VC organisms and the biocontrols is not illuminated in this case. Sahni et al. (2008) found that combining *Pseudomonas syringae* Van Hall strain PUR46 with high concentrations of VC suppressed *Sclerotium rolfsii* on chickpea better than each treatment alone. The VC used in the aforementioned study reduced mortality by between 12-40%. VC fortified with *Pseudomonas fluorescens* and *B. subtilis* was found to reduce bacterial wilt incidence in three crops, significantly more than when fortified with *Trichoderma viride* (Bora & Deka 2007). Unlike the Basco et al. (2017) study above, this earlier study did not include a VC-only control to test the contribution of the biological control agent to disease reduction: this would be a useful addition to future studies. Pereira et al. (1998) experimented with amending VC with carbon sources to increase biocontrol agent survival, with mixed results. We previously demonstrated that

C. rosea reduces the growth of Forc *in vitro* better than VC alone, and that a combined VC-*C. rosea* treatment is not significantly better than *C. rosea* alone (Chapter 4, Wylie& Punja, 2020). A uniform approach to performing experiments in this area would allow comparison between studies. The lack of strong additive effects in fortified compost and VC experiments could be explained in part if the composts are suppressing the biocontrol agents.

Table 2.4 Studies of disease suppression using fortified vermicompost (VC).

Feedstock for VC	Fortified with:	Worm Species	Substrate	Crop	Pathogen(s), Significant suppression (Y/N) or other, Reference
Municipal sewage sludge with 30% coniferous sawdust	Pre-composted	*Eisenia fetida*	Peat	Tomato (*Lycopersicon esculentum* 'Remiz')	*Phytophthora nicotianae* var. *nicotianae* **N** (Szczech & Smolinska 2001)
N/A	*T. harzianum, B. subtilis*, and *P. fluorescens*	N/A	Soil mixtures	Tomato (*Lycopersicon esculentum*) 'Herra'	*Fusarium oxysporum* f. sp. *lycopersici* ***not given*** (Basco et al. 2017)
Thermocompost	Eco-enzymes	*Eisenia fetida*	Adams and Windsor loamy sands	Radish (*Raphanus sativus*)	*Rhizoctonia solani* **Y** (Neher et al. 2017)
N/A	*B. subtilis* IIHR BS-2	N/A	N/A	Carrot (*Daucus carota*)	Root knot nematode (*Meloidogyne incognita*) and *Pectobacterium carotovorum* subsp. *Carotovorum complex* **Y** (Rao et al. 2017)
Autoclaved vermicompost 1:1 mix with talc based biocontrol formulation	*Pseudomonas fluorescens* strain Pf1 in combination with either *Trichoderma asperellum* strain TTH1 or *Bacillus subtilis* strain EPCO-16	N/A	Autoclaved pot mixture (Red soil: sand:garden soil)	Potted sugar beet *Beta vulgaris* L. Field sugar beet *Beta vulgaris* L.	Sugarbeet root rot (*Sclerotium rolfsii*) **Y** (Thilagavathi et al. 2012)

2.7. Future Directions: Predicting Vermicompost Suppressiveness

VC has demonstrated disease suppressiveness but the results are inconsistent and unpredictable, and despite initial success with FDA hydrolysis, this test has been shown to not be a good predictor of suppressiveness of organic amendments compared with other enzyme assays (Bonanomi et al. 2010). Studies of enzymatic tests such as these, as well as factors such as pathogen population, chemical and physical variables, and microbiological parameters were analyzed on an index of suppression, and reviewed by Bonanomi et al. (2010; 2018). Neher et al. (2017) found that although plate competition assays were not reliable predictors of suppressiveness, assays for activity of the ecoenzymes phosphatase, β-Glucosidase and β-1,4-N-Acetylglucosaminidase did predict suppressiveness to *R. solani* better than respiration and other tests. Current methods for predicting suppressiveness include examining compost respiration rates using laboratory methods or the Solvita® test (Woods End Laboratories, Mount Vernon ME) to determine compost stability, but plant bioassays, despite being expensive and time consuming, remain the gold standard for ruling out phytotoxicity (Wichuk & McCartney 2010). In order to be able to make comparisons between studies, Jack (2011) recommends the following factors be reported in studies using VC: feedstock, commercial source, vermicomposting system, worm species, storage conditions, as well as application details: rate, method, substrate, other media components, soil type & location so that these factors can be correlated to disease suppression. Bonanomi et al. (2018) also recommend that 13C NMR spectroscopy could be a good suppression prediction tool once correlated with other data. A minimum data set for predicting suppressiveness as proposed by Bloem et al. (2006) has not been completed.

2.7.1. Improving *in vitro* tests of biocontrol efficacy

In vitro assays are an important tool for screening biological control agents ahead of time-consuming and expensive plant trials, although these do not always predict biocontrol efficacy on plants in pots or in the field (Knudsen et al. 1997). For example, *B. bacillus* B068150 is known for its ability to suppress fusarium wilt of cucumber but shows

no obvious antagonistic activity to *F. oxysporum* f. sp. *cucumerinum* in dual-culture assays on PDA (Li et al. 2012).

In vitro dual culture assays are heavily biased towards antagonists using toxin production as a single mode of action and there is a need for an *in vitro* method that is less biased (Köhl et al. 2011). Early work by Davet et al. (1986) used biocontrol-inoculated soil mixed with agar to screen *Trichoderma* isolates for saprophytic competitiveness. The technique was validated by correlating the *Trichoderma* isolates' performance in the agar test to parasitism of *Sclerotium rolfsii* in non-sterile soils (Davet & Roure 1986), and the technique was improved by Naar & Kecskes (1998). Compared with studies of enzyme production and antibiotic production *in vitro*, this type of assay has received little attention. Previous *in vitro* work has focused on testing the compatibility between different biocontrol agents such as different strains of *Pseudomonas* (De Boer et al. 2003; De Boer et al. 1999), and combining controls with chitosan (Khan et al. 2005). There is room for the existing techniques such as those proposed by Davet et al. (1986) to be improved through the use of composted materials such as VC.

2.7.2. Predicting the effect of adding biocontrols to generally suppressive plant media

Information is lacking on the effect of an introduced biocontrol on the disease suppressive abilities of VC microbial communities, especially those on a short time scale as suggested by Handelsman (2002). A comparison between the effect of different biocontrols on the same microbiome, in combination with the field-soil framework outlined by Poudel et al. (2016) would show whether the disease suppression ability of a generally suppressive consortia were being impacted by the addition of a biocontrol agent. Conversely, an expedient assay for the screening of biocontrols for use in biologically active substrates such as VC, could fit into a strategy for identifying the best biocontrols for organic substrates, and for identifying candidate biocontrol organisms (Köhl et al. 2011). Ideally such a screen would strike a balance between existing bioassay strategies, where field or greenhouse trials are considered too cumbersome, and *in vitro* assays such as dual culture/inhibition zone, spore count, or lytic enzyme and

metabolic assays too rudimentary (Verma et al. 2007). A model system that is easily reproducible would allow for comparison between biocontrols. VC provides an active suite of microbes that are selected by the worm gut (Gómez-Brandón et al. 2011). Preparing compost tea from VC exerts a second selective step on the population, and cuts down on the amount of extra carbon that is added to treatments. Understanding the interaction between specific biological suppression exerted by an introduced biocontrol agent, and general and/or specific biological suppression of plant disease by microbially rich plant media will allow for better use of biological control in organic greenhouse vegetable production, and other production systems that have a biologically rich media such as field crops and some hydroponics. Plant-free tests could be performed using media that is rhizosphere-like (i.e. with slow-release carbohydrates and organic acids), with the incorporation of a VC tea derived microbiome.

2.7.3. High throughput screening, systems approach for complex microbial assemblages

Complex microbial communities such as soils, the bovine rumen, and the human microbiome are studied using several approaches: among these soil shares the most properties with VC. The advantages and disadvantages of traditional biochemical and low-throughput molecular methods for soil community analysis are reviewed in Kirk et al. (2004). Since this review, metagenomic approaches have been used to investigate soil (Tiedje et al. 2009; Gilbert et al. 2010; Torres-Cortés et al. 2011; Unterseher et al. 2011; Pylro et al. 2014; Delmont et al. 2015) using techniques that are transferrable to VC. Some challenges in soil microbial community analyses are applicable to VC studies: successful integration of soil community diversity and function studies is challenging, and there is no validated benchmark for soil microbial community composition (Nesme et al. 2016). DNA extraction from soil is challenging despite the development of many technologies to perform this task (Daniel, 2005). Additionally, VC contains high concentrations of humic and fulvic acids (Atiyeh et al. 2002): high levels of humic compounds can affect DNA extraction from samples (Alaeddini 2012; Green et al. 2009). Functional analyses developed for soils could also be used to investigate disease suppression by VC: in order to develop a picture of which microbes are performing certain tasks in a community, analyses such as DNA and RNA stable isotope probing

need to be layered on top of metagenomic, transcriptomic, phylogenetic, and functional gene DNA microarray data (Trevors and Masson, 2010).

Although there is longstanding commercial interest in VC as a plant growth media amendment (Edwards and Bohlen 1996) disease suppression by VC remains unpredictable. VC suffers from a lack of consistency in its preparation, and in the methods used to study its properties and use. A coordinated approach to understanding VC would increase confidence in its use among growers and IPM practitioners. Furthermore, VC is a good candidate for developing model systems for investigations into the interactions between disease-suppressive microbial consortia and biological control agents. This area of research could examine fundamental questions about the interplay of specific- and general suppression of plant disease.

Chapter 3.

Microbial activity, diversity and disease suppressive activity of five vermicomposts

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3.1. Abstract

Vermicomposts are produced from the breakdown of waste organic materials by worms and their associated gut microorganisms. The composting conditions and starting materials used to produce vermicomposts can influence the final microbial communities present. Composts were compared to determine whether starting material types and worm species correlated to final microbial communities and to disease suppressiveness. Fluorescein diacetate analysis, denaturing gradient gel electrophoresis and 18s rRNA sequencing were used to characterize the microbial populations of five vermicomposts produced commercially from two different starting material types, and using two different worm species. The effect of vermicompost on pathogen growth and on disease development on cucumber plants from *Fusarium oxysporum* f. sp. *radicis-cucumerinum* was also assessed. Microbial communities were found to remain stable over a four-month period, and varied according to starting materials and worm species. A species in the genus *Mortierella* was found across all vermicomposts. All five vermicomposts demonstrated a level of disease suppression *in vitro* and in cucumber trials. Stable microbial community profiles suggest that vermicomposts provide a consistent suite of disease-suppressive organisms that could be used for biological control in greenhouse vegetable production.

3.2. Introduction

Vermicomposting is a widely used mesothermic method for converting organic residues into finer particles with higher levels of nutrient availability, microbial populations, and desirable porosity, aeration and water holding capacity (Edwards

1995). The organic matter is digested by worms and subjected to a relatively anoxic environment in the worm gut that contains a subset of ingested microorganisms (Drake & Horn 2007). Two common vermicomposting worms are the temperate species *E. andrei* (Bouché) (red wiggler) and the tropical *Eudrilus eugeniae* (Kinberg) (African night crawler) (Arancon & Edwards 2004).

Thermal composting and vermicomposting are effective strategies for converting organic waste materials into agricultural inputs. Prior research has established the changes in nutrient and microbial profiles that occur in these processes. López-González et al. (2015) conducted extensive analysis of fungi present in composts from agricultural residues. They found that thermocomposting had a strongly selective effect on the fungal populations: only members of the Eurotiomycota were detectable at the late stages of composting. In contrast, vermicomposts harbor a wider diversity of bacteria (Neher et al. 2013; Vivas et al. 2009) and fungi (Lv et al. 2015). Bacterial communities found in vermicomposts are of interest because of their broad uses in agriculture, and for potential applications in biotechnology (Fernández-Gómez et al. 2012); for example, aqueous vermicompost extracts have been shown to suppress a wide range of phytopathogens and other fungal species *in vitro* (Arancon & Edwards 2004; Marín et al. 2013).

The effect of different starting materials and composting conditions on the bacterial and fungal communities in vermicomposts has also been studied. Microbial communities are affected by the earthworm gut (Gómez-Brandón et al. 2011), the vermicompost starting materials (Fracchia et al. 2006; Knapp et al. 2009; Yakushev et al. 2011; Fernández-Gómez et al. 2012; Grantina-Ievina et al. 2013; Koubová et al. 2015; Aira et al. 2016) or both (Lores et al. 2006). Previous studies have used denaturing gradient gel electrophoresis (DGGE) (Vivas et al. 2009; Yakushev et al. 2011; Knapp et al. 2009), sometimes incorporating quantitative PCR (Huang et al. 2013; Huang et al. 2014) or the COMPOCHIP microarray (Fernández-Gómez et al. 2012; Fritz et al. 2012) to characterize microbial communities. Vermicomposts from different starting materials have been compared with respect to their disease suppressive abilities, e.g. (Szczech & Smolinska 2001); however, further work is needed in this area (Jack 2011).

Information is lacking on a comparative analysis of different vermicompost sources with regard to microbial diversity, stability and how the vermicomposts could

influence the development of plant diseases. The objectives of the present study were to:

1. Compare five vermicomposts with regard to microbial activity and diversity using FDA, DGGE and cultural analyses.

2. Determine vermicompost microbial community stability using DGGE,

3. Assess the disease-suppressive activity of five vermicomposts against *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *radicis-cucumerinum* D.J. Vakalounakis (Forc) on greenhouse cucumbers (*Cucumis sativus* L.) which causes fusarium root and stem rot (Punja and Parker 2000).

3.3. Materials and Methods

3.3.1. Vermicompost

Samples were obtained in the Fraser Valley of British Columbia (Table 1). Random subsamples (20 x 50g grab-samples = 1 kg) of finished products were composited and stored at ambient temperature (21-23°C) in non-airtight containers at ambient RH of 60-70%. Water was added every second week (5% v/v) to compensate for moisture loss.

Table 3.1. Characteristics of the vermicomposts used in this study.

Identifier	Compost name	Starting substrate	Worm species
TVC	Transform Plant Products Vermicompost	Municipal yard waste and dairy solids	Red Wiggler (*Eisenia fetida*)
TCVC	Transform Plant Products Coffee Vermicompost	Coffee grounds	Red Wiggler (*Eisenia fetida*)
W%WC	West Creek Farms 100% Worm Castings	Municipal yard waste and dairy solids amended with humic acid, fulvic acid, and cytokinins	African Nightcrawler (*Eudrilus eugeniae*)
WVC	West Creek Farms Transform Vermicompost	Municipal yard waste and dairy solids	Red Wiggler (*Eisenia fetida*)
OVC	Origin Organic Farms Transform Vermicompost	Municipal yard waste and dairy solids	Red Wiggler (*Eisenia fetida*)

3.3.2. Culturable microbes

Vermicompost (1 g) was serially diluted in sterile distilled water to 10^{-7} and 100μL plated on potato dextrose agar (PDA) (Difco Laboratories) plates and placed on the laboratory bench at 21-23°C with ambient fluorescent light. Colonies were identified based on their frequency of occurrence over 3 weeks, subcultured, and were identified by the University of Guelph Laboratory Services Agriculture and Food Laboratory by PCR using the 18S rRNA region

3.3.3. Fluorescein diacetate (FDA) assay

FDA hydrolysis measures (Green et al. 2006): samples (1 g air dried) were tested for fluorescein diacetate (FDA) hydrolysis ability using the final soil-optimized method of Green et al. (2006).

3.3.4. DGGE

DNA was extracted from 0.25 g samples using the Powersoil DNA Isolation Kit (mobio.com) with a beadbeater (Biospec, Bartlesville, Oklahoma). DNA quality was evaluated by NanoDrop ND-2000c spectrophotometer (Thermofisher.com). DNA was amplified using the eukaryotic primers R-CUF1 and F-S1-DGGE or prokaryotic primers R-1401 and F-968 GC (Yu et al. 2004) (Table 3.2). Annealing at 53°C and a 30 min final elongation at 72°C (Janse et al. 2004) were used. PCR mixtures included ~200 ng of template, 0.2 μmol primers, 0.2 mmol dNTPs and 1.25 U of taq and 5 μL 10x taq buffer (Quiagen.com) in a volume of 50 μL. Amplicon quality and size was assessed by nanodrop and by electrophoresis on 1% agarose in TBE buffer. A DGGE ladder was constructed by amplifying DNA of *Saccharomyces cerevisiae*, *Candida tropicalis* and *Aspergillus fumigatus* with CUF1/S1-DGGE (A. Hadwin and L. Pinto, pers. comm.) DGGE was performed using the DGGEK-2001 DGGE system with GM-040 gradient maker (CBS Scientific, Del Mar, California). Fresh high-purity formamide (Invitrogen: thermofisher.com) was used. Preliminary results showed that 6% acrylamide provided the best resolution with the R-1401/F-968 GC primer set, whereas 7% and 8% resolved smaller R-CUF1/F-S1-DGGE fragments. Excised bands of interest were eluted in TE buffer, re-amplified with non-GC primers, and sequenced by Operon/Eurofins (eurofinsgenomics.com/). Sequences were cleaned in 4Peaks

(nucleobytes.com/4peaks). Sequence alignment used the Muscle algorithm (Edgar 2004) in Seqotron (Fourment & Holmes 2016). Gel images were analyzed in PyElph (Pavel & Vasile 2012): unsupported modules were replaced using anaconda (continuum.io).

Table 3.2. Primers used for DGGE.

Primer	Reference	Position, Fragment Length	Sequence	Tm 50nM NaCl
R-CUF1	(Kappe *et al.* 1998)	GC + 263-279	5'- CAA GGC CAT GCG ATT CG -3'	54.5°C
F-S1-DGGE	Modified from (Kappe *et al.* 1998)	86-110, 194bp	5'- CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG-GGG GAC TGC GAA TGG CTC ATT AAA TCA -3'	80.6°C
R-1401	(Yu *et al.* 2004)	1392-1406	5'- CGGTGTGTACAAGACCC -3'	53.0°C
F-968 GC	(Yu *et al.* 2004) (Muyzer, De Waal, and Uitterlinden 1993)	GC +968-984, 438bp	5'- CGC CCG GGG CGC GCC CCG GGC GGG GCG-GGG GCA CGG GGG GAA CGC GAA GAA CCT TAC -3'	82.5°C

3.3.5. Forc inoculum preparation

Forc was isolated from cucumber seedlings showing symptoms of damping-off and crown rot. Stem segments (2 mm length) were surface-sterilized in 70% ethanol for 30 s and 0.5% NaOCl for 5 min, rinsed and blotted on sterile filter paper. Sections were plated on PDA and after 7 days, growing hyphal tips from emerging colonies were isolated. Pathogen identification was confirmed by morphological characteristics (Vakalounakis 1996; Punja & Parker 2000). Inoculum was prepared by blending mycelium from a 2-week old colony in a Waring blender with sterile water, verified to yield ~2 x 10^6 CFU/mL using dilution plating.

3.3.6. *In vitro* pathogen suppression

A mycelial plug (0.5 cm diameter) taken from the edge of an actively growing colony of Forc was placed in the center of a 9 cm diameter petri dish containing PDA (half-strength or 19 gm/ L) which was mixed with vermicompost (2 g /100 mL of molten cooled agar) and incubated under ambient conditions for 11 days or until the Forc colonies grew to within 0.5 cm from the edge of the dish. Colony area of Forc was measured by tracing the colony outline (which was always a reddish-purple) as a hand drawn line and then using the measure tool in ImageJ v1.4 software (rsb.info.nih.gov/ij/index.html) to obtain cm^2. Area measurements (Ma) were converted to a value (r) for increase in colony radius per hour (h) using the following formula with rate expressed in micrometers per hour (μmh^{-1}) (Equation 1):

$$r_{(\mu mh^{-1})} = \frac{\sqrt{M_{a(cm^2)}/\pi} \times 10^5}{h} \qquad (3.1)$$

The experiment was conducted twice.

3.3.7. Cucumber growth trials

Vermicompost was incorporated (2% (vol/vol) in 450 mL plastic pots) into twice-autoclaved Sunshine #4 Mix (Sun Gro Horticulture, Abbotsford, BC), incubated for 48 h under ambient conditions and inoculated with $1x10^7$ CFU of Forc prepared as described above. Four surface-sterilized seeds of cucumber (*Cucumis sativus* L. 'Green Dragon')(West Coast Seeds, Delta, BC) were added to each pot, placed in a Conviron growth chamber maintained at 17°C day/20°C night, 16/8 light cycle at 60% relative humidity, watered to saturation every third day with distilled water. Disease severity was recorded for each seedling on day 24, 28, 45 and 61 on a scale of 1-10: 1 = mild wilting of any leaf, 2 = mild wilting of all leaves, 3 = mild wilting and any yellowing, 4 = severe wilting and yellowing, 5 = green stem, leaves yellow and browning, 6 = brown stem, 7 = stem collapse, stem intact, 8 = stem collapse, stem split, 9 = death, some stem intact, 10 = death, sporulation on stem. Aboveground plant parts were collected on day 61 by cutting the stem at the soil line. Plants were dried at 50°C for 48 h and weighed.

3.3.8. Statistical analysis

Area under the disease progress curve (AUDPC) was calculated using the automated method described in Appendix A in R 3.2.2 (R Core Team, 2016). Means separation for FDA data, colony growth, AUDPC and dry weight was performed using Tukey's honestly significant difference (HSD) test in R 3.2.2 (R Core Team, 2016).

3.4. Results and Discussion

Vermicomposts all exhibited enzymatic activity according to the FDA assay (Figure 3.1) reflecting presence of an active population of microbes (Schnürer & Rosswall 1982). The vermicompost with the highest FDA rating was derived from the breakdown of coffee waste: coffee grounds are a very rich source of both organic compounds and nitrogen (Campos-Vega et al. 2015). Coco coir (Coco_WC, West Creek Farms, Fort Langley, BC) and autoclaved Sunshine #4 Mix were included for comparison. Autoclaved Sunshine Mix was found to have low enzymatic activity as expected. Coco coir was found to have a high level of enzymatic activity, presumably because of the high levels of microbial species present in this substrate (Montagne et al. 2017). Vermicomposts are considered to be quite stable during storage (Dr. John Paul,

pers. comm.). Although most samples in our study exhibited similar FDA ratings when tested in February and in April, OVC increased and TVC, WVC, and TCVC decreased. This is significant because fluorescein diacetate hydrolysis has been correlated to the suppression of disease (Hoitink & Boehm, 1999; Scheurell, 2002) and composts remain microbially active during storage, sometimes decreasing in FDA activity, and sometimes not (Boulter-Bitzer et al., 2006). The large increase in FDA activity in compost OVC could be explained by an increase in microbial respiration: this material was drier than the other composts when samples were collected. This material was then treated similarly to the other samples, with water added to compensate for evaporation in the lab which may have increased microbial activity.

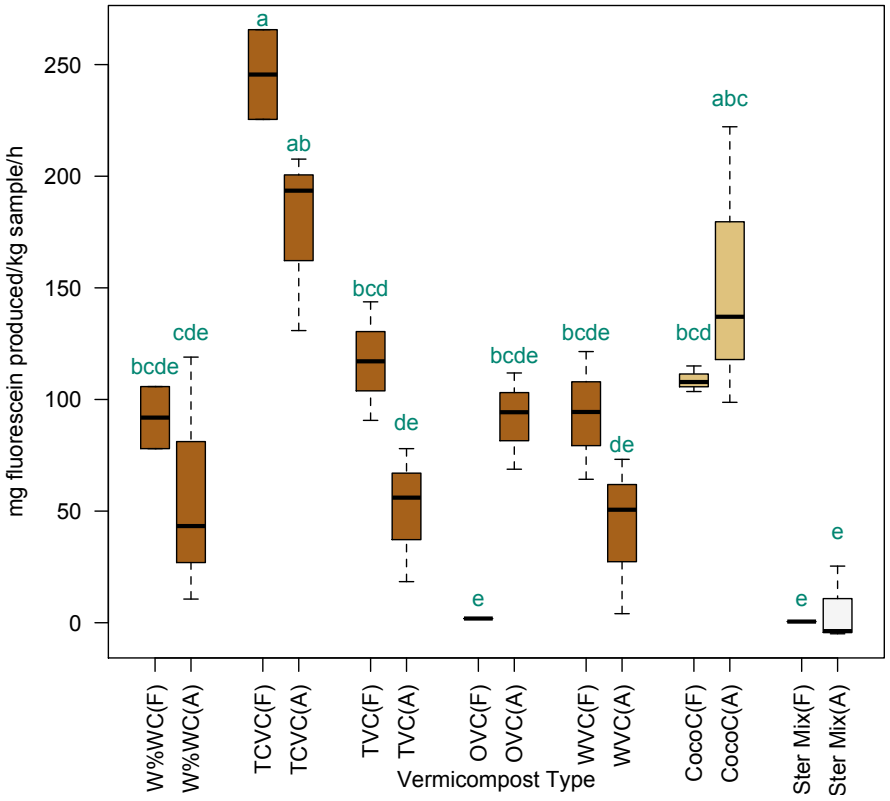


Figure 3.1. Fluorescein diacetate hydrolysis analyses of several vermicomposts listed in Table 3.1 from the Fraser Valley of British Columbia: West Creek Farms 100% Worm Castings (W%WC), Transform Plant Products Coffee Vermicompost (TCVC), Transform Plant Products Vermicompost (TVC), Origin Organic Farms Vermicompost (OVC), West Creek Farms Transform Vermicompost (WVC), as well as coco coir (CocoC) and autoclaved potting mix (Ster_mix). Different letters indicate that treatments were statistically different according to Tukey's HSD test.

DGGE analysis showed consistency of banding patterns of OVC vermicompost at several sampling times during a 4-month period (Figure 3.2). This vermicompost has also shown a stable recovery of microbes on PDA up to 2 years of storage (unpublished observations). In contrast, vermicomposts produced using different starting materials and worm species had different DGGE banding patterns (Figure 3.3). Cluster analysis (Figure 3.4) was used to differentiate between the DGGE banding patterns: the very similar vermicompost replicates taken over several months (OVC) are clustered closely together and are similar to the TVC and WVC samples of similar origin. The TCVC

(coffee) and W%WC (*Eudrilus*) were the most dissimilar from the composts produced from municipal yard waste and dairy solids using *E. fetida*: the samples with the most different banding patterns used either a different substrate or a different worm species. TCVC was the most dissimilar from other vermicomposts and outperformed the other composts in the FDA test.

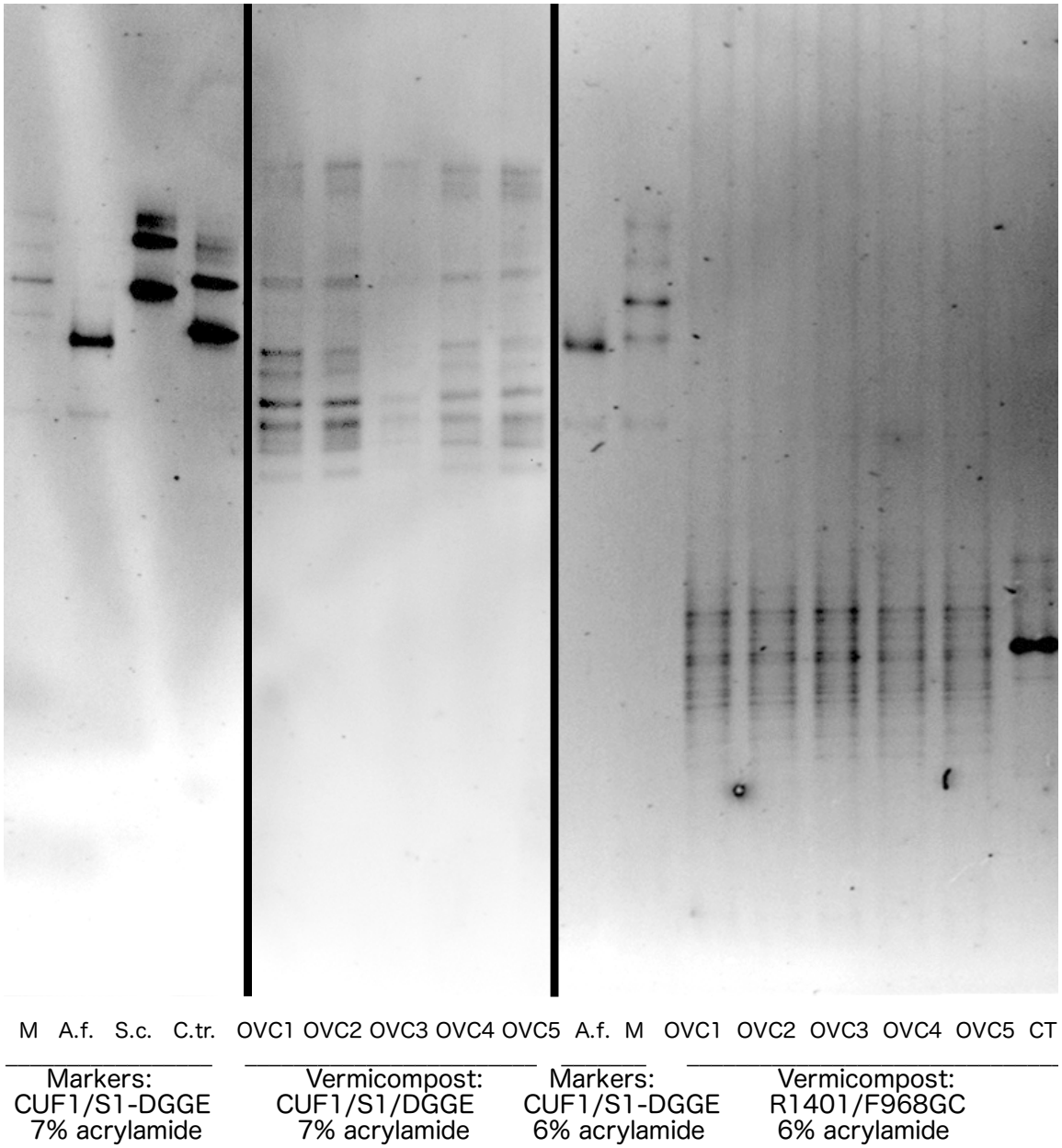


Figure 3.2. DGGE of ladder (M), *A. flavus* (A.f.), *S. cerevisiae* (S.c.), *C. tropicalis* (C.tr.), five vermicomposts (OVC 1-5) over four months and compost tea (CT).

35-70% denaturant gradient, 80V,30mA for 16h. Two gels (7% and 6% acrylamide) were run simultaneously.

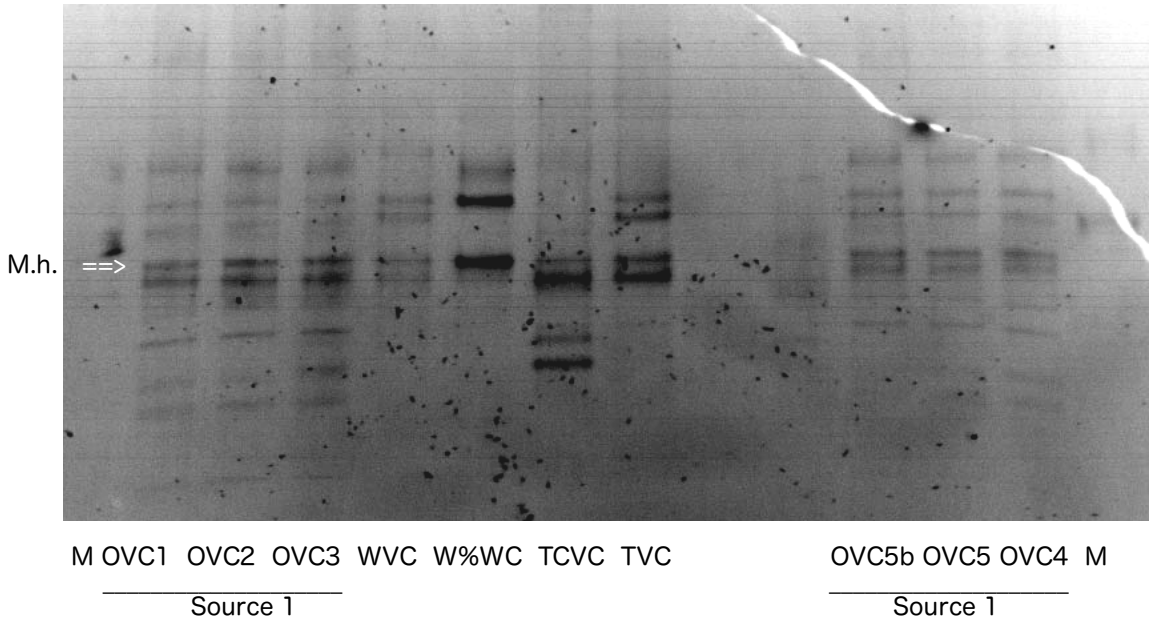


Figure 3.3. DGGE of several vermicompost samples described in Table 3.1 using CUF1/S1-DGGE.
35-70% denaturant, 8% acrylamide. Gel run at 80V, 30mA for 16h.

BLASTn searches revealed the most frequently occurring sequences belonged to Mucoraleans in the *Mortierella* genus. The determination of species was not possible because of the short fragments obtained in the DGGE analysis but the sequences were most similar to *M. hyalina*, *M. alpina*, *M. humulis* and *M. echinosphaera*. DGGE is a very sensitive technique that can detect single-nucleotide polymorphisms (SNPs) and individual bands representing a single phylotype can be directly excised from the gel and identified. It is important to note that each DGGE band does not necessarily represent a different microbe because many prokaryotes contain more than one rRNA gene, thus multiple bands could represent a single microbial species. Our DGGE results support the findings of Grantina-Ievina et al. (2013) that microbial communities in vermicomposts can differ according to worm species and manufacturer but are consistent with regard to similar starting materials. Maintaining semi-moist vermicompost under ambient conditions did not alter the microbial communities over a 4-month period, consistent with Fracchia et al. (2006) who showed stable SSCP profiles of bacterial DNA from different windrows of vermicompost, as well as composts stored over a 12-year period. The consistency was confirmed by the recurrence of a phylotype present across several

samples i.e. *Mortierella*. This genus has a widespread distribution in composts (Anastasi et al. 2005; Novinscak et al. 2009), is found in vermicomposts (Neher et al. 2013) and a wide variety of decomposing organic matter (Wagner et al. 2013).

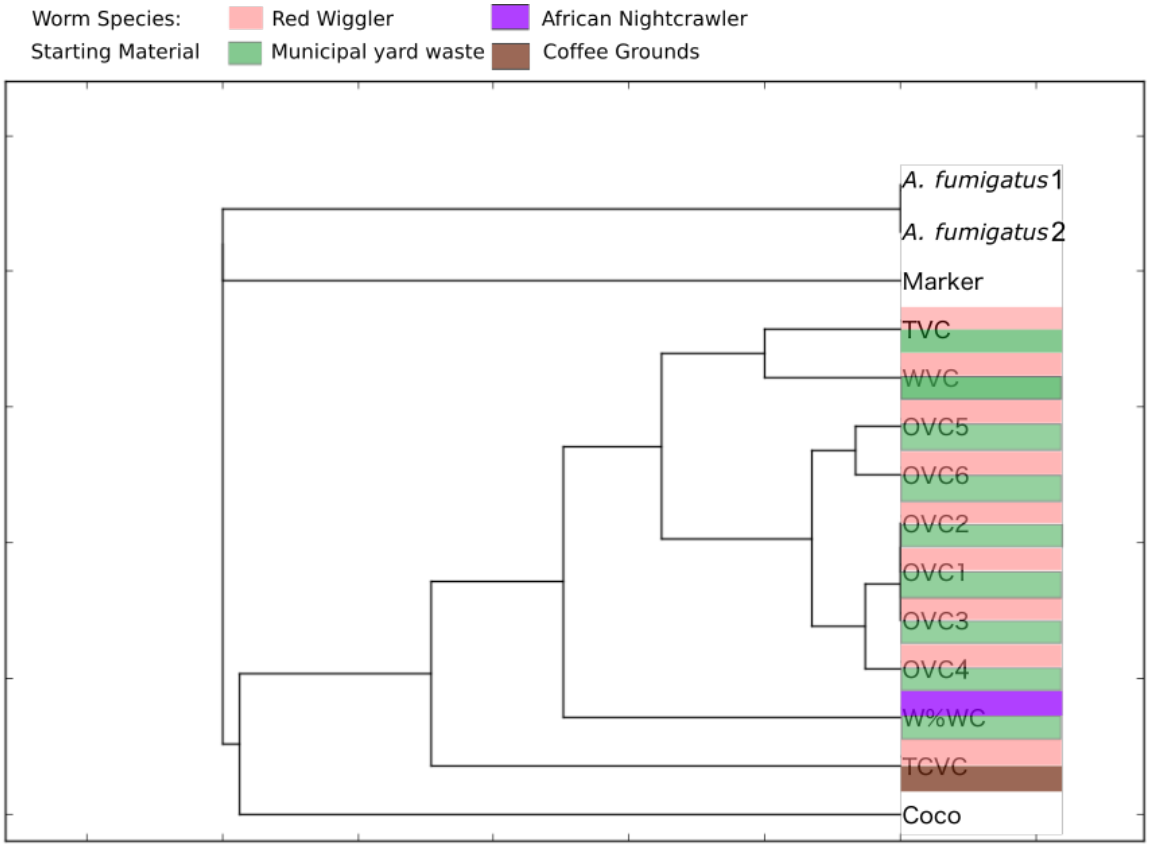


Figure 3.4 Cluster analysis of DGGE gel banding pattern (Figure 3.3), from several samples of vermicompost described in Table 3.1.
Computed using unweighted pair linkage with mathematical average in PyElph.

Double banding can be a problem for DGGE analyses, thus the method of Janse et al. (2004) was used to reduce artefactual double banding. DGGE suffers from widely acknowledged problems characteristic of PCR-based fingerprinting and inter-gel variation (Tourlomousis et al. 2010): to mitigate problems related to variation, all comparisons were run on individual gels after running pilot gels. Vermicompost contains high concentrations of humic and fulvic acids which can positively affect plant growth (Atiyeh et al. 2002). Because high levels of humic compounds can affect DNA extraction (Alaeddini 2012; Green et al. 2009), the MoBio Powersoil DNA extraction kit was used,

which has been shown to yield high amounts of DNA from soil samples containing these compounds (Dineen et al. 2010).

The most commonly recovered microbes on PDA were identified as *Rhodotorula araucariae*, *Mucor circinelloides*, *Mucor racemosus*/*plumbeus* and a *Penicillium* sp. These are cosmopolitan fungi, *R. araucariae* is known from *Araucaria araucana* (Molina) K.Koch in Chile (Grinbergs & Yarrow 1970) and has been isolated in Europe (Sampaio 2011). *A. araucana* is grown as an ornamental in the Fraser Valley of BC, but a connection is not assumed. The cultural and DGGE results were different, which reinforces the importance of using both cultural and culture-independent methods to characterize microbial communities.

All five vermicomposts tested in this study suppressed colony growth of Forc *in vitro* compared with the control not receiving compost (Figure 3.5). There was no significant difference (adjusted p values > 0.05) in suppressive ability between any of the composts. Autoclaved vermicompost added to PDA increased the pathogen colony size suggesting that the mechanism of suppression is biological.

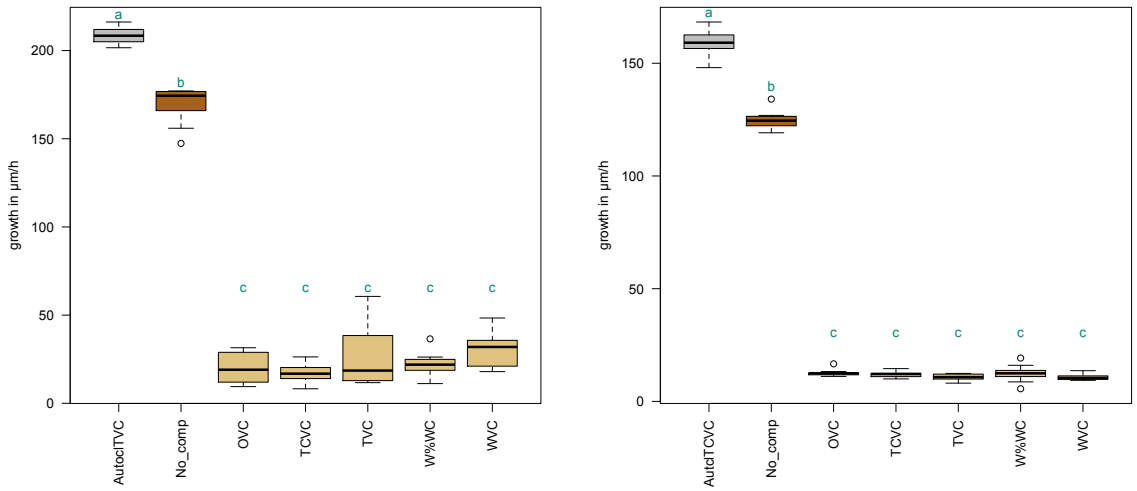


Figure 3.5. *Fusarium oxysporum* f. sp. *radicis-cucumerinum* growth rate on ½ strength PDA (No_comp) or mixed with 2% autoclaved vermicompost (AutoclTVC, AutclTCVC) or 2% vermicomposts (OVC, TCVC, TVC, W%WC, WVC described in Table 3.1)

N=10. Means separated using Tukey's HSD test.

All vermicomposts significantly (max adjusted p = 0.0027 for AUDPC, Figure 3.6 a,b) reduced Forc development on cucumber seedlings and were comparable in efficacy. In one trial, however (Figure 3.6a), the inoculated control had few disease symptoms as measured by AUDPC and plant dry weight, and two vermicomposts (TVC1B and WC1001A) developed disease symptoms, although neither had significantly greater disease in repeated trials (TVC1A and WC1001B, respectively).

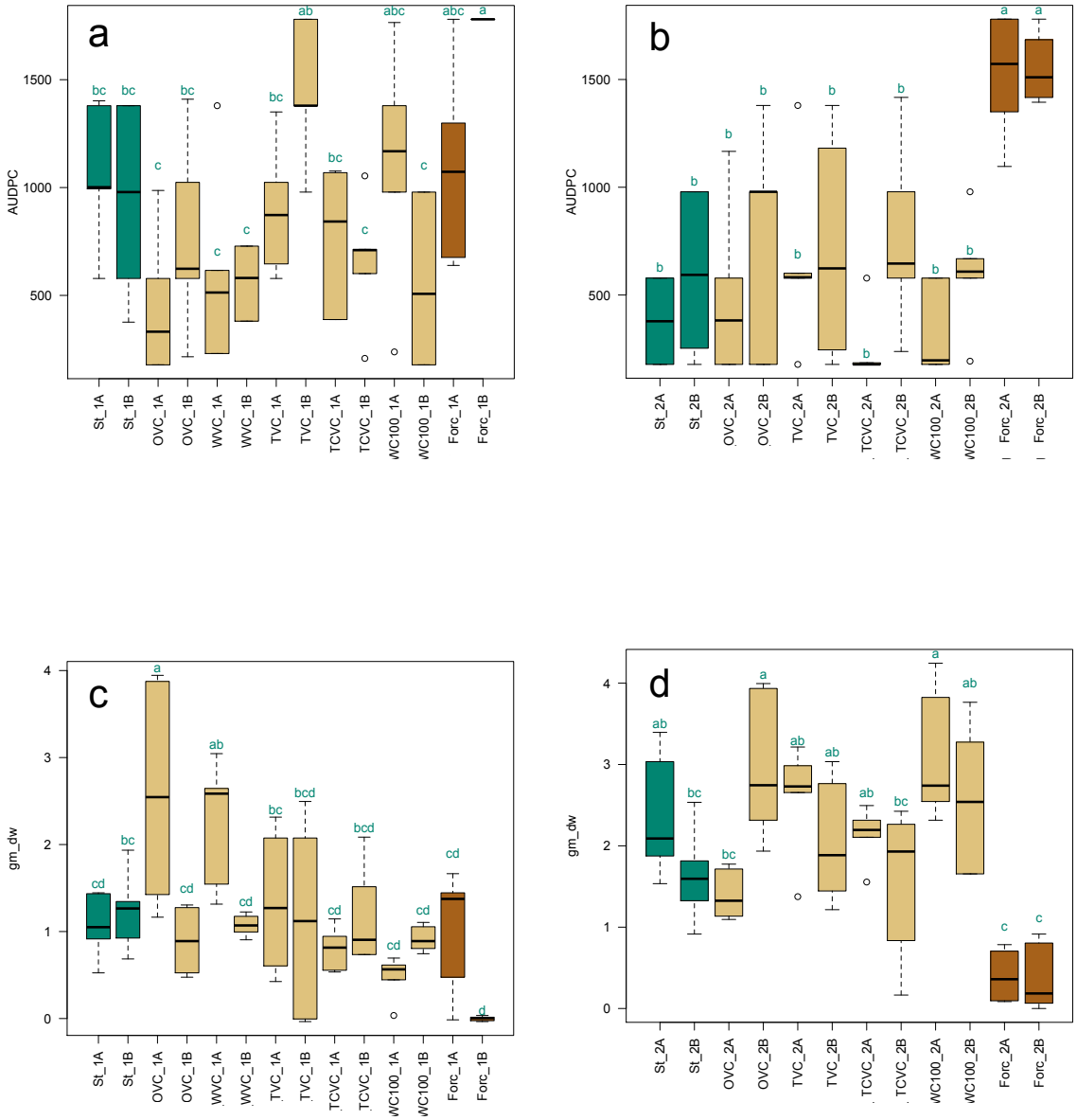


Figure 3.6. Ability of vermicomposts described in Table 3.1 to suppress *Fusarium oxysporum* f. sp. *radicis-cucumerinum* causing disease on cucumber plants grown in twice-autoclaved Sunshine # 4 mix supplemented with 2% vermicompost.

"St" refers to "Sterile control", which received vermicompost sterilized by autoclaving and no Forc pathogen, "Forc" treatments received no compost. Disease was measured using AUDPC (a,b) and shoot dry weight of experimental unit of four plants (c,d) N=6. Means separated using Tukey's HSD test.

The plant pathogen suppressive effects of organic amendments and thermophilic composts has been well studied (Hoitink et al. 1997) but suppression of plant diseases by vermicomposts has received less attention. Interestingly, some starting materials can suppress plant diseases when thermophilically composted but not when vermicomposted (Noble & Coventry 2005) e.g. Forc was suppressed by windrow composted dairy solids but not by a vermicompost of the same substrate (Kannangara et al. 2000). In the present study, all five different vermicomposts showed suppression towards Forc *in vitro* and in cucumber growth trials. There is considerable interest in utilizing vermicomposts as a plant growth medium amendment (Edwards & Bohlen 1996), and our results demonstrate that vermicomposts can provide disease suppression similar to what has been reported for thermophilic composts. In Canada, vermicomposts are acceptable for use in organic agriculture with some stipulations regarding feedstocks and pathogen levels (Canadian General Standards Board 2018).

Vermicomposts were found to contain active microbial populations by FDA, DGGE showed that these populations are consistent over time, but differ according to starting materials and worm species. A *Mortierella* sp. was found across all vermicomposts, and cultural analyses yielded cosmopolitan fungi including a dominant *Rhodotorula* sp. All vermicomposts suppressed Forc *in vitro* by a mechanism lost by autoclaving, and all vermicomposts suppressed disease caused by Forc

Chapter 4.

Assessing aerated vermicompost tea (ACT) combined with microbial biological control agents for suppression of *Fusarium* and *Rhizoctonia*

Published: Wylie, A. C. & Punja, Z. K. 2020. Assessing aerated vermicompost tea (ACT) combined with microbial biological control agents for suppression of *Fusarium* and *Rhizoctonia*. Phytopathology. Published Online:11 Nov 2020 doi:10.1094/PHYTO-05-20-0156-R

4.1. Abstract

Biological control of plant diseases is of particular importance in organic greenhouse vegetable production where fungicide use is limited. Organic producers employ microbially-diverse substrates, including composts, as media for plant growth. Previous research into the impact of a microbially-rich substrate such as vermicompost on the efficacy of applied biocontrol agents is limited. The purpose of our study was to test for an interaction between a competitive microbial background and an introduced biocontrol agent; therefore, we tested the efficacy of two biological control agents in the presence of aerated vermicompost tea using both *in vitro* and *in planta* assays. Suppression of the pathogen *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (Forc) by *Clonostachys rosea* f. *catenulata* (*Gliocladium catenulatum* strain J1446 (Prestop®) and *Bacillus subtilis* strain QST 713 (Rhapsody®), was assessed on agar media amended with aerated vermicompost tea (ACT). Pathogen growth was reduced more by *C. rosea* than ACT alone, and *C. rosea* was equally effective when combined with ACT. In contrast, *B. subtilis* reduced pathogen growth less than than ACT, and when combined, reduced pathogen growth not more than ACT alone. Both biocontrol agents were also tested singly or in combination with ACT against Forc and *Rhizoctonia solani* on cucumber and radish plants, respectively. Additive, neutral, and antagonistic responses, depending on the host, pathogen, and biocontrol agent, were observed. ACT alone provided more consistent disease suppression on cucumber compared with *B. subtilis* or *C. rosea*. In combination, disease suppression was better in most cases than each

biocontrol alone but not better than ACT alone. ACT had antagonistic or additive interactions with *C. rosea* in trials using the radish/*R. solani* pathosystem, depending on the experiment. Our study shows that the fate of biological control agents in microbially-rich environments, and on general and specific biological suppression of plant diseases, is variable. Individual biocontrol agents/composts should be tested for each specific condition (growth substrate, pathosystem) to determine efficacy.

4.2. Introduction

Organic greenhouse production often involves the addition of composted plant materials to the growing substrate. Composts harbor biologically active and diverse microbes compared with, for example, rockwool substrates used in conventional hydroponic greenhouse systems which are an initially sterile substrate (Stanghellini & Rasmussen 1994). Although hydroponic media are not as microbially-rich as composts, they do contain their own native microflora that can suppress disease, or can be inoculated with disease suppressive agents (Vallance et al. 2011; McCullagh et al. 1996; Hultberg et al. 2000; Utkhede et al. 2000; Paulitz & Bélanger 2001; Khan et al. 2003; Punja & Yip 2003). Calvo-Bado et al. (2006) suggested that the microbial communities found in hydroponic systems could interfere with the disease suppressive abilities of introduced biological controls. Biological control agents have been developed for greenhouse producers to suppress damaging fungi and pests and are an important tool for organic production where synthetic fungicide and pesticide use is limited (Whipps & Lumsden 2001). The challenge with the addition of a biological control agent to a composted substrate is the potential for microbial competition that may reduce its efficacy or lead to unpredictable effects. In natural field soils, for example, Mazzola & Freilich (2016) observed a significant decline in biocontrol populations after initial inoculation (Nihorimbere et al. 2011). Organic greenhouse media may be intermediate in microbial diversity between field soils and hydroponic systems. This poses an interesting challenge when evaluating the efficacy of biocontrol agents in compost-amended growing substrates.

Vermicomposts generally contain a greater diversity of microbes than thermocomposts (Scheuerell et al. 2005; Vivas et al. 2009; Neher et al. 2013; Lv et al.

2015; López-González et al. 2015). Aerated vermicompost teas (ACT) have been shown to suppress a wide range of phytopathogens and other fungal species *in vitro* (Marín et al. 2013; Tian & Zheng 2013). As such, ACT should contain a suite of organisms that can perform general suppression of pathogens. Previous studies from our laboratory demonstrate that vermicomposts can provide excellent suppression of plant pathogens *in vitro* and in plant trials (Wylie & Punja 2021). We hypothesized that 1) application of an inundative biological control agent would inhibit pathogen growth more than vermicompost alone, and 2) that when a biological control agent was combined with vermicompost, a sub-additive suppression of disease development would result that was greater than each treatment alone, because a synergistic effect seemed unlikely *a priori*, for example considering nutrient competition for saprophytic growth between the biological control organisms their efficacies might be reduced.

To test these hypotheses, we used the pathogens *F. oxysporum* Schlectend∴Fr f.sp. *radicis-cucumerinum* D.J. Vakalounakis (Forc), the causal agent of fusarium root and stem rot on cucumber (Vakalounakis 1996; Punja & Parker 2000) and *Rhizoctonia solani* Kühn, which causes pre- and post-emergence damping off of radish (Baker & Martinson 1970) and the biocontrol agents *Clonostachys rosea* f. *catenulata* Schroers, Samuels, Seifert & Gams strain J1446 [syn. *Gliocladium catenulatum* Gilman & Abbott] commercially formulated as Prestop® (Verdera Oy, Finland) and *Bacillus subtilis* (Ehrenberg) Cohn strain QST 713 formulated as Rhapsody® were selected to study the potential interactions *in vitro* and *in vivo*. *C. rosea* was chosen as a model biocontrol because it has been shown to reduce root disease caused by Forc on greenhouse cucumber (Rose et al. 2003). *Bacillus subtilis* (Ehrenberg) Cohn QST 713 (Rhapsody®) was selected because *Bacillus subtilis* strains have been demonstrated to suppress *F. oxysporum* causing diseases of cucumber: *F. oxysporum* f.sp. *cucumerinum* alone and in conjunction with composted materials (Chung et al. 2008; Cao et al. 2011; Yang et al. 2014; Huang et al. 2017), and *F. oxysporum* f.sp. *radicis-cucumerinum* (Al-Tuwaijri 2009).

4.3. Materials and Methods

4.3.1. Vermicompost source

Vermicompost for these trials was supplied by Transform Plant Products, Abbotsford, British Columbia and was prepared from municipal yard waste and dairy solids using the worm "Red Wiggler" (*Eisenia fetida*). Dairy cattle manure from a farm in Mission, British Columbia was collected by flushing the barn floors with water, then using a screw press to separate the manure solids from the water. The solid portion has a moisture content of about 75%. This material was allowed to further decompose in a pile for about 3 months before blending it with yard waste compost from the District of Mission for vermicomposting (Dr. John Paul, pers. comm.). Samples were stored at ambient room temperature (22-25°C) in non-airtight containers until compost tea production. Water was added every second week (5% by volume) to compensate for evaporative loss. Vermicompost tea was produced by adding vermicompost (10%) (vol/vol), molasses (0.1%) and humic acid (0.01%) (vol/vol) to water in an aerated reactor for 48 hours maintaining >6mgO_2/L dissolved oxygen. Total microbial counts were assessed in samples of finished compost tea using the method described by Ingham (2005). Inoculum amount was calculated to approximate natural soil microbial populations (~10^7 CFU/mL) and cultivable microbial populations were determined to stabilize at a carrying capacity of approximately 1x10^7 CFU/g of media after one week (Appendix E).

4.3.2. Pathogen and biocontrol agents

Fusarium oxysporum f.sp. *radicis-cucumerinum* (Forc) was isolated from diseased cucumber seedlings as described by Punja and Parker (2000). Inoculum was prepared by blending a 2-week old culture (¼ colony) in a Waring blender with 300 ml of water which yielded ~2 x 10^6 CFU/ml. *Rhizoctonia solani* (ISH-CC-22) was a gift from Kwantlen Polytechnic University, Institute for Sustainable Horticulture, Langley, British Columbia. A 3-week old culture (¼ PDA petri plate) ISH-CC-22 was blended as above yielding ~1x10^2 CFU/ml hyphal fragment slurry. *Clonostachys rosea* f. *catenulata* strain J1446 was prepared by suspending the commercial product Prestop® (Verdera Oy,

Finland) in sterile distilled water and serial dilutions to achieve isolated colonies on PDA. Colonies were cut from the agar plates, and blended at high speed to produce a slurry of conidia and hyphal fragments at 1x10^7 CFU/ml. *Bacillus subtilis* QST 713 inoculum was prepared directly from the commercial product Rhapsody (Bayer Cropscience, Calgary, AB) using the high label rate of 2%, an inoculum strength of ~2 x 10^7 CFU/ml. All isolates were maintained at room temperature (21-24°C) under ambient fluorescent light with indirect sunlight on PDA. In all cases, inoculum strength was determined by serial dilution plate assay.

4.3.3. Pathogen growth inhibition assays

Aerated vermicompost tea (ACT) was added to the surface of solidified half-strength potato dextrose agar (19 g/L) at 50 μL per 9 cm diameter Petri dish. Biocontrol agents (BC) were prepared at label rates and similarly added. Combinations of ACT and BC (1:1) were also included. A 0.5 cm diameter mycelial plug taken from the edge of an actively growing colony of Forc was placed in the center of the Petri dish and incubated for 11 days under ambient laboratory conditions or until the pathogen-only control reached a diameter of 8.5 cm. Colony area of Forc was then measured by hand-tracing the reddish-purple colony outline and then converting to cm^2 using the measure tool in ImageJ v1.4 software (rsb.info.nih.gov/ij/index.html) or using Matlab v 9.0.0 (The MathWorks, Inc.). Area measurements (Ma) were converted to a value (r) for increase in colony radius per hour (h) using the following formula with rate expressed in micrometers per hour (μmh^{-1}):

$$r_{(\mu mh^{-1})} = \frac{\sqrt{M_{a(cm^2)}/\pi} \times 10^5}{h} \qquad (4.1)$$

Where r = the increase in colony radius in micrometers (μm) per hour (h), M = the measurement of area (a) in square centimeters (cm^2). Each experiment was conducted at least twice with ten replicates. Analysis of variance was performed in R version 3.3.2 (R Core Team 2016), means separation was performed using Tukey's HSD test in the

agricolae package version 1.1-3 (de Mendiburu 2016) on a linear model comparing pathogen growth as a function of background lawn composition in R version 3.3.2 (R Core Team 2016). The Type 1 error rate (α) was set at 0.05 for all statistical tests.

Table 4.1. A guide to the *in planta* treatment combinations: all trials included an aerated vermicompost tea (ACT) treatment, a biocontrol agent treatment, and a combined ACT + biocontrol agent treatment, each with a negative (no pathogen) control, and a positive (no biocontrol agent or ACT) control.

Experiment	Pathogen	Host Plant	Biocontrol agent
RRC1	*Rhizoctonia solani*	radish var. "French Breakfast"	*Clonostachys rosea* f. *catenulata* strain J1446
RRC2	*Rhizoctonia solani*	radish var. "French Breakfast"	*Clonostachys rosea* f. *catenulata* strain J1446
RRB1	*Rhizoctonia solani*	radish var. "French Breakfast"	*Bacillus subtilis* QST 713
RRB2	*Rhizoctonia solani*	radish var. "French Breakfast"	*Bacillus subtilis* QST 713
FCC1	*Fusarium oxysporum* f.sp. *radicis-cucumerinum*	cucumber var. "Green Dragon"	*Clonostachys rosea* f. *catenulata* strain J1446
FCC2	*Fusarium oxysporum* f.sp. *radicis-cucumerinum*	cucumber var. "Green Dragon"	*Clonostachys rosea* f. *catenulata* strain J1446
FCB1	*Fusarium oxysporum* f.sp. *radicis-cucumerinum*	cucumber var. "Green Dragon"	*Bacillus subtilis* QST 713
FCB2	*Fusarium oxysporum* f.sp. *radicis-cucumerinum*	cucumber var. "Green Dragon"	*Bacillus subtilis* QST 713

4.3.4. Plant disease suppression assays

The growth medium used was Sunshine #4 Mix (Sun Gro Horticulture, Abbotsford, BC) which was autoclaved twice and mixed with various liquid inocula described in Table 4.1 or an equivalent volume of sterile distilled water (control). Cucumber seeds Green Dragon or radish French Breakfast were surface sterilized by immersion in 70% ethanol for 30 sec and 0.5% NaOCl for 5 min, then rinsed three times with sterile distilled water. They were blotted dry on sterile filter paper and placed in a laminar flow hood for 30 min. Amended potting medium was distributed into autoclaved 450 ml pots and received either 10 seeds of radish var French Breakfast or 4 seeds of cucumber var Green Dragon, which were covered with 1cm of amended potting mix. Pots were placed in growth chambers maintained at 17°C day, 20°C night, 16/8 light cycle at 60% relative humidity and treatments were randomly assigned within the growth chamber. Pots were initially watered to saturation with sterile distilled water, thereafter distilled water was used. Pots were watered every three days during the experiment. The treatment combinations are listed in Table 4.1. Any necessary changes in inoculation schedules were tested to ensure they did not affect the results (Appendix D). Autoclaved vermicompost tea was tested against non-autoclaved vermicompost tea to ensure that plant nutrition was not being significantly affected by vermicompost microbes compared to the sterile control (Appendix D).

4.3.5. Plant growth assessments

Cucumber seed germination was assessed after 20 days. Disease severity data was assessed on days 20, 24, 28, 45, and 61 according to the following scale: 1= mild wilting, 2= wilting and yellowing, 3= yellowing and browning, 4= stem collapse, 5= death. Radish seed germination data was assessed after 5 days. Disease severity was assessed on days 8, 13, 16 and 21 according to the following scale: 1= healthy plant (no stem damage), 2= plant with light damage on the stem and standing, 3= plant infected and fallen over, 4= plant is dead. After disease assessments, aboveground plant parts were collected by cutting the stem at the soil line and dried for 48 hours in a 50°C oven

and dry weights were determined. The data were used to calculate Area Under the Disease Progress Curve (AUDPC). AUDPC calculations were performed using the method described in Appendix A in R 3.3.2 (R Core Team 2016): an automated method that preserved statistical power throughout the analysis. Germination, AUDPC, and plant dry weight means were separated using Tukey's HSD test. Repeated treatments that did not differ according to the HSD test were combined, except where noted.

4.4. Results

4.4.1. Pathogen growth inhibition assays

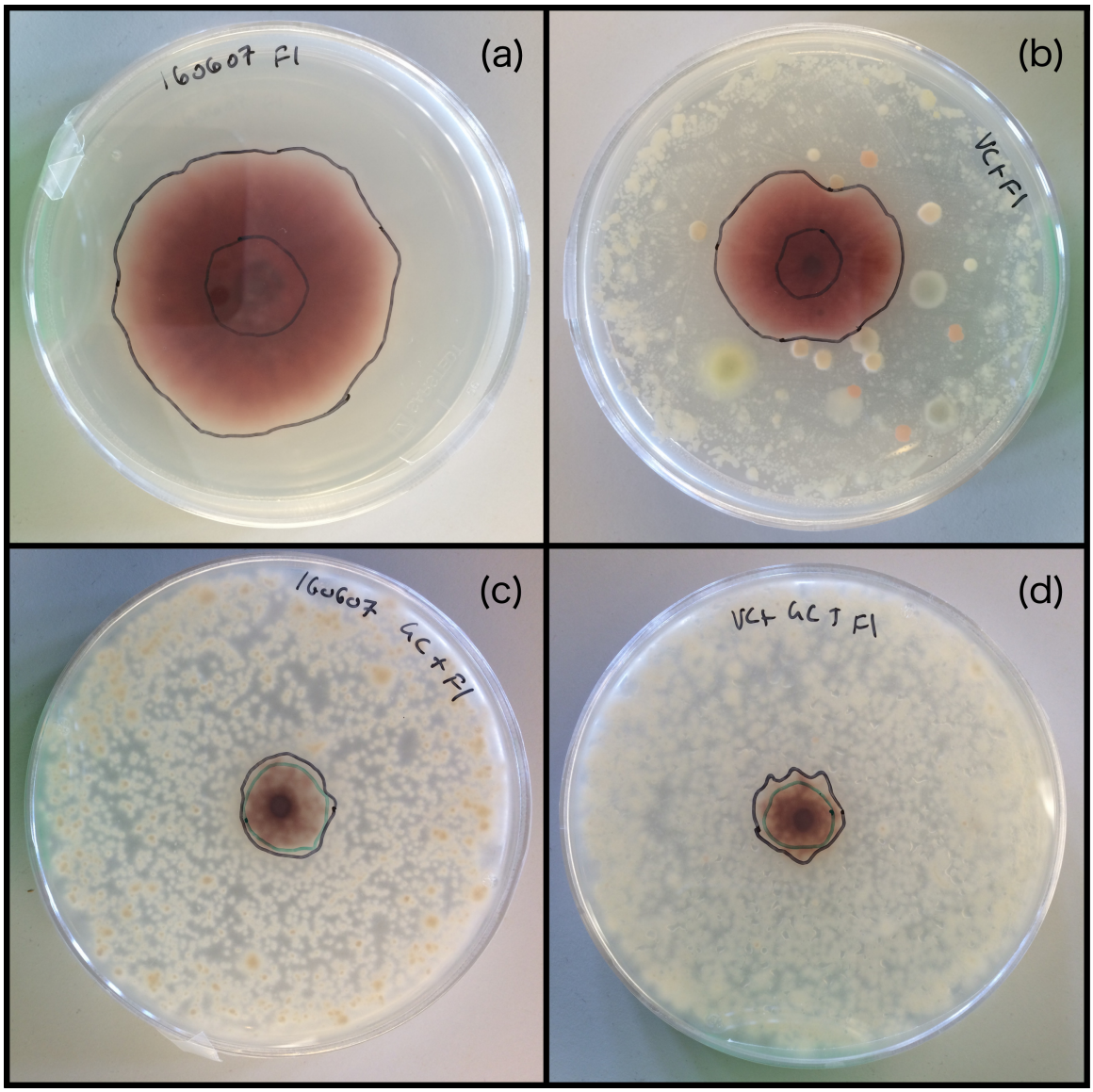


Figure 4.1. ***In vitro*** **bioassay comparing growth of** ***F. oxysporum*** **f. sp.** ***radicis-cucumerinum*** **on 1/2 strength PDA (a), on a lawn of 48 h aerated vermicompost tea (b), on a lawn of** ***C. rosea*** **f.** ***catenulata*** **(c), and on a lawn of both (d).**

Aerated vermicompost tea (ACT) provided a significant (Tukey's HSD, $P<0.05$) reduction of *F. oxysporum* f.sp. *radicis-cucumerinum* (Forc) growth compared with the

control in both assays (Figures 4.2 and 4.3), reducing its growth by approximately 50% and 60%, respectively. The biocontrol agent *Clonostachys rosea* f. *catenulata* significantly reduced growth of Forc compared with ACT in every trial, although mean Forc growth reduction was not different between trial 2 with *C. rosea* alone and trial 3 of ACT alone according to Tukey's HSD (Figure 4.2). The combination of ACT and *C. rosea* provided the same Forc growth reduction as *C. rosea* alone, with some variation.

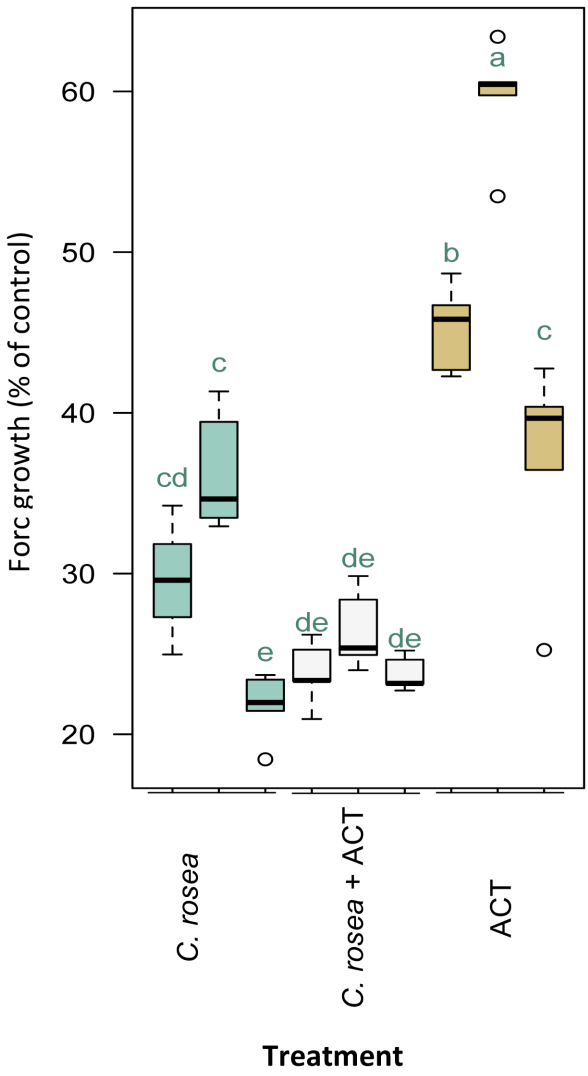


Figure 4.2. **IA1: Growth of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (Forc) on 1/2 strength PDA: on a lawn of *Clonostachys rosea* f. *catenulata* strain J1446 (Prestop®) (*C. rosea*), on a lawn of 48 h aerated vermicompost tea (Vermicompost), and on a combined lawn of *C. rosea* and vermicompost (Combined).**

Means separated using Tukey's HSD test in R. The experiment was performed three times, each bar represents results from a single experimental treatment of N=10 petri dishes , the first, second and third bars for each treatment represent the first, second and third repeat of the experiment.

The biocontrol agent *Bacillus subtilis* and ACT reduced the growth of Forc (Figure 4.3) but ACT provided significantly (Tukey's HSD, $P<0.05$) greater reduction of Forc growth than *B. subtilis* alone. The combined treatment was similar or less effective than ACT alone according to the HSD test.

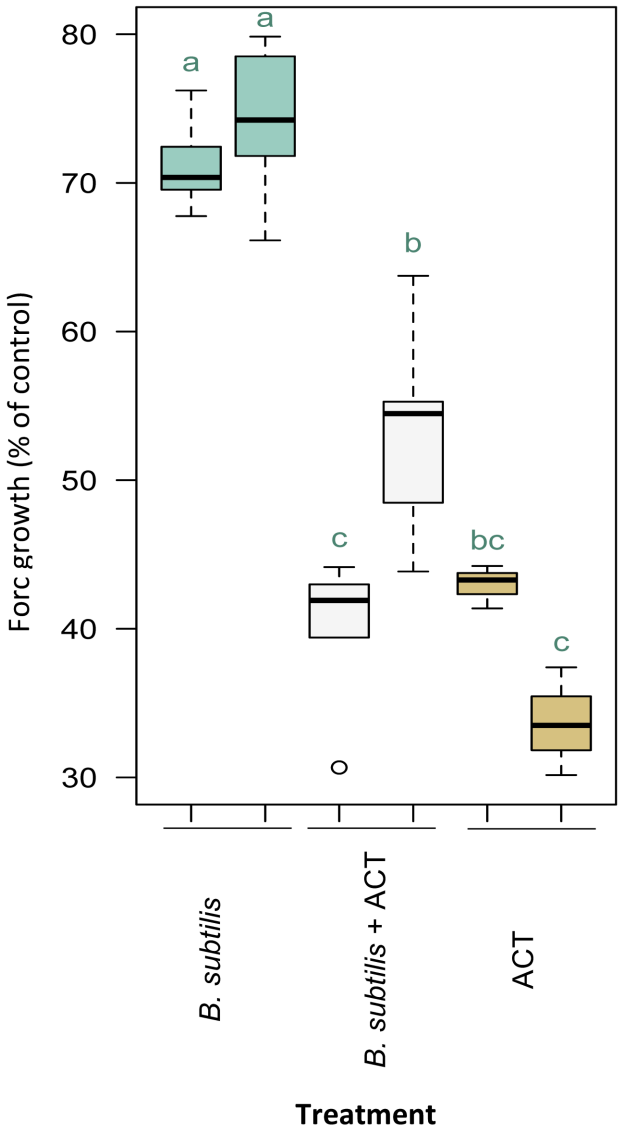


Figure 4.3. IA2: Growth of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (Forc) on 1/2 strength PDA: on a lawn of *Bacillus subtilis* strain QST 713 (Rhapsody®)(*B. subtilis*), a lawn of 48 h aerated vermicompost tea (Vermicompost), and on a combined lawn of both *B. subtilis* and vermicompost (Combined).

Means separated using Tukey's HSD test in R. The experiment was performed twice, each bar represents results from a single experimental treatment of N=10 petri dishes , the first and second bars for each treatment represent the first and second repeat of the experiment.

4.4.2. Plant disease suppression assays

Significant reduction of plant disease by inoculation with aerated vermicompost tea (ACT) was found in all experiments. Suppression of disease by inundative treatment with specific biocontrol agents varied from not significantly different from the positive control to significantly better than the positive control and was never significantly better than ACT alone. The combined biocontrol + ACT treatments were similar to the ACT only treatment in most experiments. The results of the *in planta* experiments are summarized in Table 4.2.

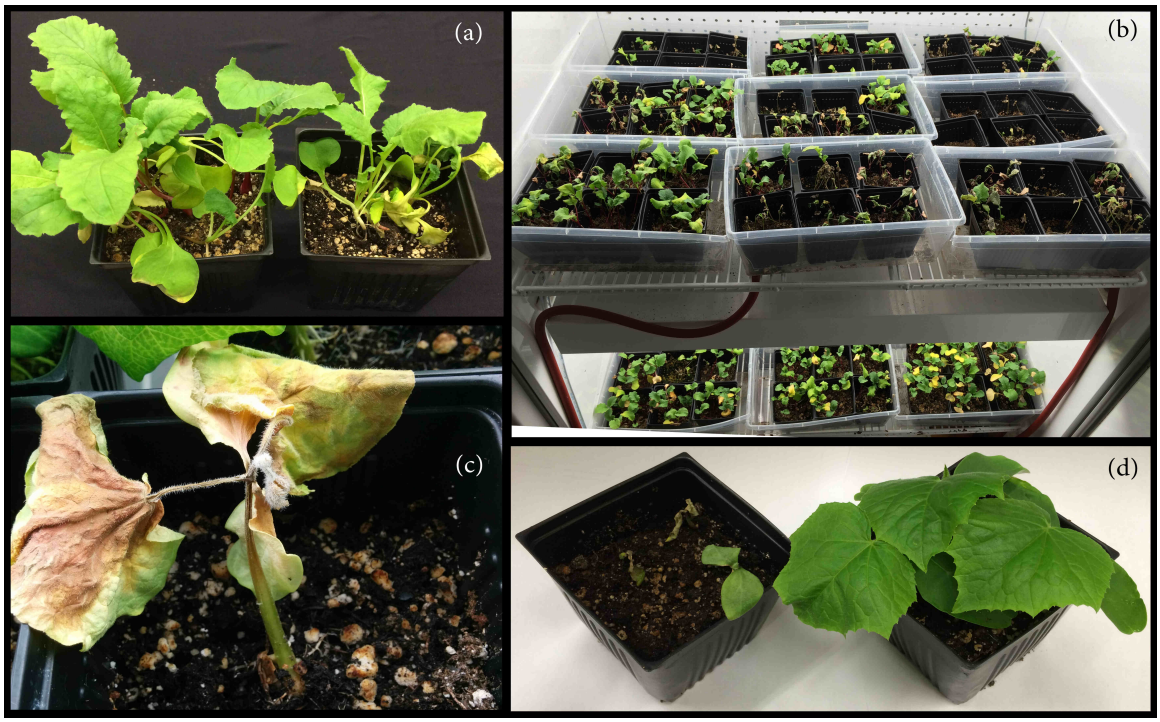


Figure 4.4. **Growth chamber system showing disease symptoms of (a) healthy radish (left) and *Rhizoctonia solani* infected (right), (b) degrees of infection of *R. solani* on radish pots, (c) severe symptoms of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (Forc) infection on an individual cucumber plant, and (d) cucumber pots infected with Forc (left) and uninfected (right)**

4.4.3. *Rhizoctonia solani* / radish pathosystem

In the *R. solani* / radish pathosystem, *C. rosea* and aerated vermicompost tea (ACT) showed significant suppression of disease, whereas *B. subtilis* did not. Vermicompost tea provided the most consistent disease suppression across treatment blocks in this system.

Rhizoctonia solani / *radish* / *Clonostachys rosea* (RRC1 & RRC2)

C. rosea and ACT showed significant (Tukey's HSD $p<0.05$) suppression of disease caused by *R. solani*, alone and in combination. All negative control treatments showed significantly lower levels of disease compared with the treatments using the AUDPC calculation with the exception of one block of combined biocontrol / vermicompost in RRC2 (Figure 4.6a). In experiments RRC1 and RRC2, the positive control had significantly greater disease severity than the treatments. In experiment 1, (RRC1 Figure 4.5 a,b) there was no difference in disease between the treatments, however in experiment 2 (RRC2 Figure 4.6) the combined *C. rosea* + ACT combination reduced disease more than vermicompost alone, which in turn reduced disease more than *C. rosea* alone (Tukey's HSD $p<0.05$) (RRC2 Figure 4.6 b). Examination of individual blocks (Figure 4.6 a) supports these results: the blocks for the *C. rosea* treatment and the combined *C. rosea* and vermicompost treatment cannot technically be combined according the Fisher's LSD test but they can be combined according to Tukey's HSD test: the LSD mean separation is used here to illustrate overall differences between treatments, but HSD is more meaningful for combining blocks, thus these data are combined in Figure 4.6 b. Close examination of the treatment blocks in Figure 4.5 a shows two cases where the individual biocontrol *C. rosea* and vermicompost alone gave better disease suppression than when the treatments were combined, however, these results were not repeated in their corresponding replicates, and because the blocks could be combined they do not influence the results illustrated in Figure 4.5 b where all treatments show similar disease suppression. In both experiments RRC1 and RRC2,

vermicompost provided similar and consistent disease suppression with little difference between treatment blocks (Figure 4.5a and Figure 4.6a.). Germination rates did not indicate any significant effects between the three treatments (Figure 4.5 c), all treatments improved germination significantly compared with the positive control, and although they consistently appear to have lower germination than the disease-free (and the biocontrol-free) control treatments, this difference is not statistically significant according to Tukey's HSD, with some variation: pathogen free negative control treatments were sometimes found to have significantly better germination than their pathogen-treated counterparts. In experiment 2 (RRC2), germination rates show that *C.rosea* did not improve germination as well as the other two treatments (Figure 4.6 c), consistent with the disease data (Figure 4.6 b). All treatments improved germination significantly compared with the positive control, however, and the combined treatment was not significantly different from the disease-free control treatments. Pathogen free negative control treatments were found to have significantly better germination than their pathogen-treated counterparts for the biocontrol and vermicompost alone. Plant dry weight measurements (Figure 4.5 d) suggest that the combination of vermicompost and *C. rosea* was significantly worse at improving plant health than was vermicompost alone, with *C. rosea* alone as an intermediate, however in the second experiment, this pattern is reversed (Figure 4.6 d): dry weight is highest in the combined *C. rosea* and vermicompost treatment.

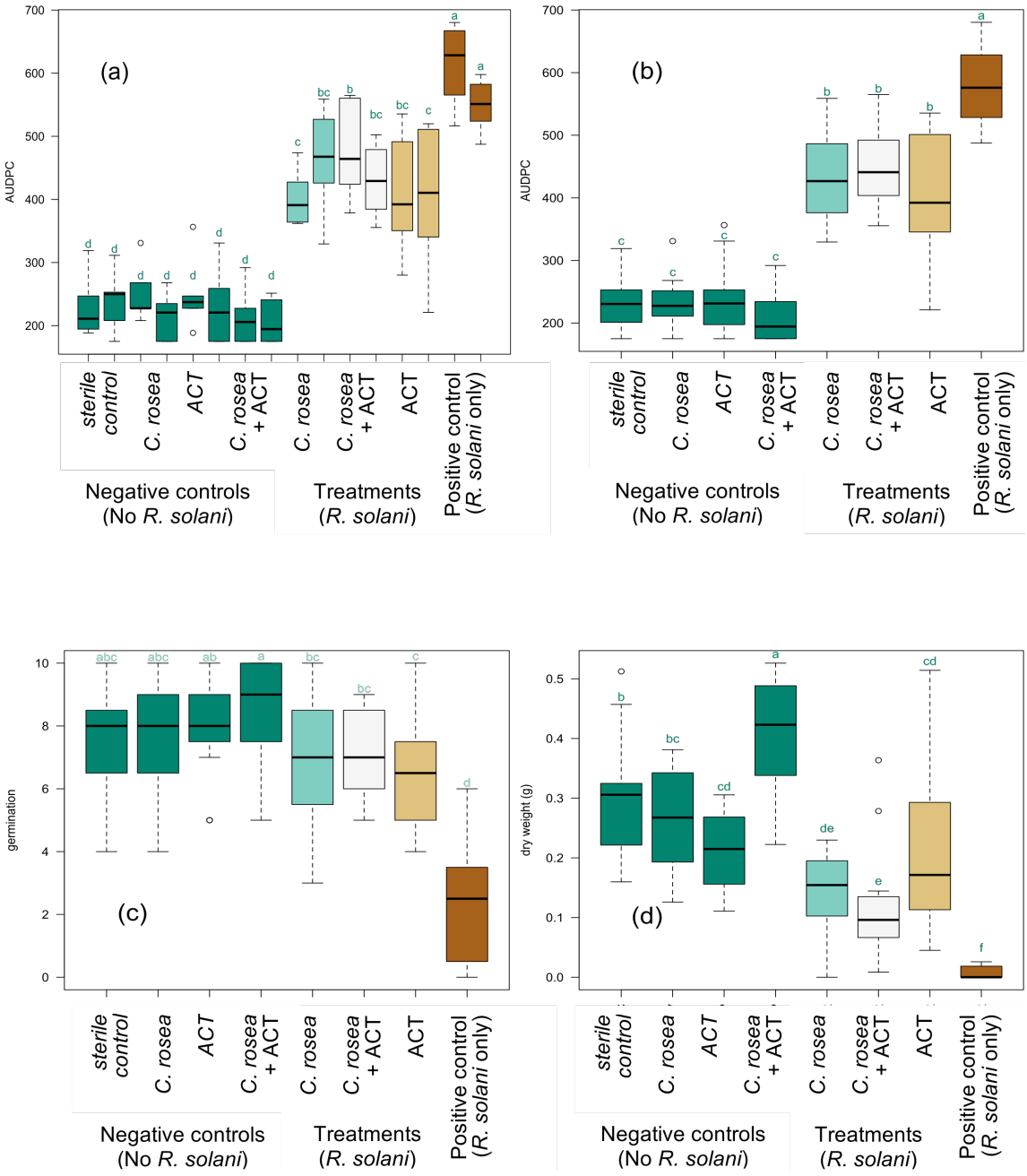
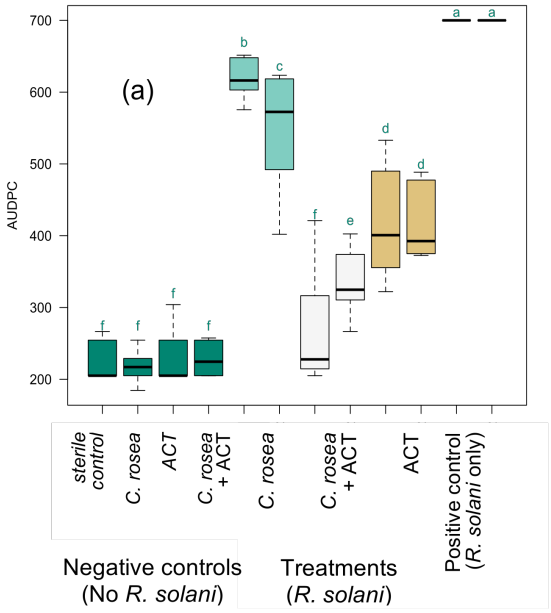
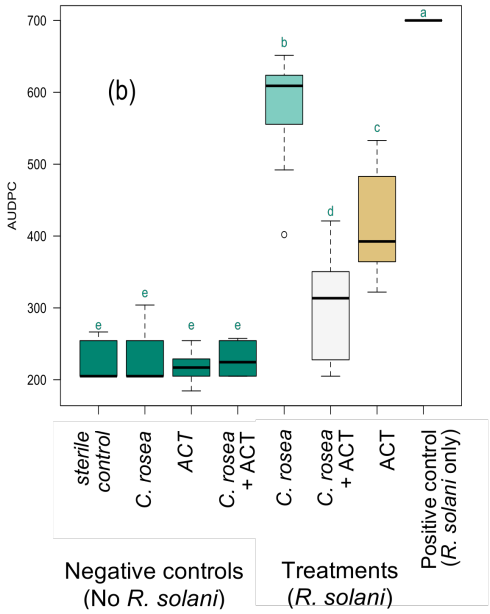


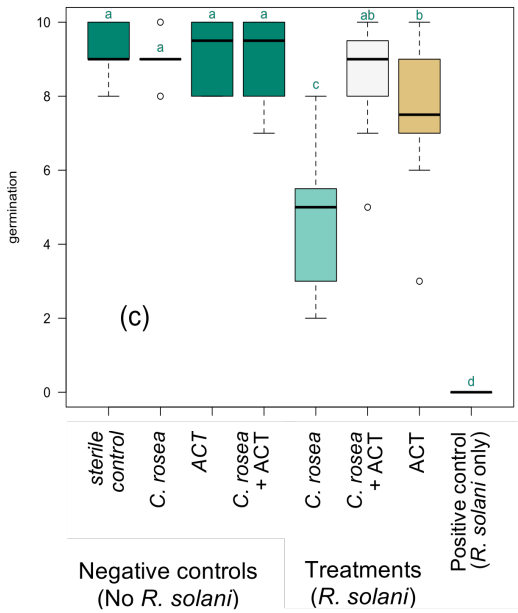
Figure 4.5. RRC1: Growth chamber bioassay measuring the reduction in disease of radish caused by *Rhizoctonia solani* (*R. solani*) when treated with *Clonostachys rosea* f. *catenulata* (*Gliocladium catenulatum*) strain J1446 (Prestop®) (*C. rosea)* aerated vermicompost tea (ACT), or both (*C. rosea* + ACT, *C.r.* + ACT*).*

Different letters indicate that treatment means could be separated using Fisher's LSD test in R. Experimental units (N=1) consisted of a pot with 10 radish plants. There were 6 experimental units per block, 2 blocks per treatment. Disease was measured using area under the disease progress curve (AUDPC) in individual treatment blocks (N=6) (a) and pooled by treatment (N=12) (b), germination rate per experimental unit after five days by treatment (N=12) (c), and shoot dry weight per experimental unit by treatment (N=12) (d). AUDPC was calculated based on repeated

measurements of radish disease severity on the following scale: 1= healthy plant (no stem damage), 2= plant with light damage on the stem and standing, 3= plant infected and fallen over, 4= plant dead.







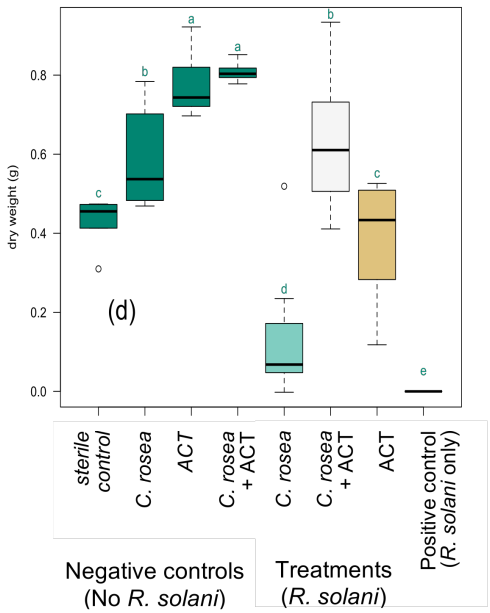


Figure 4.6. RRC2: Growth chamber bioassay measuring the reduction in disease of radish caused by *Rhizoctonia solani* (*R. solani*) when treated with *Clonostachys rosea* f. *catenulata* (*Gliocladium catenulatum*) strain J1446 (Prestop®) (*C. rosea)* and/or aerated vermicompost tea (ACT).

Different letters indicate that treatment means could be separated using Fisher's LSD test in R. Experimental units (N=1) consisted of a pot with 10 radish plants. There were 6 experimental units per block, 2 blocks per treatment. Disease was measured using area under the disease progress curve (AUDPC) in individual treatment blocks (N=6) (a) and pooled by treatment (N=12) (b), germination rate per experimental unit after five days by treatment (N=12) (c), and shoot dry weight per experimental unit by treatment (N=12) (d). AUDPC was calculated based on repeated measurements of radish disease severity on the following scale: 1= healthy plant (no stem damage), 2= plant with light damage on the stem and standing, 3= plant infected and fallen over, 4= plant dead.

Rhizoctonia solani / radish / *Bacillus subtilis* (RRB1 & RRB2)

In contrast to the *C. rosea* results in RRC1 and RRC2, *B. subtilis* did not control disease alone in sterile potting mix in the *R. solani* / radish pathosystem (RRB1 and RRB2 Figures 4.7 a,b and Figure 4.8 a,b) compared with the positive (pathogen only) control. There was no difference between the ACT treatment and the combined *B. subtilis* + ACT treatment in experiment RRB2 (Figure 4.8 b), but despite the *B. subtilis* treatments not showing any disease control when applied alone, in experiment RRB1 (Figure 4.7 b) the combined treatment provided a significantly (Tukey's HSD $p<0.05$) greater reduction in disease than ACT alone. All negative controls in these experiments showed lower levels of disease compared with the positive controls according to all of the dependent variables, and the vermicompost and combined vermicompost + *B. subtilis* treatments approach the pathogen-free negative controls in RRB1 (Figure 4.7 b). Germination rates show that *B. subtilis* did not improve germination compared with the *R. solani* positive control (Figure 4.7 c and 4.8 c), whereas the biocontrol and combined biocontrol and vermicompost treatments yielded excellent germination: in both RRB1 and RRB2 these were only marginally different from the pathogen-free negative controls (Figure 4.7 c and Figure 4.8 c). Plant dry weight measurements (Figure 4.7 d) suggest that the combination of vermicompost and *B. subtilis* was significantly better at improving plant health than was vermicompost alone, although as was the case for the disease data, this was not repeated in RRB2 (Figure 4.8 d). *B. subtilis* alone fared poorly with

respect to plant dry weight measures (Figure 4.7 d and 4.8 d): consistent with the other metrics in these experiments.

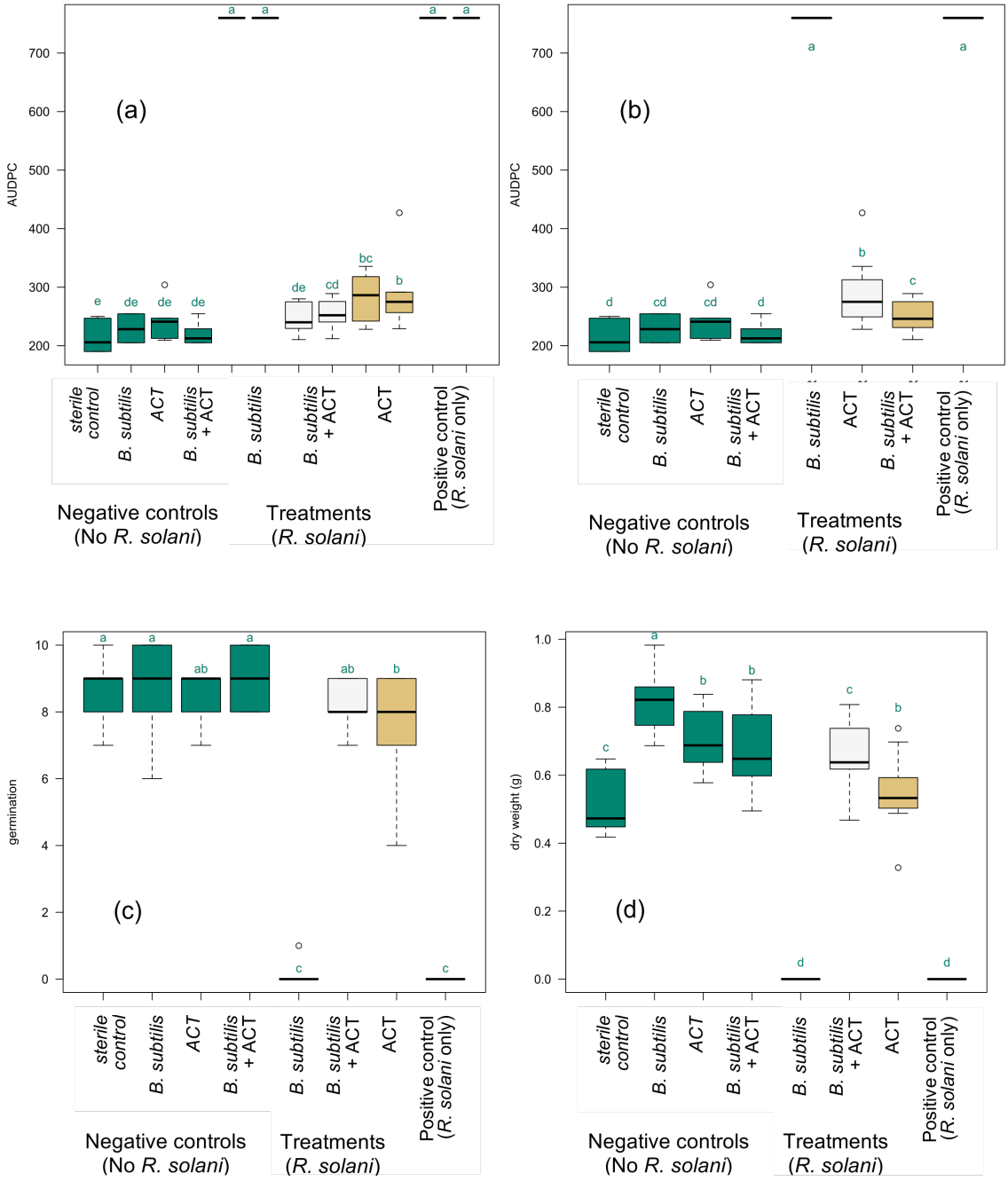


Figure 4.7. RRB1: Growth chamber bioassay measuring the reduction in disease of radish caused by *Rhizoctonia solani* (*R. solani)* when treated with *Bacillus subtilis strain QST 713 (Rhapsody)* (*B. subtilis)* and/or aerated vermicompost tea (ACT).

Different letters indicate that treatment means could be separated using Fisher's LSD test in R. Experimental units (N=1) consisted of a pot with 10 radish plants. There were 6 experimental units per block, 2 blocks per treatment. Disease was measured using area under the disease progress curve (AUDPC) in individual treatment blocks (N=6) (a) and pooled by treatment (N=12) (b), germination rate per experimental unit after five days by treatment (N=12) (c), and shoot dry weight per experimental unit by treatment (N=12) (d). AUDPC was calculated based on repeated measurements of radish disease severity on the following scale: 1= healthy plant (no stem damage), 2= plant with light damage on the stem and standing, 3= plant infected and fallen over, 4= plant dead.

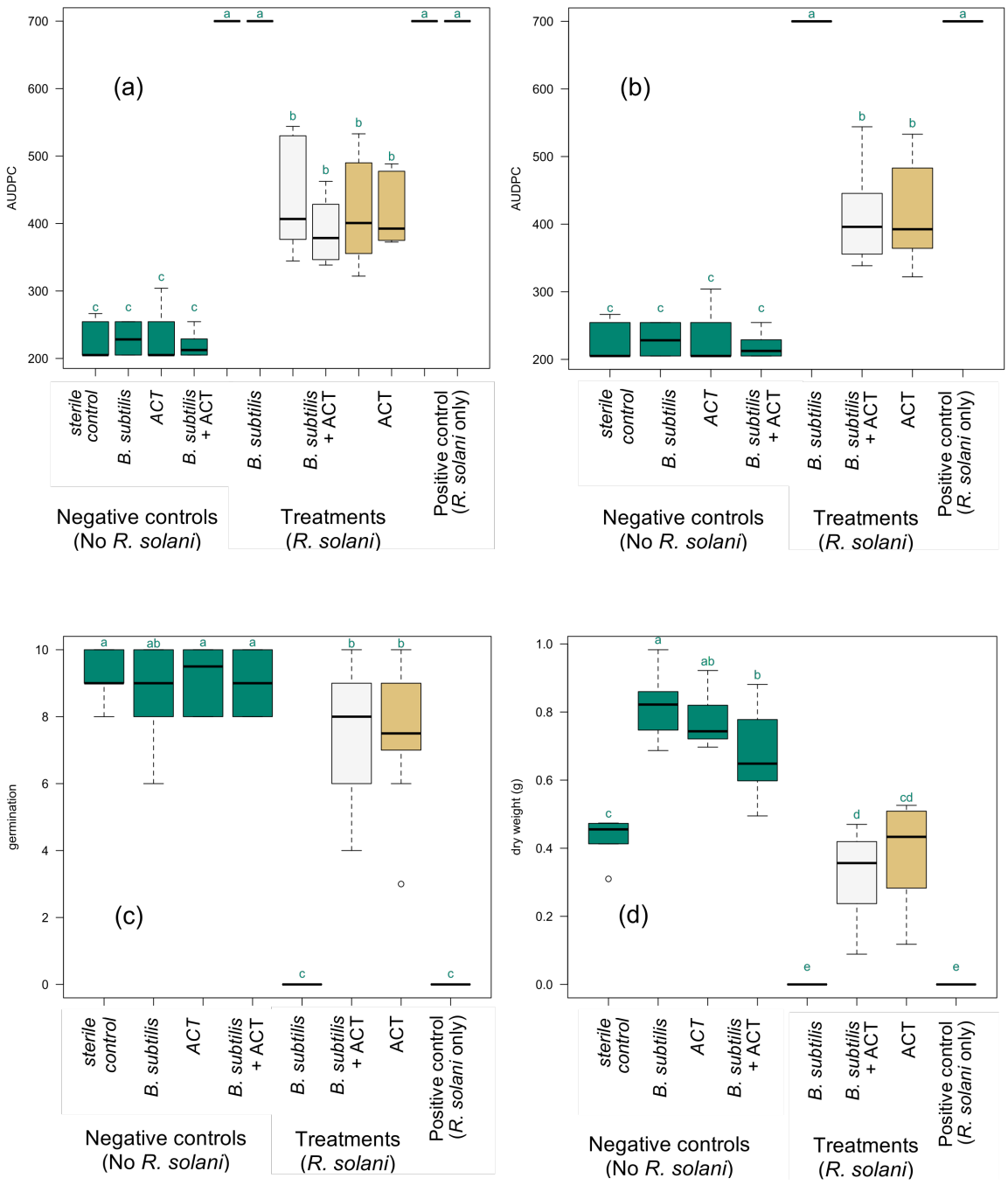


Figure 4.8. RRB2: Growth chamber bioassay measuring the reduction in disease of radish caused by *Rhizoctonia solani* (*R. solani)* when treated with *Bacillus subtilis strain QST 713 (Rhapsody)* (*B. subtilis)* and/or aerated vermicompost tea (ACT).

Different letters indicate that treatment means could be separated using Fisher's LSD test in R. Experimental units (N=1) consisted of a pot with 10 radish plants. There were 6 experimental units per block, 2 blocks per treatment. Disease was measured using area under the disease progress curve (AUDPC) in individual treatment blocks (N=6) (a) and pooled by treatment (N=12) (b), germination rate per experimental unit after five days by treatment (N=12) (c), and shoot dry weight per experimental unit by treatment (N=12) (d). AUDPC was calculated based on repeated

measurements of radish disease severity on the following scale: 1= healthy plant (no stem damage), 2= plant with light damage on the stem and standing, 3= plant infected and fallen over, 4= plant dead.

4.4.4. *Fusarium oxysporum* f.sp. *radicis-cucumerinum* / cucumber pathosystem

C. rosea and *B. subtilis* provided significant (Tukey's HSD, $P<0.05$) reduction in disease compared with the positive control in one replicated trial each (FCC1, Figure 4.9 a,b, FCB1, Figure 4.11 a,b) but not in another (FCC2, Figure 4.10 a,b,d, FCB2, Figure 4.12 a,b,c). There was no statistically significant difference among the treatment combinations with the exception of FCB2 (figure 4.12 a,b) where *B. subtilis* had lower efficacy than ACT and than two blocks of combined *B. subtilis* + ACT (Tukey's HSD, $P<0.05$).

Fusarium oxysporum f.sp. *radicis-cucumerinum* / cucumber / *Clonostachys rosea* (FCC1 & FCC2)

All negative controls showed significantly lower levels of disease compared with the treatments using the AUDPC calculation with the exception of one particularly effective combined *C. rosea* + vermicompost treatment block in FCC1 (Figure 4.9 a), and one combined *C. rosea* + vermicompost block and one vermicompost-only block in FCC2 (Figure 4.10 a). Positive controls had significantly higher disease severity than all treatments with the exception of one of the combined *C. rosea* and vermicompost blocks in FCC2 (Figure 4.10 a), and the *C. rosea* treatment in FCC2 (Figure 4.10 a,b). As in experiment RRC2 (Figure 4.6 a,b), the combined biological control + vermicompost treatment blocks cannot be combined according to Fisher's LSD test but they can be combined according to Tukey's HSD test and thus are combined in Figure 4.10 b. There were some significant differences between the biocontrol and vermicompost treatments in FCC1 (Figure 4.9 b): *C. rosea* provided significant disease reduction compared with the positive control, but marginally worse than vermicompost alone, and significantly worse than the combination of biocontrol agent and vermicompost according to Tukey's HSD. Vermicompost thus provided an intermediate level of suppression in this

experiment, and *C. rosea* combined with vermicompost provided the best suppression of Forc disease in FCC1. Closer examination of the individual blocks (Figure 4.9 a) supports these results. There is some variation between blocks within treatments, but they are similar enough that the data can be combined (Figure 4.9 b). In FCC2, there was a significant difference between the *C. rosea*-only treatment and the vermicompost-only treatment when the blocks were combined (Figure 4.10 b): vermicompost provided significant disease suppression whereas *C. rosea* did not. The combined biocontrol agent and vermicompost treatment was intermediate between these and not significantly different from either (Figure 4.10 b), however as discussed above the blocks of this treatment cannot be combined, and in Figure 4.10 a it is clear that the variance in the combined treatment is quite large. Germination rates in FCC2 show a significant difference between pathogen-free negative and pathogen-only positive controls, and suggest that the biocontrol alone was effective in reducing pre-emergence damping off, although not significantly more than the combined treatment and the vermicompost alone (Figure 4.10 c). Plant dry weight measurements (Figure 4.10 d) amplify the effect seen in the disease data: *C. rosea* alone was ineffective at improving cucumber plant health in this experiment, but these data differ from the disease data in that vermicompost treatment led to only marginal gains in plant dry weight, whereas the combined treatment of *C. rosea* and vermicompost led to significantly greater plant dry weight.

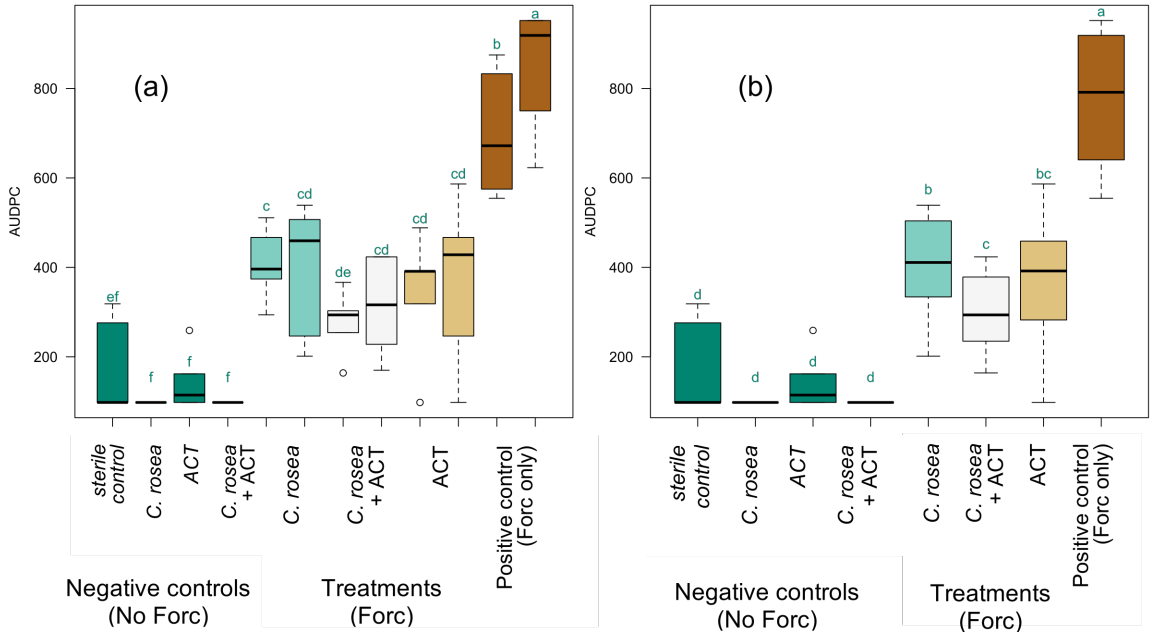


Figure 4.9. FCC1: Growth chamber bioassay measuring the reduction in disease of cucumber caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (Forc) when treated with *Clonostachys rosea* f. *catenulata* (*Gliocladium catenulatum*) strain J1446 (Prestop®) (*C. rosea)* and/or aerated vermicompost tea (ACT).

Different letters indicate that treatment means could be separated using Fisher's LSD test in R. Experimental units (N=1) consisted of a pot with 4 cucumber plants. There were 6 experimental units per block, 2 blocks per treatment. Disease was measured using area under the disease progress curve (AUDPC) in individual treatment blocks (N=6) (a) and pooled by treatment (N=12) (b), germination rate per experimental unit after five days by treatment (N=12) (c), and shoot dry weight per experimental unit by treatment (N=12) (d). AUDPC was calculated based on repeated measurements of cucumber disease severity on the following scale: 1= mild wilting, 2= wilting and yellowing, 3= yellowing and browning, 4= stem collapse, 5= death.

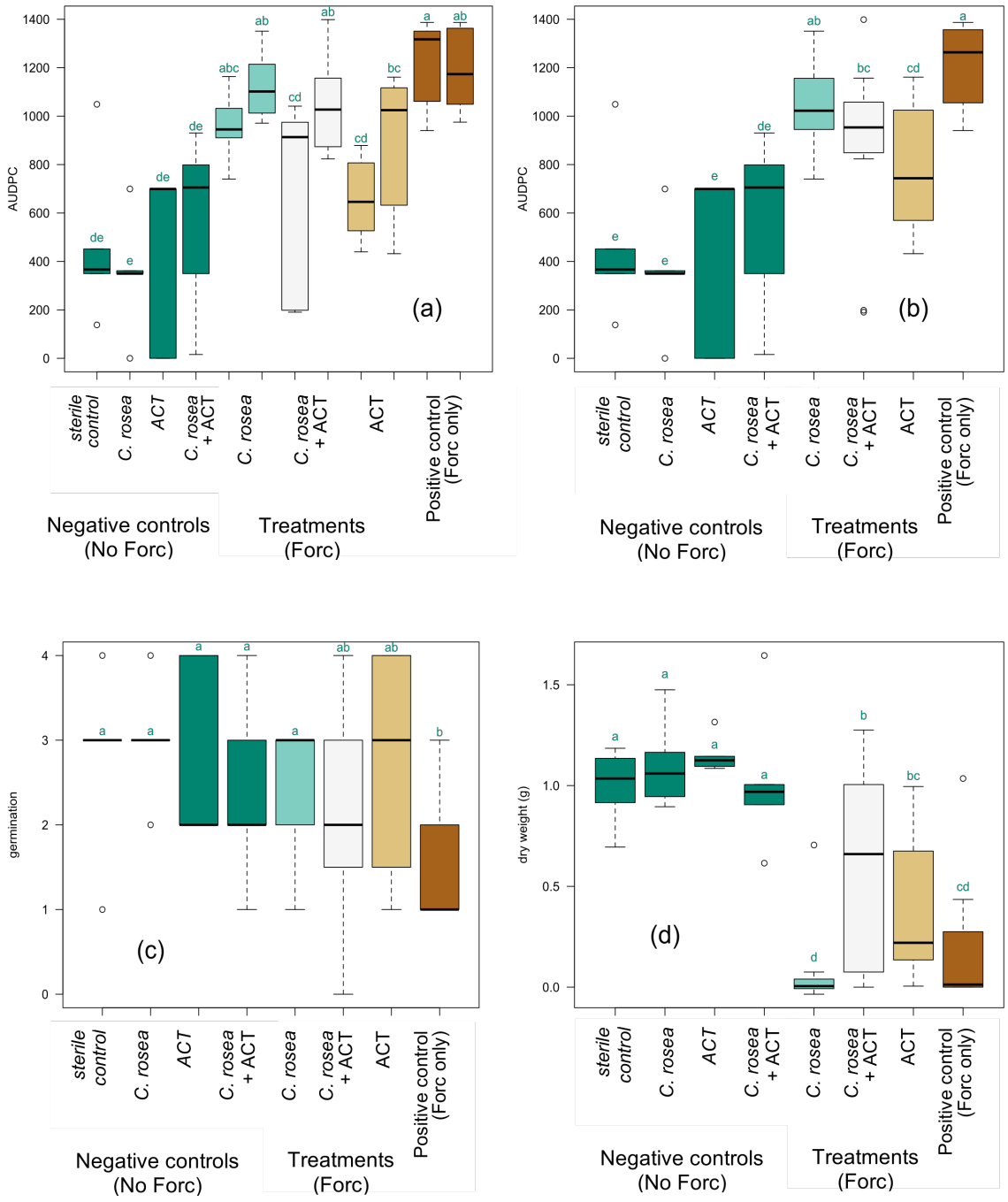


Figure 4.10. FCC2: Growth chamber bioassay measuring the reduction in disease of cucumber caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (Forc) when treated with *Clonostachys rosea* f. *catenulata* (*Gliocladium catenulatum*) strain J1446 (Prestop®) (*C. rosea*) and/or aerated vermicompost tea (ACT).

Different letters indicate that treatment means could be separated using Fisher's LSD test in R. Experimental units (N=1) consisted of a pot with 4 cucumber plants. There were 6 experimental units per block, 2 blocks per treatment. Disease was measured using area under the disease progress curve (AUDPC) in individual treatment blocks (N=6) (a) and pooled by treatment (N=12) (b), germination rate per experimental unit after five days by treatment (N=12) (c), and shoot dry

weight per experimental unit by treatment (N=12) (d). AUDPC was calculated based on repeated measurements of cucumber disease severity on the following scale: 1= mild wilting, 2= wilting and yellowing, 3= yellowing and browning, 4= stem collapse, 5= death.

Fusarium oxysporum f.sp. *radicis-cucumerinum* / cucumber / *Bacillus subtilis* (FCB1 & FCB2)

All negative controls in these experiments showed significantly lower levels of disease compared with the treatments using the AUDPC calculation with the exception of one particularly effective combined *B. subtilis* + vermicompost treatment block per experiment (Figure 4.11 a, Figure 4.12 a), and one of the vermicompost blocks in FCB2 (Figure 4.12 a). Positive controls had significantly higher disease severity than all treatments in FCB1 (Figure 4.11 b), whereas in FCB2 *B. subtilis* used alone did not provide any significant disease control, although in combination with vermicompost *B. subtilis* did appear capable of providing an increase in disease suppression over vermicompost alone (Figure 4.12 a, Table 4.2), however inconsistently. This effect is also seen in Figure 4.12 c and d: plant dry weight is increased in the combined treatment, as is germination rate. When the treatment replicate blocks of FCB1 were combined they did not differ significantly from each other (Figure 4.11 b): *B. subtilis* and vermicompost provided significant suppression of Forc, individually and in combination. Closer examination of the individual blocks (Figure 4.11 a and 4.12 a) reveals that the combined biocontrol agent and vermicompost treatments had greater inter-block variation than the individual treatments in FCB1, and high variation in FCB2 although the vermicompost-only treatment also had some variation (Figure 4.12 a). In contrast to experiments RRC2 (Figure 4.6 a) and FCC2, (Figure 4.10 a) where the combined *C. rosea* and vermicompost blocks could be combined as they did not differ according to Tukey's HSD test, the combined *B. subtilis* + vermicompost blocks in FCB2 (Figure 4.12a) cannot be combined as they do differ according to the Tukey's HSD test and thus they are combined in Figure 4.12 b for illustrative purposes only.

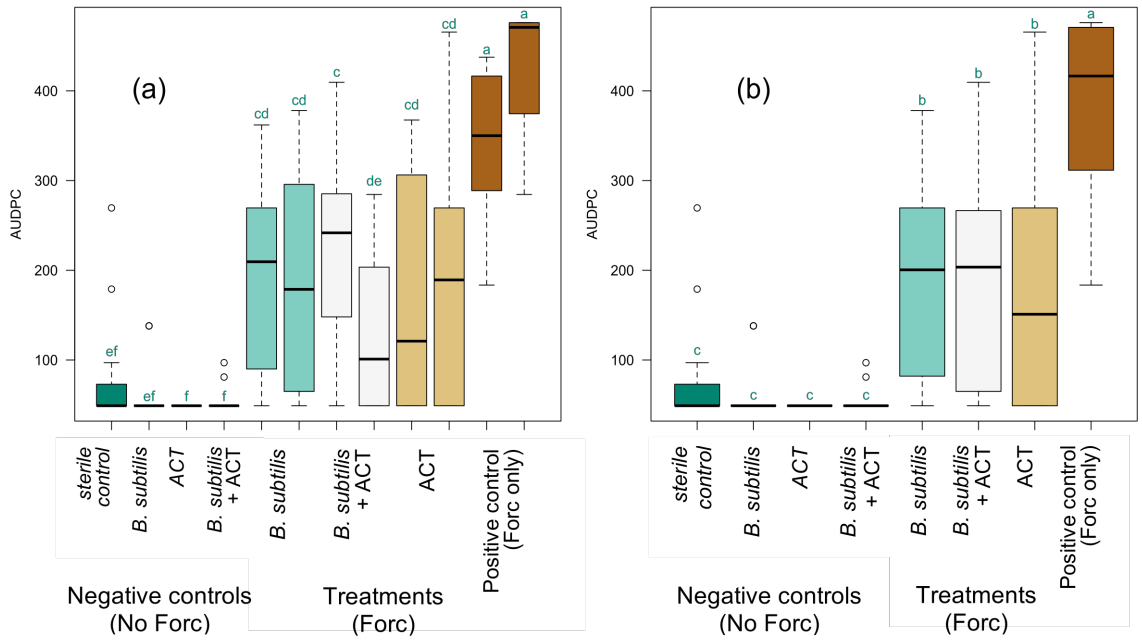


Figure 4.11. FCB1: Growth chamber bioassay measuring the reduction in disease of cucumber caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (Forc) when treated with *Bacillus subtilis strain QST 713 (Rhapsody)* (*B. subtilis)* and/or aerated vermicompost tea (ACT).

Different letters indicate that treatment means could be separated using Fisher's LSD test in R. Experimental units (N=1) consisted of a pot with 4 cucumber plants. There were 6 experimental units per block, 2 blocks per treatment. Disease was measured using area under the disease progress curve (AUDPC) in individual treatment blocks (N=6) (a) and pooled by treatment (N=12) (b). AUDPC was calculated based on repeated measurements of cucumber disease severity on the following scale: 1= mild wilting, 2= wilting and yellowing, 3= yellowing and browning, 4= stem collapse, 5= death.

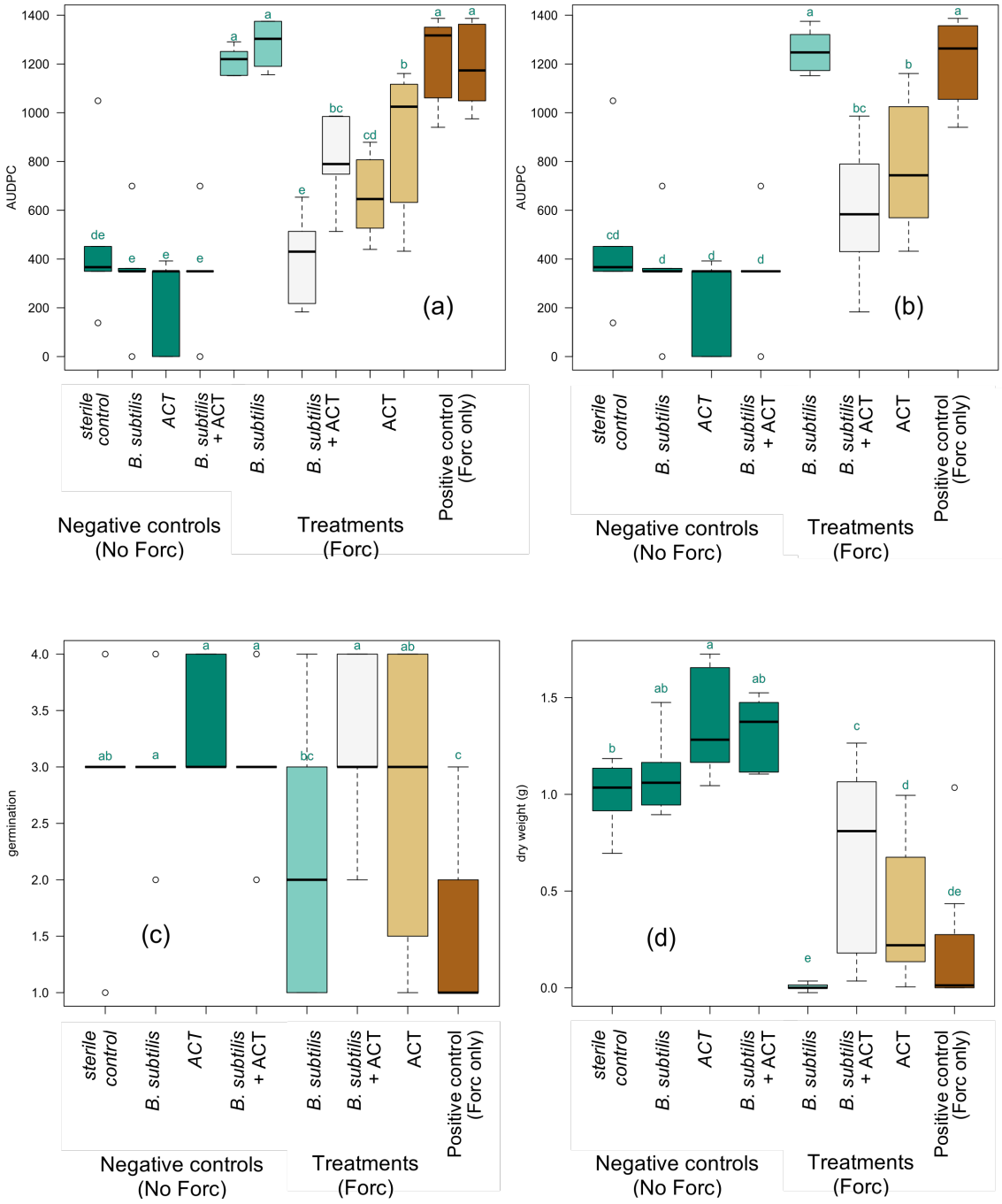

AUDPC
(a)
(b)
germination
(c)
dry weight (g)
(d)
sterile control
B. subtilis
ACT
B. subtilis + ACT
B. subtilis
B. subtilis + ACT
ACT
Positive control (Forc only)
Negative controls (No Forc)
Treatments (Forc)

Figure 4.12. FBC2: Growth chamber bioassay measuring the reduction in disease of cucumber caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (Forc) when treated with *Bacillus subtilis strain QST 713 (Rhapsody)* (*B. subtilis)* and/or aerated vermicompost tea (ACT).

AUDPC is area under the disease progress curve, germination is per 4 seedling pot, and dry weight is shoot dry weight. Different letters indicate that treatment means could be separated using Fisher's LSD test in R. Experimental units (N=1) consisted of a pot with 4 cucumber plants. There were 6 experimental units per block, 2 blocks per treatment. Disease was measured using area under the disease progress curve (AUDPC) in individual treatment blocks (N=6) (a) and pooled by treatment (N=12) (b), germination rate per experimental unit after five days by treatment (N=12) (c), and shoot dry weight per experimental unit by treatment (N=12) (d). AUDPC was calculated based on repeated measurements of cucumber disease severity on the following scale: 1= mild wilting, 2= wilting and yellowing, 3= yellowing and browning, 4= stem collapse, 5= death.

Table 4.2. Summary results of four sets of experiments (Figures 5.1-5.8) testing the disease suppressive effects of biocontrol agents *Bacillus subtilis* strain QST 713 (Rhapsody) and *Clonostachys rosea f. catenulata* (*Gliocladium catenulatum*) strain J1446 (Prestop®) on the pathogens *Fusarium oxysporum* f. sp*. radicis-cucumerinum* (Forc) and *Rhizoctonia solani* on cucumber and radish.

Pathogen/host/biocontrol	Experiment	AUDPC % reduction		
		BC agent	Vermicompost	Combined
R. solani/radish/*C. rosea*	RRC1	42 a	50 a	36 a
	RRC2	25 a	60 b	84 c
R. solani/radish/*B. subtilis*	RRB1	0 a*	89 b	96 b
	RRB2	0 a*	60 b	61 b
Forc/cucumber/*C. rosea*	FCC1	57 a	64 a	74 a**
	FCC2	22 a*	58 a**	44 a*
Forc/cucumber/*B. subtilis*	FCB1	61 a	64 a	64 a
	FCB2	-5 a*	50 b	93 c**, 47 b

Different letters indicate that treatment means could be separated according to Tukey's HSD test, P=0.05. Disease reduction was significant unless indicated by *. Double values indicate that repeated experiment means differed according to Tukey's HSD and could not be combined. **indicates that this treatment could not be separated from the negative (no pathogen) control treatments.

4.5. Discussion

Developing assays applicable to organic greenhouse agriculture which use media including composts to provide a diverse and active microbiota requires research. Disease suppression by composts is variable (Bonanomi et al. 2007; Noble & Coventry 2005; Termorshuizen et al. 2006; Larkin 2015; Yogev et al. 2006). The practice of adding biocontrol agents to composts to produce fortified composts is still in its infancy (Pugliese et al. 2011). It is not known whether biocontrol products are reliably compatible with compost-containing systems such as organic greenhouse media. Several mechanisms are reported to contribute to plant disease suppression by composts: antibiosis, parasitism, nutrient competition, induced resistance, as well as by providing better plant nutrition (Hoitink et al. 1997). Introduced biocontrol agents can provide disease suppression by these same mechanisms, but could adversely affect a suppressive compost microbial community, reducing its ability to provide disease suppression. Alternatively, it would be reasonable to predict that the ability of composts to suppress pathogens and impact soil microbial communities could also translate into the ability of compost to suppress a biocontrol agent. The application of a biocontrol product to a compost-containing media can be viewed as an interplay between general biological suppression and specific biological suppression. Although specific biological suppression has been shown to be the mode of action for some composts (Bonanomi et al. 2007; Suárez-Estrella et al. 2013), in other cases the suppressive effect cannot be narrowed to one agent or mechanism (Pane et al. 2011; Hadar & Papadopoulou 2012) and can be considered an emergent property of the microbial community (Jack 2011).

I hypothesized that more disease would be seen in the vermicompost alone plus pathogen treatment, a model for general biological suppression of disease in a model system, than in a treatment with a commercial biological control agent, a stand-in for specific biological suppression of disease. This is consistent with data from studies that demonstrate high biocontrol agent efficacy in sterile situations where the biocontrol is allowed to colonize without competition. In combination a sub-additive effect was hypothesized, where the two systems in competition worked less effectively than the sum of the two efficacies because of an interaction. Previous work on the compatibility of biocontrols with vermicomposts has been summarized by (Jack 2011), who recommends that each biocontrol agent and vermicompost system must be tested for

compatibility because of inconsistencies in studies combining vermicomposts with biocontrol agents. For example, (Sahni et al. 2008) found that combining *Pseudomonas syringae* strain PUR46 with high concentrations of vermicompost suppressed *Sclerotium rolfsii* on chickpea better than each treatment alone. The vermicompost used in the aforementioned study reduced mortality by between 12-40%. Conversely, vermicompost was found to decrease populations of *Pseudomonas fluorescens* strain Pf-D substantially during storage (Bora & Deka 2007), although this latter study did not examine whether the biocontrol agent lowers the disease suppressiveness of the vermicompost.

4.5.1. *In vitro* assays of biocontrol agent and vermicompost efficacy alone and in combination

In vitro assays can be used to screen biological control agents to precede time-consuming and costly plant trials, although they do not always predict biocontrol efficacy *in vivo* or in the field (Knudsen et al. 1997). Early work by Davet et al. (1986) used biocontrol-inoculated soil mixed with agar to screen *Trichoderma* isolates for saprophytic competitiveness. The technique was validated by correlating the *Trichoderma* isolates' performance in the agar test to parasitism of *Sclerotium rolfsii* in non-sterile soils (Davet & Roure 1986), and the technique was improved by Naar & Kecskes (1998). Compared with studies of enzyme production and antibiotic production *in vitro*, the utility of this type of assay has received little attention. Previous *in vitro* work has focused on testing the compatibility between different biocontrol agents such as different strains of *Pseudomonas* (De Boer et al. 2003; De Boer et al. 1999), and combining them with chitosan (Khan et al. 2005). The effect of compost extract on plant pathogens *in vitro* has also been studied (Marin et al. 2015). Other studies have examined the compatibility of biocontrol agents with composts in plant media: for example, Pereira et al. (1998) reported incompatibility between *B. subtilis* and *T. harzianum* in vermicompost (Larkin & Tavantzis 2013). Bernard et al. (2014) tested combinations of *Bacillus subtilis*, hypovirulent *Rhizoctonia solani*, *Trichoderma virens*, and composts to reduce soilborne diseases of potato in the field and on greenhouse cucumber, tomato, bean and basil, and found limited synergistic effects. Incompatibility between antagonists has been reported on fruit surfaces (Leibinger et al. 1997). To our knowledge no previous study has addressed the compatibility of biocontrol agents and vermicompost extract *in vitro*.

The results of *in vitro* dual culture assays are read with caution because of their inherent bias towards toxin production (Köhl et al. 2011), and their sensitivity to factors such as the Ca concentration of various agar compositions (Bosmans et al. 2016). Using a rich medium such as ½ strength PDA, and providing a suite of competing microbes, helps a simple *in vitro* assay such as the one presented here to provide results that could model the use of biocontrols in real-world applications. An *in vitro* method like the one presented here is useful beyond its application for screening compost/biocontrols systems for organic greenhouse production, and can be used to address some of the concerns of Köhl et al. (2011), providing an *in vitro* assay that is less biased towards toxin-producing antagonists, and extending the early soil competitiveness screening work by Davet et al. (1986). Furthermore, some investigators believe the main effects of vermicomposts to be from plant growth hormones (Tajbakhsh et al. 2011), whereas others believe disease protection to be at least partially biological (Edwards & Arancon 2004). If the biological disease suppression effect is subtle, and being masked in pot trials by increased plant vigour because of hormones in vermicompost, an *in vitro* test that excludes a plant component should be more sensitive to biological effects leading to biocontrol by vermicomposts. This test could also be used to screen composts for compatibility with biocontrols. This would be particularly important for pathogens such as *R. solani* that are considered to be suppressed by specific antagonists in composts, or composts intentionally fortified with antagonists (Scheuerell et al. 2005). My *in vitro* results did not always predict the outcomes of the *in planta* pot trials: the *in-vitro* trial for *C. rosea* indicated that the combined C. rosea + ACT treatments demonstrated the best control of Forc, however in the pot trial there were no differences between treatments. The *in vitro* trials for *B. subtilis* suggested that ACT alone had the strongest effect on Forc, however in the pot trial I found either no difference between treatments where *B. subtilis* was efficacious, or that the combination *B. subtilis* + ACT was somewhat similar to ACT alone, or better. The next step would be to determine whether the *in vitro* or *in planta* assays best predict results from field trials.

In the *in vitro* trials, adding *C. rosea* to a generally suppressive *in vitro* environment provided by vermicompost produced a significantly greater disease agent suppression than not adding *C. rosea*. *C. rosea* combined with vermicompost produced a sub-significant decrease in pathogen growth compared with *C. rosea* alone: less concerning is that this increase is not significantly greater than the theoretical scenario of

adding a biocontrol to an otherwise sterile environment (eg. a hypothetical sterile greenhouse), or that the effect is less than the additive effects of the vermicompost and the biocontrol together. Rather, these results suggest that it would be useful to add *C. rosea* to a plant growth media that is already providing general biological suppression of disease.

B. subtilis provided poor suppression of Forc growth *in vitro*, but in contrast to the plant trials where its combined efficacy was equal or greater, the combination of *B. subtilis* and vermicompost may have provided poorer control of Forc than vermicompost alone. This is the worst-case scenario for biocontrol application: addition of the biocontrol has rendered the biologically rich substrate more conducive to disease. This reinforces the complication and importance of performing tests of biocontrol compatibility before deployment in the field.

4.5.2. *In planta* assays of biocontrol agent and vermicompost efficacy alone and in combination

In the *in planta* experiments, as expected, all autoclaved potting mix was disease conducive, and all autoclaved potting mix inoculated with vermicompost tea was suppressive to the two diseases studied. Biocontrol agents, which provided excellent suppression (*C. rosea*) or some suppression (*B. subtilis*) of pathogen growth *in vitro*, were less consistent *in planta*. These results for the two biocontrols and the two chosen pathosystems do not make a compelling economic case for adding biocontrol products to reduce these diseases in unsterilized substrates containing compost. In all four cases vermicompost tea inoculation provided reliable reduction in disease, whereas the biocontrol agents' effects were erratic, and combining biocontrols with vermicompost only increased disease suppression over vermicompost tea alone in one of eight experiments. In experiments RRB1 and RRB2, *B. subtilis* did not provide any control of disease caused by *R. solani* when used alone, and in combination with vermicompost it did not appear to compromise vermicompost's ability to provide excellent disease suppression (Figure 4.7 and Figure 4.8). In experiments RRC1 (Figure 4.5a) and FCC2 (Figure 4.10a) some blocks showed worse suppression of *R. solani* and Forc, respectively with combined *C. rosea* + vermicompost treatment than the vermicompost sole treatment, although the effect was not significant across all blocks. In all of experiments FCC2 (Figure 4.10 a), RRC2 (Figure 4.6 a), FCB1 (Figure 4.11a), and

FCB2 (Figure 4.12a), the combined *C. rosea* + vermicompost or *B. subtilis* + vermicomposts treatments had a statistically significant difference between treatment blocks according to Fisher's LSD test, suggesting that the addition of a biocontrol reduces the stability of these systems. in FCB2 (Figure 4.12 and Table 4.2) this difference was significant according to Tukey's HSD test. The only other treatments where a significant difference according to Fisher's LSD test was observed occurred between the blocks of *C. rosea* in RRC2 (Figure 4.6 a), and the blocks of vermicompost alone in FCB2 (Figure 4.12 a), meaning that within sole treatments the inter-block variation was apparent according to the LSD test in 12.5% of experimental treatments overall, whereas in the combined treatment this frequency was 50%. The contrasting results found in the germination data of experiment RRC1 and RRC1 (Figure 4.5d and Figure 4.6d), taken together, and in addition to some negative interactions found in blocks of Figure 4.5 a and Figure 4.10 a, as well as the larger variation in combined treatments compared with sole treatments discussed above suggests that disease suppression is less predictable with combinations of biological control agents and vermicompost. As was the case for *B. subtilis in vitro*, negative interactions suggest the possibility of a scenario where addition of the biocontrol agent reduces the vermicompost microbiota's ability to suppress disease. It would be useful to control for this effect in future studies of biocontrol in competitive environments.

4.5.3. Organic and Hydroponic microbial consortia

This assay was intended to apply to organic production substrates, but initially sterile plant growth substrates such as rockwool are theoretically at greater risk than organic substrates containing composts, because if pathogens gain access to the organismal vacuum, they can spread rapidly and cause epidemics (Stanghellini & Rasmussen 1994). Suppressiveness has been found in soilless hydroponic systems using rockwool (Postma et al. 2000), and perlite and perlite-peat (Clematis et al. 2009). The communities that persist in these systems are dominated by unculturable bacteria (Calvo-Bado et al. 2006) and the mechanism of suppression has not been elucidated (Clematis et al. 2009). Biocontrols have been shown to colonize well under these conditions, for example *C. rosea* colonizes cucumber plants readily under hydroponic conditions (Chatterton & Punja 2010). It has been suggested that the suppressive microbes found in hydroponic media could interfere with biocontrol (Calvo-Bado et al.

2006), and also that biofilms could aid biocontrol colonization (Scheuerell 2002), at least on plant surfaces. An assay based on the one presented here could be useful for testing this effect.

4.5.4. Why use compost tea?

Composts have been shown to be variable in their disease suppressive abilities (Larkin 2015): one vermicompost was chosen to use throughout the trials and aerated vermicompost tea (ACT) was used to inoculate the media. Using compost tea rather than direct compost inclusion has several benefits in bioassay experiments. Brewing compost tea not only enriches the numbers of active microbes present compared with stored compost (Ingham 2005), but it should also exert a selective pressure towards microbes that are easily enriched by the brewing process: humic acid and molasses were used during brewing, foregoing some of the other amendments such as glacial rock dust that are sometimes added to compost teas for this reason. Narrowing microbial diversity should help to increase the experimental reproducibility by providing a consistent suite of enriched microbes. Additionally, the brewed compost tea has relatively low amounts of extra nutrients compared with compost. Although nutritional effects were controlled for in our experiments using a large set of negative control treatments, this might not always be practical.

4.5.5. Limitations of plant-free assays

The *in vitro* assay described here tests a biocontrol organism's ability to compete and provide reduction of pathogen growth in a competitive milieu which may predict its ability to do so in growth media or soil, a very difficult environment for introduced microorganisms (Van Veen et al. 1997). With no plant present, our assay necessarily ignores the importance of microhabitats on plant roots, which can be of greater importance to the colonization of biocontrol organisms than survival in the bulk soil (Normander & Hendriksen 2002). I tested an in vitro assay using the Radish/R. solani pathosystem and the vermicompost/*C. rosea* biocontrol combination with inconclusive

results (Appendix C). Biocontrol ability is related to spermosphere competence in *Pseudomonas chlorographis* and rhizosphere competence in *Trichoderma* spp., (Whipps 2001), and in *C. rosea* (Chatterton & Punja 2009), making this an important area for further study, perhaps by using media that more closely simulates the rhizosphere. I tested the *in vitro* assay using V8 media which is often used to grow plant pathogens, hypothesizing that favouring pathogen growth would increase the separation between treatment responses. The results were inconsistent and the image analysis was problematic (Appendix C).

Using this model system to test specific biological suppression of a disease agent within a generally suppressive milieu, different effects were found between the two biocontrol agents tested. With care taken to maintain consistency between experiments, this assay could prove to be a useful screen for biological control agents bound for application to substrates already containing a generally suppressive population such as organic greenhouse media or fortified composts.

Chapter 5.

Interactions between the biological control agents *Clonostachys rosea* and *Bacillus subtilis* and vermicompost. A conceptual framework and mixed effects analysis in two experimental model systems: *in vitro* and the radish / *Rhizoctonia solani* and cucumber / *Fusarium oxysporum* f.sp. *radicis-cucumerinum* pathosystems

5.1. Abstract

Microbial biocontrol agents are important tools for disease reduction, and little research has been done to determine how these agents interact with the simultaneous application of composts such as vermicompost. To study this, we developed a conceptual framework for these interactions, and *in vitro* Petri dish assays and *in planta* growth chamber assays of the radish / *Rhizoctonia solani* and cucumber / *Fusarium oxysporum* f.sp. *radicis-cucumerinum* pathosystems to investigate whether the efficacy of two inundative biological control agents: *Clonostachys rosea* and *Bacillus subtilis*, was affected by simultaneous application of vermicompost with respect to pathogen growth and the extent of plant disease. We analyzed the interactions using a mixed-effects model to quantify biocontrol success in growth media. Consistent interactions were found *in vitro* and a range of interaction was found between the biocontrols tested *in planta* suggesting that the interaction between a biocontrol agent and a competitive milieu is not additive. The analysis presented here provides a useful tool for quantifying biocontrol success in plant growth media.

5.2. Introduction

The interaction between an inundatively applied biocontrol agent and an existing competitive microbial environment has not been tested under controlled conditions that simulate the addition of a biocontrol to organic greenhouse plant growth media

containing compost (See Figure 5.1). It is not known whether this interaction is additive, synergistic, or antagonistic with respect to disease suppression. For example, (Sahni et al. 2008) found that combining *Pseudomonas syringae* strain PUR46 with high concentrations of vermicompost suppressed *Sclerotium rolfsii* on chickpea better than each treatment alone. Conversely, vermicompost was found to decrease populations of *Pseudomonas fluorescens* strain Pf-D substantially during storage (Bora & Deka 2007). A better understanding of the effect of interactions between biocontrol agents and plant growth media microbes on disease suppression will help make decisions in integrated disease management using biocontrols, and will aid the development of future biocontrol agents for sustainable agriculture. In the model (Figure 5.1) vermicompost microbes will be considered to represent general biological suppression of disease attributed to total microbial biomass and is not transferable between media (Weller et al. 2002). The inundative application of a single biological control agent represents specific biological suppression attributed to particular microbes and is transferrable between media.

Mixed effects analyses are used to investigate data where there is variation among and by groups (Knowles 2013), and experimental plant growth data can be structured this way in controlled environments such as greenhouses (and growth chambers) where experimental units are grouped within larger blocks to prevent cross-contamination of other pots, rows, irrigation valves, etc.

The pathogens *Fusarium oxysporum* Schlectend.: Fr f.sp. *radicis-cucumerinum* D.J. Vakalounakis (Forc), the causal agent of fusarium root and stem rot on cucumber (Vakalounakis 1996; Punja & Parker 2000) and *Rhizoctonia solani* Kühn, which causes pre- and post-emergence damping off of radish (Baker & Martinson 1970) were selected, as were the biocontrol agents *Clonostachys rosea* f. *catenulata* Schroers, Samuels, Seifert & Gams strain J1446 [syn. *Gliocladium catenulatum* Gilman & Abbott] commercially formulated as Prestop® (Verdera Oy, Finland) and *Bacillus subtilis* (Ehrenberg) Cohn strain QST 713 formulated as Rhapsody®, to study the potential interactions *in vitro* and *in planta*. Application of an inundative biological control agent was hypothesized to increase pathogen growth inhibition to a greater degree when combined with vermicompost than either treatment alone, potentially resulting in sub-additive suppression of disease development ie: there would be an interaction between the biocontrol agent and the vermicompost population.

Extrapolated to a real-world scenario, the hypothesis is that there is an interaction between the microbial communities present in greenhouse media and soils that prevents the disease suppression efficacies of general and specific biological suppression as represented by vermicompost communities and BCAs, respectively, from being simply additive. Furthermore, the magnitude of this interaction varies between BCAs, and this difference can be measured by holding the background microbial population constant in a model experimental system using vermicompost microbes. Thus our null hypothesis was that there is no interaction between the microbial communities present in greenhouse media and soils that prevents the disease suppression efficacies of general and specific biological suppression, the efficacies are simply additive. The theoretical model used to test this hypothesis also stated that BCAs would provide superior suppression of disease compared with vermicompost and that the combination of a BCA and vermicompost would provide an intermediate level of disease suppression, which we found to not always be the case in Chapter 4. Aerated vermicompost tea was found to provide an excellent background population upon which to test biocontrol efficacy, as it was consistent in composition over time, and between replicates. In all of our trials (Chapters 3 and 4) we found that vermicompost also provided very consistent suppression of disease in both our pathosystems, whereas the biological controls were less consistent.

5.3. Methods

Pathogen growth and disease reduction data was collected as described in Chapter 4. Experimental units for the *in vitro* assays consisted of a 9cm Petri dish inoculated with pathogen and a biological control agent, vermicompost, or both. Five replicates were used per treatment, and each experiment was repeated at least twice. The dependent variable was increase in pathogen colony radius in umh^{-1} based on measurement of the colony area. Experimental units for the growth chamber trials consisted of a 450 mL pot with four cucumber or ten radish seeds, inoculated with pathogen and a biological control agent, vermicompost, or both. Six pots were included in each block, two blocks of each treatment per experiment, and each experiment was performed twice. The dependent variable was Area Under the Disease Progress Curve

(AUDPC) calculated using the method described in appendix A in R 3.3.2 (R Core Team 2016): an automated method that preserved statistical power throughout the analysis.

(Equation 5.1) was applied using the lme4 version 1.1-13 (Bates et al. 2015) package in R 3.3.2. This was used to test for an interaction between the biocontrol agent and vermicompost using the lmer function with block as a random effect. Our blocks needed to be physically separated from each other to prevent microbial cross-contamination during irrigation. A Shapiro-Wilk test was used to check residuals for normality in R.

lmer(AUDPC ~ P + V + P:V + (1|Block) (5.1)

Equation 5.1. a mixed-effects model using lmer in R testing the interaction between the fixed effects: biocontrol agent (P) and vermicompost microbes (V), with block (Block) held as a random effect, with the response variable area under the disease progress curve (AUDPC).

5.4. Results

5.4.1. Conceptual framework for combinations of biocontrol agents and composts/media

A conceptual framework for understanding the possible interactions between biological control agents and vermicompost was developed as a model of the interactions between biological control agents and any environment rich in microbes, for example: soil, biofilm or rhizosphere. The framework contains the following hypotheses

a. Vermicompost can provide consistent biocontrol of disease, equivalent to general biological suppression of disease (D_{0M} in Figure 5.1).

b. A biocontrol agent can provide excellent suppression of disease in a sterile substrate, a stand-in for specific biological control of disease. (D_{A0} in Figure 5.1).

c. The combined treatment of biocontrol + vermicompost provides superior disease reduction than either control alone, but less than a purely additive amount because of an interaction between the biocontrol agent and the vermicompost agents, a model for the mutual antagonism between specific and general suppressive systems. (D_{AM} in Figure 2).

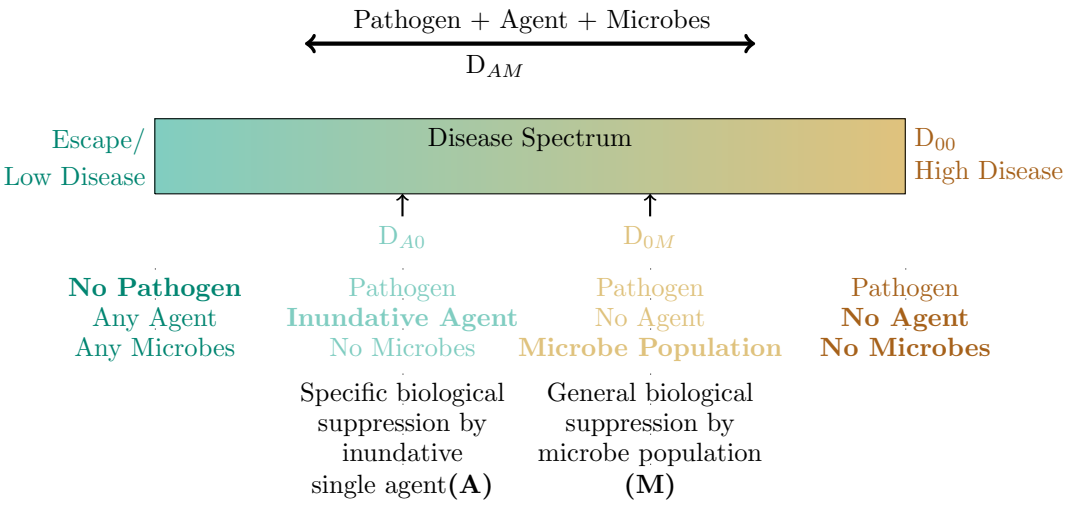


Figure 5.1. A conceptual framework for plant disease level (Dxx) in the presence of an inundatively applied biocontrol agent (A, Agent), an active microbial population (M, Microbes), or both (AM).

Disease is illustrated on a spectrum from escape at the left (no disease where the pathogen is not present or cannot live) through various combinations of pathogen, biological control agent, microbial populations. In the condition on the far right, no microbes are present except the pathogen, resulting in high disease.

5.4.2. Interactions found using the statistical model

A significant interaction (t=6.12, t=3.32) between the biocontrol agent and vermicompost was found in both of the *in vitro* experiments (Table 1): the relationship between the biocontrol agents and the vermicompost was never found to be additive in these experiments (Table 5.1, Figure 4.2-4.3).

A strong and significant (t=3.93) interaction effect was found between *C. rosea* and vermicompost in the growth chamber experiment RRC1 (Table 5.1, Figure 4.5) but no interaction was seen in RRC2 (t=0.04) (Table 5.1), consistent with the data visualized in (Figure 4.6 a,b). A significant (lmer t=3.77) interaction was found between *C. rosea* and vermicompost in the cucumber / Forc trial FCC1, whereas in FCC2 the effect was much smaller (t=1.17).

Table 5.1. Interactions between biocontrols and vermicompost populations *in vitro* and *in planta*

Experiment	Biocontrol	Experiment code[4], corresponding figure.	Pathosystem	Interaction +/-, t-value[1], Effect size, Standard Error	Interaction type
In vitro	*C. rosea*	IA1, 4.3	n/a	**+, 6.12, 88.8, 14.5**	**Antagonistic**
	B. subtilis	IA2, 4.2	n/a	**+, 3.32, 53.9, 16.2**	**Antagonistic**
In planta	*C. rosea*	RRC1, 4.5	Radish / *Rhizoctonia*	**+, 3.93, 198, 50.4**	**Antagonistic**
		RRC2, 4.6		-, 0.04, 1.96, 48.4	Additive
		FCC1, 4.9	Cucumber / Forc	**+, 3.77, 305, 80.9**	**Antagonistic**
		FCC2, 4.10		-, 1.17, 267, 228	Neutral
	B. subtilis	RRB1, 4.7	Radish / *Rhizoctonia*	-, (-2.13, -37.8, 17.8)	Neutral
		RRB2, 4.8		-, (-0.18, -4.92, 27.9)	Neutral
		FCB1, 4.11	Cucumber / Forc	**+, 3.26, 195, 59.9[2]**	**Antagonistic**
		FCB2, 4.12		-, -0.92, -216, 236	Additive[3]

[1]t-value is significant if $|t| \geq 1.96$ at $\alpha = 0.05$

[2]FCB1 fails the Shapiro-Wilk test, residuals have a bimodal distribution.

[3]In this test the combined *B. subtilis* + vermicompost blocks cannot be combined according to Tukey's HSD

[4]Experiment Codes are eg RRC1: (RR) pathosystem (C) biocontrol (1) Experiment #.

()RRB1 and RRB2 do not have normally distributed data for the biocontrol-only treatment: all plants died from pre-emergence damping off: the biocontrol had no measureable effect.

In experiments RRB1 and RRB2 there appears to be no interaction between the *B. subtilis* treatment and vermicompost (Figure 4.7a,b; 4.8a,b). *B. subtilis* did not appear to provide any control of disease: all plants in this treatment had maximum AUDPC values and thus the biocontrol-only data is not normally distributed. Thus the interaction can be described as neutral. In contrast, FCB1 showed significant (Tukey's HSD, $P<0.05$) biocontrol by *B. subtilis* (Figure 4.11) and an interaction between the biocontrol and vermicompost (Table 5.1, $t=3.26$): equal levels of biocontrol were seen with the addition of biocontrol and vermicompost alone and in combination. In FCB2 *B. subtilis* did not provide significant biocontrol (Figure 4.12) however according to the lmer analysis the small amount of disease reduction attributed to *B. subtilis* is additive when combined with the disease reduction from vermicompost (Table 5.1). As discussed in Chapter 4, the biocontrol + vermicompost treatments cannot be combined because they differ according to Tukey's HSD.

5.5. Discussion

We tested whether there was an interaction between an inundatively applied biocontrol agent and an existing competitive microbial environment derived from vermicompost to simulate the addition of a biocontrol to organic greenhouse plant growth media. Using data collected from *in vitro* measurements of the growth of the pathogen *Fusarium oxysporum* f.sp. *radicis-cucumerinum* (Forc) on a lawn of vermicompost organisms and/or the biocontrols *Clonostachys rosea* f. *catenulata* strain J1446 or *Bacillus subtilis* QST 713, and disease data collected from growth chamber experiments using the radish / *Rhizoctonia solani* and cucumber / Forc pathosystems with the aforementioned biocontrols, we tested for an interaction between the biocontrol agents and the vermicompost populations using the lme4 package in R. These interactions between biocontrol agents and vermicompost were consistently found to be significant *in vitro*, and were also found to be siginificant in some *in planta* experiments. In practice this suggests that vermicompost and microbial biocontrols lose some efficacy when combined compared to the efficacy that they demonstrate in isolation under the same conditions. There is still biological control occurring in this scenario. We hypothesized that an inundatively applied biological control agent would always provide better disease suppression than a microbial milieu from vermicompost and this was not always the case. Furthermore, biocontrol agents do not have additive effects when

combined with a competitive microbial mileu that is also exhibiting suppression of pathogens or disease. We did not find synergistic (i.e. more biological control efficacy than the sum of two treatments) in our experiments. The magnitude of the interaction between biocontrol agents and the substrates to which they are applied should be taken into account when applying microbial biocontrols to competitive substrates such as organic plant production media which contain composts. Vermicompost microbes provided consistent reduction of plant disease caused by *Rhizoctonia solani* and Forc.

The practice of deliberately adding biocontrol agents to composts to produce fortified composts is still being developed (Pugliese et al. 2011; Noble & Coventry 2005), and several studies have been conducted using thermocomposts and vermicomposts. Hypovirulent *Rhizoctonia* combined with composts yielded some limited synergistic effects (Larkin & Tavantzis 2013). Marginally additive effects of combining composts and the biocontrol agents *Trichoderma virens*, *Bacillus subtilis*, and hypovirulent *Rhizoctonia solani* for disease reduction on potato were reported in a later study by the same group (Bernard et al. 2014). Limited synergistic effects were also found in a study using *Trichoderma* and non-pathogenic *Fusarium* added to thermocompost to reduce disease of greenhouse cucumber, tomato, bean and basil. In this study the *Trichoderma* enriched compost was found to be effective against *R. solani*, but not *P. ultimum* and *P. nicotianae* (Pugliese et al. 2011). (Ros et al. 2017) found different levels of suppression depending on the biocontrol with which their thermocompost was fortified: *Trichoderma asperellum* was more effective than *T. harzianum*. Similar results have been demonstrated using vermicomposts. (Basco et al. 2017) tested the combination of vermicompost with three biocontrol agents: *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis*, finding that the fortified vermicompost treatment provided better suppression than vermicompost alone, although it is not clear what the interaction between the biocontrols and vermicompost may have been. (Amooaghaie & Korrani 2018) found that combinations of vermicompost and *B. subtilis* were more effective at lowering disease incidence caused by *F. oxysporum* on psyllium than either *B. subtilis* or vermicompost substitutions alone. (Simsek Ersahin et al. 2009) found no beneficial cucumber growth or rhizoctonia damping-off disease reduction effects when adding *T. harzianum* to vermicompost. The studies outlined above used a variety of methods from the initial preparation of their composts to their data analysis. An analysis

such as the one presented herein could help standardize biocontrol testing to enable comparisons between biocontrol/compost combinations.

In our model we made a necessary simplification: vermicompost is suppressing plant disease by general biological suppression. Some composts have been found to suppress disease by specific biological suppression (Bonanomi et al. 2007; Suárez-Estrella et al. 2013), however, in other cases the suppressive effect cannot be narrowed to one agent or mechanism (Pane et al. 2011; Hadar & Papadopoulou 2012) and can be considered an emergent property of the microbial community (Jack 2011). A limitation of this analysis is that the interaction term does not differentiate between suppression of the vermicompost by the agent, and suppression of the agent by the vermicompost. This can, however, be easily determined by comparing the means of the different treatments, which should be taken into account if this process is to be automated.

In our plant growth trials, negative (pathogen-free) controls showed that the treatments were not causing disease symptoms and only the treatments with pathogens were considered for this analysis, reducing the dimensionality of the experiment. These negative controls are important: vermicomposts have been shown to be deleterious in some cases (Kannangara et al. 2000; Szczech & Smolinska 2001; Lim et al. 2015), and these effects need to be screened before meaningful comparisons between different disease suppressive treatments can be made.

In experiments RRC1 and FCC2 (See Figures 4.5 and 4.10) some blocks showed worse suppression of *R. solani* and Forc, respectively with combined biocontrol+vermicompost treatment than the vermicompost sole treatment (data not shown), although the effect was not significant across all blocks. As was the case for *B. subtilis in vitro*, this negative interaction suggests the possibility of a scenario where addition of the biocontrol agent reduces the vermicompost microbiota's ability to control disease. It would be useful to control for this effect in future studies of biocontrol in competitive environments.

We found consistently antagonistic interactions *in vitro* and a range of interaction from antagonistic to additive between the biocontrols and vermicompost organisms that we tested *in planta*. Addition of biocontrol agents to competitive environments is not simply additive and this area requires further study. Extrapolating our results to the

inundative application of a biocontrol agent to a competitive microbial environment such as soil suggests this would also reduce the efficacy of a biocontrol agent, an effect that has been demonstrated (Mazzola & Freilich 2016; Nihorimbere et al. 2011), and appears stronger in organically managed soils than conventionally managed soils (Hiddink et al. 2005). We observed instances where disease suppression was similar between vermicompost alone, biocontrol agent alone, and combined vermicompost and biocontrol. If this were the case in a compost-containing plant growth media this implies that the addition of a biocontrol agent would have no effect on disease suppression.

Chapter 6.

Discussion and Future Directions

Organic greenhouse vegetable production is a promising contributor to the sustainable intensification of agriculture. This type of production system limits pesticide use, making biological control agents (BCAs) an important disease suppression tool. Improved assays of disease suppression efficacy are required to screen bacterial and fungal BCAs for this type of system because of the use of plant growth media containing composts. These assays must take into account the interactions between compost microbial populations and BCAs, which can be generalized as an interplay between general and specific biological suppression of plant disease. We introduced a framework to investigate this relationship as it pertains plant disease suppression. Recent literature on suppression of plant pathogens by vermicomposts was surveyed in Chapter 2. Five vermicomposts were compared using a variety of techniques in Chapter 3, and *in vitro* and *in planta* assays to test the general vs. specific suppression framework were developed and tested in Chapter 4, using two important pathosystems and two important BCAs. These methods balance the meaningful results of laborious and expensive pot and field studies, and of the higher throughput of rudimentary *in vitro* assays using a simple *in vitro* biocontrol assay that more closely simulates greenhouse or field conditions using a reproducible microbial background, and a similar assay in a simple pot study that mimics aspects of organic greenhouse production. Chapter 5 investigated the interactions between the various components of these assays using a mixed-effects analysis.

6.1.1. Vermicompost is a suitable inoculum for a model experimental system that is generally suppressive to plant disease

Composts have been shown to be variable in their disease suppressive abilities (Larkin 2015): To ensure that our microbial community would be consistently suppressive to disease, we used vermicompost, of the same batch, as a starting material for aerated compost tea (ACT), brewed under identical conditions, for each

experiment in Chapter 4. Using compost tea rather than direct compost inclusion has several benefits in bioassay experiments. Brewing compost tea not only enriches the numbers of active microbes present compared with stored compost (Ingham 2005), but it should also exert a selective pressure towards microbes that are easily enriched by the brewing process: we used humic acid and molasses, foregoing some of the other amendments such as glacial rock dust that are sometimes added to compost teas for this reason. Narrowing microbial diversity should help to increase experimental reproducibility by providing a consistent suite of enriched microbes. Additionally, the brewed compost tea has relatively low amounts of extra nutrients compared with compost. Although we controlled for nutritional effects in our experiments using a large set of negative control treatments, this might not always be practical. Vermicompost was also found to remain very consistent over time by DGGE (Chapter 3), and by repeatedly culturing it on PDA (eg. Chapter 4, Figure 4.1, Appendix B Figure B.2.2.). In addition to the consistency in composition after 4 months reported in Chapter 3, we found that vermicompost provided similar patterns of colonies on PDA after storage over 4 years, consistent with Fracchia et al. (2006) who showed stable SSCP profiles of bacterial DNA from different windrows of vermicompost, as well as composts stored over a 12 year period. The compost that we chose was mature, derived from dairy solids and yard waste, that had been thermophilically composted before vermicomposting. Other composts from Chapter 3 would have been suitable as well, for example the highest enzyme activity by FDA was from a coffee waste vermicompost. This material had been thermophilically stabilized for a month, and then vermicomposted for 4-6 weeks, these were shorter times than other composts in our study (Dr. John Paul, pers. comm.). This compost was the most dissimilar from the others according to DGGE and despite the short production time, did not have significantly different disease suppression properties compared with the other vermicomposts (Figure 4-6a,b), nor did it appear to have phytotoxic effects (Figure 4-6c,d). Autoclaved vermicompost was used as a control in Chapters 3 and 4 and the results suggested that the mechanism of suppression is biological, consistent with many studies discussed in Chapter 2.

6.1.2. Compatibility of composts and biocontrol agents

The practice of deliberately adding BCAs to composts to produce fortified composts is in its infancy (Pugliese et al. 2011; Noble & Coventry 2005), but several studies have been conducted to test compatibility in this area. Previous work on the compatibility of biocontrols with vermicomposts has been summarized by (Jack 2011). For example, (Sahni et al. 2008) found that combining *Pseudomonas syringae* strain PUR46 with high concentrations of vermicompost suppressed *Sclerotium rolfsii* on chickpea better than each treatment alone. The vermicompost used in the aforementioned study reduced mortality by between 12-40%. Conversely, vermicompost was found to decrease populations of *Pseudomonas fluorescens* strain Pf-D substantially during storage (Bora & Deka 2007), although this latter study did not examine whether the BCA lowers the disease suppressiveness of the vermicompost.

Hypovirulent *Rhizoctonia* with composts yielded some combined effects (Larkin & Tavantzis 2013). Marginally additive effects of combining composts and the BCAs *Trichoderma virens*, *Bacillus subtilis*, and hypovirulent *Rhizoctonia solani* for disease reduction on potato were reported in a later study (Bernard et al. 2014), although Pereira et al. (1998) had earlier reported incompatibility between *B. subtilis* and *T. harzianum* in vermicompost (Larkin & Tavantzis 2013). Limited combined effects were also found in a study using *Trichoderma* and non-pathogenic *Fusarium* added to thermocompost to reduce disease of greenhouse cucumber, tomato, bean and basil. In this study the *Trichoderma* enriched compost was found to be effective against *R. solani,* but not *P. ultimum* and *P. nicotianae* (Pugliese et al. 2011). (Ros et al. 2017) found different levels of suppression depending on the biocontrol with which their thermocompost was fortified: *Trichoderma asperellum* was more effective than *T. harzianum*. Similar results have been demonstrated using vermicomposts. (Basco et al. 2017) tested the combination of vermicompost with three BCAs: *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis*, finding that the fortified vermicompost treatment provided better suppression than vermicompost alone, although it is not clear what the interaction between the biocontrols and vermicompost may have been. (Amooaghaie & Korrani 2018) found that combinations of vermicompost and *B. subtilis* were more effective at lowering disease incidence caused by *F. oxysporum* on psyllium than either *B. subtilis* or vermicompost substitutions alone. (Simsek Ersahin et al. 2009) found no beneficial cucumber growth or rhizoctonia damping-off disease suppression effects

when adding *T. harzianum* to vermicompost. The studies outlined above used a variety of methods from the initial preparation of their composts to their data analysis and thus it is difficult to make comparisons between them. An analysis such as the one presented herein could help standardize biocontrol/compost testing to enable such comparisons.

6.1.3. Mechanisms of disease suppression by composts: Specific vs general suppression

Although the model for understanding the suppression of plant disease by composts incorporates both specific and general suppression, given that in Chapter 1 even the emblematic specific biological suppressive soil system for take-all decline was shown to be more complicated than previously assumed, we chose to make a simplification necessary for our model: vermicompost would serve as a representative of general biological suppression of plant disease. BCAs could then represent specific biological suppression, and we could test the theoretical model outlined in Chapter 1 using the tests in Chapter 4. This allows us to control the microbes representing general suppression in our experimental model more reproducibly than, for example, the pioneering work by Davet et al. (1986), who used biocontrol-inoculated soil mixed with agar to screen *Trichoderma* isolates for saprophytic competitiveness.

We observed that our preparations of vermicompost consistently suppressed pathogen growth and disease, consistent with the studies described in Chapter 2 section 2.2. Vermicompost varied in ability to suppress the efficacy of biocontrol organisms *in vitro* and *in planta*, and the microbial communities in the vermicompost inoculum that we used remained stable over several months.

6.1.4. Theoretical model

Introduced BCAs can provide disease suppression but could adversely affect a suppressive compost microbial community, reducing its ability to provide disease suppression. Alternatively, it would be reasonable to predict that the ability of composts to suppress pathogens and impact soil microbial communities could also translate into

the ability of compost to suppress a BCA. We hypothesized that more disease would be seen in the vermicompost alone plus pathogen treatment, a model for general biological suppression of disease in our model system, than in a treatment with a commercial BCA, a stand-in for specific biological suppression of disease. This is consistent with data from studies that demonstrate high biocontrol efficacy in sterile situations where the agent is allowed to colonize without competition. In combination we expected to see a sub-additive effect, where the two systems in competition worked less effectively than the sum of the two efficacies because of an interaction.

Interactions between BCAs and vermicompost were consistently found *in vitro*, and often encountered *in planta*. Because BCAs do not always have additive effects when combined with a competitive microbial milieu that exhibit suppression of pathogens or disease, the reduction in biocontrol efficacy because of the substrates to which they are applied should be taken into account when applying microbial biocontrols to competitive substrates such as organic plant production media which contain composts. This is similar to the effect that has been demonstrated in soils (Mazzola & Freilich 2016; Nihorimbere et al. 2011), in particular if they are organically managed (Hiddink et al. 2005). Conversely, vermicomposts suppressed disease very consistently throughout our experiments, reinforcing previous findings that vermicompost is an excellent disease reduction tool. The worst-case scenario for application of a BCA in such a system is one in which the BCA harms the plant growth media's inherent disease suppressiveness, an effect which we saw in some rare instances, discussed in Chapter 4. This concept is aligned with the debate around non-target effects of microbial biocontrols, where the displacement of non-target microorganisms, allergenicity, toxigenicity, and pathogenicity are of concern to some (Brimner & Boland, 2003; Brimner & Boland 2004), and not to others (Kiss 2004; Koch et al. 2018). The interactions between biocontrol agents and indigenous soil microbial communities can and has been monitored using techniques such as SSCP (Grosch et al. 2006) who found little effect of *Trichoderma* isolates antagonistic to *R. solani* on the root-associated microbial communities of potato and lettuce. With some exceptions, fungal and bacterial biocontrol agents have minimal and transient effects on soil microbial communities (Scherwinski et al. 2007; Scherwinski et al. 2008; Cordiera & Alabouvette 2009; Griffiths & Philippot 2013; Scheepmaker & Kassteele 2011). These would generally not be a concern in greenhouse systems

because the media is isolated from the natural environment, however, if media suppressiveness were damaged, this could be a costly side-effect of an already costly biocontrol application. The *in planta* assay in this study could help to screen for this effect in a specific host / pathogen / biocontrol / vermicompost system so that it could be avoided. Although not a significant effect in this study, the results of assays IA2, RRC1, and FCC2 (Figure 4.3, Table 4.2, Figure 4.5 a, and Figure 4.10 a) suggest that the biocontrol agent can reduce a vermicompost's ability to suppress disease, as disease suppression is greater when vermicompost acts alone, rather than in combination with a biocontrol in some of the blocks of these experiments.

6.1.5. Future Directions: Biocontrol effects on the vermicompost microbiome

Future work in this area could examine the effect of an introduced biocontrol on the vermicompost-derived microbial community, on a short time scale as suggested by (Handelsman 2002). Studies have been performed to examine the effect of biocontrols on existing communities, and basing such a study on the assay presented here, would allow a direct comparison between the effect of different biocontrols on the same microbiome, and combining this with the field-soil framework outlined by (Poudel et al. 2016) would show whether the disease suppression related members of the generally suppressive consortia were being impacted by the addition of a BCA. The tools are available to elucidate the interactions between an added BCA and a background soil microbiome. For example, to extend the findings of the current study, microbial community profiles could be compared before and after perturbation by a BCA using a number of techniques such as those listed in Section 2.6.3. Understanding this relationship will allow for better use of biological control in organic greenhouse vegetable production, and in other systems that have a biological background such as hydroponics and field crops.

6.1.6. Future directions: Molecular work on *Mortierella*

Approaches for characterizing compost microbial communities have ranged from chemical and cultural analyses (Janzen et al. 1995; Anastasi et al. 2005; Hénault-Ethier 2007; Grantina-Ievina et al. 2013; Marín et al. 2013), to high throughput sequencing (Neher et al. 2013; Lv et al. 2015; Yu et al. 2015; Aira et al. 2016). We chose DGGE for our analyses in Chapter 3, a very sensitive technique that can detect single-nucleotide polymorphisms (SNPs) and individual bands representing a single phylotype can be directly excised from the gel and identified. Other techniques that yield longer sequences would allow future work to narrow organism identifications: the short DGGE fragments limited our identification by BLASTn searches of *Mortierella* to *M. hyalina*, *M. alpina*, *M. humulis* or *M. echinosphaera*-like. It would be interesting to learn more about the populations of Mortierella in vermicomposts. Species such as *M. alpina* produce arachidonic acid, which can induce plant defences (Dedyukhina et al. 2014) and it would be interesting to see what the contribution of this organism makes to the disease suppressiveness of vermicomposts. This genus has a widespread distribution in composts (Anastasi et al. 2005; Novinscak et al. 2009), is found in vermicomposts (Neher et al. 2013), and in forest soils (Buée et al. 2009), grassland soils (States & Christensen 2001), a wide variety of decomposing organic matter (Wagner et al. 2013) and occasionally causes fungal infection of animals eg. *M. wolfii* (Davies et al. 2010). *Mortierella* spores can be found in air and rainwater (Fröhlich-Nowoisky et al. 2015) and from environmental samples (Nagy et al. 2011).

6.1.7. The cultivable portion of a vermicompost microbiome can improve *in vitro* pre-screening of microbial antagonists, and this assay can be expanded to test other systems

Our goal with the *in vitro* assay was to test a biocontrol organism's ability to compete and provide reduction of pathogen growth in a competitive milieu which may predict its ability to do so in growth media or soil, a very difficult environment for introduced microorganisms (Van Veen et al. 1997). The results of *in vitro* dual culture assays are biased towards toxin production (Köhl et al. 2011), and are sensitive to factors such as the calcium concentration of various agar compositions (Bosmans et al.

2016). Thus, many potential biocontrols are missed during *in vitro* screening, and those that succeed are not guaranteed to be adept at surviving in competitive environments. Using a rich medium such as ½ strength PDA, and providing a suite of competing microbes, helps a simple *in vitro* assay such as the one presented here to provide results that could better select potential biocontrols. We found in Chapter 3 that the populations discovered through molecular analyses differed from those detected in culture on PDA, and it was originally established that the cultivable fraction of soil microbial populations was less than 1% (Torsvik et al. 1990), and the prevailing microorganisms from composts cannot be cultured (Ivors et al. 2017). Hence our *in vitro* tests in Chapter 4 that use the same media as the cultural analyses of Chapter 3 are only using a subset of the vermicompost microbiome. It would be reasonable to speculate that most of the effects seen in the Chapter 4 trials were because of organisms that grew well on PDA. The goal of these assays was to examine BCA efficacy in a competitive milieu, and the *in vitro* assays necessarily miss some possible interactions with unculturable microbes. The BCAs and the vermicompost organisms were, however, in close contact: spread plates were prepared with a combined lawn of the two inocula. The observed effect was solely the reduction in growth of the third participant, the pathogen, which was inoculated only at the center of the petri dish and thus not as intimately introduced to the vermicompost microbes as were the biocontrols. This possible selectivity to organisms that readily grow on PDA could explain some of the discrepancies between Chapter 4's *in vitro* trial results and those of the *in planta* trials which were performed in potting mix.

The subset of microbes described here is providing a competitive environment, evidenced by a consistent reduction in pathogen growth and disease suppression in Chapters 3 and 4. Moreover, this competitive environment is reproducible, because of the stability over time of vermicompost found in Chapter 3, and through repeated testing throughout Chapters 3 and 4. It is our hope that an *in vitro* method like the one presented here is useful beyond its application for screening compost/biocontrols systems for organic greenhouse production, and can be used to address some of the concerns of Köhl et al. (2011), providing an *in vitro* assay that is less biased towards toxin-producing antagonists. Whereas previous *in vitro* work has mainly focused on testing the compatibility between different agents such as strains of *Pseudomonas* (De

Boer et al. 2003; De Boer et al. 1999), combining them with chitosan (Khan et al. 2005), or examining the effect of compost extract on plant pathogens (Marín et al. 2015), these assays could extend the early soil competitiveness screening work by Davet et al. (1986) discussed above. Furthermore, some investigators believe the main effects of vermicomposts to be from plant growth hormones (Tajbakhsh et al. 2011), whereas others believe disease protection to be at least partially biological (Edwards & Arancon 2004). If the biological disease suppression effect is subtle, and being masked in pot trials by increased plant vigour from hormones present in vermicompost, an *in vitro* test that excludes a plant component should be more sensitive to biological effects leading to biocontrol by vermicomposts. With no plant present, our assay also ignores the importance of microhabitats on plant roots, which can be of greater importance to the colonization of biocontrol organisms than survival in the bulk soil (Normander & Hendriksen 2002). Biocontrol ability is related to spermosphere competence in *Pseudomonas chlorographis* and rhizosphere competence in *Trichoderma* spp., (Whipps 2001), and in *C. rosea* (Chatterton & Punja 2009), making this an important area for further study, perhaps by using media that more closely simulates the rhizosphere. This test could also be used to screen composts for compatibility with biocontrols. This would be particularly important for pathogens such as *R. solani* that are considered to be controlled by specific antagonists in composts, or composts intentionally fortified with antagonists (Scheuerell et al. 2005).

Our assays could be applied to other pathosystems and BCAs, with modifications based on individual disease histories. For example, we combined the effect of pre- and post-emergence damping off of radish caused by *R. solani* in our AUDPC calculations. This might not be appropriate to other systems. The amount by which a pathosystem is affected by a vermicompost is expected to differ, as has been found for composts (Termorshuizen et al. 2006), which must be accounted for in the interpretation of results. In particular, suppressive products for Oomycete diseases of organic greenhouse crops should be assayed: these organisms are good candidates for suppression using vermicomposts (Jack 2011), as well as being well adapted to the hydroponic greenhouse environment. They cause problems in organic greenhouses where chemical controls such as metalaxyl available to hydroponic growers are not permitted. We intended this assay to apply to organic production substrates, but initially sterile plant

growth substrates such as rockwool are theoretically at greater risk than organic substrates containing composts, because if pathogens gain access to the organismal vacuum, they can spread rapidly and cause epidemics (Stanghellini & Rasmussen 1994). If the microbial communities found in conventional hydroponic systems are suppressive to disease, as found by in soilless hydroponic systems using rockwool (Postma et al. 2000), and perlite and perlite-peat (Clematis et al. 2009), and if these interfere with the disease suppressive abilities of introduced BCAs as suggested by Calvo-Bado et al. (2006), or aid their colonization as suggested by Scheuerell (2002), a variation of this assay could test which biocontrols are most affected before application. Intensification of agriculture has traditionally been linked to environmental harm (Pretty et al. 2018): using vermicomposts and other composted materials together with BCAs for the reduction of plant diseases in intensive agriculture such as conventional hydroponics can help reduce deleterious effects.

References

Agrios, G. N. (2005). Plant Pathology. 5th ed. Elsevier. San Diego, CA.

Aira, M., Olcina, J., Pérez-Losada, M., & Domínguez, J. (2016). Characterization of the Bacterial Communities of Casts from *Eisenia Andrei* Fed with Different Substrates. *Applied Soil Ecology, 98,* 103–111. doi:http://dx.doi.org/10.1016/j.apsoil.2015.10.002

Al-Mazroui, S. S., & Al-Sadi, A. M. (2015). Highly Variable Fungal Diversity and the Potential Occurrence of Plant Pathogenic Fungi in Potting Media, Organic Fertilizers and Composts Originating from 14 Countries. *Journal of Plant Pathology, 97*(3), 529–534. doi:10.4454/JPP.V97I3.033

Al-Sa'di, A. M., Drenth, A., Deadman, M. L., Al-Said, F. A., Khan, I. and Aitken, E. A. B. (2008). Potential sources of *Pythium* inoculation into greenhouse soils with no previous history of cultivation. *Journal of Phytopathology, 156*, 502-505. doi:10.1111/j.1439-0434.2008.01396.x

Al-Tuwaijri, M. M. (2009). Role of the Biocontrol Agents *Trichoderma viride* and *Bacillus subtilis* in Elimination of the Deteriorative Effects of the Root-Rot Pathogens, *Fusarium oxysporum* and *F. solani*, on Some Metabolic and Enzyme Activities of Cucumber Plants. *The Egyptian Society of Experimental Biology (Botany), 5*(0), 29–35.

Alabouvette, C., Lemanceau, P., & Steinberg, C. (1996). Biological Control of Fusarium Wilts: Opportunities for Developing a Commercial Product. In R. Hall (Ed.), *Principles and Practice of Managing Soilborne Plant Pathogens* (pp. 192–212). St Paul, US: APS Press.

Alabouvette, C., Olivain, C., L–Haridon, F.L., Aimé, S., & Steinberg, C. (2007). Using strains of *Fusarium oxysporum* to control Fusarium wilts: dream or reality? In M. Vurro and J. Gressel Eds. *Novel Biotechnologies for Biocontrol Agent Enhancement and Management.* Springer, US.

Alabouvette, C., Olivain, C., & Steinberg, C. (2006). Biological Control of Plant Diseases: The European Situation. *European Journal of Plant Pathology, 114*(3), 329–341. doi:10.1007/s10658-005-0233-0

Alaeddini R. (2012). Forensic Implications of PCR Inhibition - A Review. *Forensic Science International: Genetics, 6*(3), 297–305. doi:10.1016/j.fsigen.2011.08.006

Alfano, G., Lustrato, G., Lima, G., Vitullo, D., & Ranalli, G. (2011). Characterization of Composted Olive Mill Wastes to Predict Potential Plant Disease Suppressiveness. *Biological Control, 58*(3), 199–207. doi:10.1016/j.biocontrol.2011.05.001

Amooaghaie, R., & Golmohammadi, S. (2017). Effect of Vermicompost on Growth , Essential Oil , and Health of *Thymus vulgaris*. *Compost Science & Utilization, 25*(3), 166-177. doi:10.1080/1065657X.2016.1249314

Amooaghaie, R., & Korrani, F. M. (2018). *Bacillus subtilis* and Vermicompost Suppress Damping-off Disease in Psyllium through Nitric Oxide-Dependent Signaling System. *Russian Journal of Plant Physiology, 65*(3), 435–445. doi:10.1134/S1021443718030093

Anastasi A., Varese G. C., Marchisio V. F. (2005). Isolation and Identification of Fungal Communities in Compost and Vermicompost. *Mycologia, 97*(1), 33–44. doi:10.1080/15572536.2006.11832836

Arancon, N. & Edwards, C., (2004). The Use of Earthworms in the Breakdown of Organic Wastes to Produce Vermicomposts and Animal Feed Protein. In Earthworm Ecology. CRC Press, pp. 345–379.

Arancon, N. Q., Edwards, C. A., Atiyeh, R., & Metzger, J. D. (2004). Effects of Vermicomposts Produced from Food Waste on the Growth and Yields of Greenhouse Peppers, *Bioresource Technology, 93*(2), 139–144. doi:10.1016/j.biortech.2003.10.015

Arancon, N. Q., Edwards, C. A., Yardim, E. N., Oliver, T. J., Byrne, R. J., & Keeney, G. (2007). Suppression of Two-Spotted Spider Mite (*Tetranychus urticae*), Mealy Bug (*Pseudococcus* sp) and Aphid (*Myzus persicae*) Populations and Damage by Vermicomposts. *Crop Protection, 26*(1), 29–39. doi:10.1016/j.cropro.2006.03.013

Arancon, N. Q., Galvis, P. A., & Edwards, C. A. (2005). Suppression of Insect Pest Populations and Damage to Plants by Vermicomposts. *Bioresource Technology, 96*(10), 1137–1142. doi:10.1016/j.biortech.2004.10.004

Asaka, O., & Shoda, M. (1996). Biocontrol of *Rhizoctonia solani* Damping-Off of Tomato with *Bacillus subtilis* RB14. *Applied and Environmental Microbiology, 62*(11), 4081–4085.

Asciutto, K., Rivera, M. C., Wright, E. R., Morisigue, D., & López, M. (2006). Effect of Vermicompost on the Growth and Health of *Impatiens wallerana*. *International Journal of Experimental Botany, 75,* 115–123.

Atiyeh, R. M., Lee, S., Edwards, C. A., Arancon, N. Q., & Metzger, J. D. (2002). The Influence of Humic Acids Derived from Earthworm-Processed Organic Wastes on Plant Growth. *Bioresource Technology, 84*(1), 7–14. doi:10.1016/S0960-8524(02)00017-2

Bae, Y. S., & Knudsen, G. R. (2005). Soil Microbial Biomass Influence on Growth and Biocontrol Efficacy of *Trichoderma harzianum*. *Biological Control, 32*(2), 236–242. doi:10.1016/j.biocontrol.2004.10.001

Baker, R., & Martinson, C. A. (1970). Epidemiology of Diseases Caused by *Rhizoctonia solani*. In J. R. J. Parmeter (Ed.), *Rhizoctonia solani, Biology and Pathway* (pp. 172–188). Berkeley: University of California Press.

Bao, J. R. & Lazarovits, G. (2001). Differential Colonization of Tomato Roots by Nonpathogenic and Pathogenic *Fusarium oxysporum* Strains may Influence Fusarium Wilt Control. *Phytopathology, 91*, 449-456. doi:10.1094/PHYTO.2001.91.5.449

Barthod, J., Rumpel, C., & Dignac, M. (2018). Composting with Additives to Improve Organic Amendments. A Review. *Agronomy for Sustainable Development, 38*(2), doi:10.1007/s13593-018-0491-9

Basco, M. J., Bisen, K., Keswani, C., & Singh, H. B. (2017). Biological Management of Fusarium Wilt of Tomato Using Biofortified Vermicompost. *Mycosphere, 8*(3), 467–483. doi:10.5943/mycosphere/8/3/8

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software, 67,* 1–48. doi:10.18637/jss.v067.i01

Bélanger, R. R., Labbé, C., Lefebvre F. & Teichmann, B. (2012). Mode of Action of Biocontrol Agents: all that Glitters is not Gold. *Canadian Journal of Plant Pathology, 34*(4), 469-478. doi:10.1080/07060661.2012.726649

Benhamou, N., Kloepper, J. W., & Tuzun, S. (1998). Induction of Resistance Against Fusarium Wilt of Tomato by Combination of Chitosan with an Endophytic Bacterial Strain: Ultrastructure and Cytochemistry of the Host Response. *Planta, 204*(3), 153–168. doi:10.1007/s004250050242

Berg, G. (2009). Plant–Microbe Interactions Promoting Plant Growth and Health: Perspectives for Controlled use of Microorganisms in Agriculture. *Applied Microbiology and Biotechnology, 84*(1), 11–18. doi:10.1007/s00253-009-2092-7

Bernard, L., Chapuis-Lardy, L., Razafimbelo, T., Razafindrakoto, M., Pablo, A.-L., Legname, E., Poulain, J., Brüls, T., O'Donohue, M., Brauman, A., Chotte, J.-L., & Blanchart, E. (2011). Endogeic Earthworms Shape Bacterial Functional Communities and Affect Organic Matter Mineralization in a Tropical Soil. *The ISME Journal, 6*(1), 213–222. doi:10.1038/ismej.2011.87

Bernard, E., Larkin, R. P., Tavantzis, S., Erich, M. S., Alyokhin, A., & Gross, S. D. (2014). Rapeseed Rotation, Compost and Biocontrol Amendments Reduce Soilborne Diseases and Increase Tuber Yield in Organic and Conventional Potato Production Systems, *Plant Soil, 374*(1-2), 611–627. doi:10.1007/s11104-013-1909-4

Bertrand, M., Blouin, M., Barot, S., Charlier, A., Marchand, D., & Roger-Estrade, J. (2015). Biocontrol of Eyespot Disease on Two Winter Wheat Cultivars by an Anecic Earthworm (*Lumbricus terrestris*). *Applied Soil Ecology, 96,* 33–41. doi:10.1016/j.apsoil.2015.07.006

Bloem, J., Hopkins, D. W., & Benedetti, A. (2006). *Microbiological Methods for Assessing Soil Quality.* Cambridge, MA: CABI Publishing.

Boehm, M. J., & Hoitink, H. A. J. (1992). Sustenance of Microbial Activity in Potting Mixes and its Impact on Severity of Pythium Root Rot of Poinsettia. *Phytopathology, 82*(3), 259-264. doi:10.1094/Phyto-82-259

Boulter- Jeanine I. Boulter-Bitzer, J. I., Trevors, J. T., & Boland, G. J. (2006). A polyphasic approach for assessing maturity and stability in compost intended for suppression of plant pathogens. *Applied Soil Ecology,* 34, 65–81 doi:10.1016/j.apsoil.2005.12.007

De Boer, M., Bom, P., Kindt, F., Keurentjes, J. J. B., Sluis, I. Van Der, Loon, L. C. Van, & Bakker, P. A. H. M. (2003). Control of Fusarium Wilt of Radish by Combining *Pseudomonas putida* Strains that have Different Disease-Suppressive Mechanisms. *Phytopathology, 93*(5), 626–632. doi:10.1094/PHYTO.2003.93.5.626

De Boer, M., Sluis, I. Van Der, Loon, L. C. Van, & Bakker, P. A. H. M. (1999). Combining Fluorescent *Pseudomonas* spp. Strains to Enhance Suppression of Fusarium Wilt of Radish, *European Journal of Plant Pathology, 105*(2), 201–210.

Bonanomi, G., Antignani, V., Capodilupo, M., & Scala, F. (2010). Identifying the Characteristics of Organic Soil Amendments that Suppress Soilborne Plant Diseases. *Soil Biology and Biochemistry, 42*(2), 136–144. doi:10.1016/j.soilbio.2009.10.012

Bonanomi, G., Antignani, V., Pane, C., & Scala, F. (2007). Supression of Soilborne Fungal Diseases with Organic Amendments. *Journal of Plant Pathology, 89*(3), 311–324. doi:10.2307/41998410

Bonanomi, G., Lorito, M., Vinale, F., & Woo, S. L. (2018). Organic Amendments , Beneficial Microbes , and Soil Microbiota : Toward a Unified Framework for Disease Suppression. *Annual Review of Phytopathology, 56*(1), 1–20. doi:10.1146/annurev-phyto-080615-100046

Bora, L. C., & Deka, S. N. (2007). Wilt Disease Suppression and Yield Enhancement in Tomato (*Lycopersicon esculentum*) by Application of *Pseudomonas fluorescens*-Based Biopesticide (Biofor-Pf) in Assam. *Indian Journal of Agricultural Science, 77*(8), 490–494.

Bosmans, L., Bruijn, I. De, Mot, R. De, Rediers, H., & Lievens, B. (2016). Agar Composition Affects *in vitro* Screening of Biocontrol Activity of Antagonistic Microorganisms. *Journal of Microbiological Methods, 127,* 7–9. doi:10.1016/j.mimet.2016.05.004

Bowen, G. D. & Rovira, A. D. (1999). The Rhizosphere and Its Management to Improve Plant Growth. *Advances in Agronomy, 66,* 1-102. doi:10.1016/S0065-2113(08)60425-3

Brewer, M. T., & Larkin, R. P. (2005). Efficacy of Several Potential Biocontrol Organisms Against *Rhizoctonia solani* on Potato. *Crop Protection, 24*(11), 939–950. doi:10.1016/j.cropro.2005.01.012

Brimner, T. A & Boland, G. J. (2003). A Review of the Non-Target Effects of Fungi Used to Biologically Control Plant Diseases. *Agriculture Ecosystems and Environment, 100*, 3-16. doi:10.1016/S0167-8809(03)00200-7

Brimner, T. A & Boland, G. J. (2004). Nontarget Effects of Biological Control Agents. *New Phytologist, 163,* 455–457.

Buée, M., Reich, M., Murat, C., Morin, E., Nilsson, R. H., Uroz, S., & Martin, F. (2009). 454 Pyrosequencing Analyses of Forest Soils Reveal an Unexpectedly High Fungal Diversity. *New Phytologist, 184*(2), 449–456. doi:10.1111/j.1469-8137.2009.03003.x

Calvo-Bado, L. A., Petch, G., Parsons, N. R., Morgan, J. A. W., Pettitt, T. R., & Whipps, J. M. (2006). Microbial Community Responses Associated with the Development of Oomycete Plant Pathogens on Tomato Roots in Soilless Growing Systems, *Journal of Applied Microbiology, 100*(6), 1194–1207. doi:10.1111/j.1365-2672.2006.02883.x

Campos-vega R., Loarca-Piña G., Vergara-Castañeda H.A., Oomah B.D. (2015). Spent Coffee Grounds: A Review on Current Research and Future Prospects. *Trends in Food Science and Technology, 45*(1), 24-36. doi:10.1016/j.tifs.2015.04.012

Canadian General Standards Board (2018). Organic Production Systems: General Principles and Management Standards. http://www.publications.gc.ca/pub?id=9.854643&sl=0

Cao, Y., Zhang, Z., Ling, N., & Yuan, Y. (2011). *Bacillus subtilis* Sqr 9 can Control Fusarium Wilt in Cucumber by Colonizing Plant Roots. *Biology and Fertility of Soils, 47*(5), 495–506. doi:10.1007/s00374-011-0556-2

Chatterton, S., & Punja, Z. K. (2009). Chitinase and β-1,3-glucanase Enzyme Production by the Mycoparasite *Clonostachys rosea* f . *catenulata* Against Fungal Plant Pathogens, *Canadian Journal of Microbiology, 55*(4), 356–367. doi:10.1139/W08-156

Chatterton, S., & Punja, Z. K. (2010). Factors Influencing Colonization of Cucumber Roots by *Clonostachys rosea* f. *catenulata*, a Biological Disease Control Agent. *Biocontrol Science and Technology, 20*(1), 37–55. doi:10.1080/09583150903350253

Chen, M., & Nelson, E. B. (2012). Microbial-Induced Carbon Competition in the Spermosphere Leads to Pathogen and Disease Suppression in a Municipal Biosolids Compost. *Phytopathology, 102*(6), 588-596. doi:10.1094/PHYTO-08-11-0241

Chitarra, W., Cogliati, E., Pugliese, M., Gilardi, G., Gullino, M. L., & Garibaldi, A. (2014). Effect of Silicates and Electrical Conductivity on Fusarium Wilt of Tomato Grown Soilless*. Acta Horticulturae*, 1044, 53–55. doi:10.17660/actahortic.2014.1044.4

Chung, S., Kong, H., Buyer, J. S., Lakshman, D. K., Lydon, J., Kim, S., & Roberts, D. P. (2008). Isolation and Partial Characterization of *Bacillus subtilis* ME488 for Suppression of Soilborne Pathogens of Cucumber and Pepper. *Applied Microbiology and Biotechnology, 80*(1), 115–123. doi:10.1007/s00253-008-1520-4

Clapperton, M. J., Lee, N. O., Binet, F., & Conner, R. L. (2001). Earthworms Indirectly Reduce the Effects of Take-All (*Gaeumannomyces graminis* var . *tritici*) on Soft White Spring Wheat (*Triticum aestivum* cv. Fielder). *Soil Biology & Biochemistry, 33*(11), 1531–1538. doi:10.1016/s0038-0717(01)00071-2

Clematis, F., Minuto, A., Gullino, M. L., & Garibaldi, A. (2009). Suppressiveness to *Fusarium oxysporum* f. sp. *radicis lycopersici* in Re-Used Perlite and Perlite-Peat Substrates in Soilless Tomatoes. *Biological Control, 48*(2), 108–114. doi:10.1016/j.biocontrol.2008.10.001

Contreras-Blancas, E., Ruíz-Valdiviezo, V. M., Santoyo-Tepole, F., Luna-Guido, M., Meza-Gordillo, R., Dendooven, L., & Gutiérrez-Miceli, F. A. (2014). Evaluation of Worm-Bed Leachate as an Antifungal Agent against Pathogenic Fungus, *Colletotrichum gloeosporioides*. *Compost Science & Utilization, 22*(1), 23–32. doi:10.1080/1065657x.2013.870944

Cordiera, C., Alabouvette, C. (2009). Effects of the introduction of a biocontrol strain of *Trichoderma atroviride* on non target soil micro-organisms. *European Journal of Soil Biology, 45*, 267–274. doi:10.1016/j.ejsobi.2008.12.004

Cotxarrera, L., Trillas-gay, M. I., Steinberg, C., & Alabouvette, C. (2002). Use of Sewage Sludge Compost and *Trichoderma asperellum* Isolates to Suppress Fusarium Wilt of Tomato. *Soil Biology & Biochemistry, 34*(4), 467-476. doi:10.1016/s0038-0717(01)00205-x

Coventry, E., Noble, R., Mead, A., Marin, F. R., Perez, J. A., & Whipps, J. M. (2006). Allium White Rot Suppression with Composts and *Trichoderma viride* in Relation to Sclerotia Viability, *Phytopathology, 96*(9), 1009–1020. doi:10.1094/PHYTO-96-1009

Cummings, J. A., Miles, C. A., & Toit, L. J. (2009). Greenhouse Evaluation of Seed and Drench Treatments for Organic Management of Soilborne Pathogens of Spinach. *Plant Disease, 93*(12), 1281–1292. doi:10.1094/pdis-93-12-1281

Davet, P., & Roure, C. (1986). Activité Parasitaire des *Trichoderma* vis-à-vis des Champignons à Sclérotes ; Corrélation avec l'Aptitude à la Compétition dans un Sol Non Stérile. *Agronomie, 6*(9), 863–867. doi:10.1051/agro:19860911

Davet, P., Camporota, P., & Roure, C. (1986). Etude Comparative de Quelques Méthodes d'Estimation de l'Aptitude à la Compétition Saprophytique Dans le Sol des *Trichoderma*. *Agronomie, 6*(6), 575–581. doi:10.1051/agro:19860610

Davies, J. L., Ngeleka, M., & Wobeser, G. A. (2010). Systemic Infection with *Mortierella wolfii* Following Abortion in a Cow. *Canadian Veterinary Journal, 51*(12), 1391–1393.

de Mendiburu F. (2016). agricolae: Statistical Procedures for Agricultural Research. R package version 1.2-4. https://CRAN.R-project.org/package=agricolae

DeCeuster, T. J. J., & Hoitink, H. A. J. (1999). Prospects for Composts and Biocontrol Agents as Substitutes for Methyl Bromide in Biological Control of Plant Diseases Prospects for Composts and Biocontrol Agents as Substitutes for Methyl Bromide in Biological Control of Plant Diseases. *Compost Science & Utilization, 7*(3), 6–15. doi:10.1080/1065657X.1999.10701970

Dedyukhina, E. G., Kamzolova, S. V., & Vainshtein, M. B. (2014). Arachidonic Acid as an Elicitor of the Plant Defense Response to Phytopathogens. *Chemical and Biological Technologies in Agriculture, 1*(1), 1-6. doi:10.1186/s40538-014-0018-9

Van Diepeningen, A. D., Vos, O. J., De, Korthals, G. W., & Van Bruggen, A. H. C. (2006). Effects of Organic Versus Conventional Management on Chemical and Biological Parameters in Agricultural Soils. *Applied Soil Ecology, 31,* 120–135. doi:10.1016/j.apsoil.2005.03.003

Daniel, R. (2005). The Metagenomics of Soil. *Nature Reviews Microbiology, 3*(6), 470–478. doi:10.1038/nrmicro1160

Delmont, T. O., Eren, A. M., Maccario, L., Prestat, E., Esen, Ö. C., Pelletier, E., Le Paslier, D., Simonet, P., Vogel, T. M. (2015). Reconstructing Rare Soil Microbial Genomes Using in situ Enrichments and Metagenomics. *Frontiers in Microbiology, 6*, 356. doi:10.3389/fmicb.2015.00358

Dineen, S. M., Aranda, R., Anders, D. L., & Robertson, J. M. (2010). An Evaluation of Commercial DNA Extraction Kits for the Isolation of Bacterial Spore DNA from Soil. *Journal of Applied Microbiology, 109*(6), 1886–1896. doi:10.1111/j.1365-2672.2010.04816.x

Dionne, A., Tweddell, R. J., Antoun, H., & Avis, T. J. (2012). Effect of Non-aerated Compost Teas on Damping-off Pathogens of Tomato. *Canadian Journal of Plant Pathology, 34*(1), 51–57. doi:10.1080/07060661.2012.660195

Doan, T. T., Ngo, P. T., Rumpel, C., Nguyen, B. Van, & Jouquet, P. (2013). Interactions Between Compost, Vermicompost and Earthworms Influence Plant Growth and Yield: A One-Year Greenhouse Experiment. *Scientia Horticulturae, 160,* 148–154. doi:10.1016/j.scienta.2013.05.042

Dominguez, J. (2011). The Microbiology of Vermicomposting. In C. A. Edwards, N. Q. Arancon, & R. Sherman (Eds.), *Vermiculture Technology: Earthworms, Organic Wastes, and Environmental Management* (pp. 53–66). Boca Raton, FL: CRC Press Taylor & Francis Group. doi:10.1201/b10453

Dominguez, J. & Edwards, C. A. (2011). Relationships between Composting and Vermicomposting. In C. A. Edwards, N. Q. Arancon, & R. Sherman (Eds.), *Vermiculture Technology: Earthworms, Organic Wastes, and Environmental Management* (pp. 12-25). Boca Raton, FL: CRC Press Taylor & Francis Group. doi:10.1201/b10453

Dorais, M., & Alsanius, B. (2015) Advances and Trends in Organic Fruit and Vegetable Farming Research. *Horticultural Reviews, 43,* 185-268. doi:10.1002/9781119107781.ch04

Doube, B. M., Stephens, P. M., Davoren, W., & Ryder, M. H. (1994). Interactions Between Earthworms, Beneficial Soil Microorganisms and Root Pathogens. *Applied Soil Ecology, 1*(1), 3–10. doi:10.1016/0929-1393(94)90018-3

Doyle, O. P. E. (2017). Suppressive Composts in Organic Horticulture: Fact or Fiction ? *European Journal of Horticultural Science, 82*(6), 263–276. doi:10.17660/ejhs.2017/82.6.1

Drake, H. L., & Horn M. A. (2007). As the Worm Turns: The Earthworm Gut as a Transient Habitat for Soil Microbial Biomes. *Annual Review of Microbiology, 61,* 169–189. doi:10.1146/annurev.micro.61.080706.093139

Dunne, C., Moënne-Loccoz, Y., McCarthy, J., Higgins, P., Powell, J., Dowling, D. N., & O'Gara, F. (1998). Combining Proteolytic and Phloroglucinol-Producing Bacteria for Improved Biocontrol of *Pythium*-Mediated Damping-Off of Sugar Beet. *Plant Pathology, 47*(3), 299–307. doi:10.1046/j.1365-3059.1998.00233.x

Eastman, B. R., Kane, P. N., Edwards, C. A., Trytek, L., Gunadi, B., Stermer, A. L., & Mobley, J. R. (2001). The Effectiveness of Vermiculture in Human Pathogen Reduction for USEPA Biosolids Stabilization. *Compost Science & Utilization, 9*(1), 38–49. doi:10.1080/1065657X.2001.10702015

Ebrahimi, E. (2018). Suppressive Effect of Composts from Residual Biomass on *Pythium ultimum*. *Journal of Plant Diseases and Protection, 125*(5), 443–449. doi:10.1007/s41348-018-0163-7

Edgar, R. C. (2004). MUSCLE: Multiple Sequence Alignment with High Accuracy and High Throughput. *Nucleic Acids Research, 32*(5), 1792–1797. doi:10.1093/nar/gkh340

Edwards, C. A. (1995). Historical Overview of Vermicomposting. *BioCycle, 36(6),* 56.

Edwards, C. A., & Bohlen, P. J. (1996). *Biology and Ecology of Earthworms*. London, Chapman & Hall.

Edwards, C. A., & Arancon, N. Q. (2004). The Use of Earthworms in the Breakdown of Organic Wastes to Produce Vermicomposts and Animal Feed Protein. In Edwards, C. A. (Ed). *Earthworm Ecology, Second Edition*. Boca Raton, FL: CRC Press. doi:10.1201/9781420039719

Edwards, C. A., Arancon, N. Q., & Greytak, S. (2006). Effects of Vermicompost Teas on Plant Growth and Disease. *Biocycle, 47,* 28.

Edwards, C. A., Arancon, N. Q., & Sherman, R. L. (Eds.). (2011). Vermiculture Technology.CRC Press, Boca Raton, FL. doi:10.1201/b10453

El-Tarabily, K. A. (2006). Rhizosphere-Competent Isolates of Streptomycete and Non-Streptomycete Actinomycetes Capable of Producing Cell-Wall-Degrading Enzymes to Control *Pythium aphanidermatum* Damping-Off Disease of Cucumber. *Canadian Journal of Botany, 84*(2), 211–222. doi:10.1139/b05-153

Elad, Y., Sadowsky, Z. & Chet, I. (1987). Scanning electron microscopical observations of early stages of interaction of *Trichoderma harzianum* and *Rhizoctonia solani. Transactions of the British Mycological Society, 88*, 259–263. DOI:10.1016/S0007-1536(87)80223-1

Elmer, W. H. (2009). Influence of Earthworm Activity on Soil Microbes and Soilborne Diseases of Vegetables. *Plant Disease, 93*(2), 175–179. doi:10.1094/pdis-93-2-0175

Eparvier, A., & Alabouvette, C. (1994). Use of ELISA and GUS-Transformed Strains to Study Competition Between Pathogenic and Non-Pathogenic *Fusarium oxysporum* for Root Colonization. *Biocontrol Science and Technology, 4*(1), 35–47. doi:10.1080/09583159409355310

FAO. (2018). The Future of Food and Agriculture - Alternative Pathways to 2050. Rome. 224 pp. Licence: CC BY-NC-SA 3.0 IGO.

Fedi, S., Tola, E., Moënne-Loccoz, Y., Dowling, D. N., Smith, L. M., & O'Gara, F. (1997). Evidence for Signaling Between the Phytopathogenic Fungus *Pythium ultimum* and *Pseudomonas fluorescens* F113: *P. ultimum* Represses the Expression of Genes in *P.fluorescens* F113, Resulting in Altered Ecological Fitness. *Applied and Environmental Microbiology, 63*(11), 4261. doi:10.1128/aem.63.11.4261-4266.1997

Fernández-Gómez, M. J., Nogales, R., Insam, H., Romero, E., & Goberna, M. (2012). Use of DGGE and COMPOCHIP for Investigating Bacterial Communities of Various Vermicomposts Produced from Different Wastes Under Dissimilar Conditions. *Science of the Total Environment, 414,* 664–671. doi:10.1016/j.scitotenv.2011.11.045

Fess, T. L., & Benedito, V. (2018). Organic Versus Conventional Cropping Sustainability : A Comparative System Analysis. *Sustainability, 10*(1), 272. doi:10.3390/su10010272

Fourment, M., & Holmes, E. C. (2016). Seqotron: A User-Friendly Sequence Editor for Mac OS X. *BMC Research Notes, 9*(1), 106. doi: 10.1186/s13104-016-1927-4

Fracchia, L., Dohrmann, A. B., Martinotti, M. G., & Tebbe, C. C. (2006). Bacterial Diversity in a Finished Compost and Vermicompost: Differences Revealed by Cultivation-Independent Analyses of PCR-Amplified 16S rRNA Genes. *Applied Microbiology and Biotechnology, 71*(6), 942–952. doi:10.1007/s00253-005-0228-y

Fravel, D. R. (2005). Commercialization and Implementation of Biocontrol. *Annual Review of Phytopathology, 43*(1), 337–359. doi:10.1146/annurev.phyto.43.032904.092924

Fritz J. I., Franke-Whittle I. H., Haindl S., Insam H., & Braun R. (2012). Microbiological Community Analysis of Vermicompost Tea and its Influence on the Growth of Vegetables and Cereals. *Canadian Journal of Microbiology, 58*(7). 836-847. doi:10.1139/w2012-061

Fröhlich-Nowoisky, J., Hill, T. C. J., Pummer, B. G., Yordanova, P., Franc, G. D., & Pöschl, U. (2015). Ice Nucleation Activity in the Widespread Soil Fungus *Mortierella alpina*. *Biogeosciences, 12*(4), 1057–1071. doi:10.5194/bg-12-1057-2015

Gamliel, A., & van Bruggen, A. H. C. (2016). Maintaining Soil Health for Crop Production in Organic Greenhouses. *Scientia Horticulturae, 208,* 120–130. doi:10.1016/j.scienta.2015.12.030

Ghini, R., Fortes, N. L. P., Navas-Cortés, J. A., Silva, C. A., & Bettiol, W. (2016). Combined Effects of Soil Biotic and Abiotic Factors, Influenced by Sewage Sludge Incorporation, on the Incidence of Corn Stalk Rot. *PLOS ONE, 11*(5), e0155536. doi:10.1371/journal.pone.0155536

Gilbert, J. A., Meyer, F., Antonopoulos, D., Balaji, P., Brown, C. T., Brown, C. T., Desai, N., Eisen, J. A., Evers, D., Field, D., Feng, W., Huson, D., Jansson, J., Knight, R., Knight, J., Kolker, E., Konstantindis, K., Kostka, J., Kyrpides, N., Mackelprang, R., McHardy, A., Quince, C., Raes, J., Sczyrba, A., Shade, A., & Stevens, R. (2010). Meeting Report: The Terabase Metagenomics Workshop and the Vision of an Earth Microbiome Project. *Standards in Genomic Sciences, 3*(3), 243–248. doi:10.4056/sigs.1433550

Gómez-Brandón, M., Aira, M., Lores, M., & Domínguez, J. (2011). Epigeic Earthworms Exert a Bottleneck Effect on Microbial Communities Through Gut Associated Processes. *PLoS ONE, 6*(9), e24786. doi:10.1371/journal.pone.0024786

Gómez-Brandón, M., Lores, M., & Domínguez, J. (2013). Changes in Chemical and Microbiological Properties of Rabbit Manure in a Continuous-Feeding Vermicomposting System. *Bioresource Technology, 128,* 310–316. doi:10.1016/j.biortech.2012.10.112

Gopal, M., Gupta, A., Sunil, E., & Thomas, G. V. (2009). Amplification of Plant Beneficial Microbial Communities During Conversion of Coconut Leaf Substrate to Vermicompost by *Eudrilus* sp . *Current Microbiology, 59*(1), 15–20. doi:10.1007/s00284-009-9388-9

Gopalakrishnan, S., Girish, I., Kannan, K., & Alekhya, G. (2010). Efficacy of Jatropha , Annona and Parthenium Biowash on *Sclerotium rolfsii* , *Fusarium oxysporum* f . sp . *ciceri* and *Macrophomina phaseolina* , Pathogens of Chickpea and Sorghum. *African Journal of Biotechnology, 9*(47), 8048–8057. doi:10.5897/AJB10.1276

Gopalakrishnan, S., Pande, S., Sharma, M., Humayun, P., Kiran, B. K., Sandeep, D., Vidya, M. S., Deepthi, K., & Rupela, O. (2011). Evaluation of Actinomycete Isolates Obtained from Herbal Vermicompost for the Biological Control of Fusarium Wilt of Chickpea. *Crop Protection, 30*(8), 1070–1078. doi:10.1016/j.cropro.2011.03.006

Grantina-Ievina, L., Andersone, U., Berkolde- Pīre, D., Nikolajeva, V., & Ievinsh, G. (2013). Critical Tests for Determination of Microbiological Quality and Biological Activity in Commercial Vermicompost Samples of Different Origins. *Applied Microbiology and Biotechnology, 97*(24), 10541–10554. doi: 10.1007/s00253-013-4825-x

Grantina-Ievina, L., Nikolajeva, V., Rostoks, N., Skrabule, I., Zarina, L., Pogulis, A., & Ievinsh, G. (2015). Impact of Green Manure and Vermicompost on Soil Suppressiveness, Soil Microbial Populations, and Plant Growth in Conditions of Organic Agriculture of Northern Temperate Climate. In M. K. Meghvansi & A. Varma Eds., *Organic Amendments and Soil Suppressiveness in Plant Disease Management.* Springer International Publishing: 381–399. doi:10.1007/978-3-319-23075-7_18

Grebus, M. E., Feldman, K. A., Musselman, C. A., & Hoitink, H. A. J. (1993). Production of Biocontrol Agent-Fortified Compost-Amended Potting Mixes for Predictable Disease Suppression. *Phytopathology, 83,* 1406.

Green, S. J., Leigh, M. B., & Neufeld, J. D. (2009). Denaturing Gradient Gel Electrophoresis (DGGE) for Microbial Community Analysis. In K.N. Timmis Ed., *Microbiology of Hydrocarbons, Oils, Lipids, and Derived Compounds.* Heidelberg, Germany, Springer: 4137–4158. doi:10.1007/978-3-540-77587-4

Green, V. S., Stott, D. E., & Diack, M. (2006). Assay for Fluorescein Diacetate Hydrolytic Activity: Optimization for Soil Samples. *Soil Biology and Biochemistry, 38*(4), 693–701. doi:10.1016/j.soilbio.2005.06.020

Griffiths, B.S. & Philippot, L. (2013). Insights into the Resistance and Resilience of the Soil Microbial Community. *FEMS Microbiology Reviews, 37*(2), 112–129. doi:10.1111/j.1574-6976.2012.00343.x

Grinbergs, J., & Yarrow, D. (1970). *Rhodotorula araucariae* sp.n. *Antonie van Leeuwenhoek, 36*(1), 455–457. doi:10.1007/bf02069046

Grosch, R., Junge, H., Krebs, B., and Bochow, H. (1999). Use of *Bacillus subtilis* as a Biocontrol Agent III. Influence of *Bacillus subtilis* on Fungal Root Diseases and on Yield in Soilless Culture. *Journal of Plant Diseases and Protection, 106(6),* 568-580.

Grosch, R., Scherwinski, K., Lottmann, J., & Berg, G. (2006). Fungal Antagonists of the Plant Pathogen *Rhizoctonia solani*: Selection, Control Efficacy and Influence on the Indigenous Microbial Community. *Mycological Research, 110(12),* 1464–1474. doi:10.1016/j.mycres.2006.09.014

Hadar, Y. (2011). Suppressive compost: When Plant Pathology Met Microbial Ecology. *Phytoparasitica, 39*(4), 311–314. doi:10.1007/s12600-011-0177-1

Hadar, Y., & Papadopoulou, K. K. (2012). Suppressive Composts : Microbial Ecology Links Between Abiotic Environments and Healthy Plants. *Annual Review of Phytopathology, 50*(1). doi:10.1146/annurev-phyto-081211-172914

Handelsman, J. (2002). Future Trends in Biocontrol. In S. S. Gnanamanickam Ed., *Biological Control of Crop Diseases*: 443–448. Boca Raton, FL: CRC Press.

Harman, G. E. (2000). Myths and Dogmas of Biocontrol: Changes in Perceptions Derived from Research on *Trichoderma harzianum* T-22. *Plant Disease, 84*(4), 377–393. doi:10.1094/pdis.2000.84.4.377

Harman, G. E., Howell, C. R., Viterbo, A., Chet, I. & Lorito, M. (2004). *Trichoderma* Species – Opportunistic, Avirulent Plant Symbionts. *Nature Reviews Microbiology, 2,* 43-56. doi:10.1038/nrmicro797

Hénault-Ethier, L. (2007). Vermicomposting: From Microbial and Earthworm Induced Effects in Bacterial Sanitation to the Chemistry of Biodegradation Under Batch or Continuous Operation. MSc Thesis, Concordia University. https://spectrum.library.concordia.ca/976165/

Hénault-Ethier, L., Martin, V. J. J., & Gélinas, Y. (2016). Persistence of Escherichia Coli in Batch and Continuous Vermicomposting Systems. *Waste Management, 56,* 88–99. doi:10.1016/j.wasman.2016.07.033

Héry, M., Singer, A. C., Kumaresan, D., Bodrossy, L., Stralis-Pavese, N., Prosser, J. I., Thompson, I. P., & Murrell, J. C. (2007). Effect of Earthworms on the Community Structure of Active Methanotrophic Bacteria in a Landfill Cover Soil. *The ISME Journal, 2*(1), 92–104. doi:10.1038/ismej.2007.66

Hiddink, G. A., van Bruggen, A. H. C., Termorshuizen, J. M., Raaijmakers, & A. V., Semenov. (2005). Effect of Organic Management of Soils on Suppressiveness to *Gaeumannomyces graminis* var. *tritici* and its Antagonist, *Pseudomonas fluorescens*. *European Journal of Plant Pathology, 113*(4), 417–435. doi:10.1007/s10658-005-5402-7

Hill, G. B., & Baldwin, S. A. (2012). Vermicomposting Toilets , an Alternative to Latrine Style Microbial Composting Toilets , Prove far Superior in Mass Reduction , Pathogen Destruction , Compost Quality , and Operational Cost. *Waste Management, 32*(10), 1811–1820. doi:10.1016/j.wasman.2012.04.023

Hobson, A. M., Frederickson, J., & Dise, N. B. (2005). CH4 And N2O from Mechanically Turned Windrow And Vermicomposting Systems Following In-Vessel Pre-Treatment. *Waste Management, 25*(4) 345–352. doi:10.1016/j.wasman.2005.02.015

Hoitink, H. A. J., Grebus, M. E., Hayes, M. H. B., & Wilson, W. S. (1997). Composts and the Control of Plant Diseases. In M. H. B. Hayes & W. S. Wilson, Eds. *Humic Substances, Peats and Waters : Health and Environmental Aspects*. Cambridge, UK, Woodhead Publishing: 359–366. doi:10.1016/B978-1-85573-805-8.50040-9

Hoitink, H. A. J., & Boehm, M. J. (1999). Biocontrol Within the Context of Soil Microbial Communities : A Substrate-Dependent Phenomenon. *Annual Review of Phytopathology, 37*(1), 427-446.

Hoitink, H. A. J., Stone, A. G., & Han, D. Y. (1997). Suppression of Plant Diseases by Composts. *HortScience, 32*(2), 184-187. doi:10.21273/hortsci.32.2.184

Howell, C. R. (1987). Relevance of Mycoparasitism in the Biological Control of *Rhizoctonia solani* by *Gliocladium virens. Phytopathology, 77*(6), 992–994. doi:10.1094/phyto-77-992

Howell, C. R. (2006). Understanding the Mechanisms Employed by *Trichoderma virens* to Effect Biological Control of Cotton Diseases. *Phytopathology, 96,* 178–180. doi:10.1094/phyto-96-0178

Huang, K., Li, F., Wei, Y., Chen, X., & Fu, X. (2013). Changes of Bacterial and Fungal Community Compositions During Vermicomposting of Vegetable Wastes by *Eisenia foetida*. *Bioresource Technology, 150*: 235–241. doi:10.1016/j.biortech.2013.10.006

Huang, K., Li, F., Wei, Y., Fu, X., & Chen, X. (2014). Effects of Earthworms on Physicochemical Properties and Microbial Profiles During Vermicomposting of Fresh Fruit and Vegetable Wastes. *Bioresource Technology, 170*: 45–52. doi: 10.1016/j.biortech.2014.07.058

Huang, N., Wang, W., Yao, Y., Zhu, F., Wang, W., & Chang, X. (2017). The Influence of Different Concentrations of Bio-Organic Fertilizer on Cucumber Fusarium Wilt and Soil Microflora Alterations, *PLOS ONE 12*(2) e0171490. doi:10.1371/journal.pone.0171490

Huang, Y., Wu, Z., He, Y., Ye, B., & Li, C. (2017). Rhizospheric *Bacillus subtilis* Exhibits Biocontrol Effect against *Rhizoctonia solani* in Pepper (*Capsicum annuum*). *BioMed Research International,* 1-9. doi:10.1155/2017/9397619

Hultberg, M., Alsanius, B., & Sundin, P. (2000). *In Vivo* and *in Vitro* Interactions Between *Pseudomonas fluorescens* and *Pythium ultimum* in the Suppression of Damping-off in Tomato Seedlings. *Biological Control, 19*(1), 1–8. doi:10.1006/bcon.2000.0840

Hussain, N. (2018). Efficacy of the Vermicomposts of Different Organic Wastes as " Clean " Fertilizers : State-of-the-Art. *Sustainability, 10*(4), 1205. doi:10.3390/su10041205

Ingham, E. R. (2005). *The Compost Tea Brewing Manual* (5th ed.) Corvallis: Soil Foodweb Incorporated.

Ishii, K., & Takii, S. (2003). Comparison of Microbial Communities in Four Different Composting Processes as Evaluated by Denaturing Gradient Gel Electrophoresis Analysis. *Journal of Applied Microbiology, 95*(1), 109–119. doi:10.1046/j.1365-2672.2003.01949.x

Ivors, K. L., Collopy, P. D., Beyer, D. M., & Kang, S. (2017). Identification of Bacteria in Mushroom Compost Using Ribosomal RNA Sequence. *Compost Science & Utilization,* 8(3), 247-253. doi:10.1080/1065657X.2000.10701997

Jack, A. L. H. (2011). The Suppression of Plant Pathogens by Vermicomposts. In C. A. Edwards, N. Q. Arancon, & R. Sherman (Eds.), *Vermiculture Technology Earthworms, Organic Wastes, and Environmental Management.* New York: CRC Press: 165–182. doi:10.1201/b10453-13

Jack, A. L. H., & Nelson, E. B. (2018). A Seed-Recruited Microbiome Protects Developing Seedlings from Disease by Altering Homing Responses of *Pythium aphanidermatum* Zoospores, *Plant and Soil, 422*(1-2), 209–222. doi:10.1007/s11104-017-3257-2

Janse, I., Bok, J., & Zwart, G. (2004). A Simple Remedy Against Artifactual Double Bands in Denaturing Gradient Gel Electrophoresis. *Journal of Microbiological Methods, 57*(2), 279–281. doi:10.1016/j.mimet.2003.12.006

Janzen, R. A., Cook, F. D., & McGill, W. B. (1995). Compost Extract Added to Microcosms may Simulate Community-Level Controls on Soil Microorganisms Involved in Element Cycling. *Soil Biology and Biochemistry, 27*(2), 181–188. doi:10.1016/0038-0717(94)00165-w

Kannangara, T., Utkhede, R. S., Paul, J. W., & Punja, Z. K. (2000). Effects of Mesophilic and Thermophilic Composts on Suppression of Fusarium Root and Stem Rot of Greenhouse Cucumber. *Canadian Journal of Microbiology, 46*(11), 1021–8. doi:10.1139/cjm-46-11-1021

Kappe, R., Okeke, C. N., Fauser, C., Maiwald, M., & Sonntag, H. G. (1998). Molecular Probes for the Detection of Pathogenic Fungi in the Presence of Human Tissue. *Journal of Medical Microbiology, 47*(9), 811–820. doi:10.1099/00222615-47-9-811

Khabbaz, S. E., Zhang, L., Cáceres, L. A., Sumarah, M., Wang, A., & Abbasi, P. A. (2015). Characterisation of Antagonistic *Bacillus* and *Pseudomonas* Strains for Biocontrol Potential and Suppression of Damping-Off and Root Rot Diseases, *Annals of Applied Biology, 166*(3), 456–471. doi:10.1111/aab.12196

Khan, A., Sutton, J. C., & Grodzinski, B. (2003). Effects of *Pseudomonas chlororaphis* on *Pythium aphanidermatum* and Root Rot in Peppers Grown in Small-Scale Hydroponic Troughs. *Biocontrol Science and Technology, 13*(6), 615–630. doi:10.1080/0958315031000151783

Khan, M. R., Fischer, S., Egan, D., & Doohan, F. M. (2005). Biological Control of Fusarium Seedling Blight Disease of Wheat and Barley. *Phytopathology, 96*(4), 386–394. doi:10.1094/phyto-96-0386

Kim, Y. C., Jung, H., Kim, K. Y., & Park, S. K. (2008). An Effective Biocontrol Bioformulation Against Phytophthora Blight of Pepper Using Growth Mixtures of Combined Chitinolytic Bacteria Under Different Field Conditions. *European Journal of Plant Pathology, 120*(4), 373–382. doi:10.1007/s10658-007-9227-4

Kirk, J. L., Beaudette, L. A., Hart, M., Moutoglis, P., Klironomos, J. N., Lee, H., & Trevors, J. T. (2004). Methods of Studying Soil Microbial Diversity. *Journal of Microbiological Methods, 58*(2), 169–188. doi:10.1016/j.mimet.2004.04.006

Kiss, L. (1998). Natural Occurrence of *Ampelomyces* Intracellular Mycoparasites in Mycelia of Powdery Mildew Fungi. *New Phytologist, 140,* 709-714. doi:10.1046/j.1469-8137.1998.00316.x

Kiss, L. (2004). How Dangerous is the Use of Fungal Biocontrol Agents to Nontarget Organisms? *New Phytologist 163*, 453-455. doi:10.1111/j.1469-8137.2004.01129.x

Knapp, B. A., Podmirseg, S. M., Seeber, J., Meyer, E., & Insam, H. (2009). Diet-Related Composition of the Gut Microbiota of *Lumbricus rubellus* as Revealed by a Molecular Fingerprinting Technique and Cloning. *Soil Biology and Biochemistry, 41*(11), 2299–2307. doi:10.1016/j.soilbio.2009.08.011

Knowles, J. (2013). Getting Started with Mixed Effect Models in R. Retrieved August 20, 2019, from https://www.r-bloggers.com/getting-started-with-mixed-effect-models-in-r/

Knudsen, I. M. B., Hockenhull, J., Jensen, D. F., Gerhardson, B., Hökeberg, M., Tahvonen, R., Teperi, E., Sundheim, L. & Henriksen, B. (1997). Selection of Biological Control Agents for Controlling Soil and Seed-Borne Diseases in the Field, *European Journal of Plant Pathology, 103*(9)*,* 775–784.

Koch, E., Becker, J.O., Berg, G., Hauschild, R., Jehle, J., Köhl, J., & Smalla, K. (2018). Biocontrol of Plant Diseases is not an Unsafe Technology! *Journal of Plant Diseases and Protection. 25*, 121–125. doi:10.1007/s41348-018-0158-4

Köhl, J., Postma, J., Nicot, P., Ruocco, M., & Blum, B. (2011). Stepwise Screening of Microorganisms for Commercial Use in Biological Control of Plant-Pathogenic Fungi and Bacteria. *Biological Control, 57*(1), 1–12. doi:10.1016/j.biocontrol.2010.12.004

Koubová, A., Chroňáková, A., Pižl, V., Sánchez-Monedero, M. A., & Elhottová, D. (2015). The Effects of Earthworms Eisenia spp. on Microbial Community are Habitat Dependent. *European Journal of Soil Biology, 68,* 42–55. doi:10.1016/j.ejsobi.2015.03.004

Krause, M. S., Madden, L. V., & Hoitink, H. A. J. (2001). Effect of Potting Mix Microbial Carrying Capacity on Biological Control of Rhizoctonia Damping-Off of Radish And Rhizoctonia Crown and Root Rot of Poinsettia. *Phytopathology, 91*(11), 1116–1123. doi:10.1094/PHYTO.2001.91.11.1116

Larkin, R. P. (2015). Soil Health Paradigms and Implications for Disease Management. *Annual Review Phytopathology, 53,* 199–221. doi:10.1146/annurev-phyto-080614-120357

Larkin, R. P., & Fravel, D. R. (1999). Mechanisms of Action and Dose-Response Relationships Governing Biological Control of Fusarium Wilt of Tomato by Nonpathogenic *Fusarium* spp. *Phytopathology, 89*(12), 1152–1161. doi:10.1094/phyto.1999.89.12.1152

Larkin, R. P., & Tavantzis, S. (2013). Use of Biocontrol Organisms and Compost Amendments for Improved Control of Soilborne Diseases and Increased Potato Production, *American Journal of Potato Research, 90*(3), 261–270. doi:10.1007/s12230-013-9301-8

Lazcano, C., & Dominguez, J. (2010). Effects of Vermicompost as a Potting Amendment of Two Commercially-Grown Ornamental Plant Species. *Spanish Journal of Agricultural Research, 8*(4), 1260–1270. doi:10.5424/sjar/2010084-1412

Lazcano, C., & Domínguez, J. (2011). The Use of Vermicompost in Sustainable Agriculture : Impact on Plant Growth and Soil Fertility. *Soil Nutrients, 10*(1-23), 187.

Lecomte, C., Alabouvette, C., Edel-Hermann, V., Robert, F., & Steinberg, C. (2016). Biological Control of Ornamental Plant Diseases Caused by *Fusarium oxysporum*: A review. *Biological Control, 101,* 17–30. doi:10.1016/j.biocontrol.2016.06.004

Leibinger, W., Breuker, B., Hahn, M., & Mendgen, K. (1997). Control of Postharvest Pathogens and Colonization of the Apple Surface by Antagonistic Microorganisms in the Field. *Phytopathology, 87*(11), 1103–1110. doi:10.1094/phyto.1997.87.11.1103

Lemanceau, P., Bakker, P. A. H. M., De Kogel, W. J., Alabouvette, C., & Schippers, B. (1993). Antagonistic Effect of Nonpathogenic *Fusarium oxysporum* Fo47 and Pseudobactin 358 upon Pathogenic *Fusarium oxysporum* f. sp. *dianthi*. *Applied and Environmental Microbiology, 59*(1), 74–82. doi:10.1128/aem.59.1.74-82.1993

Li, L., Ma, J., Li, Y., Wang, Z., Gao, T., & Wang, Q. (2012). Screening and Partial Characterization of *Bacillus* with Potential Applications in Biocontrol of Cucumber Fusarium Wilt. *Crop Protection, 35,* 29–35. doi:10.1016/j.cropro.2011.12.004

Li, D. W. and LaMondia, J. (2010). Airborne Fungi Associated with Ornamental Plant Propagation in Greenhouses. *Aerobiologia, 26,* 15–28. doi:10.1007/s10453-009-9139-1

Lim, S. L., Lee, L. H., & Wu, T. Y. (2016). Sustainability of Using Composting and Vermicomposting Technologies for Organic Solid Waste Biotransformation : Recent Overview , Greenhouse Gases Emissions and Economic Analysis. *Journal of Cleaner Production, 111*, 262-278. doi:10.1016/j.jclepro.2015.08.083

Lim, S. L., Wu, T. Y., Lim, P. N., & Shak, K. P. Y. (2015). The Use of Vermicompost in Organic Farming: Overview, Effects on Soil and Economics. *Journal of the Science of Food and Agriculture, 95*(6), 1143–1156. doi:10.1002/jsfa.6849

Liu, K., McInroy, J. A., Hu, C.-H., & Kloepper, J. W. (2017). Mixtures of Plant Growth-Promoting Rhizobacteria Enhance Biological Control of Multiple Plant Diseases and Plant Growth Promotion in the Presence of Pathogens. *Plant Disease, 102*(1), 67—72. 10.1094/pdis-04-17-0478-re

Lockwood, J. L. (1988). Evolution of Concepts Associated with Soilborne Plant Pathogens. *Annual Review of Phytopathology, 26*(1), 93—121. 10.1146/annurev.py.26.090188.000521

López-González, J. A., Vargas-García, M. C., López, M. J., Suárez-Estrella F., Jurado, M. M., & Moreno, J. (2015). Biodiversity and Succession of Mycobiota Associated to Agricultural Lignocellulosic Waste-Based Composting. *Bioresource Technology, 187.* 305–313. 10.1016/j.biortech.2015.03.124

Lores, M., Gómez-Brandón, M., Pérez-Díaz, D., & Domínguez, J. (2006). Using Fame Profiles for the Characterization of Animal Wastes and Vermicomposts. *Soil Biology and Biochemistry, 38*(9), 2993–2996. doi:10.1016/j.soilbio.2006.05.001

Lorito, M., Farkas, V., Rebuffat, S., Bodo, B. & Kubicek, C. P. (1996). Cell Wall Synthesis is a Major Target of Mycoparasitic Antagonism by *Trichoderma harzianum*. *Journal of Bacteriology, 178*, 6382–6385. doi:10.1128/jb.178.21.6382-6385.1996

Lumsden, R. D., & Locke, J. C. (1989). Biological Control of Damping-Off Caused by *Pythium ultimum* and *Rhizoctonia solani* with *Gliocladium virens* in Soilless Mix. *Phytopathology, 79*(3), 361–366. doi:10.1094/Phyto-79-361

Luna, E., Bruce, T. J. A., Roberts, M. R., Flors, V., & Ton, J. (2012). Next-Generation Systemic Acquired Resistance. *Plant Physiology, 158*(2), 844–853. doi:10.1104/pp.111.187468

Lv, B., Xing, M., Yang, J., & Zhang, L. (2015). Pyrosequencing Reveals Bacterial Community Differences in Composting and Vermicomposting on the Stabilization of Mixed Sewage Sludge and Cattle Dung. *Applied Microbiology and Biotechnology, 99*(24). 10703–10712. doi:10.1007/s00253-015-6884-7

Mäder, P., Fliessbach, A., Dubois, D., & Gunst, L. (2002). Soil Fertility and Biodiversity in Organic Farming. *Science, 296*(5573), 1694–1697. doi:10.1126/science.1071148

Manandhar, T., & Yami, K. D. (2008). Biological Control of Foot Rot Disease of Rice Using Fermented Products of Compost and Vermicompost. *Scientific World, 6*(6), 52-57. doi:10.3126/sw.v6i6.2634

Marín, F., Diánez, F., Gea, F. J., Navarro, M. J., & Santos, M. (2015). Effect of Compost Tea on Plant Growth and Plant Disease Management. In S. Ganesan, K. Vadivel, & J. Jayaraman Eds., Sustainable Crop Disease Management using Natural Products. CAB International: 234–264. doi:10.1079/9781780643236.0234

Marín, F., Santos, M., Diánez, F., Carretero, F., Gea, F. J., Yau, J. A., & Navarro, M. J. (2013). Characters of Compost Teas from Different Sources and their Suppressive Effect on Fungal Phytopathogens. *World Journal of Microbiology and Biotechnology, 29*(8), 1371–1382. doi:10.1007/s11274-013-1300-x

Mathre, D. E., Cook, R. J., & Callan, N. W. (1999). From Discovery to Use: Traversing the World of Commercializing Biocontrol Agents for Plant Disease Control. *Plant Disease, 83,* 972-983. doi:10.1094/pdis.1999.83.11.972

Mazzola, M., & Freilich, S. (2016). Prospects for Biological Soil-Borne Disease Control: Application of Indigenous Versus Synthetic Microbiomes. *Phytopathology, 107*(3, 256-263. doi:10.1094/PHYTO-09-16-0330-RVW

McCullagh, M., Utkhede, R., Menzies, J. G., Punja, Z. K., & Paulitz, T. C. (1996). Evaluation of Plant Growth-Promoting Rhizobacteria for Biological Control of Pythium Root Rot of Cucumbers Grown in Rockwool and Effects on Yield. *European Journal of Plant Pathology, 102*(8), 747–755. doi:10.1007/BF01877149

McKellar, M. E., & Nelson, E. B. (2003). Compost-Induced Suppression of Pythium Damping-Off Is Mediated by Fatty-Acid-Metabolizing Seed-Colonizing Microbial Communities, *Applied and Environmental Microbiology, 69*(1), 452–460. doi:10.1128/AEM.69.1.452

McNeil, J. N., Leroux, T., & Laprade, R. (2010). A Canadian National Survey on the Public Perception of Biological Control, *Biocontrol, 55*(4), 445–454. doi:10.1007/s10526-010-9273-2

McQuilken, M., Gemmell, J., & Lahdenpera, M.-L. (2001). *Gliocladium catenulatum* as a Potential Biological Control Agent of Damping-off in Bedding Plants. Journal of *Phytopathology, 149,* 171–178. doi:10.1046/j.1439-0434.2001.00602.x

Mehta, C. M., Palni, U., Franke-whittle, I. H., & Sharma, A. K. (2014). Compost : Its Role, Mechanism and Impact on Reducing Soil-Borne Plant Diseases. *Waste Management, 34*(3), 607–622. doi:10.1016/j.wasman.2013.11.012

Minuto, A., Clematis, F., Gullino, M. L., & Garibaldi, A. (2007). Induced Suppressiveness to *Fusarium oxysporum* f. sp. *radicis lycopersici* in Rockwool Substrate Used in Closed Soilless Systems. *Phytoparasitica 35*(1), 77–85. doi:10.1016/j.biocontrol.2008.10.001

Montagne, V., Capiaux, H., Barret, M., Cannavo, P., Charpentier, S., Claire, G., & Lebeau, T. (2017). Bacterial and Fungal Communities Vary with the Type of Organic Substrate: Implications for Biocontrol of Soilless Crops. *Environmental Chemistry Letters, 15*(3), 537–545. doi:10.1007/s10311-017-0628-0

Moody, S. A., Piearce, T. G., & Dighton, J. (1996). Fate of Some Fungal Spores Associated with Wheat Straw Decomposition on Passage Through the Guts of *Lumbricus terrestris* and *Aporrectodea longa*. *Soil Biology & Biochemistry, 28*(4/5), 533–537. 10.1016/0038-0717(95)00172-7

Muyzer, G., De Waal, E. C., & Uitterlinden, A. G. (1993). Profiling of Complex Microbial Populations by Denaturing Gradient Gel Electrophoresis Analysis of Polymerase Chain Reaction-Amplified Genes Coding for 16S rRNA. *Applied and Environmental Microbiology, 59*(3), 695–700. doi:0099-2240/93/030695-06$02.00/0

Naar, Z., & Kecskés, M. (1998). Factors Influencing the Competitive Saprophytic Ability of *Trichoderma* Species. *Microbiological Research, 153*(2), 119–129. doi:10.1016/S0944-5013(98)80029-3

Nagy, L. G., Petkovits, T., Kovács, G. M., Voigt, K., Vágvölgyi, C., & Papp, T. (2011). Where is the Unseen Fungal Diversity Hidden? A Study of *Mortierella* Reveals a Large Contribution of Reference Collections to the Identification of Fungal Environmental Sequences. *New Phytologist, 191*(3), 789–794. doi:10.1111/j.1469-8137.2011.03707.x

Nakasone, A. K., Bettiol, W., & de Souza, R. M. (1999). The Effect of Water Extracts of Organic Matter on Plant Pathogens. *Summa Phytopathologica, 25,* 330–335.

National Research Council (2010). Toward Sustainable Agricultural Systems in the 21st Century. Washington, DC: The National Academies Press. doi:10.17226/12832

Neher, D. A., Fang, L., & Weicht, T. R. (2017). Ecoenzymes as Indicators of Compost to Suppress *Rhizoctonia Solani*. *Compost Science and Utilization, 25*(4), 251-261. doi:10.1080/1065657X.2017.1300548

Neher, D. A., Weicht, T. R., Bates, S. T., Leff, J. W., & Fierer, N. (2013). Changes in Bacterial and Fungal Communities Across Compost Recipes, Preparation Methods, and Composting times. *PLoS ONE, 8*(11), e79512. doi:10.1371/journal.pone.0079512

Nesme, J., Achouak, W., Agathos, S. N., Bailey, M., Baldrian, P., Brunel, D., Frostegård, Å., Heulin, T., Jansson, J. K., Jurkevitch, E., Kruus, K. L., Kowalchuk, G. A., Lagares, A., Lappin-Scott, H. M., Lemanceau, P., Le Paslier, D., Mandic-Mulec, I., Murrell, J. C., Myrold, D. D., Nalin, R., Nannipieri, P., Neufeld, J. D., O'Gara, F., Parnell, J. J., Pühler, A., Pylro, V., Ramos, J. L., Roesch, L. F. W., Schloter, M., Schleper, C., Sczyrba, A, Sessitsch, A., Sjöling, S., Sørensen, J., Sørensen, S. J., Tebbe, C. C., Topp, E., Tsiamis, G., van Elsas, J. D., van Keulen, G., Widmer, F., Wagner, M., Zhang, T., Zhang, X., Zhao, L., Zhu, Y.-G., Vogel, T. M., and Simonet, P. (2016). Back to the Future of Soil Metagenomics. *Frontiers in Microbiology, 7*, 1–5. doi:10.3389/fmicb.2016.00073

Nihorimbere, V., Ongena, M., Smargiassi, M., & Thonart, P. (2011). Beneficial Effect of the Rhizosphere Microbial Community for Plant Growth and Health. *Biotechnologie, Agronomie, Société et Environnement, 15*(2), 327-337.

Noble, R. (2011). Risks and Benefits of Soil Amendment with Composts in Relation to Plant Pathogens, *Australasian Plant Pathology, 40*(2), 157–167. doi:10.1007/s13313-010-0025-7

Noble, R., & Coventry, E. (2005). Suppression of Soil-Borne Plant Diseases with Composts: A review. *Biocontrol Science and Technology, 15*(1), 3–20. doi:10.1080/09583150400015904

Noble, R., & Roberts, S. J. (2004). Eradication of Plant Pathogens and Nematodes During Composting: A Review. *Plant Pathology, 53*(5), 548–568. doi:10.1111/j.1365-3059.2004.01059.x

Normander, B., & Hendriksen, N. B. (2002). Effective Dose of a Microbial Inoculant is One to Four Cells in the Rhizosphere. *Canadian Journal of Microbiology, 48*(10), 940–944. doi:10.1139/w02-088

Novinscak, A., DeCoste, N. J., Surette, C., & Filion, M. (2009). Characterization of Bacterial and Fungal Communities in Composted Biosolids over a 2 Year Period Using Denaturing Gradient Gel Electrophoresis. *Canadian Journal of Microbiology, 55*(4), 375–87. doi:10.1139/W08-152

Olivain, C., Humbert, C., Nahalkova, J., Fatehi, J., L'Haridon, F., & Alabouvette, C. (2006). Colonization of Tomato Root by Pathogenic and Nonpathogenic *Fusarium oxysporum* Strains Inoculated Together and Separately into the Soil. *Applied and Environmental Microbiology, 72*(2), 1523–1531. doi:10.1128/aem.72.2.1523-1531.2006

On, A., Wong, F., Ko, Q., Tweddell, R. J., Antoun, H., & Avis, T. J. (2015). Antifungal Effects of Compost Tea Microorganisms on Tomato Pathogens. *Biological Control, 80,* 63–69. doi:10.1016/j.biocontrol.2014.09.017

Pal, K. K., & Mc Spadden Gardener, B. (2006). Biological Control of Plant Pathogens. *The Plant Health Instructor*. doi:10.1094/PHI-A-2006-1117-02.

Pane, C., Spaccini, R., Piccolo, A., Scala, F., & Bonanomi, G. (2011). Compost Amendments Enhance Peat Suppressiveness to *Pythium ultimum* , *Rhizoctonia solani* and *Sclerotinia minor*. *Biological Control, 56*(2), 115–124. doi:10.1016/j.biocontrol.2010.10.002

Pane, C., Villecco, D., & Zaccardelli, M. (2013). Short-Time Response of Microbial Communities to Waste Compost Amendment of an Intensive Cultivated Soil in Southern Italy. *Communications in Soil Science and Plant Analysis, 44*(15), 2344-2352. doi:10.1080/00103624.2013.803566

Pant, A., Radovich, T. J. K., Hue, N. V, & Arancon, N. Q. (2013). Effects of Vermicompost Tea (Aqueous Extract) on Pak Choi Yield , Quality , and on Soil Biological Properties. Compost Science and Utilization, 19(4), 279-292. doi:10.1080/1065657X.2011.10737010

Pathma, J., & Sakthivel, N. (2013). Molecular and Functional Characterization of Bacteria Isolated from Straw and Goat Manure Based Vermicompost. *Applied Soil Ecology, 70,* 33–47. doi:10.1016/j.apsoil.2013.03.011

Paulitz, T. C. (1997). Biological Control of Root Pathogens in Soilless and Hydroponic Systems. *HortScience, 32*(4), 750. doi:10.21273/hortsci.32.2.193

Paulitz, T. C., & Bélanger, R. R. (2001). Biological Control In Greenhouse Systems. *Annual Review of Phytopathology, 39*(1), 103–133. doi: 10.1146/annurev.phyto.39.1.103

Paulitz, T. C., Ahmad, J. S., & Baker, R. (1990). Integration of *Pythium nunn* and *Trichoderma harzianum* Isolate T-95 for Biological Control of Pythium Damping-Off of Cucumber. *Plant and Soil, 121*(2), 243–250. doi: 10.1007/bf00012318

Pavel, A. B., & Vasile, C. I. (2012). PyElph - A Software Tool for Gel Images Analysis and Phylogenetics. *BMC Bioinformatics, 13*(1), 9. doi:10.1186/1471-2105-13-9

Pereira, J. C. R., Chaves, G. M., Zambolim, L., Matsuoka, K., Acuna, R. S., & do Vale, F. X. R. (1998). Sobrevivência de *Trichoderma harzianum* e de *Bacillus subtilis* em Vermicomposto. *Summa Phytopathologica, 24*(3-4), 231–238.

Pérez-García, A., Romero, D., & de Vicente, A. (2011). Plant Protection and Growth Stimulation by Microorganisms: Biotechnological Applications of Bacilli in Agriculture. *Current Opinion in Biotechnology, 22(*2), 187–193. doi:10.1016/j.copbio.2010.12.003

Pérez-Piqueres, A., Edel-Hermann, V., Alabouvette, C., & Steinberg, C. (2006). Response of Soil Microbial Communities to Compost Amendments. *Soil Biology and Biochemistry, 38*(3), 460–470. doi:10.1016/j.soilbio.2005.05.025

Petersen, B., & Snapp, S. (2015). What is Sustainable Intensification ? Views from Experts. *Land Use Policy, 46,* 1–10. doi:10.1016/j.landusepol.2015.02.002

Pilkington, L. J., Messelink, G., Lenteren, J. C. Van, & Le Mottee, K. (2010). “Protected Biological Control” – Biological Pest Management in the Greenhouse Industry. *Biological Control, 52*(3), 216–220. doi:10.1016/j.biocontrol.2009.05.022

de Ponti, T., Rijk, B., van Ittersum, M. K. (2012). The Crop Yield Gap Between Organic and Conventional Agriculture. *Agricultural Systems*, 108, 1–9. doi:10.1016/j.agsy.2011.12.004

Postma, J. (2010). The Status of Biological Control of Plant Diseases in Soilless Cultivation. In Gisi, U., Chet, I., & Gullino, M. L. Eds. *Recent Developments in Management of Plant Diseases.* Springer Netherlands: 133–146. doi:10.1007/978-1-4020-8804-9_11

Postma, J., van Os, E., Bonants, P. J. M. (2007). Pathogen Detection and Management Strategies in Soilless Plant Growing Systems. In M. Raviv & J. H. Leith Eds. *Soilless Culture: Theory and Practice*. Elsevier Science & Technology, Amsterdam; London. 425-457.

Postma, J., Stevens, L. H., Wiegers, G. L., Davelaar, E., & Nijhuis, E. H. (2009). Biological Control of *Pythium aphanidermatum* in Cucumber with a Combined Application of *Lysobacter enzymogenes* Strain 3.1T8 and Chitosan. *Biological Control, 48*(3), 301–309. doi:10.1016/j.biocontrol.2008.11.006

Postma, J., Willemsen-de Klein, M. J., & van Elsas, J. D. (2000). Effect of the Indigenous Microflora on the Development of Root and Crown Rot Caused by *Pythium aphanidermatum* in Cucumber Grown on Rockwool. *Phytopathology, 90*(2), 125–133. doi:10.1094/PHYTO.2000.90.2.125

Poudel, R., Jumpponen, A., Schlatter, D. C., Paulitz, T. C., Gardener, B. B. M., Kinkel, L. L., & Garrett, K. A. (2016). Microbiome Networks: A Systems Framework for Identifying Candidate Microbial Assemblages for Disease Management. *Phytopathology, 106*(10), 1083–1096. doi:10.1094/PHYTO-02-16-0058-FI

Pretty, J., Benton, T. G., Bharucha, Z. P., Dicks, L. V, Flora, C. B., Godfray, H. C. J., Goulson, D., Hartley, S., Lampkin, N., Morris, C., Pierzynski, G., Vara Prasad, P. V., Reganold, J, Rockström, J., Smith, P., Thorne, P., & Wratten, S. (2018). Global Assessment of Agricultural System Redesign for Sustainable Intensification. *Nature Sustainability, 1*(8), 441–446. doi:10.1038/s41893-018-0114-0

Procházková, P., Hanč, A., Dvořák, J., Roubalová, R., Drešlová, M., Částková, T., Šustr, V., Škanta, F., Pacheco, N. I. N., & Bilej, M. (2018). Contribution of *Eisenia andrei* Earthworms in Pathogen Reduction During Vermicomposting. *Environmental Science and Pollution Research, 25*(26), 26267–26278. doi:10.1007/s11356-018-2662-2

Pugliese, M., Liu, B., Gullino, M. L., & Garibaldi, A. (2011). Microbial Enrichment of Compost with Biological Control Agents to Enhance Suppressiveness to Four Soil-Borne Diseases in Greenhouse, *Journal of Plant Diseases and Protection, 118*(2), 45–50. doi:10.1007/bf03356380

Punja, Z. K., & Parker, M. (2000). Development of Fusarium Root and Stem Rot, a New Disease on Greenhouse Cucumber in British Columbia, Caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum*. *Canadian Journal of Plant Pathology, 22*(4), 349–363. doi:10.1080/07060660009500453

Punja, Z. K., & Yip, R. (2003). Biological Control of Damping-Off and Root Rot Caused by *Pythium aphanidermatum* on Greenhouse Cucumbers. *Canadian Journal of Plant Pathology, 25*(4), 411–417. doi:10.1080/07060660309507098

Pylro, V. S., Roesch, L. F. W., Ortega, J. M., do Amaral, A. M., Tótola, M. R., Hirsch, P. R., Rosado, A. S., Góes-Neto, A., da Costa da Silva, A. L., Rosa, C. A., Morais, D. K., Andreote, F. D., Duarte, G. F., de Melo, I. S., Seldin, L., Lambais, M. R., Hungria, M., Peixoto, R. S., Kruger, R. H., Tsai, S. M., & Azevedo, V. (2014). Brazilian Microbiome Project: Revealing the Unexplored Microbial Diversity—Challenges and Prospects. *Microbial Ecology, 67*(2), 237–241. doi:10.1007/s00248-013-0302-4

Quarles, B. W. (2018). Regenerative Agriculture Can Reduce Global Warming, *The IPM Practitioner: Monitoring the Field of Pest Management, XXXVI*(1/2), 1-8.

R Core Team (2016). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C., & Moënne-Loccoz, Y. (2009). The Rhizosphere : a Playground and Battlefield for Soilborne Pathogens and Beneficial Microorganisms. *Plant and Soil, 321*(1-2), 341–361. doi:10.1007/s11104-008-9568-6

Rankin, L., & Paulitz, T.C. (1994). Evaluation of Rhizosphere Bacteria for Biological Control of Pythium Root Rot of Greenhouse Cucumbers in Hydroponic Culture. *Plant Disease, 78*(5), 447. doi:10.1094/pd-78-0447

Rao, M. S., Kamalnath, M., Umamaheswari, R., Rajinikanth, R., Prabu, P., Priti, K., Grace, G. N., Chaya, M. K., & Gopalakrishnan, C. (2017). *Bacillus subtilis* IIHR BS-2 Enriched Vermicompost Controls Root Knot Nematode and Soft Rot Disease Complex in Carrot. *Scientia Horticulturae*, *218*, 56–62. doi:10.1016/j.scienta.2017.01.051

Rattink, H. (1996). Root Pathogens in Modern Cultural Systems: Assessment of Risks and Suggestions for Integrated Control. *IOBC wprs Bulletin, 19*, 1-10.

Raupach, G. S., & Kloepper, J. W. (1998). Mixtures of Plant Growth-Promoting Rhizobacteria Enhance Biological Control of Multiple Cucumber Pathogens. *Phytopathology, 88*(11), 1158–1164. doi:10.1094/phyto.1998.88.11.1158

Raviv, M. & Lieth, J. H. (2008). Soilless Culture: Theory and Practice. Elsevier, San Diego, CA.

Ray, D. K., Mueller, N. D., West, P. C., & Foley, J. A. (2013). Yield Trends Are Insufficient to Double Global Crop Production by 2050. *PLoS ONE, 8*(6), e66428. doi:10.1371/journal.pone.0066428

Reddell, P., & Spain, A. V. (1991). Transmission of Infective *Frankia* (actinomycetales) Propagules in Casts of the Endogeic Earthworm *Pontoscolex corethrurus* (Oligochaeta:Glossoscolecidae). *Soil Biology and Biochemistry, 23*(8), 775–778. doi:10.1016/0038-0717(91)90148-d

Reddy, S. A., Bagyaraj, D. J., & Kale, R.D. (2015). Management of Tomato Bacterial Spot Caused by *Xanthomonas campestris* Using Vermicompost. *JBiopest 5*(1): 10-13.

Reganold, J. P., & Wachter, J. M. (2016). Organic Agriculture in the Twenty-First Century. *Nature Plants, 2*(2), 1–8. doi:10.1038/nplants.2015.221

Roberts, D. P., Lohrke, S. M., Meyer, S. L. F., Buyer, J. S., Bowers, J. H., Jacyn Baker, C., Li, W., de Souza, J. T., Lewis, J. A., & Chung, S. (2005). Biocontrol Agents Applied Individually and in Combination for Suppression of Soilborne Diseases of Cucumber. *Crop Protection, 24*(2), 141–155. doi:10.1016/j.cropro.2004.07.004

Roberts, P., Edwards, C. A., Edwards-Jones, G., & Jones, D. L. (2007). Responses of Common Pot Grown Flower Species to Commercial Plant Growth Media Substituted with Vermicomposts. *Compost Science & Utilization, 15*(3), 159–166. doi:10.1080/1065657X.2007.10702328

Ros, M., Raut, I., Santisima-Trinidad, A. B., & Pascual, J. A. (2017). Relationship of Microbial Communities and Suppressiveness of *Trichoderma* Fortified Composts for Pepper Seedlings Infected by *Phytophthora nicotianae*. *PLoS ONE, 12*(3), e0174069. doi:10.1371/journal.pone.0174069

Rose, S., Parker, M., & Punja, Z. K. (2003). Efficacy of Biological and Chemical Treatments for Control of Fusarium Root and Stem Rot on Greenhouse Cucumber. *Plant Disease, 87*(12), 1462–1470. doi:10.1094/PDIS.2003.87.12.1462

Rose, S. & Punja, Z. K. (2004). Greenhouse Cucumber Cultivars Differ in Susceptibility to Fusarium Root and Stem Rot. *HortTechnology, 14,* 240–242. doi:10.21273/horttech.14.2.0240

Sahni, S., Sarma, B. K., & Singh, K. P. (2008). Management of *Sclerotium rolfsii* with Integration of Non-Conventional Chemicals , Vermicompost and *Pseudomonas syringae*. *World Journal of Microbiology and Biotechnology, 24*(4), 517–522. doi:10.1007/s11274-007-9502-8

Sampaio, J. P. (2011). *Rhodotorula* Harrison (1927). In C. Kurtzman, J. W. Fell, & T. Boekhout Eds., *The Yeasts, A Taxonomic Study*. Elsevier B.V: 1873–1927. doi:10.1016/B978-0-444-52149-1.00155-5

Sanguin, H., Sarniguet, A., Gazengel, K., Moënne-Loccoz, Y., & Grundmann, G. L. (2009). Rhizosphere Bacterial Communities Associated with Disease Suppressiveness Stages of Take-All Decline in Wheat Monoculture. *New Phytologist, 184*(3), 694–707. doi:10.1111/j.1469-8137.2009.03010.x

Santos, M., Diánez, F., del Valle, M. G., & Tello, J. C. (2007). Grape Marc Compost: Microbial Studies and Suppression of Soil-Borne Mycosis in Vegetable Seedlings. *World Journal of Microbiology and Biotechnology, 24*(8), 1493–1505. doi:10.1007/s11274-007-9631-0

Scheepmaker, J.W.A. & Kassteele, & J. Van De. (2011). Effects of Chemical Control Agents and Microbial Biocontrol Agents on Numbers of Non-Target Microbial Soil Organisms: A Meta-Analysis, *Biocontrol Science and Technology, 21*(10), 1225-1242, doi:10.1080/09583157.2011.594952

Scherwinski, K., Wolf, A., & Berg, G. (2007). Assessing the Risk of Biological Control Agents on the Indigenous Microbial Communities: *Serratia plymuthica* HRO-C48 and *Streptomyces* sp. HRO-71 as Model Bacteria. *BioControl, 52,* 87–112. doi:10.1007/s10526-006-9006-8

Scherwinski, K., Grosch, R., & Berg, G. (2008). Effect of Bacterial Antagonists on Lettuce: Active Biocontrol of *Rhizoctonia solani* and Negligible, Short-Term Effects on Nontarget Microorganisms. *FEMS Microbiol Ecology, 64*, 106–116. doi:10.1111/j.1574-6941.2007.00421.x

Scheuerell, S. J. (2002). Compost Teas and Compost Amended Container Media for Plant Disease Control. PhD Thesis: Oregon State University.

Scheuerell, S. J., & Mahaffee, W. F. (2004). Compost Tea as a Container Medium Drench for Suppressing Seedling Damping-Off Caused by *Pythium ultimum*. *Phytopathology, 94*(11), 1156–1163. doi:10.1094/phyto.2004.94.11.1156

Scheuerell, S. J., Sullivan, D. M., & Mahaffee, W. F. (2005). Suppression of Seedling Damping-Off Caused by *Pythium ultimum* , *P . irregulare* , and *Rhizoctonia solani* in Container Media Amended with a Diverse Range of Pacific Northwest Compost Sources. *Phytopathology, 95*(3), 306–315. doi:10.1094/phyto-95-0306

Schnürer J., & Rosswall T. (1982). Fluorescein Diacetate Hydrolysis as a Measure of Total Microbial Activity in Soil and Litter. *Applied and Environmental Microbiology, 43*(6). 1256–1261.

Schrama, M., de Haan, J. J., Kroonen, M., Verstegen, H., & Van der Putten, W. H. (2018). Crop Yield Gap and Stability in Organic and Conventional Farming Systems. *Agriculture, Ecosystems, & Environment, 256*, 123–130. doi:10.1016/j.agee.2017.12.023

Schroers, H. (2001). A Monograph of *Bionectria* (Ascomycota, Hypocreales, Bionectriaceae) and its *Clonostachys* anamorphs. *Studies in Mycology 46*. Utrecht, The Netherlands: Centraalbureau voor Schimmelcultures.

Schroers, H., Samuels, G. J., Seifert, K. A., & Gams, W. (1999). Classification of the Mycoparasite *Gliocladium roseum* in *Clonostachys* as *C . rosea* , Its Relationship to *Bionectria ochroleuca* , and Notes on Other *Gliocladium*-Like Fungi. *Mycologia, 91*(2), 365–385. doi:10.2307/3761383

Seaby, D. A. (1996). Investigation of the Epidemiology of Green Mould of Mushroom (*Agaricus bisporus*) Compost Caused by *Trichoderma harzianum*. *Plant Pathology, 45(*5), 913–923. doi:10.1111/j.1365-3059.1996.tb02902.x

Sen, B., & Chandra, T. S. (2009). Do Earthworms Affect Dynamics of Functional Response and Genetic Structure of Microbial Community in a Lab-Scale Composting System? *Bioresource Technology, 100*(2), 804–811. doi:10.1016/j.biortech.2008.07.047

Shang, H., Chen, J.-J., Handelsman, J., & Goodman, R. M. (1999). Behavior of *Pythium torulosum* Zoospores During their Interaction with Tobacco Roots and *Bacillus cereus*. *Current Microbiology, 38,* 199-204. doi:10.1007/pl00006787

Sid Ahmed, A., Ezziyyani, M., Pérez Sánchez, C., & Candela, M. E. (2003). Effect of Chitin on Biological Control Activity of *Bacillus* spp. and *Trichoderma harzianum* Against Root Rot Disease in Pepper (*Capsicum annuum*) Plants. *European Journal of Plant Pathology, 109*(6), 633–637. doi:10.1023/A:1024734216814

Simon, H. M., Smith, K. P., Dodsworth, J. A., Guenthner, B., Handelsman, J., & Goodman, R. M. (2001). Influence of Tomato Genotype on Growth of Inoculated and Indigenous Bacteria in the Spermosphere. *Applied and Environmental Microbiology, 67*(2), 514–520. doi:10.1128/aem.67.2.514-520.2001

Simsek Ersahin, Y., Haktanir, K., & Yanar, Y. (2009). Vermicompost Suppresses *Rhizoctonia solani* Kühn in Cucumber Seedlings. *Journal of Plant Diseases and Protection, 116*(4), 182–188. doi:10.1007/bf03356308

Simsek-Ersahin, Y. (2015). Suggested Mechanisms Involved in Suppression of Fusarium by Vermicompost Products. In M. K. Meghvansi & A. Varma Eds., *Organic Amendments and Soil Suppressiveness in Plant Disease Management* Switzerland: Springer International Publishing: 331–351. doi:10.1007/978-3-319-23075-7_15

Slaughter, A., Daniel, X., Flors, V., Luna, E., Hohn, B., & Mauch-mani, B. (2012). Descendants of Primed Arabidopsis Plants Exhibit Resistance to Biotic Stress. *Plant Physiology, 158*(2), 835–843. doi:10.1104/pp.111.191593

Soobhany, N. (2018). Preliminary Evaluation of Pathogenic Bacteria Loading on Organic Municipal Solid Waste Compost and Vermicompost. *Journal of Environmental Management, 206,* 763–767. doi:10.1016/j.jenvman.2017.11.029

Soobhany, N., Mohee, R., & Kumar, V. (2017). Inactivation of Bacterial Pathogenic Load in Compost Against Vermicompost of Organic Solid Waste Aiming to Achieve Sanitation Goals : A review. *Waste Management, 64,* 51–62. doi:10.1016/j.wasman.2017.03.003

Soresi, D., Zappacosta, D., Garayalde, A., Miranda, R., & Carrera, A. (2015). An *In Vitro* Assay for Pre-Screening Resistance to Fusarium Head Blight in Durum Wheat. *Phytopathologia Mediterranea*, 54(2), 253−264. doi:10.14601/Phytopathol_Mediterr-14986

Spadaro, D., & Gullino, M. L. (2005). Improving the Efficacy of Biocontrol Agents Against Soilborne Pathogens. *Crop Protection, 24*(7), 601–613. doi:10.1016/j.cropro.2004.11.003

St. Martin, C. C. G. (2014). Potential of Compost Tea for Suppressing Plant Diseases. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 9*(32). doi:10.1079/pavsnnr20149032

Stanghellini, M. E., & Rasmussen, S. L. (1994). Hydroponics: A Solution for Zoosporic Pathogens. *Plant Disease, 78*(12), 1129–1138. doi:10.1094/pd-78-1129

Stanghellini, M. E., Rasmussen, S. L., Kim, D. H., & Rorabaugh, P. A. (1996). Efficacy of Nonionic Surfactants in the Control of Zoospore spread of *Pythium aphanidermatum* in a Recirculating Hydroponic System. *Plant Disease, 80,* 422-428. doi:10.1094/PD-80-0422

States, J. S., & Christensen, M. (2001). Fungi Associated with Biological Soil Crusts in Desert Grasslands of Utah and Wyoming. *Mycologia, 93*(3), 432–439. doi:10.2307/3761728

Stein, T. (2005). *Bacillus subtilis* Antibiotics: Structures, Syntheses and Specific Functions. *Molecular Microbiology, 56*(4), 845–857. doi:10.1111/j.1365-2958.2005.04587.x

Stephens, P. M., & Davoren, C. W. (1997). Influence of the Earthworms *Aporrectodea trapezoides* and *A. rosea* on the Disease Severity of *Rhizoctonia solani* on Subterranean Clover and Ryegrass. *Soil Biology and Biochemistry, 29*(3-4), 511–516. doi:10.1016/s0038-0717(96)00108-3

Stephens, P. M., Davoren, C. W., Doube, B. M., & Ryder, M. H. (1994). Ability of the Lumbricid Earthworms *Aporrectodea rosea* and *Aporrectodea trapezoides* to Reduce the Severity of Take-All Under Greenhouse and Field Conditions. *Soil Biology and Biochemistry, 26*(10), 1291–1297. doi:10.1016/0038-0717(94)90209-7

Suárez-Estrella, F., Arcos-Nievas, M. A., López, M. J., Vargas-García, M. C., & Moreno, J. (2013). Biological Control of Plant Pathogens by Microorganisms Isolated from Agro-Industrial Composts. *Biological Control, 67*(3), 509–515. doi:10.1016/j.biocontrol.2013.10.008

Swati, A., & Hait, S. (2017). Fate and Bioavailability of Heavy Metals During Vermicomposting of Various Organic Wastes — A Review. *Process Safety and Environmental Protection, 109*, 30–45. doi:10.1016/j.psep.2017.03.031

Swati, A., & Hait, S. (2018). Greenhouse Gas Emission During Composting and Vermicomposting of Organic Wastes – A Review. Clean Soil Air Water, 46(6), 1700042. doi:10.1002/clen.201700042

Szczech, M. (2008). Mixtures of Microorganisms in Biocontrol. In M.-B. Kim (Ed.), *Progress in Environmental Microbiology.* Hauppauge, New York: Nova Science Publishers, Inc.: 69–110.

Szczech, M. M. (1999). Suppressiveness of Vermicompost Against Fusarium Wilt of Tomato. *Journal of Phytopathology, 147*(3), 155–161. doi:10.1046/j.1439-0434.1999.147003155.x

Szczech, M., & Smolinska, U. (2001). Comparison of Suppressiveness of Vermicomposts Produced from Animal Manures and Sewage Sludge against *Phytophthora nicotianae* Breda de Haan var . *nicotianae*. Journal of Phytopathology, 149(2), 77–82. doi:10.1046/j.1439-0434.2001.00586.x

Tajbakhsh, J., Goltapeh, E. M., & Varma, A. (2011). Vermicompost as a Biological Soil Amendment. In A. Karaca Ed., *Biology of Earthworms.* Heidelberg, Germany: Springer-Verlag: 215–228. doi:10.1007/978-3-642-14636-7_13

Termorshuizen, A. J., Rijn, E. Van, Gaag, D. J. Van Der, Alabouvette, C., & Chen, Y., Lagerlöf, J., Malandrakis, A. A., Paplomatas, E.J., Rämert, B., Ryckeboer, J., & Steinberg, C. (2006). Suppressiveness of 18 Composts Against 7 Pathosystems : Variability in Pathogen Response, 38, 2461–2477. doi:10.1016/j.soilbio.2006.03.002

Thilagavathi, R., Rajendran, L., Nakkeeran, S., Raguchander, T., Balakrishnan, A., & Samiyappan, R. (2012). Vermicompost-Based Bioformulation for the Management of Sugarbeet Root Rot Caused by *Sclerotium rolfsii, Archives of phytopathology and plant protection, 45*(18), 2243-2250. doi:10.1080/03235408.2012.724974

Tian, X., & Zheng, Y. (2013). Compost Teas and Reused Nutrient Solution Suppress Plant Pathogens *in vitro*, *Hortscience, 48*(4), 510–512. doi: 10.21273/hortsci.48.4.510

Tognetti, C., Laos, F., Mazzarino, M. J., & Hernández, M. T. (2005). Composting vs. Vermicomposting: A Comparison of End Product Quality. *Compost Science & Utilization, 13*(1), 6–13. doi:10.1080/1065657x.2005.10702212

Torres-Cortés, G., Millán, V., Ramírez-Saad, H. C., Nisa-Martínez, R., Toro, N., & Martínez-Abarca, F. (2011). Characterization of Novel Antibiotic Resistance Genes Identified by Functional Metagenomics on Soil Samples. *Environmental Microbiology, 13*(4), 1101–1114. doi:10.1111/j.1462-2920.2010.02422.x

Torsvik, V., Goksyr, J., & Daae, F. L. (1990). High Diversity in DNA of Soil Bacteria, 56(3), 782–787.

Tourlomousis, P., Kemsley, E. K., Ridgway, K. P., Toscano, M. J., Humphrey, T. J., & Narbad, A. (2010). PCR-Denaturing Gradient Gel Electrophoresis of Complex Microbial Communities: A Two-Step Approach to Address the Effect of Gel-to-Gel Variation and Allow Valid Comparisons Across a Large Dataset. *Microbial Ecology, 59*(4), 776–786. doi:10.1007/s00248-009-9613-x

Trevors, J. T., & Masson, L. (2010). DNA Technologies: What's Next Applied to Microbiology Research? *Antonie van Leeuwenhoek, 98*(3), 249–262. doi:10.1007/s10482-010-9480-y

DESA, UN. (2017). World Population Prospects: The 2017 Revision. New York: United Nations. Department of Economic and Social Affairs, Population Division.

Unterseher, M., Jumpponen, A., Öpik, M., Tedersoo, L., Moora, M., Dormann, C. F., & Schnittler, M. (2011). Species Abundance Distributions and Richness Estimations in Fungal Metagenomics - Lessons Learned from Community Ecology. *Molecular Ecology, 20*(2), 275–285. doi:10.1111/j.1365-294x.2010.04948.x

Utkhede, R. S., Lévesque, C. A., & Dinh, D. (2000). *Pythium aphanidermatum* Root Rot in Hydroponically Grown Lettuce and the Effect of Chemical and Biological Agents on its Control. *Canadian Journal of Plant Pathology, 22*(2), 138–144. doi:10.1080/07060660009500487

Utkhede, R., & Koch, C. (2004). Biological Treatments to Control Bacterial Canker of Greenhouse Tomatoes. *BioControl, 49*(3), 305–313. doi:10.1023/b:bico.0000025373.69584.08

Vakalounakis, D. J. (1996). Root and Stem Rot of Cucumber Caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum* f. sp. nov. *Plant Disease, 80*, 313–316. doi:10.1094/pd-80-0313

Vallance, J., Déniel, F., Floch, G., Guérin-Dubrana, L., Blancard, D., & Rey, P. (2011). Pathogenic and Beneficial Microorganisms in Soilless Cultures. *Agronomy for Sustainable Development, 31*(1), 191–203. doi:10.1051/agro/2010018

van der Lans, C. J. M., Meijer, R. J. M., & Blom, M. (2011). A View of Organic Greenhouse Horticulture Worldwide. In: *International Conference on Organic Greenhouse Horticulture, Acta Horticulturae 915,* 15-22. doi:10.17660/ActaHortic.2011.915.1

Van Lenteren, J. C. (2000). A Greenhouse Without Pesticides: Fact or Fantasy? *Crop Protection, 19(*6), 375–384. doi:10.1016/S0261-2194(00)00038-7

van Lenteren, J.C. (2006). The Area Under Biological Control and IPM in Greenhouses is Much Larger than we Thought. *Sting, 29*, 7.

Van Loon, L. C. (1997). Induced Resistance in Plants and the Role of Pathogenesis-Related Proteins. *European Journal of Plant Pathology, 103*(9), 753–765.

Van Veen, J. A., Van Overbeek, L. S., & Van Elsas, J. D. (1997). Fate and Activity of Microorganisms Introduced into Soil. *Microbiology and Molecular Biology Reviews, 61*(2), 121–135.

Veeken, A. H. M., Blok, W. J., Curci, F., Coenen, G. C. M., Termorshuizen, A. J., & Hamelers, H. V. M. (2005). Improving Quality of Composted Biowaste to Enhance Disease Suppressiveness of Compost-Amended, Peat-Based Potting Mixes. *Soil Biology and Biochemistry, 37*(11), 2131–2140. doi:10.1016/j.soilbio.2005.03.018

Verma, M., Brar, S. K., Tyagi, R. D., Surampalli, R. Y., & Valéro, J. R. (2007). Antagonistic Fungi, *Trichoderma* spp.: Panoply of Biological Control. *Biochemical Engineering Journal, 37*(1), 1–20. doi:10.1016/j.bej.2007.05.012

Vilich V. & Sikora, R. A. (1998). Diversity in Soil-Borne Microbial Communities: A Tool for Biological System Management of Root Health. In G. J. Boland & L. D. Kuykendall, Eds. *Plant-Microbe Interactions and Biological Control.* Marcel Dekker Inc., New York, USA.

Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Marra, R., Woo, S. L., Lorito, M. (2008). *Trichoderma*-Plant-Pathogen Interactions. *Soil Biology and Biochemistry, 40*, 1–10. doi:10.1016/j.soilbio.2007.07. 002

Viterbo A., Ramot, O., Chemin L. & Chet, I. (2002). Significance of Lytic Enzymes from *Trichoderma* spp. in the Biocontrol of Fungal Plant Pathogens*. Antonie Van Leeuwenhoek, 81*, 549-56. doi:10.1023/A:1020553421740

Vivas, A., Moreno, B., Garcia-Rodriguez, S., & Benitez, E. (2009). Assessing the Impact of Composting and Vermicomposting on Bacterial Community Size and Structure, and Microbial Functional Diversity of an Olive-Mill Waste. *Bioresource Technology, 100*(3), 1319–1326. doi:10.1016/j.biortech.2008.08.014

Vogel, T. M., Simonet, P., Jansson, J. K., Hirsch, P. R., Tiedje, J. M., van Elsas, J. D., Bailey, M. J., Nalin, R., & Philippot, L. (2009). TerraGenome: a Consortium for the Sequencing of a Soil Metagenome. *Nature Reviews Microbiology, 7*(4), 252–252. doi:10.1038/nrmicro2119

von Grebmer, K., Bernstein, J., Hossain, N., Brown, T., Prasai, N., Yohannes, Y., Sonntag, A., Zimmerman, S. M., Towey, O., & Foley, C. (2017). 2017 Global Hunger Index: The Inequalities of Hunger. Bonn, Washington, DC, and Dublin: Welthungerhilfe, International Food Policy Research Institute, and Concern Worldwide. doi:10.2499/9780896292710

Wagner L., Stielow B., Hoffmann K., Petkovits T., Papp T., Vágvölgyi C., de Hoog G.S., Verkley G., & Voigt K. (2013). A Comprehensive Molecular Phylogeny of the Mortierellales (Mortierellomycotina) Based on Nuclear Ribosomal DNA. *Persoonia, 30*, 77–93. doi:10.3767/003158513X666268

Walters, D. R. (2009). Are Plants in the Field Already Induced? Implications for Practical Disease Control. *Crop Protection, 28,* 459-465. doi:10.1016/j.cropro.2009.01.009

Warman, P. R. & AngLopez, M. J. (2010). Vermicompost Derived from Different Feedstocks as a Plant Growth Medium. *Bioresource Technology, 101,* 4479-4483. doi: 10.1016/j.biortech.2010.01.098

Welc, M., Ravnskov, S., Kieliszewska-rokicka, B., & Larsen, J. (2010). Suppression of Other Soil Microorganisms by Mycelium of Arbuscular Mycorrhizal Fungi in Root-Free Soil. *Soil Biology & Biochemistry, 42*(9), 1534–1540. doi:10.1016/j.soilbio.2010.05.024

Weller, D. M., Raaijmakers, J. M., McSpadden Gardener, B. B., & Thomashow, L. S. (2002). Microbial Populations Responsible for Specific Soil Suppressiveness to Plant Pathogens. *Annual Review of Phytopathology, 40*(1), 309–348. doi:10.1146/annurev.phyto.40.030402.110010

Weltzien, H. C. (1992). Biocontrol of Foliar Fungal Diseases with Compost Extracts. In J. H. Andrews & S. S. Hirano Eds., *Microbial Ecology of Leaves*. New York: Springer-Verlag: 430–450. doi:10.1007/978-1-4612-3168-4_22

Whipps, J. M. (2001). Microbial Interactions and Biocontrol in the Rhizosphere. Journal of *Experimental Botany, 52*(suppl_1), 487–511. doi:10.1093/jexbot/52.suppl_1.487

Whipps, J. M., & Lumsden, R. D. (2001). Commercial Use of Fungi as Plant Disease Biological Control Agents: Status and Prospects. In T. M. Butt, C. Jackson, & N. Magan. *Fungi as Biocontrol Agents: Progress, Problems and Potential*, 9–22. CABI Publishing, Wallingford, Oxon, UK. doi:10.1079/9780851993560.0009

Wichuk, K. M., & McCartney, D. (2010). Compost Stability and Maturity Evaluation — a Literature Review. *Canadian Journal of Civil Engineering, 37*, 1505–1523. doi:10.1139/L10-101

Wichuk, K. M., Tewari, J. P., & McCartney, D. (2011). Plant Pathogen Eradication During Composting: A Literature Review. *Compost Science & Utilization, 19*(4), 244–266. doi:10.1080/1065657x.2011.10737008

Windstam, S., & Nelson, E. B. (2008). Differential Interference with *Pythium ultimum* Sporangial Activation and Germination by *Enterobacter cloacae* in the Corn and Cucumber Spermospheres. *Applied and Environmental Microbiology, 74*(14), 4285–4291. doi:10.1128/aem.00263-08

Wylie, A. C., & Punja Z. K. (2020). Assessing aerated vermicompost tea (ACT) combined with microbial biological control agents for suppression of *Fusarium* and *Rhizoctonia*. *Phytopathology* (November 11, 2020) doi:10.1094/phyto-05-20-0156-r

Wylie, A. C., & Punja, Z. K. (20xx) Microbial activity, diversity and disease suppressive activity of five vermicomposts. Submitted March 19, 2019

Yakushev, A. V, Bubnov, I. A., & Semenov, A. M. (2011). Estimation of the Effects of Earthworms and Initial Substrates on the Bacterial Community in Vermicomposts. *Eurasian Soil Science*, *44,* 1117–1124. doi:10.1134/S1064229311100164

Yang, Q.-Y., Jia, K., Geng, W.-Y., Guo, R., & Li, S.-D. (2014). Management of Cucumber Wilt Disease by *Bacillus subtilis* B006 Through Suppression of *Fusarium oxysporum* f. sp. *cucumerinum* in Rhizosphere. *Plant Pathology Journal, 13*(3), 160–166. doi:10.3923/ppj.2014.160.166

Yasir, M., Aslam, Z., Kim, S. W., Lee, S.-W., Jeon, C. O., & Chung, Y. R. (2009). Bacterial Community Composition and Chitinase Gene Diversity of Vermicompost with Antifungal Activity. *Bioresource Technology, 100*(19), 4396–4403. doi:10.1016/j.biortech.2009.04.015

Yogev, A., Raviv, M., Hadar, Y., Cohen, R., & Katan, J. (2006). Plant Waste-Based Composts Suppressive to Diseases Caused by Pathogenic *Fusarium oxysporum*. *European Journal of Plant Pathology, 116*(4), 267–278. doi:10.1007/s10658-006-9058-8

Yu, Z., & Morrison, M. (2004). Comparisons of Different Hypervariable Regions of rrs Genes for Use in Fingerprinting of Microbial Communities by PCR-Denaturing Gradient Gel Electrophoresis. *Applied and Environmental Microbiology, 70*(8), 4800–4806. doi:10.1128/aem.70.8.4800-4806.2004

Yu, D., Sinkkonen, A., Hui, N., Kurola, J. M., Kukkonen, S., Parikka, P., Vestberg, M., Romantschuk, M. (2015). Molecular Profile of Microbiota of Finnish Commercial Compost Suppressive Against Pythium Disease on Cucumber Plants. *Applied Soil Ecology, 92,* 47–53. doi:10.1016/j.apsoil.2015.03.005

Zhang, W., Han, D. Y., Dick, W. A., Davis, K. R., & Hoitink, H. A. J. (1998). Compost and Compost Water Extract-Induced Systemic Acquired Resistance in Cucumber and Arabidopsis. *Phytopathology, 88*(5), 450–455. doi:10.1094/phyto.1998.88.5.450

Zheng, J., Sutton, J. C., & Yu, H. (2000). Interactions among *Pythium aphanidermatum,* roots, root mucilage, and microbial agents in hydroponic cucumbers. *Canadian Journal of Plant Pathology, 22*(4), 368–379. doi:10.1080/07060660009500455

Appendix A.

Automated AUDPC R script

Current AUDPC scripts require a single set of values, averaging values then performing this test removes statistical power.

The original APS R code example can be found at https://www.apsnet.org/edcenter/disimpactmngmnt/topc/EcologyAndEpidemiologyInR/DiseaseProgress/Pages/AUDPC.aspx

The following R script modifies the example to calculate an AUDPC value for each experimental unit individually though an automated process. This allows us to perform statistical analyses on the AUDPC data itself. The script outputs a PDF file containing a batch of AUDPC curves in the familiar APS format (Figure A.1), as well as a .csv file with all of the results for the batch.

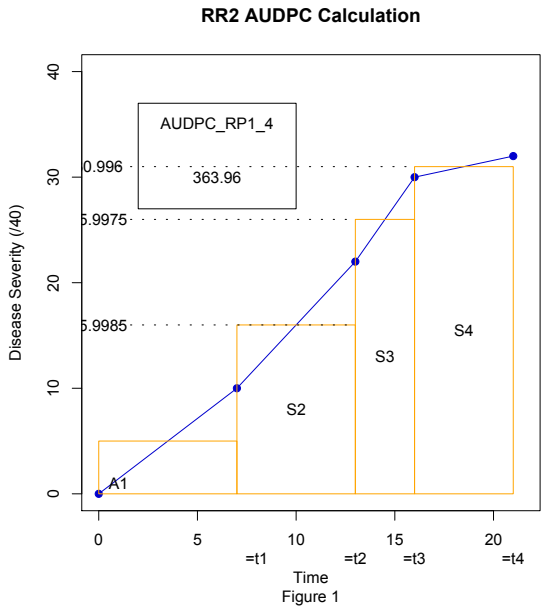


Figure A.1. An example AUDPC curve generated by the script.

This is an example R script for plants where “pot” is the experimental unit, sampled for disease severity at day 8, 13, 16, 21, and 23, on a ds scale from 0-40:

```
setwd("my_disease_experiment")
sheet=read.csv("My_data.csv")
head(sheet)
#set up data frame
frame<-as.data.frame.matrix(sheet)

#call an individual cell eg
ds1<-frame[2,"disease_severity_1"]
ds1

pdf()
pot<-sheet $Pot

for (row in c(pot)){

#this is for row one (P1_1)
ds0<-0
ds1<-frame[row,"ds_8_40"]
ds2<-frame[row,"ds_13_40"]
ds3<-frame[row,"ds_16_40"]
ds4<-frame[row,"ds_21_40"]
ds5<-frame[row,"ds_23_40"]

#Put these values into a vector without making any changes
disease.severity<-c(ds0,ds1,ds2,ds3,ds4,ds5)

#Time points at which disease severity
#   measurements are made,
#change these in subsequent analyses to
#see how it affects the AUDPC Value
t0<-0
t1<-8
t2<-13
t3<-16
t4<-21
t5<-23

#Put time period into a vector
## Do not change these values
time.period<-c(t0,t1,t2,t3,t4,t5)

#Create the plot of disease severity over time
plot(time.period,
     disease.severity,
     ylim=c(0,(40)),
     xlim=c(0,(t5+0.5)),
     xlab="Time",
     ylab="AUDPC (/40)",
     type="o",
     pch=19,
     col="mediumblue")
```

```
#Add a title and subtitle to our plot
title(main="RR4 AUDPC Calculation D23",
      sub="Figure 1")

#Add text to x labels defining time periods
#   defined in text

mtext("=t1",1,at=8,2)
mtext("=t2",1,at=13,2)
mtext("=t3",1,at=16,2)
mtext("=t4",1,at=21,2)
mtext("=t5",1,at=23,2)

#Illustrate the area under disease progress
#   curve with rectangles.
## Do not change these values
rect(t0,0,t1,((ds0+ds1)/2),border="orange")
# Add text to rectangle to describe rectangle
text(1,1,"A1")
#Add segment to Y axis
#And so-on
rect(t1,0,t2,((ds1+ds2)/2),border="orange")
text(((t1+t2)/2),(((ds1+ds2)/2)/2),"S2")
#Draw line to axis and label with value
segments(.4,((ds1+ds2)/2),t2,((ds1+ds2)/2),
         col="black",lty="18")
text(0,((ds1+ds2)/2),((ds1+ds2)/2))

rect(t2,0,t3,((ds2+ds3)/2),border="orange")
text(((t2+t3)/2),(((ds2+ds3)/2)/2),"S3")
segments(0.4,((ds2+ds3)/2),t2,((ds2+ds3)/2),
         col="black",lty="18")
text(0,((ds2+ds3)/2),((ds2+ds3)/2))

rect(t3,0,t4,((ds3+ds4)/2),border="orange")
text(((t3+t4)/2),(((ds3+ds4)/2)/2),"S4")
segments(0.4,((ds3+ds4)/2),t2,((ds3+ds4)/2),
         col="black",lty="18")
text(0,((ds3+ds4)/2),((ds3+ds4)/2))

rect(t4,0,t5,((ds4+ds5)/2),border="orange")
text(((t4+t5)/2),(((ds4+ds5)/2)/2),"S5")
segments(0.4,((ds4+ds5)/2),t2,((ds4+ds5)/2),
         col="black",lty="18")
text(0,((ds4+ds5)/2),((ds4+ds5)/2))

#Build a function for AUDPC calculation
#the left curly bracket indicates the beginning
#   of the function
audpc <- function(disease.severity,time.period){

  #n is the length of time.period, or
  #  the total number of sample dates
  n <- length(time.period)
  #meanvec is the vector (matrix with one dimension)
  #that will contain the mean percent infection
  #it is initialized containing -1 for all entries
```

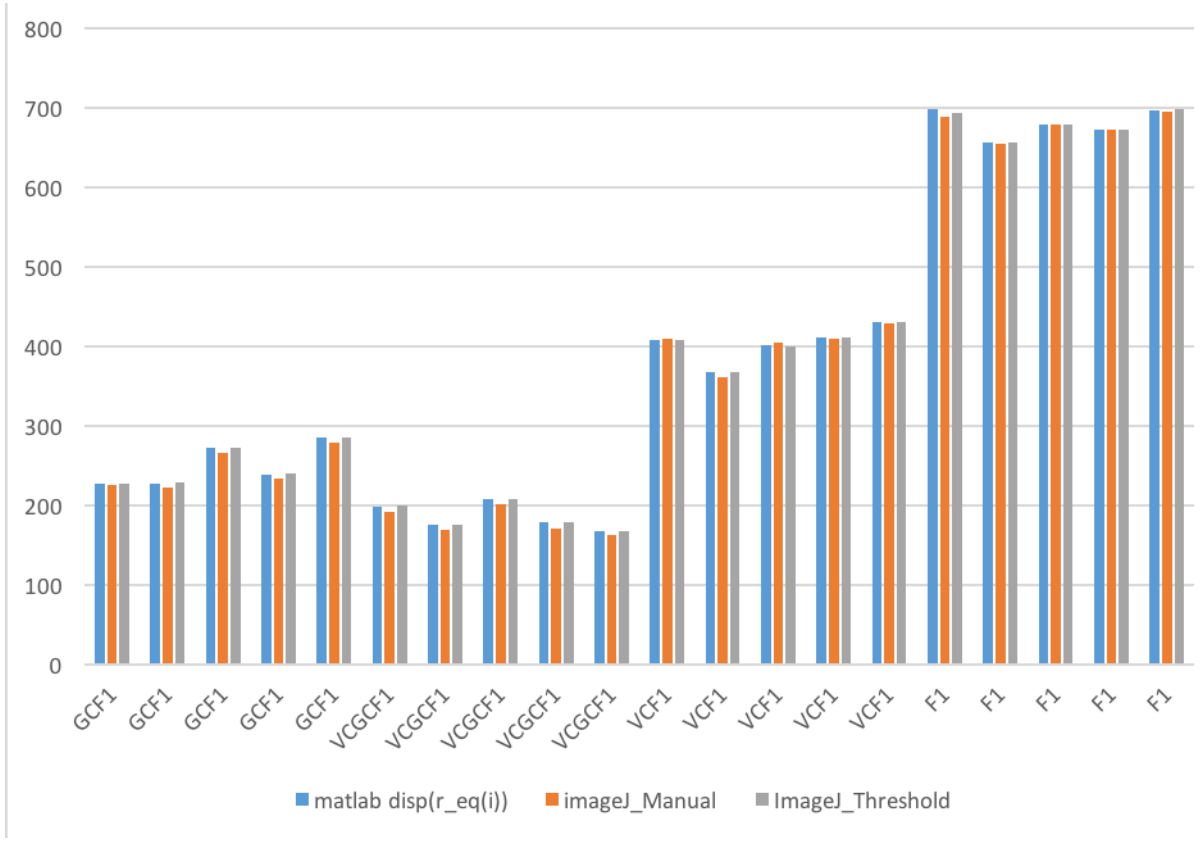
```
  #this sort of initialization is sometimes useful
  #  for debugging
  meanvec <- matrix(-1,(n-1))

  #intvec is the vector that will contain the length of
  #   time between sampling dates
  intvec <- matrix(-1,(n-1))
  #the loop goes from the first to the penultimate entry
  #the left curly bracket indicates the beginning of
  #   commands in the loop
  for(i in 1:(n-1)){

    #the ith entry in meanvec is replaced with the
    #   mean percent infection
    #between sample time i and sample time i+1
    meanvec[i] <- mean(c(disease.severity[i],
                         disease.severity[i+1]))

    #the ith entry in intvec is replaced with the length
    # of the time interval between time i and time i+1
    intvec[i] <- time.period[i+1] - time.period[i]

    #the right curly bracket ends the loop
  }

  #the two vectors are multiplied together
  #  one entry at a time
  infprod <- meanvec * intvec

  #the sum of the entries in the resulting vector
  #   gives the AUDPC
  sum(infprod)

  #the right curly bracket ends the function
}
#Now apply the function to the example data and put
# the result in a new object called 'AUDPCexample'
audpc(disease.severity,time.period) -> AUDPCexample
#Display AUDPC Value
#Draw rectangle around value
rect(2,(27),10,(37),border="black")
#AUDPC Text
text(6,(30),row[pot])
text(6,(32),AUDPCexample)

write.table(AUDPCexample, file = "results.csv", append = TRUE, quote = TRUE, sep = "
 ",
            eol = "\n", na = "NA", dec = ".", row.names = FALSE,
            col.names = FALSE, qmethod = c("escape", "double"),
            fileEncoding = "")
}
dev.off()
```

Appendix B.

In vitro quality control tests

B.1. Area measurement and calculation procedure validation

Several methods of analysis were explored for the image analysis in these trials: a manual method where the colony circumference is traced in ImageJ, a semi-automated method where the colony circumference is determined by thresholding in ImageJ, and an attempt to fully automate the procedure in Matlab with code courtesy of Darren Sutton, an engineer working in the Punja lab. All three methods are capable of achieving statistically similar results, and the fully automated method shows promise although the code must be tweaked for each trial run whereas the ImageJ methods works regardless of the differences between assays and the thresholding method can be relatively expedient.


800
700
600
500
400
300
200
100
0
GCF1
GCF1
GCF1
GCF1
GCF1
VCGCF1
VCGCF1
VCGCF1
VCGCF1
VCGCF1
VCF1
VCF1
VCF1
VCF1
VCF1
F1
F1
F1
F1
F1
matlab disp(r_eq(i))
imageJ_Manual
ImageJ_Threshold

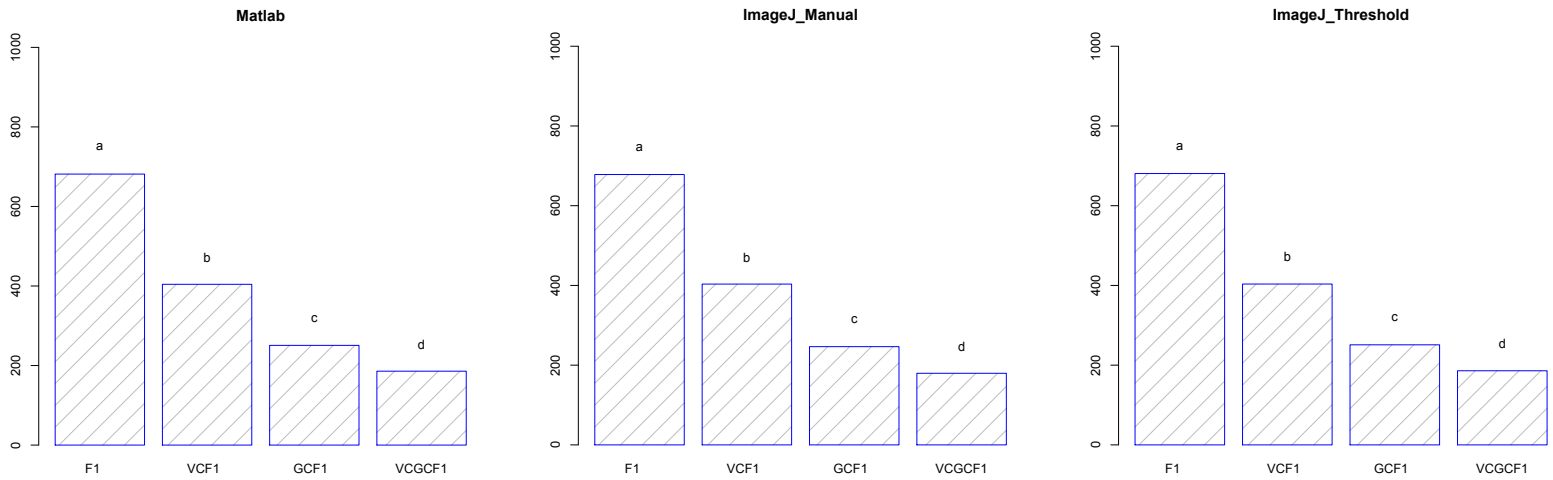


Figure B.1.1. Comparison of three different colony size calculation methods. Side by side bar graph is presented to illustrate the similarity between measurements of individual plates. Multiple comparisons yielding identical results were performed using Tukey's HSD test in R. n=5.

B.2. Non viable *Clonostachys rosea* (Prestop®) tests

A control experiment determined the effect of the proprietary Prestop® carrier compounds on the assay: suppression of pathogen growth was no different between the treatments that received the carrier compounds (GCF and VCGCF) and their counterparts that did not (F and VCF). Vermicompost suppressed the growth of Forc. I am interested in the organism in the biocontrol formulation providing disease suppression efficacy. This is also important for future experiments which will not use the carrier compounds. The results were as expected. I re-isolated the *Clonostachys* fungus and produced fresh inoculum for further trials.

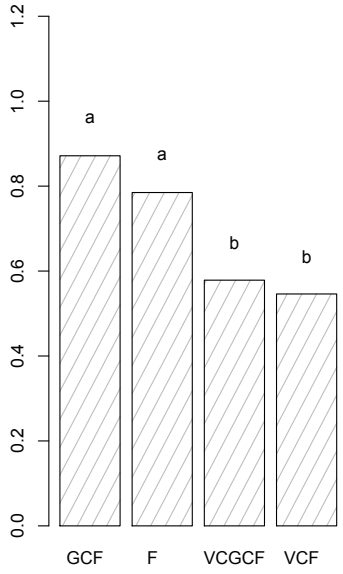


Figure B.2.1. *In vitro* assay using non-viable *Clonostachys* (GC) formulated as Prestop ® trials. n=5.

Viable *Clonostachys* reduces the growth of fungi from the vermicompost population:

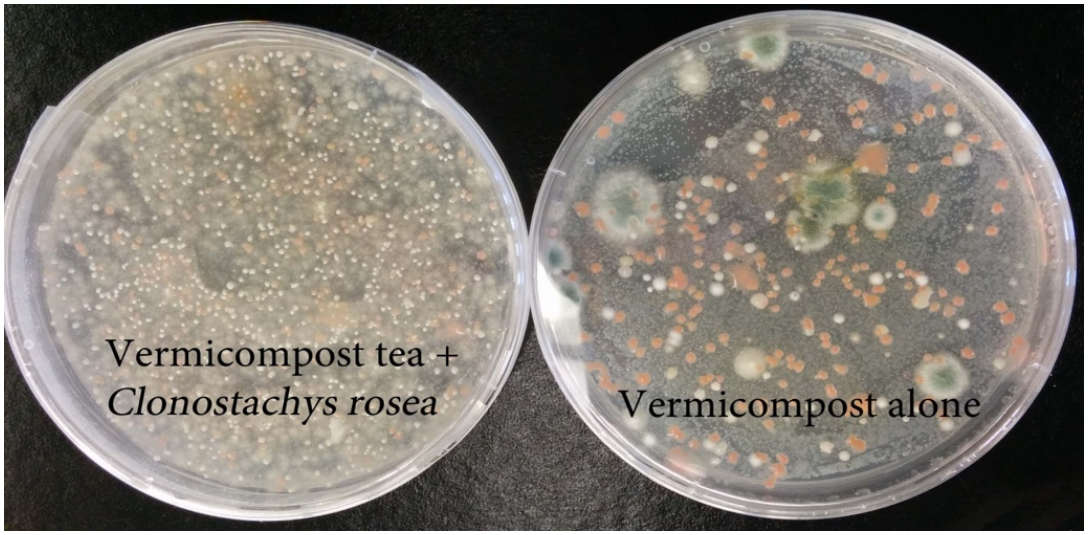


Figure B.2.2. Comparison of lawns of 100 μL 48h aerated vermicompost tea organisms with or without 100 μL 0.05% *Clonostachys rosea* (Prestop®).

3. *In vitro* tests using *R. solani*

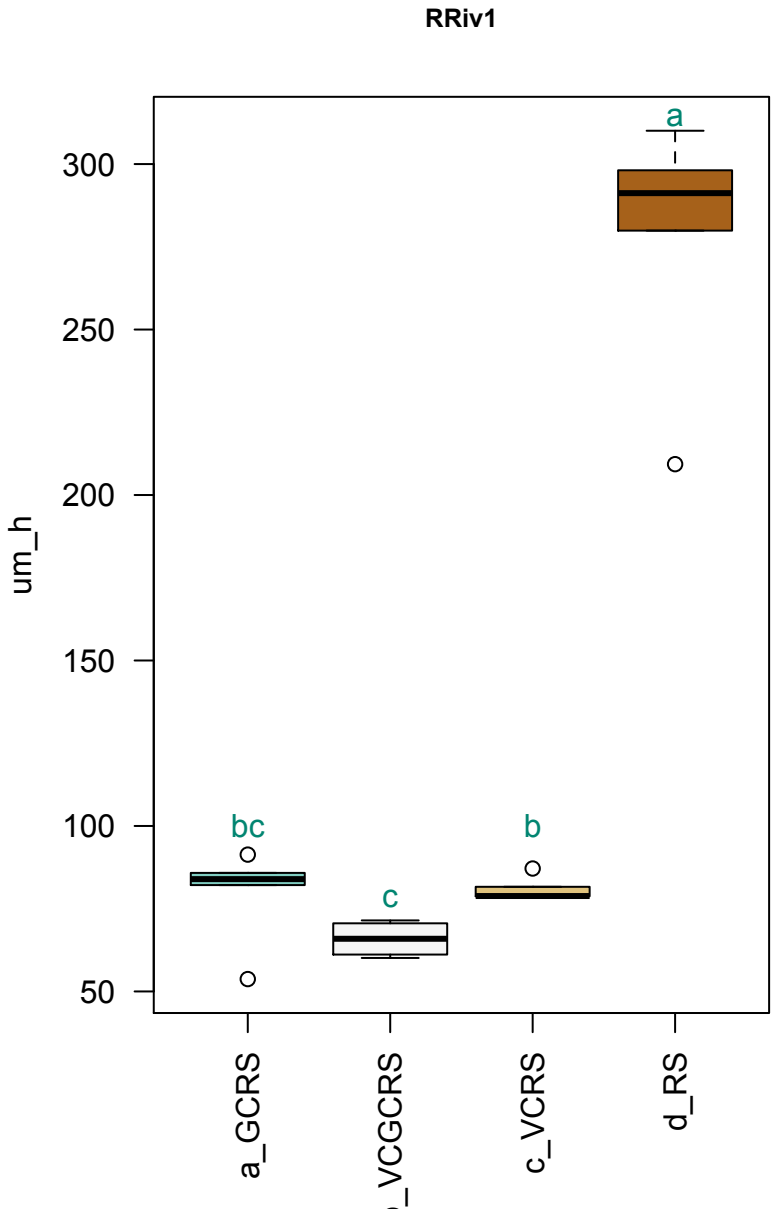


Figure B.3.1. Growth of *Rhizoctonia solani* (RS) on 1/2 strength PDA: on a lawn of *Clonostachys rosea* f. *catenulata* (aka *Gliocladium catenulatum*) strain J1446 (Prestop®) (GCRS), on a lawn of 48 h aerated vermicompost tea (VCRS), and on a combined lawn of *C. rosea* and vermicompost (VCGCRS). Means of n=5 separated using Tukey's HSD test in R.

C. rosea was tested against *R. solani* using the same conditions as the Forc trials described in Chapter 4, presenting some issues illustrative of challenges for wider

application of the assay. Whereas Forc produces pigmented mycelia that are easy to perform image analysis of, even using automated methods (Appendix B.1), *Rhizoctonia* and many other fungi produce more hyaline hyphae and although the colony margin is distince in pure culture, it is more difficult to detect the margin when the colony is growing on a lawn of other organisms.

Furthermore, both vermicompost and *C. rosea* provided excellent suppression of *R. solani* at the experimental rates (Figure B.3.1) and thus there was not a large degree of separation between the different treatment combinations. This experiment did, however, suggest that the combination of vermicompost and the biological control agent provided better suppression of *R. solani* than vermicompost alone, an effect also seen in the growth chamber experiment RRC2 (Figure 4.6). Future trials could use more dilute controls to look for a larger difference between treatment combinations when using *C. rosea* and *R. solani.*

Appendix C.

Alternate *in vitro* systems: 1. *in vitro* plant disease and 2. V8 media.

C.1. *In vitro* plant disease assay

Intermediate between the *in vitro* assay and the growth chamber assay presented in Chapter 4, we developed an *in vitro* assay to quantify the effects seen in the plating assays based on disease levels of seedlings from surface-sterilized seeds, rather than pathogen growth (Figures C.1.1-C.1.2). These types of assays have been performed previously, for example, using *Fusarium graminearum* (Soresi 2015).

Figure C.1.1. A radish seedling with typical signs and symptoms of *Rhizoctonia solani* infection *in vitro*

Rhizoctonia solani infection of radish seedlings causes a characteristic spotting pattern that can be quantified on a four point disease scale: 1. Healthy seedling: green cotyledons and no brown spots, 2. <50% infected: brown/black spots over <50% of

seedling, 3. >50% infected: brown/black spots over >50% of seedling, 4. Plant completely dead. This data can be analyzed using the AUDPC method described in Appendix A.

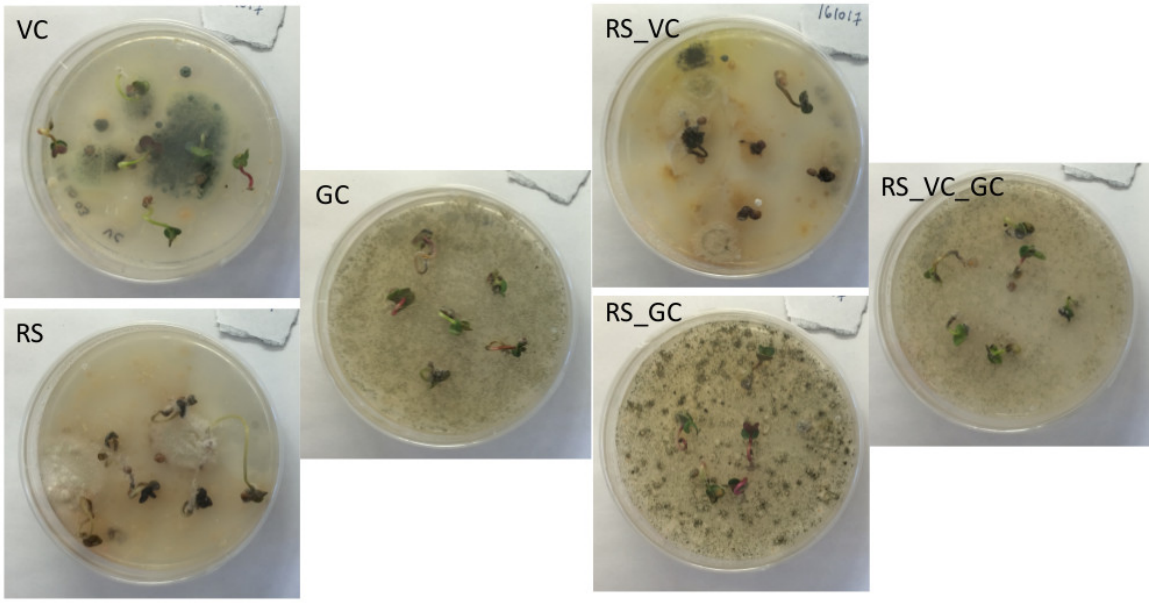


Figure C.1.2. Infection of radish (*Raphanus sativus*) "French Breakfast" by *Rhizoctonia solani* (RS) on 1/2 strength PDA: on a lawn of *Clonostachys rosea* f. *catenulata* (aka *Gliocladium catenulatum*) strain J1446 (Prestop®) (RS_GC), on a lawn of 48 h aerated vermicompost tea (RS_VC), and on a combined lawn of *C. rosea* and vermicompost (RS_VC_GC).

C. rosea was able to provide statistically significant suppression of disease caused by *R. solani* compared with a *R. solani* only control (Figure C.1.3 and C.1.4). *B. subtilis* was also able to do so, although apparently not in the presence of vermicompost tea (Figure C.1.4), although this experiment was not replicated. Under these conditions, the biological control agent *C. rosea* also produced disease symptoms that could not be distinguished according to the disease scale, an effect not seen in the growth chamber tests (Chapter 4). Despite the seedling damage caused by *C. rosea*, when combined with *R. solani* the disease levels became statistically similar to those of the pathogen-free *C. rosea*-containing controls. Damage to seedlings by *C. rosea* was not found to be as severe as *R. solani*. Vermicompost inhibited seedling germination in some cases, when used alone. This is a dose-dependent effect seen elsewhere (Grantina-Ievina et al., 2013). Warman & AngLopez (2010) found that vermicompost extracts inhibited

germination of marigold, radish, and upland cress in Petri dish germination experiments, and in vermicompost/soil mixes. This effect did not appear to be significant when we used vermicompost teas in plant growth media in the growth chamber (Figures 4.5-4.12).

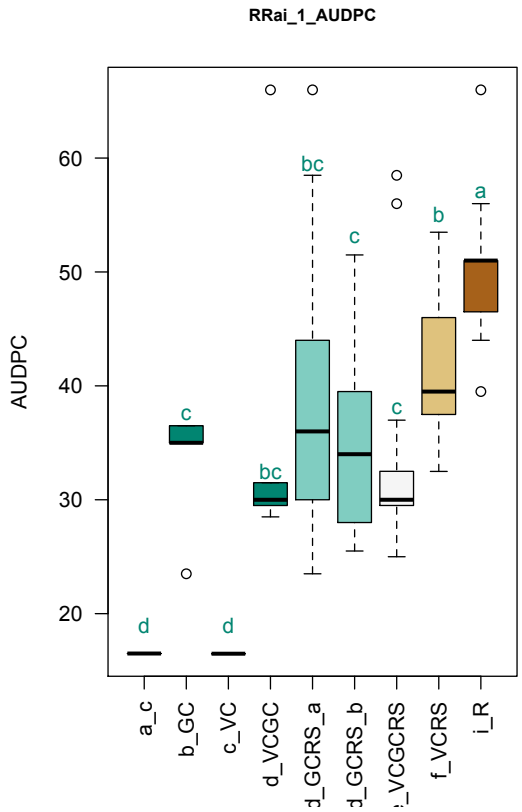


Figure C.1.3. Infection of radish (*Raphanus sativus*) “French Breakfast” by *Rhizoctonia solani* (RS) on 1/2 strength PDA: on a lawn of *Clonostachys rosea* f. *catenulata* (aka *Gliocladium catenulatum*) strain J1446 (Prestop®) (GCRS), on a lawn of 48 h aerated vermicompost tea (VCRS), and on a combined lawn of *C. rosea* and vermicompost (VCGCRS).

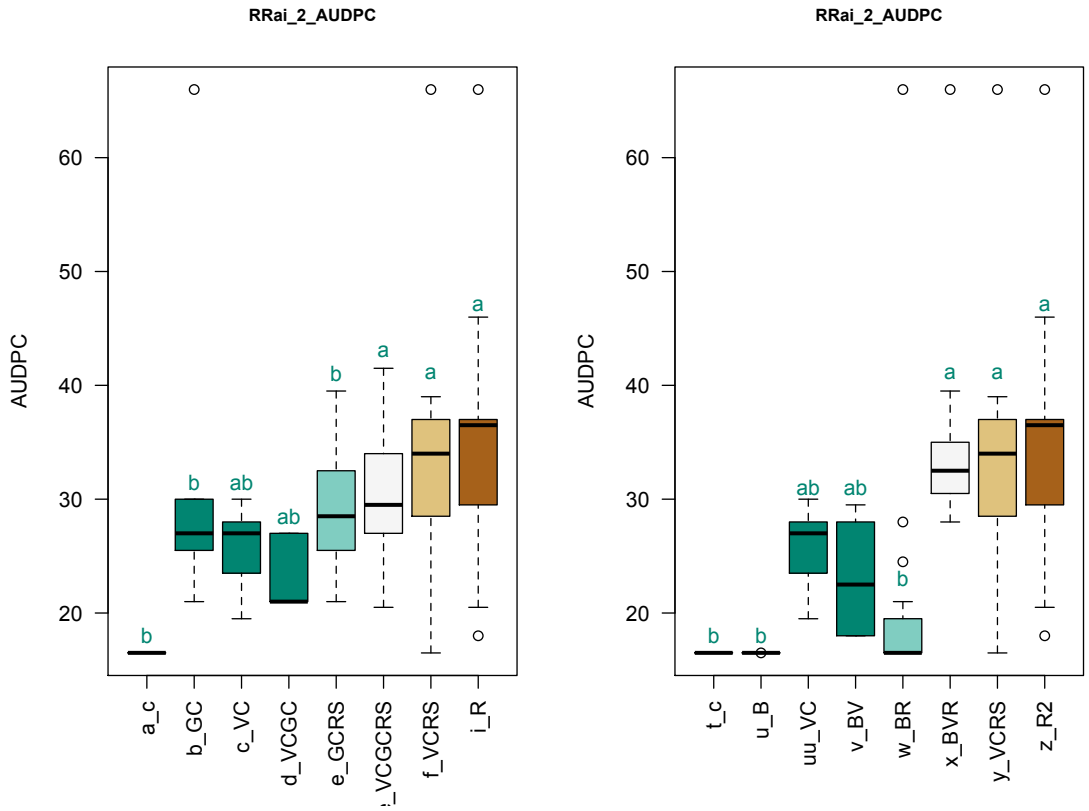


Figure C.1.3. Infection of radish (*Raphanus sativus*) "French Breakfast" by *Rhizoctonia solani* (RS or R) on 1/2 strength PDA: on a lawn of *Clonostachys rosea* f. *catenulata* (aka *Gliocladium catenulatum*) strain J1446 (Prestop®) (GCRS), on a lawn of *Bacillus subtilis* strain QST 713 (Rhapsody) (BR), on a lawn of 48 h aerated vermicompost tea (VCRS), and on a combined lawn of either *C. rosea* or *B. subtilis* and vermicompost (VCGCRS or BVR).

In these experiments, disease symptoms appeared worse when the plant contacted the petri dish, especially where condensed water was present. Magenta jars may be a better choice for future assays as the plants may exhibit a greater range of symptoms, and the criterion "plant has fallen over" can be used to quantify stem damage, not possible in a Petri dish.

C.2. V8 Media

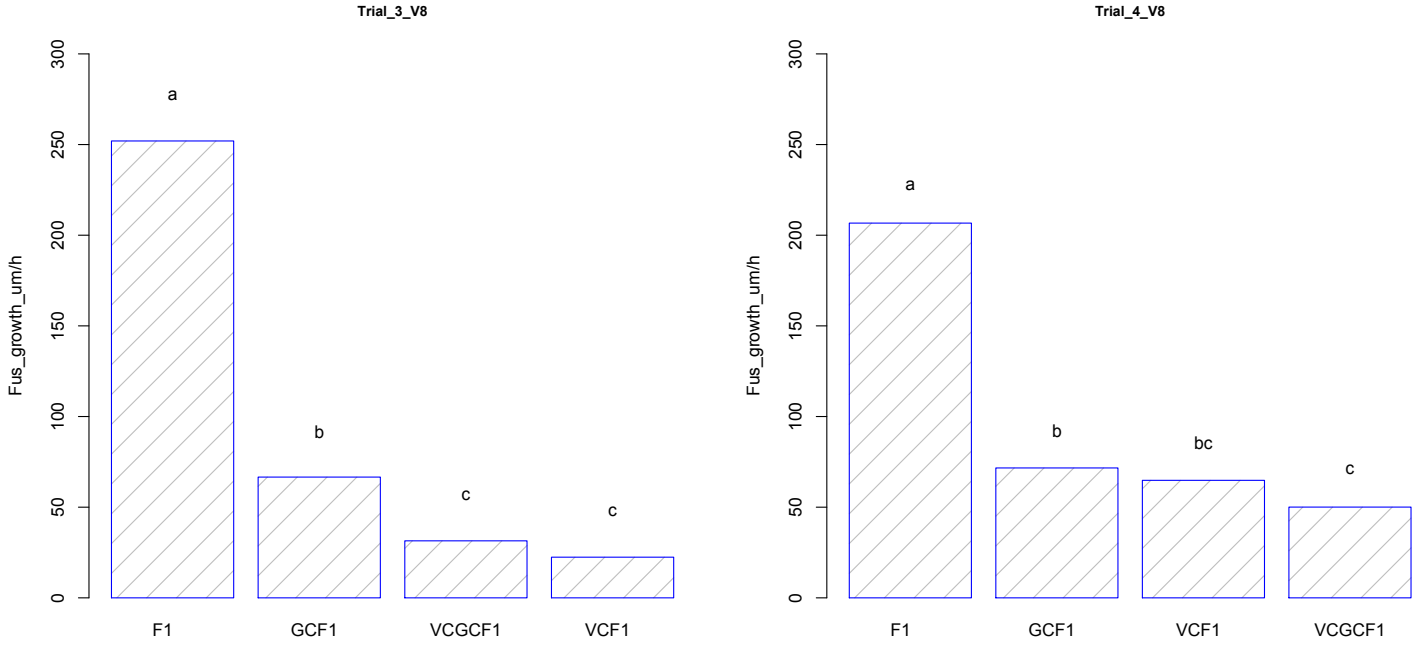


Figure C.2.1. Growth of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* on V8 agar on a lawn of 48 h vermicompost tea (VCF1), on a lawn of *Clonostachys rosea* f. *catenulata* (*Gliocladium catenulatum*) strain J1446 (Prestop®) (GCF1), and on a combined lawn of both vermicompost tea and Prestop® (VCGCF1). Means separated using Tukey's HSD test in R.

V8 media should favour pathogen growth. My hypothesis was that the increased pathogen growth on V8 media would increase the spread between treatments, but this rich medium seemed to have the opposite effect of masking the differences (Figure C.1) and producing inconsistent results (Figure C.2). Furthermore, image analysis was difficult because of the media's opacity and its colour similarity to Forc. This was the reason that ½ PDA was chosen for the trials: this would seem to validate the assumption that a weaker nutrient media would bring out competitive effects. This is similar to the reasoning that biological control of plant pathogens occurs where available nutrients are at an intermediate level, for example in peats that have a relatively high carrying capacity (light peats) compared with those that are fully degraded (dark peats) (Boehm & Hoitink 1992; Hoitink & Boehm 1999).

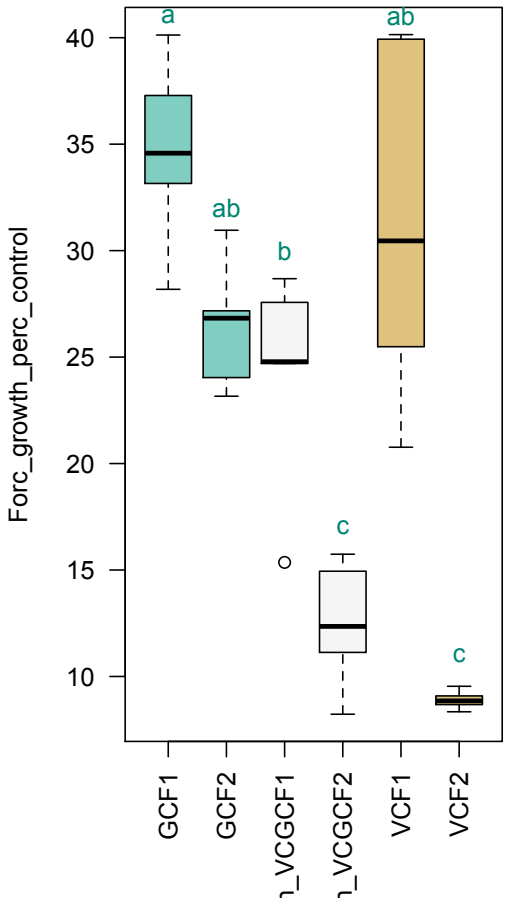


Figure C.2.2. Growth of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* on V8 agar on a lawn of 48 h vermicompost tea (VCF1), on a lawn of *Clonostachys rosea* f. *catenulata* (*Gliocladium catenulatum*) strain J1446 (Prestop®) (GCF1), and on a combined lawn of both vermicompost tea and Prestop® (VCGCF1). Means of n=5 separated using Tukey's HSD test in R.

The V8 media did favour the growth of *Fusarium* in isolation compared with other media, but when vermicompost and the biological control agent were added, suppression levels were similar to those found on half-strength PDA. Interestingly, compared with the ½ PDA where the biological control agent provided the best suppression, in this test vermicompost provided the best suppression, either alone or in combination with the biocontrol agent, although the effect was not statistically significant.

Appendix D.

In planta inoculation method sequence test (D1) and autoclaved vermicompost comparison (D2)

1. Changes in plant growth trial inoculation schedule do not affect disease severity

Summary:

Experiment logistics dictated that the inoculation schedule differed between plant trials. This experiment demonstrates that differing the hold times at various stages in the procedure does not affect the interpretation of data in the plant trials.

Materials and Methods

48 hour vermicompost tea and *Rhizoctonia solani* inoculum was prepared as described in Chapter 4. Groups of 6 pots planted with 10 radish seeds per pot were inoculated with both vermicompost tea and *R. solani* with the following differences: media was inoculated with both and immediately seeded (RS), inoculated with both and then seeded 24 hours later (SD), or inoculated with vermicompost tea, incubated 24 hours, then inoculated with *R. solani* and seeded (VW). All treatments were seeded simultaneously to each other. Area under the disease progress curve (AUDPC) was calculated in R 3.2.2 (http://www.r-project.org/) according to the automated method described in Appendix A from disease severity measurements on days 8, 16, 21, and 23. On day 23 aboveground plant parts were collected, dried in a 50ºC oven for 48 hours, and weighed. Germinated seedlings were counted on day 5. Statistical significance was determined using Fisher's least significant difference test in R, α=0.05.

Results

Among the variables measured in this experiment, only dry weight measurements showed any significant difference according to the inoculation sequence using the Fisher's LSD test. None of these differences were consistent among

treatments. No difference between treatments was found for AUDPC, disease severity, or germination rate (Figure D.1).

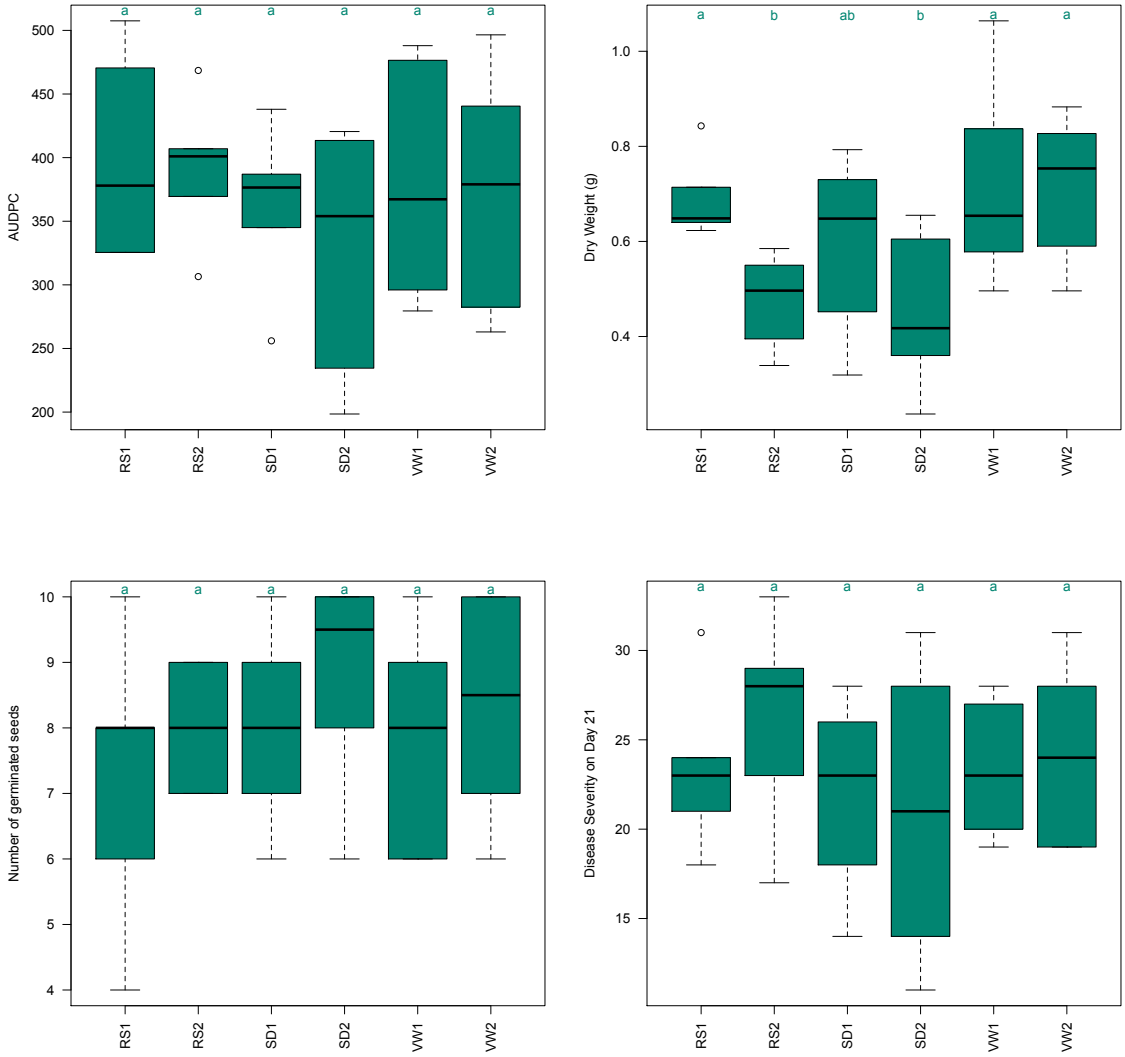


Figure D.1. Area under the disease progress curve (AUDPC), dry weight, germination, and disease severity values for groups of 6 pots planted with 10 radish seeds each, and inoculated with 48 hour vermicompost tea and *Rhizoctonia solani*. Media was inoculated with both and immediately seeded (RS), inoculated with both and then seeded 24 hours later (SD), or inoculated with vermicompost tea, incubated 24 hours, then inoculated with *R. solani* and seeded (VW). Statistical significance was determined using Fisher's least significant difference test in R, α=0.05.

Conclusion

We hypothesized that delays in the inoculation procedure could make our media more or less disease conducive based on differences in the amount of time that inoculum was allowed to colonize ahead of seeding. Inconsistency in the inoculation schedule was inevitable so we tested changes in the timeline independently to ensure that these possible effects were accounted for. No consistent, significant differences in disease data were found for all variables. The increased time that the VW treatment was allowed to incubate with vermicompost before introduction of *R. solani* produced a higher plant dry weight value than the other two treatments, suggesting that pre-inoculation of the media with vermicompost helped the radish plants grow in the presence of the pathogen. This effect was significant according to Fisher's LSD test, by comparison the more stringent Tukey HSD test found a significant difference only between the replicates SD2 and VW2 so further testing would be needed to determine whether this is a meaningful difference. Caution should be used when comparing between trials using the dry weight variable alone because of this possible effect.

2. Autoclaved Vermicompost

Summary

Does the addition of viable vermicompost provide a nutritional or other plant growth effect on the plants compared with non-viable (autoclaved) vermicompost? Vermicompost tea potentially constitutes a strong fertilizer effect which would affect disease measurements, this was found to not be the case in our experimental system in Chapter 4. This quality control experiment examines the effect of autoclaving on vermicompost's potential plant growth promotion.

Materials and Methods.

48 hour vermicompost tea and cucumber pots were prepared as per Chapter 4. Autoclaved vermicompost tea (noVC) was added to all treatments not receiving vermicompost tea (VC). Cucumber seedlings were grown under the same conditions given in Chapter 4.

Results.

No significant difference was found according to Fisher's LSD test in R:

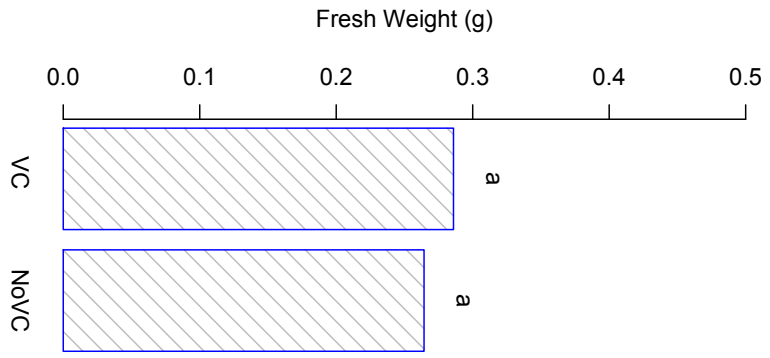


Figure D.2. Shoot fresh weight of cucumber seedlings grown in media inoculated with 50ml of 48h aerated vermicompost tea (VC) vs. 50 ml of autoclaved vermicompost tea (noVC). The vermicompost addition treatment does not produce an effect at this stage according to Fisher's LSD test. N=50.

Conclusion

Adding viable vermicompost does not significantly affect the shoot weight of cucumber seedlings in our experimental system compared with autoclaved vermicompost. The presence/absence of vermicompost tea is controlled for by the negative control treatments in all of the plant trial experiments.

Appendix E.

Carrying capacity of inoculated potting mix

We hypothesized that increasing concentrations of organisms from vermicompost water extract would produce a greater interaction with the biological control agents in our plant disease assay. Increasing microbial populations in plant substrates is usually performed by adding available carbon. Including greater concentrations of microbial substrate in the media would affect the growth of the treatment biological control organisms in the experiments, indeed adding substrates that are available to the biocontrol agent or pathogen have been demonstrated to have profound effects on biocontrol (Hoitink & Boehm 1999; Li et al. 2012). We inoculated potting media substrate with a wide range of concentrations of vermicompost organisms, to determine whether the greater concentrations would persist long enough to perform experiments. To quantify microbial populations we used the FDA method described in Chapter 3, as well as direct plating of dilution series. Pots were filled with 2x autoclaved potting mixture, and inoculated with 0, 1, 10, 100, or 500 mL of 48 h aerated vermicompost tea, the same batch that was used the *in vitro* Forc / Vermicompost / *C. rosea* assay in Chapter 4. Samples were collected over a two-week period and processing and analysis for FDA (Figure E.1) and plating (Figure E.2). We found that the high concentration treatments quickly crashed, and all microbial populations equalized within a week (Figure E.3). Future trials could address this issue by utilizing a substrate for the microbial population that is not available to the biocontrol agent or pathogen.

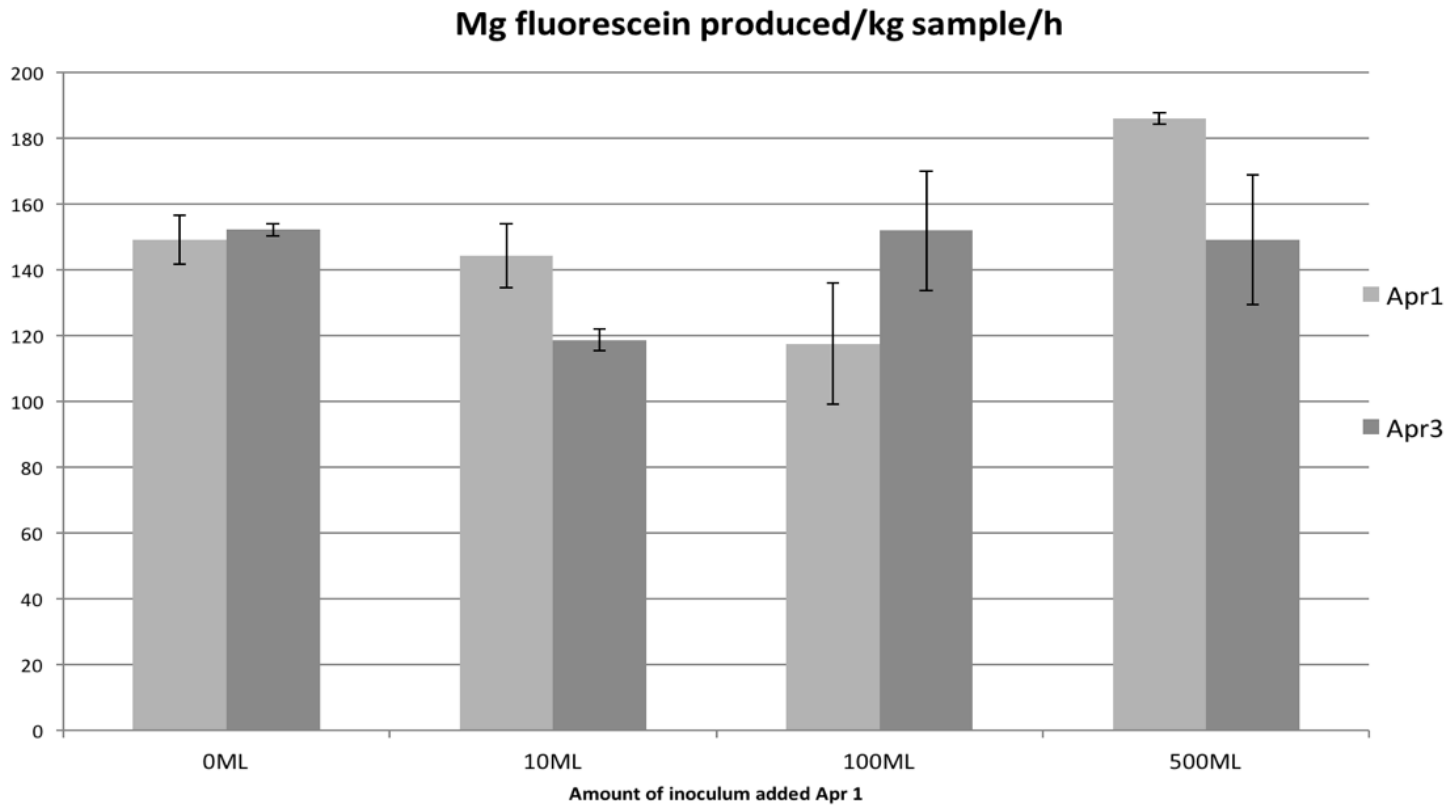


Figure E.1. FDA analysis of sterile potting mix inoculated with four levels of aerated vermicompost tea immediately after inoculation and two days later

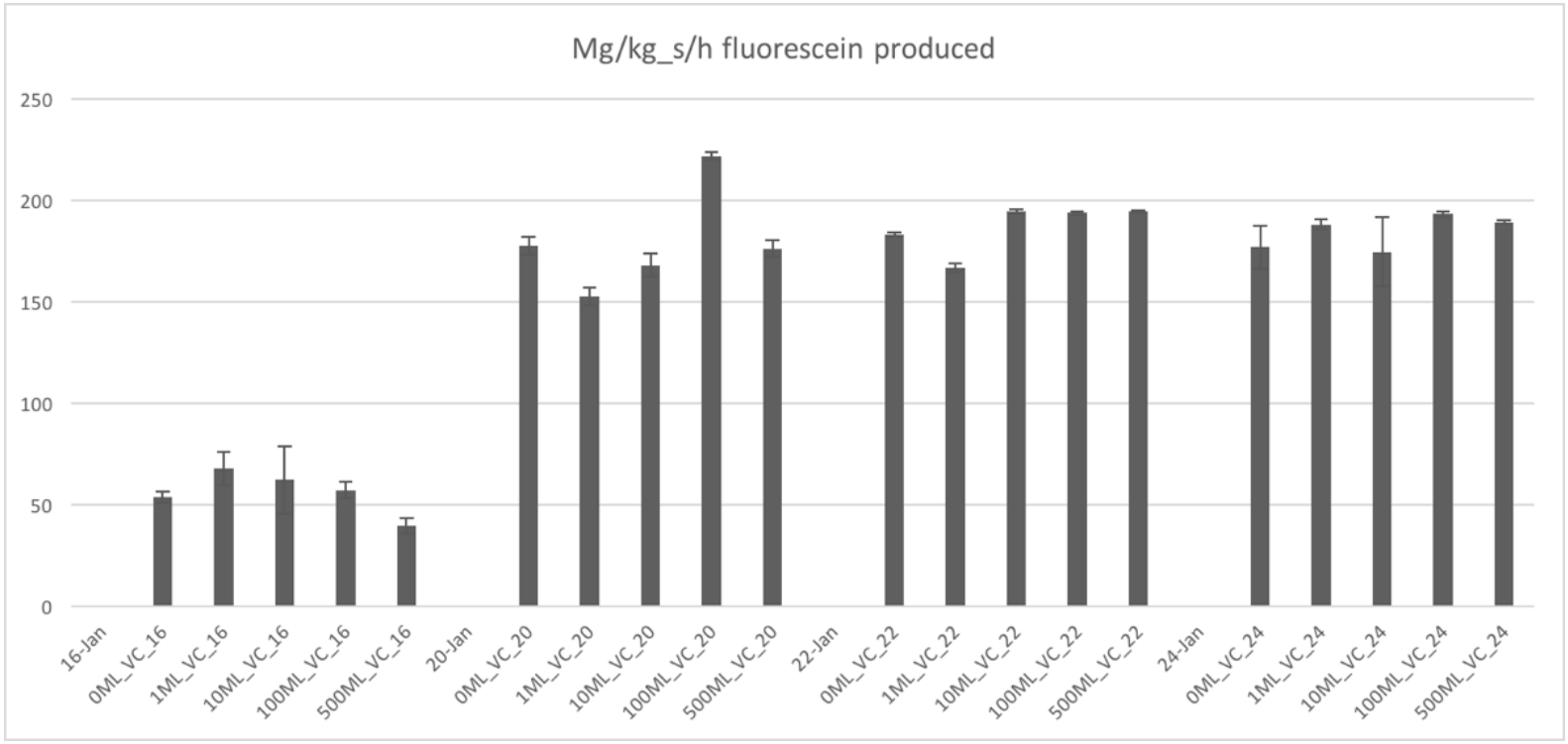


Figure E.2. FDA analysis of sterile potting mix inoculated with four different amounts of aerated compost tea over eight days.

Dilution-series plating showed that soils inoculated with compost tea have a much greater microbial activity than sterilized soils. The results from the carrying capacity trial showed that over time this does not remain the case and the trend appears to become reversed within a week (Figure E.3). It appears that the higher inoculum levels might be expending the nutrition in the system within the first four days, and remaining at low levels thereafter, whereas the uninoculated control takes until the eighth day to decline in population because of expended nutrients, with the lower inoculum levels presenting an intermediate condition.

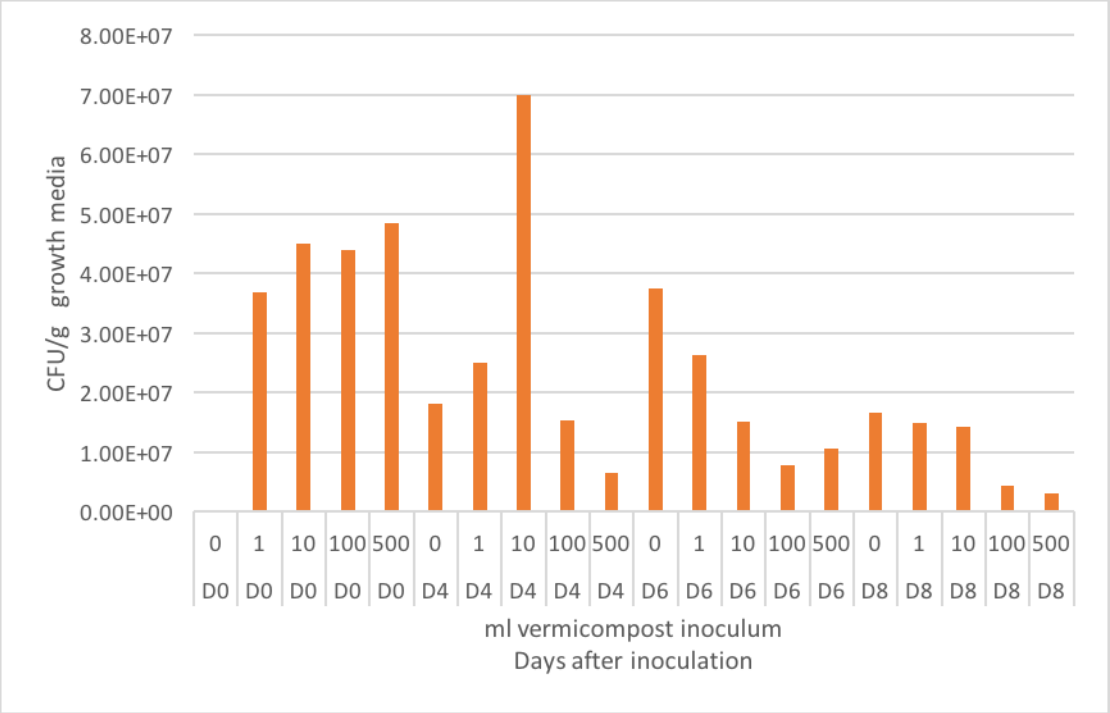


Figure E.3. Culturable organism counts from spread-plate assay of growth media inoculated with 0-500 mL of vermicompost tea. D value indicates days after inoculation.