COMPOST WATER EXTRACTS AND SUPPRESSION OF ROOT ROT (F. SOLANI F. SP. PISI) IN PEA: FACTORS OF SUPPRESSION AND A POTENTIAL NEW MECHANISM

by

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As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Stacy Joy Tollefson, titled Compost Water Extracts and Suppression of Root Rot (*F. solani* f. sp. *pisi*) in Pea: Factors of Suppression and a Potential New Mechanism and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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DEDICATION

This dissertation is dedicated to my dad, Richard Lebowitz, who lost his battle with multiple myeloma cancer on March 14, 2012, while I was still working on my degree. I wish he was still here to see me graduate.

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ABSTRACT

One of the motivating reasons for the development of hydroponics was avoidance of root pathogens. Hydroponics involves growing crops in relatively sterile media, isolated from the underlying soil which may have disease pressure. However, even when hydroponics is coupled with controlled environments such as high tunnels and climate-controlled greenhouses, soil-borne pathogens can enter the growing area and proliferate due to optimal environmental conditions for pathogen growth. Control of root pathogens is difficult and usually achieved through synthetic fungicides since few biocontrol options are available. Compost water extracts (CWE) have recently been gaining the attention of greenhouse growers because they may be a low-cost, environmentally friendly approach to control root disease. CWE are mixtures of compost and water incubated for a defined period of time, either with or without aeration, and with or without additives intended to increase microbial populations, which in turn suppress disease. Much anecdotal, but very little scientific, evidence exists describing CWE effect on suppressing soil-borne pathogens. The present study 1) examined the effect of an aerated CWE on disease suppression at the laboratory scale and in container studies using different soilless substrates, 2) investigated a phenotypic change at the root level caused by CWE that may be associated with disease suppression, and 3) isolated some factors in the production of CWE that affect the ability of a CWE to suppress disease. The common model pathogen-host system of *Fusarium solani* f.sp. *pisi* and pea was used to examine CWE-induced disease suppression, with information then being translatable to similar patho-systems involved in greenhouse crop production .

In the first study, laboratory-based root growth and infection assays resulted in 100% suppression of *F. solani* when roots were drenched in CWE. These protected seedlings were then taken to a greenhouse and transplanted into fine coconut coir, watered with hydroponic nutrient solution, and grown for five weeks. At the end of the experiment, 23% of the shoots of the pathogen-inoculated, CWE-drenched seedlings remained healthy while only 2% of the inoculated seedlings without CWE drench remained healthy. All of the roots of the inoculated seedlings developed lesions, even those drenched in CWE. However, 29% of the CWE drenched roots were able to recover from disease, growing white healthy roots past the lesion, while only 2% recovered naturally. A shorter-term container study was conducted in the laboratory to determine the effects of CWE-induced suppression when peas were grown in different substrates and to determine if the hydroponic nutrient solution had an effect on the suppression. Peas were grown in sterilized fine and coarse coconut coir fiber and sand irrigated with water, with a second set of fine coir irrigated with hydroponic nutrient solution. Pea seeds with 20-25mm radicles were inoculated with pathogen and sown directly into CWE-drenched substrate and grown for three weeks. At the end of the experiment, 80%, 60%, 90%, and 50% of the shoots of the inoculated, CWE-drenched seedlings remained healthy when grown in fine coir, coarse coir, sand, and fine coir irrigated with hydroponic nutrient solution, respectively. Nearly 100% of the roots grown in coconut coir substrates again developed necrotic lesions but 83%, 87%, 100%, and 87% grew healthy roots beyond the disease region. The hydroponic nutrient solution had a negative effect on suppression, with a reduction of at least 30 percentage points. Sand demonstrated a natural ability to suppress *F. solani*. Only 23% of inoculated seedlings

had dead or dying shoots by the end of the experiment (compared to 77-80% in coir substrates) and although all but one of the roots developed lesions, all were able to recover on their own with CWE. CWE further increased shoot health and also prevented 57% of the roots from developing lesions.

In a second study, two different CWE were used to examine the effect on root border cell dispersion and dynamics in pea, maize, cotton, and cucumber and its relation to disease suppression. Dispersal of border cells after immersion of roots into water or CWE was measured by direct observation over time using a compound microscope and stereoscope. Pictures were taken and the number of border cells released into suspension were enumerated by counting the total number of cells in aliquots taken from the suspension. Border cells formed a mass surrounding root tips within seconds after exposure to water, and most cells dispersed into suspension spontaneously. In CWE, >90% of the border cell population instead remained appressed to the root surface, even after vigorous agitation. This altered border cell phenomena was consistent for pea, maize, and cotton and for both CWE tested. For most cucumber roots (n=86/95), inhibition of border cell dispersal in both CWE was similar to that observed in pea, maize, and cotton. However, some individual cucumber roots (8±5%) exhibited a distinct phenotype. For example, border cells of one root immersed into CWE remained tightly adhered to the root tip even after 30 minutes while border cells of another root immersed at the same time in the same sample of CWE expanded significantly within 5 minutes and continued to expand over time. In a previous study, sheath development over time in growth pouches also was distinct in cucumber compared with pea, with detachment of the sheaths over time, and root infection was reduced by only 38% in

cucumber compared with 100% protection in pea (Curlango-Rivera et al. 2013). Further research is needed to evaluate whether this difference in retention of border cell sheaths plays a role in the observed difference in inhibition of root infection.

In the third study, a series of investigations were conducted to isolate different factors that contribute to the suppression ability of a CWE by changing incrementally changing some aspect of the CWE production process. The basic aerated CWE recipe (with molasses, kelp, humic acid, rock phosphate, and silica) provided 100% protection of pea from root disease while the non-aerated basic recipe CWE provided 72% protection. Aerated CWE made of only compost and water resulted in 58% protection. It was found that molasses did not contribute to the suppression ability of the ACWE, while kelp contributed strongly. When soluble kelp was added by itself to the compost and water, the CWE provided 80% suppression. However, when all additives were included except molasses and kelp, suppression remained high (93%) indicating that humic acids, rock phosphate, and/or silica were also major contributors toward the suppression effect. Optimal fermentation time for ACWE was 24 hr to achieve 100% suppression, with increased time resulting in inconsistent suppression results. Optimal fermentation time for NCWE was 3 days or 8 days.

These studies are important contributions to understanding the differences that might be expected in CWE suppression when growing in different substrates, some of the factors in the production of CWE that affects the ability of a CWE to suppress disease, and the phenotypic effect CWE has on the root zone of plants and the possible relationship between that effect and disease suppression.

Key words: compost water extract, compost tea, disease suppression, *F. solani*, peas, soilless substrate, border cells

1. INTRODUCTION

1.1 Statement of the Problem

Soil-borne pathogens cause about 90% of the 2000 major diseases in the principal crops grown in the U.S. (MSU, 2011), resulting in 10% of all vegetable crop losses in the U.S. in 2009 (SAAESD, 2013). One of the motivating reasons for the development of hydroponics was avoidance of soil-borne pathogens (Stanghellini and Rasmussen, 1994). Hydroponics involves growing crops in relatively sterile media, isolated from the underlying soil which may have disease pressure, or fertility, salt, or drainage inadequacies (Jensen, 1991). However, even when hydroponics is coupled with controlled environments such as climate-controlled greenhouses, soil-borne pathogens can enter in many ways: through contaminated soil on workers' shoes or equipment; seeds may be contaminated or transplants produced off-site may bring pathogens with them; propagation materials such as sand and peat may be infested; and, airborne spores can enter through cracks in the greenhouse structure, vents, and open doors (Paulitz and Belanger, 2001; Stanghellini and Rasmussen, 1994). Once introduced to the system, these pathogens often reproduce rapidly because the environmental conditions maintained for the crop are conditions that also favor growth of the pathogens. Constant warm air and water temperatures, high humidity, abundant nutrition and water at the root zone, and usually low oxygen levels all support growth of root pathogens (Takushi, 2014). In addition, sterilization and sanitation are stressed in hydroponic systems, thus there is potentially very little beneficial biota in the growing media or nutrient water to oppose the growth of disease organisms as there might be in a robust organic soil (Paulitz and Belanger, 2001). Disease can spread rapidly, especially in liquid hydroponic systems

such as NFT (nutrient film technique) and deep flow systems since all the plants are subjected to the same water that may be infested with a pathogen. Root pathogens can also spread quickly through aggregate systems when a trough, raised bed, or bag culture system is used since many plants share the same nutrient water and substrate. If water comes in contact with a pathogen from one plant root, it may transmit spores or hyphae to the roots of plants downgradient. Recirculating systems in conjunction with aggregrate systems can also spread disease rapidly if sterilization is inadequate or inefficient.

Fungal pathogens cause the most destructive root diseases in hydroponic systems, resulting in up to 100% loss of a crop (Duffy and Defago, 1999; Estevez de Jensen and Abad, 2009; Stanghellini and Rasmussen, 1994). Species in the *Fusarium, Pythium, Phytophthora*, *Plasmospara*, and *Olpidium* genera are the most destructive, causing seedling damping-off, crown and root rot, and vascular wilt in a wide range of crops including tomatoes, peppers, cucumbers, lettuce, strawberries (Stanghellini and Rasmussen, 1994).

Root pathogens in hydroponic systems are difficult to prevent and control. Few resistant cultivars are available for greenhouse vegetable crops (Stanghellini and Rasmussen, 1994). If recycling nutrient solution, sterilization is critical. But once a root pathogen has been established, it is difficult to eradicate. In the field, solarization, fumigation, or chemical treatment is common to kill existing pathogenic (and beneficial) fungi in the soil before seeding or transplantation. In the greenhouse, these practices are not employed since soilless substrates are assumed to be relatively sterile before use. Chemical control is sometimes used as a curative but primarily preventative requiring chemical application before the signs of disease, but is problematic in greenhouse crop

production for multiple reasons. First, there are very few fungicides registered for use in greenhouse crops and they generally have a re-entry period and a post- harvest interval of one week or more so they cannot be used in continuous harvest situations. Fungicides can be phytotoxic to plants even at levels approved for use. They have been found to be unreliable, and prolonged use of the relatively limited chemical options can lead to resistance in pathogens (Duffy and Defago, 1999; Paulitz and Belanger, 2001).

At the same time, most greenhouses use integrated pest management (IPM) plans in which pest problems are solved in ways that are the most economical and least harmful to human health and the environment. Biocontrol is used whenever possible, including adding natural enemies to control arthropod pests, and plant-based insecticides such as neem oil and pyrethrin rather than synthetic pesticides which may leave a more lasting harmful residue on the edible products. Using any synthetic pesticides in the greenhouse is considered a last resort.

For all of these reasons, manipulating the root environment with beneficial microorganisms that might naturally protect the plant roots from disease has been studied. Much research has been done, and continues to be done, on suppressive soils. Some of the most abundant microorganisms associated with the suppressive of the soils have been isolated and used in biocontrol products. However, still relatively few biocontrol products have been approved for use (Shipp et al., 1994; Van Lenteren, 2000). *Trichoderma harzianum*, *Bacillis subtillis*, *Pseudomonas* sp., and nonpathogenic *Fusarium oxysporum* are some of the common species used in the biocontrol of fungal root pathogens (Duffy and Defago, 1999). These beneficial microorganisms can be added to a substrate mix, applied as a drench, or added to a nutrient solution so that their

populations increase as they colonize the rhizosphere and protect the roots before a pathogen is encountered.

Compost water extracts (CWE) may be a viable alternative to synthetic fungicides in greenhouse situations because of the relatively low-cost and low-impact on human health and the environment. CWE are mixtures of compost and water incubated for a defined period of time, either with or without aeration, and with or without additives intended to increase microbial populations which in turn are thought to suppress disease. CWE have been shown to suppress a wide range of foliar and soil-borne plant diseases (Litterick et al., 2004; Noble and Coventry, 2005; St. Martin and Brathwaite, 2012; Santos et al., 2011; Scheuerell and Mahaffe, 2002). More research has been done on suppressive composts than CWE. However, the use of suppressive composts in hydroponic crop production is more problematic than CWE. Compost needs to be incorporated as part of the root-zone substrates, changing the texture and water-holding capacity, while CWE made from a suppressive compost, can be used as a drench or in the nutrient solution without making much change to the root-zone system dynamics. Before CWE can be used on a large scale, however, much more needs to be known about the mechanisms of suppression and factors contributing to the suppression induced by CWE so that more reliable and consistent prediction can be made for growing systems.

Many research approaches involve characterizing, isolating, and testing the most abundant microbe species within a suppressive compost or CWE, and using those species (as described above) as biocontrol agents. While some single species have been found to be helpful in suppressive soil-borne pathogens, there is evidence that microbial communities can have more effect on suppression (McKellar and Nelson, 2003; Chen et

al., 2012). Many composts and compost teas, which contain a complex consortia of microorganisms, have indeed, been found to be suppressive to various fungal pathogens (Hoitink and Boehm, 1999; Litterick et al., 2004; Mehta et al., 2014; Noble and Coventry, 2005; St. Martin and Brathwaite, 2012; Santos et al., 2011; Scheuerell and Mahaffe, 2002). Using CWE, therefore, may result in better control than a single species biocontrol formulation.

A recent discovery in the root zone of CWE-drenched seedlings may provide an explanation of how CWE induce disease suppression. Curlango-Rivera et al. (2013) discovered a phenotypic change in the root zone border cells when the root is exposed to CWE. Root border cells are specialized cells that are only produced by the root tip. The cells quickly release in the presence of free water, yet in the presence of CWE, border cells remain intact on the root tip and also move into the zone of elongation, which is most susceptible to infection by pathogens. While the mechanism behind this change is not known, it may be a viable predictor of suppression ability of a CWE and needs additional research.

Any successes that have been reported with CWE use in the field have been largely anecdotal (Ingham, 2002). While research investigating the suppression ability of CWE is increasing, there are relatively few studies presently in the literature. The majority of the studies conducted with CWE have been laboratory-based and reported success in using CWE to suppress a number of pathogens. These studies involved adding CWE to petri dishes of pathogen and measuring the affect on mycelial growth or spore germination. However, very few of these laboratory studies were followed by container or field studies to determine the corresponding efficacy of CWE suppression in plants.

When they have, the level of suppression found in the laboratory did not often correspond to similar suppression in plants. Further, the few field and container studies conducted with CWE report variable degrees of success. It is evident that the physical and chemical properties of the growing substrate and addition of fertilizers and organic amendments can affect the ability of pathogens or beneficial microorganisms to flourish or parish (Alabouvette et al., 1996; Curlango et al., 2013; Hoitink and Boehm, 1999; Janvier et al., 2007; Lozano et al., 2009). Therefore, more studies need to be conducted to understand the interplay between CWE, substrate, and fertilizers.

Research that has been done on CWE has been complicated by the myriad of variables involved: the variety of compost sources and composting methods, compost maturity, different extract methods, extraction time, and dilution rates. Very few studies have explored the effects of these factors on the ability of CWE to suppress disease (Scheuerell and Mahaffe, 2002, 2004; St. Martin et al., 2012). These factors need to be understood in order to be able to produce CWE that will most consistently provide disease suppression. The sources and characteristics of the compost used in CWE production needs to be examined closely. Since the compost sources are always variable, prediction of their ability to suppress disease depends on how well understanding of the mechanisms of disease, and learning characteristics of the composts and its subsequent CWE with disease suppression.

Prediction of the suppressiveness of a given CWE has largely been attributed to the number and distribution of the microbial flora in the CWE. Elaine Ingham (2003) claimed that a CWE must have large numbers and diversity of bacteria, fungi, and protozoan to be suppressive. Ingham started a business of conducting microbial testing

of CWEs for growers (Ingham, 2014). However, the research does not necessarily support this predictive model (Scheuerell and Mahaffee, 2002). Curlango-Rivera et al. (2013) used a more promising approach. They used a growth pouch assay to test a given CWE on a specific plant and specific pathogen. Roots are drenched in CWE and then inoculated with the pathogen, and then observed for sign of disease. These pouches provide an ideal environment for the growth of pathogens, including high humidity, and a much better condition than in the field. After repeated trials, these researchers observed 98%-100% protection of pea seedlings whose roots, inoculated with a highly pathogenic strain of Fusarium (*F. solani* f.sp. *pisi*), were drenched in CWE. However, when seedlings were inoculated and grown in soil-drenched with CWE, results were variable, depending on soil type. Seedlings grown in sand exhibited 100% protection while seedlings grown in a heavy clay soil exhibited only 55% of the seedlings were protected. Hoitink and Boehm (1999) and Lozano (2009) discuss how important the nature and properties of the root-zone substrate were in their relation to the ability of a CWE to sustain a suppressive effect on disease (Hoitink & Boehm, 1999; Lozano et al., 2009).

The study by Curlango-Rivera et al. (2013) illustrated the complexities to successfully predict the suppression ability of a single CWE. The majority of studies on CWE have been *in vitro*, demonstrating much success in producing CWE that suppresses a variety of soil-borne pathogens (Santos et al., 2011; Scheuerell and Mahaffee, 2002; St. Martin and Brathwaite, 2012). However, few studies have been conducted using container root-zone media with soilless substrate in particular, or field investigation and all of those studies have had varied results. There is a need to further investigate the use

of CWE to control soil-borne pathogens in real-world field studies or container studies for greenhouse crops.

In summary, there is a much work that needs to be done to further the knowledge base in the area of CWE and disease suppression, particularly in regards to its use to suppress soil-borne fungal pathogens. There is a need to determine mechanisms for the disease suppression, to evaluate factors contributing to suppression, and to evaluate predictive models in relation to real-world behavior of CWE in the field. St. Martin and Brathwaite (2012) sum up the significance of the research done in this study as follows:

> "… compost teas as soil drenches may be an effective control strategy for root diseases in soil-less production systems. However, further research is needed in order to gain a greater understanding of the factors affecting suppressivity of compost teas and mechanisms used to effect control. This may prove useful in assessing the utility of *in vitro* pathogen screening results as predictors of disease under *in vivo* and field conditions. As it stands, testing compost teas for soil-borne disease suppression under simulated field conditions, with the crop growing in pathogen-inoculated soil or growing media, might be a better predictor of field suppression than *in vitro* assays."

The present study examined the effect of a CWE on disease suppression at the laboratory scale and in container studies, and determined some factors involved in

suppression including changes in CWE production methods and a description of a phenotypic change at the root level that may be associated with disease suppression.

First, the effect of a CWE on suppression of *F. solani* in pea was determined using root growth and infection assays. These laboratory-based assays resulted in 100% suppression of *F. solani* when roots were drenched in CWE. These protected seedlings were then taken to a greenhouse and transplanted into fine coconut coir, watered with hydroponic nutrient solution, and grown for five weeks. Disease incidence and plant health were compared to the original laboratory-based findings and potential reasons for the difference in suppression were described. A separate smaller-scale container study was conducted in the laboratory to determine the effect of different substrates and the use of hydroponic nutrient solution on disease suppression of the same CWE. Fine and coarse coconut coir and sand were used as media and all were irrigated with water. One set of fine coconut coir was irrigated with hydroponic nutrient solution so that the results could be compared to the greenhouse study and to the fine coir irrigated with water.

Second, a cursory finding by Curlango-Rivera et al. (2013) was further investigated, dealing with a phenotypic change in the root zone of seedlings drenched in CWE. They found that in the presence of CWE, border cells remain aggregated to the root tip and also move onto the zone of elongation, which is most susceptible to infection by pathogens. This continual coverage of the root by border cells was correlated with enhanced disease suppression but was not systematically researched until the study included herein. Two different CWE were used to investigate their effects on root border cells of peas, corn, cotton, and cucumber. All of these plants have numbers of border cells that range from many to few, and have been study previously in relation to

border cell effects on pathogens. Cucumber, in particular, was studied because of the variability seen in the disease suppress by CWE in this crop compared to more consistent results with pea.

Lastly, different factors associated with the ability of a CWE to suppress disease were determined by incrementally changing some aspect of the CWE production process. Some factors evaluated included aeration, heat sterilization, addition of chlorine, increasing compost/water contact time, and eliminating additives.

1.2 Role of the Researcher

I was entirely responsible for the study included in Appendix A: *Effect of a Compost Water Extract on Growth and Root Disease Suppression in Pea Grown in Different Substrates*. I was the primary researcher, writer, and first author. I planned, designed, and implemented the studies myself, including the collection and analysis of the data, and writing the results. Professor Martha C. Hawes and post-doc Dr. Gilberto Curlango-Rivera provided laboratory space and materials and advised and assisted as needed. Dr. Curlango-Rivera trained me in the laboratory protocols used in the study and prepared the pathogen in the concentrations needed for the experiments. Dr. Gene Giacomelli provided greenhouse space and materials, as well as advisement and assistance as needed.

My role in the study included in Appendix B: *Altered Carbon Delivery from Roots: Rapid, Sustained Inhibition of Border Cell Dispersal in Response to Compost Water Extracts* was less active. I provided an independent new source of CWE (CWE

#2) to test in addition to the CWE provided by Tom Pew. I assisted in developing the laboratory protocols and participated in the writing, review, and feedback for the paper.

I was entirely responsible for the study included in Appendix C: *Factors Affecting the Ability of Compost Water Extracts to Suppress Root Disease in Pea*. I was the primary researcher, getting assistance from post-doc Dr. Gilberto Curlango-Rivera as needed. Dr. Curlango-Rivera provided the pathogen for the studies. I planned, designed, and implemented the studies myself, including the collection and analysis of the data, and writing up the results. Again Professor Martha C. Hawes provided laboratory space and materials.

1.3 Review of the Literature

Compost and compost teas have attracted attention of farmers for their potential as a low-cost, environmentally-friendly alternative to chemical pesticides. Beginning in the mid-1990's, largely through the work of Elaine Ingham (2002), claims were made (and were still at this writing) that compost "tea", or more technically compost water extracts (CWE), can be used to suppress disease. Compost water extracts (CWE) are mixtures of compost and water incubated for a defined period of time, either with or without aeration, and with or without additives intended to increase microbial populations (Scheuerell and Mahaffee, 2002). Theses mixtures, which also contain plant nutrients and plant hormones, are applied to the soil or foliage in order to enhance growth or suppress disease.

While promising effects have been claimed by many regarding the disease suppression abilities of CWE, relatively few scientific research studies can be found in

the literature (Scheurell and Mahaffee, 2002; St. Martin and Brathwaite, 2012). It is difficult to reproduce studies on CWE and generalize results from such studies, because of the variability in compost source material, inconsistencies from batch to batch, inconsistencies in CWE production methods, and incomplete descriptions of source materials and methods.

Most of the research conducted on CWE involved spraying it on plant leaves suppress foliar diseases. There has been limited work regarding CWE and soil-borne pathogens, and the majority of that research has been done in the laboratory. Very few studies have been conducted using CWE as a drench to suppress soil-borne pathogens in container media or in the field. A few literature reviews have been conducted to date, summarizing the studies involving CWE and disease suppression (Litterick et al., 2004; St. Martin and Brathwaite, 2012; Scheurell and Mahaffee, 2002, 2006). Pertinent studies from these reviews and others will be briefly described below.

1.3.1 CWE-induced disease suppression in the laboratory and field

The limited research that has been done on CWE has provided evidence that CWE have the ability to suppress foliar and soil-borne diseases. Most of the research involving CWE and soil-borne pathogens have been conducted *in vitro*. Decreased germination of spores or decreased growth of hyphae in concert with CWE have been reported in several laboratory studies of soil-borne pathogens in the fungal genera including *Fusarium, Verticillum, , Rhizoctonia,* and *Sclerotium,* as well as those in the sub-group of oomycetes such as *Pythium, Alternaria, Phytophthora* (Alfano et al., 2011; Curlango-Rivera et al., 2013; Dianez at al., 2006; Dionne et al., 2012; El-Masry et al.,

2002; Kirkeni et al., 2007; Marin et al., 2013; Pane et al., 2014). However, very few of these laboratory studies were followed by container or field studies to determine the corresponding efficacy of CWE suppression in the subsequent growing plants. When they have, the level of suppression found in the laboratory did not always correspond to similar suppression in plants. Pane at al. (2012) found ten CWE that inhibited *A. alternaria*, *B. cinerea*, and *P. lycopersici* both *in vitro* and *in vivo*, but the ones that were most disease inhibiting *in vitro* were not often the ones that reduced disease the most in tomato plants. In a study of the effect on non-aerated CWE on foliar pathogen *B. cinerea*, Kone et al. (2010) found a higher degree of suppression when a CWE was applied to plants compared to the inhibition of mycelial growth found in the laboratory. The effectiveness of foliar application of CWE on gummy stem blight (*D. bryonae*) was studied by Marin et al. (2013), who found 100% suppression *in vitro*, but overall poor control of disease in melon plants. Curlango-Rivera et al. (2013) reported 98-100% protection of pea seedlings from *F. solani* f. sp. *pisi* in growth pouches and 100% protection when grown in sand but only 55% were protected when grown in a heavy clay soil.

The few field and container studies conducted with CWE report varying degrees of success. Field studies using CWE as a soil drench have resulted in decreased incidence of *Streptomyces scabiei* in potato, *Ralstonia solanacearum* in eggplant, *Fusarium moniliforme* in rice, and *Fusarium solani*, *Rhizoctania solani*, and *M. Phaseolina* in bean plants (Al-Mughrabi et al., 2008; Islam et al., 2014; Larkin et al., 2008; Manandhar et al.,2008; Mansour at al., 2011), although no effect on *Fusarium sambucinum* and *Rhizoctonia solani* in potato was observed (Al-Mughrabi et al., 2008;

Larkin et al., 2008). CWE drenching decreased seedling damping-off and wilt incidence in cucumber caused by *P. aphanidernatum* and *P. ultimum* differentially depending on type of compost, level of aeration, and additives used (Jack & Nelson, 2010; Scheuerell & Mahaffee, 2004). Sang (2010, 2011) found CWE from four different composts reduced severity of disease caused by *Phytophthora* sp. and *Colletotrichum* sp. in pepper and cucumber when grown in standard potting mix. In a study of the effect of CWE on *F. oxysporum* f.sp. *radicis lycopersici* in tomato, Hibar at al. (2006) found that four different CWE reduced the severity of disease when grown in soilless media with hydroponic nutrient solution.

Two studies soaked seeds in CWE before sowing and measured the suppressive effect. In a study by Ozer et al. (2006) onion seeds were soaked in two different CWE for 30 minutes, in pathogen suspension (*Aspergillis niger*) for 12 hours, and then air-dried. When the seeds were germinated in the laboratory, there was no difference in disease severity using either CWE that was tested, but when the seeds were sown into a sterilized soil, incidence of black mold was reduced in the bulbs. Mansour at al. (2011) soaked bean seeds in three different CWE for 48 hours, air-dried the seeds, and then sowed them into soil inoculated with *F. Solani*, *R. solani*, and *M. Phaseolina.* All three CWE tested increased bean seedlings survival compared to controls.

The physical properties of the root-zone growing substrate were important, albeit often overlooked, factors in the ability of a CWE to sustain a suppressive effect (Hoitink & Boehm, 1999; Lozano et al., 2009). Soil texture and structure, clay type, porosity, water potential, and resulting oxygen availability were all factors affecting the ability of pathogens or beneficial microorganisms to flourish (Alabouvette et al., 1996; Janvier et

al., 2007; Lozano et al., 2009;). Some pathogens were naturally more conducive in some textured soils than others. For instance, *Fusarium wilts, R. solani*, and *P. sorghi* have been shown to be more conducive in sand and more suppressive in clay (Amir et al., 1993; Cook and Papendick, 1972; Gill et al., 2000; Hoeper et al., 1995; Schuh et al., 1987). However, Scher and Baker (1980) found a clay loam that was naturally conducive to Fusarium wilt and a fine sandy loam that was suppressive. Likewise, Curlango-Rivera (2013) reported sand being suppressive and a clay loam being conducive to Fusarium root rot when a CWE was used for biocontrol. Hibar (2006) found that perlite was more conducive than peat in its ability to develop *F. oxysporum* f.sp. *radicis lycopersici* in tomato. Lozano et al. (2009) reported that a compost particle size of 2-4mm increased the suppression of *F. oxysporum, P. cinnamomi*, and *M. hapla*.

1.3.2 Biotic and abiotic factors of disease suppression

CWE can be produced using a wide variety of methods. All methods involve mixing a volume of compost into a volume of water and allowing it to remain undisturbed to process for a given amount of time. The process is sometimes called fermentation or brewing. CWE can be prepared with different compost to water ratios, using aeration or not, adding microbial foods or not, and allowing varying amounts of processing time prior to application. Sufficiently detailed descriptions of the production methods of CWE in the literature were often lacking, and if when they were described, the wide differences in methods made it difficult to generalize across studies. Even so, there were some commonalities reported.

There has been much debate about whether aerated CWE (ACWE) or non-aerated CWE (NCWE) were more suppressive. Ingham (2002) claimed that aerated compost teas were the most disease suppressive because they tend to have higher microbial populations and diversity. Yet, only one study in the literature has demonstrated that ACWE outperformed NCWE (Manandar and Yami, 2008). While Marin et al. (2013) claimed that all ACWE outperformed NCWE in inhibition of mycelial growth of all eight pathogens tested, their data show that out of 64 comparisons, ACWE provided better protection than NCWE in only two cases. Other than these instances, the majority of the research supports that non-aerated CWE is more suppressive than aerated CWE or that there was no difference between the performance of the two (Litterick et al., 2004; St. Martin and Brathwaite, 2012; St. Martin et al., 2012; Scheurell and Mahaffee, 2002, 2006).

The majority of studies use NCWE with a fermentation time ranging from 3 days to 14 days and with the vast majority of them reporting suppression (Scheuerell and Mahaffee, 2002). Ingham (2002) claims that brewing ACWE for 18-36 hrs is optimal for disease suppression while Cantisano (1998) advocates at least 24 hr, with 7-14 days being optimal. St. Martin et al. (2012) found maximum suppression of *P. ultimum in vitro* could be obtained in 18 hours for ACWE and 56 hours for NCWE. Dianez et al. (2006), however, found that an ACWE increased when fermented 1 day, 7 days, and 14 days for nine different pathogens. Weltzien (1990) found maximum suppression of *P. infestans* on detached tomato leaves to occur using 7-14 day fermented NCWE. ACWE has been studied much less than NCWE so optimal fermentation times have not been determined.

These variable results have at least partially been contributed to different types of compost (St. Martin et al., 2012).

Ingham (2002) promoted the addition of microbial foods during the production of ACWE. Unsulfured, black strap molasses or fish emulsion was added to promote bacterial growth, while soluble kelp, humic acids, and rock dust powder are used to promote the growth of beneficial fungi. Despite the use of these additives by many backyard gardeners and some smaller scale growers, the vast majority of the peer-reviewed research studies do not include any of these additives. Only one study could be found which compares the suppression of a CWE with and without nutrient additives. Scheuerell and Mahaffee (2004) produced ACWE (36 hours of aeration) with three different compost sources, incorporating non additives, bacterial additive (molasses), of fungal additives (kelp, humic acids, and rock dust.) They found that incorporating no additives or bacterial additives resulted in inconsistent suppression while the fungal additives provided 100% suppression consistently across all three compost sources. Combining the bacterial and fungal additives resulted in no suppression. When making the bacterial ACWE, increasing the concentration of molasses decreased the suppression. Also, adding just 0.01% molasses to a suppressive fungal ACWE significantly decreased suppression. Taking out the rock dust in the fungal CWE recipe did not decrease suppression.

Heat sterilization and micro-filter sterilization have been shown to eliminate or decrease the suppression ability of CWE. Heat sterilization autoclaving resulted in elimination of suppression for both ACWE and NCWE (Dionne et al., 2012; El-Masry et al., 2002; Kone et al., 2010; Pane, et al., 2012; McQuilken et al., 1994). Szczech (1999)

observed enhanced growth of pathogen mycelial growth after autoclaving and microfilter sterilization most likely due to the nutrients in the CWE. Several other researchers found there was still moderate suppressive ability of ACWE and NCWE after autoclaving, although less than that observed with no heat treatment (Cronin et al., 1996; Dianez et al., 2006; Dionne et al., 2012; Siddiqui et al., 2009). Likewise, some studies found that microfilter sterilization eliminated suppression of CWE (El-Masry et al., 2002; Jack and Nelson, 2010; Kone et al., 2010; Pane et al., 2012) while others found reduced, but moderate levels of suppression with microfiltered CWE (Alfano et al., 2011; Cronin et al., 1996; Dianez et al., 2006; Siddiqui et al., 2009). Residual suppressive effect after heat or microfiltration, especially in the cases where it was verified that biological activity ceased, indicated that a chemical by-product produced by the microbes may be responsible for the suppression.

The ratios of compost to water found in the literature to make CWE range anywhere from 1:1 to 1:50 with dilutions made from there. In a review by Weltzien (1990), dilutions ranging from 1:3 to 1:10 all supported suppression for the host-patho systems studied. Marin et al. (2013) found no statistical difference in the suppression of an ACWE and an NCWE using a 1:3 or 1:4 dilution. In a study using CWE as a foliar spray, Welke (2005) found no difference in disease severity when using a single strength or double strength CWE. Barman et al. (2013) and Scheuerell and Mahaffee (2004) found that increasing concentrations of CWE decreased disease severity.

The sources of compost and their maturity also affect their ability to suppress disease. Weltzien (1990, 1991) found that manure composts produced more effective NCWE than composts made only of vegetative materials. More recently, however,

others have found composts made of vegetative wastes such as grape marc, spent mushroom waste and seaweed, to be effective suppressors (Dianez et al., 2006; Marin et al., 2013; Siddiqui et al., 2009; Dionne et al., 2012). Tranker (1992) suggested that composts should be two to six months old when used. Winterscheidt et al. (1990) found that nonaerated CWE produced from horse manure compost was more effective when used at six months than after one year. More recently, Zmora-Nahum et al. (2008) made CWE from various ages of municipal sewage sludge (biosolids) and yard waste. They piled the 3-month old compost in a bin and periodically wet it and turned it, taking samples over time (from 11 to 95 days). They assessed the CWE ability to suppress sclerotia development *in vitro*. They found that sclerotia were suppressed when composts up to 45 days were used but germination of sclerotia were similar to water from days 67 and later.

Abiotic factors in the CWE may also play a role in the ability of a CWE to suppress disease. Temperature, electrical conductivity, pH, carbon to nitrogen ratios, lignin content, iron concentrations, concentrations of different forms of nitrogen have shown to impact suppression (Litterick et al., 2004; Scheuerell and Mahaffee, 2002). For instance, Nelson and Boehm (2002) found that producing CWE at temperatures above 18°C - 21°C reduced biological activity. Scher and Baker (1980) studied two soils that were naturally suppressive to *Fusarium oxysporum* f. sp. *dianthi*. When they adjusted the pH of the soils from their natural pH of 8.0 down to 7.0 and 6.0, there was no difference in the suppressive ability of the clay loam, but disease incidence in the fine sandy loam increased from 38% to 61% to 87% of the test plants.

The physical properties of the growing substrate were important factors in the ability of a CWE to sustain a suppressive effect (Hoitink and Boehm,1999; Lozano et al., 2009). Soil texture and structure, clay type, porosity, water potential, and resulting oxygen availability were all factors in the ability of pathogens or beneficial microorganisms to flourish (Alabouvette et al., 1996; Janvier et al., 2007; Lozano et al., 2009;). Some pathogens were naturally more conducive to growth in some textured soils than others. For instance, *Fusarium wilts*, *R. solani*, and *P. sorghi* have been shown to be more conducive in sand and more suppressive in clay (Amir et al., 1993; Cook and Papendick, 1972; Gill et al., 2000; Hoeper et al., 1995; Schuh et al., 1987). Hibar (2006) found that perlite was more conducive than peat in its ability to develop *F. oxysporum* f.sp. *radicis lycopersici* in tomato. Lozano et al. (2009) reported that a compost particle size of 2-4mm increased the suppression of *F. oxysporum, P. cinnamomi*, and *M. hapla*. Large water potentials have been associated with increased disease incidence (Cook and Papendick, 1972; Sterne et al., 1977).

The effect of inorganic fertilizers on beneficial microorganisms in CWE, that in turn impact disease suppression, has not been widely studied. CWE proponents argue that increased application of inorganic fertilizers has been killing natural soil biota (Ingham, 2002). It is unknown if routine fertilizing with inorganic nutrients would have an acute detrimental effect on microbes added through CWE drench application. Ingham and Rollins (2006) suggested that when adding CWE to a hydroponic nutrient, the electrical conductivity should be maintained between 0.01 and 1.0 dS/m. Most of the studies using CWE as a drench either do not provide additional nutrition, or they use

organic fertilizers. When inorganic fertilizers were used, they were added to all treatments making it impossible to determine its effects.

1.3.3 Prediction and mechanisms of disease suppression

Ingham (2002, 2003) promoted the idea that CWE must be aerated and contain threshold numbers of diversity of microorganisms to have a suppressive effect on pathogens. Thresholds suggested include: active bacteria = 2 to 10mg, total bacteria = 150-300mg, active fungi = 2 to 10 mg, total fungi = 150-300mg, protozoa = 1,000 individuals, and nematodes – 5 to 30 beneficials. The research, however, did not necessarily support these predictive criteria (Scheuerell and Mahaffee, 2002). Nelson and Boehm (2002) report of *Pythium* suppressive composts with relatively low populations of bacteria, actinomycetes, and fungi. Methods by which microorganisms were measured can drastically affect the resulting concentrations of microbes present. It was proposed that direct counts be used to estimate microbe numbers since plate count select only for culturable microbes. However, estimating total populations in this way still does not take into account genetic or functional diversity that might be more important in suppression than mere numbers.

In vitro methods are commonly used to predict disease suppression of CWE. These typically consist of adding CWE to petri dishes with agar and pathogen and observing the growth or inhibition of the pathogen. As discussed earlier, the majority of studies showed that the CWE tested will inhibit growth of the pathogen. However, the results did not often predict similar suppression when a disease-pressured plant was grown. Curlango-Rivera et al. (2013) used a different laboratory-based test to determine

the ability of a CWE to suppress disease. A root growth pouch and infection assay (RGIA) was used to test the effect a specific CWE on a specific pathogen of a specific type of plant. While up to 100% protection was observed when pea roots were inoculated with *F. solani* and drenched with CWE, peas grown in sand were 100% protected but peas grown in a heavy clay soil were only 55% protected. Therefore, prediction of the suppression of a CWE was very limited and could not be concluded until more knowledge of suppression mechanisms were determined.

A number of mechanisms of CWE induced disease suppression have been reported, although none have been universally accepted. It is widely acknowledged that the suppression was biological in nature since heat and microfiltration sterilization reduced or eliminated suppression (Dianez et al., 2006; Dione et al., 2012; El-Masry et al., 2002; Kone et al., 2010; Litterick et al., 2004; Pane et al., 2012; St. Martin and Brathwaite, 2012; Santos et al., 2011; Scheuerell and Mahaffe, 2002).

1.4 Objectives of the Study

The objective of this study was to use a model pathogen and host to examine mechanisms, factors of suppression, and effects of different growing media on the ability of compost water extracts to suppress soil-borne disease to be able to apply the findings to similar patho-systems involved in greenhouse crop production. The most common soil-borne pathogens worldwide are *Fusarium* species, infecting a wide range of hosts and causing substantial crop losses (Saremi et al., 2011). *Fusarium* species, most commonly *F. oxysporum* and F. *solani*, have historically been used to model the mechanisms of disease resistance of soil-borne pathogens because of the commonality of

host-pathogen interactions between *Fusarium* and other soil-borne pathogens, the ease of experimental manipulation, and the short time required to obtain results (Hadwiger, 2008; Roncero et al., 2003). In particular, *Fusarium solani* f. sp. *pisi* teleomorph *Nectria haematococca* Mating Population VI, pathogenic on pea, has been studied extensively to elucidate mechanisms of disease resistance (Coleman et al., 2009; Gunawardena and Hawes, 2002; Hadwiger, 2008). In addition, the Hawes/Van Etten plant pathology laboratories at the University of Arizona where this study was undertaken had extensive experience using the *F. solani* f. sp. *pisi* and pea pathosystem. Therefore, this study used the model pathosystem of *F. solani* f. sp. *pisi* teleomorph *Nectria haematococca* Mating Population VI and pea (*P. sativum* L.) cultivar "Alaska", to determine some factors involved in the ability of a CWE to suppress a soil-borne pathogen and potential mechanisms of suppression.

A traditional greenhouse crop, such as tomato, pepper, or cucumber was not used in this study because those crops are not as widely studied as pea in terms of examining mechanisms of soil-borne pathogens. In addition, the roots of tomato seedlings were discovered to be too fragile for the rigors of the growth pouch assay technique used in this study, and pepper seedlings grow too slowly. Cucumber was not used because of the inconsistent disease resistance obtained in response to CWE in past studies (Curlango-Rivera et al., 2013; Scheuerell and Mahaffee, 2002). Although pea is not a common crop in climate-controlled greenhouses, it is common in high tunnel production (Cornell University, 2014; Roger et al., 2014). Pea has a sturdy root system, grows quickly, has resulted in consistent disease resistant with CWE (Curlango-Rivera et al., 2013), and

when used with *F. solani* f. sp. *pisi*, has an extensive research history toward understanding disease resistance.

The specific objectives of this study were to: 1) examine the effects of a CWE on growth and disease suppression in pouch assays and in seedlings studies using different types of growing media; 2) examine and quantify the effect of two different CWE on border cell dispersion, its relation to its ability to suppress *F. solani*; and, 3) to isolate different factors that contribute to the suppression ability of a CWE by changing methods of production one factor at a time.

The journal article found in Appendix A, *Effect of a Compost Water Extract on Growth and Root Disease Suppression in Pea Grown in Different Substrates*, was submitted to *Compost Science & Utilization*, a peer-reviewed journal. Appendix B contains a peer-reviewed article accepted by the journal *Plant and Soil* for publication in 2015 entitled: *Altered Carbon Delivery from Roots: Rapid, Sustained Inhibition of Border Cell Dispersal in Response to Compost Water Extracts*. In Appendix C, is a compilation of a series of studies that have not yet been developed into a journal article for publication, and is entitled: *Factors Affecting the Ability of Compost Water Extracts to Suppress Root Disease in Pea*.

2. PRESENT STUDY

2.1 Overall Summary

The methods, results, and conclusions of this study were presented in each of the two manuscripts and the additional study appended to this dissertation. The following is a summary of the primary results of the research.

In Appendix A, an aerated Compost Water Extract (CWE) produced from vegetable and green waste was used to evaluate the efficacy of a CWE for suppressing root rot (*F. solani* f. sp. *pisi*) in pea when grown in various substrates under water irrigation and hydroponic nutrient irrigation. A greenhouse transplant experiment and a laboratory container study were conducted. The greenhouse transplant experiment tested the ability of root growth and infection assays (RGIA) to predict suppression of disease when plants were grown in substrate and evaluated suppression in coconut coir fiber. First, efficacy of the CWE to suppress the pathogen was conducted using RGIA as described in Curlango-Rivera et al. (2013). When seedlings were drenched with CWE and then inoculated, 100% remained free of infection. These findings were consistent across six individual batches of CWE across both experiments. These same seedlings were taken to a controlled environment greenhouse, transplanted in fine coconut coir fiber, and irrigated with hydroponic nutrient solution for 5 weeks. At the end of the experiment, 23% of the shoots of the pathogen-inoculated, CWE-drenched seedlings remained healthy while only 2% of the inoculated seedlings without CWE drench remained healthy. All of the roots of the inoculated seedlings developed lesions, even those drenched in CWE. However, 29% of the CWE drenched roots were able to recover

from disease, growing white healthy roots past the lesion, while only 2% recovered naturally.

Results from the first study raised questions to be addressed in the second. Why did the seedlings show protection in the pouches, yet most of them became diseased once they were transplanted into substrate, with no addition of pathogen? Was there something about the substrate that negated the suppression or did the salt content of the hydroponic nutrient solution affect the suppression? A second shorter-term experiment was designed to test suppression in two different textures of the same source of coconut coir fiber and in sand. All were irrigated with water. The fine coconut coir fiber was the same as that used in the greenhouse experiment. To compare the effect observed in the greenhouse experiment and to test the effect of hydroponic nutrient solution on CWE-induced disease suppression, an additional set of containers were filled with fine coir and watered with hydroponic nutrient solution. This experiment was conducted at room temperature (24°C) and typical laboratory room lighting. In this experiment, pea seeds with 20-25mm radicles were inoculated with pathogen and sown directly into CWE-drenched substrate and grown for three weeks. At the end of the experiment, 80%, 60%, 90%, and 50% of the shoots of the inoculated, CWE-drenched seedlings remained healthy when grown in fine coir, coarse coir, sand, and fine coir, respectively, and irrigated with hydroponic nutrient solution. Nearly 100% of the roots grown in coconut coir substrates again developed necrotic lesions but 83%, 87%, 100%, and 87% grew healthy roots beyond the disease region. Comparing the inoculated CWE drenched seedlings grown in fine coir watered with hydroponic nutrient solution, the laboratory experiment resulted in 50% healthy shoots while the greenhouse experiment results were

23% healthy shoots. Comparing the percentage of healthy shoots of plants in fine coir irrigated with water (80%) to the healthy shoots of plants in fine coir irrigated with HNS (50%), it is evident that the HNS had a significantly negative effect on shoot health. Sand demonstrated a natural ability to suppress *F. solani*. Only 23% of inoculated seedlings had dead or dying shoots by the end of the experiment (compared to 77-80% in coir substrates) and although all but one of the roots developed lesions, all were able to recover on their own with CWE. CWE further increased shoot health and also prevented 57% of the roots from developing lesions.

In Appendix B, two different CWE were used to examine the effect on root border cell dispersion and dynamics in pea, maize, cotton, and cucumber and its relation to disease suppression. In a previous study (Curlango-Rivera et al., 2013), CWE applied to pea seedlings resulted in >95% protection against root infection. The protection was correlated with retention of a sheath of root border cells surrounding each root tip. A transient exposure to CWE was correlated with 80% reduction in infection, and with retention of border cell sheaths. Therefore, this study was conducted to further describe and quantify this phenomenon. A vermicompost and vegetable and green waste thermophillic compost were used to produce two aerated CWE using similar methods. Dispersal of border cells after immersion of roots into water or CWE was measured by direct observation over time using a compound microscope and stereoscope. Pictures were taken and the number of border cells released into suspension were enumerated by counting the total number of cells in aliquots taken from the suspension.

Border cells formed a mass surrounding root tips within seconds after exposure to water, and most cells dispersed into suspension spontaneously. In CWE, >90% of the

border cell population instead remained appressed to the root surface, even after vigorous agitation. In cotton, for example, >25,000 border cells dispersed within seconds of immersion in water, but <100 border cells dispersed after >24 hours in CWE. This altered border cell phenomena was consistent for pea, maize, and cotton and for both CWE tested. For most cucumber roots (n=86/95), inhibition of border cell dispersal in both CWE was similar to that observed in pea, maize, and cotton. However, some individual cucumber roots (8±5%) exhibited a distinct phenotype. For example, border cells of one root immersed into CWE remained tightly adhered to the root tip even after 30 minutes while border cells of another root immersed at the same time in the same sample of CWE expanded significantly within 5 minutes and continued to expand over time. In a previous study, sheath development over time in growth pouches also was distinct in cucumber compared with pea, with detachment of the sheaths over time, and root infection was reduced on 38% of the test seedlings in cucumber compared with 100% protection in pea (Curlango-Rivera et al., 2013). Further research was required to evaluate whether this difference in retention of border cell sheaths was the cause of the observed differences in inhibition of root infection.

In Appendix C, information from a series of studies that were conducted to determine the different factors that contributed to the suppression ability of a CWE was provided. By incrementally changing aspects of the CWE production process in a prescribed manner, then the effect of each test factor could be determined. The same compost source and basic CWE recipe was used in this study and all of the other studies in this dissertation. Some factors evaluated included aeration, heat sterilization, chlorine addition, increasing compost/water contact time, and eliminating additives. Each

different CWE produced in this study was tested for its ability to suppress *F. solani* using root growth and infection assays (RGIA).

The basic aerated CWE recipe (with molasses, kelp, humic acid, rock phosphate, and silica) provided 100% protection while the non-aerated basic recipe CWE only provided 72% protection with the same 24 hours of aeration. Aerated CWE made of only compost and water resulted in 58% protection. Compost wash (CW), which is compost and water with only 30 minutes of contact time, provided no protection.

Molasses did not contribute significantly to the suppression ability of the ACWE. When only molasses was added, disease suppression was reduced to 7% of the test plants and when molasses was taken out of the basic recipe, suppression remained at 100%. Kelp contributed significantly to suppression. For example, kelp added to the compost and water resulted in 80% suppression. Although, when all additives were included EXCEPT molasses and kelp, suppression remained high (93%) indicating that humic acids, rock phosphate, and/or silica were major contributors toward the suppression effect, as well as kelp. More work needs to be completed to determine the contributions of those additives.

Low levels of total chlorine (1-3ppm) did not inhibit the suppression ability of the CWE, as it provided as much as 88-100% protection. However, at 4ppm chlorine, suppression was reduced by 60%. Autoclave sterilization eliminated the suppression effects for ACWE-BASIC and ACWE-compost and water, while NCWE maintained minimal protection (5%).

The effects of fermentation time were examined. Suppression decreased after 24 hours of brewing for ACWE, with decreases of an average of 35%, 26%, 20% on Days 2,

3, and 8. By Day 10, suppression recovered to about 88%. Results for NCWE were inconsistent. One trial resulted in 100% suppression for all days except Day 2 when suppression was 93%. Another trial resulted in 73% protection on Day 1, with increasing protection on Days 2, 3, and 8 up to 100% but then down to 47% protection on Day 10. A third trial resulted in decreasing protection for Days 1 through 3 (57% to 40%) and then 47% on Day 10. Aerated compost mixed with water and no additives maintained an average of about 30% protection over time.

2.2 Overall Conclusions and Recommendations

Compost water extracts have potential to be used as an alternative to fungicides in greenhouse crop production. However, more work remains to understand the factors and mechanisms affecting CWE-induced suppression to provide consistent and reliable control. Laboratory results were almost overwhelmingly positive, demonstrating suppression of numerous root pathogens with numerous different composts but suppression with the same CWE tested in the laboratory varied considerably when that CWE was used to treat plants in a production situation in the greenhouse. Successfully predicting the CWE response to pathogens in crop production situations remained highly variable. For greenhouse vegetable growers whose economic margins are slim, this uncertainty is too risky and rather chemical fungicides will continue to be used.

The greatest uncertainty in the suppression of disease using CWE was the compost source. Composts created from biomass waste products available in a given region were highly variable in their source materials. This provided an unknown starting point when creating the CWE. Each research study conducted used a given compost or

composts for the duration of the study to ensure consistency and to keep the variable of "compost" constant. However, experimental results obtained for that compost may completely change when a compost comprised of different sources was tested. Therefore, it remains highly important to research and understand the underlying mechanisms and factors leading to disease suppression by CWE. To obtain any consistency in suppression when using CWE, these mechanisms and factors must be known so that they can be applied to each individual compost or CWE.

Through additional research into CWE-induced suppression, it is possible that another bacteria or fungal species may be discovered which assists in suppression and can be used to create more reliable biocontrol products. The discovery of specific antimicrobial substances produced by beneficial microorganisms in CWE may be able to be harvested and applied as biocontrol agents.

The research in this dissertation increased the understanding of some factors related to CWE-induced disease suppression. It highlighted the importance of the nature of the substrate on the ability of a CWE to suppress disease and it examined the effects of different conditions in the production of CWE that affected suppression. Most importantly, though, a change in phenotype of CWE-drenched roots was described which may be correlated with the ability to suppress disease. This newly observed phenomena may be a potential mechanism of CWE-induced disease suppression never before studied.

APPENDIX A

EFFECT OF A COMPOST WATER EXTRACT ON ROOT DISEASE SUPPRESSION IN PEA GROWN IN DIFFERENT SUBSTRATES

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ABSTRACT

Aerated compost water extract (CWE) produced from vegetable and green waste was used to evaluate the efficacy of a CWE for suppressing root rot (*F. solani* f. sp. *pisi*) in pea when in grown in coconut coir fiber and sand. Efficacy of the CWE to suppress the pathogen was first conducted in growth pouches, where in the presence of CWE, 100% of inoculated seedlings were protected from infection. However, when these protected seedlings were transplanted into fine coconut coir fiber and grown for 5 weeks in a controlled environment greenhouse, only 23% of the shoots remained healthy and all roots developed necrotic lesions which the healthy plants overcame. In a second experiment, pea seeds with 20-25mm radicles were inoculated with pathogen and sown into CWE-drenched fine or coarse coconut coir, sand, or fine coir watered with hydroponic nutrient solution. After 3 weeks, 80%, 60%, 90%, and 50% of shoots remained healthy respectively. Nearly 100% of the roots grown in coconut coir substrates developed necrotic lesions but 83%, 87%, 100%, and 87% grew healthy roots beyond the disease region. Sand naturally protected seedlings from disease, even without the addition of CWE. Irrigating seedlings with hydroponic solution rather than water decreased the ability of the CWE to maintain shoot health by 30%. This study highlighted the complex interactions of CWE, substrate, and fertilizer, among other variables, that have an effect on the success rate of using CWE to suppress disease in actual growing situations.

Introduction

Soil-borne pathogens cause about 90% of the 2000 major diseases in the principal crops grown in the U.S. (Michigan State University 2010), resulting in 10% of all vegetable crop losses in the U.S. in 2009 (Delheimer 2014). Fungi are the most prevalent, causing seedling damping-off, root rot, and vascular wilt in a wide range of crops including legumes, strawberries, tomatoes, and peppers.

Control of soil-borne pathogens is difficult. Soil amendments, crop rotation, and field sterilization have only limited success because of resilient overwintering structures (Oyarzun et al. 1994; Schwartz 2011). Plants develop resistance to fungicides, fungicides are not consistently effective, and they pose hazards to human and environmental health (Delheimer 2014; Oyarzun et al. 1994).

Compost and compost teas have attracted attention of farmers for their potential as a low-cost, environmentally-friendly alternative to suppress disease. Compost teas, or compost water extracts (CWE), are mixtures of compost and water incubated for a defined period of time, either with or without aeration, and with or without additives intended to increase microbial populations (Scheuerell and Mahaffee 2002). Decreased germination of spores or decreased growth of hyphae in concert with CWE have been reported in several laboratory studies of soil-borne pathogens in the fungal genera including *Fusarium, Verticillum, , Rhizoctonia,* and *Sclerotium*, as well as those in the sub-group of oomycetes such as *Pythium*, *Alternaria*, *Phytophthora* (Alfano et al. 2011; Curlango-Rivera et al. 2013; Dianez at al. 2006; Dionne et al. 2012; El-Masry et al. 2002; Kirkeni et al. 2007; Marin et al. 2013; Pane et al. 2014). However, very few of these laboratory studies were followed by container or field studies to determine the

corresponding efficacy of CWE suppression in growing plants. When they have, the level of suppression found in the laboratory did not always correspond to similar suppression in plants. This difference in performance is attributed to the abundance of nutrients provided by the agar and ideal temperature and humidity for both the beneficial microbes and pathogens to develop. When plants grow *in vivo*, microbes and pathogens must adapt to non-ideal environmental and nutrient conditions which have varying affects on the pathogens and beneficials, changing the balance between the two populations, (Duffy and Defago 1999; Marin et al. 2013; Scheuerell and Mahaffee 2002). Pane at al. (2012) found ten CWE that inhibited *A. alternaria*, *B. cinerea*, and *P. lycopersici* both *in vitro* and *in vivo*, but the ones that were most disease inhibiting *in vitro* were not often the ones that reduced disease the most in tomato plants. The effectiveness of foliar application of CWE on gummy stem blight (*D. bryonae*) was studied by Marin et al. (2013), who found 100% suppression *in vitro*, but overall poor control of disease in melon plants. Curlango-Rivera et al. (2013) reported 98-100% protection of pea seedlings from *F. solani* f. sp. *pisi* when roots were drenched in CWE in growth pouches. However, they found that 100% of the inoculated seedlings were protected when grown in sand drenched in CWE but only 55% of the inoculated seedlings were protected when grown in a heavy clay soil drenched in CWE.

The few field and container studies conducted with CWE report varying degrees of success. Field studies using CWE as a soil drench have resulted in decreased incidence of *Streptomyces scabiei* in potato, *Ralstonia solanacearum* in eggplant, *Fusarium moniliforme* in rice, and *Fusarium solani*, *Rhizoctania solani*, and *M. Phaseolina* in bean plants (Al-Mughrabi et al. 2008; Islam et al., 2014; Larkin et al. 2008;

Manandhar et al. 2008; Mansour et al. 2011;) although no effect on *Fusarium sambucinum* and *Rhizoctonia solani* in potato (Al-Mughrabi et al. 2008; Larkin et al. 2008). CWE drenching decreased seedling damping-off and wilt incidence in cucumber caused by *P. aphanidernatum* and *P. ultimum* differentially depending on type of compost, level of aeration, and additives used (Jack and Nelson 2010; Scheuerell & Mahaffee 2004). Sang (2010, 2011) found CWE from four different composts reduced severity of disease caused by *Phytophthora* sp. and *Colletotrichum* sp. in pepper and cucumber when grown in standard potting mix. In a study of the effect of CWE on *F. oxysporum* f.sp. *radicis lycopersici* in tomato, Hibar (2006) found that four different CWE reduced the severity of disease when grown in soilless media with hydroponic nutrient solution.

The physical properties of the growing substrate are important, albeit often overlooked, factors in the ability of a CWE to sustain a suppressive effect (Hoitink and Boehm1999; Lozano et al. 2009). Soil texture and structure, clay type, porosity, water potential, and resulting oxygen availability are all factors in the ability of pathogens or beneficial microorganisms to flourish (Alabouvette et al. 1996; Janvier et al. 2007; Lozano et al. 2009;). Some pathogens are naturally more conducive in some textured soils than others. For instance, *Fusarium wilts, R. solani,* and *P. sorghi* have been shown to be more conducive in sand and more suppressive in clay (Amir et al. 1993; Cook and Papendick 1972; Gill et al. 2000; Hoeper et al. 1995; Schuh et al. 1987). Suppressiveness of clay soils has been contributed to increased surface area for adsorption and resproduction of microorganisms which compete with pathogens for limited nutrients

(Amir and Alabouvette 1993). More studies need to investigate the effect that different substrate types and textures have on the ability of a CWE to suppress disease in soils.

The effect of inorganic fertilizers on beneficial microorganisms in CWE, that in turn impact disease suppression, has not been widely studied. CWE proponents argue that increased application of inorganic fertilizers has been killing natural soil biota (Ingham, 2002). It is unknown if routine fertilizing with inorganic nutrients would have an acute detrimental effect on microbes added through CWE drench application. Ingham and Rollins (2006) suggest that when adding CWE to a hydroponic nutrient, the electrical conductivity should be maintained between 0.01 and 1.0 dS/m. Most of the studies using CWE as a drench either do not provide additional nutrition or they use organic fertilizers. When inorganic fertilizers were used, they were added to all treatments making it impossible to determine its effects.

The present study evaluated 1) the efficacy of a CWE for controlling root rot in pea grown in sand and coconut-coir-based substrates and 2) the effect that irrigating with hydroponic nutrient solution has on disease suppression.

Materials and Methods

Plant Material and Pathogen

Pea seeds (*Pisum sativum* L.) cv "Alaska" were treated as described previously (Curlango-Rivera et al. 2013). Seeds were immersed for 10 minutes in 95% ethanol, for 60 minutes in 5.0% sodium hypochlorite, rinsed six times in sterilized distilled DI water, and then imbibed in sterilized distilled DI water for 6 hours. Seeds with damaged seed coats or that floated to the surface were discarded. Imbibed seeds were placed on the

surface of sterile germination paper which lay on top of sterilized 1% agar (Bacto TM Agar, Becton Dickinson and Co., Baltimore, MD, USA). These seeds were placed in an incubator at 24°C in the dark for 48 hours. Seedlings with 20-25 mm roots were either placed in pouches as described in Curlango-Rivera et al. (2013) for the greenhouse experiment or planted in Cone-tainers (Ray Leach Cone-tainers, www.greenhousemegastore.com) for the laboratory experiments.

The fungal pathogen *Nectria haematococca* (anamorph *Fusarium solani* f. sp. *pisi*), pathogeni on pea, was provided by the University of Arizona Hawes/VanEtten plant pathology laboratory. T8 cultures were maintained and processed as described in Gunawardena and Hawes (2002).

CWE Preparation

CWE source and preparation was the same as described in CWE #2 preparation in Curlango-Rivera et al. (2013). Thermally-produced compost made of fruit, vegetable, wood, and bark waste was obtained for use throughout this study (Ecoscraps, Phoenix, AZ, http://ecoscraps.com). A fresh batch of aerated compost water extract plus additives was prepared for each trial based on an equal ratio fungi to bacteria tea recipe (1/30 v/v) recommended in Ingham (2002). CWE was produced using a 18.9 L bucket brewer. Constant aeration was achieved using an 18 Watt, 38 L/min, 20 kPa air pump (Commercial 1 Air Pump, www.sea-of-green.com) with two airlines extending into the water. One airline had a 2.5cm x 1.25 cm airstone connected to the end; the other airline remained open. Before a batch of CWE was made, the 18.9 L bucket was filled with 15 L of municipal tap water and aerated for 24 h to remove any residual chlorine in the water. The following ingredients were added to the water and mixed: 29.6 ml unsulfured

blackstrap molasses (Plantation Brand, www.vitacost.com), 14.8 ml kelp meal (Ohrstrom's Maxicrop Soluable Seaweed Powder, http://maxicrop.com/), 14.8 ml liquid humic acid (BioAg Brand Ful-Power, www.bioag.com), 14.8ml soft rock phosphate (Dr. Earth Brand, http://drearth.net) and 14.8 ml silicates (Silica Blast, Botanicare, www.botanicare.com). Compost (473 ml) was placed in a 250 micron mesh bag (Pentair Aquatic Ecosystems, http://pentairaes.com) and placed in the bucket. The mixture was continuously aerated for 22 h at 18°C and was used within 30 minutes after the aeration was turned off.

Chemical and biological properties of compost and compost teas

All compost used in this study was taken from a single bag of Ecoscraps Brand compost purchased in Phoenix, Arizona. A sample of the compost was sent to Penn State Analytical Services Laboratories for compost analysis using U.S. Compost Council's Test Methods for the Examination of Composting and Composts (2002). A sample was also sent to International Ag Labs, Inc for Formazan analysis. Formazan is a measure of biological activity of a soil or compost obtained by adding a specific amount of calcium carbonate as food supply, waiting a specific amount of time, and then measuring amount of metabolic enzymes given off (Frank 2014). Table 1 shows the results of the compost analyses. This compost had very high biological activity as compared to several other thermal composts and vermicomposts tested, as reflected in the Formazan test results (Table 2).

Immediately after aeration was stopped, the pH, alkalinity, and electrical conductivity (EC) was determined using a handheld pH meter (Model 102 Milwaukee Instruments, Rocky Mount, NC), handheld EC meter (Truncheon Nutrient Meter,

Bluelab, New Zealand), and a colormetric drop test (HTH 6 Way Test Kit, Lonza Company, Switzerland). The pH of each CWE was between 7.4-8.1, electrical conductivity was between 1.2 - 1.5 dS/m, and the alkalinity ranged from 440 – 640 mg/L as $CaCO_3$. An HTH 6 Way Test Kit was used to measure total chlorine concentration in the start water to be sure that chlorine had been off-gassed prior to adding compost. Each CWE preparation was placed under the microscope, confirming the presence of bacteria and protozoa.

Root growth and infection assays

Root growth and infection assays (RGIA), as described in Curlango-Rivera et al. (2013), were conducted in the laboratory for both the greenhouse and laboratory experiments. Before implementing each trial of the greenhouse transplant experiment, 14 RGIA (N=70 seedlings) were conducted for each treatment to determine the effectiveness of the CWE to suppress the pathogen, to determine if the CWE was phytotoxic to the seedlings, and to measure root growth and lateral development. Before implementing the laboratory study, three RGIA (N=15 seedlings) were conducted for each treatment to determine the effectiveness of the CWE to suppress the pathogen.

A growth pouch consisting of a clear cellophane envelope inserted with germination paper (Mega International, St. Paul, MN) was used to expose roots to a CWE treatment and/or pathogen and view and measure root length and disease response over time. Fifteen milliliters of CWE or sterilized water was placed in each pouch using a syringe. Once the germination paper was soaked, five pea seedlings with root length of 20-25mm were placed at the top of the pouch. Pouches were laid flat and liquid was pushed up the pouch to make sure it had complete contact with the roots. Roots were

then inoculated with sterilized distilled water or a 0.15-ml spore suspension (10^6 spores/ml) of *F. solani* pathogen by uniform application from base to tip using a micropipette. Pouches were left in a horizontal position for 15 minutes and were then placed upright and maintained at 24°C for up to 8 days on a laboratory bench. The position of the tip of each root was marked on the cellophane envelope at the time the experiment was initiated. The tip of the root was marked again at the same time the next day and the length recorded, with the procedure repeated on Day 5. On Day 5, the number of laterals extending from the main root whose length was greater than 3mm were also recorded. On Day 8, infection levels were obtained by observation of lesion development on seedlings in pouches. Seedlings were assigned a rating based on the presence of tan (1), brown (2), or black (3) sections of root with internal fungal hyphae, as described (Gunawardena and Hawes 2002), with data later collapsed into two categories: 0 = not infected and 1 = infected (1, 2, 3 above).

The four treatments (drench/inoculants) were: Treatment 1 = sterilized distilled water/sterilized distilled water (negative control), Treatment 2 = sterilized distilled water/pathogen (positive control), Treatment 3 = CWE/sterilized distilled water, and Treatment 4 = CWE/pathogen.

Plant infection levels

The shoot and roots of every plant in both experiments were examined for health and disease symptoms. Each plant shoot was assigned a shoot health index (SHI). For the greenhouse experiment, SHI was determined by assigning a health value to each plant as follows: 1) healthy plant, 2) 2-5 dead leaves, some leaves yellowing, 3) more than 5 dead leaves, stem drying, extensive yellowing, and 4) dead plant. For the laboratory

study, health values were assigned as follows: 1) healthy plant, 2) 2-3 drying leaves, 3) wilting, stunted growth, and 4) dead plant. Criteria for the two experiments were slightly different because the shoot characteristics were somewhat different in the younger plants. Data was then collapsed into a shoot health index (SHI) with an index of 1 for plants with a health value of 1 or 2, and an index of 2 for plants with a health value of 3 or 4. Plant roots were separated from the growing media, rinsed with water, and examined for infection symptoms. Roots were assigned a root disease index (RDI) of 0 if non-infected or 1 if they had any visible grey, dark brown, or black lesions. Roots of plants inoculated with pathogen and having an RDI = 1 were also assigned a root recovery index (RRI) where an RRI of 0 was assigned to infected roots that showed no signs of recovering infection and a value of 1 was assigned to infected roots that grew some amount of healthy white roots past the infection point.

Plant growth measurements

At the end of each trial, shoots from 12 random plants in each treatment (6 from each block) and shoots and roots from three random plants in each treatment in the greenhouse and laboratory experiments respectively, were sampled and analyzed for fresh and dry weight. Sample plants were cut at the soil surface. Shoots were weighed immediately and then after drying at 80°C for at least 48 hours as described in Hernandez and Kubota (2012). Drying time was extended to 72 hr because of the larger size of plant material obtained in this study. In the greenhouse study, pea pods were included in the shoot measurements. In the laboratory study only, roots were separated from the media, rinsed with water, blotted dry with a paper towel, and weighed for fresh weight. Roots were then dried at 80°C for at least 72 hr. Because of the longer grow time and larger

amount of substrate, it was difficult separate root mass and substrate to obtain a complete root mass to weight for plants in the greenhouse study

At the end of the greenhouse transplant experiment, stem diameter and two internode lengths were measured for 12 random plants in each treatment (6 from each block). Stem diameter was measured using calipers approximately halfway between the second and third node from the growing head. Internode length 1 was measured as the distance between the first and second nodes from the growing head and internode 2 was measured between the second and third node.

Experimental Set-Up and Design

Greenhouse transplant experiment

Before conducting each trial of the greenhouse study, 14 RGIA (N=70 seedlings) were conducted for each treatment to determine the effectiveness of the CWE to suppress the pathogen. In each case, all 70 pathogen-inoculated CWE- drenched seedlings were protected from disease in the pouches, while all 70 pathogen-inoculated water-drenched seedlings became infected. The purpose of the greenhouse transplant study was to determine if this CWE-induced disease suppression in the pouches would be maintained if the seedlings were planted and grown for 5 weeks, with no additional pathogen or CWE.

This experiment was carried out in an 1148 square foot research greenhouse located at the University of Arizona Campus Agriculture Center and Controlled Environment Agriculture Center in Tucson, Arizona. The greenhouse was equipped with a pad and fan cooling system for cooling, natural gas fired heater for heating. The

greenhouse roof and walls were covered with double layer acrylic glazing. A 30% shade cloth covered the roof. The greenhouse climate, mainly air temperature, was controlled using a climate controller (MicroGrow). Three separate trials were conducted: Trial 1 (February 16 - March 23, 2013), Trial 2 (April 16 - May 21, 2013) and Trial 3 (April 18 – May 23, 2013). Trials 2 and 3 were conducted at the same time in the same greenhouse, with Trial 2 in the south end of the greenhouse and Trial 3 in the north. Greenhouse temperatures were set for a daytime high of 21°C and nighttime low of 10°C. Actual average temperatures and standard deviations for Trial 1 were 20.6°C ± 8.1°C, and were 27.9°C ± 4.4°C for Trials 2 and 3 which occurred simultaneously. The average relative humidity and standard deviations for Trial 1were 43.3% ± 18.9%, and were 37.3% ± 10.8% for Trials 2 and 3. Irrigation was conducted by hand, by adding a 3-4 cm reservoir of nutrient solution to the seedling trays every two days allowing for sub-irrigation as the plant needed water.

The laboratory conducted RGIAs were transported 10 days post-inoculation to the greenhouse. Seedlings from each treatment were carefully taken out of the pouches and transplanted into 10 cm by 10 cm plastic pots filled with Riococo 200, expanded untreated coconut coir fiber (Riococo, Irving, TX, www.riococo.com/index.php/hydroponic-coir-growbags), one seedling per pot. Riococo 200 is a fine textured substrate made of 15mm cocopeat (80%) and a small amount of crushed coco chips (20%). Manufacturer specifications indicate the air-filled porosity as 21-22% and water holding capacity as 6.5-7.0 L/kg. Seedlings with roots that cracked or became damaged during transplanting were discarded. A set of eight containers were placed in each standard 28 cm by 53 cm seedling tray with no holes. Seedlings were top-

watered by hand to soak the media immediately after transplantation using hydroponic tomato nutrient solution (HNS) for fruiting crops (Rorabaugh, et al. 2009) with EC = 2.4 dS/m and pH = 6.3. This high EC was used because in preliminary trails, the leaves of the seedlings quickly began showing signs of nitrogen deficiency (older leaves turning yellow) when a lower EC was used. Likewise, when pod development occurred, nutrient deficiencies were evident again. Seedlings were sub-irrigated every two days, thereafter, by adding 3-4 cm of nutrient solution to the seedling trays. The concentration of nutrient solution was incrementally increased from 2.4 to 3.6 dS/m at the end of the experiment to keep up with plant growth and nutrient demand. Concentrated nutrient solution was taken from stock tanks each watering day and diluted with tap water in a 68L tote. EC and pH were monitored and the solution adjusted as needed to meet setpoints. The fertilizer brought the solution naturally to pH 6.3-6.4 so acid was not needed to decrease pH. Plants were supported by twisting stems around string hanging from hooks on a high wire above the plants. Seedlings were grown for a total of five weeks in the greenhouse.

A 2x4 randomized block design was used for the greenhouse experiment. Each trial consisted of 192 plants with 48 plants in each treatment. Each treatment was split into duplicates, one set of 24 plants in each block. The four treatments were conducted on pea seedlings from the pouches, which were transplanted into the pots, described here in the form of drench/inoculate: Treatment 1 = sterilized distilled water/sterilized distilled water (negative control), Treatment 2 = sterilized distilled water/pathogen (positive control), Treatment 3 = CWE/sterilized distilled water, and Treatment 4 = CWE/pathogen.

Laboratory experiment

The purpose of the laboratory experiment was to conduct a smaller scale study to determine if different textured substrates and watering with a hydroponic nutrient solution have an effect on the ability of a CWE to suppress disease. The laboratory experiment was conducted at the University of Arizona, Department of Plant Pathology, Marley Building, Tucson, AZ. Three trials were conducted, with data pooled: Trial 1 (September 26 – October 17, 2013), Trial 2 (October 30 – November 20, 2013), and Trial 3 (December 18, 2013 – January 8, 2014). The substrates used were fine coconut coir fiber (Riococo 200), coarse coconut fiber (Riococo 300), and washed river sand (obtained from the Controlled Environment Agriculture Center, University of Arizona). According to manufacturer specifications, (http://www.riococo.com/), the fine and coarse coconut coir consisted of 80% and 30% fine cocopeat (15mm) and 20% and 70% coco chips and the air-filled porosity and water holding capacity of the fine and coarse coconut are 21-22% and 24-26%, and 6.5-7.0 L/kg and 5.0-5.5 L/kg respectively. Properties of the sand are unknown. Sterilized, germinated pea seedlings with 20-25mm length roots were sown directly into Cone-tainers containing 150 ml of sterilized substrate. Before sowing, substrate was top-drenched with 15 ml of sterilized water or CWE. Each pea root was held above its media using sterilized tweezers and its radical was inoculated with 0.15 ml sterilized water or 0.15-ml spore suspension (10^6 spores/ml) of *F. solani* pathogen by uniform application from base to tip using a micropipette and then sowed. Seedlings were top watered every three days with equal amounts of sterilized DI water. A second set of seedlings were sown into fine coconut coir but were watered with equal amounts of hydroponic tomato nutrient solution, which is a modified Hoagland's solution

(Rorabaugh et al., 2009). The pH ranged from 6.7 - 6.8. The EC of the solution began at 0.8 dS/m and was incrementally increased up to 2.2 dS/m to keep up with plant growth and nutrient demand. The EC used in this experiment was lower than in the greenhouse study because the seedlings started out much smaller and did not grow long enough to develop pods.

Each trial consisted of a set of 40 seedlings per substrate type, 10 plants per treatment group. The four treatments were identical to those described above for the greenhouse experiment. Each set of 10 seedlings per treatment was placed side-by-side the next treatment within a substrate type. The seedlings were grown for three weeks on laboratory benches at room temperature (~23°C). Plants were placed as close to open windows as possible and therefore experienced natural day/night lighting as well as additional artificial light from the lab space during the day. Sufficient light and nutrient was available for plant growth as evidenced by relatively strong stems and no discoloring of the leaves.

Statistical Analysis

Quantitative data for all collected from lab and greenhouse experiments were analyzed using one-way analysis of variance (ANOVA) at $p<0.05$ with treatment differences compared using Tukey's HSD post-hoc tests at the 5% probability level. Categorical data (SHI, RDI, and RRI) were analyzed using chi-square analysis at $p<0.05$. All statistics were performed using the software package IBM SPSS Statistics for Windows (version 22.0; IBM Corp., Armonk, NY).

Results and Discussion

Disease incidence in laboratory conducted RGIA

None of the seedlings in the control pouches (water-drench, water inoculation) became infected and none of the CWE-drenched seedlings inoculated with water became infected. There were no physical changes in the roots of the CWE-drenched seedlings to indicate any phytotoxic effects of CWE. When seedlings were inoculated with *F. solani* in the growth pouches, 100% developed lesions. Despite uniform inoculation along the entire root surface, infection occurred only in the zone of elongation, leaving the root tip itself unharmed. This finding is consistent with previous studies that found that 90% of infection by *F. solani* f. sp. *pisi* was localized to this zone of elongation, with the root tips escaping infection (Gunawardena and Hawes 2002; Gunawardena et al. 2005). When seedlings were drenched with CWE and then inoculated, 100% remained free of infection. These findings were consistent across six individual batches of CWE across both experiments. Curlango-Rivera et al. (2013) found similar results with pea and *F. solani* in RGIA (98% protection) using a CWE made with worm castings, Alaskan humus, rock phosphate dust, fish hydrolysate, kelp, and humic acids, aerated for 18 hr.

Disease incidence and plant health in fine coconut coir fiber

In the greenhouse transplant study, 98% of the inoculated seedlings resulted in shoots which were dead or dying by the end of the 5 week experiment, while 77% of the CWE-drenched inoculated seedlings were dead or dying (Table 3). This 21% increase in health was statistically significant indicating that the CWE had a positive effect on plant health. Despite this increase in shoot health, all roots of inoculated plants developed necrotic lesions at the roots, even the CWE-drenched seedlings. However, as indicated

by the root recover index (RRI), 2.1 % and 28.5% of the water-drenched inoculated and CWE-drenched inoculated seedlings, respectively, developed some level of healthy root mass past the infection point, corresponding to the approximate percentage of plants with healthy shoots. In plants whose roots recovered, it was evident that the lesion developed at the point where inoculation had occurred in the growth pouch (within 20-25 mm from the pea seed) and expanded from there. In those cases, it is most likely that the lesion started in the zone of elongation at the time of inoculation because previous have shown that roots infected in that region often continue to grow and develop past such lesions. On the other hand, roots whose root tips become infected will experience cessation of growth and development (Gunawardena and Hawes 2002). It was impossible to determine the initial point of infection in the roots that did not recover from disease because there were very few roots left and the roots that were left were thin and completely black and necrotized. Therefore, it is likely that those root tips became infected.

In the laboratory study, the same fine coconut coir substrate was used and either irrigated with sterilized distilled water or with hydroponic nutrient solution (HNS) made with sterilized distilled water. When irrigated with HNS, 87% of the inoculated seedlings resulted in shoots which were dead or dying by the end of the 3 week experiment, while 50% of the CWE-drenched inoculated seedlings were dead or dying (Table 4). When irrigated with water, 77% of the inoculated seedlings resulted in shoots which were dead or dying, while 20% of the CWE-drenched inoculated seedlings were dead or dying. The source of irrigation did not make statistical difference in the shoot health of inoculated seedlings not drenched in CWE. However, HNS had a significant negative effect on the

ability of CWE to protect shoots from disease, decreasing protection by 30%. The nutrient solution made no difference in shoot health for roots drenched in CWE or roots inoculated with pathogen but not exposed to CWE but it improved shoot health for seedlings not treated with CWE or pathogen. One might expect that the additional nutrition and growth experienced by non-treated plants with the addition of HNS would positively impact the plants' ability to fight off infection but it did not. The pathogen by itself caused the same percentage of plants to die whether they were irrigated with HNS or not so HNS did not exasperate the effect of the pathogen. However, HNS did have an effect on the interaction between the CWE and the pathogen, inhibiting the ability of the CWE to be able to keep the shoots healthy.

The decrease in the suppression ability of the CWE when seedlings were watered with HNS may be due to the salt content of the HNS which could be detrimental to the beneficial microorganism in the CWE which help suppress disease. Most of the studies using CWE as a drench either do not provide additional nutrition or they use organic fertilizers. When inorganic fertilizers were used, they were added to all treatments making it impossible to determine its effects. Ingham and Rollins (2006) suggest that when adding CWE to a hydroponic nutrient, the electrical conductivity should be maintained between 0.01 and 1.0 dS/m to "make sure that the solution is the right environment for microorganisms to thrive. Testing needs to be done to determine how much of an inorganic salt-based nutrient can be added before harming the microbiology." In the greenhouse experiment, the EC of the nutrient solution was between 2.4 dS/m and 3.6 dS/m and in the laboratory experiment it was between 0.8 dS/m and 2.2 dS/m.

Like in the greenhouse experiments, all of the inoculated seedlings in the laboratory experiment developed lesions on the roots, with the exception of one plant treated with CWE. Again, more root recovery was seen in roots treated with CWE which in turn correlated with the percentage of healthy shoots. In the laboratory experiment, more inoculated roots were able to self-recover than in the greenhouse experiment with 60% recovery in fine coir, 47% in fine coir with HNS, and only 2% recovery in the greenhouse experiment. Even so, CWE improved the percentage recovering, from 60% to 83% and 47% to 87%.

In the greenhouse experiment, even though CWE increased the number of inoculated plants with healthy roots, the stem diameter, internodes lengths, and shoot fresh and dry weights were not significantly different than those of inoculated plants not treated with CWE (Table 6). However, in the laboratory experiment, the shoot fresh weight was greater for inoculated CWE-treated plants than the non-CWE-treated counterparts for both water irrigated and HNS irrigated plants and they were statistically the same as the weights of the non-inoculated seedlings (Table 7). Dry shoot weight and fresh and dry root weights of inoculated and CWE-drenched inoculated plants were the same for both water and HNS, except fine coir irrigated with water showed a larger fresh root weight for CWE-treated plants.

The anomaly of sand

Different substrates, even soilless substrates, have a natural ability to suppress disease depending on factors such as their texture, organic matter and nitrogen content, pH, porosity, water potential, and oxygen availability (Alabouvette et al. 1996; Janvier et al. 2007; Lozano 2009). For instance, *Fusarium wilts*, *R. solani*, and *P. sorghi* have been

shown to be more conducive in sand and more suppressive in clay (Amir et al. 1993; Cook and Papendick 1972; Gill et al. 2000; Hoeper et al. 1995; Schuh et al. 1987). Hibar (2006) found that perlite was more conducive than peat in its ability to develop *F. oxysporum* f.sp. *radicis lycopersici* in tomato. Lozano et al. (2009) reported that a compost particle size of 2-4mm increased the suppression of *F. oxysporum*, *P. cinnamomi,* and *M. hapla*.

In this study, it was found that sand was naturally suppressive to *F. solani* f.sp. *pisi* in pea. Only 23% of the inoculated seedlings grown in sand without CWE drench developed dead or dying shoots by the end of experiment, as compared to 80% in coarse coir, 77% in fine coir, and 87% in fine coir irrigated with HNS (Table 4), with corresponding fresh shoot weight being largest in sand (Table 8). At the same time, 97% of the roots developed disease symptoms but all of the roots recovered naturally, allowing roots and shoots to grow as if no pathogen were present. CWE provided a significant amount of protection beyond the natural suppression ability of the sand, reducing the dead or dying shoots to 10% and protecting 54% of the roots from infection. Overall, *F. solani* caused minimal damage to pea plants when they were grown in sand, with fresh and dry shoot and root weights being equivalent across all four treatments.

The finding that sand is suppressive to *F. solani* is contradictory to the research described above. It is consistent, however, with a study by Scher and Baker (1980) who found a fine sandy loam that was suppressive to Fusarium wilt and a clay loam that was naturally conducive. Interestingly, Curlango-Rivera et al. (2013) used the same materials and methods used in this laboratory study, including the same sand, and found no natural disease suppression. The only difference in method was that they sub-irrigated

periodically rather than top-irrigated. During periods of sub-irrigation, the substrate would be saturated with water, increasing the water potential and perhaps decreasing available oxygen to the roots. Large water potentials have been associated with increased disease incidence (Cook and Papendick 1972; Sterne et al. 1977), perhaps accounting for the 100% incidence of disease found by those researchers.

Results of this study indicated 90% protection of pea seedlings grown in sand when roots were drenched in CWE and inoculated with *F. solani* while Curlango-Rivera et al. (2013) found 100% protection. With materials and methods as a constant, the difference is most likely due to the nature of the CWE and its interaction with the sand. The compost sources were different and the microbial food additives were mostly the same (kelp, humic acid, rock phosphate and other minerals) but from different sources. Other than the compost source, the main difference in the CWE recipe was the inclusion of molasses in the CWE in this study. Scheuerell and Mahaffee (2004) found that adding as little as 0.01% molasses to an aerated CWE significantly increased damping-off caused by *P. ultimum*. This slight decrease in suppression in sand could be due to the inclusion of the molasses to the CWE.

Comparison of shoot health across substrates

Drenching substrate with CWE resulted in significant reduction of dead or dying shoots in all substrates tested, as shown in Table 4. Inoculated seedlings growing in fine coir, coarse coir, sand, and fine coir watered with HNS, resulted in 77%, 80%, 23%, and 87% of the shoots dying or dead, while drenching with CWE lowered these percentages to 20%, 40%, 10%, and 50% respectively. These numbers represent an increase in shoot health of 26%, 50%, 43%, and 57% respectively, as compared to inoculated plants which

were not treated with CWE. The protection provided by the CWE was also evident by the fact that the fresh shoot weights for inoculated CWE-drenched seedlings were equivalent to fresh shoot weights for non-inoculated seedlings within each substrate group, except for sand (Table 7). For sand, all fresh and dry shoot and roots weights were the same, as though no pathogen were ever present. The protection provided to the coconut coir substrates by the CWE was most pronounced for the fine coir and fine coir plus HNS, where the shoot weights were significantly larger than for the inoculated seedlings.

CWE-drenched fine coir and sand resulted in the highest percentage of healthy shoots, with health levels being equivalent statistically (Table 5). However, the fine and coarse coir provided statistically the same level of shoot health even though coarse coir had double the amount of dead and dying plants as fine coir. Despite the variability in shoot health, all four substrates had equivalent fresh and dry shoot and root weights for CWE-drenched inoculated seedlings, except the dry root weight in sand was slightly higher (Table 8).

Comparison of root health across substrates

Table 4 shows the root infection and recovery levels for all substrates in the laboratory experiments. When roots became infected, it was evident that the infection started in the area where the roots were first inoculated with pathogen in the pouches or before being sowed, and then expanded down the taproot to a variable degree. All of the pathogen-inoculated roots growing in coconut coir became infected and 97% were infected when growing in sand. Similar to results found in the greenhouse experiment, close to 100% of the CWE-drenched inoculated roots in coconut coir became infected as

well but only 43% of the roots became infected in sand. While 100% of the diseased seedlings growing in sand recovered naturally, 47% to 60% of the inoculated seedlings in the coconut coir substrates had some level of natural recovery, growing some amount of white roots past the lesion. However, the level of that recovery was less than that experienced by the sand, as evidenced by the greater percentage of dead and dying seedlings. CWE drenching increased the percentage of seedlings in coir that were able to recover from the disease, subsequently resulting in increased shoot health, but root fresh and dry weights were no different from inoculated seedlings without CWE drench. The only exception was that the root fresh weight of CWE drenched, inoculated roots was higher than for non-CWE-drenched inoculated roots.

Effect of CWE on growth

One explanation for disease suppression induced by CWE is that enhanced nutrition or effects of growth promoting hormones in the CWE may improve the health of the seedlings such that they can defend themselves better from pathogens (Pane and Zaccardelli 2014; Scheuerell and Mahafee 2002). In this study, the compost water extract did not have a significant effect on early root growth in the RGIA (Table 9) except that it resulted in a greater lateral development. These results are in agreement with Curlango-Rivera et al. (2013) who used an aerated vermicompost CWE and obtained similar results, except they found no difference in lateral development.

Tables 6 and 7 show the results of the plant growth parameters for both experiments. CWE alone had no effect on the growth of the plants in the greenhouse, as shown in Table 4. Mean fresh and dry weights, stem diameter, and internode lengths were not significantly different in plants whose roots were originally drenched in water or

CWE. Inoculation with *F. solani* decreased growth of the plants across the board. Mean growth was not significantly different for inoculated plants whether they were drenched in water or CWE. In the laboratory experiment, fresh and dry weights of shoots and roots of water-drenched and CWE-drenched substrates were not significantly different for any of the substrates tested. These results indicate that improved plant health caused by CWE was not a factor in the suppression induced by the CWE in this study.

Most studies involving CWE and plant growth have reported enhanced growth or yields (Fritz et al. 2012; Pane et al. 2014; Pant et al. 2009; Radin and Warman 2011; Xu et al. 2012). However, CWE was applied at higher concentration and involved multiple applications. These results are consistent with Knewston et al. (2009) who found no difference in fresh mass of collard greens when an aerated manure tea was applied as a drench three or more times in three different fertilization schemes. The concentration of CWE used in that study was close to that used in the current study. This study intentionally used only one early drench of CWE applied to the roots, without subsequent CWE application. The intent was to determine if one application was sufficient to induce systemic resistance or other resistance mechanisms in the plants. Because only one dose of a dilute CWE was used in this study, the results obtained are consistent with the research demonstrating that more frequent applications of CWE are needed to enhance growth and biomass.

Translation of laboratory suppression to container studies

The ability of a CWE to suppress disease is often attributed to the numbers or types of bacteria, fungi, and protozoa present even though there is little research to support threshold levels that work across all pathosystems (Ingham 2003; Scheurell and

Mahaffee 2002). One CWE may be suppressive to one pathogen but not another or it may be suppressive to that pathogen on one type of crop but not another. Many suppression mechanisms have been suggested, but none are completely understood or predictive (Litterick et al. 2004; Mehta et al. 2014; Noble and Coventry 2005; St. Martin and Brathwaite 2012; Santos et al. 2011; Scheuerell and Mahaffe 2002). Root growth and infection assays (RGIA) may be a tool used to predict whether a specific CWE will be effective at suppressing a specific disease for a specific type of plant. This study, together with Curlango-Rivera et al. (2013), tested two different CWE using RGIA and demonstrated approximately 100% protection of pea from *F. solani*. Although it is a laboratory-based test, a growth pouch assay may be useful as a predictor of suppression by a CWE because it provides an ideal high humidity environment for a pathogen to grow. If a CWE is able to suppress disease in the growth pouch environment where the pathogen is most virulent, it should be able to suppress disease in a non-ideal growing environment where it is harder for the pathogen to grow, but this would have to be tested.

The greenhouse transplant study tested the predictability of the pouch assay by taking the CWE-protected seedlings out of the laboratory and planting them in soilless substrate to determine if the protection would endure longer term without additional application of CWE. Despite 100% protection of roots from the pathogen when drenched in CWE in the pouches, all of the roots developed lesions when grown in the fine coconut coir substrate and watered with HNS. However, about 29% of the plants overcame the disease, growing some amount of healthy root mass beyond the lesions. In some cases, the healthy root mass was substantial and in others minimal (Figure 1). The reflection of the overall vitality of the root system was apparent by an overall slight reduction in

healthy shoots compared to healthy roots (29% of the roots showed some recovery from disease while 23% of the plants maintained healthy shoots).

The roots developed disease once transplanted. The previously protected seedlings may have sustained damaged during the transplant process, although care was taken during transplantation and any plants with broken roots were discarded. Roots are most susceptible to fungal pathogens when they are wounded and even with careful handling, roots may be wounded inadvertently (Doling 1963; Tooley et al. 2014). However, the fact that all the lesions observed occurred at the point of inoculation negates the idea of random wounds being the infection entry points.

Altered root border cell dispersion may provide an alternative explanation. Border cells are living cells produced only by the root tip and disperse readily in free water. Hawes et al. (1998) and Gunawardena et al. (2005) describe how these cells produce an extracellular DNA mucilage which attracts, traps, and immobilizes pathogens. In these studies, roots of peas were placed in a growth pouch, an environment with high humidity but not free water so that border cells remained at the root tip. Roots that were inoculated with *F. solani* f. sp. *pisi* developed lesions in the zone of elongation but the root tip remained uninfected. However, when examining the root tip under the microscope, it was discovered that the border cells trapped the spores, the spores germinated, and hyphae grew around the border cells but within the mucilage. When immersed in free water and agitated, these aggregates of border cells and hyphae, or "mantles", detached as an intact entity from the root tip. They also observed a microscopic lesion on the root cap which also came away with the mantle, subsequently leaving the root tip uninfected.

Root border cells, and the protection mechanism described above, only occur at the root tip. Because border cells are not produced in the region of soft tissue just behind the root tip, or the zone of elongation, this is where fungal infections are most often initiated (Gunawardena et al. 2005). However, Curlango-Rivera et al. (2013) and Tollefson et al. (2014) microscopically examined the roots of peas drenched in CWE and observed that root border cells did not disperse as they do in free water but remained tightly adhered to the root tip, forming a sheath which remains intact for hours even after agitation. Furthermore, this sheath of border cells extended into the zone of elongation.

In the greenhouse experiment, drenching the seedlings in CWE would have resulted in border cells forming a sheath around the zone of elongation and protecting it, just as it would the root tip. This would explain why 100% of those seedlings never developed necrotic lesions in the pouches.

Gunawardena and Hawes (2002) determined that the amount of time these mantles stay as a sheath around the root is critical to the level of protection obtained. Roots must be in the presence of enough free water to induce separation of the mantles to take the pathogens away from the root tip. In that study, root tips were observed three days after inoculation in non-reduced and reduced free water environments. In the presence of 15 ml of water in a growth pouch, mantles detached and 78% of the root tips remained uninfected, while 7 ml of water inhibited detachment and resulted in only 45% of the root tips being protected from infection. Since CWE inhibits the detachment of mantles similarly to a reduced water environment, and seedlings in the greenhouse transplant study remained in growth pouches for 8 days, it is possible that microscopic lesions occurred that were not seen by the naked eye. If the mantles remained intact on

the roots as they were transplanted, the additional contact time with the root would increase the chances of pathogen penetration. When a lesion first develops on the root, plant defense mechanism genes are turned on, including rcpme1 which signals the root to release border cells (Gunawardena and Hawes 2002). This signaled release, coupled with increased watering of the transplant would move the mantles with the pathogen away from the roots to prevent any secondary infections. Without supplemental addition of pathogen and with its defense mechanisms turned on, the root would be free of pathogen pressure and continue to grow healthy roots with the assistance of nutrients from the hydroponic fertilizer.

Inoculated, water-drenched roots in the pouches would not have the initial advantage of border cell protection at the zone of elongation, where in fact 100% of those seedlings developed lesions. Because the pathogen was applied along the entire length of the root, more root surface area could be infected in a shorter amount of time, in a root of smaller size than when the CWE-drenched roots first became infected. With the bombardment of pathogen during an earlier period in the seedling's life, the plant would have less chance to recover. The disease could quickly expand throughout the small root system. Even if plant defense mechanisms were initiated, the disease would already have taken hold and destroyed the root.

Conclusions

In conclusion, this study has found that CWE produced from a vegetable and green waste compost may provide significant protection to peas from fungal root rot infection. Sand and fine coconut coir fiber provided the highest levels of protection.

Laboratory experiment results show an increase in shoot health of 26%, 50%, 43%, and 57% for inoculated peas grown in CWE-drenched fine coir, coarse coir, sand, and fine coir watered with HNS. In the greenhouse experiment using fine coir watered with HNS, increase in shoot health of CWE-drenched inoculated seedlings was 21%. Despite the number of healthy shoots, nearly 100% of the roots grown in coconut coir substrates developed necrotic lesions but 86-87% grew healthy roots beyond the disease region when the substrate was drenched in CWE, resulting in healthier roots for those plants. Sand used in this study naturally protected seedlings from disease, even without the addition of CWE. Irrigating seedlings with hydroponic solution rather than water decreased the ability of the CWE to maintain shoot health by 30%, highlighting the potential for traditional fertilizer to thwart the benefits of using CWE to suppress disease.

This study was consistent with previous studies which examined the effect of CWE on disease in plants. This study and others found differences in the level of disease suppression shown by a CWE during *in vitro* testing compared to suppression observed when CWE was applied to pathogen-pressured plants. It is unclear, especially in a situation where soilless substrates are used which have with more consistent physical and chemical properties than natural soil, why less than 100% suppression occurs. More research needs to be carried out to determine the variables which cause the variability so that CWE and/or substrates can be engineered to induce more consistent disease suppression.

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TABLE 1. Experimental compost material characterization

pH	7.8	P (mg/kg)	568
EC (dS/m)	1.16	K (mg/kg)	2656
C (%)	18	Ca (mg/kg)	5600
C/N Ratio	52.8	Mg (mg/kg)	800
Moisture (%)	52.1	Na (mg/kg)	334
OM (%)	34.1	Al (mg/kg)	1429
Formazan (ppm)	41097	Fe (mg/kg)	1899
Total N (mg/kg)	3000	Mn (mg/kg)	57
Organic N (mg/kg)	3000	Cu (mg/kg)	6.3
Nitrate N (mg/kg)	2.3	Zn (mg/kg)	14.9

TABLE 2.
Microbial activity in different composts as measured by the Formazan method

Company/Product	Company location	Compost type/sources	Formazan (ppm)
Cascade Compost	Puyallup, WA	Thermal/unknown	399
Fairfax Companies	Tucson, AZ	Thermal/green waste	4072
Tanque Verde Farms	Tucson, AZ	Thermal /green waste, manure	9710
Ecoscraps	Sandy, Utah	Thermal/fruit, veg, wood, bark	41097
Wormgold	San Diego, CA	Vermicompost/unknown	363
Black Gold	Agawam, MA	Vermicompost/unknown	384
Unco Industries/Wiggleworm	UnionGrove, WI	Vermicompost/unknown	399
Nature's Solution	Greenbrae, CA	Vermicompost/vegetative	716
Worm Power	Avon, NY	Vermicompost/cow manure	2022
Organic Gardening Supply	Madison, WI	Vermicompost/fruit, vegetable	4421
Alaska Organics/Denali Gold Humus	Forestville, CA	Alaskan humus	330

TABLE 3.
Influence of *F. Solani* and CWE on final shoot and root health in greenhouse experiment[y]

	Shoot Health Index (SHI)			Root Infection Index(RII)			Root Recovery Index (RRI)		
Treatment[z]	Healthy	Dying or dead	% Dead or Dying	Not infected	Infected	% Infected	No recovery	Recovery	% Recovery
Water	140	4	3a	144	0	0a	NA	NA	NA
CWE	125	19	13b	144	0	0a	NA	NA	NA
F. Solani	3	141	98c	0	144	100b	141	3	2a
CWE + *F. Solani*	33	111	77d	0	144	100b	103	41	29b

[y] Values reflect results from 144 samples for each treatment combined over three trials. Percentages with the same letter are not significantly different according to chi-square tests (P<0.05).

[z] CWE = compost water extract

TABLE 4. Effect of *F. Solani* and CWE on final shoot and root health by substrate type[x]

	Shoot Health Index (SHI)			Root Infection Index(RII)			Root Recovery Index (RRI)		
Treatment[y]	Healthy	Dying or dead	% Dying or dead	Not infected	Infected	% Infected	No recovery	Recovery	% Recovery[z]
Fine coir									
Water	23	7	23a	30	0	0a	NA	NA	NA
CWE	28	2	7a	30	0	0a	NA	NA	NA
F. Solani	7	23	77b	0	30	100b	12	18	60a
CWE + *F. Solani*	24	6	20a	1	29	97b	4	25	86b
Coarse coir									
Water	23	7	23a	30	0	0a	NA	NA	NA
CWE	22	8	27a	30	0	0a	NA	NA	NA
F. Solani	6	24	80b	0	30	100b	16	14	47a
CWE + *F. Solani*	18	12	40a	0	30	100b	4	26	87b
Sand									
Water	30	0	0a	30	0	0a	NA	NA	NA
CWE	30	0	0a	30	0	0a	NA	NA	NA
F. Solani	23	7	23b	1	29	97b	0	29	100a
CWE + *F. Solani*	27	3	10a	17	13	43c	0	13	100a
Fine coir + Hoag									
Water	30	0	0a	30	0	0a	NA	NA	NA
CWE	30	0	0a	30	0	0a	NA	NA	NA
F. Solani	4	26	87b	0	30	100b	16	14	47a
CWE + *F. Solani*	15	15	50c	0	30	100b	4	26	87b

[x] Values reflect results from all 30 replicates for each treatment combined over three trials. Percentages with the same letter are not significantly different according to chi-square tests ($P<0.05$).

[y] CWE = compost water extract

[z] Reflect percentage of roots that were infected.

TABLE 5. Effect of *F. Solani* and CWE on final shoot and root health by treatment[y]

	Shoot Health Index (SHI)			Root Infection Index(RII)			Root Recovery Index (RRI)		
Treatment[z]	Healthy	Dying or dead	% Dead or Dying	Not infected	Infected	% Infected	No recovery	Recovery	% Recovery
F. Solani									
Fine coir	7	23	77a	0	30	100a	12	18	60a
Coarse coir	6	24	80a	0	30	100a	16	14	47a
Sand	23	7	23	1	29	97a	0	30	100
Fine coir + Hoag	4	26	87a	0	30	100a	16	14	47a
CWE + *F. Solani*									
Fine coir	24	6	20ac	1	29	97a	4	26	87a
Coarse coir	18	12	40ab	0	30	100a	4	26	87a
Sand	27	3	10c	17	13	43	0	30	100a
Fine coir + Hoag	15	15	50b	0	30	100a	4	26	87a

[y] Values reflect results from all 30 replicates for each treatment combined over three trials. Percentages with the same letter are not significantly different according to chi-square tests ($P<0.05$).

[z] CWE = compost water extract

TABLE 6.

Effect of *F. Solani* and CWE on plant growth in fine coconut coir irrigated with hydroponic nutrient solution in greenhouse experiment[y]

Treatment[z]	Shoot Fresh weight (g)	Shoot Dry weight (g)	Stem diameter (mm)	Internode 1 length (cm)	Internode 2 length (cm)
Water	23.79a	3.65a	3.06ac	8.61a	8.70a
CWE	25.20a	4.00a	3.30a	8.36a	8.56a
F. Solani	1.28b	0.53b	2.51bc	4.02b	4.05b
CWE + *F. Solani*	6.63b	1.34b	2.20b	5.01b	5.39b

[y] Mean values represent results from 36 samples out of the total 144 plants per treatment combined over 3 trials, except stem diameters were results from 16 samples per treatment. Values with the same letter are not significantly different according to ANOVA and Tukey post-hoc tests ($p<0.05$).

[z] CWE = compost water extract

TABLE 7.
Effect of *F. Solani* and CWE on plant growth by substrate[y]

	Shoot		Root	
Treatment[z]	Fresh Weight (g)	Dry Weight (g)	Fresh Weight (g)	Dry Weight (g)
Fine coir				
Water	0.754a	0.058a	0.922a	0.059a
CWE	0.759a	0.057a	0.873a	0.054a
F. Solani	0.256	0.056a	0.545	0.036b
CWE + *F. Solani*	0.676a	0.057a	0.813a	0.048ab
Coarse coir				
Water	0.668a	0.054ab	0.829a	0.052a
CWE	0.682a	0.056a	0.825a	0.056a
F. Solani	0.418b	0.038b	0.654a	0.058a
CWE + *F. Solani*	0.526ab	0.050ab	0.703a	0.047a
Sand				
Water	0.900a	0.065a	0.769a	0.073a
CWE	0.879a	0.063a	0.660a	0.056a
F. Solani	0.762a	0.061a	0.641a	0.064a
CWE + *F. Solani*	0.827a	0.063a	0.739a	0.074a
Fine coir + Hoag				
Water	0.871a	0.057ab	0.881a	0.049a
CWE	0.950a	0.063a	0.794a	0.042ab
F. Solani	0.078	0.045b	0.460b	0.030bc
CWE + *F. Solani*	0.635a	0.055ab	0.692ab	0.036ac

[y] Values reflect results from 9 samples for each treatment combined over three trials. Percentages with the same letter are not significantly different according to ANOVA and Tukey HSD post-hoc tests ($P<0.05$).

[z] CWE = compost water extract

TABLE 8.
Effect of *F. solani* and CWE on plant growth by treatment[y]

Treatment[z]	Shoot		Root	
	Fresh Weight (g)	Dry Weight (g)	Fresh Weight (g)	Dry Weight (g)
Water				
Fine coir	0.754a	0.058a	0.922a	0.059ab
Coarse coir	0.668a	0.054a	0.829a	0.052a
Sand	0.900a	0.065a	0.769a	0.073b
Fine coir + Hoag	0.871a	0.057a	0.881a	0.049a
CWE				
Fine coir	0.759ab	0.057a	0.873a	0.054b
Coarse coir	0.682b	0.056a	0.825a	0.056b
Sand	0.879ab	0.063a	0.660a	0.056b
Fine coir + Hoag	0.950a	0.063a	0.794a	0.042b
F. Solani				
Fine coir	0.256ab	0.056a	0.545a	0.036ab
Coarse coir	0.418a	0.038a	0.654a	0.058ac
Sand	0.762c	0.061a	0.641a	0.064c
Fine coir + Hoag	0.078b	0.045a	0.460a	0.030b
CWE + *F. Solani*				
Fine coir	0.676a	0.057a	0.813a	0.048a
Coarse coir	0.526a	0.050a	0.703a	0.047a
Sand	0.827a	0.063a	0.739a	0.074b
Fine coir + Hoag	0.635a	0.055a	0.692a	0.036a

[y] Values reflect results from 9 samples for each treatment combined over three trials. Percentages with the same letter are not significantly different according to ANOVA and Tukey post-hoc tests ($P<0.05$).

[z] CWE = compost water extract

TABLE 9.
Influence of *F. Solani* and CWE on root growth in RGIA[y]

	Root Length (mm)		
Treatment[z]	Day 1	Day 5	Number of laterals
Water	14.16a	61.87a	10.99a
CWE	12.16b	61.3a	13.31cd
F. Solani	13.32ab	66.98ab	11.83ab
CWE + *F. Solani*	10.92c	57.93ac	12.87bd

[y] Mean values reflect results from 210 seedlings per treatment combined over 3 trials. Values with the same letter are not significantly different according to ANOVA and Tukey post-hoc tests ($P<0.05$)

[z] CWE = compost water extract

FIGURE 1. Comparison of roots from greenhouse transplant experiment. A) No pathogen, healthy root, B) Complete necrosis C) Disease region with minimal recovery D) Disease region with extensive recovery

APPENDIX B

ALTERED CARBON DELIVERY FROM ROOTS: RAPID, SUSTAINED INHIBITION OF BORDER CELL DISPERSAL IN RESPONSE TO COMPOST WATER EXTRACTS

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Abstract

Background and aims In a previous study, a compost water extract (CWE) applied to pea seedlings resulted in >95% protection against root infection. The protection was correlated with retention of a sheath of root border cells surrounding each root tip. A transient exposure to CWE was correlated with 80% reduction in infection, and with retention of border cell sheaths. Early effects of CWE on border cell dispersal therefore were examined.

Materials and methods Temporal and spatial dynamics of pea, maize, cotton, and cucumber border cell dispersal into water or CWE were measured.

Results Border cells formed a mass surrounding root tips within seconds after exposure to water, and most cells dispersed into suspension spontaneously. In CWE, >90% of the border cell population instead remained appressed to the root surface, even after vigorous agitation. In cotton, for example, >25,000 border cells dispersed within seconds of immersion in water, but <100 border cells dispersed after >24 hours in CWE.

Conclusions Border cells can contribute >90% of carbon released from young roots, and a single border cell can trap hundreds of bacteria within minutes. The impact of altered border cell dispersal on soil properties, plant nutrition, and root disease development warrants further study.

Keywords: Root border cells, Rhizosphere microbiome, Carbon deposition, Belowground C, Root-derived C, Compost

Abbreviations
CWE: compost water extract
ANOVA: analysis of variance
DI: deionized

Introduction

The importance of microbial community growth and development at the root-soil interface, and its role in plant growth and development, has long been recognized (Rovira 1991). Burgeoning interest in defining how patterns of colonization occur is reflected in the publication of more than seventy reviews on rhizosphere microbiology in the last year alone (webofknowledge.com). The rhizosphere develops as the root tip penetrates the soil environment, and a well-characterized complex of carbohydrates, proteins and small metabolites is released from the root cap into the extracellular matrix (Baetz and Martinoia 2013; Vermeer and McCully 1982). Defining how this plant-controlled process contributes to rhizosphere microbiome patterns may have been hindered by a long-standing presumption that the 'sloughed' cells released within this matrix are dead cells passively leaking their contents into the soil and providing a general source of nutrients (Hawes et al. 2012). Instead, cells that detach from the root cap of most species are viable upon dispersal into hydroponic culture or the soil surrounding the elongating root, where they can survive and even continue to grow (Guinel and McCully 1987; Knudson 1919). Populations of these detached root 'border cells' have a high rate of metabolic activity, and their gene expression patterns are distinct from those of progenitor cells in the root cap (Wen et al. 2007).

Host- and microbe-specific patterns of growth inhibition and stimulation by border cells have been documented, but the impact of the cell populations in plant health has remained unclear (Gochnauer et al. 1990; Hawes and Brigham 1992). As summarized by Rogers et al. (1942), *'Early recognition of the sloughing away of certain root tissues as a normal process during root growth led to little more than mere speculation as to what the role of such a process might be in the nutrition of the plant. The process was assigned, quite summarily it seems, the function of lubricating the advancing root tip as it forced its way into the soil.'* Emerging evidence now has revealed key parallels with an extracellular trapping process that occurs in mammalian immune responses (Brinkmann and Zychlinsky 2012; Hawes et al 2011). As with neutrophils, border cell production is a tightly controlled process that can be stimulated or suppressed in response to distinct signals (Curlango-Rivera et al. 2010). Upon contact with pathogens, neutrophils and border cells export a slime matrix that immobilizes the invader; extracellular DNA is an integral component of this 'extracellular trap' which disintegrates in response to addition of DNase, resulting in loss of resistance to infection (Brinkmann et al. 2004; Wen et al. 2009). Border cell slime layers also are induced within minutes by exposure to aluminum, copper, and other metals, which are trapped within the matrix (Cai et al. 2013; Curlango-Rivera et al. 2013a). Conversely, border cells can stimulate protozoa, nitrogen fixing bacteria and other soil-borne organisms (Somasundarum et al. 2008; Zhu et al. 1997). Understanding how border cells influence rhizosphere development under

divergent conditions may yield new insights into sustainable crop production methods (Odell et al. 2008).

A growth pouch assay previously was used to measure impact of a compost water extract (CWE) on fungal infection of pea during early stages of root development (Curlango-Rivera et al. 2013b). Even under optimal conditions for disease development, which normally result in infection of >95% of inoculated seedlings within 2-3 days, no lesions developed when CWE was added during inoculation of seedlings with spores of *Fusarium solani* f. sp. *pisi* in pouches or in sand. The protection was found to be correlated with retention of border cells that remained as a sheath covering the root tip after several weeks. In the current study, we report that immersion of maize, pea, cucumber and cotton root tips into two different CWE mixtures made by independent sources results instantaneously in a dramatically altered pattern of border cell dispersal.

Materials and methods

Plant material

Pea seeds (*Pisum sativum* L.) cv 'Little Marvel' (Meyer Quality Seeds, Baltimore, MD) and 'Alaska' (Chesmore Seed Company, St. Joseph MO) were treated as described (Brigham et al. 1995; Curlango-Rivera et al. 2010, 2013a,b): after surface-sterilization by immersion in 95% (v/v) ethanol followed by 30 minutes in 0.5% sodium hypochlorite, seeds were rinsed 5 times with sterile deionized water followed by a 6 h imbibition in sterile water. Seeds that floated to the surface were discarded, and the remaining seeds were maintained at 99% humidity during germination by placing onto 1% agar (Bacto TM Agar, Becton Dickinson and Co., Baltimore MD, USA) overlaid with sterile germination paper (Anchor Paper Co., Hudson WI, USA) at 24 C for 48 h, until emergent radicles were 20-25 mm in length. Cucumber (*Cucumis sativa*) cv 'Tendergreen' (Ferry Morse Seeds Company, Fulton KY), maize (*Zea mays* L.) cv 'Golden Cross Bantam,' and cotton (*Gossypium hirsutum* L.) cv PHY499 (Dow AgroSciences) were surface sterilized sterilized by immersion in 95% ethanol for 5 minutes followed by 10 minutes in 0.5% sodium hypochlorite, then rinsed 6 times in sterile water before germination on 1% water agar overlaid with sterile paper and incubated until radicles were 20-25 mm in length..

Compost preparation and handling

Two distinct preparations (CWE#1, CWE#2) were provided by independent sources (authors TP, SJT, respectively). Fresh batches were prepared for each experiment.

CWE#1 (TP) components and preparation were as described in Curlango-Rivera et al. 2013b: The following ingredients were added to a 18.9-l brewer (www.simplici-

tea.com) filled with well water: 250 ml Alaskan humus (Earthfort, www.earthfort.com/), 250 ml worm castings (Acme Worm Farm, Tucson AZ), 15 ml rock phosphate dust (Peaceful Valley, www.groworganic.com), 57 g fish hydrolysate (Earthfort), 28 g Algamin (Cold Processed Kelp--Peaceful Valley), 28 g "organic Turf Pro" (Earthfort); 15 ml "Compost Tea Catalyst " (Peaceful Valley). the mixture was maintained at 26 C for 18 h, 40 minutes, and the presence of a mixed population of bacteria, fungi, and protozoa was confirmed by direct microscopic examination of representative samples. Prior to initiation of assays, pH (5.2 +0.6) was measured.

For CWE#2 (SJT), an 18.9 L container was filled with municipal tap water, which was continuously aerated for 24 h to remove chlorine prior to use in compost preparation. Two air lines from an 18 Watt, 38 L/min, 20 kPa commercial air pump were placed into the water . A 2.5 cm x 1.25 cm airstone was attached to one line and the other line remained open. The following ingredients were then added and mixed: 29.6 ml unsulfured blackstrap molasses (Plantation, www.vitacost.com), 14.8 ml kelp meal (Maxicrop, www.maxicrop.com), 14.8 ml liquid humic acid (Ful-Power, www.bioag.com), 14.8 ml soft rock phosphate (Dr. Earth, www.drearth.net) and 14.8 ml silicates (Silica Blast, Botanicare, www.botanicare.com), 473 ml wood- and vegetable-based thermally produced compost from Ecoscraps, Phoenix, AZ (http://ecoscraps.com) in a 250-micron mesh bag (Pentair Aquatic Ecosystems, www.aquaticeco.com) was placed in the container. The mixture was continuously aerated for 22 h at 21°C. Each CWE preparation was examined microscopically to confirm the presence of bacteria and protozoa. Immediately after aeration was turned off, the pH was between 7.6-8.1, electrical conductivity ranged from 1.4 and 1.5 dS/cm, and the alkalinity from 440-560 mg/L as $CaCO_3$. CWE was taken to the test laboratory and aerated until use in assays.

Altered border cell dispersal

Dispersal of border cells after immersion of roots into water or CWE preparations was measured by direct observation over time using an Olympus BX60 F5 compound microscope, and a Zeiss STMI SV8 stereoscope. Photographs to illustrate altered dispersal patterns over time were taken using a Leica DFC290 HD digital camera equipped with Leica Application Suite Version 4.0.0 2011. Border cell number was measured by direct counts after removing the root from suspension. The number of cells released into suspension was obtained by counting the total number of cells within at least three sample aliquots taken from each of at least ten individual roots in at least three independent tests. For CWE-treated roots in which dispersal was reduced to a degree that most border cells remained adhered to the root surface, the entire liquid sample was collected after removal of the root tip, and was centrifiged to pellet border cells so that all dispersed cells could be counted. Border cell viability was measured based on the presence of cytoplasmic streaming in samples of at least 100 individual cells (Hawes and

Wheeler 1982). All values are based on evaluation of at least 30 replicate roots in at least 3 independent experiments.

Statistical analysis Analysis of variance (ANOVA) followed by Tukey-Kramer HSD test to compare means were performed using JMP 11, Second Edition (SAS Institute Inc. 2014. Cary, NC. USA).

Results

Border cell dispersal on legume roots immersed into water or bacterial suspensions

On roots of pea seedlings germinated at >90% humidity, in the absence of free water, and viewed with a dissecting microscope, border cells were invisible (Fig. 1a). The surface of the root cap was smooth, with no protuberances or other evidence of cellular material extending beyond the epidermis. Only when viewed using scanning electron microscopy were the contours of the individual border cells on the root surface evident (Fig. 1b). Upon exposure to water, including even a single droplet forming and falling onto the root, mucilage encasing border cells began to absorb water instantaneously and within 30-60 sec the cell populations became obvious in photographs as an expanding mass surrounding the root tip (Fig. 1c).

When immersed into a suspension of plant pathogenic bacteria (*Pectobacterium carotovora*), border cell dispersal also began instantaneously, and chemotaxis toward detaching border cells was immediately evident (Fig. 1d, arrow). At higher magnification, dozens of bacteria could be seen to be trapped within an extracellular layer $\geq$15 micrometers in diameter, approximately the width of a single cell, within 2-3 minutes (Fig. 1e, arrow).

Dynamics of border cell dispersal from pea roots immersed into water or CWE

In a previous study, protection of pea roots from fungal infection during a 13-day period of observation in growth pouches was found to be correlated with retention of a sheath of border cells surrounding root tips throughout the test period (Curlango-Rivera et al. 2013b). Preliminary tests revealed that even a transient exposure to CWE#1, by a 15-minutes immersion of roots in the CWE before placing seedlings into pouches and inoculating with fungal spores, resulted in reduced frequency of root infection at 9 days after inoculation, from 100% (10/10) to 20% (2/10). To test the possibility that border cell sheaths surrounding the root tip might develop during this brief exposure to CWE, a time course measuring pea border cell dispersal in water was compared with border cell dispersal from pea roots immersed in CWE#1 (Fig. 2).

Within the period of time needed to insert a root into water and focus the microscope camera (<10 sec), expansion of the border cell-mucilage was evident (Fig. 2a, arrows). Increased expansion and dispersal of distinctive clumps on each side of the root tip (arrows) continued over a 60-sec period of observation (Fig. 2b, c, d). When the root was gently lifted from the water with forceps and then reinserted, the cell masses (arrows) detached from the root surface (Fig. 2e). When the root tips were removed and the cells remaining in suspension were enumerated by direct counts, each root yielded cell numbers consistent with values obtained by gentle agitation of pea roots in water in previous studies (Table 1) (Curlango-Rivera et al. 2010). To assess the possibility that this dispersal pattern is an artifact of immersion in sterile deionized water, dispersal was measured using alternative sources. Mean cell numbers for roots immersed in Luria broth, municipal water, or in water mixed with sand, coconut coir or clay loam soil did not vary significantly from cell numbers dispersed into sterile deionized water (Table 1).

When pea root tips were immersed into CWE#1, an instantaneous swelling of the border cell-mucilage surrounding the root tip occurred as in water (Fig. 2f, arrow). In contrast with root tips in water, however, the mucilage swelling ceased within 10 minutes and border cell dispersal did not occur (Fig. 2g,h). Even over a 5-hour period of observation, the root tip appearance remained nearly identical to the initial image (Fig. 2i). When the root was lifted from the liquid and reimmersed, and border cells remained adhered to the root tip (Fig. 2i). The granular background appearance of the CWE remained unchanged over the test period (Fig. 2f-i).

The altered border cell dispersal phenomenon was found to be consistent among multiple replicate experiments tested using three separate batches of CWE#1 (n=92 roots). When roots were removed from the liquid samples and the border cells remaining in suspension were counted, the highest number of cells obtained from individual roots was 320 (Table 1. Even when the root was subjected to repeated vigorous agitation with water squirted from a Pasteur pipette directly onto the root surface, and mechanical scraping of the root against the plate, the highest number of border cells that detached from the root surface was 1200, and nearly all (>95%) were present in aggregates of >100 cells rather than dispersed single cells.

To assess the possibility that this phenomenon was a function of distinctive physical, biological or chemical properties unique to CWE#1, a second mixture with different ingredients, preparation, and pH, and provided by an independent source was tested. Results with CWE#2 were similar to those obtained with CWE#1: within seconds of immersion, swelling of the mucilage became evident but, , no further expansion occurred over a 6-hour period of observation When border cells from individual roots (n=43) in suspension were counted, the highest number obtained was 500 (range 73-500) (Table 1). Even after vigorous agitation and mechanical scraping of root tips (n=49), the highest number of dispersed border cells was 850 (range 79-850).

Possible explanations for the altered response include killing or disruption of border cells by toxins or other products within the CWE, resulting in altered mucilage production and function (Carballo et al. 2009). When roots immersed into CWE#1 were observed at higher magnification, most border cells were found to be within layers adhered closely to the root surface (Fig. 3a, arrows). In some cases larger clumps of border cells were present, but still remained adhered to the root surface (Fig. 3b, arrows). The morphology of the cells was normal, and cell viability in CWE#1 (95 $\pm$3%) and CWE#2 (92 $\pm$5%) was not significantly different from that of cells in water (93 $\pm$4%).

An alternative explanation of the change in mucilage expansion and border cell dispersal is that locally altered osmoticum within root tip-compost mixtures could inhibit uptake of water into the mucilage; this change would be predicted to result in plasmolysis of cells and altered cell viability (Hawes and Wheeler 1982). Direct observation of cells adhered to the root surface revealed no evidence of plasmolysis in living cells (n=300 cells), and cell viability was 94$\pm$5%.

Dynamics of border cell dispersal from maize roots immersed into water, bacterial suspension, or CWE

Border cells of maize dispersed within minutes upon immersion of root tips into water (Fig. 4a). Mean cell viability was 97$\pm$3% (n=753 cells). When pathogenic bacteria were added to the water, chemotaxis and trapping by border cells was evident within minutes (Fig. 4b-d).

In contrast with pea border cells which disperse completely into suspension, on maize root tips immersed into water a circular mass of border cells formed and expanded within 60 minutes to a diameter of >1 cm but without agitation the mass remained in place (Fig. 5a). Border cell dispersal in response to immersion in water results in activation of cell cycle within the root cap meristem within 10 minutes (Ponce et al. 2005). New cells began to emerge from maize root tips within 30 minutes after immersion into water (Fig. 5a, arrow). In contrast, on roots immersed into CWE#1, the surface remained smooth and slightly irregular (arrows), and no border cells were detectable apart from the root even after overnight incubation in the liquid (Fig. 5b). Similar results occurred on roots immersed into CWE#2 (not shown).

Altered border cell dispersal on cotton roots immersed into CWE#1 and CWE#2

Cotton border cells dispersed so quickly upon immersion of root tips into water that photographing the mass was a challenge (Fig. 6). Lifting the root after 10 sec, without agitation, yielded >20,000 cells in suspension (Table 2. None remained adhered to the root tip. Upon immersion into CWE#1 or CWE#2, in contrast, each root exhibited complete absence of border cell dispersal even after extended incubation periods of >5 h (Fig. 6, inset) (Table 2).

Dynamics of border cell dispersal from cucumber roots immersed into water or CWE

Swelling of border cells from cucumber roots was evident within 10 sec after immersion of the roots into water (Fig. 7a), and became increasingly obvious at 30-120 (Fig. 7b). After gently lifting the root and reinserting it into the sample of water, all border cells were released into suspension (Fig. 7c). The mean number of cells dispersed from individual roots (n=57), without agitation, was 3498 ±855 (Table 2)

For most cucumber roots (n=86/95), inhibition of border cell dispersal in CWE#1 and CWE#2 was similar to that observed in pea, maize, and cotton (as in Figs. 2-6). Within 30 sec after immersion in CWE#1, a roughened surface was evident as mucilage absorbed water and dispersing border cells formed a visible sheath (Fig. 7d). A slight increase in the dimensions was evident after 5 minutes (Fig. 7e), but the appearance remained virtually indistinguishable even after 90 minutes (Fig. 7f). After lifting and reinserting these roots, the mean number of cells dispersed into solution was only 212 ±179

In contrast with the consistent dispersal responses of all tested pea, maize, and cotton roots to CWE, some individual cucumber roots (8 ±5%) exhibited a distinct phenotype. For example, border cells on one root immersed into CWE#1 remained tightly adhered to the root tip even after 30 minutes (Fig. 7g). Border cells on a second, adjacent root immersed at the same time into same sample of CWE, however, expanded to a significant degree within 5 minutes (Fig. 7h) and continued to expand over time (Fig. 7i). Among 37 roots which exhibited this phenotype, when lifted and reimmersed into the liquid, significantly higher numbers of border cells (1226 ±645 cells) were found to be dispersed in solutio. No other obvious differences in root phenotype (e.g. growth rate, morphology, development, etc) were detected among these seedlings.

In a previous study, sheath development over time in growth pouches also was distinct in cucumber compared with pea, with detachment of the sheaths over time, and root infection was reduced by only 38% in cucumber compared with 100% protection in pea (Curlango-Rivera et al. 2013). Further research is needed to evaluate whether this difference in retention of border cell sheaths plays a role in the observed difference in inhibition of root infection.

Discussion

We report here for the first time that the number of root border cells that disperse into the extracellular environment from roots with a full set of border cells present on the tip, can be altered by orders of magnitude in response to even a transient exposure to certain compost mixtures. To our knowledge, altered border cell dispersal in response to

compost or other treatments has not been described previously, but altered appearance of microbial colonization on root tips was shown to occur in response to compost on plants grown on metalliferous mine tailings (Iverson and Maier 2009). Complex cytological barriers also were reported to occur on roots inoculated with *Fusarium* in the presence of compost (Pharand et al. 2002). The need to define underlying mechanisms by which compost influences aspects of crop production is recognized (e.g. Bailey and Lazarovits 2003; Ben-Yephet and Nelson 1999; Carballo et al. 2009; Craft and Nelson 1996; Cummings et al. 2009; Elmer and McGovern 2004; Everts et al. 2006, Fichtner et al. 2004; Kavroulakis et al. 2010; Larkin 2008; Litterick et al. 2002; McKellar and Nelson 2003; Xu et al. 2012; Zhang et al. 1996). Understanding factors that influence the effectiveness of compost could make it feasible to measure the impact of specific mixtures on specific crops and facilitate wider commercial application, especially under controlled conditions in which the growth matrix and environment can be engineered for optimal performance (Marin et al. 2013; Noble and Coventry 2005; Sabeh et al. 2011). Improved precision in tracking carbon deposition sources and their fate in situ, over time, also will facilitate field studies of plant-regulated factors controlling soil microbiology at the root-soil interface (Chalk et al. 2012; Duong et al. 2012; Ebid et al. 2008; Pausch et al. 2013).

Root border cells can contribute up to 98% of the total weight of root exudates that condition the development of the rhizosphere (Griffin et al. 1976). The function of the cell populations and their excretions has remained unclear. Recent recognition that an extracellular trapping process that occurs in animal cells also functions in border cells has provided new insight into host-microbe recognition and response at the root-soil interface (Brinkmann and Zychlinsky 2012). The discovery that DNA is a component of the extracellular matrix surrounding border cells may offer insight into nutritional aspects of the rhizosphere, considering the long-standing observation that extracellular phosphatases also are present (Rogers et al. 1942). The fact that individual border cells can specifically stimulate or inhibit growth of distinct microbial populations could in part underlie previously inexplicable variation in rhizosphere community structure (Raajmakers et al. 2009). Variation in border cell production and dispersal in response to signals from treatments such as compost could be an even more important factor in defining how populations develop, by creating islands of microbial activity or quiescence within the rhizosphere.

Conclusions

Further research is needed to define mechanisms by which CWE causes border cells to aggregate as an apparent protective sheath tightly bound to the root surface,

instead of dispersing into the environment enmeshed with trapped metals, bacteria, fungi, nematodes and other pathogens (e.g. Hawes et al. 2012).

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Table 1. Pea border cell dispersal in CWE and other media*

Medium	Border cell number	Range
DI water	3855 ± 457[a]	2800-4500
CWE #1	117 ± 102[b]	7-320
CWE #2	277 ± 125[c]	75-540
Luria Broth	3876 ± 402[a]	2790-4600
Tap water	3745 ± 370[a]	2900-4250
Sand	3818 ± 501[a]	2900-4600
Coconut coir	3597 ± 478[a]	2800-4200
Clay loam soil	3440 ± 501[a]	2300-4200

*Values reflect the mean ± standard deviation from at least 30 replicates samples for each treatment. For each column, numbers followed by the same letter are not significantly different ($p < 0.05$).

DI = Deionized water

CWE = Compost water extract.

Table 2. Corn, cucumber, and cotton border cell dispersal in water or CWE.

Treatment*	Corn	Cucumber	Cotton
DI water	3840 ± 404[a]	3563 ± 944[a]	27,492 ± 2169[a]
CWE #1	19 ± 13[b]	217 ± 183[b]	124 ± 114[b]
CWE #2	22 ± 15[b]	393 ± 458[b]	166 ± 196[b]

*Values reflect the mean ± standard deviation from at least 19 replicates samples for each treatment. For each column, numbers followed by the same letter are not significantly different ($p < 0.05$).

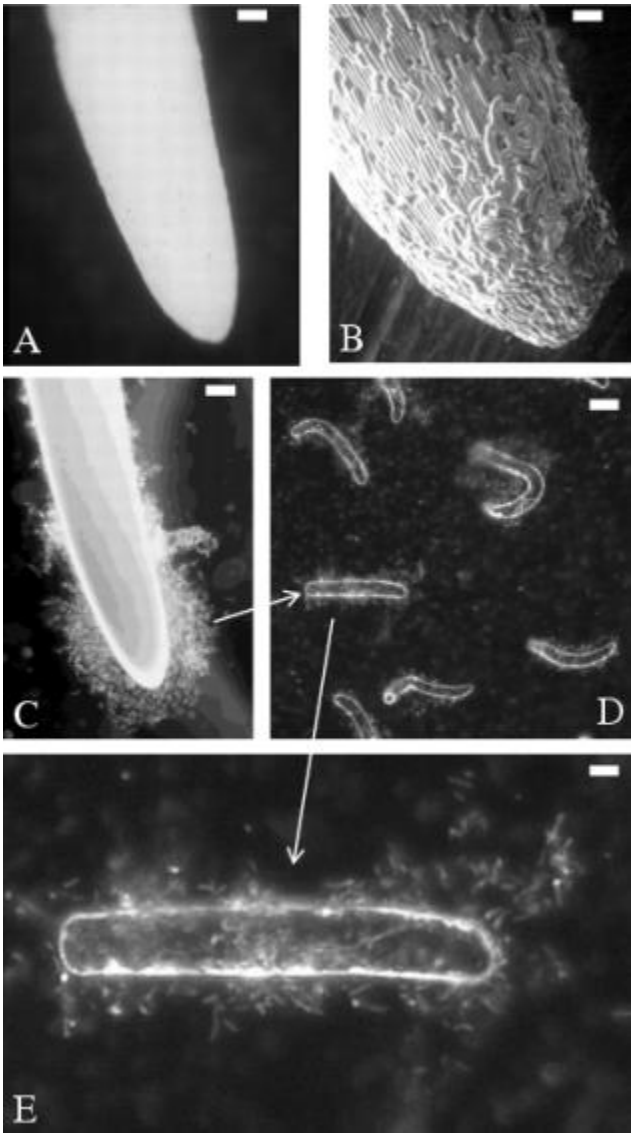

Fig. 1. **a**, Appearance of a pea root tip maintained at >95% humidity, in the absence of free water, viewed with a Zeiss stereoscope without fixation or other treatment. Scale bar: 250 microns. **b**, Use of scanning electron microscopy to view a soybean root tip, in the absence of free water (from Hawes and Brigham, 1992). Scale bar: 100 microns. **c,** Altered appearance of root tips as border cells dispersed from the root surface within 30 seconds of immersing the root into water. Scale bar: 350 microns. **d,** When pea root tips were immersed in a suspension of *Pectobacterium carotovorum*, chemotaxis was evident as masses aggregating around individual border cells (arrow). Scale bar: 20 microns. **e,** When viewed at higher magnification, the masses surrounding border cells could be seen to be trapped bacteria (arrow). Scale bar: 5 microns.

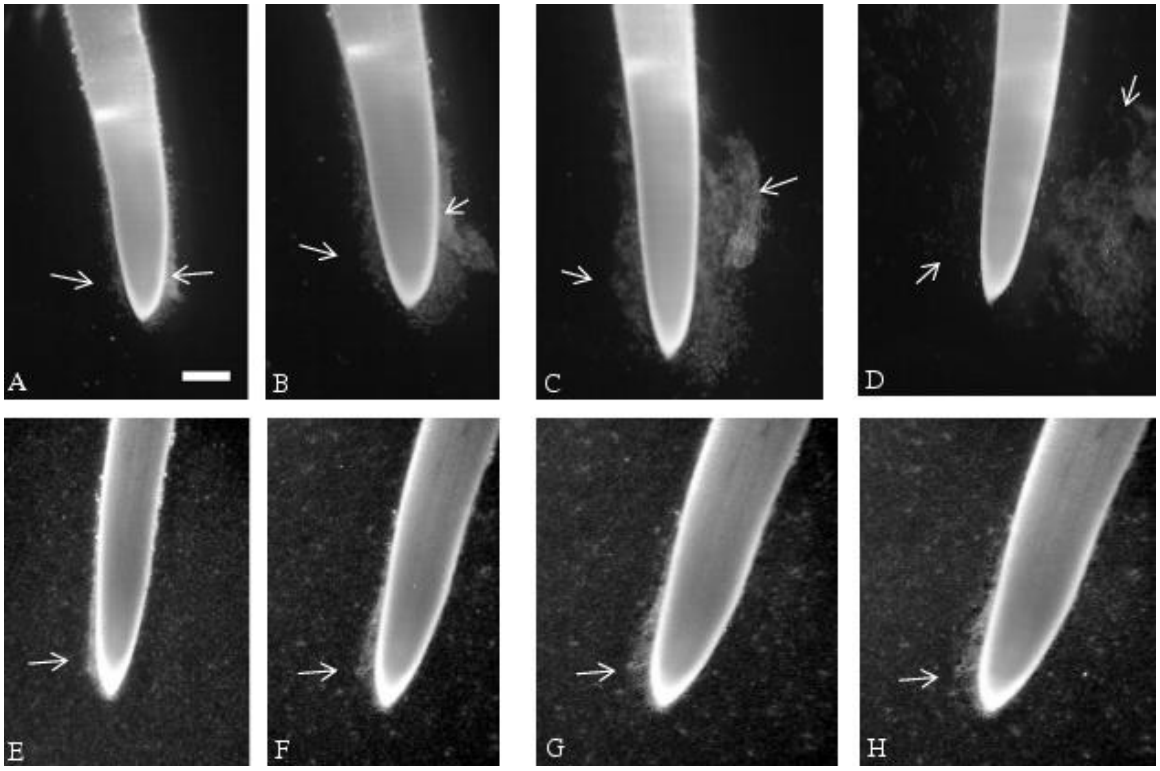

Fig. 2. Time course for pea border cell dispersal into suspension, upon immersion of root tips into water (**a-de**), or CWE#1 (**e-h**). Swelling of border cell mucilage (arrows) after immersion into water for **a,** 5 sec; **b,** 10 sec; **c,** 60 sec; **d,** dispersal of border cells into suspension after lifting the root gently at 65 seconds after immersion, then reinserting into the water, without additional agitation. Border cells on pea roots immersed in CWE#1 for **e**, 10 sec; **f,** 10 min; **g** 2.5 hours; **h**, 5 hours, after lifting and reinserting into CWE#1.

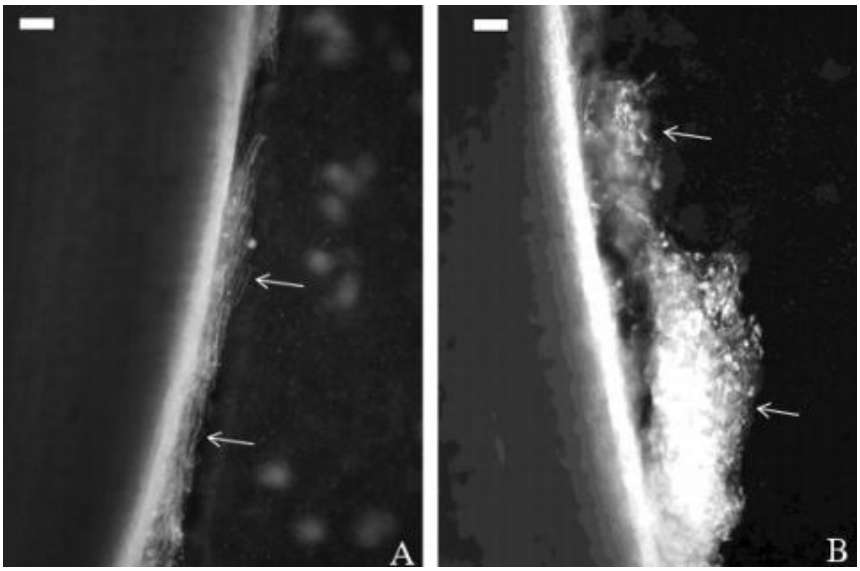

Fig. 3. Retention of border cells at the surface of pea roots. **a,**4 hours after immersion in liquid CWE#1, despite lifting the root, repeatedly applying water directly to the root tip surface using a pasteur pipette, and rubbing the root along the surface of the dish**; b,** An aggregate of border cells adhered to the root surface after 4 hours' immersion in CWE#1, subsequent to repeated agitation**.** Scale bars: 50 microns.

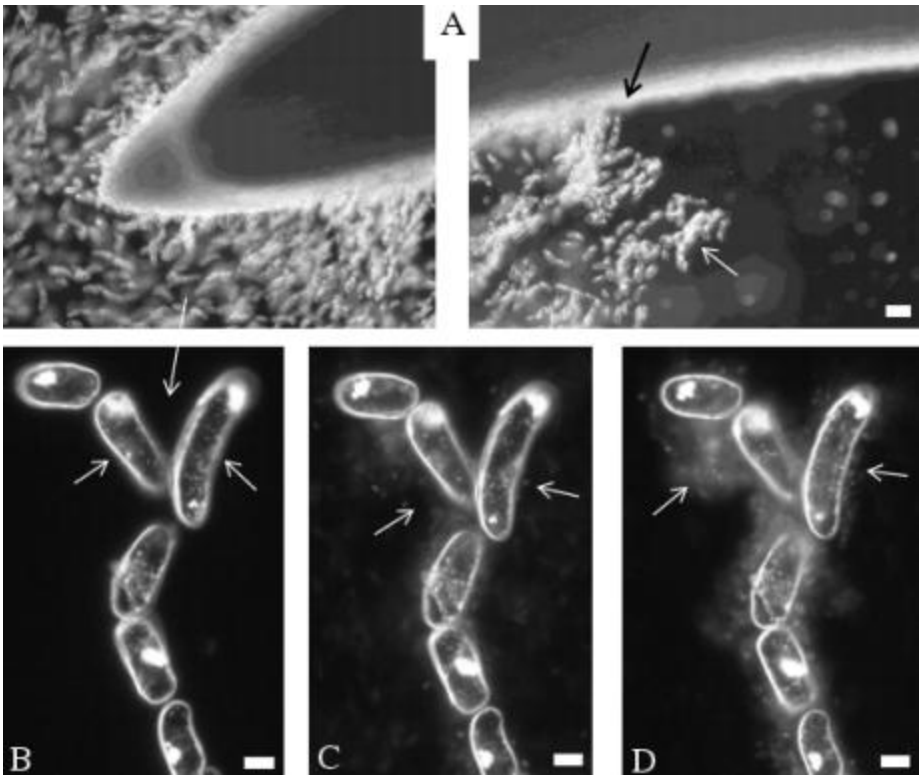

Fig. 4. Dispersal of maize border cells in water or bacterial suspension. **a,** Within 60 sec of immersing maize root tips into water, the root tip was covered in a sheath of border cells that ended abruptly (black arrow), between the apical meristem and the region of elongation. The cells in suspension ranged from single cells to small clumps (white arrow). Scale bar: 30 microns. **b**, When root tips were immersed into water containing *P. carotovorum*, dispersal of border cells began within 60 sec, and chemotaxis of the bacteria toward the dispersing border cells was evident within 5 min as layers of bacteria accumulated on the cell surfaces (arrows), **c**, Increased extracellular trapping of the bacteria after 10 min and **d**, 20 min. Scale bar: 10 microns.

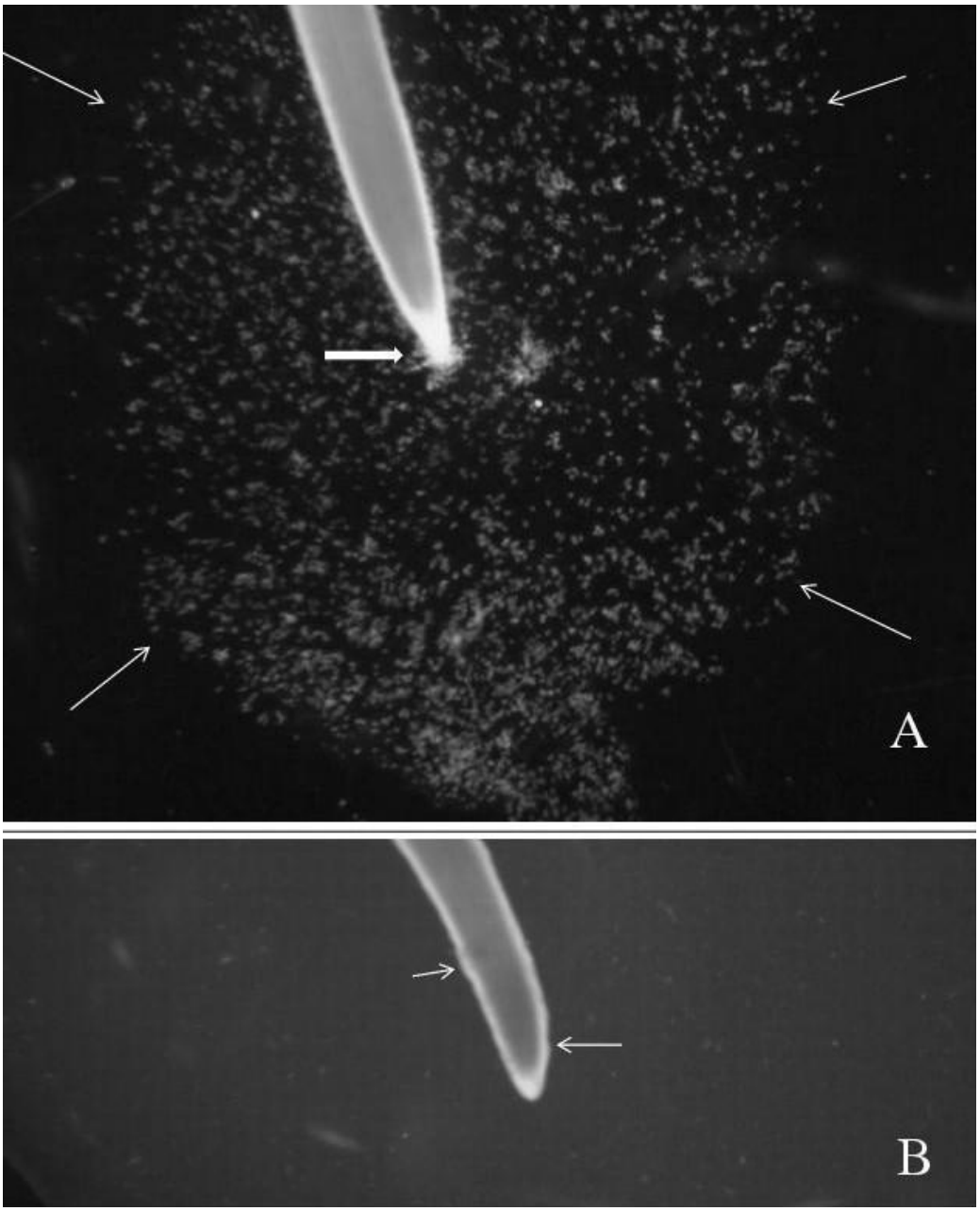

Fig. 5. a, Expansion of maize border cells from root tips (1 mm in diameter) immersed into water into a mass >2 cm in diameter (arrows) that remained in place for >4 h, . **b,** Border cells of roots immersed into CWE#1 for 24 h remained tightly appressed to the root surface, and no dispersal occurred.

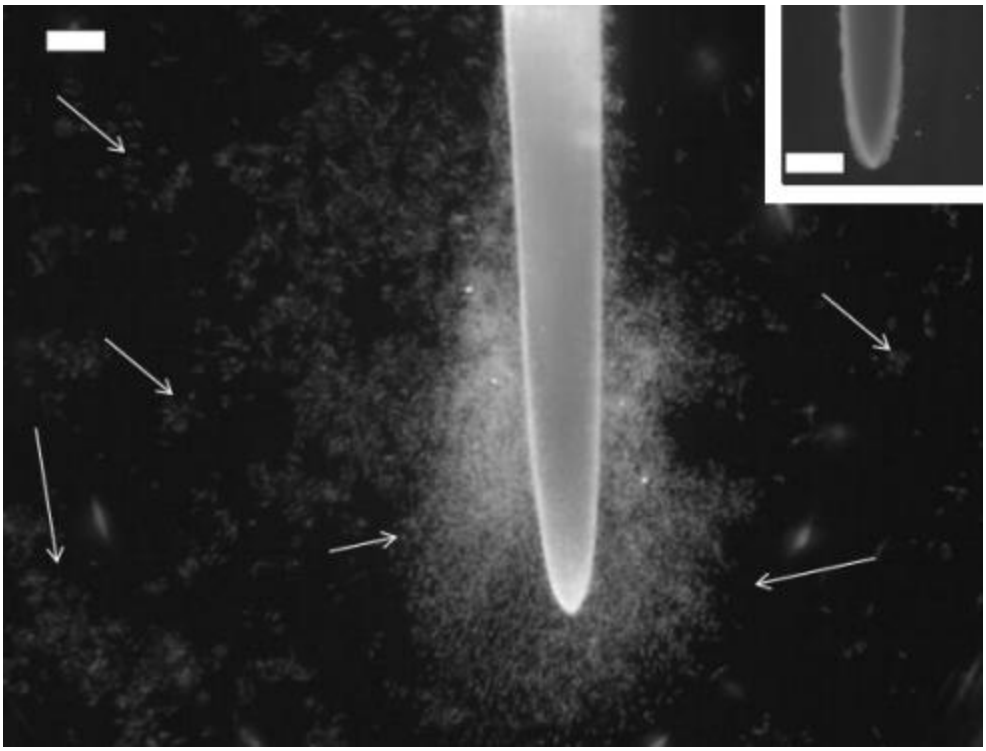

Fig. 6. Border cell dispersal (arrows) from a cotton root immersed for 30 sec in water. Inset: Lack of border cell dispersal from a cotton root immersed in CWE#1 for >4 hours.

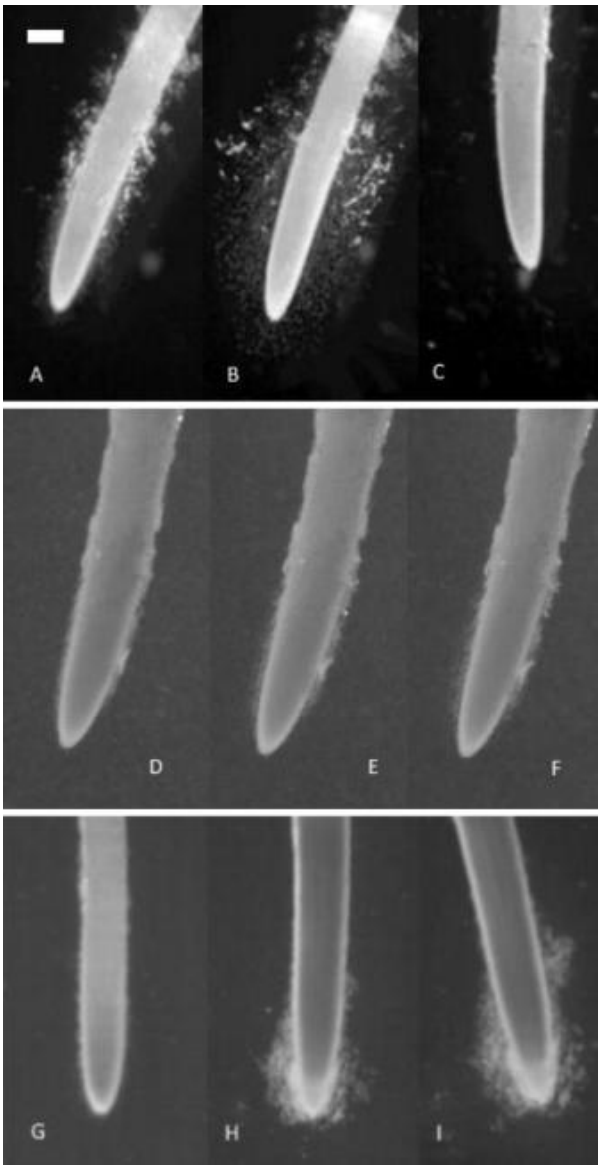

Fig. 7. Dispersal of border cells from cucumber root tips immersed in water (**a-c**), CWE#1 (d-**f**), or CWE#2 (**g-i**). **a,** Rapid dispersal of border cells from a single cucumber root tip immersed into water for 10 sec**; b,**60 sec;. **c,** Complete dissociation of border cells from root tips into suspension in response to lifting the root and gently reimmersing it into the water. **d,** Altered border cell dispersal on a cucumber root immersed in CWE#1 for 30 sec**; e,** 5 min; **f,** 90 min**. g-i,** Variability in border cell dispersal on two adjacent cucumber roots immersed in one sample of CWE#1. **g,** Near-complete retention of border cells on the surface of a cucumber root immersed in CWE#1 for 30 min. **h,** Partial dispersal of border cells on the surface of an adjacent cucumber root immersed in the same 1.5-ml sample of CWE#1 for 5 min and **i,** 30 min

APPENDIX C

FACTORS AFFECTING THE ABILITY OF COMPOST WATER EXTRACTS TO SUPPRESS ROOT DISEASE IN PEA

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This study is unpublished but was of great value in understanding some critical aspects of the CWE production process that may affect the ability of a CWE to suppress disease.

INTRODUCTION

The purpose of this series of studies was to isolate specific variables that have an effect on the suppression of a given compost water extract (CWE). Factors examined included the effects of aeration, specific additives, residual chlorine, refrigeration, heat sterilization by autoclaving (Table 1), and brew time (Table 2). Root growth and infection assays (RGIA) as described in Curlango-Rivera et al. (2013) were used to test the suppression of *Fusarium solani* f. sp. *pisi* on pea seedlings.

MATERIALS AND METHODS

Plant material. Pea seeds (*Pisum sativum* L.) cv "Alaska" were treated as described previously (Curlango-Rivera et al. 2013). Seeds were immersed for 10 minutes in 95% ethanol, for 60 minutes in 5.0% sodium hypochlorite, rinsed six times in sterilized distilled DI water, and then imbibed in sterilized distilled DI water for 6 hours. Seeds with damaged seed coats or that floated to the surface were discarded. Imbibed seeds were placed in on the surface of sterile germination paper which lay on top of sterilized 1% agar (Bacto TM Agar, Becton Dickinson and Co., Baltimore, MD, USA). These seeds were incubated at 24°C in the dark for 48 hours. Seedlings with 20-25 mm roots were either placed in pouches as described in Curlango-Rivera et al., 2013 for the greenhouse experiment or planted in Cone-tainers (Ray Leach Cone-tainers, www.greenhousemegastore.com) for the laboratory experiments.

Pathogen isolates. The fungal pathogen *Nectria haematococca* (anamorph *Fusarium solani* f. sp. *pisi*), pathogenic on pea, was used in this study. T8 cultures were maintained and processed as described in Gunawardena & Hawes, 2002.

Compost source. CWE source and preparation is the same as described in CWE #2 preparation in Curlango-Rivera et. al 2014. Thermally-produced compost made of fruit, vegetable, wood, and bark waste was obtained for use throughout this study (Ecoscraps, Phoenix, AZ, http://ecoscraps.com).

CWE preparation. All CWE were prepared in the laboratory at room temperature (~23°C). The basic CWE (BASIC) recipe consisted of adding the following five ingredients to a 18.9 L bucket with 15L of tap water in it, after being de-chlorinated for 24 hrs using forced aeration: 29.6 ml unsulfured blackstrap molasses (Plantation), 14.8 ml kelp meal (Maxicrop), 14.8 ml liquid humic acid (Ful-Power), 14.8ml soft rock phosphate (Dr. Earth) and 14.8 ml silicates (Silica Blast, Botanicare). Compost (473 ml) was placed in a 250 micron mesh bag (Pentair Aquatic Ecosystems) and placed in the bucket producing a 1:30 v/v CWE. This mixture was either aerated (ACWE) or not aerated (NCWE) for 22 hr. If aerated, an 18 Watt, 38 L/min, 20 kPa commercial air pump was used to continuously force air into the mixture using two airlines extended into the water, one with a 2.5cm x 1.25 cm airstone connected to the end with the other airline remaining open. Compost wash (CW) was prepared at the same ratio as above by adding 30 ml compost to 1 L of de-chlorinated tap water. The mixture was stirred and left to sit undisturbed for 15 minutes, then stirred once more and left undisturbed another 15 minutes at which time the supernatant was used for the experiment. For the chlorine experiment, chlorine bleach (6%) was added to the tap water as needed to obtain each chlorine concentration desired, as tested with an HTH 6 Way Test Kit (total chlorine test). Once adjusted, compost and all additives were added to the water and the mixture was aerated for 22 hr. CWE was autoclaved at 121°C for 30 minutes and cooled to room

temperature before use. Refrigerated ACWE was allowed to come to room temperature before used in testing.

Root growth and infection assays. Root growth and infection assays (RGIA) as described in Curlango-Rivera et al. (2013) were conducted in the laboratory. A growth pouch consisting of a clear cellophane envelope inserted with germination paper (Mega International, St. Paul, MN) was used to expose roots to a CWE treatment and/or pathogen and view and measure root length and disease response over time. Fifteen milliliters of CWE or sterilized water was placed in each pouch using a syringe. Once the germination paper was soaked, five pea seedlings with root length of 20-25mm were placed at the top of the pouch. Pouches were laid flat and liquid was pushed up the pouch to make sure it had complete contact with the roots. Roots were then inoculated with sterilized distilled water or a 0.15-ml spore suspension (10^6 spores/ml) of *F. solani* pathogen by uniform application from base to tip using a micropipette. Pouches were left in a horizontal position for 15 minutes and were then placed upright and maintained at 24°C for up to 8 days. On Day 8, infection levels were obtained by observation of lesion development on seedlings in pouches. Seedlings were recorded as "not infected" or "infected" if tan, brown, or black sections of root were observed, as described (Gunawardena & Hawes, 2002).

RESULTS AND DISCUSSION

Aeration. The basic aerated CWE recipe resulted in 100% of the inoculated seedlings being protected while the non-aerated basic recipe CWE only resulted in72% of the seedlings being protected.

Chlorine. Low levels of total chlorine (1-3ppm) did inhibit the suppression ability of the CWE by very much (88-100% protection). However, at 4ppm chlorine, suppression was reduced by 60%. Infection percentages for 1, 2, and 3 ppm were 0%, 12%, and 7%. This inconsistency could have been due to the low precision of the chlorine test kit, causing inaccurate chlorine concentrations measurements at such small concentration differences.

Variation in recipe. The compost wash (CW), with only 30 minutes contact time between compost and water, provided no protection. ACWE made of only compost and water resulted in only 58% protection, as compared to 100% protection with all of the additives included. Molasses did not contribute significantly to the suppression ability of the ACWE, in agreement with Scheuerell & Mahaffee (2004) who found that increasing the amount of molasses decreased suppression. When only molasses was added, suppression was reduced to 7% and when molasses was taken out of the basic recipe, suppression remained at 100%. Kelp contributed significantly to suppression. When kelp was added by itself to the compost and water, it resulted in 80% suppression. When all additives were includes EXCEPT molasses and kelp, suppression remained high (93%) indicating that humic acids, rock phosphate, and/or silica were the major contributors toward the suppression effect.

Autoclaving. Autoclave sterilization eliminated the suppression effects for ACWE-BASIC and ACWE-compost and water, while NCWE maintained minimal protection (5%).

Refrigeration. Refrigeration ACWE – BASIC reduced suppression drastically from 100% to 20% protection.

Time. Suppression decreased after 24 hrs of brewing for ACWE, with decreases of an average of 35%, 26%, 20% on Days 2, 3, and 8. By Day 10, suppression recovered to about 88%. Results for NCWE were inconsistent. One trial resulted in 100% suppression for all days except Day 2 when suppression was 93%. Another trial resulted in 73% protection on Day 1, with increasing protection on Days 2, 3, and 8 up to 100% but then down to 47% protection on Day 10. A third trial resulted in decreasing protection for Days 1 through 3 (57% to 40%) and then 47% on Day 10. Aerated compost mixed with water and no additives maintained an average of about 30% over time.

Table 1. Effect of various factors on CWE ability to suppress *F. solani* f.sp. *pisi* in pea tested by growth pouch

	CWE			CWE + *F. solani*		
	Number of Seedlings			Number of Seedlings		
Treatment	NI	I	I (%)	NI	I	I (%)
Controls						
[x]Water Only or Water + *F. Solani*	315	0	0%	0	315	100%
Variation in recipe						
[x]ACWE -BASIC	330	0	0%	330	0	0%
NCWE - BASIC	60	0	0%	43	17	28%
CW (no additives- stirred, sit for 15 min)	30	0	0%	0	30	100%
ACWE - Compost and water (no additives)	45	0	0%	26	19	42%
ACWE - Compost and molasses	15	0	0%	1	14	93%
ACWE - Compost and kelp	30	0	0%	25	5	20%
ACWE - Compost, all except molasses and kelp	15	0	0%	14	1	7%
ACWE - BASIC with all additives, no molasses	15	0	0%	15	0	0%
Chlorine						
1 ppm	45	0	0%	45	0	0%
2 ppm	60	0	0%	53	7	12%
3 ppm	60	0	0%	56	4	7%
4 ppm	15	0	0%	9	6	40%
Autoclaving						
ACWE - BASIC				0	60	100%
ACWE - Compost and water (no additives)				0	30	100%
CW (no additives- stirred, sit for 30 min)				0	30	100%
NCWE - BASIC				3	57	95%
Refrigeration						
ACWE-BASIC Refrigerated for 5 days				3	12	80%

[x] Over 10 or more trials

Table 2. Effect of various factors on CWE ability to suppress *F. solani* f.sp. *pisi* in pea tested by growth

	Day 1 (24 hr brew)			Day 2 (48 hr brew)			Day 3 (72 hr brew)			Day 8 (192 hr brew)			Day 10 (240 hr brew)		
	No. of Seedlings			No. of Seedlings			No. of Seedlings			No. of Seedlings			No. of Seedlings		
Treatment	NI	I	I (%)	NI	I	I (%)	NI	I	I (%)	NI	I	I (%)	NI	I	I (%)
Controls															
Water	30	0	0%	30	0	0%	30	0	0%	15	0	0%	30	0	0%
F. Solani	0	30	100%	0	30	100%	0	30	100%	0	15	100%	0	30	100%
ACWE - BASIC															
"Alaska" Pea															
ACWE	45	0	0%	45	0	0%	45	0	0%	15	0	0%	45	0	0%
ACWE + *F. Solani*	15	0	0%	14	1	7%	13	2	13%	12	3	20%	14	1	7%
ACWE + *F. Solani*	30	0	0%	17	13	43%	13	17	57%				27	3	10%
"Little Marvel" Pea															
ACWE	15	0	0%	15	0	0%	15	0	0%	15	0	0%	15	0	0%
ACWE + *F. Solani*	15	0	0%	11	4	27%	14	1	7%	15	0	0%	15	0	0%
NCWE -BASIC															
"Alaska" Pea															
NCWE	45	0	0%	45	0	0%	45	0	0%	15	0	0%	45	0	0%
NCWE + *F. Solani*	15	0	0%	14	1	7%	15	0	0%	15	0	0%	15	0	0%
NCWE + *F. Solani*	17	13	43%	18	12	40%	12	18	60%	NA	NA	NA	14	16	53%
"Little Marvel" Pea															
NCWE	15	0	0%	15	0	0%	15	0	0%	15	0	0%	15	0	0%
NCWE + *F. Solani*	11	4	27%	13	2	13%	15	0	0%	15	0	0%	7	8	53%
ACWE - Compost + Water (no additives)															
"Alaska" Pea															
CW	30	0	0%	30	0	0%	30	0	0%	NA	NA	NA	30	0	0%
CW + *F. Solani*	21	9	30%	23	7	23%	17	10	37%*	NA	NA	NA	23	7	30%

* Only 27 total seedlings, did not have enough pathogen for all 30.

APPENDIX D

FACTORS INFLUENCING DISEASE SUPPRESSION BY COMPOST WATER EXTRACT (CWE) UNDER CONTROLLED CONDITIONS

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Poster presented at the American Phytopathological Society Caribbean/Pacific Joint Meeting, June 2013, Tucson, AZ.

FACTORS INFLUENCING DISEASE SUPPRESSION BY COMPOST WATER EXTRACTS (CWE) UNDER CONTROLLED CONDITIONS

Abstract. Compost potentially offers a sustainable approach to control of disease caused by important soil-borne pathogens, but variability in composition and efficacy has hampered application. As one example, factors that result in altered extracellular pH at the root tip can alter growth and development of the root system. Production of compost water extracts (CWE) provides a reproducible means to develop homogeneous mixtures whose effects on growth, development, and susceptibility to disease can be quantified under controlled environmental conditions. A growth pouch assay was used to measure root infection by *Fusarium solani* in pea (*Pisum sativum* L) seedlings, which normally develop local lesions within 24-48 h after inoculation with the fungal pathogen. When seedlings were co-inoculated with the pathogen and CWE, no infection occurred (Phytopathology 103:255). Protection was sustained when plants were transplanted from pouches into 4-inch containers of coconut coir in a greenhouse and watered with hydroponic nutrients: The purpose of this study is to explore the potential for using a growth pouch assay to predict ability of specific CWEs to suppress disease on specific crop plants in greenhouse conditions engineered to facilitate optimal compost-root interaction.

Introduction: Root caps of most plant species are programmed to deliver specialized cells, termed 'root border cells,' into the environment in response to signals including water (Fig. 1a-c and 2f). Pathogenic nematodes, bacteria, and zoospores are

chemotactically attracted to host border cell surfaces, where they aggregate and are immobilized (Fig. 2a-e). The significance of these host-microbe specific interactions has been unclear. Recent studies using *Fusarium solani* f. sp. *pis*i as a model system suggest that border cells function in root tip resistance to fungal infection by an extracellular DNA (exDNA) based immune response that is parallel to that operating in mammalian innate immunity (3). White blood cells now are known to release extracellular 'traps' (ET) which immobilize and kill pathogens before they can invade host tissues (2). exDNA is a component of the ETs and its importance in innate immunity was shown by demonstrating that extracellular DNase causes ET disintegration and that mutation of exDNases in bacterial pathogens impairs virulence (5). In the current study we report inhibition of root infection in seedlings treated with CWE, in correlation with altered dynamics of border cell dispersal into the rhizosphere surrounding the growing root tip.

Conclusion: Results suggest that even under controlled conditions, disease suppression varies according to growth medium. Factors in the substrate may affect the colonization of CWE microorganisms, which may modify the success of disease suppression. Further research focused on studying the specific substrate microenvironment conditions for the establishment of CWE microorganisms during early root development is needed.

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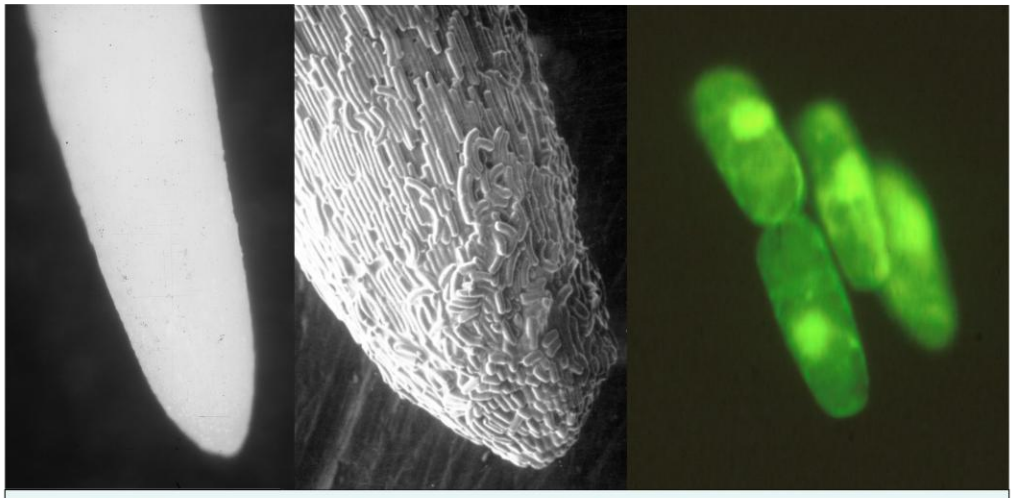

Fig. 1. Root cap and border cells (4). At 99% humidity, the presence of border cells is undetectable (A) except with scanning electron microscopy (B). Addition of a droplet of water results in swelling of border cells and immediate dispersal (Fig. 2F). The vital stain fluorescein diacetate reveals border cell viability of 95–100 % (C).

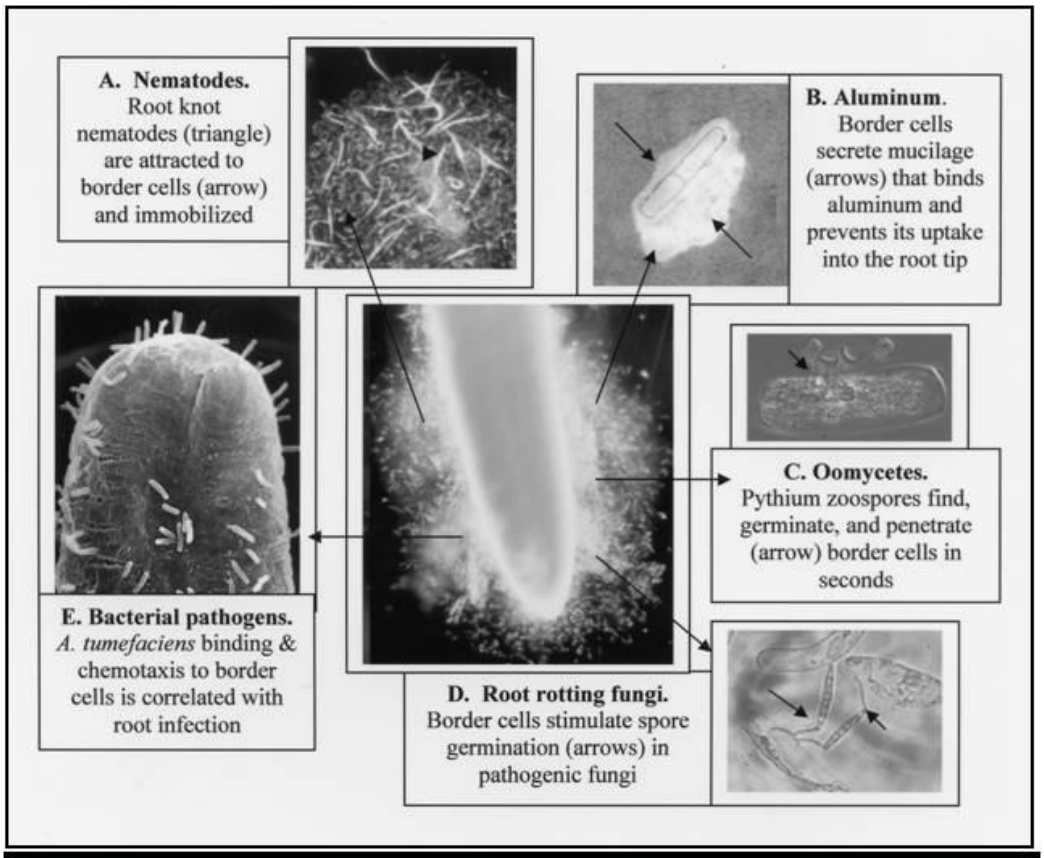

Fig. 2. Recognition of pea root border cells by plant pathogens (4).

Fig. 3. Impact of CWE on pea root infection by *F. solani* (1). A. Water control. Arrow denotes the site of the root tip at the time of inoculation. B Pea roots grown in CWE alone reveal no changes in root growth or development, compared with control samples grown in water. C, Pea roots inoculated uniformly with *F. solani* spores and maintained in growth pouches at 25 C for 13 days. Localized lesion development is evident at the site that was the region of elongation at the time of inoculation. Most roots do not become infected at the root tip containing the rot cap and apical meristems; therefore, root growth continues as the same rate as controls. D, Pea roots inoculated uniformly with *F. solani* spores in the presence of CWE develop no lesions. E, Pea roots grown in the presence of filtered CWE and *F. solani*. When pea seedlings inoculated with *F. solani* and drench with CWE were grown in sand as a substrate, protection remained 100%, however when were grown in heavy clay soil protection was reduced to 45% (1).

Fig. 4. Longevity of disease suppression by CWE on pea root infection by *F. solani*. Pea seedlings were transplanted from CWE-treated growth pouches into coconut coir substrate at 10 days post-inoculation and grown for 6 weeks under controlled environment conditions. (A and B) Pea plants and root system irrigated with control nutrient solution (98-100% survival). (C and D) Pea plants and root system inoculated with *F. solani* (2% survival). (E and F) Pea plants and root systems inoculated with *F. solani* in the presence of CWE (17-31% survival). All roots developed legions at point of inoculation but many overcame disease and developed healthy roots thereafter, including healthy shoots. Pea plants grown in the presence of CWE showed a 79-100% survival (pictures not shown).

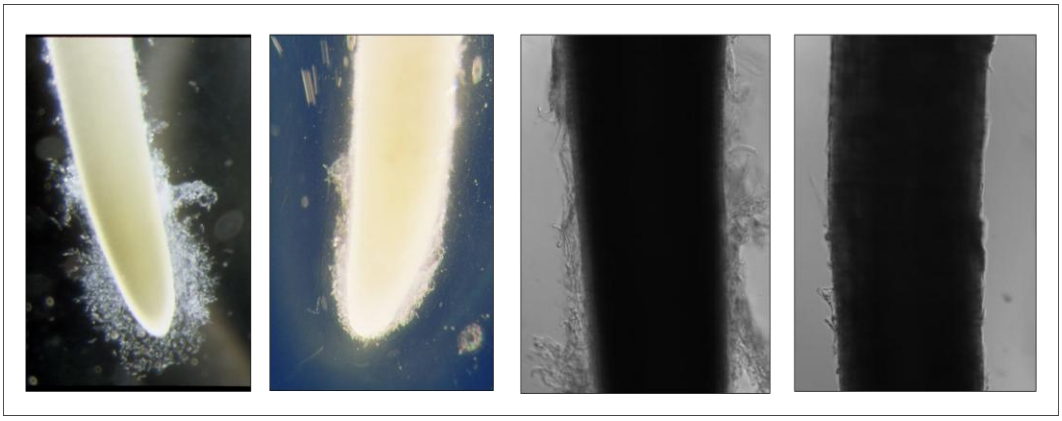

Fig. 5. Mechanism of root disease suppression. (A) Separation of pea border cells in the presence of water. (B) Pea border cells do not separate and instead remain as a sheath covering the root tip in the presence of CWE. (C) Pea border cell sheath extending past the region of elongation on pea roots inoculated with *F. solani* in the presence of CWE. This aggregate remains adhered to the root surface after 14 days of growth in moist pouches maintained at 25 C (1). (D) On roots of water-treated control pea seedlings, only a few border cells can be seen near the surface (1).

FACTORS INFLUENCING DISEASE SUPPRESSION BY COMPOST WATER EXTRACTS (CWE) UNDER CONTROLLED CONDITIONS

Stacy Joy Tollefson[1], Gilberto Curlango-Rivera[2], Thomas Pew[3], Gene Giacomelli[1], Martha C. Hawes[2]

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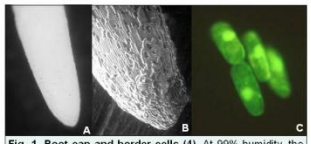

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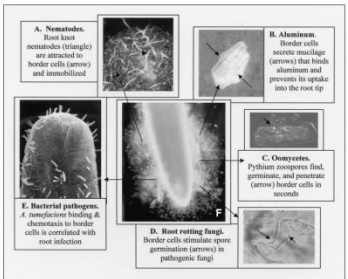

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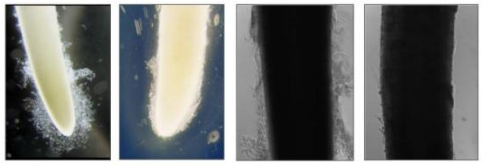

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APPENDIX E

PHD PROGRAM ACTIVITIES

S.J. Tollefson

PHD PROGRAM ACTIVITIES

There are numerous experiences during my degree program, that fall outside of the research presented in this dissertation, that have significantly contributed to my knowledge and professional development as an Agricultural and Biosystems Engineer. My professional goal is to use the knowledge of controlled environment agriculture I have learned within this program to teach others how to design growing systems, grow crops, and develop businesses. Below I will discuss activities I have participated in that have helped prepare me as more forward into my career.

In 2001, I took PLS 217: Introduction to Hydroponics and Controlled Environment Agriculture with Dr. Rorabaugh. I was in my last year of my Master's program in Hydrology and Water Resources at the UA. I did not need course credit, but I had intense interest in hydroponics so I audited the course and learned a lot. After leaving the University for a while, my interest in hydroponics intensified and in 2009, I returned to complete the PhD in Agricultural and Biosystems Engineering. Dr. Rorabaugh welcomed me back and immediately trained me and placed me in a Greenhouse Manager position in her teaching greenhouse. I remained Greenhouse Manager for two full years, learning under Dr. Rorabaugh all the ins and outs of managing a commercial style hydroponic tomato greenhouse. The hands-on, behind-the-scenes knowledge of the day-to-day operations was invaluable! I worked closely with the greenhouse crew who took care of the maintenance of the greenhouse structure, systems, and pest control to learn about what they did.

After taking a break to conduct my research, I came back to the teaching greenhouse as a pest manager and to help work on the new state-of-the-art re-circulating fertigation system. I worked with the installer and the fertigator company (Hortimax) to develop protocols with the new software and then documented the methods for using the software and fertigator system. Once we receive the UV filter in January, I will be learning how to run a re-circulating system. This is excellent real-world experience that I did not get in the classroom.

Through my pest management class and throughout my years working in the teaching greenhouse, I developed my expertise in pest identification and control. This past year working as the pest manager for the teaching greenhouse and another research greenhouse, I have honed these skills even more. Through the short course, I befriended Karin Tifft, an entomologist who is a consultant with Greenhouse Vegetable Consultants Company, and have sought her advice often and learned a great deal. I also talked with several beneficials companies, obtaining added knowledge.

When I worked in the teaching greenhouse, I assisted Dr. Rorabaugh's students when they were working in the greenhouse and sometimes guest lectured for her on topics within my research interests. When funding decreased, I develop a marketing strategy for our produce by having the student club (mostly me) sell produce at the local farmer's market and manage a few wholesale accounts in order to bring money back to our program. I personally sold our produce for an entire year at the local farmer's market. It gave me experience interfacing with the public and educating them about our growing practices. I got experience talking with other farmers and examining costs and price points.

As a student, I participated in the annual Engineering and Crop Production Short Course conducted at the CEAC. I listened to lectures by professors and guest speakers and networked with new and experienced growers, suppliers, and educators. I assisted Dr. Rorabaugh in teaching the hands-on portion of the short course, as well as her week long Tomato Intensive Course and a Nutrient Management Course. In 2014, I presented a session on Pest Identification. Through the Short Course, I learned about both small and large greenhouse operations through guest speakers and tours to two commercial hydroponic greenhouses in Arizona: Eurofresh Farms (now Nature Sweet) and Sunizona Family Farms.

During the 2011-2012 school year, I accepted an NSF Graduate Teaching Fellowship where I worked for 10-15 hrs per week with a local high school agriculture teacher to help increase the scientific rigor in her classes and assist her in running her hydroponic greenhouse. I brought my CEA knowledge to the students through multiple lectures, assistance in the greenhouse, and giving them a tour of the UA-CEAC facilities. I was able to gain experience bringing complicated subject matter to younger students and excite them about growing food with high technology.

Another part of my program that was invaluable was my interaction with fellow students. I participated in the Controlled Environment Agriculture Student Association (CEASA) for many years, acting as treasurer and then president. We had access to some greenhouse space where we could experiment with different types of hydroponic systems. But the best part was talking to each other about our common interests and future plans. Even outside of the club, it was valuable talking to fellow students, sharing knowledge and experiences. One particular graduate student, Myles Lewis, created his own business

called Arizona Vegetable Company and shared his business expertise and experiences with trying to find funding, buying land, dealing with zoning issues, and learning about food safety protocols.

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