AN ABSTRACT OF THE DISSERTATION OF

Steven J. Scheuerell for the degree of Doctor of Philosophy in Botany and Plant Pathology presented on November 22, 2002.

Title: Compost Teas and Compost Amended Container Media for Plant Disease Control.

Abstract approved Signature redacted for privacy.

Walter F. Mahaffee

The primary goal of this dissertation research was to assess the use of compost for the control of several foliar and soil borne diseases commercially important in the Pacific Northwest. The use of compost teas to control of gray mold (*Botrytis cinerea*) on geraniums, powdery mildew (*Podospheara pannosa* var. *rosae*), rust (*Phragmidium* spp.), and black spot (*Diplocarpon rosae*) on field grown roses, and damping-off caused by *Pythium ultimum* was examined. The goal was to optimize control through manipulation of compost tea production parameters of compost source, fermentation nutrients, fermentation duration, stirring, depth of liquid, aeration, and spray adjuvants.

No one optimal set of compost tea production practices could be determined for control of grey mold, however, empirical evidence indicated that the probability of disease suppression could be increased through compost tea production choices, especially compost source and addition of fermentation nutrients.

Incorporating compost into container media resulted in variable suppression of seedling damping-off across compost sources. Damping-off caused by *P. irregulare* was suppressed by 66% of the compost samples, *P. ultimum* by 56% of the samples, and *Rhizoctonia solani* by 17% of the samples. *R. solani* damping-off was made worse by 42% of the compost samples. Damping-off of the three pathogens was suppressed by 11% of the compost samples. Twenty-two percent of the samples did not significantly suppress damping-off disease caused by any pathogen.

Manipulating compost production to consistently attain *P. ultimum* damping-off suppression was investigated. By placing hot compost removed from curing piles into sterile storage, it became clear that compost required recolonization by exogenous mesophyllic microflora for the rapid development of *Pythium* damping-off.

Compost can be used to assist in plant disease management resulting in significant disease control. However, as with most biological methods, inconsistency is still an issue. Research on application methodology and further refinement of composting and compost tea production practices (i.e. compost source and spray adjuvants) will likely increase the potential for consistently suppressing plant disease with these technologies.

Compost Teas and Compost Amended Container Media for Plant Disease Control

by
Steven J. Scheuerell

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented November 22, 2002
Commencement June 2003

ACKNOWLEDGEMENTS

I would like to thank my graduate committee for supporting my academic and research goals at Oregon State University. Acting as my major professor, Walter Mahaffee deserves a warm thank you for his abilities to help when needed, give me enough freedom to learn things the hard way, and stand 100% behind my alternative research choices. Walter understood the value of pursuing both foliar and soil-borne disease projects, it is a rare opportunity to be supported in following ones scientific imagination. Walter helped focus each project, discuss the results, and edited each manuscript. His rapid attention to editing each manuscript is a rare trait according to graduate student lore.

Dr. Chris Mundt has been an inspiration for teaching and researching things that actually matter to farmers. Additionally, Chris gave valuable input on data analysis and general guidance for the entire committee. Dr. Dan Sullivan was invaluable for keeping my compost research focused and proved to be a humorous outlet for those of us who really love compost. Dan and his graduate student, Jeff Chaote, performed the compost respiration potential work that proved to be very informative for interpreting disease suppression by compost. Dr. Cindy Ocamb is appreciated for her advice on handling multiple experiments and good humor. Dr. Sven Svenson provided valuable insight into the Oregon nursery industry. He could also appreciate the need to deliver practical results to the people paying taxes. Dr. Linda Blythe, acting as the Graduate School representative, is greatly appreciated for taking on this extra responsibility.

Funding for this work was provided by USDA CRIS # 303-5358-22000-024-00D. Funding was also provided by the Oregon Association of Nurserymen and Oregon Department of Agriculture through the competitive Nursery Research grants program. In addition to direct financial support, this research would not have been possible without the logistical support of the entire USDA ARS Horticultural Crops

Research Laboratory. Within the HCRL, my Foliar Pathology lab mates gave immeasurable support, through labor, suggestions, and the mutual will to survive.

The support of the Department of Botany and Plant Pathology was instrumental to the maintenance of my entire graduate program. Faculty and staff worked diligently to provide a quality educational experience and shield graduate students from the financial turmoil facing the entire system of higher education in Oregon.

A number of industry people and businesses gave valuable support by donating information and materials. Bear Creek Gardens donated the roses used for research on foliar disease suppression. Soil Soup Inc. donated Bio-Blenders™ for making aerated compost tea. The Composting Council of Oregon helped spread the word to commercial composters that my research was worth supporting. Numerous composting facilities donated compost and information that greatly facilitated this work. In particular, Jack Hoeck at Rexius Inc. and Tom Campbell at Lane Forest Products provided a great deal of access to their composting facilities. Without their assistance, and the assistance recieved at all other facilities, this work would not have been possible.

Like good seed, I have saved the best for last. These words would never have been written without the loving support of my wife ,Anne Cameron Barrett. Our son, River, has been an inspiration to complete this process and I thank him for loving me. I thank my brother Mark D. Scheuerell for inspiring me to finish this process. My parents, Judy and Bob Scheuerell, have always been proponents of higher education and encouraged me to get the best education possible. As my father often said, "education is one of the few things that they can't take away from you."

CONTRIBUTION OF AUTHORS

Dr. Dan Sullivan assisted with data collection and analysis for Chapter 6. He directed the incubation of compost in soil to determine the compost respiration potential. His laboratory also processed compost samples to prepare the samples for NH_4-N and NO_3-N analysis. He assisted with interpretation of the compost chemical data and edited the manuscript content.

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DEDICATION

I dedicate this dissertation to Annie, River and our wonderful families.
Thank you for supporting me along the way!

Compost Teas and Compost Amended Container Media for Plant Disease Control

Chapter 1: Introduction

Historically, the impact of plant diseases has been a limiting factor in the production of agricultural crops (Agrios, 1997). Before the advent of synthetic pesticides for plant disease control, farmers relied on selecting for plant resistance and maintaining or increasing natural pathogen suppression through cultural practices. These practices included sanitation measures, crop rotation, ley fields, cover cropping and adding organic matter via manures, mulches, and compost. The widespread adoption of synthetic pesticides was initially viewed with great awe for the power to control a range of plant diseases. Over time however, the benefits of certain chemistries were overshadowed by the health risks associated with applying or consuming the materials. This realization helped spur investigations into the biological control of plant pathogens (Cook and Baker, 1983). Passage of the Food Quality Protection Act further obviated the need to develop biological alternatives for chemistries that could be lost through regulatory action (Ragsdale, 2000). Along with health concerns, growers have become interested in alternative disease control measures due to pathogens developing pesticide resistance, phytotoxic potential of chemistries, and reduced worker efficiency during the re-entry interval after pesticide application. Additionally, growers are increasingly transitioning acreage into certified organic production to meet growing consumer for certified organic products (Klonsky, 2000).

Using compost in plant production systems has been developed into an effective control practice for some soil borne pathogens, particularly in the greenhouse and nursery production of container plants (Hoitink and Fahy, 1986; Hoitink *et al*, 1993). Incorporating compost has also been demonstrated to reduce foliar diseases through induced resistance, however, this has not been refined into a predictable

disease control method. Effective foliar disease control has been reported with compost extracts or non-aerated compost teas when applied directly on plant surfaces (reviewed by Weltzien, 1991; Scheuerell and Mahaffee, 2002). Compost teas and compost amended container media have been utilized in commercial plant production in various locations worldwide, yet few producers in the Pacific Northwest region of the United States have adopted these practices. In Oregon, despite a thriving container plant industry and abundant compost resources, data supporting the use of compost for root rot pathogen control is lacking. Likewise, for compost teas there is a lack of data relevant to Oregon.

The primary goal of this dissertation research was to assess the use of compost teas and compost amended container media for the control of several plant diseases that affect agriculture in Oregon and elsewhere. Chapter two provides the historical and scientific background on the uses of compost tea for plant disease control. It introduces the factors of compost tea production that have been shown to influence plant disease control including compost source, use of aeration, fermentation nutrients, duration of fermentation, and the addition of spray adjuvants. In particular, the role of aeration is addressed because of the recent popular surge of producing aerated compost tea.

The use of compost teas for the control of gray mold, caused by *Botrytis cinerea,* on geraniums under greenhouse conditions is investigated in Chapter three. The goal was to optimize gray mold control by manipulating the compost tea production factors of compost source, fermentation nutrients, fermentation duration, stirring, depth of liquid, aeration, and spray adjuvants. No one optimal set of compost tea production practices could be determined, however, empirical evidence indicated that the probability of disease suppression could be increased through compost tea production choices, especially choice of compost.

Chapter four investigates the use of compost teas under field conditions for control of the three most prevalent foliar fungal diseases of rose in Western Oregon, powdery mildew (*Podospheara pannosa* var. *rosae*), rust (*Phragmidium* spp.), and

black spot (*Diplocarpon rosae*). The compost tea production factors of compost source, aeration, and fermentation nutrients were assessed for their impact on disease suppression. In addition, disease control by compost teas was compared to a commercial biocontrol formulation of *Trichoderma harzianum* T-22 and the fungicides triflorine and neem oil. The results indicate that choice of compost had the greatest effect on disease suppression.

Transitioning from foliar disease control to soil borne disease control, Chapter five examines the application of compost tea as a container media drench. The compost tea production factors of compost source, aeration, and fermentation nutrients were investigated for the control of damping-off of cucumber caused by *Pythium ultimum*. Very little research has explored the potential for compost tea to suppress root rot disease. This research demonstrates that compost tea can consistently suppress damping-off in soilless media that is naturally conducive to the disease.

Directly incorporating compost into container media was also examined for the suppression of seedling damping-off disease. Chapter six investigates the potential for composts produced at Pacific Northwest commercial composting facilities to suppress seedling damping-off disease caused by *P. ultimum*, *P. irregulare* and *Rhizoctonia solani*. Thirty six compost samples were analyzed for a number of physical, chemical, and biological properties, with these properties related to damping-off suppression. This research indicates that there is immediate commercial potential for amending soilless container media with select composts to effectively suppress damping-off caused by *Pythium* spp. This research also provides important information on the variability of compost properties across samples. This information can assist both producers and users of compost to better match compost products with appropriate end uses.

To optimize the use of compost for damping-off disease suppression, the need to inoculate compost after peak heating for the rapid development of suppression towards *P. ultimum* damping-off was examined in Chapter seven. Compost removed from the hot (>55 C) core of yard trimmings compost piles was incubated under sterile

conditions to observe if suppression could develop due to cooling, or if inoculation with organic matter is a critical factor. This has practical implications for optimizing the use of compost for damping-off suppression. It is indicated that inoculation of compost is necessary for the rapid and consistent production of *Pythium* suppressive compost for use in soilless container media.

Chapter eight summarizes the conclusions of this dissertation research. These finding indicate that compost teas and compost can provide a valuable measure of plant disease suppression. The challenge remains to effectively integrate these practices into commercial production. Hopefully this research will provide guidance for those seeking to use compost and compost teas for plant disease control.

Compost Tea: Principles and Prospects for Plant Disease Control

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Compost Science and Utilization
419 State Avenue, Emmaus, PA, 18049
Vol. 10(4):313-338

Chapter two: Compost Tea: Principles and Prospects for Plant Disease Control

ABSTRACT

An increasing body of experimental evidence indicates that plant disease can be suppressed by treating plant surfaces with a variety of water-based compost preparations, referred to in the literature as watery fermented compost extracts or compost teas. The terms non-aerated compost teas (NCT) and aerated compost teas (ACT) are used in this review to refer to the common production methods that diverge in the intent to actively aerate. Very little data directly compares the efficacy of NCT and ACT for plant disease suppression. A variety of foliar plant pathogens and/or diseases have been suppressed by applications of NCT while few controlled studies have examined ACT. For some diseases the level of control would be considered inadequate for conventional agriculture; organic producers with limited control options consider partial disease control to be an important improvement. For both compost tea production methods, decisions that influence pathogen suppression include choice of compost feedstocks, compost age, water ratio, fermentation time, added nutrients, temperature and pH. Application technology choices include the dilution ratio, application equipment, timing, rates, spray adjuncts and adding specific microbial antagonists. Increased understanding of compost tea microbiology and the survival and interactions of microbes on plants surfaces should make it possible to modify compost tea production practices and application technology to optimize delivery of a microflora with multiple modes of pathogen suppression. Innovative growers and practitioners are leading the development of new compost tea production methods and uses, generating many potential research opportunities. The use of compost tea as part of an integrated plant health management strategy will require much additional whole systems research by a cohesive team of farmers and experts in composting, plant pathology, phyllosphere biology, molecular microbial ecology, fermentation science, plant physiology, plant breeding, soil science, and horticulture.

INTRODUCTION

Soil and plant sprays based on compost and various plant materials have been in practice since the 1920's (Koepf, 1992). Applying compost in liquid form has roots in an old gardening practice of soaking seeds and drenching plants or soil with 'compost water' for fertilization and to help prevent damping-off (Rodale, 1967). An increasing body of experimental evidence indicates that plant disease can be suppressed by applying a variety of water-based compost preparations (reviewed in Weltzien (1991) and Diver (1998)). There has been a recent surge of popular interest in the potential for improving plant health through the use of water-based compost sprays, typically called compost teas.

There are several reasons why compost tea use is expanding (Touart, 2000; Scheuerell, personal observation). Garden writers are exposing the idea to a wide audience. Professional landscapers are using compost tea and educating clients. Golf courses are assessing compost tea for fertility and disease control. Municipal parks and recreation departments are using compost tea for grounds maintenance. A number of individuals and companies are selling compost tea at farmers markets, retail outlets, internet sites, and through application services.

Major reasons why organic farmers are experimenting with compost tea include the lack of approved disease management tools and their utility for integration into existing plant fertility and microbial inoculation programs. As the number of growers using compost tea expands, so has the number of unconfirmed reports of foliar plant disease control. Several examples include *Botrytis* on green beans; strawberries, grapes, and geranium, powdery mildew on apples; black spot and powdery mildew on roses; late blight on greenhouse tomatoes; and downy mildew on *Brassica* seedlings. Testimonials attributing compost tea to soil borne disease control include reduced damping-off of direct sown crops by drenching the seed row and drenching transplanted seedling plugs. Dipping potato seed pieces and ornamental flower bulbs is thought to reduce root rots and is commercially practiced. At least one

greenhouse grower has observed reduced fusarium root rot of cyclamen through the use of compost tea drenches.

Despite growers' concerns over the lack of information available to guide on-farm trials with compost tea, they continue to experiment with compost tea due to the lack of available control measures. Anecdotal success stories abound, but many lack good experimental design, objective assessment strategies or supportive data. In spite of this, many practitioners have continued to expand their capacity to make and use compost tea. This is strong evidence that measurable benefits are being perceived and that a better understanding of compost tea and its uses is needed.

Innovative growers and practitioners are leading the development of new compost tea production methods and uses, generating many potential research opportunities. The purpose of this review is to summarize much of the current knowledge of plant disease suppression with compost tea and indicate future directions. It is particularly important to properly attribute the role of aeration in documented cases of disease suppression, as there appears to be confusion in recent publications (Ingham and Alms, 1999; Merril and McKeon, 2001). In addition, disease suppression has been attributed to compost tea when the cited literature describes the incorporation of solid compost (Merril and McKeon, 2001; Quarles, 2001). It should become clear that our understanding of compost tea is in its infancy and more research is needed. This review aims to help establish a common knowledge base to facilitate communication and collaboration between practitioners and researchers.

TERMINOLOGY

During the 1990's, water-based compost preparations received increasing attention from growers and researchers resulting in a proliferation of preparation methodologies and terminologies (Brinton, 1995; Diver, 2001). Numerous terms have been used to describe compost fermentations - compost tea, aerated compost tea, organic tea, compost extracts, watery fermented compost extract, amended extracts,

steepages, and slurries - and each needs to be clarified. Many are synonymous with each other or are easily confused with other concepts.

'Compost tea' has been described as the product of showering recirculated water through a porous bag of compost suspended over an open tank with the intent of maintaining aerobic conditions (Riggle, 1996). The product of this method has also been termed 'aerated compost tea' (Pscheidt and Wittig, 1996) and 'organic tea' (Merrill and McKeon, 2001). Several companies have developed various units for making compost tea under highly aerated conditions. Each company describes the end product as 'compost tea' thus effectively cementing the term 'compost tea' into common usage.

These use of the term 'compost extract' poses a particular challenge due to the widespread use of this term in studies on the chemical properties of compost (Krogstadt and Solbraa, 1975; Chanyasak and Kubota, 1981); in studies examining the *in vitro* inhibition of soil fungal pathogens by organic materials (Sivasithamparam, 1981; Kai *et al*, 1990; Hardy and Sivasithamparam, 1991); and in the *in vitro* immobilization of nematodes by toxic compounds extracted from composted municipal refuse (Hunt, 1973). These studies use 'extract' to indicate that the samples were obtained by pressure, distillation, evaporation or treatment with a solvent (Cayne, 1989). The term 'compost extract' should be reserved for describing the filtered product of compost mixed with any solvent, but not fermented, when used for analytical or assay work.

Compost extracts, watery fermented compost extracts, amended extracts, compost steepages, compost slurry, and compost tea have been used to refer to non-aerated fermentations. 'Compost extract' (Weltzien, 1989), 'watery fermented compost extracts'(Weltzien, 1991) and 'steepages' (Hoitink *et al*, 1997) are synonyms, defined as a 1:5 to 1:10 (v:v) ratio of compost to water that is fermented without stirring at room temperature in an open container for a defined length of time. 'Amended extracts' are compost extracts that have been fermented with the addition of specific nutrients or combined with isolated microorganisms before application

(Welztien, 1991). 'Compost slurry' has been used to describe NCT prior to filtration (Cronin *et al*, 1996). Brinton (*et al*, 1996) defines compost extracts or teas as a "deliberate production of specific (water) extracts based on composts of known properties and age" without distinguishing between non-aerated and aerated production.

Since the term 'compost tea' has not always been uniformly associated with an aerated fermentation process (Brinton *et al.*, 1996; Quarles, 2001; Sideman, 1996;Yohalem, 1996a) further clarification is needed. It is tempting to use aerobic or anaerobic pre-fixes to label compost tea. However, without actually measuring oxygen concentrations, it is unclear how to define the aerobic, micro-aerobic, and anaerobic gradients of oxygen found in open fermentation vessels (Johnson,1999). For clarity, we will use the terms non-aerated compost teas (NCT) and aerated compost teas (ACT) throughout this review to refer to the two dominant compost fermentation methods. ACT will refer to any method in which water is actively aerated during the fermentation process. NCT will refer to methods that do not disturb or only minimally disturb the fermentation after initial mixing. The term compost tea is retained because of the ubiquitous use of the term among practitioners.

METHODS FOR PRODUCING COMPOST TEAS

Two dominant approaches being advocated in compost tea production are aerated and non-aerated methods. Irrespective of aeration, both methods intentionally ferment well-characterized compost in water for a defined period of time. Throughout this review fermentation is used in the common way, meaning the cultivation of microorganisms (Hilton, 1999). Both methods of compost tea production (Table 2.1) require a fermentation vessel, compost, water, incubation, and filtration. Nutrients may be added before or after fermentation and various spray adjuvants can be added prior to application of undiluted or diluted tea.

Table 2.1. Process Steps for production of compost teas.

Process Step	Non-aerated (NCT)	NCT issue	Aerated (ACT)	ACT issue	common issue
Fermentation vessel	Open container	Inexpensive Reliable	Make or Buy	Expense and Reliability	Free of biocide residues
Compost source	Mixed with water in the vessel		Typically held in perforated container within the vessel		Pathogen Free. Feedstocks and age affect suppression
Water source and ratio	1:4 to 1:10 compost:water ratio		Ratios of 1:10 to 1:50 in commercial units		De-chlorinated
Fermentation nutrients		Foul odor issue		Some nutrients foam excessively eg saponin. Need defoamers	Can increase microbes, disease control, also pathogens
Fermentation duration	Range from 1-21 days	Optimum must be determined experimentally	Range from 18 hours to weeks, depending on technology	Fast times may have residual nutrients that stimulate pathogens	Longer times prevent timing flexibility
Filtration			Integrated in some commercial units		Nozzle or emitter dependent
Dilute for use		Most work done with undiluted NCT		Proposed guidelines, see text	Depends on intended use and experience
Tank Mix **Nutrients** **Surfactants** **Stickers** **UV stabilizers**					Can improve disease control, nutrients may increase pathogens
Spray equipment					PSI, velocity, sheer, pressure drop effects
Spray Timing **Rate**					Use preventively, total coverage needed

There is debate over the necessity to aerate during compost tea production (Brinton *et al*, 1996; Ingham, 1999 and 2000b). Aerated production methods are associated with reduced production time. Non-aerated production is associated with

low cost, low energy input and many documented reports of plant disease control (Weltzien, 1991). NCT production has been suggested to cause phytotoxicity and provide an optimal environment for human pathogen re-growth. However, we are not aware of any documented evidence to substantiate this claim, nor have we observed phytotoxic symptoms when NCT was used as a foliar spray or potting mix drench (Scheuerell and Mahaffee, unpublished data). There are no biological grounds to substantiate the claim that low oxygen conditions are ideal for most human pathogens to grow (Murray, 1999). Well-designed experiments that directly compare both production methods are necessary to determine the utility of aeration.

Aerated production requires mechanics and energy for continuous air addition; a number of designs are currently in use. Common aeration designs include showering recirculated water through a porous bag of compost that is suspended over an open tank (Cantisano, 1995; Riggle, 1996; Merril and Mckeon, 2001), recirculating water through a vortex nozzle mounted above a tank (Ingham and Alms, 1999), injecting air through a hollow propeller shaft (Soilsoup.com), venturi nozzles (Composttea.com), aquarium stones (Ingham, 2000a), or fine bubble diffusion mats (Growingsolutions.com, Compara.com). There are a growing number of companies offering units that produce aerobic 'compost tea' by suspending compost in a fermentation vessel and actively aerating and/or recirculating the liquid (Diver, 2001).

NCT has traditionally been made by mixing 1 volume compost with 4-10 volumes of water in an open container, initially stirring the mixture, and then leaving it undisturbed at 15-25°C for at least 3 days (Weltzien, 1991). Brinton (*et al*, 1996) advocates stirring NCT every 2-3 days during the fermentation to possibly facilitate the release of microbes from compost particles. Container sizes range from several thousand liters down to small buckets. However, to avoid compost sampling error, at least 500g compost should be used when considering experimental designs for *in vitro* inhibition screening with NCT (Yohalem *et al* 1996b).

Other preparation methods are in limited practice and have not been assessed in controlled studies for disease suppression. One method involves straining water

through compost-filled sacks directly into a spray tank for use (Diver, 1998). Another method is to make instant compost tea by mixing water with finely ground compost without fermentation or filtration before use (Don Cranford, personal communication). Gardening lore also recommends hanging burlap bags filled with compost in water barrels to produce a plant drench (Peavy, 1992). Several other related preparations have been made including herbal or manure teas (Diver, 2001), but will not be discussed further. Regardless of preparation method, compost teas are typically applied with conventional pesticide spray equipment after filtering out material that would clog the nozzles (Brinton *et al*, 1996, Ingham, 2000b).

For clarity, research reports on the use of compost tea should include detailed information on several fermentation and application parameters (Table 2.2). As a group, the fermentation parameters will influence the composition and population of microbial species in the final product. The application parameters influence the extent of target coverage and establishment of the applied microorganisms on plant surfaces.

Table 2.2. Fermentation and application parameters. Influence on compost tea production and efficacy.

Fermentation Parameters	
Fermentation vessel	Dimensions, manufacturer and model if applicable
Compost	Producer, feedstocks, age, stability, % moisture, available nutrients, microbial analysis, either volume and bulk density used or weight
Water source	volume, initial and final temperature
Fermentation nutrients	Source, quantity and timing
Oxygen content in ppm	Include any stirring, agitation, or aeration; indicate time of reading(s) during production
Fermentation duration	Method of storage if not used immediately
Application Parameters	
Filtration	Material used for filtering
Dilution ratio	Water source used
Adjuncts	Nutrients, surfactants, stickers, UV stabilizers, microorganisms
Application equipment	Make, model, nozzle specifications, PSI
Application	Rate, time of day, weather, interval between applications

EFFECT ON PLANT DISEASE

Very little data directly compares non-aerated and aerated production methodologies for plant disease control. Cronin *et al* (1996) used a manure-based spent mushroom compost to compare NCT and an air-bubbled ACT for *in vitro* effects on germination of conidia of *Venturia inaequalis*. They concluded that 7-day NCT inhibited germination while the ACT had no effect. Conidial inhibition was induced after the 7-day aerated fermentations were allowed to incubate for an additional 7 days without aeration.

Scheuerell and Mahaffee (2000b) examined the role of aeration and 3 compost types (yard debris, chicken manure/sawdust, CMC mixed source) in producing compost teas for controlling powdery mildew (*Sphaerotheca pannosa*) on field grown roses. The ACT were fermented for 24 hours in commercial compost tea 'brewers'. The NCT's were fermented in buckets for 7 -10 days. Applications were made every 7-11 days over a 5-month season. All 6 compost teas significantly reduced powdery mildew incidence on leaflets compared to a water spray control; within each compost type there was no difference between the ACT and NCT. The composted chicken

manure produced the most suppressive compost teas. They concluded that compost source was more important than aeration for maximizing disease control.

A variety of foliar diseases have been suppressed by applications of NCT. A range of experimental approaches and scales have been utilized including *in vitro* inhibition, seedling assays, detached leaves, growth chambers, production greenhouses, and field studies (Table 2.3). The large number of studies supporting the use of NCT for pathogen suppression indicates that it is a viable tool. A number of these studies have been reviewed previously (Weltzien, 1991).

Table 2.3. Non-aerated compost tea (NCT) for plant disease control. Summary of experiments.

Pathogen	Host Tissue	Scale[1]	Control[2]	Pathogen Inoculated	Compost Type	Optimum Fermentation Duration	Fermentation Nutrients	Spray Adjuvants	Source
Alternaria panax	ginseng	IV SA	- -	5 x 10^5 spores/ml	Spent Mushroom	7 days	none	none	Yohalem et al 1994
Alternaria solani	tomato plants	Field	+	conidia, amount not stated	cattle manure	14 days	none	none	Tsror, 1999
Botrytis cinerea	bean	IV DL	+ +	2 x 10^6 spores/ml	horse bedding, chicken litter	8 days	none	none	McQuilken et al 1994
Botrytis cinerea	bean	DL	+	2 x 10^6 spores/ml	cattle manure	24 hours	0.5 - 1.0% yeast extract	none	Urban and Trankner 1993
Botrytis cinerea	bean	DL	+	2 x 10^6 spores/ml	horse manure	24 hours	0.5 - 1.0% yeast extract	none	Urban and Trankner 1993
Botrytis cinerea	grape	DL berries	+ +	2 x 10^6 spores/ml	horse-straw-soil	8 days	none	none	Ketterer et al 1992
Botrytis cinerea	grape berries	Field	+	2 x 10^6 spores/ml	horse-straw-soil	2 and 4 months	none	0.5 % casein + 0.05 pine oil	Ketterer et al 1992
Botrytis cinerea	lettuce	GH	+	2 x 10^6 spores/ml	horse bedding, chicken litter	8 days	none	none	McQuilken et al 1994
Botrytis cinerea	strawberry	Field	-	natural	Cattle manure	7-21 days	none	none	Welke 1999
Botrytis cinerea	strawberry	Field	-	natural	Chicken manure	7-21 days	none	none	Welke 1999
Botrytis cinerea	strawberry	Field	+	natural	Cattle manure	16 days	none	none	Stindt 1990
Botrytis cinerea	strawberry	Field	+	natural	Horse manure	12 weeks	none	none	Stindt 1990
Botrytis cinerea	strawberry	Field	+ early - late season	2 x 10^6 spores/ml	horse-straw-soil	24 hours	1.0% yeast extract	none	Urban and Trankner 1993
Botrytis cinerea	tomato pepper grape	DL DL berries	+ + +	2 x 10^5 spores/ml	Cattle manure	14 days	nutrient broth didn't increase suppression of 10 day fermentations	none	Elad and Shtienberg 1994
Botrytis cinerea	tomato pepper grape	DL DL berries	+ + +	2 x 10^5 spores/ml	horse manure	14 days	nutrient broth didn't increase suppression of 10 day fermentations	none	Elad and Shtienberg 1994
Botrytis cinerea	tomato pepper grape	DL DL berries	- + +	2 x 10^5 spores/ml	Grape marc	14 days	nutrient broth didn't increase suppression of 10 day fermentations	none	Elad and Shtienberg 1994

Table 2.3 (Continued).

Pathogen	Host Tissue	Scale[1]	Control[2]	Pathogen Inoculated	Compost Type	Optimum Fermention Duration	Fermentation Nutrients	Spray Adjuvants	Source
Botrytis cinerea	tomato foliage	GH	+	natural	cattle manure	14 day	none	none	Elad and Shtienberg 1994
Cochliobolus carbonum	maize	IV SA	+ +	5 x 10^5 spores/ml	Spent Mushroom	7 days	none	none	Yohalem et al 1994
Erysiphe polygoni	bean	GH	+	Not stated	Not stated	7-14 days	none	0.5% casein	Ketterer and Schwager, 1992
Phytophthora infestans	potato	Field Field	- +	natural	horse-straw-soil	7 day	controlled only by adding pure cultures of microbial antagonists to tea just before spraying		Ketterer 1990
Phytophthora infestans	potato	SA Field	+ -	?	?	?	none	none	Jongebloed et al 1993
Phytophthora infestans	tomato	GH	+	Not stated	Not stated	7-14 days	none	0.5% casein	Ketterer and Schwager, 1992
Phytophthora infestans	tomato	DL	+	8 x 10^4 sporangia/ml	horse-straw-soil	14 day	none	none	Ketterer 1990
Plasmopara viticola	grape	DL	+	8 x 10^4 sporangia/ml	horse-straw-soil	3	none	none	Weltzien and Ketterer 1986a
Plasmopara viticola	grape	Field	+	natural	horse-straw-soil	3	adding pure cultures of microbial antagonists just before spraying significantly increased control		Ketterer 1990
Plasmopora viticola	grape	DL GH	+ +	1 x 10^4 sporangia/ml	fresh cow dung, soil	14 day	none	none	Achimu and Schlosser 1992
Pseudopeziza tracheiphila	grape	Field	+	ND	horse-straw-soil	3	none	none	Weltzien 1989
Psuedomonas syringae	Arabidopsis	SA	+	1 x 10^8 cfu/ml	pine bark	7 day	none	none	Zhang et al 1998
Sphaeropsis sapinea	red pine	IV SA	+ +	5 x 10^5 spores/ml	Spent Mushroom	7 days	none	none	Yohalem et al 1994
Sphaerotheca fuliginea	cucumber	DL	+	yes, ND	Various	various	none	none	Samerski and Weltzien 1988
Sphaerotheca pannosa	Rose	Field	+	natural	Chicken Manure	7-11 days	0.3% molasses	none	Scheuerell and Mahaffee 2000b
Sphaerotheca pannosa	Rose	Field	+	natural	Yard debris	7-11 days	0.3% molasses	none	Scheuerell and Mahaffee 2000b

Table 2.3 (Continued).

Pathogen	Host Tissue	Scale[1]	Control[2]	Pathogen Inoculated	Compost Type	Optimum Fermention Duration	Fermentation Nutrients	Spray Adjuvants	Source
Sphaerotheca pannosa	Rose	Field	+	natural	Mixed source	7-11 days	0.3% molasses	none	Scheuerell and Mahaffee 2000b
Uncinula necator	grape	GH	+	ND	horse-straw-soil	3	none	none	Weltzien 1989
Uncinula necator	grape	Field	+ +	natural	Cattle manure	3 days	none	none	Sackenheim 1993
Uncinula necator	grape	Field	+	natural	horse manure	3 days	none	none	Sackenheim 1993
Uncinula necator	grape	Field	+	natural	horse manure	3 days	none	caso bouillon, rape oil 0.5%	Sackenheim 1993
Venturia inaequalis	apple	IV SA	+ +	5 x 10^5 spores/ml	Spent Mushroom	7 days	none	none	Yohalem et al 1994
Venturia inaequalis	apple	Field	-	natural	Spent Mushroom	7 days	none	none	Andrews 1993
Venturia inaequalis	apple	Field	-	natural	Cattle manure	7 days	none	none	Andrews 1993
Venturia inaequalis	apple	Field	+	natural	Spent Mushroom	7 days	none	Latron B1956 0.06% or Fish Oil 0.025%	Yohalem *et al* 1996
Venturia inaequalis	apple	Field	+	yes, ND	manure-straw-soil	5-7 days	none	none	Trankner and Kirchner-Bierschenk 1988
Xanthomonas campestris	tomato	SA Field	+ -	1 x 10^8 cfu/ml	how Manure	7 day	none	none	Ai-Dahmani and Hoitink 1999

1 - Experimental scale: IV - in vitro, DL - detached leaf, SA - seedling assay, GH - commercial greenhouse setting, Field - outdoor agronomic conditions

2 - Control: + treatments statistically less disease (minimum p = 0.05) than control treatment; - treatment no difference from control treatment

Research on the use of ACT to control foliar and fruit diseases is summarized in Table 2.4. The limited number of controlled studies using ACT have not been widely circulated and therefore will be covered in more detail here. In the Willamette Valley Oregon, Pscheidt and Wittig (1996) did not observe significant control of powdery mildew of apple or grape, apple scab, pear scab, brown rot of peach, peach leaf curl, and cherry leaf spot when ACT was applied in the field on regular intervals. One significant result, reduced incidence of brown rot blossom blight (*Monilinia laxa*) on sweet cherry was observed. The ACT was stored in containers for 12-15 hours overnight, and it is unknown if this could have negatively influenced the observed level of suppression for all host-pathogen combinations.

Table 2.4. Aerated Compost Tea (ACT) for plant disease control. Summary of published studies.

Pathogen	Host Tissue	Control[1]	Scale	Pathogen inoculated	Compost type	Fermention Duration	Fermentation Nutrients	Source
Alternaria + Septoria	Tomato foliage	-	Field	natural	Vermicompost	24 hours	Soil Soup solution[2]	Barker-Plotkin 2000
Alternaria alternata	Tomato foliage	-	Field	natural	Not reported	24 hours	1.25% molasses, rock flour	Granatstein 1999
Blumeriella jaapii	cherry leaves	-	Field	natural	Not reported	24 hours	0.5% molasses rock dust	Pscheidt and Wittig 1996
Drop rot, pathogen not reported	lettuce	-spring +summer	Field	natural	Not reported	24 hours	1.25% molasses, rock flour	Granatstein 1999
Monilinia fructicola	Peach fruit - postharvest	-	Field	natural	Not reported	24 hours	0.5% molasses rock dust	Pscheidt and Wittig 1996
Monilinia taxa	cherry blossom	+	Field	natural	Not reported	24 hours	0.5% molasses rock dust	Pscheidt and Wittig 1996
Podosphaera leucotricha	apple terminals	-	Field	natural	Not reported	24 hours	0.5% molasses rock dust	Pscheidt and Wittig 1996
Post harvest loss	blueberry fruit	+	Field	natural	Not reported	24 hours	1.25% molasses, rock flour	Granatstein 1999
Sphaerotheca pannosa	Rose	+	Field	natural	Chicken manure	24 hours	0.3% molasses	Scheuerell and Mahaffee 2000b
Sphaerotheca pannosa	Rose	+	Field	natural	Mixed source	24 hours	0.3% molasses	Scheuerell and Mahaffee 2000b
Sphaerotheca pannosa	Rose	+	Field	natural	Yard debris	24 hours	0.3% molasses	Scheuerell and Mahaffee 2000b
Taphrina deformans	Peach leaves	-	Field	natural	Not reported	24 hours	0.5% molasses rock dust	Pscheidt and Wittig 1996
Uncinula necator	grape - leaves - clusters	- +	Field	natural	Not reported	24 hours	0.5% molasses rock dust	Pscheidt and Wittig 1996
Venturia inaequalis	apple - leaves - fruit	- -	Field	natural	Not reported	24 hours	0.5% molasses rock dust	Pscheidt and Wittig 1996
Venturia inaequalis	conidia germination	-	in vitro		Spent Mushroom	bubbled for 7 days	none	Cronin et al 1996
Venturia pirina	pear fruit	-	Field	natural	Not reported	24 hours	0.5% molasses rock dust	Pscheidt and Wittig 1996

1 - Control: + treatments statistically less disease (minimum $p = 0.05$) than control treatment; - treatment no difference from control treatment

2 - Commercial product containing molasses, kelp, bat guano, citric acid, $MgSO_4$ (Soil Soup Inc., Edmonds, WA).

Other farm trials used ACT on a variety of crops with variable yield and disease control (Granatstein, 1999). No effect of ACT applications on early blight of tomato was observed. Lettuce drop incidence was reduced in a summer but not a spring crop. Post-harvest fruit rot of blueberries was significantly reduced, but offset by reduced yields. Spinach yield also decreased, but spring and summer broccoli yields increased. It is apparent that impacts on plant health and yield can be crop specific and general inferences about disease suppression or yield cannot be made.

While relatively little research has been conducted on soil-borne disease suppression with compost tea drenches, this technique is practiced in the organic agriculture community. NCT was investigated for use as a seed treatment to prevent pea seedling damping-off caused by *Pythium ultimum* (Tränkner, 1992). NCT's prepared from either cattle manure or grape marc and fermented for 5 or 10 days were effective in suppressing *in vitro Pythium* mycelial growth. They also significantly increased seed germination, root length, and root dry weight when seeds were soaked, redried, and sown 2 days later in soil inoculated with *P. ultimum*. Weltzien (1991) reports that *Rhizoctonia solani* has been suppressed *in vivo* by NCT and that heat sterilizing the NCT increased radial growth of Rhizoctonia colonies relative to an untreated control. Significant control of Fusarium wilt of pepper (*F. oxysporum* f. sp. *vasinfectum*) and cucumber (*F. oxysporum* f. sp. *cucumerinum*) by drenching NCT in greenhouse tests has recently been reported (Ma *et al*, 1999; Ma *et al*, 2001). The NCT had an *in vitro* mycolytic effect on *Fusarium* microspores and chlamydospores, indicating that destruction of pathogen propagules could be playing a role in disease suppression. The potential of using compost tea for controlling soil borne disease, especially as a potted plant drench, deserves further research.

There is increasing interest among ACT practitioners to have their product tested by commercial laboratories for *in vitro* suppression of various soil-borne pathogens; some growers have reportedly used these assays to select for improvements in the suppressive qualities of their compost tea (Vicki Bess, BBC laboratories, personal communication). However, it is well established that *in vitro* inhibition is not

always a good predictor of disease suppression when used as a screen for microbial antagonists (Cook and Baker, 1983). For compost tea, assessing the utility of *in vitro* pathogen screening will require data correlating *in vitro* results to suppression under field conditions. Testing compost tea 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. However, all assays suffer from the complication that the tested batch of compost tea is ready for use well before assay results are available. Testing multiple batches over time could establish a probability that the same effect would be observed from a particular tea production process, but this requires significant time and cost for each pathogen.

MODE OF ACTION

Multiple modes of activity are involved in suppressing plant disease with NCT; yet to date no studies have determined the mechanisms involved with ACT. Induced resistance, antibiosis, and competition have been used to explain suppression of foliar pathogens by NCT. Besides the report of *Fusarium* spore lysis (Ma *et al*, 2001), direct destruction of pathogen structures has not been reported with compost tea whereas this observation has been made for control of root rot pathogens with compost (Hadar and Gorodecki, 1991). Considering the diverse microbial community in compost tea, it is likely that multiple modes of activity associated with microbial antagonists are involved in disease suppression.

Compost teas can induce plant defense responses. *In vitro* germination of *Sphaerotheca fuliginea* conidia was not inhibited by NCT, yet treated cucumber leaves responded to infecting conidia by increased papillae formation, lignification and necrotic reactions compared to nontreated leaves (Samerski and Weltzien, 1988). These observations indicate that host responses to the pathogen were altered. Similary, Zhang and coworkers (1998) used beta-1,3-glucuronidase (GUS) activity as a marker of plant defense gene induction when studying Arabidopsis bacterial speck caused by *Pseudomonas syringae* pv. *maculicola*. GUS activity was induced equally

in Arabidopsis plants by topical sprays of a composted pine bark NCT or salycylic acid. These results indicate that application of compost teas can stimulate plant defense reactions.

Several studies have determined that antibiosis is a mechanism of suppression based on observations that filter or heat sterilized NCT retain suppressive qualities (Elad and Shtienberg 1994, Yohalem *et al* 1994, Cronin et al 1996). Cronin *et al* (1996) elucidated that antibiosis was the mechanism of inhibiting *in vitro* conidia germination of *Venturia inaequalis* by spent mushroom NCT. When the compost was sterilized and then fermented, no suppressive activity was found. However, fermenting non-sterilized compost produced NCT that had equally suppressive activity after 0.1*u*m filtration, and it maintained most of the suppressive activity after autoclaving. Using microconcentrators, the major inhibitory agent was determined to be a low molecular weight (<3 kDa), heat stable, non-protein metabolite produced by microorganisms during NCT fermentation.

There is evidence that some antibiotic metabolites present in compost tea originate from the compost source. Al-Dahmani *et al* (1998) reported significant but inconsistent control of tomato bacterial spot (*Xanthamonas campestris* pv. *vesicatoria*) with NCT; 7-day fermentations made from either pine bark, cow manure, or yard waste composts varied in control between batches of the same compost source. After experimenting with various compost tea production methods it was suggested that control was due to an extractable, heat-stable metabolite produced within the compost pile. In this case, fermentation was not needed for suppression and the antibiotic agent(s) were inconsistently produced by identical composting practices. However, suppression did require the compost to be maintained at 55% minimum moisture content to generate suppression (Al-Dahmani, personal communication).

Numerous studies have shown that reducing the microbial component of NCT can negatively impact suppressive properties. When filter or heat sterilization results in the loss of disease suppression, it has been concluded that microbial competition for nutrients or space is the mode of action. Plant-pathogen systems demonstrating

experimental evidence to support this conclusion include *Phytophthora infestans* on tomato and potato (Weltzien and Ketterer, 1986b), *Uncinula necator* and *Plasmopora viticola* on grapes (Weltzien and Ketterer, 1986a), and *Botrytis cinerea* on bean (Stindt, 1990) and strawberries (Urban and Tränkner, 1993). For example, NCT was filtered through increasingly smaller pore sizes (50, 10, 5, 1, 0.45, and 0.2 *u*m) with each filtrate sprayed onto detached tomato leaves followed 3 days later by *P. infestans* inoculation (Ketterer, 1990). Suppression of *P. infestans* was not affected by 50 or 10 *um* filtration, but the 5.0 *u*m pore size reduced suppressive activity with further stepwise losses of suppression observed with smaller pore sizes. These studies indicate that applying the microbial component of compost tea is necessary for disease suppression. However, it is not clear whether pathogen inhibition is due to parasitism, competition for nutrients and colonization sites, or if applied organisms produce antibiotics *in situ* once established on plant surfaces.

MICROBIAL DYNAMICS

Regardless of the mode of action, the total microbial population in NCT has been correlated to increased disease suppression. Ketterer (*et al*, 1992) related the suppression of *B. cinerea* on detached grape leaves to the total culturable microbial populations in the applied NCT. Three composts were fermented for 1,3, 7 and 14 days with 7 days being the most suppressive and having the greatest population as determined by plating on caso agar. Heat sterilizing the NCT significantly reduced and nearly eliminated suppression.

The relative importance of the living microbial community for disease control can change as the duration of NCT fermentation increases. Stindt (1990) observed an inverse relationship between fermentation time and the role of microbial competition for suppressing *B. cinerea* on detached bean leaves. Fermentation periods of 4 to 16 days were equally suppressive, but heating the 4-day fermentation reduced suppression relative to heating the 16-day fermentation. In this example, microorganisms are necessary for antagonism early in the non-aerated fermentation

process, whereas longer fermentations likely accumulate metabolic byproducts that play an increasing role in disease suppression.

A number of phylloplane studies have examined populations of microbes recovered from NCT treated leaves in relation to foliar disease levels. While investigating apple scab leaf severity in the field, Andrews (1993) compared culturable populations of fungi and bacteria from leaves treated with NCT to untreated leaves throughout 2 growing seasons. During a wet year no increase in phylloplane microbial population was detected, bacterial populations generally increased 1 log/ g leaf during a dry year. However, no significant disease control was observed either season. Another study recovered phylloplane bacteria from apple trees treated with NCT mixed with 0.06% Latron B1956 spreader-sticker. Bacterial cfu/g leaf were 10-fold higher for 12 days post application compared to water treated leaves (Yohalem *et al*, 1996a). Lange and Weltzien (unpublished, reported in Tränkner and Brinton (1998)) observed a general increase in total culturable grape phylloplane populations 3 days post NCT application. Aerobic spore forming bacteria increased significantly by 1 log/g fresh leaf, coinciding with a 50% reduction in powdery mildew leaf disease severity.

The relationship of culturable leaf populations of yeasts and fungi, Enterobacteria, Pseudomonads, aerobic Bacillus spp., and total epiphytes to *Plasmopara viticola* (growth chamber) and *Uncinula necator* (vineyard) infected grape plants was studied (Sackenheim *et al*, 1994). Growth chamber and field plants were sprayed with various NCT, fermented with or without nutrients, and applied with or without methyl cellulose as a tank mixed surfactant. The growth chamber study was conducted at 2 relative humidity levels, 50-60% and 90-95%. Under lower humidity, leaf populations of all microbe groups except spore forming bacilli were drastically reduced across treatments and only treatments fermented with nutrients significantly reduced disease severity. This illustrates the need to screen potential treatments under a variety of environments. Under field conditions, all treatments significantly reduced powdery mildew severity compared to a water control. The

combination of fermentation nutrients with methyl cellulose generated the greatest number of recovered organisms per leaf area, and only this treatment reduced disease significantly more than the basic NCT. Further evidence that increasing leaf microbial populations may be related to disease suppression has been shown in greenhouse experiments with bean rust (*Erisiphe polygoni*) and tomato late blight (*P. infestans*) (Ketterer and Schwager, 1992). Plants treated with NCT had significantly increased leaf populations (cfu) of *Pseudomonas* spp., Enterobacteriaceae, and spore-forming bacteria, and these elevated populations were related to reduced disease. The greatest increases in culturable leaf populations were observed when casein (0.5% w:v) was added to the NCT just before application. Increasing microbial populations through the addition of fermentation nutrients, and using spray adjuvants to increase phylloplane survival, likely optimizes conditions for multiple modes of antagonistic activity.

UNDERSTANDING VARIABLE EFFICACY

Microbial populations in compost tea are considered the most significant factor contributing to disease suppression. However, despite their importance, there is a very limited understanding of the microbial species composition of compost tea and how these organisms survive on plant surfaces. This limited knowledge likely contributes to the variable success reported for controlling plant pathogens with compost tea. Additionally, standardized methods for reporting on compost tea microbiology have not been established and this hinders comparison across experimental systems. Examining our current knowledge of compost tea microbiology, its impact on leaf surface microbial ecology, and the methods used thus far for studying these populations can help identify causes for variability in disease control efficacy and point to future research needs.

It is possible that efficacy is linked to the total microbial population or specific sub-populations in compost tea. In theory, if all microbial species in compost tea could function towards disease suppression, then higher total microbial counts or biomass should correlate to more consistent disease control or allow greater dilution

rates to be used. The total culturable bacteria reported for suppressive NCT vary over several orders of magnitude with a range of 10^7 to 10^{10} cfu/ml (Table 2.5.) This data could suggest that 10^7 cfu/ml total bacteria in compost tea is a minimum population threshold for foliar disease suppression to function. However, we have observed variable suppression of *Botrytis cinerea* on geranium foliage with both ACT and NCT that ranged from 10^7 to 10^9 cfu/ml total bacteria (Scheuerell and Mahaffee, unpublished data). This leads us to believe that the total culturable bacterial population in compost tea does not necessarily correlate to suppression. Variability in bacterial species might be a cause of variable efficacy results but more work is needed to establish a relationship between populations of specific organisms and disease control.

Table 2.5. Aerobic colony forming units/ml. Non-aerated compost tea[a]

Compost type	Total cfu	Aerobic bacteria	Entero-bacteriaceae	Actino-mycetes	Fungi and yeasts	Source
Horse manure		1×10^7	2×10^5		1×10^5	Stindt, 1990
Horse manure	1.4×10^{10}					Ketterer, 1990
Horse manure	7.6×10^7					Ketterer et al 1992
Cattle manure		6×10^8	2×10^6		5×10^5	Stindt, 1990
Cattle manure	2.8×10^8					Ketterer, 1990
Cattle manure	8.2×10^8					Ketterer et al 1992
Grape marc		2×10^8	3×10^5		9×10^5	Stindt, 1990
Grape marc	3.1×10^{10}					Ketterer, 1990
Grape marc	7.4×10^7					Ketterer et al 1992
Horse-Chicken		5.6×10^{10}		2.4×10^5	1.1×10^2	McQuilken et al 1994
Chicken manure			0.8			Welke, 1999
Cattle manure			35			Welke, 1999

[a]Compost:water ratios range from 1:5 to 1:9. Data are from 7-8 day fermentations with no added fermentation nutrients.

Attachment and survival of the microbes after application may be equally or more important than the initial microbial populations in compost tea for disease suppression. Little is known about the underlying principles that govern the adhesion and survivability of various types of compost tea organisms in the phyllosphere and rhizosphere. Applying NCT can have immediate impact on phylloplane microbial populations, but the longevity of microbial change is likely influenced more by environmental conditions than by properties of the NCT. Tränkner (1992) detected an increase of 10^3 cfu/cm^2 total microorganisms on greenhouse grown bean leaves 1 hour after NCT treatment. Under moist conditions, culturable phylloplane populations were maintained for at least 5 days, while total populations were significantly reduced under dry conditions to 10 cfu/cm^2. Applying NCT on field grown potato plants resulted in significant increases of Pseudomonads and Enterobacteria compared to a water control; however, total culturable microbial epiphytes did not differ (Tränkner, 1992). These studies indicate that under changing environmental conditions the total microbial carrying capacity of foliage is not elevated for extended periods after applying compost tea when spray adjuvants are not used. However, it is possible for

specific groups of organisms from compost tea to increase as a proportion of the total culturable epiphytic population. If these selected groups are suppressive against the targeted pathogen, then the likelihood of disease control increases.

Techniques utilized thus far for the study of compost tea microbial ecology have limited our understanding of the variability associated with suppression. To date, published investigations have relied on enumeration of culturable components of compost tea and phyllosphere microflora. While it is understood that using plate counts for estimating total microbial populations biases the results to culturable organisms and does not provide information on metabolic state (Tate, 2000), plate counts are useful for tracking specific groups of organisms and provide a framework to relate findings to the existing phyllosphere and phytopathology literature.

It has been proposed that direct counts are the best method to assess compost tea bacteria and fungal populations, and that the addition of fluorescent stains can be used to assess metabolic state (Ingham, 2000b). While they are useful for enumerating total populations, they do not allow for estimates of genetic or functional diversity. Thus impacts of various compost tea production methods on microbial diversity cannot be measured.

Interestingly, we have observed that estimates of total bacterial cells in ACT fermented with a variety of nutrients are statistically the same when measured by enumeration on agar media or by staining and direct microscopic counts (unpublished data). These results appear to indicate that the ACT fermentation process selects for culturable bacteria. However, when NCT is fermented with nutrients, aerobic plate counts are typically significantly lower than the total bacterial population estimated using staining and direct microscopic counts (unpublished data). This indicates that a large portion of the microbial population may be strict anaerobes or non-culturable facultative aerobes. Regardless, both direct count techniques and culture methods have severe limitations for tracking the changing microbial ecology of compost tea during production and after application to plant surfaces.

The role of microbial diversity in the efficacy of compost teas has not been examined. If specific microbial types can be linked to disease suppression then monitoring microbial diversity and abundance will be crucial for achieving consistent biological control. An integrated assessment combining culture methods with extensive molecular studies is necessary to determine the diversity and abundance of microbes present in compost tea, and most importantly, to understand how different compost tea production practices affect the microbial community and how these communities function in pathogen and/or disease suppression.

Regardless of the mode of action or source of microorganisms, preventative application before pathogen infection appears necessary for optimal control through all known modes of action. Blakeman and Fokkema (1982) state that most foliar pathogens susceptible to antagonism exhibit some superficial growth on the leaf surface from which penetration of the leaf surface takes place. This is a likely reason why many successful examples of *Botrytis cinerea* suppression with NCT have been documented. The many examples of powdery and downy mildew suppression (Tables 2.3 and 2.4) could also relate to the large portion of exposed hyphae and reproductive structures accessible to applied microbes and metabolites. However, Stindt (1990) reduced *B. cinerea* infections after spraying NCT 24 hours post pathogen inoculation, indicating that eradicative treatments with compost teas may be possible. Maximizing the interaction time between pathogens and resident antagonists on plant surfaces will likely increase disease suppression. Once we have developed a better understanding of compost tea microbiology and the survival and interactions of microbes on plants surfaces, it should be possible to modify compost tea production practices and application technology to optimize delivery of a microflora with multiple modes of pathogen suppression.

PRODUCTION PRACTICES AND APPLICATION TECHNOLOGY

Besides the role of aeration in compost tea production, several of the process steps (Table 2.1) can impact the suppressive properties of NCT. Influential production decisions include choice of compost feedstocks, compost age, water ratio, fermentation time, added nutrients, temperature and pH. Application technology choices include the dilution ratio, application equipment, timing, rates, spray adjuvants and adding specific microbial antagonists. As the body of published research expands it becomes obvious that there is no one ideal management level across all host-pathogen systems for the compost tea production and application factors. Studies are highlighted that indicate where similarities and inconsistencies exist within these factors in relation to optimizing disease suppression with NCT. The emphasis is on NCT since few of these steps have been investigated for impacts on production of ACT. Less studied factors will be addressed in relation to the potential impact on disease suppressive qualities and indicate future directions of research.

Compost feedstocks. Compost feedstocks can include animal manures and bedding, landscape and agricultural plant material, and soil. Each have characteristics that influence the biological and physical characters of a mature compost, which could in turn impact the efficacy of compost tea made from the compost. Early reports on NCT indicate that the most efficacious control was attained using animal manure composts as opposed to compost made solely from vegetative material (Weltzien 1990;1991).

The superiority of manure containing compost was supported when 32 different composts were screened for the *in vitro* inhibition of *V. inequalis* conidia; only composts containing undigested plant material were not efficacious (Andrews, 1993). Contrary to this, Elad and Shtienberg (1994) determined that plant-based compost produced from grape marc was equally effective as manure based compost to make NCT that inhibited *B. cinerea* on foliage in greenhouse assays. While differences in compost source used for compost tea do translate to different levels of

disease suppression in the field (Scheuerell and Mahaffee, 2000b), the level of suppression could not be predicted by microbial enumeration on selective media.

Due to the potential for transferring detrimental effects, compost for compost tea should be certified free of human pathogens and residual herbicides. Raw manures should be avoided because of the potential of human pathogens being present. Herbicide contamination of compost tea is becoming a potential issue with the increasing occurence of clopyralid and picloram contaminated compost (Bezdecek *et al*, 2001; Rynk, 2001). However, we are unaware of any reports indicating contamination of compost tea from herbicides in compost.

Compost age. There is increasing knowledge on how old compost made from particular feedstocks can be before it is no longer useful for making suppressive NCT. Tränkner (1992) reviewed German studies that claim composts should be 2-6 months old when selected for use. In summarizing work by Dittmer *et al* (1990) and Dittmer (1991), Brinton *et al* (1996) indicate that compost made only with plant material such as leaves, yard trimmings and straw is not useful after aging 3 months while horse and dairy manure compost can be used until 9-12 months old. In a cucumber downy mildew assay using NCT prepared with horse manure compost, 6-month-old compost was significantly more effective than 1-year-old compost ((Winterscheidt *et al*, 1990) cited in Weltzien, 1991)). Andrews (1993) reported that the efficacy of NCT for *in vitro* inhibition of *V. inaequalis* germination declined as cattle manure-straw compost aged from 12 to 18 to 24 months. In trying to extend the useful life of effective compost sources, air dried compost has been used to produce NCT that was equally effective as fresh compost for *B. cinerea* suppression (Urban and Tränkner, 1993). Further work of this nature might allow effective compost to be dried and stored large quantities for future use. Thus, the effect of compost age on efficacy of compost tea is a factor of feed stocks and storage conditions. Although no compost tea research has documented the stability of compost used in trials, compost stability could be a more useful parameter to report than compost age.

Compost to water ratio. The ratio of compost to water on a volume:volume basis in published studies starts at 1:1 (Zhang *et al* 1998) and reaches 1:50 Weltzien (1990). Most studies have followed the methodology developed by Weltzien's laboratory that uses a 1:3 – 1:10 ratio. Weltzien (1990) reviewed a number of host-pathogen systems that had significant foliar suppression with NCT, no difference in suppression was observed for fermentation ratios between 1:3 and 1:10. However, for the suppression of *Phytophthora infestans*, increasing the fermentation ratio to 1:50 resulted in loss of activity (Weltzien, 1990). In general, diluting the final spray would likely have a different effect than diluting the initial fermentation ratio because the initial ratio can influence the rate of oxygen depletion during fermentation (Cronin *et al*, 1996; Merril and McKeon, 2001). It is still unclear how the compost to water ratio of NCT impacts disease suppression, but limiting the ratio to 1:10 is apparently effective.

Fermentation time. For NCT, several studies have indicated that disease suppression varies widely in relation to the fermentation time (Weltzien, 1990). Optimum fermentation times are listed in Table 2.3. Minimum effective fermentation time has been as short as 1 day for *in vitro B. cinerea* inhibition (Urban and Tränkner, 1993), or 3 days for *in vitro* inhibition of *V. inaequalis* conidia (Andrews, 1993). Usually, a 5-8 day and up to a 16 day fermentation time is needed for any level of disease control, which has been hypothesized to allow sufficient time for facultative anaerobes to dominate and for their metabolites to accumulate (Weltzien 1991). Several studies have indicated that suppressiveness increases with increasing fermentation time to a maximum and then declines. Ketterer (1990) indicated a 3 day fermentation time peaked inhibition of downey mildew (*Plasmopara viticola*) on detached grape leaves. Weltzien *et al* (1987, cited in Weltzien, 1988) found that a 4 to 7-day fermentation time was optimal for suppressing powdery mildew (*E. betae*) on sugar beet in laboratory studies. Ketterer *et al* (1992) examined *Botytis* suppression on detached grape leaves with 1, 3, 7 and 14-day fermentations of 3 composts, and suppression was uniformly maximized at 7 days. These same composts uniformly

suppressed grape berry infection by *B. cinerea* after 8 days of fermentation. However, Elad and Shtienberg (1994) observed that NCT fermented for 14 days was consistently more suppressive towards *B. cinerea* than 7-day fermentations. Weltzien (1990) showed that late blight (*P. infestans*) lesions on detached tomato leaves were inhibited to the greatest degree by 7 or 14-day fermentation as compared to 1, 2 or 28 days. The maximum NCT fermentation times reported for significant disease suppression were 2 and 4-months, this was observed in a commercial vineyard experiment that evaluated grape bunch rot (*B. cinerea*) control (Ketterer *et al,* 1992). The general trend for maximizing suppression depends primarily on the host-pathogen system and secondarily on the compost feedstock, but the ideal fermentation time may need to be determined for each host-pathogen-compost system.

Much less is known about the effect of fermentation time on efficacy of ACT. Cantisano (Cantisano, 1998) states that 1-day aerated fermentations are used for foliar feeding while maximum disease control is achieved with 7-14 day ACT. On the other hand, Ingham (1999, 2000b) states that the optimum ACT fermentation time coincides with maximum active microbial biomass during fermentation, often 18-24 hours with commercial aerobic compost tea makers.

Fermentation nutrients. Optional nutrients can be added at the beginning or during fermentation resulting in an unknown selective enrichment of the fermenting community (Bess, 2000). Several manufactures of compost tea equipment also offer prepackaged fermentation nutrients; these typically contain molasses, soluble kelp, humic materials and lesser amounts of organic materials and minerals. While practitioners use a wide range of fermentation nutrients, including molasses, kelp, fish emulsion, rock dusts, and plant extracts (personal observation), it is not known what effect these nutrients have on disease suppression.

Reductions in disease levels have been attained by using fermentation nutrients in NCT production (Table 2.3), with added concentrations generally ranging up to 1%, but 3% sucrose has been used (Sackenheim, *et al* 1994). Malt (1%) was fermented in horse manure NCT to increase suppression of *P. infestans* (Ketterer, 1990). Urban and Tränkner (1993) report that fermenting with 5-7 g/l peptone or yeast extract inhibited *B. cinerea* up to 100% while starch and sucrose additions were less effective. However, Elad and Schtienberg (1994) found no significant increase in *B. cinerea* control with the addition of an unstated quantity of nutrient broth (Difco). One issue to using NCT fermented with added nutrients is that an offensive odor is often quite evident. We have observed that odor production is directly tied to nutrient addition, if no nutrient is added there is little offensive odor. A user friendly research focus for NCT production would be exploring nutrients that minimally increase offensive odors.

ACT production frequently uses fermentation nutrients (Table 2.1), with a number of recipes and commercial blends being used (Ingham, 2000b). Ingham (Ingham, 2000b) states that the final balance between bacteria and fungi in ACT can be predetermined by selecting appropriate compost and fermentation nutrients. However, after trying various recipes, we have been unable to produce ACT dominated by fungi. We have also encountered loss of suppression associated with the addition of nutrients. It is also possible that residual fermentation nutrients could stimulate pathogens that have an efficient saprophytic phase, thus negating

suppressive effects of the compost tea. Batch fermentations that are terminated at the maximum metabolic activity level likely leave unfermented nutrients available to all organisms. This could counteract nutrient competition-mediated biocontrol. Identification of nutrients that facilitate multiplication of antagonists while not supporting growth of animal or plant pathogens is needed.

Other fermentation factors that could affect disease suppression include fermentation temperature and pH. For NCT, fermentation temperature has been reported to be within 15-21C. No studies have manipulated the fermentation temperature to observe the effect on disease suppression, but temperature non-uniformly influences growth rate of microorganisms. It is also possible that matching the fermentation temperature to the targeted environment temperature could reduce the stress experienced by applied organisms.

The pH of ACT or NCT could impact the growth and diversity of organisms in compost tea, thereby influencing efficacy. In general, bacterial growth is favored by neutral pH while yeast and fungi are favored in alkaline and acid pH ranges (Schlegel, 1993). Yohalem *et al* (1994) reported that the pH of their NCT was consistently between pH 8.0 and 8.5 in an *in vitro* assay that assessed *V. inaequalis* conidia germination. Urban and Tränkner (1993) determined that the pH should be above 6 to optimize *in vitro* inhibition of *B. cinerea.* Nothing has been reported about the relationship between compost tea pH and field suppression. Once specific microbial antagonists are identified, it is possible that manipulation of pH during compost tea production could assist their enrichment and survival after application.

Application technology and timing. In considering how to most economically and conveniently use compost tea for disease suppression, it is important to know what types of application technology can be used, and whether tank mix fertilizers, dilution prior to spraying, storing before spraying or decreased spray frequencies are viable options. Virtually nothing is known about how conventional pesticide sprayers impact the delivery and viability of these complex microbial communities. It is possible that the mechanical action, rapid pressure changes, and sheer forces

associated with sprayers can selectively affect components of the applied community. Various types of application equipment need to be tested for detrimental effects while strategies for application methodology and timing need to be optimized.

Fertilizers are sometimes added to compost tea before spraying. It is not known if fertilizer tank mixes cause undue osmotic stress on the microbial fraction of compost tea. It is also possible that a portion of the chelated micronutrients intended for foliar absorption are sequestered by microorganisms between mixing and application.

There is minimal data on the potential to dilute compost tea before use. All field trials have used NCT in undiluted form, while two reports (Elad and Shtienberg, 1994; Yohalem *et al*, 1994) have examined dilution in greenhouse and *in vitro* studies. In a greenhouse study on pepper and tomato foliage, Elad and Shtienberg (1994) diluted various NCT's 5-fold and 25-fold. They found that changes in efficacy varied across compost sources. Yohalem *et al* (1994) determined in an *in vitro V. inaequalis* conidia germination assay that 10 and 100-fold dilutions of spent mushroom NCT maintained the inhibitory effect.

The possibility of storing NCT before use has been investigated. For suppression of conidia germination (*V. inaequalis*), NCT could be stored for up to 4 months at - 20 C with no loss of efficacy, while decreasing efficacy was observed with –4 C storage, and further loss at room temperature storage (Yohalem *et al*, 1994). Urban and Tränkner (1993) freeze dried NCT, then used this material in 4-hour fermentations to produce NCT suppressive to *B. cinerea*. Length of storage could differentially affect disease suppression depending on the mode of action for a particular compost tea. If the mode of action is mainly due to competition, it could be more susceptible to reduction in efficacy with storage than if the mode of action is due to stable metabolites secreted into the water.

The minimum frequency of applying compost tea for effective disease suppression has not been systematically examined. It will probably depend more on plant growth, pathogen reproduction rates and dispersal mechanism, and

environmental conditions than on characteristics of the compost tea. In a greenhouse study, Malathrakis *et al* (1995) attained significant control of *B. cinerea* on all tomato plant parts by weekly applications of several NCT treatments made from different manures. In field studies, disease suppression has relied on regular applications, either weekly on annuals (Tsror, 1999), or on 7-14 day intervals from 5-10 times per season during periods of high disease pressure in perennial crops (Ketterer 1990, Samerski 1989, Weltzien 1991). While spraying frequency has not been a variable in these studies, this intense frequency of application portrays a biological pesticide type of deployment, and does not indicate a sustained microbial shift on the phylloplane that is capable of self-regulating biological control. Timing applications with periods of low environmental stress, such as early morning, might help establish microbial epiphytes. The use of disease forecasting models that incorporate weather forecasts could also decrease the spray frequency by applying compost tea just before conditions become favorable for pathogen infection.

Perhaps the most promising application factors that can be modified for decreasing the spray frequency, and variability associated with suppression, are adding spray adjuvants and specific microbial antagonists. The use of surfactants, sticking agents, and UV inhibitors is a common practice in chemical pesticide formulation and application (Backman, 1978), but it has received scant attention in the biological control literature. The potential antagonistic efficacy of NCT can be increased with commercial spreader-sticker agents, as recommended by Brinton *et al* (1996). However, spray adjuvants can inhibit microbial activity and this could affect the targeted pathogen and/or antagonists (Brinton *et al*, 1996). We have observed more uniform distribution and adherence of ACT bacteria on leaf surfaces due to the addition of spreader-stickers (Scheuerell and Mahaffee, 2000a). The addition of methyl cellulose to NCT has already been discussed in relation to increasing suppression of grape vine powdery mildew (Sackenheim *et al*, 1994). However, under severe disease pressure, further reduction in apple scab severity by NCT was not attained by adding either Latron B1956 (0.06% v:v) spreader-sticker or fish oil

(0.025%) to NCT (Yohalem *et al*, 1996a). Tränkner and Brinton (1998) reported that field control of grape powdery mildew was reduced from 62% to 8% by adding 0.5% CASO bouillon and 0.05% rape seed oil prior to applying a horse manure NCT. Using microbial nutrient substrates to increase adhesion of compost tea organisms, and subsequent phylloplane growth, deserves more attention. By tank mixing 0.5% casein as a protein source for organisms in NCT, Ketterer and Schwager (1992) observed decreased levels of *E. polygoni* on bean and *P. infestans* on tomato equal to that obtained by applying the fungicides propineb or sulfur.

The general foliar biological control literature indicates that a number of studies have applied specific bacterial antagonists with nutrients to enhance leaf colonization and survival (Andrews, 1992). Most often, addition of foliar nutrients has resulted in a transient spike of the total culturable microbial epiphyte population without selecting for the applied antagonist. However, increased control of early leafspot (*Cercospora arachidicola*) of peanut was observed by applying chitinolytic *Bacillus cereus* with chitin and a sticking adjuvant. The population of both the applied organism and the total chitin degrading bacterial community were selectively increased and maintained above background levels (Kokalis-Burrelle *et al* 1992). It is also possible that the effect of adding microbial growth substrates on pathogen suppression depends on the life strategy of individual pathogens. Thus far, adding nutrient adjuvants to compost tea to increase the suppression of foliar pathogens has been limited to biotrophic pathogens. These biotrophs are not enhanced by added foliar nutrients. Other pathogens with significant saprophytic activity, or those requiring exogenous nutrients for germination, could be enhanced by nutrient addition. Since the addition of various adjuvants can have either beneficial or detrimental effects on the efficacy of compost teas, each type needs to be assessed for their effects on the compost tea community, as well as on the plant and pathogen.

The addition of specific antagonistic microorganisms to compost tea can potentially increase the disease suppressive properties. Cultured antagonists were combined with NCT immediately before spraying in field studies on *P. viticola* on

grapevine and *P. infestans* on potato (Ketterer 1990). In both cases, seven pure cultures of antagonists (4 fungi, 2 bacteria, 1 yeast) that had been isolated from NCT treated potato leaves, were increased, mixed, and then added 2% by volume to the NCT. Both trials achieved suppression equal to a fungicide control, although for *P. viticola*, 10 applications of the microbial amended NCT were comparable to 5 fungicide applications. It is possible that combining commercial biological control strains with compost tea could provide more consistent suppression than either component alone. This could be the case if the compost tea microflora assists the colonization and survival of the biocontrol strain through biofilm formation on plant surfaces.

FUTURE DEVELOPMENTS

There are a number of developments unrelated to plant disease control that will potentially impact how practitioners make and use compost tea. Two of the most pertinent issues are the development of compost tea standards and the potential for human pathogen growth during fermentation.

Compost tea standards. An increasing number of businesses are selling compost tea to gardeners and growers. However, it is difficult for buyers to be assured of the product contents or functions because there are no standards for determining the suitability of compost tea for a particular use. Thus far only one set of standards has been proposed (Table 2.6), this proposal addresses three main criteria, minimum oxygen content, passing *in vitro* pathogen inhibition assays, and minimal populations of organisms (Ingham, 2001b). Whether a minimum oxygen level needs to be set for ACT is not clear; it does not appear to be needed for NCT since they are produced without aeration and are highly efficacious against a wide range of plant diseases (Table 2.3).

Table 2.6. Proposed standards for assessment of compost tea quality. Reprinted from Ingham (2001b).

Proposed compost tea standard	Standard specifications
Oxygen Concentration during compost tea production	Remain above 5.5 ppm, or 60% dissolved oxygen (15% O2 as part of total gases) when at sea level and room temperature.
In vitro Pathogen Inhibition Assay - BBC Labs (www.bbclabs.com) The pathogens to be tested need to be specified based on foliar or soil applications. A report from BBC Labs indicating that at least 75% of the pathogens in the test group were inhibited.	Foliar pathogens are Alternaria, Botrytis, Colletotrichium, Drechslera, Erwinia and Verticillium. Soil pathogens are Armillariella or Sclerotinia, Fusarium Gaumannomyces or Sclerotium, Phytophthora, Pythium, Rhizoctonia.
Total and Active Bacteria; Total and Active Fungi - SFI (www.soilfoodweb.com) Data from Soil Foodweb Inc. indicating that the desired range of both bacteria and fungi were produced in the tea. Minimum levels of all necessary organisms, per ml, when 5 gallons compost tea to the acre is applied, are:	10 to 150 ug active bacteria 150 to 300 ug total bacteria 2 to 10 ug active fungi 5 to 20 ug total fungi 1000 flagellates* 1000 amoebae* 10 ciliates* 10 nematodes* *Protozoa and nematodes may not be critical in foliar applications

It is well known that *in vitro* inhibition often correlates poorly with field performance of foliar biological control agents (Andrews, 1985; Cook and Baker, 1983). Assessing germination of pathogenic spores by compost tea has limitations; *In vitro* inhibition of conidia germination of *V. inaequalis* did not correlate to apple scab control in the field (Andrews, 1993). The nutrient agars typically used in *in vitro* assays do not represent the distribution and abundance of leaf surface nutrients (Derridj, 1996). Antibiotic production observed in agar culture may not be expressed in various environments (Bonsall *et al*, 1997; Duffy and Defago, 1999). No laboratories performing *in vitro* inhibition assays have published their database on the relationship between *in vitro* inhibition and field performance of compost tea, therefore, it is difficult to independently assess the utility of these assays.

In relation to minimum microbial populations, it has been stated that microscopic examination of leaf surfaces treated with compost tea is a superior method of predicting foliar disease control (Ingham, 2000b and 2001a). It is stated that if leaf surfaces are covered by at least 60-70 percent active bacteria and 2-5% active fungi (determined by epifluorescent examination of leaf sections stained by fluorescein diacetate) no colonization of the plant surface by a plant pathogen can occur (Ingham, 2000b and 2001a).

Direct visualization of treated leaf surfaces will likely be an important research tool for understanding the spatial distribution of applied microorganisms and pathogens. However, assays that analyze spatial distribution of applied organisms do not account for the potential of metabolites, produced during fermentation, to affect pathogens or for the potential of induced resistance. Before minimum microbiology standards are set, it is necessary to have replicated field data from a diverse range of production systems and environments to assess how proposed standards correlate to field performance.

Potential for supporting human pathogen growth. Yohalem *et al* (1994) raised the concern that fermenting compost could potentially support the growth of enteric pathogens, evidenced by the Enterobacteriaceae cfu from NCT reported by Urban and Tränkner (1993). Enterobacteriaceae cfu were greater with the addition of 10g/l yeast extract at the start of the 24 hour fermentation (Urban and Tränkner, 1993). While not all Enterobacteriaceae are human pathogens, their populations are often used as indicators that human pathogens may be present (Murray, 1999). Welke (1999) tracked fecal coliform and *Salmonella* populations from the source compost, through NCT fermentation, to samples of broccoli and leek sprayed and grown under field conditions (Table 2.7). The data suggests that human pathogens can be transferred from naturally contaminated compost to food surfaces with NCT.

Table 2.7. Fecal coliform and Salmonella data from Welke (1999).

	Chicken manure compost		Cattle manure compost	
sample material	Fecal coliforms	Salmonella	Fecal coliforms	Salmonella
Compost	< 3 MPN/g	Not detected	930 MPN/g	Not detected
8 day Non-aerated compost tea, no fermentation nutrients added	0.8 cfu/ml	Not detected	35 cfu/ml	Not detected
Broccoli tissue	3 MPN/g	Not detected	<3 MPN/g	Not detected
Leek tissue	43 MPN/g	Not detected	<3 MPN/g	Not detected

Statements have been made that human pathogens generally grow better under anaerobic or reduced oxygen tension, and that pathogen re-growth will not occur in highly aerated (>5.5 ppm O_2) ACT systems in the presence of competing microflora (Ingham 2000b, 2001a). However, the majority of enteric human pathogens - particularly common gastrointestinal infecting genera such as *Escherichia, Salmonella, Shigella,* and *Yersinia* - are most commonly isolated and grown under aerated conditions (Murray, 1999). Additionally, we have preliminary evidence indicating a variety of enteric pathogens can increase during ACT and NCT production, if fermentation nutrients are used in conjunction with compost that has been inoculated with pathogens (Scheuerell, Mahaffee, Millner and Ingram, unpublished data). Pathogen growth does not appear to be supported when ACT or NCT is made without fermentation nutrients. Similar results have been observed with ACT made from compost that naturally contained a low level of *E. coli* (Bess *et al*, 2002). Their results suggest that naturally occurring *E. coli* can be reduced or eliminated by avoiding the addition of sugars during ACT production. Other work by Duffy (*et al*, 2002) examined the growth potential of *Salmonella enterica* and *E. coli* O157:H7 inoculated at 1 cfu/ml into flasks containing 20 g compost, 180 ml sterile water, and 0-1% molasses, then rotary shaken (100 rpm, 20 C) for three days. Pathogen growth was not observed in the absence of molasses. There was a positive correlation between the growth of pathogens and molasses concentration.

It appears that adding fermentation nutrients, especially those high in sugar, is a greater factor than oxygen concentration governing the growth of enteric pathogens

in compost tea. Detailed studies are needed to determine if recalcitrant, complex fermentation nutrients can be used that increase antagonists while not supporting the growth of pathogens. However, if enteric pathogens were to inadvertently grow in compost tea, the fate of these applied enterics needs to be determined, on plant surfaces under field conditions, in order to judge the potential risks of exposing consumers to pathogens (Suslow, 2002). Enteric pathogens would pose a potential health risk to spray applicators and appropriate personal protection equipment should be used. As a precautionary measure, compost used for compost tea production should be tested for the presence of human pathogens. The need to further assess the human health risk posed by the use of compost teas has been addressed in the final rules of the National Organic Program administered by the USDA Agricultural Marketing Service (NOP, 2002). While no specific recommendations regarding the production or use of compost tea are included in the final rule, previous recommendations by the Compost Task Force of the National Organic Standards Board stated that compost tea be made with compost that has met criteria for pathogen destruction (131 F for 3 days) or contain less than 3 MPN salmonella per 4 g dry wt compost and less than 1000 MPN fecal coliforms. In addition, the recommendation stated that readily available carbon sources such as sugars and molasses not be used as fermentation nutrients for compost tea production (NOSB, 2002).

CONCLUSIONS

There is a pressing need to find sustainable approaches to managing plant diseases for both conventional and organic producers. Use of compost teas is being pursued as such an approach. Numerous reports from both practitioners and the scientific community reveal disease suppression using compost teas. For some plant diseases the level of control is considered inadequate for conventional agriculture (Yohalem *et al*, 1996); however, organic producers with limited control options consider partial disease control to be an important improvement. Since most of the recent observations on beneficial effects are from growers, following their

observations with replicated field trials will illuminate the extent that compost teas will provide a reliable disease management tool in the future.

We speculate that stabilizing suppressive activity will require identification of the active microbes, modification of compost tea production steps to ensure their presence, and the use of application technology including spray adjuvants to optimize delivery and survival of the desired organisms. Understanding modes of antagonistic activity could help combine compost tea with other biological and chemical agents in integrated control programs. In particular, fungicide resistance management programs could benefit from observations that NCT made from both cattle and horse manure composts suppressed fungicide resistant strains of *B. cinerea* (Stindt, 1990).

Ultimately, compost tea is only one tool, and must be used within a system that incorporates plant resistance, optimal nutrition, sanitation, disease forecasting and minimizes plant stress. As recently recognized by the USDA Cooperative State Research, Education, and Extension Service, "Research on agricultural production components such as biocontrol and cropping systems has been of limited value to organic farmers, since the components are generally not developed and tested in an organic agro-ecosystem, and research results and recommendations thus can not be applied directly to organic farms" (Federal Register, 2001). Thus, the use of compost tea as part of an integrated plant health management strategy will require much additional whole systems research by a cohesive team of farmers and experts in composting, plant pathology, phyllosphere biology, molecular microbial ecology, fermentation science, plant physiology, plant breeding, soil science and horticulture. The first step is having all interested groups review the range of reports and published research on compost tea use in order to facilitate an informed discussion to prioritize future collaboration.

ACKNOWLEDGEMENTS

We thank Caroline Press and Alexandra Stone for critical review of the manuscript. Funding from USDA CRIS # 303-5358-22000-024-00D and the Oregon association of Nurserymen and Oregon Department of Agriculture. Special

acknowledgement must be given to the trillions of unpaid microbes that made this work possible.

Chapter Three: Variability Associated with Suppression of Gray Mold (*Botrytis cinerea*) on Geranium (*Pelargonium* x *hortorum*) by Non-aerated and Aerated Compost Teas

ABSTRACT

Compost tea can significantly reduce leaf infection severity on geranium caused by *B. cinerea* under environmental conditions that favor disease development. Although, the majority of compost teas did not significantly suppress *B. cinerea* infection of geranium. For non-aerated compost tea, compost source and increasing fermentation time had the most consistent significant effects ($P < 0.05$), applying surface liquid had a consistent but not significantly ($P < 0.1$) effect, while stirring and adding fermentation nutrients had little effect. Aerating compost tea did not significantly ($P < 0.05$) increase disease suppression compared to NCT. Adding fermentation nutrients to ACT did not significantly ($P < 0.05$) increase disease suppression. Tank mixing spray adjuvants with ACT significantly ($P < 0.05$) increased disease suppression.

INTRODUCTION

Gray mold disease, caused by *Botrytis cinerea* Pers.:Fr., affects a wide range of ornamental and edible crops. Commercial control of the disease has been principally attained by fungicide applications and to a lesser extent through environmental modification. Due to increased frequency of fungicide resistant strains (Yourman and Jeffers, 1999; Johnson *et al*, 1994), potential regulatory loss of fungicides, and reduced worker productivity during the re-entry interval after application, alternative disease control measures are needed.

One alternative approach has been to apply non-aerated compost teas (NCT) onto plants, originally referred to as compost extracts (Weltzien, 1989) or watery fermented compost extracts (Weltzien, 1991). NCT has significantly suppressed gray mold on bean leaves (McQuilken et al 1994; Urban and Tränkner, 1993), grape leaves and berries (Elad and Shtienberg 1994; Ketterer et al 1992), lettuce (McQuilken et al 1994), strawberry fruit (Stindt, 1990; Urban and Tränkner, 1993), and tomato and

pepper foliage (Elad and Shtienberg 1994). NCT is typically produced by combining compost, water and optional fermentation nutrients in an open vessel, then fermented at room temperature without stirring for a defined length of time (Weltzien, 1991).

NCT production factors including compost source, fermentation nutrients, fermentation duration, and spray adjuvants have been modified in various combinations to optimize plant disease suppression (Scheuerell and Mahaffee, 2002, Chapter 5). Early reports on the role of compost source indicated that manure-based composts were most efficacious (Weltzien, 1991). However, Elad and Shtienberg (1994) determined that grape marc compost was equally effective as manure-based compost to make NCT that inhibited *B. cinerea* on foliage in greenhouse assays.

Using fermentation nutrients in NCT production has had variable effects on *B. cinerea* suppression. Urban and Tränkner (1993) report that fermenting with 5-7 g/l peptone or yeast extract inhibited *B. cinerea* up to 100%, while starch and sucrose additions were less effective. In contrast, Elad and Schtienberg (1994) found no significant increase in *B. cinerea* control with the addition of an unstated quantity of nutrient broth (Difco).

Various optimal NCT fermentation periods for *B. cinerea* suppression have been reported. Ketterer *et al* (1992) reported that fermenting for seven days was optimal across three compost sources for suppressing *B. cinerea* on detached grape leaves. However, Elad and Shtienberg (1994) observed that fermenting for 14 days was consistently more suppressive than seven days across three compost sources when applied to detached grape berries, pepper leaves, and tomato leaves.

Spray adjuvants added to NCT have significantly increased *B. cinerea* suppression. Casein (0.5% v/v) and pine oil (0.05% v/v) added to NCT significantly increased suppression of grape berry infection when applied over the growing season (Ketterer *et al*, 1992).

More recently, variations on compost tea production have been introduced that could further modify the potential for compost tea to suppress *B. cinerea.* One modification is to stir NCT every 2-3 days over the fermentation period to possibly

facilitate the release of microbes from compost particles (Brinton *et al*, 1996). However, the effect of stirring NCT on plant disease suppression has not been assessed. Another untested variable is the possibility that NCT removed from different depths of a open fermentation vessel could have differential disease suppression activity due to the aerobic, micro-aerobic, and anaerobic gradients of oxygen found in open fermentation vessels (Johnson,1999).

Another recently introduced compost tea production factor is actively aerating compost tea during production. Aerated compost tea (ACT) production has become widely practiced in certain areas, with a parallel increase in anecdotal reports of disease suppression (Riggle, 1996; Ingham, 2000). For ACT, it has been proposed that increasing microbial populations through the addition of fermentation nutrients will generally increase plant disease suppression (Ingham, 2000). A limited number of studies have examined the potential for ACT to suppress plant disease, none have focused on *B. cinerea* (Scheuerell and Mahaffee, 2002, chapter 5)

In order for compost tea to be adopted by growers as an alternative control for *B. cinerea*, a production protocol needs to be developed that consistently produces suppressive compost tea. The problem to date is that each report has determined a unique optimal combination of compost tea production factors suited to the plant and environment under study. Using the relatively new compost tea production factors to enhance or replace the factors known to affect suppression could help achieve a production methodology that is not specific to the crop or environment. Once a production methodology that consistently suppresses gray mold under defined conditions is developed, then broader testing would be warranted. The main objective of this study was to determine the compost tea production parameters (compost source, fermentation nutrients, fermentation time, stirring during production, and depth of liquid in the fermentation vessel) that were important for suppression of gray mold (*B. cinerea)* on geranium (*Pelargonium* x *hortorum*) foliage. Due to variability of suppression using NCT, three additional objectives were added. 1) Determine if ACT is more effective than NCT when produced from the same compost source and

fermentation nutrients; 2) Examine the impact of amending ACT with fermentation nutrients on disease suppression; and 3) Determine if spray adjuvants could be used to enhance disease suppression.

MATERIALS AND METHODS

Compost sample collection. Compost samples (N=29) were collected at commercial composting facilities in Western Oregon from stockpiled material considered ready for sale by the facility operator. These compost samples included the major types of commercially available compost in Western Oregon and Washington states. The samples encompass a wide range of compost age, pH, EC, moisture content, bacterial and fungal populations (Table 3.1). Compost feedstock ratios were provided by the facility operator and used to categorize each compost into compost types (Table 3.1). A composite sample was removed from four cores dug 1.0 m deep and placed in a 19 L bucket or 45 L plastic bin. During the study compost samples were stored in a covered building at ambient temperature.

Table 3.1. Charateristics of compost sources used to make compost tea.

ID	Compost Class	Compost Feedstocks	Sample date	Age (days)	pH[a]	EC[b]	Moisture [c]	Bacteria[d] Cfu/ml (1×10^6)	Fungi[e] Cfu/ml (1×10^3)
1	Yard Trimmings	ground landscape plants	12/16/1998	108	6.58	0.5	59	1560	585
2	Yard Trimmings	ground landscape plants	4/19/1999	40	6.98	0.78	66	282	100
3	Yard Trimmings	ground landscape plants	10/21/1998	85	6.86	1.07	46	106	56
4	Yard Trimmings	ground landscape plants	12/16/1998	105	6.75	0.52	60	1000	550
5	Yard Trimmings	ground landscape plants	2/16/1999	110	7.49	0.52	64	91	nd
6	Yard Trimmings	ground landscape plants	4/19/1999	68	7.78	0.37	62	74	21
7	Yard Trimmings	ground landscape plants	5/25/1999	280	7.22	0.37	62	118	237
8	Yard Trimmings	ground landscape plants+chicken manure (10:1 v/v)	12/16/1998	137	7.12	0.93	49	3820	275
9	Chicken	sawdust+rice hulls+chicken manure (2::1:1 v/v)	12/16/1998	105	8.34	2.42	61	1540	35900
10	Chicken	sawdust+rice hulls+chicken manure (2::1:1 v/v)	2/16/1999	165	7.61	2.01	64	147	61100
11	Chicken	sawdust+rice hulls+chicken manure (2::1:1 v/v)	2/16/1999	105	7.53	1.92	64	89	111
12	Chicken	sawdust+rice hulls+chicken manure (2::1:1 v/v)	4/19/1999	170	8.1	3.75	61	6	1
13	Chicken	sawdust+rice hulls+chicken manure (2::1:1 v/v)	5/26/1999	200	6.39	4.06	58	38	95
14	Chicken	sawdust+rice hulls+chicken manure (2::1:1 v/v)	3/22/2000	165	6.19	3.69	68	625	11300
15	Chicken	sawdust+yard trimmings+chicken manure (1:1:1)	10/21/1998	70	8.15	3.69	59	2	nd
16	Chicken	sawdust+yard trimmings+chicken manure (1:1:1)	12/16/1998	67	7.25	1.84	61	795	1490
17	Dairy	straw, dairy manure, eggshells (20:10:1 v/v)	10/20/1998	95	7.85	4.15	63	1460	92
18	Dairy	dairy manure, bedding, fish waste (2:2:1 v/v)	4/23/1999	65	8.84	10.05	69	903	nd
19	CMC	straw, sawdust, pome fruit, manure, clay soil (2:2:2:2:1 v/v)	2/16/1999	160	7.1	1.42	31	26	6
20	CMC	straw, sawdust, pome fruit, manure, clay soil (2:2:2:2:1 v/v)	5/27/1999	65	8.1	0.53	48	3	nd
21	Micronized	ground plants, hay, horse and cow manure, lime (10:5:5:5:1)	8/3/1999	120	7.91	5.95	8	1	2
22	Mint Blend	bark, sawdust, mixed manure, mint (2:2:2:1 v/v)	12/16/1998	74	7.94	1.86	53	1190	5960
23	Steer blend	Proprietary blend, manures and bulking agents (1:1 v/v)	10/21/1998	390	7.43	2.47	48	454	1080

Table 3.1 (Continued).

ID	Compost Class	Compost Feedstocks	Sample date	Age (days)	pH[a]	EC[b]	Moisture[c]	Bacteria[d] Cfu/ml (1×10^6)	Fungi[e] Cfu/ml (1×10^3)
24	Steer blend	Proprietary blend, manures and bulking agents (1:1 v/v)	12/16/1998	90	6.14	2.64	38	345	16100
25	Blend	Proprietary blend of manure and vegetative composts	5/15/2001	120	6.9	3.52	51	135	5
26	Bark	mixed bark+landscape plants+biosolids (proprietary ratio)	8/20/1999	180	5.15	0.51	69	84	452
27	Bark	Douglas Fir bark+gravity belt separated dairy solids (3:1 v/v)	8/13/1999	75	5.35	0.1	64	167	5830
28	Vermicompost	cow manure+food waste+paper (1:1:1 v/v)	3/20/2000	90	6.43	5.56	78	155	1270
29	Vermicompost	vegetative food waste, paper (1:1 v/v)	8/28/2001	120	6.03	4.7	42	1	43
30	Vermicompost	straw, dairy manure, eggshells (20:10:1 v/v)	10/20/1998	160	7.2	3.75	67	1150	2360

[a] Compost pH was determined from a saturated paste using a portable pH meter (model 150, IQ Scientific Instruments)

[b] Electrical conductivity (EC) was determined from compost and de-ionized water (2:1 v/v) with a portable EC meter (model 933100, Hanna Instruments).

[c] Determined from weight loss of compost (50 g) dried for 36 hours at 70 C in a forced air oven.

[d] Bacteria enumerated on 5% trypticase soy broth agar (1.5 g Difco tryticase soy broth with 15 g agar) with 100 ug/ml cycloheximide. Populations reported as Cfu/dry g compost.

[e] Fungi enumerated on water agar pH 6 (18 g agar/L) with 50 ug/ml rifampicin. Populations reported as Cfu/dry g compost.

Botrytis cinerea assay. *B. cinerea* inoculum was produced by individually culturing four isolates of *B. cinerea* for two weeks on potato dextrose agar plates. Plates were flooded with sterile distilled water and gently rubbed with a sterile, bent glass rod to remove conidia. The flooded plates were poured through four layers of cheesecloth and the isolates bulked. Conidia/ml were determined with a hemacytometer, then diluted with sterile distilled water to 1×10^5 conidia/ml and applied immediately.

Compost tea treatments were assessed using an assay developed for screening biological control agents on Geranium (*Pelargonium* x *hortorum*) (Mahaffee and Di Leone, 1998). Six to eight-week-old greenhouse grown geranium seedlings ('Ringo Red 2000') with five to seven fully expanded leaves were used. Cotyledons and all non-expanded leaves were removed before treatment. Compost tea was applied to all leaves until run-off with a hand pump sprayer. Dried, ground geranium leaf powder was uniformly dusted onto abaxial leaf surfaces to increase the potential for *B. cinerea* infection. Treated plants were placed in a growth chamber for 24 hours (22 C, 16 hour photoperiod) then inoculated with *B. cinerea* conidia by misting the abaxial leaf surfaces using a hand pump sprayer. Plants were then incubated at 22 C with > 85% humidity for 5-7 days in a growth chamber (16 hour photoperiod). Disease severity was rated for each leaf, either using a four-point scale or determining the percent diseased leaf area. The four-point scale was assigned as follows. Zero points for no lesions; one point for discreet lesions occupying less than one third of the leaf area; two points for spreading lesions occupying one third to two thirds of the leaf area; three points for greater than two thirds of the leaf area covered in lesions. Plant disease severity was determined by averaging the leaf ratings of each plant.

Compost tea production. NCT was produced with 5:1 water: compost ratio (v/v) in a two L round, plastic container (1500 ml water) or in 19 L plastic buckets (10 L water). Where indicated, a limited number of NCT were produced with 15 L water and 500 g compost (wet weight, approximately 25:1, v/v) in a 19 L plastic bucket. Tap water was placed in a container and allowed to sit for 24 hours for passive reduction of chlorine in the water. Optional fermentation nutrients were added (Table 3.2), followed by addition of compost to the water. The entire contents were vigorously stirred for 20 seconds, then left undisturbed for 7 or 14 days. For application, NCT from each container was poured through eight layers of cheesecloth to prevent spray nozzle clogging.

NCT production variations. To examine the effect of stirring NCT, specified containers were stirred for 20 seconds with sterile glass rods on three and five days after the initial mixing and applied on the seventh day.

Table 3.2. Fermentation nutrient recipes used to make compost tea.

Nutrient recipe	Fermentation nutrients per liter water
No nutrient	none
Molasses	0.3% v/v (Aunt Pattie's Blackstrap; Glorybee Foods, Eugene, OR)
Yeast	hydrolyzed powder 0.3% w/v (Basic Yeast; Red Star Yeast Co., Milwaukie, WI)
Bacterial	0.5% v/v Bacterial Nutrient Solution (Soil Soup, Inc., Edmonds, WA)
Fungal[a]	1.2 g powdered soluble kelp (Maxicrop) 2.5 ml liquid humic acids (Humax) 3 g rock dust (Target Glacial Dust; Target Products Ltd., Burnaby, B.C.)

[a] Adapted from Ingham and Alms (1999).

ACT was produced with two types of commercial units designed for this purpose. Compost Tea Brewers (Growing Solutions, Inc., Eugene, OR) were filled with 50 L tap water and run for 2 hours for chlorine reduction. If used, fermentation

nutrients were added (Table 3.2). Compost (4.5 L, contained in a mesh basket) was hung into the water. After 34-36 hours filtered compost tea was removed through a valve for application.

ACT was also produced with a Bio-blender™ (Soil Soup, Inc., Edmonds, WA). 15-L tap water was placed in a 19-L bucket and aerated for 2 hours for chlorine reduction. Optional fermentation nutrients were added (Table 3.2). Compost (500 g; approximately 25:1 water:compost v/v) was immersed into the water in a 100 *u*m mesh filter bag (Soil Soup, Inc). To assist the removal of soluble material and microorganisms from the compost, the filter bag was lifted above the water and allowed to drain into the bucket for 15 seconds, then re-immersed for 30 seconds. This was done a total of 3 times, with the filter bag left in the liquid for the remainder of the 34-36-hour production cycle.

Bacterial populations in aerated compost tea were enumerated to monitor population changes associated with the addition of fermentation nutrients. A 1-ml sample of compost tea was aseptically removed from each fermentation vessel at the end of the fermentation period. After serial dilution in sterile 0.02M potassium phosphate buffer (pH 7.0), 50 *u*L was plated using an automated spiral plater (Eddy Jet; IUL Instruments, Barcelona, Spain) on 5% trypticase soy broth agar (1.5 g Difco tryticase soy broth with 15 g agar and 100 ug/ml cycloheximide). Plates were incubated at 22 C for 3 days before enumeration, results recorded as cfu/ml.

Adding spray adjuvants to aerated compost tea. Because ACT was rapid to produce, it was used as the experimental model for adding spray Adjuvants. The following adjuvants were added to specified compost tea treatments prior to application, Gum Karaya (0.05% w/v, SIGMA Chemical Co., St. Lious, MO); Thermx-70 Yuccah Extract (0.05% v/v, Cellu-con, Inc., Strathmore, CA); Nu Film 17 Spreader/Sticker (0.1% v/v, Miller Chemical & Fertilizer Company, Hanover, PA); Latron B1956 Spreader/Sticker (0.1% v/v Rohm and Haas Company, Philadelphia, PA). All other compost tea treatments had Tween 80 (0.001% v/v, Fischer Scientific, Fairhaven, NJ) added as a surfactant.

Experimental design and statistical analysis. Each experiment had two control treatments, one treated with water and one inoculated with *B. cinerea* conidia that was suspended in water. All experiments were randomized complete blocks with six to eight replications (individual plants). The individual leaf ratings were average for the entire plant and this average used for statistical analysis. Two-way analysis of variance was performed with pre-planned linear contrasts of treatment means separated by Fischer's protected least significant difference using proc mixed in SAS version 8.0 (SAS Institute Inc., Cary, North Carolina). The bacterial populations in specified categories of compost tea were compared using non-paired t-tests after $\log_{10}$ transformation to correct for non-normal variance between compost tea categories. Chi square analysis was used to compare the frequency of NCT batches that significantly suppressed disease made from different compost sources.

RESULTS

NCT production factors that influenced disease suppression. Compost source influenced the frequency of disease suppression. Twenty-seven compost samples were used to make 104 NCT batches with a seven-day fermentation period and no added fermentation nutrients (Table 3.3).

Table 3.3. Effect of compost type on suppression of *B. cinerea* by non-aerated compost-teas.

Compost type	Number of compost samples in each type	Total number of NCT batches	Percent of batches that significantly[a] suppressed disease
Yard trimmings	8	30	37
Chicken	7	35	46
Dairy	2	10	20
CMC	2	3	33
Micronized	1	2	0
Mint blend	1	5	20
Steer blend	2	7	14
Bark	2	5	0
Vermicompost	2	7	0
SUMS	27	104	31

[a] Significantly different ($p<0.05$) than the botryits control according single degree of freedom linear contrasts.

NCT produced from two compost samples (ID 4 and 9, Table 3.1) significantly suppressed disease ($P < 0.05$) greater than 50% of the time in repeated geraniums assays (Table 3.4). NCT made with these two compost samples and subsequent samples from window rows produced using the same feed stocks by the same facility (ID 10-13 and 4-7) significantly suppressed disease ($P < 0.05$) in 24 of 49 batches (Table 3.4).

Table 3.4. Variability of *B. cinerea* control. Non-aerated compost teas made with compost from sequential windrows produced at the same composting facility.

Chicken Manure Compost		Yard Trimmings Compost	
Sample[b] ID#	Percent Suppressive	Sample[b] ID#	Percent Suppressive
9	56 (9)[a]	3	0 (3)[a]
10	40 (5)	4	67 (9)
11	40 (10)	5	33 (6)
12	100 (2)	6	50 (2)
13	50 (2)	7	100 (1)
Average	50 (28)	Average	48 (21)

[a] Total number of NCT batches in parenthesis for each compost sample.
[b] Compost samples characterized in Table 3.1.

Only eight of the 55 NCT batches produced with the remaining 17 compost samples significantly suppressed disease ($P < 0.05$). Chi square analysis indicated that NCT made from the yard trimmings and chicken manure composts in Table 3.4 had a significantly greater frequency ($P < 0.001$) of disease suppression than NCT made with all other compost sources.

Fermentation time influenced disease suppression by NCT applications. Increasing the fermentation time from seven to 14 days significantly ($P <0.1$) decreased disease in 29% of the direct comparisons, while disease suppression with the seven day fermentations was never significantly greater ($P <0.1$) than the 14 day fermentation (Table 3.5). Additionally, 76% of the comparisons had significantly ($P<0.05$) less disease severity with the 14-day fermentation.

Table 3.5. Effect of fermentation time on disease severity on geranium.

ID#	Compost Type	Disease Severity[a] 7 day	14 day	Linear Contrast 7 day vs. 14 day p-value
9	Chicken	1.42	1.59	0.2596
9	Chicken	0.83*	1.12*	0.1969
9	Chicken	1.23	0.88*	0.0593
4	Yard trimmings	0.87*	0.99	0.8596
4	Yard trimmings	1.43*	1.41*	0.8837
4	Yard trimmings	1.40*	1.24*	0.4281
5	Yard trimmings	1.29	1.23	0.4088
5	Yard trimmings	1.19	1.17	0.9189
5	Yard trimmings	1.69*	1.52*	0.3751
10	Chicken	0.95	0.93	0.9413
10	Chicken	1.58*	1.24*	0.0039
10	Chicken	1.15	1.07*	0.6510
10	Chicken	1.76	1.32*	0.0284
11	Chicken	0.71	0.88	0.4570
11	Chicken	2.00	1.68	0.0762
11	Chicken	1.02*	1.20	0.3211
11	Chicken	1.89	1.41*	0.0155

[a] Compost samples characterized in Table 3.1

[b] Plant disease severity was determined by averaging the leaf disease severity ratings. Leaf disease severity was rated using a 0-3 point scale, with zero points for no lesions; one point for discreet lesions occupying less than one third of the leaf area; two points for spreading lesions occupying one third to two thirds of the leaf area; three points for greater than two thirds of the leaf area covered in lesions.

* Indicates significantly less disease severity than the water control treatment for that experiment (P <0.05) separated by Fischer's protected LSD.

NCT production factors that did not influence disease suppression.

Although significant differences ($P < 0.05$) in disease severity were not detected between NCT removed at different depths, for all NCT produced with compost that contained manure (ID 13, 18, 20, Table 3.1), NCT removed from the surface of the fermentation vessel had numerically less disease severity compared to NCT removed from 15 cm deep (Table 3.6). In these experiments, only NCT removed from the surface significantly reduced disease ($P < 0.05$) compared to the water control treatment (Table 3.6).

Table 3.6. NCT[a] removed from the surface of the open fermentation vessel and NCT removed from 15 cm beneath the surface. Significance of linear contrasts for disease severity.

ID#	Compost Type	Disease Severity[b]		Linear Contrast P value
		Surface Liquid	Liquid from 15 cm depth	
18	Dairy	1.27*	1.40	0.4615
7	Yard trimmings	1.71	1.57	0.4252
7	Yard trimmings	1.71	1.42	0.1211
13	Chicken	1.37*	1.67	0.1102
13	Chicken	1.38*	1.51	0.4687
20	CMC	1.38*	1.52	0.4252
20	CMC	1.31*	1.55	0.1903

[a] Compost and water (5:1 v/v) fermented with molasses (0.3%, v/v) for seven days.

[b] Plant disease severity was determined by averaging the leaf disease severity ratings. Leaf disease severity was rated using a 0-3 point scale, with zero points for no lesions; one point for discreet lesions occupying less than one third of the leaf area; two points for spreading lesions occupying one third to two thirds of the leaf area; three points for greater than two thirds of the leaf area covered in lesions.

0.□ Indicates significantly less than the water control treatment for that experiment (P <0.05) separated by Fischer's protected LSD.

Adding fermentation nutrients to NCT, or stirring twice during production, generally did not influence disease suppression. No significant difference ($P < 0.05$) in disease severity was detected in more than 90% of the linear contrasts between NCT produced with and without added fermentation nutrients (Table 3.7) or between stirred and not-stirred NCT (Table 3.8).

Table 3.7. Effect of amending non-aerated compost teas with various nutrients. Suppression of *B. cinerea* on geranium.

ID# [a]	Compost Type	Linear Contrast	Disease Severity (0-3 point scale[b])	p-value
6	Yard trimmings	No nutrient vs. molasses	0.7* vs. 0.93	0.3370
6	Yard trimmings	No nutrient vs. molasses	1.90 vs. 1.47	0.0548
12	Chicken	No nutrient vs. molasses	0.66* vs. 1.03	0.1118
12	Chicken	No nutrient vs. molasses	1.6* vs. 1.97	0.0957
26	Bark	No nutrient vs. molasses	1.46 vs. 1.76	0.1267
26	Bark	No nutrient vs. molasses	1.38 vs. 2.15	0.0028
21	Micronized	No nutrient vs. molasses	1.68 vs. 1.63	0.7797
21	Micronized	No nutrient vs. molasses	2.17 vs. 1.84	0.2074
28	Vermicompost	No nutrient vs. molasses	2.63 vs. 2.60	0.9247
28	Vermicompost	No nutrient vs. molasses	1.78 vs. 1.98	0.3592
28	Vermicompost	No nutrient vs. molasses	1.75 vs. 1.17*	0.0145
26	Bark	No nutrient vs. yeast	1.46 vs. 1.35	0.5761
26	Bark	No nutrient vs. yeast	1.38 vs. 2.13	0.0039
26	Bark	No nutrient vs. yeast	2.28 vs. 2.29	0.9458
21	Micronized	No nutrient vs. yeast	1.68 vs. 1.93	0.2103
21	Micronized	No nutrient vs. yeast	2.17 vs. 2.13	0.8686
28	Vermicompost	No nutrient vs. yeast	2.63 vs. 2.96	0.2101
28	Vermicompost	No nutrient vs. yeast	1.78 vs. 1.88	0.6591
28	Vermicompost	No nutrient vs. yeast	1.75 vs. 1.63	0.6276
			Disease Severity (percent scale)	
25	Blend	No nutrient vs. Bacterial Solution	30.0* vs. 35.2	0.4100
25	Blend	No nutrient vs. Bacterial Solution	36.1 vs. 38.1	0.7800
7	Yard trimmings	No nutrient vs. Bacterial Solution	57.8 vs. 63.8	0.4100
7	Yard trimmings	No nutrient vs. Bacterial Solution	49.0 vs. 45.5	0.7800
29	Vermicompost	No nutrient vs. Bacterial Solution	35.9* vs. 46.5	0.2100
29	Vermicompost	No nutrient vs. Bacterial Solution	50.0 vs. 44.6	0.5000
25	Blend	No nutrient vs. Fungal Nutrients	30.0* vs. 28.6*	0.8300
25	Blend	No nutrient vs. Fungal Nutrients	36.1 vs. 31.6	0.5400
7	Yard trimmings	No nutrient vs. Fungal Nutrients	57.8 vs. 57.8	0.6300
7	Yard trimmings	No nutrient vs. Fungal Nutrients	49.0 vs. 36.6	0.3400
29	Vermicompost	No nutrient vs. Fungal Nutrients	35.9* vs. 41.6	0.4900
29	Vermicompost	No nutrient vs. Fungal Nutrients	50.0 vs. 31.3*	0.0230

[a] Compost samples characterized in Table 3.1.

[b] Plant disease severity was determined by averaging the leaf disease severity ratings. Leaf disease severity was rated using a 0-3 point scale, with zero points for no lesions; one point for discreet lesions occupying less than one third of the leaf area; two points for spreading lesions occupying one third to two thirds of the leaf area; three points for greater than two thirds of the leaf area covered in lesions.

* Indicates significantly less than the water control treatment for that experiment ($P < 0.05$) separated by Fischer's protected LSD.

Table 3.8. Effect of stirring non-aerated compost teas[a] on suppression of *B.cinerea* on geranium.

ID#	Compost type	Fermentation Nutrient	Disease Severity[b]		Linear Contrast P value
			Not-stirred	Stirred[c]	
11	Chicken	None	0.29*	0.79	0.0053
11	Chicken	None	1.18	1.32	0.2258
6	Yard trimmings	None	0.70*	0.68*	0.9147
6	Yard trimmings	None	1.90	1.72	0.4036
12	Chicken	None	0.66*	0.82	0.4873
12	Chicken	None	1.60*	1.98	0.0892
6	Yard trimmings	Molasses (0.3% v/v)	0.93	0.54*	0.1024
6	Yard trimmings	Molasses (0.3% v/v)	1.47*	1.75	0.2221
12	Chicken	Molasses (0.3% v/v)	1.03	0.63*	0.0903
12	Chicken	Molasses (0.3% v/v)	1.97	1.83	0.5346
18	Dairy	Molasses (0.3% v/v)	1.25	1.03	0.2319
18	Dairy	Molasses (0.3% v/v)	1.27*	1.21*	0.7586
7	Yard trimmings	Molasses (0.3% v/v)	1.85*	1.89*	0.8268
7	Yard trimmings	Molasses (0.3% v/v)	1.29	1.18	0.6578
7	Yard trimmings	Molasses (0.3% v/v)	1.71	1.53	0.3271
20	CMC	Molasses (0.3% v/v)	1.30	1.11	0.5392
20	CMC	Molasses (0.3% v/v)	1.83*	2.05*	0.3808
20	CMC	Molasses (0.3% v/v)	1.38*	1.49	0.5392

[a] Compost and water (5:1 v/v) fermented for seven days.

[b] Plant disease severity was determined by averaging the leaf disease severity ratings. Leaf disease severity was rated using a 0-3 point scale, with zero points for no lesions; one point for discreet lesions occupying less than one third of the leaf area; two points for spreading lesions occupying one third to two thirds of the leaf area; three points for greater than two thirds of the leaf area covered in lesions.

[c] Stirred for 20 seconds on days three and five.

* indicates significantly less than the water control treatment for that experiment ($P < 0.05$) separated by Fischer's protected LSD.

Impact of aerating compost tea on disease suppression. There was no significant difference ($P > 0.05$) in disease severity for 85% of the linear contrasts comparing ACT and NCT that were produced with a range of compost sources and fermentation nutrients (Table 3.3). When significant differences ($P < 0.05$) were detected, 75% indicated that NCT had less disease than ACT.

Table 3.9. NCT[a] and ACT[b] produced with or without added fermentation nutrients. Significance of linear contrasts for disease severity.

ID#	Compost Type	Fermentation Nutrients[c]	Linear Contrast	Disease Severity[d] (Percent Scale)	p-value
25	Blend	None	NCT vs. ACT	30.0* vs. 30.8*	0.8900
25	Blend	None	NCT vs. ACT	36.1 vs. 50.6	0.0550
7	Yard trimmings	None	NCT vs. ACT	57.8 vs. 51.8	0.4000
7	Yard trimmings	None	NCT vs. ACT	49.0 vs. 33.9	0.2500
29	Vermicompost	None	NCT vs. ACT	35.9* vs. 53.4	0.0380
29	Vermicompost	None	NCT vs. ACT	50.0 vs. 40.3	0.2300
25	Blend	Bacterial	NCT vs. ACT	35.2 vs. 28.1*	0.2600
25	Blend	Bacterial	NCT vs. ACT	38.1 vs. 44.6	0.3800
7	Yard trimmings	Bacterial	NCT vs. ACT	63.8 vs. 44.9*	0.0120
7	Yard trimmings	Bacterial	NCT vs. ACT	45.5 vs. 36.8	0.5100
29	Vermicompost	Bacterial	NCT vs. ACT	46.5 vs. 43.9	0.7500
29	Vermicompost	Bacterial	NCT vs. ACT	44.6 vs. 43.5	0.9000
25	Blend	Fungal	NCT vs. ACT	28.6* vs. 19.7*	0.1600
25	Blend	Fungal	NCT vs. ACT	31.6 vs. 32.1	0.9530
7	Yard trimmings	Fungal	NCT vs. ACT	57.8 vs. 55.9	0.8200
7	Yard trimmings	Fungal	NCT vs. ACT	36.6 vs. 32.1	0.7300
29	Vermicompost	Fungal	NCT vs. ACT	41.6 vs. 40.5*	0.8900
29	Vermicompost	Fungal	NCT vs. ACT	31.3* vs. 27.3*	0.6200
				Disease Severity (0-3 scale)[e]	
7	Yard trimmings	Molasses	NCT vs. ACT	0.96* vs. 1.46	0.0110
7	Yard trimmings	Molasses	NCT vs. ACT	2.20 vs. 2.35	0.5500
7	Yard trimmings	Molasses	NCT vs. ACT	1.69 vs. 1.95	0.3380
13	Chicken	Molasses	NCT vs. ACT	1.17 vs. 1.94	0.0002
13	Chicken	Molasses	NCT vs. ACT	2.23 vs. 2.17	0.7900
13	Chicken	Molasses	NCT vs. ACT	1.73 vs. 1.95	0.4100
20	CMC	Molasses	NCT vs. ACT	1.08* vs. 1.35	0.1680
20	CMC	Molasses	NCT vs. ACT	2.60 vs. 2.42	0.4680
20	CMC	Molasses	NCT vs. ACT	1.47 vs. 1.90	0.1200

[a] NCT fermented for seven days in an open 19 L bucket with 2 L compost, 10 L water and the listed fermentation nutrient.

[b] ACT made with none, bacterial, or fungal nutrients aerated for 36 hours with a Bio-blender (Soil Soup, Inc., Edmonds, WA). ACT made with molasses aerated with a Compost Tea Brewer (Growing Solutions, Inc., Eugene, OR).

[c] Fermentation nutrients described in Table 3.3.

[d] Plant disease severity was determined by averaging the leaf disease severity ratings. Leaf disease severity was rated using a 0-3 point scale, with zero points for no lesions; one point for discreet lesions occupying less than one third of the leaf area; two points for spreading lesions occupying one third to two thirds of the leaf area; three points for greater than two thirds of the leaf area covered in lesions.

* indicates significantly less than the *B. cinerea* control ($P < 0.05$) separated by Fischer's protected LSD.

Effect of adding fermentation nutrients to aerated compost tea on disease suppression. Compared to ACT produced without added nutrients, linear contrast analysis indicated that ACT made with fermentation nutrients had significantly ($P < 0.05$) less disease in only four percent of the direct comparisons (Table 3.10).

Table 3.10. ACT produced with and without fermentation nutrients. Significance of linear contrasts for disease severity.

ID#	Compost Type	Linear contrast	Disease Severity (percent scale)	P value
25	Blend	No nutrient vs. Bacterial Solution[a]	30.8* vs. 28.1*	0.6600
25	Blend	No nutrient vs. Bacterial Solution	50.6 vs. 44.6	0.4200
7	Yard trimmings	No nutrient vs. Bacterial Solution	51.8 vs. 44.9*	0.3500
7	Yard trimmings	No nutrient vs. Bacterial Solution	33.9 vs. 36.8	0.7200
29	Vermicompost	No nutrient vs. Bacterial Solution	53.4 vs. 43.9	0.2600
29	Vermicompost	No nutrient vs. Bacterial Solution	40.3 vs. 43.5	0.6900
25	Blend	No nutrient vs. Fungal Nutrients	30.8* vs. 19.7*	0.0800
25	Blend	No nutrient vs. Fungal Nutrients	50.6 vs. 32.1	0.0150
7	Yard trimmings	No nutrient vs. Fungal Nutrients	51.8 vs. 55.9	0.5600
7	Yard trimmings	No nutrient vs. Fungal Nutrients	33.9 vs. 32.1	0.8800
29	Vermicompost	No nutrient vs. Fungal Nutrients	53.4 vs. 40.5*	0.1240
29	Vermicompost	No nutrient vs. Fungal Nutrients	40.3 vs. 27.3*	0.1100
			Disease Severity (0-3 scale)[b]	
7	Yard trimmings	No nutrient vs. Molasses	1.64 vs. 1.76	0.5035
7	Yard trimmings	No nutrient vs. Molasses	1.29 vs. 1.46	0.4024
20	CMC	No nutrient vs. Molasses	1.62 vs. 1.68	0.7377
20	CMC	No nutrient vs. Molasses	1.53 vs. 1.64	0.5666
26	Bark	No nutrient vs. Molasses	2.10 vs. 2.13	0.9019
26	Bark	No nutrient vs. Molasses	1.99 vs. 1.76	0.2653
28	Vermicompost	No nutrient vs. Molasses	2.39 vs. 2.25*	0.5924
28	Vermicompost	No nutrient vs. Molasses	1.83 vs. 1.95	0.6415
28	Vermicompost	No nutrient vs. Yeast	2.39 vs. 2.60	0.4317
28	Vermicompost	No nutrient vs. Yeast	1.83 vs. 2.25	0.0692
27	Bark	No nutrient vs. Yeast	2.72 vs. 2.61	0.5453
27	Bark	No nutrient vs. Yeast	2.08 vs. 2.21	0.6402
27	Bark	No nutrient vs. Yeast	2.68 vs. 2.82	0.6105

[a] Fermentation nutrients described in Table 3.1.

[b] Plant disease severity was determined by averaging the leaf disease severity ratings. Leaf disease severity was rated using a 0-3 point scale, with zero points for no lesions; one point for discreet lesions occupying less than one third of the leaf area; two points for spreading lesions occupying one third to two thirds of the leaf area; three points for greater than two thirds of the leaf area covered in lesions.

* indicates significantly less than the water control treatment for that experiment ($P < 0.05$) separated by Fischer's protected LSD.

However, ACT made with fermentation nutrients had significantly greater ($P < 0.05$) bacterial populations than ACT made without fermentation nutrients (Table 3.11). Therefore, increasing the culturable population of bacteria did not correspond to increased suppression of gray mold.

Table 3.11. Comparison of bacterial populations[a]. Aerated compost tea produced with and without fermentation nutrients.

Fermentation Nutrient					
No Nutrient	Yeast[b]	Molasses[c]	Bacterial[d]	Fungal[e]	t-test P value
7.09 (0.89)	8.27 (0.56)				0.0355
6.47 (0.66)		8.32 (0.43)			0.0007
5.93 (0.20)			8.28 (0.55)		0.0002
5.93 (0.20)				7.66 (0.27)	<0.0001

[a] Bacterial populations $\log_{10}$ cfu/ml compost tea, enumerated on 5% TSBA. Standard deviation in parathesis.
[b] Hydrolyzed yeast powder (0.2% w/v, Red Star Yeast Co., Milwaukie, WI).
[c] Unsulfured Blackstrap Molasses (0.3% v/v, GloryBee Foods Inc, Eugene, OR).
[d] 0.5% v/v Bacterial Nutrient Solution (Soil Soup, Inc., Edmonds, WA).
[e] 1.2 g Maxicrop soluble seaweed powder (Maxicrop USA Inc., Arlington Hts, IL), 2.5 ml Humax liquid humic acids (JH Biotech Inc., Ventura, CA), 3 g rock dust (Target Glacial Dust; Target Products Ltd., Burnaby, B.C.).

Influence of adding spray adjuvants to aerated compost tea on disease suppression. Adding spray adjuvants to ACT produced with or without fermentation nutrients significantly ($P < 0.05$) reduced disease severity compared to applying either the ACT or adjuvants alone (Table 3.12).

Table 3.12. Enhancement of disease suppression by adding adjuvants to aerated compost teas prior to application.

Compost D #	Fermentation Nutrients	Adjuvant Added to ACT	Disease Severity[a] (%leaf area) Untreated Control	ACT + Adjuvants	ACT	Adjuvants	Botrytis control
29	Bacterial	Karaya gum (0.05% w/v) + ThermX 70 (0.05% v/v)	11.8 a	13.0 a	43.5 b	55.5 c	50.5 c
7	Fungal	Karaya gum (0.05% w/v) + ThermX 70 (0.05% v/v)	24.3 a	43.5 b	58.8 c	56.0 c	59.4 c
27	Yeast (0.2% v/v)	Nu-Film 17 (0.1% v/v)	21.3 a	48.5 b	85.2 de	87.4 e	69.4 c
27	None	Nu-Film 17 (0.1% v/v)	21.3 a	44.4 b	75.7 cd	87.4 d	69.4 c
			Disease Severity (0-3 scale)[b]				
27	None	Nu-Film 17 (0.1% v/v)		2.29 a	2.72 b	2.81 b	2.67 b
13	Molasses (0.3%)	Latron B1956 (0.1% v/v)	1.39 a	1.96 b	2.42 c	2.49 c	2.51 c

[a] Within a row, numbers followed by the same letter are not significantly different (P <0.05) according to Fischer's protected LSD.

[b] Plant disease severity was determined by averaging the leaf disease severity ratings. Leaf disease severity was rated using a 0-3 point scale, with zero points for no lesions; one point for discreet lesions occupying less than one third of the leaf area; two points for spreading lesions occupying one third to two thirds of the leaf area; three points for greater than two thirds of the leaf area covered in lesions.

DISCUSSION

Altering NCT production and use through compost selection, fermentation nutrients, fermentation period, stirring, and depth of liquid applied had varying effect on disease suppression. Compost source and fermentation time had the greatest effect, for most compost sources applying surface liquid had a consistent but not significantly ($P < 0.1$) different effect, while stirring and adding fermentation nutrients had little effect. Aerating compost tea did not significantly ($P < 0.05$) increase disease suppression compared to NCT. Adding fermentation nutrients to ACT did not significantly ($P < 0.05$) increase disease suppression. Tank mixing spray adjuvants with ACT significantly ($P < 0.05$) increased disease suppression.

Two compost sources (yard trimmings and manure based) produced a significantly ($P < 0.001$) greater proportion of suppressive NCT batches than the other compost sources. Similarly, grape-marc compost and manure based composts were used to suppress *B. cinerea* infecting foliage in greenhouse assays (Elad and Shtienberg, 1994). These results differ from earlier reports that indicated manure-based composts were more effective for producing suppressive NCT (Weltzien, 1991).

Increasing NCT fermentation time from seven to 14 days often resulted in significantly ($P < 0.05$) increased disease suppression (Table 3.5). Increasing the fermentation time could increase disease suppression due to accumulation of antibiotics or other secondary metabolites produced by the microbes (Cronin *et al*, 1996). Similarly, Elad and Shtienberg (1994) observed that fermenting NCT for 14 days was more suppressive than seven days for multiple compost sources for suppression of gray mold on greenhouse grown crops. However, the results presented here suggest that differences in suppression due to fermentation time appear to be related to the type of compost used to produce NCT. For chicken manure compost, fermenting for 14 days significant reduced disease ($P <0.08$) compared to fermenting for seven day in 55% of the comparisons. While for yard trimmings compost, significant differences ($P <0.1$) associated with fermentation time were not detected (Table 3.5). Other research has indicated that fermenting NCT for seven days was

optimal across three compost sources for suppressing *B. cinerea* on detached grape leaves (Ketterer *et al*, 1992). While there is no consensus on the optimal fermentation time, it appears that NCT should be fermented for longer periods in order to obtain more consistent suppression of gray mold.

Aerating compost tea during production did not result in compost tea that was significantly more suppressive than NCT. Similar results were observed for suppression of powdery mildew (*Podosphaera pannosa* var. *rosae*) of rose with ACT and NCT (Scheuerell and Mahaffee, 2000; Chapter 4). From this data it appears that compost has a greater influence on disease suppression than aeration.

Adding fermentation nutrients to ACT did not significantly increase ($P > 0.05$) disease suppression compared to ACT produced without fermentation nutrients in a large majority of the direct comparisons, although bacterial populations in the ACT were significantly ($P<0.05$) increased. This is in agreement with other research (Chapter 5) where total bacteria, and active bacteria (measured through direct cell counts) as well as culturable populations were not directly related to disease suppression when all types of fermentation nutrient additions are considered.

The use of spreader/sticker adjuvants is common practice in chemical pesticide formulation and application (Backman, 1978), but it has received little attention in the biological control literature. These experiments indicate that the enhanced suppression is independent of the compost source or fermentation nutrients used to produce ACT. Similarly, Gum Karaya, and other polysaccharides tank mixed (0.1% v/v) with microbial biocontrol agents (BCA) were recently demonstrated to significantly reduce *B. cinerea* infection of geranium compared to the BCA alone and adjuvant controls (Mahaffee *et al* 2002). They have also been shown to restore biocontrol activity of a biofilm deficient mutant of a foliar biocontrol agent against *B. cinerea* on geranium (Roche et al, 2002).

Adjuvants apparently altered the colonization of bacteria on the leaf surface. Preliminary observations with scanning electron microscopy indicated that adding Gum Karaya (0.05% w/v) and a Yucca surfactant (Thermx-70, 0.05% v/v) to ACT

increased the dispersion and adhesion of bacteria on geranium leaf surfaces. Leaves treated with non-amended ACT typically had scattered aggregations and single bacteria at the base of trichomes and leaf cell junctions, whereas ACT mixed with Karaya and Yucca had abundant aggregations of bacteria embedded in a matrix across the leaf surface (Steve Scheuerell, personal observation).

Increased disease suppression from the addition of adjuvants to compost tea could be due to increased attachment and subsequent survival of applied organisms in a modified phylloplane environment. Modifying the phylloplane environment has been shown to affect the recovery of microorganisms applied in compost tea (Sackenheim *et al*, 1994). Microbial epiphyte populations recovered from plants sprayed with various NCT were significantly reduced when the plants were maintained in a growth chamber at 50-60% relative humidity compared to 90-95% relative humidity (Sackenheim *et al*, 1994). Similarly, in our experiments, plants treated with compost tea dried completely in the 24-hour period between compost tea application and pathogen inoculation. This environment likely limited epiphytic microbial colonization and survival. Preliminary examination with scanning electron microscopy indicated that spray adjuvants increased microbial colonization from compost tea. Increased populations of bacterial epiphytes could more effectively compete for phylloplane nutrients, leading to a reduction in *B. cinerea* conidia germination (Blakeman, 1975).

Compost tea can significantly reduce leaf infection severity caused by *B. cinerea* under environmental conditions that favor disease development. However, variability from batch to batch of compost tea necessitates further development of a production protocol that consistently reduces disease. Further development of spray adjuvant technology that is compatible with compost teas appears to be a promising direction to minimize variability of disease suppression.

ACKNOWLEDGMENTS

Funding from USDA CRIS # 303-5358-22000-024-00D and the Oregon association of Nurserymen and Oregon Department of Agriculture made this work possible. Julie DiLeone and Tara Sechler provided valuable technical assistance.

Chapter four: Effect of aerated and non-aerated compost tea, and *Trichoderma harzianum* T-22 aplications for control of rose powdery mildew (*Podospheara pannosa* var. *rosae*), rust (*Phragmidium* spp.), and black spot (*Diplocarpon rosae*) on field grown roses

ABSTRACT

Compost tea and *Trichoderma harzianum* T-22 (Plant Shield, Bioworks, Inc., Geneva, NY) were evaluated under field conditions for suppressing three foliar fungal diseases affecting rose in Western Oregon, Powdery Mildew (*Podospheara pannosa* var. *rosae* [formally *Sphaerotheca pannosa var. rosae*]), Rust (*Phragmidium* spp.), and Black Spot (*Diplocarpon rosae*). Disease control was assessed in relation to the type of compost, use of aeration, and added nutrients in the compost teas. All compost teas significantly ($P<0.05$) suppressed powdery mildew, compost type had more impact on disease suppression than aeration. Few compost teas suppressed rust, differences in rust severity were not detected across compost type, aeration, or added nutrients. Black spot developed to an overall low level, no compost teas significantly ($P<0.05$) reduced disease compared to a water control. Significant ($P<0.05$) differences in black spot severity were detected across compost types. *T. harzianum* T-22 did not control powdery mildew, rust, or black spot.

INTRODUCTION

There is an increasing interest in the potential of compost teas to control plant disease (Chapter 2). Altering the materials and method used to produce compost tea, such as choice of compost (chapter 2), has been demonstrated to affect disease suppression (Chapters 3). Despite the increasing conviction that actively aerating and adding fermentation nutrients to compost tea increases plant disease suppression, few studies have examined the impact of aeration and added nutrients on disease suppression (Chapters 3 and 5).

Compost tea and *Trichoderma harzianum* T-22 (Plant Shield, Bioworks, Inc., Geneva, NY) were evaluated under field conditions for suppressing three foliar fungal diseases affecting rose in Western Oregon, Powdery Mildew (*Podospheara pannosa* var. *rosae* [formally *Sphaerotheca pannosa var. rosae*]), Rust (*Phragmidium* spp.),

and Black Spot (*Diplocarpon rosae*). In 1999, the objectives were to determine effects of aeration and compost source in compost tea production on disease suppression. In addition, the efficacy of a commercial biocontrol preparation of *Trichoderma harzianum* T-22 was examined. In 2000, the primary goals were to examine the effects of aeration and fermentation nutrients in compost tea production on disease control.

MATERIALS AND METHODS

Field plot information. The study plot was located at the Oregon State University, Department of Botany and Plant Pathology – Field Laboratory Farm, Corvallis, OR. One-year-old bare root rose bushes (*Rosa* spp. 'pink simplicity' grafted on 'Dr. Huey' rootstock; Bear Creek Gardens, Grants Pass, OR) were planted in March, 1999. The study plot had 128 rose bushes planted in 8 columns and 16 rows on 6-foot square centers. At the time of planting, 150 cm^3 of slow-release fertilizer was mixed in the planting hole (Osmocote 14-14-14, Scotts-Sierra Horticultural Products Company, Marysville, OH). Black, synthetic weed barrier was placed around the base of each plant (1-m^2). Plants were watered as needed with drip irrigation. Plants were fertilized four times each summer with 250 ml liquid fertilizer poured around the base of each plant (1 lb/10gal, Peters 20-20-20 Professional Water Soluble Fertilizer, Scotts-Sierra Horticultural Products Company, Marysville, OH)

Compost sample collection. Compost used to make compost tea was collected two weeks prior to each field season at commercial composting facilities in Western Oregon (Tables 1 and 2). Compost that was considered ready for sale by the facility operator was sampled with an excavator (1.5 m^3) and dumped onto a truck bed for transport to the USDA ARS Horticultural Crops Research Laboratory. Compost was stored in shaded bins and used over the field season for producing the compost teas.

Table 4.1. Rose powdery mildew incidence for treatments applied in 1999.

Treatment Name	Active Ingredient	Powdery Mildew[1]
Aerated chicken	ACT from Chicken manure compost[2] with 0.3% v/v molasses	2.02ab
Non-aerated chicken	NCT from Chicken manure with 0.3% v/v molasses	1.36a
Aerated Yard	ACT from Yard trimmings compost[3] with 0.3% (v/v) molasses	2.56bc
Non-aerated Yard	NCT from Yard trimmings compost with 0.3% v/v molasses	2.88cde
Aerated Mix	ACT from Mixed source compost[4] with 0.3% (v/v) molasses	2.65bcd
Non-aerated Mix	NCT from Mixed source compost with 0.3% v/v molasses	2.56bc
Topshield LR	*Trichoderma harzianum* T-22[5] (2.5 g/L) + Adjuvant	3.41ef
Topshield MR	Trichoderma harzianum T-22 (7.5 g/L) + Adjuvant	3.39ef
Topshield HR	Trichoderma harzianum T-22 (22.5 g/L) + Adjuvant	3.50ef
Adjuvant control	Latron B-1956 spreader sticker[6] (0.1% w/v)	3.31def
Water Control		3.66f
Untreated Control		4.46g

[1] Average leaflet incidence (0-5 scale) assessed on 10/15/99. Numbers followed by the same letter are not significantly different (Fischer's protected LSD, $P < 0.05$)

[2] Four-month-old composted Douglas Fir sawdust and chicken manure (3:1 v/v, Lane Forest Products, Eugene Oregon)

[3] Nine-month-old composted ground Yard trimmings (Rexius Inc, Eugene, OR)

[4] Four-month-old composted Straw, sawdust, pome fruit, manure, clay (Columbia Gorge Organics, Mt. Hood, OR)

[5] Bioworks Inc., Geneva, NY

[6] Rohm and Haas Company, Philadelphia, PA

Production and application of treatments. Non-aerated compost tea (NCT) was produced with 5:1 ratio of water and compost (v/v) in 19-L plastic buckets. Tap water (10 L) was placed in a bucket and allowed to sit for 24 hours for passive reduction of chlorine. Optional fermentation nutrients were added (Tables 1 and 2), followed by addition of compost to the water. The entire contents were vigorously stirred for 20 seconds, then left undisturbed for seven to 11 days. Before application, NCT was strained through eight layers of cheesecloth to prevent spray nozzle clogging.

Aerated Compost tea (ACT) was produced with Compost Tea Brewers (Growing Solutions, Inc., Eugene, OR). After adding 50-L tap water to each brewer unit, they were run for 2 hours to reduce chlorine concentrations. If used, fermentation nutrients were then added (Tables 1 and 2). Compost (4.5 L, contained in a mesh basket) was hung into the water. After 24 hours compost tea was removed through a valve for application.

Trichoderma harzianum T-22 (Plant Shield, Bioworks, Inc., Geneva, NY) was mixed with water at three rates (2.5 g/L, 7.5 g/L, 22.5 g/L). Due to clogging of the sprayer nozzle by the clay carrier material in Plant Shield, it was necessary to thoroughly mix the material in water, allow the carrier material to settle for 30 minutes, then pour off the liquid into another container for application. In 2000, only the 7.5 g/L Plant Shield rate was applied.

In 1999, spray adjuvants were not added to the compost tea treatments. The Topshield treatments had Latron B-1956 spreader/sticker added (0.1% v/v; Rohm and Haas Company, Philadelphia, PA) as recommended by Bioworks, Inc. In 2000, all compost tea treatments had Nu Film 17 spreader/sticker added (0.02% v/v, Miller Chemical & Fertilizer Company, Hanover, PA) just prior to application. Additionally, Nu Film 17 was added (0.02% v/v) to the Topshield treatment instead of Latron B-1956.

Treatments were applied before 10 am, with all leaf surfaces covered until run-off using a hand held sprayer (CO_2 propelled, 60 psi). Between treatments, the sprayer was flushed with 95% ethanol and rinsed with distilled water.

Disease and defoliation assessment. The mean powdery mildew leaflet incidence (MPMLI) was determined for each rose plant. Ten subsample leaves per plant were assessed. Subsample leaves were selected by randomly choosing 10 tertiary shoots, then moving down the shoot until the first fully expanded 5-leaflet leaf was encountered. The incidence (presence or absence, 0 or 1) of powdery mildew was determined for each of the five leaflets (sub-subsample) and an additive value of 0-5

was recorded for each leaf subsample. The 10 leaf subsamples values were averaged to calculate the plant powdery mildew leaflet incidence .

Rust and Black Spot severity were assessed separately for each plant by visually estimating the percent of the total leaf surface covered by lesions typical of the diseases. Defoliation was evaluated by estimating the percent defoliation on each plant.

Compost tea measurements. Temperature (model 150 pH meter, IQ Scientific Instruments) and dissolved oxygen (Model 600; Engineered Systems & Design) were recorded for each compost tea batch by immersing the probes into the fermentation vessel at the end of the fermentation period. For bacterial population enumeration, 1-ml compost tea was aseptically removed from each fermentation vessel at the end of the fermentation period. Following serial dilution in sterile 0.02M potassium phosphate buffer pH 7.0, dilutions were plated using an automated spiral plater (Eddy Jet; IUL Instruments, Barcelona, Spain) on 5% trypticase soy broth agar (1.5 g Difco tryticase soy broth with 15 g agar) with 100 ug/ml cycloheximide. Plates were incubated in the dark at 22 C and counted after three days. Populations were recorded as CFU/ml compost tea.

Experimental design and statistical analysis. The 1999 and 2000 experiments were randomized complete block designs with eight replications (individual plants). Individual plant disease ratings were used for the statistical analysis. Two-way analysis of variance was performed with treatment means separated by Fischer's protected least significant difference using proc mixed in SAS version 8.0 (SAS Institute Inc., Cary, North Carolina).

1999 Trial

In 1999, the center 12 rows of rose plants were used for treatment applications with the outer two rows on each end of the study plot left untreated. Twelve treatments were applied (Table 4.1) every 7-11 days beginning on April 20th, and ending on October 5th, 1999. Powdery mildew was assessed on October 15, 1999. Rust was assessed on October 15, but had not progressed throughout the plot. Black

Spot infections were not observed. In late November 1999, rose leaves exhibiting black spot lesions were gathered from the city of Corvallis rose garden in Avery Park, Corvallis, OR. A handful of these leaves were placed in the canopy of each rose at the field plot to assist the establishment of Black Spot for the following field season.

2000 Trial

In the summer of 2000, the Southernmost fourteen rows of plants were treated. Treatments (Table 4.2) were applied every 7-11 days, a total of 21 times between March 8 and October 5. Rust and Blackspot were assessed four times on May 4, June 8, June 22, and July 18. Defoliation resulting from rust and black spot infection was evaluated on July 18. Powdery mildew was first noted in the first week of September and was assessed on September 11. Further disease assessments were not possible because a fire destroyed the field plot on September 25, 2000.

Table 4.2. Severity of rust and black spot of rose and percent defoliation.

Treatment Name	Active Ingredient	Disease Severity[a]				Percent Defoliation
		Rust 6/20/00	Rust 7/18/00	Black Spot 6/20/00	Black Spot 7/18/00	7/18/00
Aerated chicken Mol	ACT - Chicken manure compost[b] with 0.3% v/v molasses[c]	11.58	16.88	4.32	5.60	12.98
Non-aerated chicken Mol	NCT from Chicken manure with 0.3% v/v molasses	9.12	16.37	4.87	7.00*	11.75
Aerated worm	ACT from Vermicompost[d]	14.60*	22.72	2.64	2.79	15.01
Aerated worm Mol	ACT from Vermicompost with 0.3% v/v molasses	7.07*	13.29	2.05	3.52	5.92
Aerated worm Yeast	ACT from Vermicompost with 0.2% yeast[e]	8.04*	15.74	3.13	3.52	5.83
Non-aerated worm	NCT from Vermicompost	4.67**	7.70	6.26	4.95	5.64
Non-aerated worm Mol	NCT from vermicompost with 0.3% v/v molasses	4.31**	7.83*	2.44	3.46	8.49
Non-aerated Yeast	NCT from Vermicompost with 0.2% yeast	7.73*	14.65	1.81	2.13	4.66
Topshield	Trichoderma harzianum T-22[f] (7.5 g/L) + Adjuvant[g]	20.42	16.51	5.28	5.33	24.50*
Adjuvant control	Nu-Film 17[h] (0.2 ml/L)	13.03	23.29	3.64	3.93	10.15
Neem Oil	Clarified hydrophobic extract of neem oil[g] (7.9 ml/L)	1.08***	1.16***	1.16***	0.75***	1.50
Funginex	Triforine 18.2% A.I.[h] (1.3 ml/L)	0***	0***	0***	0***	0
Untreated control		14.47	25.20	2.97	4.05	18.64
Water control		15.29	20.32	3.39	3.03	11.15

[a] Percent of plant foliage covered in lesions. Within a column * Compared to water control, treatments with * are significantly different (P<0.1) with Fischer's protected LSD; ** P<0.05; *** Treatments removed from the analysis due to unequal variance compared to the other treatments.

[b] Four-month-old composted Douglas Fir sawdust and chicken manure (3:1 v/v, Lane Forest Products, Eugene Oregon)

[c] Aunt Pattie's Blackstrap; Glorybee Foods, Eugene, OR.

[d] Three month-old casting from a vertical reactor (cow manure, food waste, paper, 1:1:1 v/v, EPM, Inc. Cottage Grove, OR).

Table 4.2 (Continued).

[e] hydrolyzed yeast powder (Basic Yeast; Red Star Yeast Co., Milwaukie, WI).
[f] Bioworks Inc., Geneva, NY.
[g] Rose Defense, Green Light Company, San Antonio, TX.
[h] Funginex, Syngenta, Wilmington, DE .
[i] Miller Chemical & Fertilizer Company, Hanover, PA.

RESULTS

Powdery mildew. All compost tea treatments significantly (P <0.05) reduced the mean powdery mildew leaflet incidence (MPMLI) compared to the water control (Table 4.1). The water control treatment significantly (P <0.05) reduced disease compared to the untreated control (Table 4.1). No significant differences (P<0.1) in MPMLI were detected between the water control, surfactant control and the three rates of *Trichoderma harzianum* T-22 (Table 4.1).

Powdery mildew disease levels significantly ($P < 0.05$) varied among compost sources. Compost teas made with the composted chicken manure had significantly (P<0.05) less MPMLI than the other compost types (Table 4.3). Aerating compost tea during production did not significantly (P<0.05) affect powdery mildew suppression. Within each compost type, there was not a significant (P<0.05) difference in powdery mildew disease levels between the aerated compost tea and the non-aerated compost tea.

Table 4.3. Influence of compost source and aeration in compost tea production on incidence of powdery mildew.

Compost Tea Production Factor	Orthogonal contrast	Disease severity[a]	P value
Compost source	Chicken manurevs. Yard trimmings	1.696 vs. 2.725	0.0003
Compost source	Chicken manure vs. Mixed source	1.696 vs. 2.606	0.0014
Compost source	Yard trimmings vs. Mixed source	2.725 vs. 2.606	0.6472
Aeration	All aerated vs. All non-aerated compost teas	2.414 vs. 2.2710	0.5124

[a] Mean powdery mildew leaflet incidence (0-5 scale). Disease assessed on 10/15/99.

Rust severity. Rust developed to a moderately high level in 2000, but was to patchy for evaluation in 1999. Compared to the water treated plants, significant ($P<0.05$) differences in disease severity were limited to NCT made with the vermicompost (Table 4.2). Orthogonal contrasts did not detect significant differences in rust severity between compost teas made from the chicken manure compost and the vermicompost (Table 4.4). Similarly, orthogonal contrast analysis indicated that the mean rust severity of all ACT was not significantly ($P<0.05$) different than all NCT (Table 4.4). In one case, aeration negatively affected suppression. NCT made from the vermicompost without added nutrients had significantly ($P<0.05$) less rust severity than the corresponding ACT. For compost teas made with vermicompost, rust severity was not significantly ($P<0.05$) different between compost teas made with and without fermentation nutrients (Table 4.4).

Table 4.4. Influence of compost source, aeration, and fermentation nutrients in compost tea production on rust and black spot severity.

Compost Tea Production Factor	Treatment Orthogonal Contrast[a]			Disease Severity Means: Rust 6/22/00	Rust 7/18/00	Black Spot 6/22/00	Black Spot 7/18/00
Compost source	Aerated chicken Mol and Non-aerated chicken Mol	vs.	Aerated worm Mol and Non-aerated worm Mol	10.36 vs. 5.69	16.63 vs. 10.56	4.60 vs. 2.25*	6.30 vs. 3.49**
Aeration	All Non-aerated compost teas	vs.	All Aerated compost teas	6.46 vs. 10.33	11.64 vs. 17.16	3.85 vs. 3.04	4.38 vs. 3.86
Fermentation Nutrients	Aerated worm and Non-aerated worm	vs.	Aerated worm Mol, Aerated worm Yeast, Non-aerated worm Mol, Non-aerated Yeast	9.64 vs. 6.79	15.21 vs. 12.87	4.45 vs. 2.36*	3.87 vs. 3.15

[a] Treatments are described in Table 4.2.

* Significantly different ($P<0.05$) with Fischer's protected LSD.

** Significantly different ($P<0.01$) with Fischer's protected LSD.

Both the neem oil and funginex fungicides effectively suppressed rust disease development (Table 4.2). Due to unequal variance of neem oil and funginex treatments, they were removed from the statistical analysis. The *Trichoderma harzianum* T-22 and adjuvant control treatments did not significantly ($P<0.05$) reduce rust severity.

Black spot severity. Black spot developed to a low overall level in 2000 and was not detected in 1999. None of the compost tea treatments had disease severity that was significantly ($P<0.05$) less than the water treated plants (Table 4.2). Orthogonal contrast analysis indicated that compost tea produced with the vermicompost had significantly ($P<0.05$) less black spot than teas made with the composted chicken manure (Table 4.4). No Significant ($P>0.05$) differences in black spot severity were detected between aerated and non-aerated compost teas (Table 4.4). For teas produced with the vermicompost, fermentation nutrients amended teas had significantly ($P<0.05$) less black spot severity than non-amended teas on one sampling date (Table 4.4).

The *Trichoderma harzianum* T-22 and adjuvant control treatments did not significantly ($P>0.05$) reduce black spot severity. Both the Neem oil and Funginex fungicides effectively suppressed rust development. Due to unequal variance compared to the other treatments, both fungicides were removed from the statistical analysis. The neem oil product caused a deforming phytotoxic reaction on some emerging shoots during the first month of spring growth. Phytotoxicity was not observed on emerging secondary and tertiary shoots.

Defoliation. Compared to the water treated plants, none of the compost tea treatments had significantly different ($P<0.05$) average percent defoliation (Table 4.2). Defoliated leaves were severely affected by rust and to a less degree black spot. There was a significant linear relationship ($P<0.0001$, R^2 0.77) between rust disease severity on 6/22/00 and average percent defoliation on 7/18/00. This indicates that the 7/18/00 rust disease severity rating underestimated the total percent of foliage that had been

infected by rust, particularly for treatments with greater amounts of rust earlier in the season.

Compost tea measurements. Significant differences in the dissolved oxygen, temperature, and bacterial populations were detected across the compost tea treatments (Table 4.5). The aerated compost teas had significantly more dissolved oxygen than the non-aerated compost tea (Table 4.5), but this did not correspond to increased disease control (Table 4.2). Likewise, disease suppression (Table 4.2) did not relate to increased populations of culturable bacteria (Table 4.5). For rust suppression, there was an inverse relationship between bacterial populations and disease suppression for ACT and NCT made from the vermicompost without added nutrients (Tables 2 and 5).

Table 4.5. Dissolved oxygen, temperature and bacterial populations for compost tea treatments applied during the 2000 field season.

Compost tea treatment[1]	Dissolved Oxygen (mg/kg)	Temperature (C)	Bacteria (Log_{10} cfu/ml)
Aerated worm	8.4 c[2]	23.3 b	6.68 b
Aerated worm Mol	8.6 c	23.5 b	8.57 d
Aerated worm Yeast	7.8 c	24.7 b	9.00 e
Aerated chicken Mol	8.2 c	24.7 b	8.30 d
Non-aerated worm	3.8 b	20.5 a	5.86 a
Non-aerated worm Mol	0.42 a	20.7 a	7.81 c
Non-aerated worm Yeast	0.45 a	20.5 a	7.53 c
Non-aerated chicken Mol	0.37 a	20.6 a	7.71 c

[1] Treatments described in Table 4.2.

[2] Numbers in each column followed by the same letter are not significantly different, means separated by Duncan's multiple range test ($P<0.05$).

DISCUSSION

For suppressing the prevalent foliar fungal diseases of rose with compost tea, choice of compost had more impact than aeration for optimizing suppression. Similarly, compost source was more important than aeration in compost tea production for control of gray mold (*B. cinerea*) on geranium foliage (Chapter 3). Actively aerating compost tea did not increase suppression of powdery mildew, rust, or black spot compared to non-aerated compost tea. Similarly, consistent differences between ACT and NCT were not observed for the control of gray mold (*B. cinerea*) on

geranium (Chapter 3). Both ACT and NCT significantly ($P<0.05$) suppressed of *Pythium ultimum* cucumber damping-off when applied as a container media drench (Chapter 5). Amending compost teas with fermentation nutrients generally did not increase control of rust or black spot. This is similar to the results observed with control of gray mold (*B. cinerea*) on geranium foliage (Chapter 3). In contrast, amending compost tea with select fermentation nutrients significantly increased the suppression of *Pythium ultimum* cucumber damping-off when applied as a container media drench (Chapter 5).

Compost teas exhibited moderate control of powdery mildew and little control of rust and black spot. The commercial formulation of *Trichoderma harzianum* did not suppress powdery mildew, rust, or black spot. In comparison, the fungicides Triflorine and neem oil resulted in plants with extremely low levels of rust and black spot. The compost teas would likely not be considered a feasible alternative to the fungicides for conventional commercial rose cultivation.

ACKNOWLEDGEMENTS

We thank Bear Creek Gardens for donating the rose plants, Bioworks, Inc. for donating the Topshield, and Lane Forest Products and EPM Inc. for donating compost. Kathryn Sackett provided valuable technical assistance. Funding for this work was provided by USDA CRIS # 303-5358-22000-024-00D.

Chapter Five: Using Compost Tea as a Container Media Drench for Suppressing Seedling Damping-off Caused by *Pythium ultimum*

ABSTRACT

Aerated and non-aerated compost tea, produced with or without fermentation nutrients, was investigated for the suppression of *P. ultimum* damping-off of cucumber. Compost tea effectively suppressed damping-off in soilless container media. Both aerated compost tea (ACT) and non-aerated compost tea significantly reduced disease only when fermentation nutrients were added. The most consistent formulation for damping-off suppression was ACT fermented with kelp and humic acids. Producing ACT with a molasses-based fermentation nutrient solution inconsistently suppressed damping-off, evidence suggests that residual fermentaton nutrients can interfere with disease suppression. Across all compost tea samples, there was not a significant relationship of bacterial populations, determined by active, total or cfu methods, to disease suppression. However, for all ACT produced without the molasse-based fermentation nutrient, there does appear to be a threshold of bacterial population level above which compost teas are suppressive.

INTRODUCTION

Compost tea is increasingly being used as an alternative plant disease control measure in commercial horticulture. Compost tea is produced by mixing compost with water and fermenting for a defined period, with or without added fermentation nutrients and either actively aerated (aerated compost tea, ACT) or not (non-aerated compost tea, NCT) (Scheuerell and Mahaffee, 2002). Compost tea applied to foliage has been demonstrated to suppress a range of foliar diseases (Weltzien, 1991), however, the use of compost tea as a soil drench for seed or root rot suppression has received very little attention.

Of particular interest to greenhouse growers is the control of damping-off disease of seedlings, commonly caused by *Pythium* spp. in Northern latitudes (Stephens and Powell, 1982; Stephens *et al,* 1983). This can be a severe problem when peat based media that is naturally conducive to the pathogen is used (Hoitink *et*

al, 1993). The only investigation to date involving *Pythium* spp. and compost tea determined that pea seeds soaked in NCT, redried, and sown two days later reduced disease symptoms on seedlings caused by *P. ultimum* (Tränkner, 1992). Heat treating the NCT negated all suppression of pathogen growth *in vitro*, indicating the likely role of the NCT microflora in disease suppression (Tränkner, 1992).

The microflora of both NCT (Weltzien, 1991) and ACT (Ingham, 2000) are typically described as being dominated by bacteria, and therefore the bacterial population of compost tea could be a useful parameter to measure in relation to plant disease suppression. It has been proposed that increasing the population of total and active bacteria in ACT will generally increase the level of plant disease suppression (Ingham, 2000). However, there is no direct evidence supporting these assumptions.

The objectives of this study were to determine if compost tea can be applied as a drench to suppress seedling damping-off of cucumber caused by *P. ultimum* in a peat-based potting mix that is naturally conducive to the disease; to examine how aeration and fermentation nutrients impact disease suppression; and determine if the level of disease suppression is related to bacterial population size (measured as active cells, total cells, and colony forming units (cfu)) in compost teas.

MATERIALS AND METHODS

Producing Compost Teas. Three commercial sources of compost were used to make compost teas (Table 5.1). Yard trimmings compost (Rexius, Inc., Eugene, OR) was produced from ground landscape trimmings in windrows turned weekly for 3 months then cured in a large pile for 9 months before sampling. One cubic yard of this material was cured an additional two years before being used for these experiments. Vermicompost was produced from mixed vegetation in a vertical flow reactor (Soil Soup, Inc., Edmonds, WA.). Tea compost is a proprietary blend of vegetative and animal manure based composts sold for making compost tea (Rexius, Inc., Eugene, OR). After sampling, approximately one cubic yard of the tea compost was cured for five months in a shaded, open container.

A Bio-blender™ (Soil Soup, Inc., Edmonds, WA) was used to produce ACT. 15-L tap water (20-22 C) was placed in a 19-L bucket and aerated for 2 hours to reduce chlorine present in the water. If used, fermentation nutrients were added (Table 5.2). Compost inoculum was added to the liquid by immersing 500 g compost (approximately 50% moisture w/w) held in a 100 *u*m mesh filter bag (Soil Soup, Inc). To assist the removal of soluble material and microorganisms from the compost, the filter bag was lifted above the water and allowed to drain into the bucket for 15 seconds, then re-immersed for 30 seconds. This was done a total of 3 times, with the filter bag left in the liquid for the remainder of the 36-hour production cycle.

NCT was started by adding 15-L tap water (20-22 C) to a 19-L bucket, this was allowed to sit for 24 hours for passive chlorine removal. If used, fermentation nutrients were added (Table 5.2), followed by pouring 500-g compost into the water. The entire contents were vigorously stirred for 20 seconds, then left undisturbed for 7-9 days until used.

Table 5.1. Chemical and biological properties of compost and compost teas.

Compost[a] and Aeration[b]	fermentation nutrients[c]	n[d]	dissolved oxygen[e] (ppm)	temperature[e] (C)[e]	pH[e]	EC[e]	Log10 Populations				
							yeast[f] cfu[i]	fungi[g] cfu[i]	bacteria[h] cfu[i]	bacteria active cells[j]	bacteria total cells[k]
Yard trimmings		4			5.7 (0.1)	0.87 (0.08)	5.10 (.11)	5.25 (.14)	7.96 (.21)		
NCT	none	5	6.4 (0.5)	15.5 (2.0)	6.7 (0.3)	0.26 (0.05)	bdl[l]	0.69 (1.4)	5.27 (.26)	5.31 (.59)	6.45 (.42)
NCT	bacterial	5	0.2 (0.1)	15.2 (1.9)	5.3 (1.0)	0.95 (0.13)	2.17 (2.5)	3.84 (.10)	6.18 (.51)	5.76 (.49)	7.49 (.32)
NCT	fungal	5	0.2 (0.1)	15.3 (2.3)	6.9 (1.1)	1.05 (0.08)	0.73 (1.5)	1.39 (1.6)	7.14 (.38)	6.11 (.39)	7.26 (.45)
ACT	none	6	8.5 (0.5)	18.1 (1.6)	7.4 (0.2)	0.27 (0.03)	bdl	0.70 (1.2)	6.11 (.41)	5.84 (.34)	6.96 (.40)
ACT	bacterial	9	8.0 (0.4)	18.5 (2.0)	7.8 (0.5)	0.96 (0.18)	2.37 (1.6)	0.58 (1.1)	8.20 (.38)	6.98 (.33)	8.18 (.36)
ACT	fungal	13	8.2 (0.4)	18.6 (2.6)	8.6 (0.3)	1.00 (0.11)	bdl	1.05 (1.3)	7.82 (.22)	6.87 (.34)	7.78 (.47)
ACT	bact-fungal	2	8.5 (0.8)	18.2 (0.7)	8.4 (0.0)	1.38 (0.06)	2.65 (.49)	bdl	8.47 (.12)	6.83 (.32)	7.59 (.47)
Vermicompost		2			6.0 (0.8)	4.7 (0.2)	4.70 (.02)	6.09 (.16)	8.33 (.33)		
ACT	none	3	9.0 (0.6)	17.8 (1.1)	7.3 (0.5)	0.77 (0.02)	bdl	1.85 (1.6)	6.02 (.30)	5.74 (.06)	7.20 (.51)
ACT	bacterial	4	8.6 (0.7)	18.1 (1.1)	8.0 (0.9)	1.51 (0.23)	2.86 (1.9)	bdl	8.74 (.16)	7.35 (.37)	8.42 (.28)
ACT	fungal	4	8.7 (0.7)	17.8 (1.9)	8.5 (0.1)	1.62 (0.18)	1.50 (1.7)	1.23 (1.4)	7.84 (.19)	7.03 (.25)	8.11 (.50)
Tea compost		2			6.9 (0.2)	3.52 (0.02)	4.60 (.64)	6.32 (.20)	9.13 (.16)		
ACT	bacterial	2	8.3 (0.6)	17.3 (2.5)	8.0 (0.4)	1.93 (0.10)	3.53 (.21)	2.10 (3.0)	8.60 (.28)	6.92 (.02)	8.17 (.24)
ACT	fungal	2	8.2 (0.9)	16.1 (1.0)	8.5 (0.0)	1.67 (0.02)	bdl	1.97 (2.8)	8.04 (.49)	6.86 (.480	8.61 (.50)

[a]Yard trimmings compost (Rexius Inc., Eugene, OR); Vermicompost - vegetative based vermicompost sold for compost tea use (Soil Soup, Inc., Edmonds, WA); Tea compost - proprietary compost blend sold for compost tea use (Rexius, Inc., Eugene, OR).

[b]ACT - aerated compost tea, made with Bio-Blenders (Soil Soup, Inc., Edmonds, WA); NCT - not aerated.

[c]nutrients - none, bacterial, fungal, and bact-fungal fermentation nutrients described in Table 5.2.

[d]sample size

[e]recorded at end of ACT (36 h) and NCT (7-10 days) fermentation period, standard deviation in parenthesis

[f] media dilute yeast agar (Benbow and Spots) with 500 ppm streptinomycin sulfate added

[g] media pH 6.0 water agar with 100 ppm rifampicin

[h] media 5% TSBA with 100 ppm cycloheximide

[i] compost cfu/ dry gram; compost tea cfu/ml

[j] Fluorescein diacetate stained after membrane filtration, cells/ml compost tea

[k] 4,6-diamidino-2-phenylindole (DAPI) stained after membrane filtration, cells/ml compost tea

[l] all samples below detection limit of $\log_{10}$ 2.3 cfu/ml compost tea

Table 5.2. Fermentation nutrient recipes used to make compost tea.

Nutrient recipe	Fermentation nutrients per liter water
No nutrient	None
Bacterial	0.5% v/v Bacterial Nutrient Solution (Soil Soup, Inc., Edmonds, WA)
Fungal[a]	1.2 g Maxicrop soluble seaweed powder (Maxicrop USA Inc., Arlington Hts, IL) 2.5 ml Humax liquid humic acids (JH Biotech Inc., Ventura, CA) 3 g rock dust (Target Glacial Dust; Target Products Ltd., Burnaby, B.C.)
Bact-fungal	combination of bacterial and fungal nutrients at the same rates

[a] Adapted from Ingham and Alms (1999).

Chemical properties of compost and compost teas

The pH and electrical conductivity (EC) was recorded twice for the Vermicompost and Tea compost, four times for the Yard trimmings compost during the course of the study. Compost pH was determined from a saturated paste using a portable pH meter (model 150, IQ Scientific Instruments) and electrical conductivity (EC) was determined from a 2:1 (v/v) of distilled water: compost using a portable EC meter (model 933100, Hanna Instruments) (Leege and Thompson, 1997). For compost teas, the pH, EC, temperature and dissolved oxygen (Model 600; Engineered Systems & Design) were recorded for each batch by immersing the probes into the fermentation vessel just before use in the *P. ultimum* bioassay.

Microbiological populations of compost. A 10 g sample of compost was added to 90 ml sterile 0.02M potassium phosphate buffer pH 7.0 (PPB) in a 250 shaker flask, shaken (300 rpm, 25 C) for 20 minutes, serially diluted, plated using an automated spiral plater (Eddy Jet; IUL Instruments, Barcelona, Spain) on selective agar, and then incubated at 22 C. Bacteria were enumerated on 5% trypticase soy broth agar (1.5 g Difco tryticase soy broth with 15 g agar) with 100 ug/ml cycloheximide (TSBAcyc^{100}). Fungi were enumerated on water agar pH 6 (18 g agar/L) with 50 ug/ml rifampicin (WArif^{50}). Yeast were enumerated on dilute,

selective yeast media (SYM; 1.5 g yeast extract, 2.5 g peptone, 5 g dextrose, 2.3 g malt agar, 17 g agar per L medium, amended with 100 ug/ml chlroramphenicol, 50 ug/ml ampicillin, 500 ug/ml streptomycin sulfate, and 2 ug/ml dichloran). Populations reported as cfu/dry g compost.

Microbiological populations of compost teas. A 1-ml sample of compost tea was aseptically removed from each fermentation vessel at the end of the fermentation period. Following serial dilution in sterile PPB, dilutions were plated on TSBAcyc^{100}, WArif^{50}, and SYM then incubated and counted as described above. Populations were recorded as CFU/ml compost tea.

Active and total bacterial cells in compost tea. Active and total bacteria cells were enumerated by staining, filtering, epifluorescent microscopy, and sequential digital imaging using the following procedure. Determination of metabolically active cells was done by staining with fluorescein diacetate (FDA; Sigma), total cells with 4,6-diamidino-2-phenylindole (DAPI, Sigma). DAPI stock solutions (0.2 mg/ml DAPI in sterile 18Ω DI H_2O) and FDA stock solutions (0.2 mg/ml FDA in DMSO) were kept frozen at -20 C with fresh stock used each day. A working solution of FDA was made by adding 1 ml stock solution to 9 ml of PPB. For staining, an appropriate 10 ml suspension of compost tea were prepared from the serial dilutions used for plating on media. DAPI stock solution (100 µl) was added to 10 ml compost tea solution resulting in 0.002 mg DAPI/ml . After 3 minutes of incubating in the dark, the sample was vacuum filtered through a black 0.22 µm filter (Millipore Isopore™ Membrane Filter 0.2µm GTBP, 25 mm dia.) on top of a Millipore MF support Pad (AP10, 25mm) in a Fisher™ 25 mm diameter glass microanalysis filter (Fisher ™ # 0-9753G), which was covered with foil to reduce light exposure throughout the process. After filtration, the vacuum was stopped and 1 ml sterile PPB gently overlaid on the filter. After 5 seconds, the PPB was vacuum filtered through. FDA working solution (1ml) was added on top of the filter, allowed to sit for 2 min. and filtered through. The filter was immediately adhered to a glass slide with a 20 ul drop of sterile PPB and examined microscopically.

Digital imaging of stained cells was done with a Lieca DMRB Microscope using a 40X long working distance objective with the cover-slide thickness adjustment set to 0.0, with a gfp filter set (480/40 nm excitation and 510 nm LP barrier filters) and UV-blue (360/40 nm excitation and 420 nm barrier filters) to observe FDA and DAPI stained cells respectively. The filter was viewed and a digital image captured (Spot™ RT Slider diagnostic camera with Spot™ version 3.1 image capture software 2000 [Diagnostic Instruments, inc., Sterling Heights, MI]) with the GFP filter cube, a second image was captured with the UV filter cube. The stage was moved randomly and the process repeated until 6 pairs of images were obtained. Image acquisition was completed before visible quenching of the FDA stained cells occurred.

Each pair of images were merged with the uv-cube image used as the source image and GFP-cube image as the active image, and semi-automated counting of the total stained cells was performed using Image Pro Plus version 4.1 software (Media Cybernetics, Silver Springs, MD.). The counted cells were verified and manually recounted if necessary. The active cells, FDA stained (green), were manually counted from the merged image.

The process was repeated for each pair of images and the number of active and total cells averaged across the six image pairs. To determine cells/ml compost tea, the average number of active and total cells per image are multiplied by 3341.995973, to extrapolate the number of stained cells in the area of the camera frame to the exposed filter area (2.1 cm^2), and multiplied by the dilution factor.

Cucumber seedling assay. The *P. ultimum* (isolated from corn roots, Willamette Valley, OR; provided by Beth Hoinacki) inoculum was produced with Ko and Hora's (1971) soil and chopped potato medium, dried, then sieved through a 1-mm^2 grid with the particles retained on a 0.25-mm^2 sieve used. For each drench treatment, 2-L of commercial peat-perlite growing media (Sunshine Mix #1, Sun Gro Horticulture, Inc., Vancouver, B.C.) was thoroughly mixed with 1.0 g *P. ultimum* inoculum in a Twin Shell Dry Blender (Patterson-kelley Co., East Straudsberg, PA) for two minutes. The inoculated media was evenly placed into six 400-ml plastic

nursery pots. Eight cucumber seeds (*Cucumus sativus* cv. Marketmore 76) per pot were sown 1 cm deep. Each compost tea drench was applied to the pots using a fine-spray watering can until the pots were saturated. The six pots for each drench were placed on separate nursery trays that served as experimental blocks. A large, clear, plastic bag was inflated and sealed around each nursery tray to simulate a germination room and maintain even moisture in the pots. The flats were placed in a 20 C growth chamber with 16-hour photoperiod. At 3 and 6 days after planting (DAP) each flat was vented to minimize changes in the atmosphere within the sealed flats. At 9 DAP the pots were assessed for the number healthy seedlings. A seedling was classified as healthy if it was growing normally and had no symptoms or signs of infection. Infection symptoms included a water soaked or yellowing stem, wilted cotyledons, stem lesion leading to seedling collapse; pathogen sign was white mycellia covering any portion of the seedling.

Effect of residual nutrients on disease development. In order test whether the presence of excess nutrients impacted Pythium suppression, nutrient concentration was manipulated in three separate ways. First, ACT was produced with 0.5, 1.0 and 1.5% bacterial fermentation nutrients (Table 5.2). Not all of the available nutrients in these treatments would have been used during the fermentation. Second, ACTs made with or without the fungal fermentation nutrients were amended with 0.1% (v/v) bacterial nutrient solution just prior to being used as a drench. Third, 0.01, 0.04, 0.125, and 0.3% molasses was added (v/v) to ACT made with the fungal fermentation nutrients immediately before drenching. The last two experiments simulated excess nutrients at quantified levels. For each experiment, the drench treatments, along with *P. ultimum* inoculated and non-inoculated peat-perlite controls were applied as above and assessed in the cucumber seedling assay (described above).

Modification for incorporating compost into container media. The three compost sources were tested for the ability to suppress damping-off when incorporated into container media. Compost was mixed with the peat-perlite growing media (1:3, v/v), inoculated with *P. ultimum* (1.0 g/L), and distributed into six 400-ml plastic

nursery pots. Eight cucumber seeds (*Cucumus sativus* cv. Marketmore 76) per pot were sown 1 cm deep. Pots were watered thoroughly, then treated exactly as described above through the assessment of healthy seedlings.

Experimental design and statistical analysis. All cucumber seedling experiments were randomized complete blocks with individual pots as the experimental units. Each experiment had both pathogen inoculated and pathogen free peat-perlite control treatments that were drenched with tap water. Two-way analysis of variance was performed with treatment and block as factors, means were separated from the water drench control using Fisher's protected least significant difference. Linear regression was used to relate compost tea bacterial populations to the percent healthy cucumber seedlings. Percent healthy seedlings for each treatment were calculated by dividing the treatment mean healthy seedlings by the mean healthy seedlings of the non-inoculated peat-perlite standard in each seedling assay. All statistical analysis done with Statgraphics 4.0 software (Manugistics, Rockville, MD).

RESULTS

Chemical and biological properties of compost and compost teas. The pH, EC, and culturable populations of bacteria, yeast, and filamentous fungi varied across the three compost sources (Table 5.1). The average dissolved oxygen content across all ACT was 8.0 ppm. For the yard trimmings compost, the average dissolved oxygen content of NCT without added nutrients was 6.4 ppm, significantly lower than ACT without added nutrients ($P<0.0001$). Adding either the bacterial or fungal fermentation nutrients to NCT decreased dissolved oxygen to 0.2 ppm. For all tea preparations, fermentation vessel temperatures closely followed ambient temperatures (data not shown). When all ACT samples are grouped by fermentation nutrient, the average pH of no nutrients (pH 7.40), bacterial nutrient (pH 7.94), and fungal nutrients (pH 8.56) are significantly different from each other ($P<0.0001$). The EC of all ACT produced without nutrients (EC 0.40 ds/m) was significantly lower ($P<0.0001$) than both ACT made with the bacterial nutrient (EC 1.23 ds/m) and with the fungal nutrient (EC 1.02 ds/m).

Microbial communities of both NCT and ACT were predominately bacteria (Table 5.1), with most bacteria occurring as individual planktonic cells (data not shown). The average population of culturable bacteria, active bacterial cells, and total bacterial cells increased with the addition of fermentation nutrients across compost sources (Table 5.1). While fungal populations were significantly lower than the source compost in all tea preparations, the highest fungal cfu were recovered from NCT fermented with the bacterial nutrient solution. A surface mat of organic material and microbial biomass partially consisting of sporulating filamentous fungi formed in these open, static fermentation vessels. However, there were no significant differences (P=0.46) in the median population of culturable fungi between the bacterial nutrient and fungal nutrient amended compost teas across all ACT samples (Table 5.1). In both NCT and ACT made without fermentation nutrients, the average culturable populations of yeast (cfu/ml) were at least 250 times lower than the source compost (cfu/dry g), while average fungi (cfu/ml) were at least 1000 times lower (Table 5.1). Adding the bacterial fermentation nutrients consistently increased the average yeast population in relation to not adding fermentation nutrients (Table 5.1).

The bacterial population of compost tea measured as cfu's, active cells, or total cells is influenced by the use of aeration and fermentation nutrients during production (Fig. 1). Cfu populations are generally statistically equal or significantly greater (P<0.05) than populations measured as active or total cells when nutrients are added to ACT.

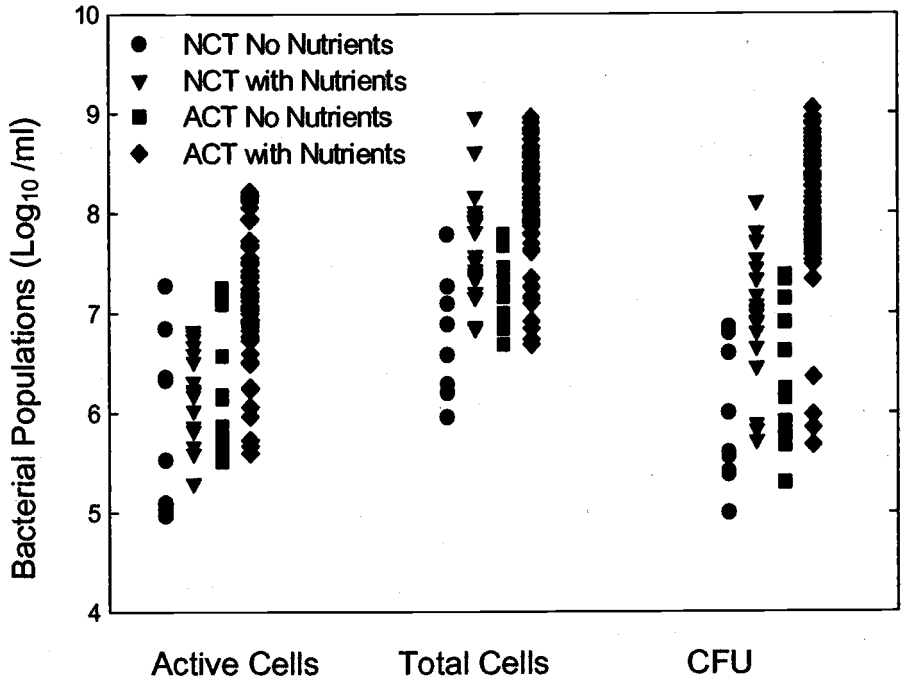

Figure 5.1. The influence of aeration and fermentation nutrients during compost tea production on bacterial population measurements. NCT- not aerated, ACT- aerated. Fermentation nutrients include combinations of 0.12% w/v soluble kelp, 0.25% v/v humic acids, 0.30% glacial rock dust or 0.5%,1.0%, and 1.5% v/v bacterial nutrient solution (Soil Soup, Inc, Edmonds, WA). Active and total cells determined by direct counting after staining with Fluorescein diacetate and DAPI, respectively. CFU determined on 5% TSBA media with 100 ppm cycloheximide.

For both ACT and NCT made without added fermentation nutrients, the average bacterial cfu population is equivalent ($P>0.1$) to the active cell population, while the total cell population is significantly greater ($P<0.001$) than the cfu population (Table 5.3).

Table 5.3. Comparison of methods to estimate bacterial populations in compost tea[g]

			Bacterial Populations (Log10)			Paired t-test *P*-value	
Aeration[a]	Fermentation Nutrients[b]	n[c]	cfu[d]	active cells[e]	total cells[f]	bacterial cfu vs. active cells	bacterial cfu vs. total cells
ACT	none	15	6.8 (6.9)	6.6 (6.8)	7.4 (7.3)	0.12	0.0002
ACT	bacterial	20	8.7 (8.5)	7.6 (7.7)	8.4 (8.3)	0.0001	0.005
ACT	fungal	24	8.0 (8.1)	7.4 (7.6)	8.3 (8.4)	0.0001	0.23
ACT	rock dust or humic acids	4	6.0 (5.9)	5.8 (5.4)	6.9 (6.5)	0.16	0.02
ACT	kelp +/- humic acids	4	7.7 (7.2)	6.3 (6.1)	7.6 (7.4)	0.01	0.41
NCT	none	10	6.3 (6.4)	6.5 (6.8)	7.1 (7.3)	0.69	0.0004
NCT	bacterial	10	7.1 (7.2)	6.2 (6.1)	8.2 (8.4)	0.01	0.0001
NCT	fungal	10	7.5 (7.6)	6.5 (6.4)	7.9 (8.1)	0.0007	0.001

[a] ACT-aerated compost tea; NCT-non aerated compost tea
[b] Fermentation nutrients from Table 5.2
[c] Sample size
[d] Cfu per ml compost tea; media 5% TSBA amended with 100 ppm cycloheximide
[e] Fluorescein diacetate stained after membrane filtration
[f] 4,6-diamidino-2-phenylindole (DAPI) stained after membrane filtration
[g] All compost teas made with yard trimmings compost, vermicompost, or tea compost; not all samples were used in the *P. ultimum* assays presented in this study.

However, NCT made with either the bacterial or fungal fermentation nutrients has significantly ($P<0.001$) greater total populations and significantly ($P<0.01$) lower active populations compared to cfu populations (Table 5.3). With active aeration and addition of fungal nutrients, the total cell population equals ($P>0.1$) the cfu count; with the bacterial nutrients the cfu count is greater ($P<0.05$) than the total cell count (Table 5.3). ACT produced with components of the fungal nutrients indicates that adding kelp with or without the humic acids results in a similar relationship of bacterial cfu to active and total cells as compared to the full fungal recipe (Table 5.3).

Damping-off suppression by compost amended container media or compost tea drench. When the yard trimmings compost, tea compost, or vermicompost was mixed (1:3 v/v) with the peat-perlite media, damping-off caused by *P. ultimum* was not consistently suppressed over repeated bioassays (Table 5.4).

Table 5.4. Frequency of significant disease suppression and average healthy seedlings. Incorporated compost or compost tea drench.

Compost[a] or Compost Tea Drench[b]	Fermentation Nutrient[d]	Yard trimmings Compost		Tea Compost		Vermicompost	
		Disease Control[e]	Healthy Seedlings[f]	Disease Control	Healthy Seedlings	Disease Control	Healthy Seedlings
compost incorporated 25% by volume[a]		1-3	2.44 (2.27)	0-2	0.91 (0.35)	0-3	2.50 (0.44)
NCT[c]	none	0-5	1.65 (0.37)	nd[g]	nd	1-1	4.83
NCT	bacterial	4-5	4.33 (1.84)	nd	nd	0-1	2.66
NCT	fungal	3-5	3.10 (1.30)	nd	nd	1-1	6.33
ACT[c]	none	1-6	2.31 (0.51)	nd	nd	1-3	3.28 (0.48)
ACT	bacterial	3-9	3.14 (1.53)	0-2	2.92 (.59)	3-4	3.27 (1.99)
ACT	fungal	13-13	4.85 (0.77)	2-2	5.25 (1.06)	4-4	4.76 (0.97)
ACT	bact-fungal	0-2	2.33 (2.59)	nd	nd	0-1	2.33
Water control		0-22	1.38 (0.58)				
Pythium free		22-22	6.92 (0.50)				

[a] Compost mixed with peat-perlite media (1:3, v/v); inoculated with *P. ultimum*, water drenched.

[b] Drenches applied to 100% peat-perlite media inoculated with *P. ultimum*.

[c] NCT-non aerated compost tea; ACT-aerated compost tea.

[d] Listed in Table 5.2.

[e] Ratio of significantly suppressive trials over repeated assays (LSD, *P* 0.05, compared to water control).

[f] Mean number of healthy seedlings averaged over assay trials (standard deviation in parenthesis). Healthy seedlings for each assay determined as the average of six pots each seeded with eight cucumber seeds grown in *P. ultimum* infested potting mix.

[g] Not done.

In contrast, compost teas made from the conducive compost resulted in damping-off suppression that ranges from not suppressive to consistently suppressive depending on the method used to produce compost tea. Both NCT and ACT made without added nutrients did not consistently suppress damping-off (Table 5.4). Using either the bacterial or fungal fermentation nutrients in NCT produced with the yard trimmings compost increased the average number of healthy cucumber seedlings and the proportion of suppressive trials over repeated bioassays compared to NCT without added nutrients (Table 5.4). ACT made with any compost and fungal nutrients suppressed damping-off in 19 out of 19 bioassays, while ACT made with the bacterial nutrients was suppressive in six out of 15 repeated *P. ultimum* bioassays (Table 5.4). ACT produced with a combination of bacterial and fungal nutrients was not suppressive over three trials. Damping-off suppression is not limited to peat-perlite growing media. Suppression has been observed with ACT fermented with fungal nutrients in composted fir bark:peat-perlite growing media (1:1 v/v) that was inoculated with *P. ultimum* (data not shown).

Linear regression analyses of the percent healthy seedlings of ACT (including no nutrients, bacterial nutrients, or fungal nutrients) against bacterial cfu, active cells, and total cells explained only 13.2, 7.07, and 7.77% of the variation, respectively. Likewise, for ACT made with the bacterial nutrients, regression analyses indicates that there is no relationship ($P>0.1$) between percent healthy seedlings and the bacterial population (Fig. 5.2).

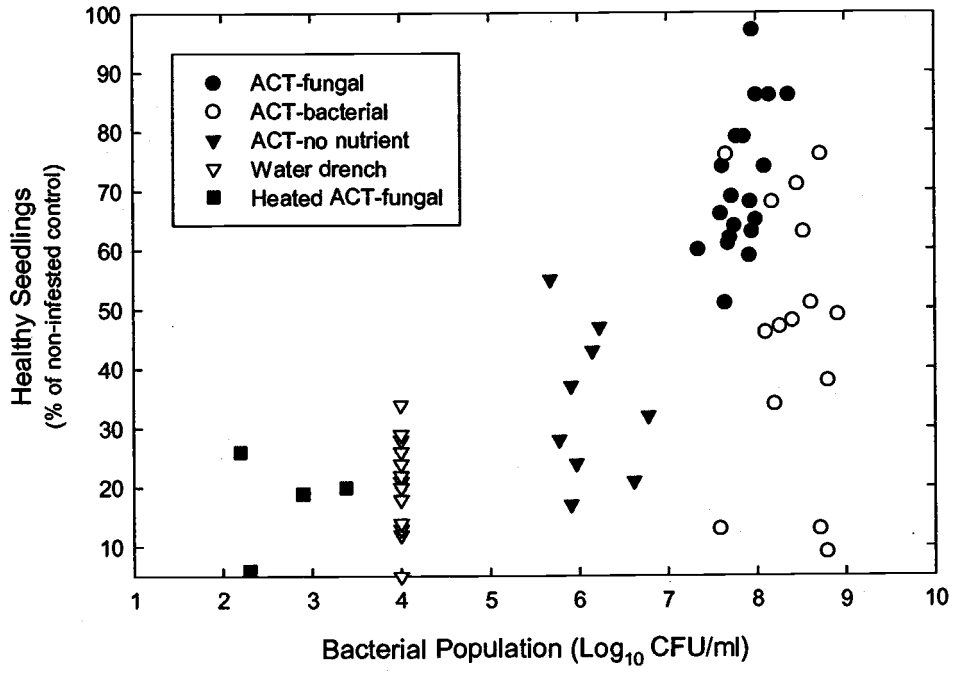

Figure 5.2. Relationship of the average number of healthy cucumber seedlings to bacterial population of aerated compost teas (ACT). Compost teas produced with different fermentation nutrients (Table 5.2). Heated ACT-fungal treatments were heated to 95 C for 30 minutes then cooled to 25 C prior to drenching. Bacteria enumerated on 5% TSBAcyc[100]. The water drench control treatments are all placed at the average cfu recorded from four tap water samples.

For the ACT in Figure 5.2, the bacterial populations metrics are related to each other; linear regression of cfu and total cells is highly significant ($P<0.0001$, $R^2=0.67$); linear regression of cfu and active cells is highly significant ($P<0.0001$, $R^2=0.77$). Therefore, a similar relationship exist between the percent healthy seedlings and all of the bacterial populations metrics. When the bacterial populations of all ACT's, except samples made with bacterial nutrient solution, are considered in relation to disease suppression, a more apparent pattern develops (Fig. 5.3).

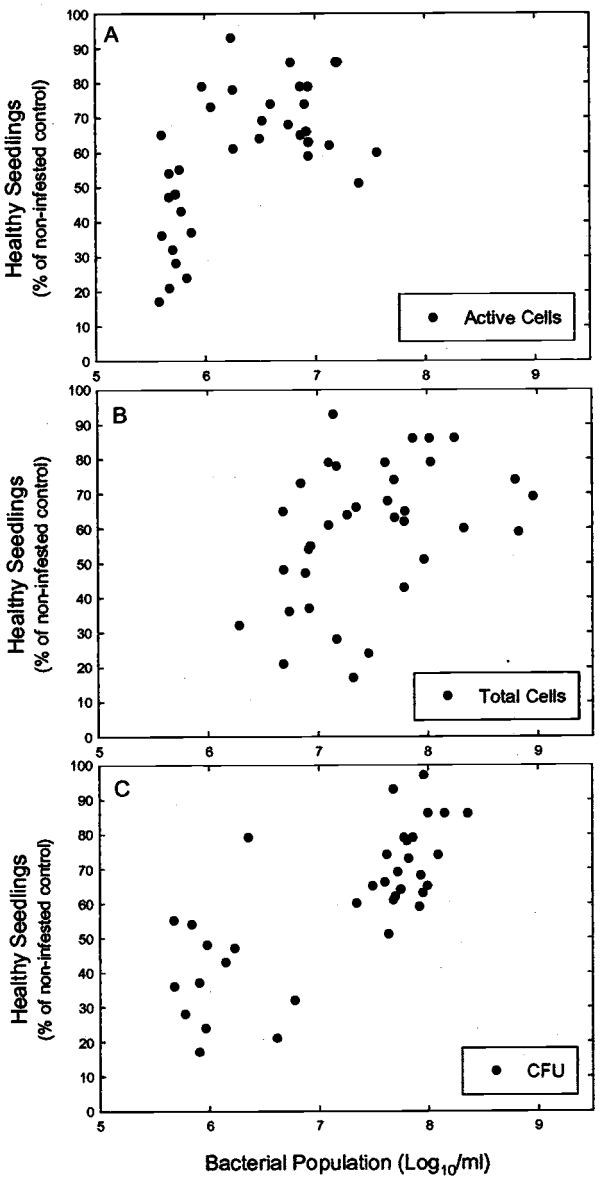

Figure 5.3. Relationship of the bacterial population of aerated compost tea (ACT) used to drench growing media to the average number of healthy cucumber seedlings in a *P. ultimum* damping-off bioassay. All ACT fermented with no nutrients, single components or combinations of the fungal nutrient mixture. These components are 0.12% w/v powdered soluble kelp, 0.25% v/v humic acids, 0.30% w/v glacial rock dust. **A)** Active bacterial cells measured by staining with fluorescein diacetate. **B)** Total bacterial cells measured by staining with DAPI. **C)** Bacterial cfu cultured on 5% trypticase soy broth agar amended with 100 ppm cycloheximide.

For the 18 NCT samples presented in Table 5.4, no positive linear relationship exists between the average number of healthy seedlings and any bacterial population metric. Linear regression of healthy seedlings and bacterial cfu, total cells, and active cells have R^2 of 0.13, 0.08, and 0.00001 respectively.

Heating NCT or ACT to 95-98 C for 30 minutes and cooling to 25 C before drenching significantly ($P<0.05$) increases damping-off compared to unheated compost tea (Table 5.5). Diluting compost tea 1:9 (v/v) with tap water significantly ($P<0.05$) reduced disease suppression in most trials, with intermediate dilution rates having variable reductions in suppression (Table 5.5).

Table 5.5. Effect of diluting and heating compost tea on seedling health

Compost[a]	Aeration[b]	Nutrients[c]	Seedling Health[d]	Dilute 1:1[e]	Dilute 1:4	Dilute 1:9	Heat treated[f]
Yard trimmings	ACT	None	36.8 b				15.8 a
Yard trimmings	ACT	Bacterial	51.2 b				0.0 a
Yard trimmings	ACT	Fungal	73.6 b				7.9 a
Yard trimmings	ACT	Fungal	61.5 b				20.5 a
Yard trimmings	ACT	Fungal	86.0 b				25.6 a
Yard trimmings	ACT	Fungal	63.6 b	34.1 a	31.8 a	25.0 a	
Yard trimmings	ACT	Fungal	96.9 b	91.1 b	70.3 ab	44.2 a	
Vermicompost	ACT	Fungal	74.3 cd	52.3 abc	40.4 abc	33.3 ab	19.0 a
Yard trimmings	NCT	Bacterial	70.4 b			15.1 a	
Yard trimmings	NCT	Bacterial	85.3 b			10.4 a	18.3 a
Yard trimmings	NCT	Bacterial	86.9 b				39.5 a
Yard trimmings	NCT	Fungal	57.8 b			49.9 b	13.1 a

[a] Compost source described in Table 5.1.
[b] ACT-aerated compost tea; NCT-non aerated compost tea.
[c] Added nutrients described in Table 5.2.
[d] Healthy seedlings scaled to percent of *Pythium* free control treatment. Numbers in same row followed by the same letter are not significantly different (p=0.05, Duncan's multiple range test).
[e] Compost tea diluted with tap water at given ratio.
[f] Compost tea heated to 95-98 C for 30 minutes, then cooled to 25 C for drenching.

Enhancing disease development with bacterial nutrient solution. When producing ACT, increasing the bacterial nutrient solution concentration from 0.5% to 1.0% or 1.5% (v/v) significantly ($P<0.05$) decreased the number of healthy cucumber seedlings (Fig. 5.4).

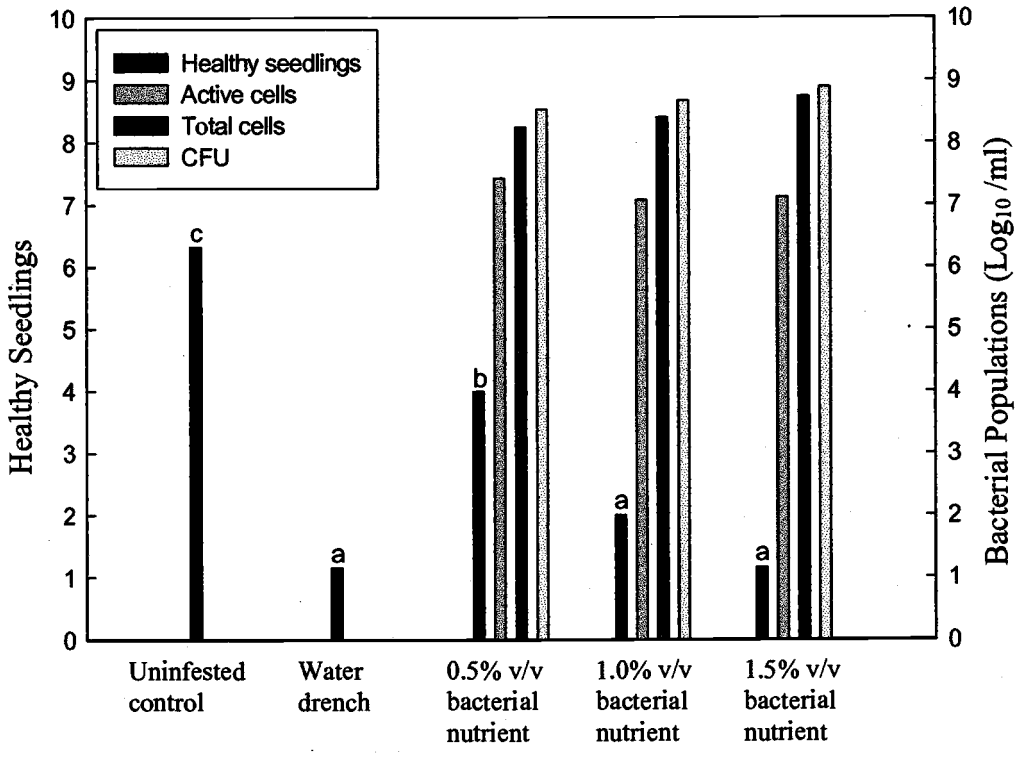

Figure 5.4. Effect bacterial fermentation nutrient concentration (Soil Soup, Inc., Edmonds, WA) used to make aerated compost tea on seedling health and bacterial populations. Active and total cells determined by direct counting after staining with Fluorescein diacetate and DAPI, respectively. CFU determined on 5% TSBA media with 100 ppm cycloheximide. Healthy seedlings determined as the average of six pots each with eight seeds. Letters labeling healthy seedlings indicate different numbers, treatments with the same letter are not significantly different ($P=0.05$, Duncan's multiple range test).

The experiment was performed twice with similar results. Mixing 0.1% (v/v) bacterial nutrient solution with suppressive ACT just before drenching negated suppression (Fig. 5.5).

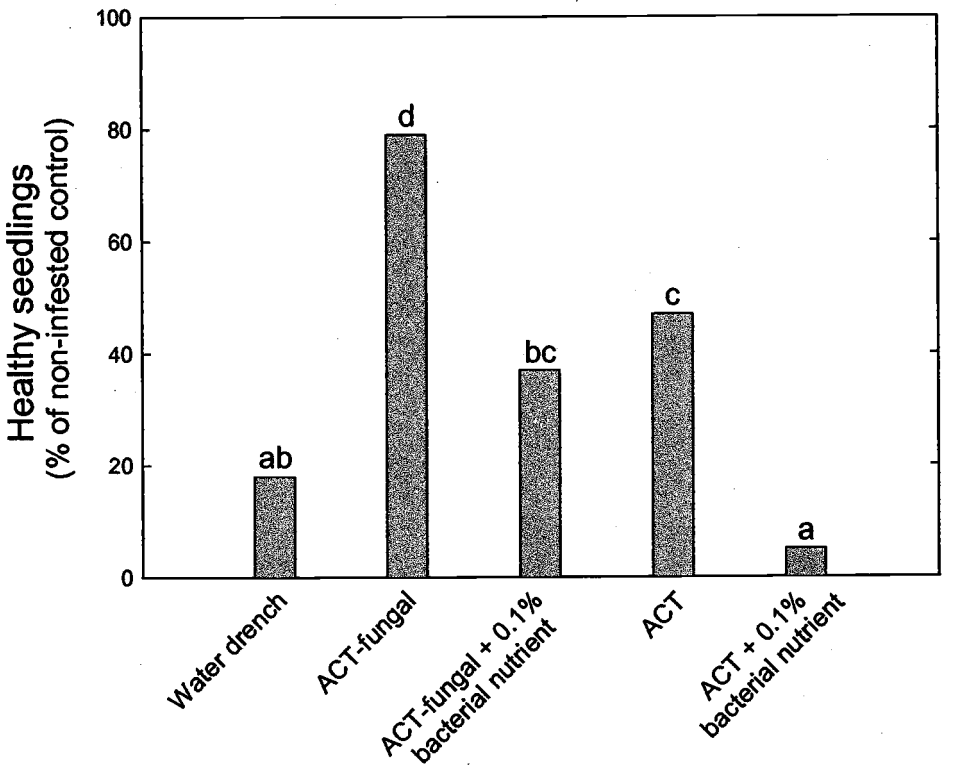

Figure 5.5. Effect on seedling health of tank mixing molasses-based nutrient solution to aerated compost tea (ACT). ACT drenched onto *P. ultimum* infested peat-perlite growing media. ACT-fungal fermented with 0.12% w/v soluble kelp, 0.25% v/v humic acids, and 0.30% w/v glacial rock dust. Columns with the same letters are not significantly different (P=0.05, Duncan's multiple range test).

Since molasses constitutes one-third of the bacterial nutrient solution by volume, the role of unused molasses in enhancing disease development was investigated. Mixing from 0.01% to 0.3% molasses with suppressive ACT just before drenching significantly ($P<0.05$) reduced suppression (Figure 6). In the absence of *P. ultimum*, the healthy seedling resulting from drenching with either bacterial nutrient

solution (0.5%) or molasses (0.3%) in water or compost tea was not different than drenching with 100% water (data not shown).

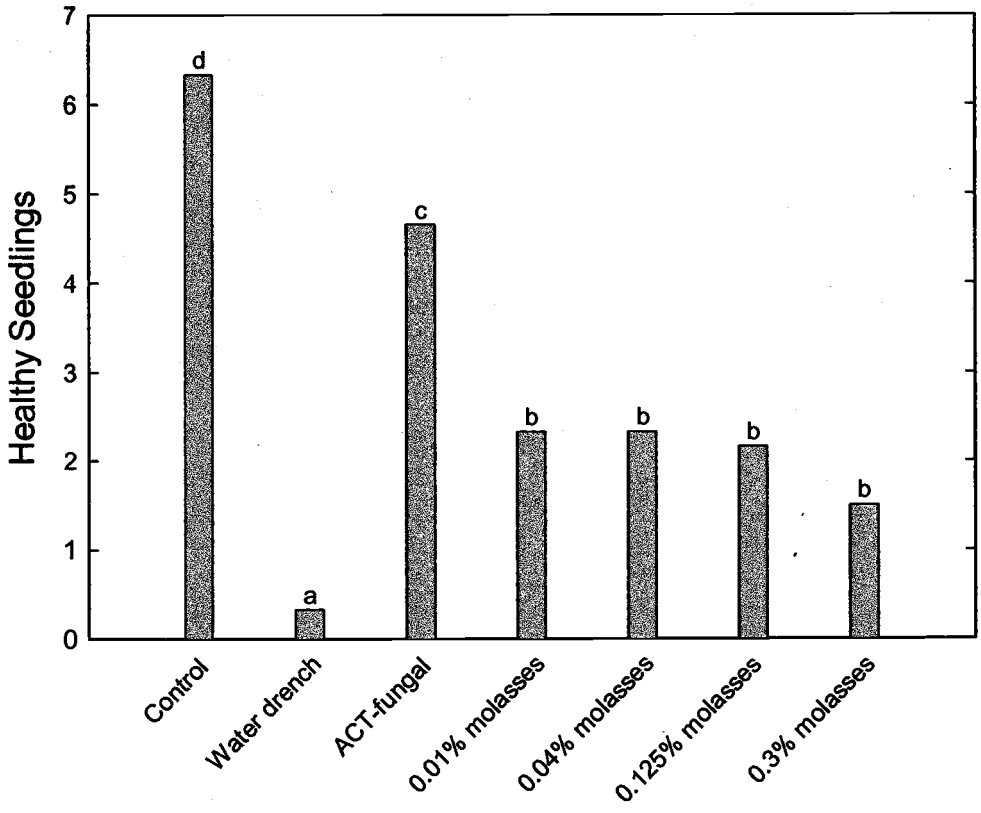

Figure 5.6. Effect on seedling health of molasses additions to aerated compost tea (ACT) prior to drenching onto *P. ultimum* infested peat-perlite growing media. ACT made with fungal fermentation nutrients (see Table 5.1). Healthy seedlings determined as the average of six pots seeded with eight seeds each. Columns with the same letters are not significantly different (p=0.05, Duncan's multiple range test).

DISCUSSION

Compost tea can effectively suppress *P. ultimum* damping-off of cucumber in soilless container media. Both ACT and NCT significantly reduced disease when fermentation nutrients were added. The most consistent formulation for damping-off suppression was ACT fermented with kelp, humic acids, and rock dust (termed fungal

nutrients). The suppressiveness of this formulation was significantly reduced by heat treating or diluting ten fold with tap water. Further characterization indicated that the rock dust component is not necessary for damping-off suppression and caused excessive wear on mechanical parts (data not shown). Suppression conferred with the fungal nutrients was independent of the compost source used in ACT production. These compost did not consistently suppress damping-off when directly incorporated into peat-perlite container media (1:3 v/v; Table 5.4). Thus indicating that the selection of fermentation nutrients is more critical than the source of compost for producing suppressive ACT.

Across all compost tea samples, there was not a significant relationship of bacterial populations, determined by active, total or cfu methods, to disease suppression. Although, for all ACT produced without the molasses fermentation nutrient, there does appear to be a threshold of bacterial population level above which compost teas are suppressive. For this group of ACT, the transition from non-suppressive compost tea drenches to suppressive drenches appears to occur at 1×10^6 active bacterial cells/ml (Fig. 3A). The relationship of total bacterial cells to disease suppression is less clear, however, 15 of 16 samples with greater than 3×10^7 total cells/ml have average healthy seedlings above 50% of the non-infested control (Fig. 3B). Nearly all suppressive samples have greater than 1×10^7 cfu/ml bacteria (Fig. 3C).

Since the ACT fermented with fungal nutrients has a pH of 8.5, the damping-off - suppression observed in this study could have been due to reduced saprophytic activity of *Pythium* by increasing the growing media pH. Increasing soil pH to 8.5 can cause increasing release of NH_4, which is known to suppress *Pythium* spp. activity (Martin and Loper, 1999). However, it is unlikely that disease suppression is primarily due to the pH, since drenching with either the fungal nutrients mixed in water (pH 8.6), or heat treated fungal nutrient ACT (pH 8.5) resulted in the same disease incidence as drenching water. In addition, all growing media had a pH of 6.3 to 6.5 at the end of the 9-day cucumber bioassay (data not shown). Laboratory

analysis of the well-cured yard trimmings compost indicated only 13 ppm NH_4, therefore, less than 1 ppm NH_4 from the compost would be in solution after diluting approximately 25-fold v/v with water in compost tea production. Lastly, ACT made with a combination of the fungal and bacterial nutrients had a pH of 8.4 and did not suppress damping-off.

There are several possible explanations why repeated trials using ACT made with the molasses-based bacterial nutrient solution had erratic damping-off suppression. The most probable explanation is that residual nutrients, most likely sucrose, varied across compost tea batches and this enhanced *Pythium* propagule germination and growth or reduced competition for seed exudates, resulting in enhanced pathogen growth and infection. It is generally accepted that bacteria can indirectly protect seeds against *Pythium* infection by metabolizing seed exudate stimulants (Dijk and Nelson, 1998) and that competition for available nutrients is a likely mechanism of soil borne pathogen suppression if adding nutrient amendments negates suppression (Lockwood, 1977). Evidence supporting this conclusion includes the inverse relationship between the concentration of bacterial nutrient solution used in ACT production and disease suppression (Fig. 4); ACT produced with increasing concentrations of sucrose as the sole fermentation nutrient corresponded to increased damping-off disease levels (data not shown); and the addition of as little as 0.01% molasses or 0.1% bacterial nutrient solution to suppressive ACT significantly increased damping-off (Fig. 5 and 6). Similarly, suppression of cucumber seedling damping-off caused by *P. ultimum* in compost amended container media was negated by adding 0.375 % sucrose and 0.075% asparagine to the growing media (Chen *et al*, 1988b).

Residual fermentation nutrients could also suppress antibiotic production or parasitic activity in favor of saprophytic metabolism. This would affect bacteria capable of producing antimicrobial compounds that reduce *Pythium* germination through fungistatic or fungicidal effects (Dijk and Nelson, 1998). Excessive nutrients has been shown to reduce hyphal lysing of *P. aphanidermatum* by antagonistic

bacteria in separated cattle manure-compost media (Mandelbaum and Hadar, 1990). Amending the container media with a glucose-asparagine mixture (3.36:1 w/w, amended at 0.5% wet weight) reduced hyphal lysis to 18% over a 24 h period compared to 80% lysis for non-amended media (Mandelbaum and Hadar, 1990). Additionally, drenching the glucose-asparagine mixture (1% solution in water) onto the compost media negated cucumber damping-off suppression (Mandelbaum and Hadar, 1990).

In addition, the use of simple sugars in producing compost tea has been linked to growth of *E. coli* in aerated compost tea makers (Bess *et al*, 2002). Other work by Duffy (*et al*, 2002) determined that *Salmonella enterica* and *E. coli* O157:H7 did not grow when inoculated into flasks containing 20 g compost and 180 ml sterile water; however, incremental additions of molasses was related to increasing growth of both organisms.

While further work is needed to directly quantify the residual sucrose concentrations in ACT produced with molasses-based fermentation nutrients to determine the effect on damping-off suppression, it is clear that the use of simple sugars as fermentation nutrients should be avoided when making compost tea.

Initial research on the use of compost teas for plant disease suppression utilized NCT that was generally produced without fermentation nutrients (Weltzien, 1991); however, recent attention has advocated for the use of aeration and fermentation nutrients (Ingham 2000). Evidence is provided here that aeration is not necessary to produce a suppressive compost tea drench. For both NCT and ACT, fermentation nutrients were needed for suppression. The choice to use aeration is mostly influenced by issues of production timing and potential putrescent odors associated with NCT made with fermentation nutrients. It could be possible to produce suppressive NCT without adding fermentation nutrients by reducing the water to compost ratio and selecting compost with greater microbial populations. NCT produced by maintaining water to compost ratios of 5:1-8:1, using compost with

greater microbial populations, and avoiding fermentation nutrients has suppressed numerous foliar diseases when applied topically. (Scheuerell and Mahaffee, 2002).

The use of aeration and fermentation nutrients in compost tea production appears to determine the proportion of the total bacterial population that is culturable. For NCT produced with or without added nutrients, the population of total bacterial cells is significantly greater than bacterial cfu, indicating the majority of cells would not grow under the culturing conditions. The same is true for ACT made without nutrients or when ACT fermented with rock dust or humic acids are considered as a group. Aggregation of bacterial cells could be a cause for lowered cfu counts, however, extensive aggregation was not evident when observing samples for total and active cell enumeration (data not shown). For the above types of compost tea, the bacterial cfu population underestimates the total bacterial cell population and the dominant bacterial types might not be readily culturable. However, in ACT made with the bacterial or fungal nutrients, bacterial cfu are statistically equivalent or greater than the total bacterial cells. Thus it appears that ACT made with either the bacterial or fungal fermentation nutrients selectively increase culturable bacteria. These findings suggest that the simpler cfu counting procedure can be used to monitor the total bacterial population in ACT produced with readily available fermentation nutrients, and that it is possible to readily culture the dominant bacterial strains from these compost teas.

Further understanding the quantitative relationship between compost tea microbial populations and disease threshold levels could help develop guidelines for producing suppressive drench formulations similar to the guidelines for minimum levels of microbial activity and biomass in compost amended container media for the suppression of *P. ultimum* damping-off (Chen et al 1988a). Information on the duration of suppression afforded by drenching container media with compost tea would also be useful to develop an application schedule for long-term suppression of Pythium damping-off in commercial production. Further experimentation on a

production scale is needed to develop this method into an effective disease control tool.

ACKNOWLEDGEMENTS

We acknowledge Soil Soup, Inc. for supplying Bio-Blenders for producing aerated compost tea. Ben Short provided superb technical assistance. Beth Hoinacki graciously provided the *P. ultimum* culture. Funding from USDA CRIS # 303-5358-22000-024-00D and the Oregon association of Nurserymen and Oregon Department of Agriculture provided for the research.

Chapter six: Suppression of Seedling Damping-off Caused by *Pythium ultimum*, *Pythium irregulare*, and *Rhizoctonia solani* in Compost Amended Container Media

ABSTRACT

Suppression of seedling damping-off disease caused by *Pythium* spp. and *Rhizoctonia solani* is a potential benefit of amending soilless container media with compost. Thirty six compost samples from Pacific Northwest commercial composting facilities were analyzed for a number of physical, chemical, and biological properties, including suppression of damping-off caused by *P. ultimum*, *P. irregulare*, and *R. solani*. The samples were produced from diverse feedstocks and composting technologies, this was reflected in a large degree of variability in the measured properties. When mixed with sphagnum peat moss and inorganic aggregates, 66% of the samples significantly suppressed *P. irregulare* damping-off of cucumber, 64% suppressed *P. ultimum* damping-off of cucumber, and 17% suppressed *R. solani* damping-off of cabbage. *Pythium* damping-off suppression was related to the potential of compost to support microbial activity and a qualitative index of ammonia volatilization. *R. solani* damping-off suppression was not related to any one compost factor. Currently available compost products could potentially provide acceptable control of damping-off caused by *Pythium* spp. while the need for inoculation with microbial antagonists for the control of *R. solani* is indicated.

INTRODUCTION

The use of sphagnum peat-based container media for germinating seedlings is a standard greenhouse industry practice (Hartmann *et al*, 1990). With peat-based container media, fungicides are routinely used in commercial greenhouses to manage damping-off disease of seedlings, commonly caused by *Pythium* spp. and *Rhizoctonia solani* (Stephens and Stebbins, 1985). While some peat sources that suppress damping-off have been identified (Tahvonen, 1982: Wolffhechel, 1988), the suppressive effect is variable and short in duration depending on the degree of peat decomposition (Boehm and Hoitink, 1992, Inbar *et al*, 1991).

Damping-off disease caused by *Pythium* spp. and *Rhizoctonia solani* can be suppressed by incorporating compost into soilless container media (Hoitink and Fahey, 1986; Hoitink *et al*, 1993). Suppression of damping-off caused by *Pythium* spp. has been associated with compost made from a broad range of raw feedstocks, with suppression related to the level of microbial activity and biomass in compost amended container media (Chen *et al*, 1987; Chen *et al*, 1988a; Craft and Nelson, 1996; Grebus *et al*, 1994; Hadar and Mandelbaum, 1986; Kuter *et al*, 1988; Mandelbaum *et al*, 1988; Schüler *et al*, 1989; Theodore and Toribio, 1995). Suppression of damping-off caused by *R. solani* has not been related to the total level of microbial activity or biomass in compost amended container media, but has been related to the presence of specific microbial antagonists (Nelson *et al*, 1983, Kuter *et al*, 1983; Kwok *et al*, 1987). For damping-off suppression with compost amended container media, the majority of studies have focused on a specific source of compost while fewer studies have surveyed multiple compost types produced at various locations (Craft and Nelson, 1996; Erhart and Burian, 1997; Ringer *et al*, 1997). Craft and Nelson (1996) found a strong correlation between microbial activity and damping-off suppression of *P. graminicola* across a wide variety of compost types. Erhart and Burian (1997) found that *P. ultimum* suppression across 21 composts, made primarily from source separated household organic waste, correlated to compost organic matter content. Ringer (*et al*, 1997) determined that *P. ultimum* suppression varied across three types of manure based compost when used in container media. Dairy manure compost was most suppressive, steer manure compost was intermediate, and poultry litter compost was least suppressive (Ringer *et al*, 1997). In the same study, the level of *R. solani* suppression did not relate to the type of compost (Ringer *et al*, 1997).

Compost amended container media has not been widely adopted by the Pacific Northwest commercial greenhouse industry. For this geographical region, there is a lack of information on compost quality characteristics, including the potential for suppressing damping-off disease. Goals of this study were to 1) develop a database of physical, chemical and biological properties of compost produced from diverse

feedstocks at commercial facilities; 2) determine if damping-off caused by *P. ultimum*, *P. irregulare* and *R. solani* is suppressed by container media amended with these composts; 3) determine if *R.solani* suppression is biological in nature; and 4) relate damping-off suppression to a database of compost properties.

MATERIALS AND METHODS

Compost sample collection. Thirty compost samples were collected at commercial compost facilities between May and September, 2000. Most samples (28) were from Western Oregon; two samples were transported from Washington. An additional six compost samples were also included in this study that were not collected during May-September, 2000. They were collected prior to the initiation of the study and had been stored at 4 C for 2 to 8 months. Compost products that had been screened to remove large particles and were considered ready-for-sale by the facility operator were sampled. Compost piles were sampled by removing five subsamples, subsamples were 0-4 foot deep composites (15 L), all placed in an 80 L plastic bin. Compost was thoroughly mixed in the bin with a shovel, then covered and transported to the laboratory. At the time of sampling, compost feedstocks, pile dimensions, turning frequency, compost age and additional processing and process control information was collected for each sample.

All sample processing, analyses, and initiation of seedling damping-off assays was completed within 24 hours of compost sampling. Compost was screened to pass 6.4 mm mesh (0.25 inch) for all analysis and seedling assays. Remaining compost was then stored at 4 C.

Compost physical properties. Compost temperature was recorded at 12 and 48 inch depths from three locations in the compost at the time of sampling. Water content was determined by drying 50 g compost for 36 h at 70 C in a forced air oven (Leege and Thompson, 1997). Particle size distribution (wet weight basis) was determined by passing 1000 g compost through 1/2", 1/4", 4mm and 2mm sieves.

Compost chemical properties. Compost pH was determined from a saturated paste with a portable pH meter (model 150, IQ Scientific Instruments) and electrical conductivity (EC) was determined from a 2:1 (v/v) of distilled water: compost (Leege and Thompson, 1997) using a portable EC meter (model 933100, Hanna Instruments).

Chemical analysis of compost samples was performed by the Oregon State University Central Analytical Laboratory using modified soil and plant analysis methods (Gavlak et al., 1994). Ammonium-N and NO_3-N were extracted from fresh (not dried) compost using 2M KCl. Ammonium-N and NO_3-N were determined using automated colorimetric methods. Compost was dried at 60 C and ground to pass 2 mm screen prior to other chemical analyses. Total C, N, and S were determined using high-temperature furnace oxidation and subsequent direct measurement of C and N by an infared detector (LECO Instruments Model CNS 2000, LECO Instruments, St. Joseph, MI; Sweeney, 1989). Other elements were determined after extraction or digestion using an inductively coupled plasma spectrophotometer (ICP). Exchangeable bases (Ca, Mg and K) were extracted from the compost sample with 1M ammonium acetate at a pH of 7. For total P analysis, the sample was digested in acid (0.03 N NH_4F and 0.025 N HCl). Iron and Mn were extracted with a chelate solution (0.025 M DTPA).

An index of compost ammonia volatilization was determined with Solvita Compost Maturity Test kits (Woods End Research Laboratory, Inc., Mt. Vernon, Maine) according to the manufacturer's instructions (Woods End Research Laboratory, 2002). A reading of "5" indicates very low NH_3 and no phytotoxicity

potential from ammonia gas (Woods End Research Laboratory, 2002). The intensity of ammonia detected increases as Solvita NH3 test score decreases from 4 to 1.

Compost respiration. Compost respiration was assessed with the Solvita Compost Maturity Test Kit (Woods End Research Laboratory, 2002).

Compost respiration potential was estimated by measuring CO2 evolution during a 7-day incubation in moist Puyallup fine sandy loam topsoil (collected from 0-15 cm depth; coarse-loamy over sandy, mixed, mesic Vitrandic Haploxerolls). A 7-day incubation is routinely used to estimate decomposition rates of organic materials in soil (Gilmour et al., 1996). Compost samples were held approximately 90 days at 4 C prior to respiration potential testing. Five of 36 samples were excluded from respiration testing because they were not refrigerated after collection.
Compost respiration potential tests were performed at 25 °C in sealed 0.9 L (1qt) mason jars. Air-dry soil was prepared for incubation by misting to a moisture content of 220 g kg^{-1}. Moist compost samples (8 g) were mixed into 50 g soil (dry wt basis) for an approximate incorporation rate of 80 g compost per kg soil (dry wt basis). During incubation, CO_2 was collected in vials containing 40 mL of 1M NaOH. For CO_2 determination, the carbonate trapped in NaOH was precipitated with excess $BaCl_2$, and the remaining NaOH was back-titrated with standardized 0.1 M HCl, using phenolphthalein as the indicator (Anderson, 1982). Compost respiration rate for the 7-day period was expressed as mg CO2-C per g compost C per day, thereby correcting for differences among compost samples in moisture content and C concentration.

Pythium ultimum and P. irregulare damping-off assay. A modified *P. ultimum* cucumber seedling bioassay was used (Chen *et al*, 1987). The *P. ultimum* (isolated from corn roots, Willamette Valley, OR; provided by Beth Hoinacki, Oregon State University) and *P. irregulare* (provided by Joe Marlow; USDA-ARS Corvallis, OR) inoculum were produced with Ko and Hora's (1971) soil and chopped potato medium, dried, then sieved through a 1-mm^2 grid with the particles retained on a 0.25-mm^2 grid used. Container media was made by mixing equal volumes compost and commercial peat-perlite media (Sunshine Mix #1, Sun Gro Horticulture, Inc.,

Vancouver, B.C.). Either *P. ultimum* or *P. irregulare* inoculum was mixed with 2-L container media in a plastic bag (1.0 g/L), then dispersed into six 400-ml square plastic nursery pots. Each experiment had pathogen inoculated and pathogen free peat-perlite treatments as standards. Each pot was sown with eight cucumber seeds (*Cucumis sativus* 'Marketmore 76') 1-cm deep, then watered to capacity with tap water. Replicate pots were placed on separate nursery trays, the trays served as experimental blocks. A large, clear plastic bag was inflated and sealed around each nursery tray to simulate a germination room and maintain even moisture in the pots. The trays were placed in a 20 C growth chamber with 16-hour photoperiod. At 3 and 6 DAP each tray was vented to minimize changes in the atmosphere within the sealed trays. At 9 DAP pots were assessed for the number of healthy cucumber seedlings. A seedling was classified as healthy if it was growing normally and had no symptoms of infection. Infection symptoms included a water soaked or yellowing stem, wilted cotyledons, stem lesions leading to seedling collapse, and white mycellia covering any portion of the seedling.

Rhizoctonia solani damping-off assay. *R. solani* AG-4 (isolated from poinsettia; provided by Marc Cubeta) was cultured on short grain brown rice that had been soaked in water for 12 hours then autoclaved in 1-L flasks on three consecutive days for 55 minutes. Actively growing hyphae on potato dextrose agar were aseptically transfered onto the rice and incubated in the dark at 22 C for 3 weeks. The rice was transferred to a sterile box, partially covered, and dried over a period of 3 days in a laminar flow hood. Dried rice was ground and sieved, with the 0.50 to 1.00 mm size fraction retained for inoculum. Inoculum was stored at 4 C until used.

Compost-amended container media was prepared by mixing 2 L compost with light sphagnum peat (Lakeland Peat Moss Ltd., Hubbard, OR) and vermiculite (5:4:1, v/v). *R. solani* inoculum was thoroughly mixed into the container media (0.75 g/L). Inoculated media was distributed into four replicate plug transplant tray sections (five plug cell by ten plug cell rows) that had been cut from 200 cell trays (each cell was 2.0 x 2.0 x 4.5 cm; Landmark Plastic Corp., Akron, OH). All cells were sown with one

cabbage seed (*Brassica oleracae* 'Cheers'), covered with 3 mm fine vermiculite, and thoroughly watered with tap water. The plug tray sections were placed in a greenhouse at approximately 23 C under natural light that was supplemented as needed to maintain a 16 h photoperiod. Trays were watered with tap water as needed. Fourteen days after planting (DAP) 24 plug cells (inner three by eight rows) of each tray section were assessed for disease incidence. The number of healthy seedlings, defined as having no visible lesions above the media-air interface, was counted (0-24). In each experiment, peat and vermiculite container media (4:1, v/v), with and without *R. solani* inoculum, were included as standards.

Compost sources that initially suppressed *R. solani* damping-off were assayed again to determine if suppression was consistent. After completion of the damping-off disease assays, three compost sources that consistently suppressed *R. solani* were again sampled and processed as described. A portion of these compost samples and peat:vermiculite media were individually heated with aerated steam to determine if disease suppression was significantly reduced by heating. Pressurized steam and ambient air were proportioned into a modified metal cabinet to maintain either 60 C or 88 C. The samples were heated in perforated metal pans and maintained at the target temperature for 30 minutes once the center of the heating material had attained either 60 or 88 C. After cooling to ambient temperature, the material was used immediately in the *R. solani* damping-off assay, modified to include heated and non-heated material with and without inoculation to ensure that heating did not induce phytotoxicity.

In one experiment, a bark-dairy solids compost was inoculated with *Trichoderma* hamatum (T 382) to determine if suppression could be biologically enhanced. Four L compost was inoculated with T 382 (5 g colonized millet seeds/L compost) five days before mixing the compost with peat and vermiculite (5:4:1 v/v). Another 4 L compost was inoculated with T 382 as described just prior to mixing with peat and vermiculite (5:4:1 v/v). The T 382 inoculated treatments were compared to compost not inoculated with T 382 in the *R. solani* damping-off assay.

Microbiological populations of compost. A 10 g compost sample was added to 90 ml sterile 0.02M potassium phosphate buffer (pH 7.0) in a 250 shaker flask, shaken (300 rpm, 25 C) for 20 minutes, then serially diluted in sterile 0.02M phosphate buffer. Dilutions were plated using an automated spiral plater (Eddy Jet; IUL Instruments, Barcelona, Spain) on selective agar and incubated at 22 C. Bacteria were enumerated on 5% trypticase soy broth agar (TSBA, 1.5 g Difco tryticase soy broth with 15 g agar) with 100 ug/ml cycloheximide. Actinomycetes were enumerated on actinomycete isolation agar (AIA, 20 g Difco actinomycete isolation agar, 5 ml glycerol per L medium, amended with 50 ug/ml cycloheximide. Fungi were enumerated on water agar (WA, pH 6, 18 g agar/L) with 50 ug/ml rifampicin. Yeast were enumerated on dilute, selective yeast media (SYM; 1.5 g yeast extract, 2.5 g peptone, 5 g dextrose, 2.3 g malt agar, 17 g agar per L medium, amended with 100 ug/ml chlroramphenicol, 50 ug/ml ampicillin, 500 ug/ml streptomycin sulfate, and 2 ug/ml dichloran). *Trichoderma* spp. were enumerated on Trichoderma selective media (Elad and Chet, 1983). Populations were recorded as cfu/dry g compost.

Experimental designs and statistical analysis. The damping-off bioassays were randomized complete block experiments with trays serving as blocks and individual pots as the experimental unit. Two-way analysis of variance was performed with container media treatment and block as factors, means were separated using Duncan's multiple range test. For each treatment, the percent healthy seedlings were calculated by dividing the mean number of healthy seedlings in each compost treatment by the mean number of healthy seedlings in the non-inoculated peat-perlite standard in each seedling assay. The measured physical, chemical, and biological properties were individually regressed against the percent healthy cucumber seedlings for each pathogen.

The percent healthy seedlings for each pathogen were grouped according to presence or absence of detectable ammonia volatilization. The groups were compared

with a T-test or the Mann Whitney W test when the assumption of equality of variance could not be satisfied.

The average populations of bacteria, actinomycetes, yeast, and fungi were compared across compost categories using one-way analysis of variance. All statistics were performed with Statgraphics 4.0 software (Manugistics Inc., Rockville, MD).

RESULTS

Samples were grouped into four categories based on feedstocks and composting process (Table 6.1). Three samples, a certified organic spent mushroom compost, a compost made with mixed seafood processing waste, and a compost made from ground plants and recycled soilless media at a commercial nursery did not fit into a general category. Selected physical, chemical , and biological properties are reported for all samples (Tables 6.1-6.5).

Table 6.1. Compost system physical properties.

#	Compost category	Compost Feedstocks	Pile volume (m^3)	Turning frequency[a]
1	bark	*Tsuga heterophylla* bark	5000	0
2	bark	*Pseudotsuga menziesii* bark	13500	0
3	bark	*P. menziesii* bark	1400	+++
4	bark	*P. menziesii* bark+gravity belt separated dairy solids (3:1 v/v)	350	+++
5	bark	*P. menziesii* bark+gravity belt separated dairy solids (3:1 v/v)	1200	+++
6	bark	mixed bark+landscape plants+biosolids (proprietary ratio)	600	+++
7	manure	steer manure+proprietary bulking agents (1:1 v/v)	5000	+
8	manure	straw, dairy manure, eggshells (20:10:1 v/v)	350	++
9	manure	gravity belt separated dairy manure	400	+
10	manure	gravity belt separated dairy manure	550	+++
11	manure	rice hulls+chicken manure (3:1 v/v)	300	+++
12	manure	sawdust+rice hulls+chicken manure (2:1:1 v/v)	2100	+
13	manure	sawdust+rice hulls+chicken manure (2:1:1 v/v)	600	++
14	manure	sawdust+rice hulls+chicken manure (2:1:1 v/v)	500	++
15	manure	sawdust+yard trimmings+chicken manure (1:1:1)	1500	0
16	mushroom	straw+chicken manure+seed meal+others (proprietary blend)	200	+++
17	seafood	sawdust+soil+fish+shrimp+crab waste (4:1:1:1:1 v/v)	450	++
18	vermicompost	cow manure+food waste+paper (1:1:1 v/v)	2	0
19	vermicompost	cow manure+food waste+paper (1:1:1 v/v)	2	0
20	vermicompost	gravity belt separated dairy manure and bedding	40	0
21	vermicompost	straw, dairy manure, eggshells (20:10:1 v/v)	180	0
22	nursery regrind	ground nursery prunings and culls	800	air
23	yard trimmings	ground landscape plants	300	+++
24	yard trimmings	ground landscape plants	300	+++
25	yard trimmings	ground landscape plants	600	air
26	yard trimmings	ground landscape plants	600	+++
27	yard trimmings	ground landscape plants	7500	+
28	yard trimmings	ground landscape plants	16500	+
29	yard trimmings	ground landscape plants	300	+++
30	yard trimmings	ground landscape plants	300	air
31	yard trimmings	ground landscape plants	600	+++
32	yard trimmings	ground landscape plants	22000	+
33	yard trimmings	ground landscape plants	20000	+
34	yard trimmings	ground landscape plants	600	++
35	yard trimmings	ground landscape plants+chicken manure (10:1 v/v)	450	+++
36	yard trimmings	landscape plants+food waste (10:1 v/v)	400	+

[a] 0 not turned, + turned less than once/month, ++ turned 2-3 times/month, +++ at least once/week, air indicates static forced aeration system.

Table 6.2. Compost physical and chemical properties.

#	Pile Age (days)	Pile temp (C)	Moisture (% w/w)	Particle size[c]	pH	EC (dS/m)
1	125	44	50	62	3.4	0.5
2	139	47	47	60	3.7	0.2
3	94	58	49	82	4.1	0.3
4	75[a]	25[b]	52	66	7.8	0.1
5	89	53	52	83	4.0	0.3
6	385[a]	25[b]	62	82	5.7	0.8
7	339	45	54	79	7.5	1.5
8	422	58	35	93	8.4	3.4
9	51[a]	25[b]	67	79	8.8	0.5
10	118	49	38	87	7.2	1.4
11	118	44	26	85	8.4	7.0
12	81	49	60	84	7.0	3.5
13	210[a]	25[b]	59	53	7.1	2.2
14	60	55	58	79	8.4	2.7
15	308	60	48	80	8.5	5.1
16	108	29	56	69	7.9	6.7
17	419	27	46	88	6.6	0.5
18	90[a]	25[b]	78	59	6.5	2.7
19	90	25	70	76	5.6	3.7
20	62	19	70	99	6.8	2.0
21	361	25	58	88	7.6	1.2
22	168	32	55	69	6.0	0.4
23	51	55	41	74	7.1	2.1
24	44	ND	49	ND	5.8	2.0
25	155	36	57	84	6.6	0.6
26	65	56	52	77	7.4	0.9
27	383	58	45	72	7.4	1.4
28	392	54	46	80	7.5	1.1
29	148	56	44	80	7.1	1.2
30	90	52	37	83	6.9	0.8
31	79	29	50	73	7.4	0.6
32	438	61	46	79	7.2	1.0
33	193	49	29	69	5.8	1.5
34	103	63	31	78	5.4	2.7
35	65	58	43	65	7.7	1.3
36	500	39	40	51	7.2	0.4

[a] compost age at sampling, then stored at 4 C, warmed to 25 C over 4 days
[b] compost temperature at time of analysis and pathogen bioassays
[c] Percent passing 6.4 mm (0.25 inch) sieve by wet weight

Table 6.3. Compost chemical properties.

#	EC (dS/m)	C g/kg	C:N ratio	S g/kg	Solvita NH3	NH_4-N mg/kg	NO_3-N mg/kg
1	0.5	503	193	3400	5	4	7
2	0.2	526	239	2000	5	4	1
3	0.3	495	159	1900	5	228	1
4	0.1	451	74	1800	5	340	2
5	0.3	469	179	5300	5	4	1
6	0.8	449	42	3000	5	100	901
7	1.5	321	22	1200	2	1036	248
8	3.4	221	14	1500	4.5	173	57
9	0.5	456	22	3400	5	17	885
10	1.4	162	14	2900	4.5	43	484
11	7.0	244	9	500	3	2003	16
12	3.5	396	24	2300	5	7	776
13	2.2	296	16	3500	5	8	420
14	2.7	344	19	4700	3	10	8386
15	5.1	322	23	500	4.5	5481	3
16	6.7	281	11	500	4	1242	10
17	0.5	185	19	400	5	4	165
18	2.7	311	11	1300	5	62	7404
19	3.7	373	10	1800	5	430	1498
20	2.0	258	15	1800	5	12	3451
21	1.2	239	15	1800	4.5	10	304
22	0.4	247	34	21200	4.5	4	620
23	2.1	212	10	1780	5	1428	3
24	2.0	185	11	5640	4	NA	NA
25	0.6	321	25	1540	5	15	1
26	0.9	334	34	500	5	11	1
27	1.4	196	14	480	4.5	446	21
28	1.1	284	20	6220	4	18	4
29	1.2	242	15	1070	5	14	5
30	0.8	253	19	1290	5	82	2
31	0.6	271	19	1065	5	7	2
32	1.0	256	19	3152	5	205	13
33	1.5	227	18	1510	4	97	2
34	2.7	215	13	1160	4.5	1361	3
35	1.3	306	21	1380	3.5	22	7
36	0.4	115	10	1090	5	7	35

Table 6.4. Compost elemental analysis.

#	P g/kg	K (g/kg)	Ca (g/kg)	Mg (g/kg)	Fe (g/kg)	Mn (g/kg)
1	0.52	4774	5792	1957	16	5
2	0.33	6888	1864	705	39	102
3	<2.00	8626	982	960	74	69
4	0.60	3557	6012	1289	28	9
5	<2.00	11700	3066	997	33	17
6	5.38	6107	3246	1434	36	21
7	5.29	3955	4930	1082	40	42
8	3.87	6412	4208	1191	34	45
9	1.77	10413	1764	1520	16	15
10	3.36	4830	5892	1933	10	8
11	17.57	1220	4108	817	624	114
12	7.73	2320	5671	1690	26	17
13	15.47	11631	29	29	19	35
14	11.92	21017	79	34	33	9
15	2.08	1760	7916	1702	526	188
16	7.42	1490	5972	1313	492	168
17	2.90	780	1986	620	63	36
18	6.02	6010	1387	284	284	232
19	4.03	5265	4629	997	NA[1]	NA
20	3.42	9360	2345	1702	NA	NA
21	2.76	3276	3828	1398	NA	NA
22	1.30	18330	12365	2553	NA	NA
23	2.83	1830	1453	477	NA	NA
24	3.18	11500	941	497	NA	NA
25	9.02	3730	3026	668	NA	NA
26	1.82	909	1012	368	NA	NA
27	2.63	1150	1623	415	NA	NA
28	1.58	14700	6263	2674	NA	NA
29	2.19	2810	2445	603	NA	NA
30	1.81	2890	3998	841	NA	NA
31	1.87	542	3467	400	NA	NA
32	1.73	8140	2014	728	NA	NA
33	1.98	1640	1693	298	NA	NA
34	1.98	2070	1112	344	NA	NA
35	1.74	2850	631	368	NA	NA
36	2.36	2020	1613	378	NA	NA

[1] NA – Not analyzed.

Table 6.5. Compost biological properties.

#	Solvita Compost Maturity Index	Compost Respiration mg CO_2-C g^{-1} C d^{-1}	Healthy Seedlings[a]		
			Pythium ultimum	*Pythium irregulare*	*Rhizoctonia solani*
1	5	0.6	67*[c]	80*[c]	84*[c]
2	8	0.8	38	38*	15
3	7	0.8	65	48	(21)[e]
4	8	0.2	44	44	69*
5	8	0.8	71*	25	68*
6	8	0.0	18	77	(3)
7	7	1.9	86*	88*	58*
8	8	1.4	80*	78*	(17)
9	6	nd[b]	72	92*	(8)
10	8	0.1	35	73	(19)
11	1	6.7	80*	93*	(33)
12	8	2.3	54	97*	23
13	7	1.3	90*	99*	(5)
14	6	nd[b]	96*	96*	(49)
15	5	6.6	104*	94*	11
16	2	5.0	92*	98*	75*
17	8	0.1	35	58	(41)
18	8	0.3	78*	91*	25
19	8	nd[b]	88*	96*	54
20	8	0.9	59	90*	80
21	7	1.0	90*	84*	27
22	2	nd[b]	98*	89*	80*
23	7	2.8	100*	92*	(35)
24	2	nd[b]	104*	96	(41)
25	6	1.1	100*	71	17
26	8	1.9	100*	88*	27
27	7	1.6	89*	89*	(0)
28	8	1.2	100*	93*	(0)
29	6	0.9	38	52	(23)
30	8	0.7	31	10	(40)
31	7	1.6	96*	96*	61
32	5	1.0	100*	93*	52
33	2	6.3	55	29	62
34	2	7.1	78*	80*	39
35	6	2.9	102*	98*	14
36	8	-0.4	15	11	(4)

[a] Number of healthy seedlings in compost amended container media inoculated with respective pathogen, expressed as percentage of the non-inoculated peat control media.

[b] Not analyzed.

[c] * Significant less (P value <0.05) disease compared to control, means separated by Duncan's multiple range test.

Compost physical properties. At the time of sampling there was no significant relationship between disease suppression and any of the measured physical properties. For compost samples that significantly suppressed damping-off caused by *Pythium* spp., compost temperature was 21 to 63 C and compost moisture content ranged from 26 % to 78% (figure 6.1).

For compost samples that significantly suppressed damping-off caused by *R. solani*, compost temperature was 25 C to 53 C and moisture content ranged from 50%-56%. Damping-off suppression did not relate to compost pile volume, turning frequency, age, or particle size distribution (data not shown).

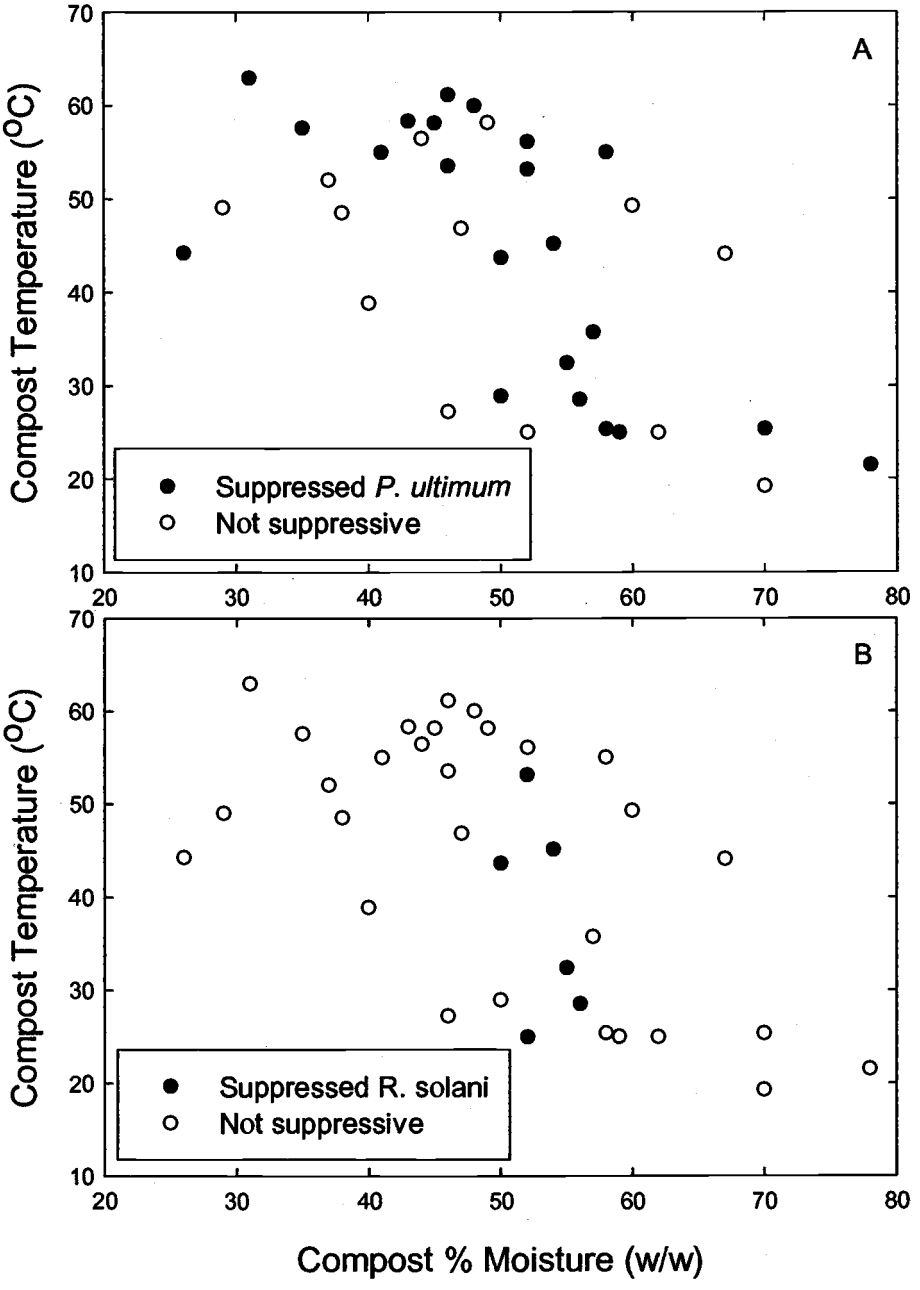

Figure 6.1. Temperature and percent moisture for compost samples that significantly suppressed damping-off disease when incorporated into container media. Figure 6.1A) *P. ultimum* inoculated. Figure 6.1B) *R. solani* inoculated.

Chemical properties. Fifteen compost samples had detectable levels of ammonia volatilization with the Solvita Compost Maturity Test kits. The percent healthy seedlings of the 15 compost samples with detectable ammonia were compared to the healthy seedlings of the 21 samples without ammonia volatilization for each pathogen (Figure 6.2).

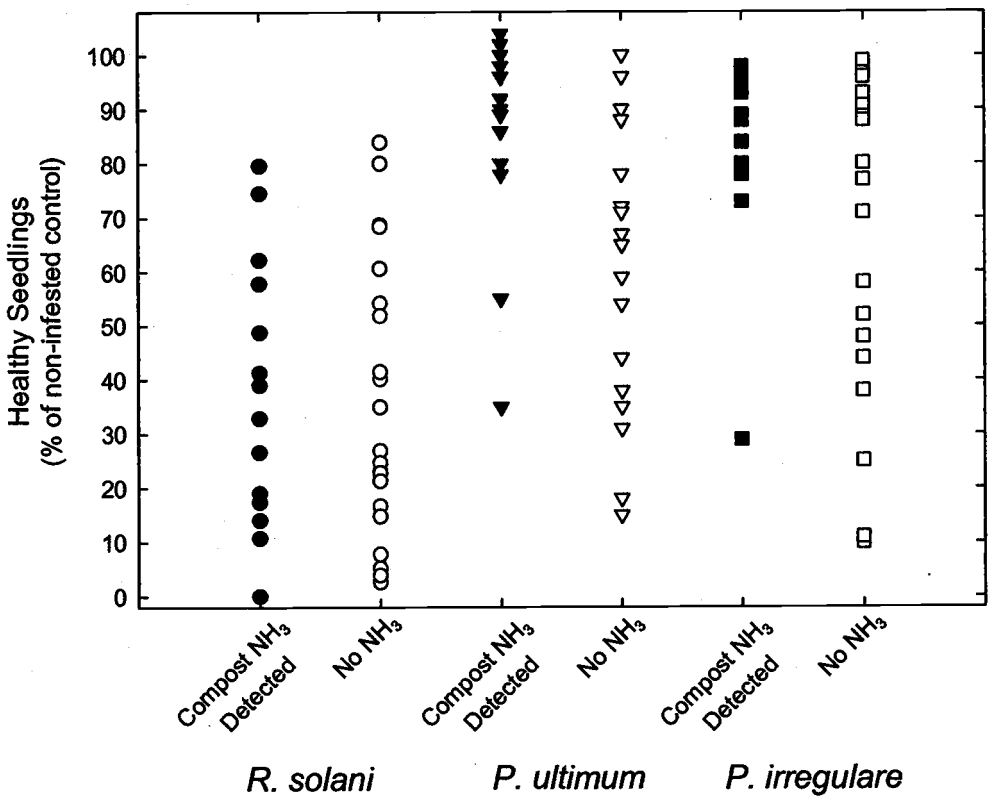

Figure 6.2. Average seedling health for three damping-off pathogens. Seedling health in relation to compost sources that had ammonia volatilization detected verses compost samples that did not have ammonia volatilization detected using the Solvita Compost Maturity Test kits.

For *P. ultimum*, the samples with ammonia volatilization had significantly greater median percent healthy seedlings than the median percent healthy seedlings of

the non-ammonia volatizing samples (*P* value 0.02). A similar, but not statistically significant (*P* value 0.13), trend was evident for *P. irregulare* (Figure 2). There was no significant difference in the mean percent healthy seedlings between compost samples with and without ammonia volatilization for *R. solani* (*P* value=0.91).

There was no consistent relationship between compost pH, EC, and average percent healthy seedlings for each pathogen.

Compost respiration. Compost respiration, measured by Solvita compost maturity test kits, indicated 36% of the 36 samples were in the category of lowest compost respiration, while seventeen percent of the samples were in the two highest respiration categories (Figure 6.3).

Figure 6.3. Relationship of compost stability and ammonia volatilization to the percentage of healthy seedlings in pathogen inoculated container media. Compost respiration potential determined by NaOH trapping CO_2 released from seven day incubation of 8 g compost with 61 g sandy loam soil, expressed as mg CO_2 released/g compost carbon/day. Solvita scale determined by the Solvita compost maturity test kit that integrates compost respiration rate and ammonia release and is inversely proportional to the rate of compost respiration and/or ammonia release. • Ammonia (NH_3) volatilization detected from compost samples with Solvita compost maturity test kits (2.0 - 4.5 Solvita scale), o Ammonia volatilization not detected. Figures 3A-D Healthy seedlings mean of six pots each with eight cucumber seeds. Container media (compost mixed with peat-perlite, Sunshine Mix #1, Sungro Horticulture 1:1 v/v) inoculated with labeled *Pythium* sp. Figures 3E-F Healthy seedlings mean of four plug flat trays each with 24 cabbage seeds. Container media (compost mixed with peat and vermiculite, 5:4:1 v/v) inoculated with *Rhizoctonia solani.*

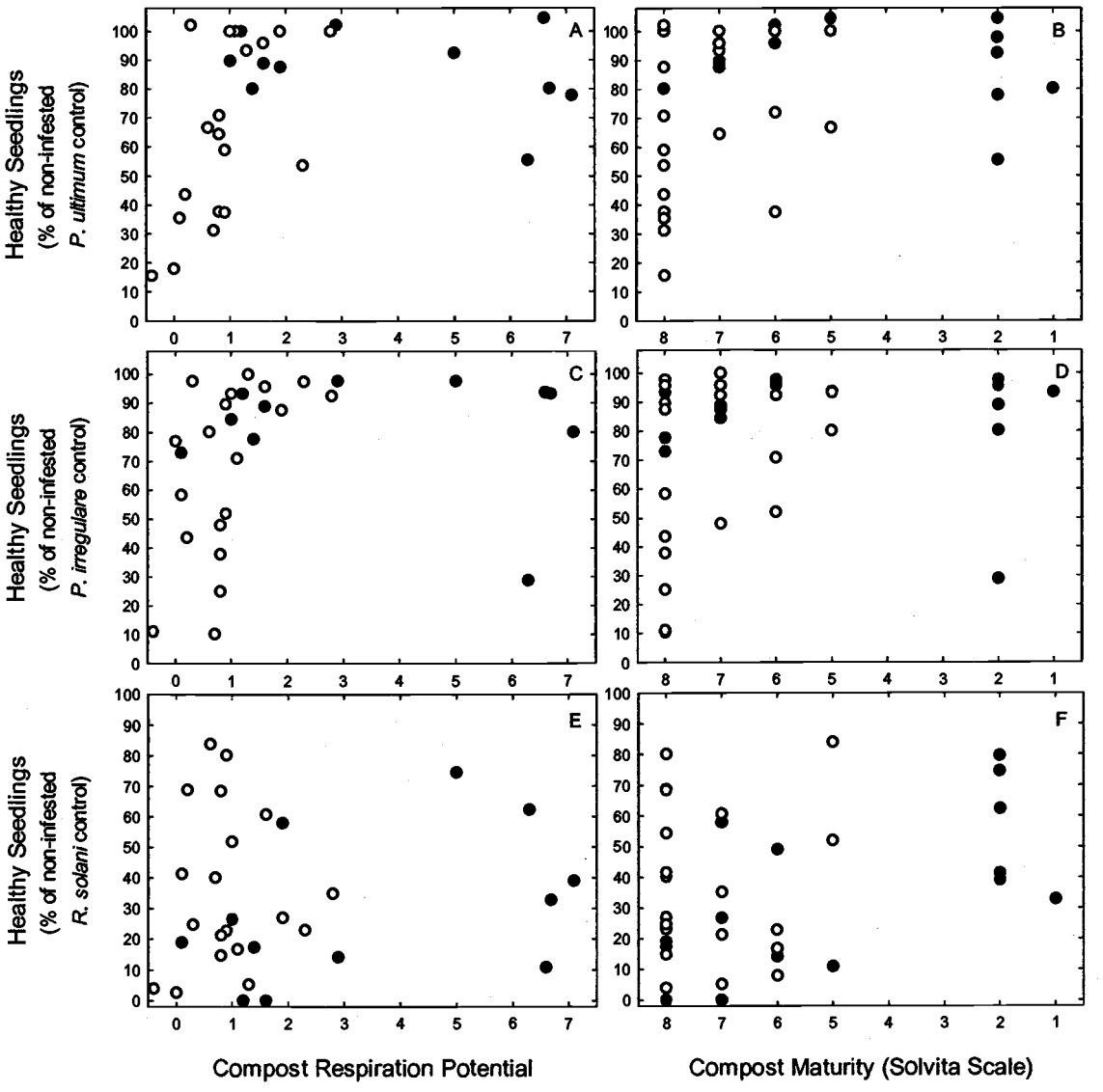
Healthy Seedlings
(% of non-infested
P. ultimum control)
Healthy Seedlings
(% of non-infested
P. irregulare control)
Healthy Seedlings
(% of non-infested
R. solani control)
A
B
C
D
E
F
Compost Respiration Potential
Compost Maturity (Solvita Scale)

Figure 6.3.

Damping-off assays. Composts varied in their ability to suppress *Pythium* spp. and *R. solani* damping-off disease. Compared to the inoculated peat-perlite media, 66% of the compost amended container media significantly reduced damping-off caused by *P. irregulare*, 64% significantly reduced damping-off caused by *P. ultimum* and 56% of the samples suppressed damping-off of both *Pythium* spp. (Table 6.5). Only 17% of the compost samples significantly reduced damping-off caused by *R. solani* when mixed with peat and vermiculite. A large portion (42%) of the compost amended container media had significantly worse damping-off caused by *R. solani* compared to the peat-vermiculite standard (Table 6.5). Damping-off caused by all three pathogens was significantly suppressed by 11% of the compost samples. Twenty-two percent of the samples did not significantly suppress damping-off disease caused by any pathogen.

Compost sources that initially suppressed *R.solani* damping-off varied both in the consistency of suppression and to the degree that heat treatment reduced suppressiveness. Of the six compost sources that significantly suppressed damping-off by *R. solani*, the hemlock bark, bark-dairy solids compost, mushroom compost, and nursery regrind compost, (numbers 1, 4, 16, and 22 in Table 6.1) were consistently suppressive over repeat bioassays (data not shown). Besides the hemlock bark, the other suppressive compost sources were sampled again and these samples treated with aerated steam. Compared to unheated compost, aerated steam treatment of the mushroom and nursery regrind composts significantly (P value <0.05) increased disease caused by *R. solani* (Table 6.6). Heating the bark-dairy solids compost did not significantly (P value <0.05) alter damping-off suppression (Table 6.6). The peat-vermiculite media had a low level of natural suppression that was significantly (P value <0.05) reduced in two out of three aerated steam heat treatments (Table 6.6).

Table 6.6. Non-heated and aerated-steam treated[c] container media components. Percent healthy cabbage seedlings[a] in *Rhizoctonia solani* infested container media[b]

Container media component treated with aerated steam	Not Heated	60°C aerated steam	88°C aerated steam	Not heated vs. 60°C aerated steam P value[d]	Not heated vs. 88°C aerated steam P value
Mushroom compost	51		10		0.008
Mushroom compost	92		39		<0.0001
Mushroom compost	56	13	16	<0.0001[d]	<0.0001
Nursery regrind compost	47		4		0.01
Dairy-fir bark compost	92		83		0.46
Dairy-fir bark compost	50	56	67	0.39	0.02
Dairy-fir bark compost	78	72	57	0.35	0.003
Peat-vermiculite (4:1 v/v)	18		2		0.03
Peat-vermiculite (4:1 v/v)	30	12	29	0.02	0.88

[a] Number of disease free seedlings 14 days after planting cabbage seeds in four plug flats each with 24 cells, reported in percent of seedlings in the pathogen free peat-vermiculite control.

[b] Composts mixed with peat and vermiculite (5:4:1 v/v) and inoculated with *R.solani* (0.075% w/v).

[c] Heated with aerated steam to specified temperature for 30 minutes.

[d] Linear contrasts separated with Duncan's multiple range test.

The potential of the bark-dairy compost to inhibit damping-off caused by *R. solani* was enhanced by inoculating the compost with *Trichoderma hamatum* (T382). Without T 382, the dairy-bark media had 50% healthy cabbage seedlings. Adding T 382 to the compost immediately before use in the damping-off assay resulted in a positive but non-significant increase in healthy seedlings (71% vs. 50%, linear contrast P value 0.25). Inoculating T 382 into the compost five days before initiating the damping-off assay significantly increased the healthy seedlings to 93% (93% vs. 50%, linear contrast P value 0.0004). The number of healthy seedlings in the inoculated and incubated treatment was not significantly different ($P > 0.1$) from the pathogen free peat-vermiculite standard treatment.

For *Pythium* spp., the percent healthy seedlings in the lowest respiration category ranged from 11% to 102%, this respiration category did not separate suppressive from non-suppressive samples (Figure 6.3). With the compost respiration potential method, 84% of the 31 samples tested released less than 3.0 mg CO_2/g

compost carbon/day, while 16 percent of the samples released at least 5.0 mg CO_2-C/g compost carbon/day (Figure 6.3). Compost respiration potential values > 1 mg CO2-C/g compost carbon/day generally suppressed *Pythium* damping-off (Figure 6.3). When only the compost samples that released less than 3.0 mg CO_2/g compost carbon/day were considered, linear regression analysis indicated a significant positive relationship between compost respiration potential and the average percent healthy seedlings for *P. ultimum* (*P* value 0.0001, R^2 0.49) and *P. irregulare* (*P* value 0.002, R^2 0.33). No significant linear relationship existed between the compost respiration potential and the average percent healthy seedlings for *R. solani* (*P* value 0.80, R^2 0.0026).

Microbial populations. One-way analysis of variance detected no significant (*P* value <0.05) difference in populations of bacteria, actinomycetes, or yeast across compost categories (Table 6.7).

Table 6.7. Microbial populations[a] by compost category.

Compost Type	n[b]	Bacteria[c]	Actinomycetes[d]	Yeast[e]	Fungi[f]	Trichoderma[g]
Bark	6	7.5 (1.2)a	7.0 (1.0)a	4.8 (2.0)a	6.7 (0.6)a	4.6 (1.9)a
Manure	9	7.8 (1.4)a	7.8 (0.8)a	3.9 (1.8)a	4.0 (1.8)b	3.0 (0.7)b
Vermicompost	4	8.2 (0.8)a	8.1 (0.7)a	5.2 (1.5)a	5.7 (0.7)a	4.8 (1.1)a
Yard trimmings	14	7.9 (0.7)a	7.8 (0.6)a	3.7 (1.3)a	4.1 (1.4)b	3.7 (1.20ab
Mushroom	1	10.14	10.05	6.19	4.96	4.70
Nursery regrind	1	9.42	9.09	6.80	6.99	3.25
Seafood	1	6.82	6.34	2.30	4.13	4.56

[a] Colony forming units (log_{10}/dry g compost), standard deviation in parenthesis. Numbers in each column followed by the same letter are not significantly different (Duncan's multiple range test, P value 0.05).
[b] Number of compost samples.
[c] Enumerated on TSBA, see materials and methods.
[d] Enumerated on AIA, see materials and methods.
[e] Enumerated on SYM, see materials and methods.
[f] Enumerated on WA, see materials and methods.
[g] Enumerated on Trichoderma selective media (Elad and Chet, 1983).

The fungal populations of the bark and vermicompost samples were significantly (*P* value <0.05) greater than in the manure or yard trimmings samples

(Table 6.7). The population of *Trichoderma* spp was significantly (*P* value <0.05) greater in the bark and vermicompost samples than in the manure samples (Table 6.7).

There was no significant (P value 0.05) positive linear relationship between the population of bacteria, actinomycetes, yeast, fungi, or *Trichoderma* spp. and the percent healthy seedlings for any pathogen. A relative scale of microbial abundance was generated by summing the bacterial, actinomycete, yeast, and fungal (each $\log_{10}$ cfu/dry g compost) populations (Figure 6.4).

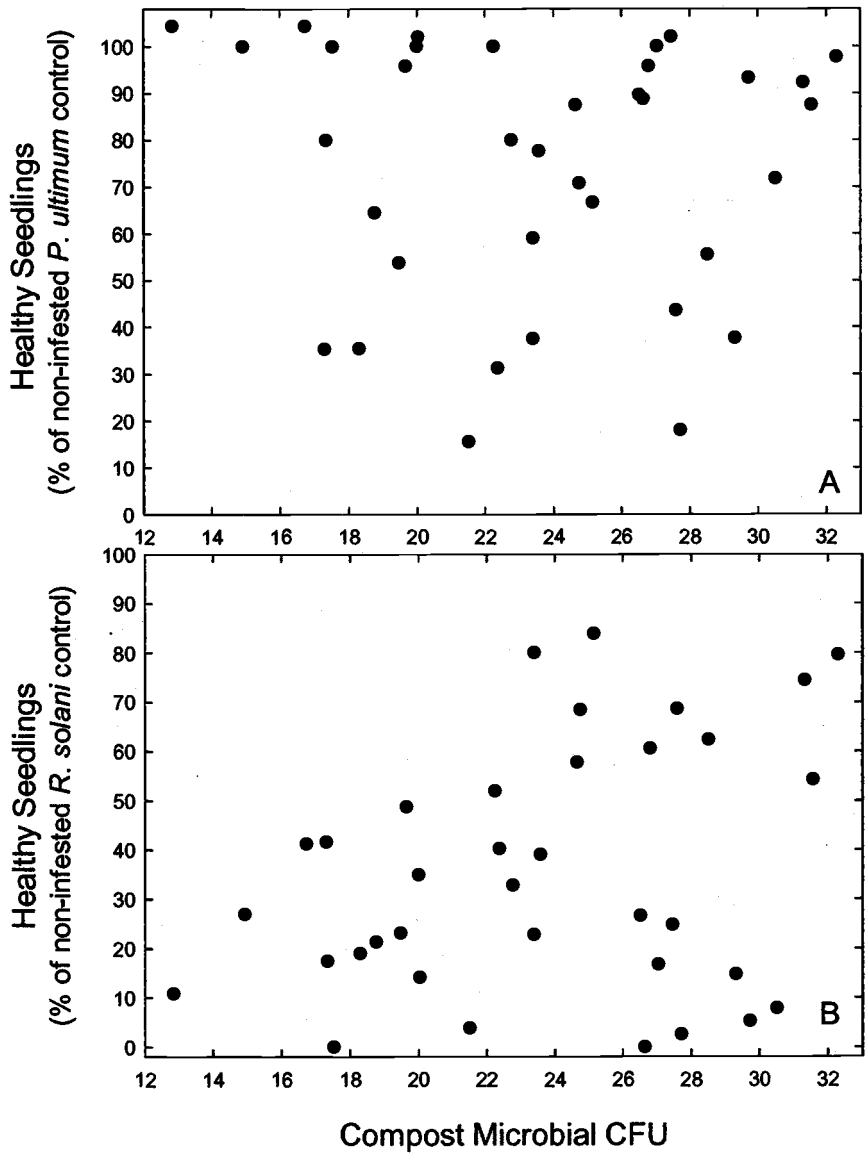

Figure 6.4. Relationship of compost microorganisms to damping-off disease suppression. Compost microbial cfu calculated as the sum of the populations of bacteria, actinomycetes, fungi, and yeast (each $\log_{10}$ cfu/g dry wt compost).

No significant positive linear relationship was detected between microbial abundance and the percent healthy seedlings for *P. ultimum* P value=0.78) or *P.*

irregulare (P value=0.87). Microbial abundance and the percent healthy seedlings for *R. solani* had a slight positive linear relationship (P value =0.086, R^2 0.084).

DISCUSSION

A majority of the compost samples suppressed damping-off caused by *P. irregulare* and *P. ultimum* when mixed with commercial peat-perlite container media. For *Pythium* spp., indirectly measuring compost microbial activity with the compost respiration potential method provided more predictive information on percent healthy seedlings than determining populations of bacteria, actinomycetes, yeast, fungi, and *Trichoderma* spp. A positive linear relationship was evident between the percent healthy seedlings and the 26 compost samples that had below 3.0 mg CO_2/g compost carbon/day according to the compost respiration potential. Similarly, increasing microbial activity, measured as the rate of fluorescein diacetate hydrolysis, has been correlated to suppression of *Pythium* damping-off in peat-based media (Boehm and Hoitink, 1992; Inbar *et al*, 1991) and compost amended container media (Chen *et al*, 1988b; Craft and Nelson, 1996). The five samples analyzed for compost respiration potential that released greater than 5.0 mg CO_2/g compost carbon/day also had ammonia volatilization detected (Figure 6.3). It is appropriate to consider them separately because compost that has a relatively high respiration rate and detectable ammonia volatilization is not considered suitable for greenhouse container production (Steve Scheuerell, personal observation).

For *Pythium* spp., damping-off suppression did not relate to compost moisture, temperature or other physical properties. Of the chemical properties examined, compost samples with detectable ammonia volatilization had significantly (*P* value <0.05) greater suppression of damping-off caused by *P. ultimum* than compost samples that did not have detectable levels of ammonia volatilization. A graphically similar, but not statistically significant, trend was observed for *P. irregulare.* In comparison to *P. ultimum*, *R. solani* damping-off did not relate to compost ammonia volatilization. Howell (*et al*, 1988) determined that *in vitro* growth of *R. solani* is less sensitive to ammonia than *P. ultimum.* Damping-off caused by *Pythium* spp. is known

to be suppressed by increasing the concentration of ammonia in field soil (Chun and Lockwood, 1985; Lin *et al*, 1990). Ammonia was suggested as a probable mechanism of turkey litter compost suppressing damping-off of creeping bentgrass caused by *P. graminicola* (Craft and Nelson, 1996). Further investigation is needed to determine the potential for ammonia in compost to suppress *Pythium* spp. However, the use of compost amended soilless media containing sufficient ammonia to suppress *Pythium* spp. might limit utilization greenhouse plant production due to ammonia phytotoxicity concerns (Schumann and Mills, 1996).

For *R. solani*, the percent healthy seedlings did not significantly (P value <0.05) relate to any of the physical, chemical or biological measurements. Additionally, *R. solani* damping-off did not correlate to total microbial activity (figures 6.3E and 6.3F) or culturable populations of bacteria, actinomycetes, yeast, fungi, or *Trichoderma* spp. (data not shown). This agrees with results from container media amended with mature composted hardwood bark, suppression of *R. solani* was not related to the general microflora populations, but rather corresponded to populations of specific antagonistic fungi and bacteria (Nelson *et al*, 1983, Kwok *et al*, 1987).

Damping-off caused by *R. solani* was suppressed by only 17 percent of the compost samples while 42 percent of the compost samples significantly (P value <0.05) increased disease compared to the peat-vermiculite media (Table 6.5). In part, this could be due to a dilution of the peat-vermiculite media. Two of three aerated steam heat treatments significantly reduced damping-off suppression of the peat-vermiculite media (Table 6.6), indicating that the peat-vermiculite media has a natural degree of damping-off suppression caused by *R. solani*. Similarly, lightly decomposed peat has been reported to suppress *R. solani* (Tahvonen, 1982).

Additionally, increased disease could also be related to the nature and quantity of decomposable substrates remaining in the compost. Insufficiently cured compost can support high populations of antagonists, but saprophytic activity prevails and suppression is usually not induced (Nelson *et al*, 1983). Further biochemical

characterization of the composts, such as cellulose content, and determining the population dynamics of *R. solani* in the inoculated container media, could indicate the potential for the composts to increase the density of the pathogen (Chung *et al*, 1988). Increasing the inoculum density of *R. solani* from 15 to 25 propagules/g in compost amended container media has been shown to significantly increase damping-off of radish seedlings (Nelson and Hoitink, 1982).

Heat treatment with aerated steam significantly reduced suppression in the mushroom and nursery regrind composts, likely indicating that damping-off suppression was biological in nature. Before sampling, these composts had cured outside, contained 55-56% moisture, and were below 33 C. These curing conditions are consistent with other investigations that have concluded that maintaining moisture between 45-55%, compost temperatures < 40 C, and curing compost outdoors are optimal for natural biological suppression of *R. solani* to develop (Kuter *et al*, 1983; Kuter *et al*, 1988, Hoitink *et al*, 1999).

Heat treatment did not reduce suppression in the bark/dairy-solids compost, likely indicating that suppression is mediated by physio-chemical properties. The suppressive nature of the bark dairy-solids compost was significantly increased by inoculation with *Trichoderma hamatum* 382 (T 382) and incubating for five days before formulating into container media. T 382 is known to suppress damping-off caused by *R. solani*, a process for the controlled inoculation of T 382 during compost production to produce a product that consistently suppresses *R. solani* has been patented (Hoitink, 1987). Suppression was not significantly increased when T 382 was inoculated just prior to using the compost in the damping-off assay, possibly indicating that the incubation period allowed for establishment of the antagonist in the already cured compost. T 382 has been demonstrated to colonize cured hardwood bark compost, yard trimmings compost and mixed biowaste compost (Nelson *et al*, 1983; Ryckeboer *et al*, 2002), further investigation with composted Douglas-fir bark is warranted.

Understanding the minimum rate of compost respiration that supports suppression of damping-off caused by *Pythium* spp. could assist soilless media blenders avoid compost that is too stable to support biological control of *Pythium* spp. The compost respiration potential data indicated that a relatively small change in the potential to support microbial activity existed between non-suppressive and suppressive composts (figures 6.3A and 6.3C). This is supported by the Solvita respiration data, the category of lowest respiration contained compost samples with a large range in damping-off suppression (figures 6.3B and 6.3D). Other minimum parameters of compost amended container media have been used to relate compost properties to damping-off suppression, a minimum rate of FDA hydrolysis and minimal level of microbial biomass has been proposed for predictable suppression of *P. ultimum* (Chen, 1988a). More recently, the potential for organic matter to support suppression of *Pythium* damping-off has been related to the substrate biological energy availability using carbon^{13} cross-polarization magic angle spinning nuclear magnetic resonance spectroscopy and diffuse reflectance fourier transform infrared spectroscopy (Boehm *et al*, 1997; Stone *et al*, 2001). These method requires laboratory equipment not readily available to compost producers and users, a more suitable method is needed. Evidence is provided here that compost respiration can be related to the potential for *Pythium* damping-off suppression, however, a more accurate quantitative measurement of compost respiration than used in this study is needed to understand the transition from suppressive to conducive compost.

Compost amended container media must consistently suppress damping-off and support optimal plant growth to be a viable alternative to current commercial greenhouse practices. The majority of the compost samples in this study suppressed damping-off caused by *Pythium* spp. when used 50% by volume in container media. For commercial application, various incorporation rates should be tested because proper physical, chemical, and biological properties of soilless container media need to be considered for optimal plant growth (Inbar *et al*, 1993). Optimal incorporation rates for disease suppression could be higher than ideal physical and chemical analysis

would indicate for a particular crop and container size. Additionally, compost sources need to be individually assessed for appropriate uses and application rates because a wide range of physical, chemical, and biological properties was evident when sources were categorized based on compost feedstocks. Using commercially available compost in container media for suppression of damping-off caused by *Pythium* spp. should be tested on a production scale. For consistent suppression of *R. solani* damping-off, inoculation of compost with specific microbial antagonists should be pursued.

ACKNOWLEDGEMENTS

We thank the compost facilities for providing compost and detailed process information and Kathryn Sackett for excellent technical assistance. Jeff Choate, graduate student in Crop and Soil Science, OSU, performed the compost respiration potential test. Funding for this work provided by USDA CRIS # 303-5358-22000-024-00D and the Oregon Association of Nurserymen and Oregon Department of Agriculture.

Chapter seven: Inoculation of Compost After Peak Heating Needed for the Rapid Development of Damping-off Suppression Caused by *Pythium ultimum*

ABSTRACT

Compost removed from the hot (>55 C) core of yard trimmings compost piles was incubated under sterile conditions to observe if suppression could develop due to cooling, or if inoculation with organic matter is a critical factor. Suppression towards damping-off caused by *P. ultimum* did not develop in the cooled compost over seven days of incubation, but compost inoculated 10% v/v with cured yard trimmings compost became suppressive after incubating three to five days. Thus indicating that inoculation of this material is needed in order to rapidly and consistently produce *Pythium* suppressive compost for use in greenhouse propagation of seedlings.

INTRODUCTION

Compost amended container media has been used for the biological control of several *Pythium* species in greenhouse crops (Hoitink *et al*, 1993). With suppression of *Pythium* spp. related to the total microbial activity and biomass (Chen *et al,* 1988a; Craft and Nelson, 1996), and the quantity and quality of microbial growth substrates (Boehm *et al,* 1993; Chen *et al,* 1988b; Mandelbaum and Hadar, 1990; Stone *et al,* 2001). It is thought that the majority of beneficial antagonists are destroyed during the hot phase (>55 C) of composting and *Pythium* suppression is due to recolonization of compost by natural antagonists after peak heating (Chen *et al* 1988b, Hadar and Mandelbaum, 1986). Compost removed from the cooler (<40 C) edges of piles is suppressive, while hot (>60 C) zones are typically conducive to *Pythium* (Chen *et al* 1987, Chen *et al* 1988b; Hadar and Mandelbaum, 1986). *Pythium* suppression has been negated by using compost or compost amended container media immediately after being heated at 60 C for five days (Chen *et al* 1987, Chen *et al* 1988b, Kuter *et al*, 1988; Ryckeboer *et al*, 1999) or short treatments at 55 C (Theodore and Toribio, 1995), 70 C (Hadar and Mandelbaum, 1986), 80 C (Mandelbaum *et al* 1988), 121 C (Craft and Nelson, 1996).

It is generally accepted that using organic matter to inoculate compost made conducive by heat treatment is associated with the development of a suppressive environment, but can require incubating 3-4 days before suppression develops (Chen *et al* 1988b). However, hot (>55 C) compost produced from either yard trimmings or sewage sludge has suppressed *P. ultimum* when incorporated into container media within 24 h after sampling (Grebus *et al* 1994; Kuter *et al* 1988; Scheuerell 2002). Antagonistic *Bacillus subtilis* strains isolated from ambient temperature compost were inoculated into waste, survived compost peak heating and was active in *P. ultimum* suppression (Phae *et al* 1990). These studies indicate that the introduction of mesophilic organisms to hot or heated compost lacking the physiochemical and biological balance necessary for suppressing *Pythium* can shift the balance to a suppressive environment (Chen et al, 1988b; Hadar and Mandelbaum, 1986; Ryckeboer *et al*, 1999). However, they cannot rule out that suppression developed from the growth of microflora surviving the heating process because heated compost was not incubated without added organisms. Thus it is not clear that *P. ultimum* suppression develops without inoculating the compost.

Yard trimmings compost was investigated, since it is abundant, widely available, and cost effective for use in container plant production. Many commercial producers of yard trimmings compost in Oregon, USA, stock pile screened, finished compost in large piles that do not allow for efficient heat removal by convection, resulting in material that often is hotter than 50 C (personal observation). A survey of these composts indicated that immediate use does not always suppress *P. ultimum* (Scheuerell, 2002). Determining if simply cooling hot compost is sufficient to induce suppression or if active inoculation is necessary would assist the composting and nursery industries to more consistently produce and utilize a natural source of disease suppressive material.

MATERIALS AND METHODS

Compost production, sampling and sterile storage. Sampled compost was produced at Rexius, Inc. (Eugene, Oregon) from mixed yard trimmings that were ground, placed in 3 m tall windrows that were turned once per week for 1 month, then composted with static forced air for 6 weeks. Compost was screened to 3/4 inch minus, then stacked in a pile 20 x 12 x 6 m (L x W x H). The sampled compost had been in this pile for approximately six months for the first experiment and approximately one month for the second experiment. Autoclaved metal shovels were used to place compost into 15 L buckets, 45 L plastic bin, or 45 L insulated cooler. Newly purchased containers and lids had been sanitized by scrubbing with 95% ethanol, followed by placement under ultraviolet light within a sterile laminar flow hood for 12 hours. Compost was sampled from the outer 10 cm of the pile into one bucket ('edge' compost). The same shovel was cleaned with 95% ethanol and used to remove the outer 80 cm depth of compost from a 1 m^2 area. A second sterile shovel was used to fill containers 1 m depth ('core' compost). After sealing each container, they were immediately transported to the laboratory, and placed in a sterile laminar flow hood. Container lids were adjusted to leave a 1 cm gap for heat and gas exchange but reduce moisture loss.

In the first experiment, a sealed 45 L insulated cooler filled with core compost was partially submerged in a 55 C water bath to reduce the rate of heat loss for the first 24 hours ('core-hot' compost). It was then transferred to the laminar flow hood and the lid adjusted as described. For both experiments, eight hours after placement in the laminar hood, a bucket of core compost had cooled below 35 C and was inoculated ('inoculated' compost) by thoroughly incorporating 10% (v/v) cured yard trimmings compost (Rexius, Inc.) previously determined to suppress *P. ultimum*. The first experiment (experiment I) only core compost was sampled on 5/29/01, with sterile storage lasting five days. The second experiment (experiment II) used edge and core compost sampled on 10/29/01, with sterile storage lasting seven days.

Abiotic compost measurements. At the time of sampling, compost temperature was recorded from 4 locations surrounding the sampled area at 10 cm and 1 m depths. In the laminar hood, compost temperature was recorded with surface sterilized stainless steel thermometers on one, two, three, and five days after sampling (DAS) for experiment I; on one, four, and seven DAS for experiment II. Compost pH and electrical conductivity (EC) were recorded one and five DAS for experiment I; on one, four, and seven DAS for experiment II. Calibrated meters were used to determine compost pH (saturated paste method; instrument model 150, IQ Scientific Instruments) and electrical conductivity (EC) (1:2 v/v method; instrument model 933100, Hanna Instruments). Percent moisture was determined each day that container media was prepared by oven drying 50-g compost for 36 hours at 70 C.

Compost microbial populations and Rrelative microbial activity. Compost microbial populations were determined one, three, and five DAS in experiment I, and one, three, four, five, and seven DAS in experiment II. 10 g compost were added to 90 ml sterile 0.02M potassium phosphate buffer (PPB)(pH 7.0) in a 250 shaker flask, shaken (300 rpm, 25 C) for 20 minutes, then serially diluted in sterile PPB. Dilutions were plated using an automated spiral plater (Eddy Jet; IUL Instruments, Barcelona, Spain) on selective agar and incubated at 22 C. Bacteria were enumerated on 5% trypticase soy broth agar (TSBA, 1.5 g Difco tryticase soy broth with 15 g agar) with 100 ug/ml cycloheximide. Actinomycetes were enumerated on alkaline water agar (AWA, pH 10.5, 18 g agar/L) amended with 50 ug/ml cycloheximide. Fungi were enumerated on water agar (WA, pH 6, 18 g agar/L) with 50 ug/ml rifampicin. Yeast were enumerated on dilute, selective yeast media (SYM; 1.5 g yeast extract, 2.5 g peptone, 5 g dextrose, 2.3 g malt agar, 17 g agar per L medium, amended with 100 ug/ml chlroramphenicol, 50 ug/ml ampicillin, 500 ug/ml streptomycin sulfate, and 2 ug/ml dichloran). Populations recorded as cfu/dry g compost.

The relative rate of microbial activity was determined one and five DAS in experiment I, and one, four, and seven DAS in experiment II. Microbial activity, based on the rate of compost respiration, was recorded with Solvita^{TM} compost

maturity test kits according to the manufactures instructions (Woods End Research Laboratory, 2002).

Container media treatments. Container media were made immediately before use in consecutive cucumber seedling damping-off assays. For each container media, 1.5 L compost was aseptically removed from the laminar hood with 500-ml reserved for the abiotic and microbial measurements. Container media were made by mixing 1 L compost and 1 L peat-perlite growing media (Sunshine Mix #1, Sun Gro Horticulture, Inc., Vancouver, B.C.) in a plastic bag. For experiment I, container media were made from core compost, core-hot compost, inoculated compost, amended compost, and suppressive compost (Table 7.1).

Table 7.1. Compost types and blends used to make container media

Compost type/blend	Description
Edge	Compost from outer 10 cm of curing yard trimmings pile.
Core	Compost from 1 m depth of curing yard trimmings pile.
Core-Hot	Compost from 1 m depth of curing yard trimmings pile that was stored in an insulated container for the first 24 hours.
Inoculated	Separate container of core compost inoculated with 10% (v/v) Suppressive compost eight hours after placement in sterile laminar flow hood.
Amended	Core compost with 10% (v/v) Suppressive compost added immediately before each *P. ultimum* bioassay.
Suppressive	Stored yard trimmings compost (Rexius, Inc., Eugene, Oregon) previously determined to suppress *P. ultimum* damping-off.

For experiment II, container media were made from core compost, edge compost, inoculated compost, amended compost, and suppressive compost (Table 7.2).

Table 7.2. Compost properties for experiment I. Initial and final temperature, relative microbial activity and microbial populations[g] for compost held in sterile storage over a five-day period.

Compost[a]	Day[b]	Temper-ature	Microbial activity[c]	Bacteria[d] ($X10^5$)	Actino-mycetes[e] ($X10^5$)	Fungi[f] ($X10^2$)	Mucoid Yeast[g] ($X10^2$)
Core-Hot	1	46	5.5	33	12	4	<2
	5	21	7.5	40	3	4	<2
Core	1	25	8	40	10	7	<2
	5	21	8	19	7	<2	<2
Amend	1		7	420	130	760	220
	5		7	350	71	1900	250
Inoculated	1	24	7	850	120	1500	96
	5	21	5	3700	520	790	390
suppressive	1	24	5.5	2800	780	13000	520
	5	23	4.5	6800	730	11000	18000

[a] Compost described in Table 7.1.
[b] Days of storage in sterile laminar hood.
[c] Relative microbial activity on a scale of 1 (most active) to 8 (least active) using Solvita test kits (Woods End Research Lab, Mt. Vernon, Maine).
[d] Bacteria enumerated on 5% trypticase soy broth agar with 100 ppm cycloheximide.
[e] Actinomycetes enumerated on water agar pH 10.5 (18 g agar/L) with 50 ppm cycloheximide.
[f] Fungi enumerated on water agar pH 6 (18 g agar/L) with 50 ppm rifampicin.
[g] Yeast enumerated on dilute, selective yeast media (1.5 g yeast extract, 2.5 g peptone, 5 g dextrose, 2.3 g malt agar, 17 g agar per L medium, amended with 100 ppm chlroramphenicol, 50 ppm ampicillin, 500 ppm streptomycin sulfate, and 2 ppm dichloran).
[h] Reported as cfu/dry g compost.

Both experiments had pathogen inoculated and pathogen free peat-perlite media treatments included as standards in each cucumber seedling damping-off assay. For experiment I, all compost container media were included as pathogen free control treatments to test for phytotoxicity. For experiment II, only the core compost was included as a pathogen free compost container media.

Cucumber seedling damping-off assay. Consecutive damping-off assays were started one, two, three, and five DAS in experiment I, and one, three, four, five, and seven DAS in experiment II. A modified *P. ultimum* cucumber seedling assay was used (Chen *et al*, 1987). The *P. ultimum* (isolated from corn roots, Willamette Valley, OR; provided by Beth Hoinacki) inoculum was produced with Ko and Hora's soil and

chopped potato medium(Ko and Hora, 1971), dried, then sieved through a 1-mm^2 grid with the particles retained on a 0.25-mm^2 grid used. *P. ultimum* inoculum was mixed with 2-L container media in a plastic bag (1.0 g/L), and then equally dispersed into six 400-ml square plastic nursery pots. Each pot was sown with eight cucumber seeds (*Cucumis sativus* 'Marketmore 76') 1-cm deep, then watered to capacity with tap water. Pots from each treatment were placed on separate nursery trays. A large, clear plastic bag was inflated and sealed around each nursery tray to simulate a germination room and maintain even moisture in the pots. The trays were placed in a 20 C growth chamber with 16-hour photoperiod. At 3 and 6 DAP each tray was vented to minimize changes in the atmosphere within the sealed trays. At 9 DAP pots were assessed for the number of healthy cucumber seedlings. A seedling was classified as healthy if it was growing normally and had no symptoms or signs of infection. Infection symptoms included a water soaked or yellowing stem, wilted cotyledons, stem lesion leading to seedling collapse; pathogen sign was white mycellia covering any portion of the seedling.

Experimental designs and statistical analysis. The cucumber damping-off assays were designed as randomized complete block experiments were trays served as experimental blocks. Two-way analysis of variance was performed; individual pots were experimental units with container media and block (trays) as factors. Means were separated using Duncan's multiple range test (Statgraphics 4.0, Manugistics, Inc., Rockville, MD). For both experiments, consecutive damping-off assays were analyzed individually.

RESULTS

Damping-off suppression. Container media made with either suppressive or edge compost had significantly greater number of healthy seedlings compared to using the core compost or the 100% peat-perlite industry standard (*P* value 0.05; figures 7.1 and 7.2).

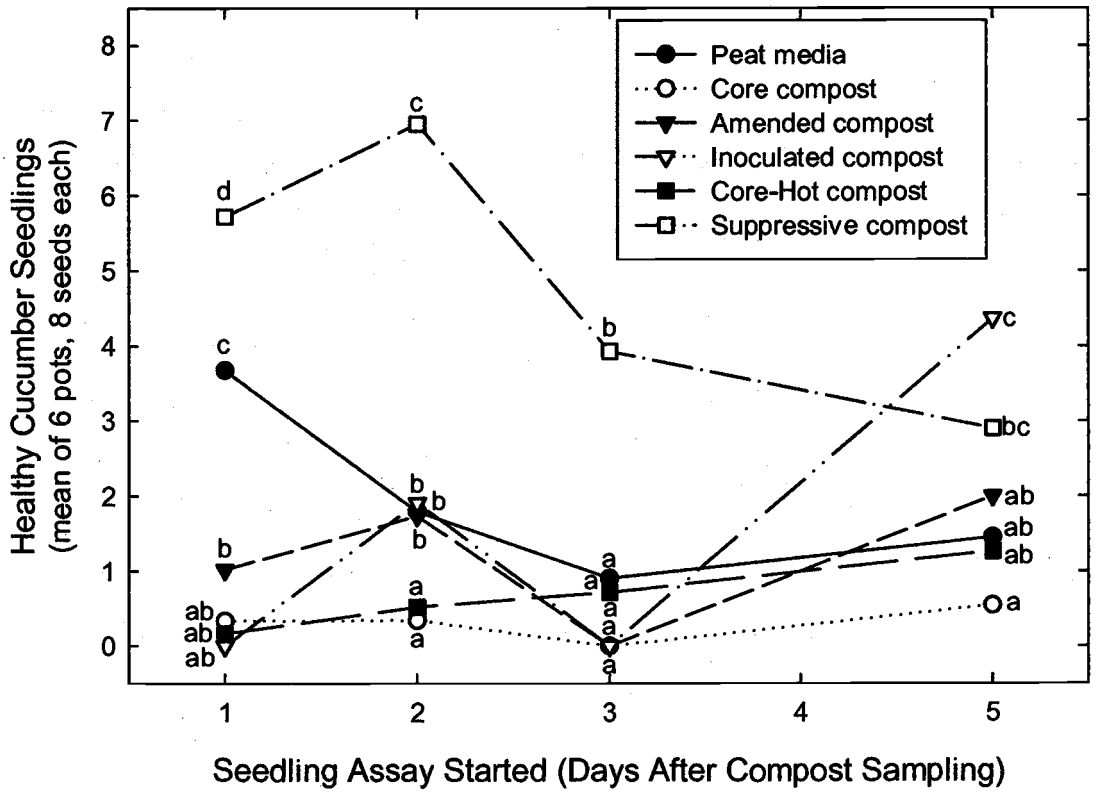

Figure 7.1. Seedling health with compost amended container media, experiment I. Container media made from yard trimmings compost incubated under sterile conditions. Container media made with compost and peat media (1:1 v/v). Peat media is Sunshine Mix #1 (Sun Gro Horticulture, Inc., Vancouver, B.C.) Core compost removed from 1 m deep in pile. Amended compost was core compost mixed with suppressive compost (9:1, v/v) immediately before each assay. Inoculated compost was core compost inoculated with suppressive compost (9:1, v/v) eight hours after put in sterile storage. Core-Hot compost was removed from 1 m deep in pile and cooled slowly over first 24 hours of sterile storage. Suppressive compost was cured yard trimmings compost previously determined to suppress cucumber seedling damping-of caused by *P. ultimum*. All container media infested with *P. ultimum*. Non-infested peat media and compost controls were symptom free and are not graphed. Within each day, data points with the same letter are not significantly different according to Duncan's multiple range test (*P* value 0.05).

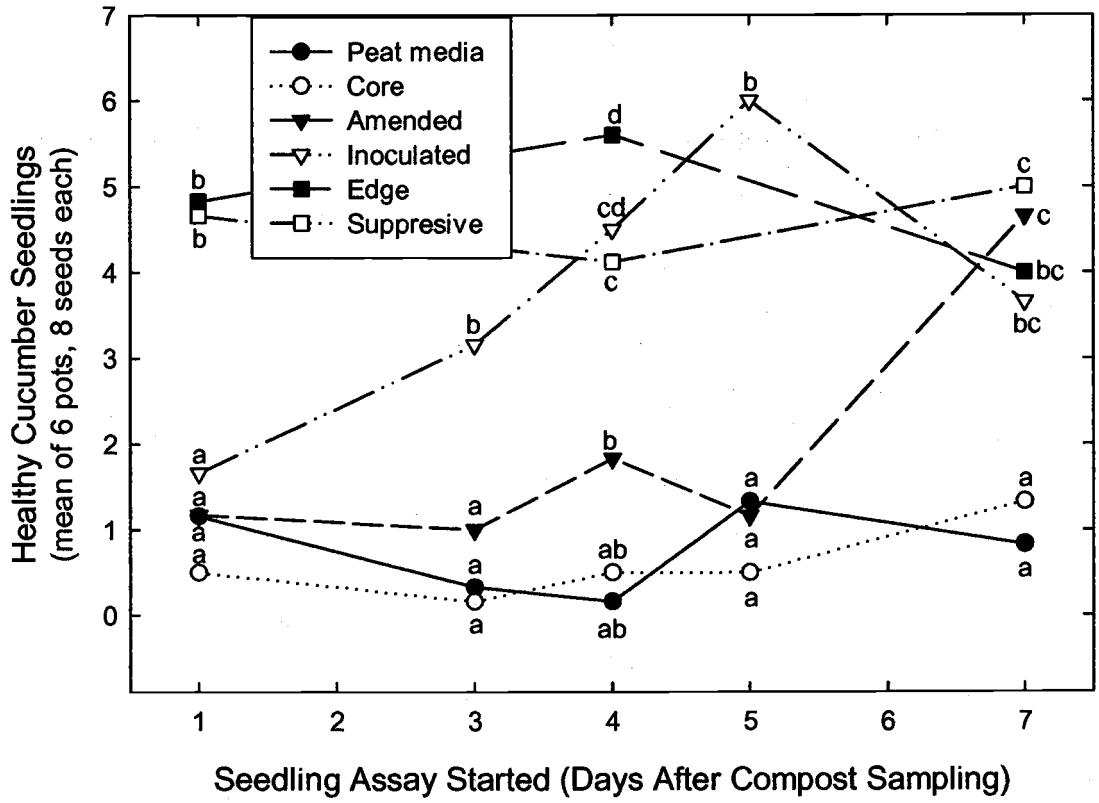

Figure 7.2. Seedling health with compost amended container media, experiment II. Container media made from yard trimmings compost incubated under sterile conditions. Container media made with compost and peat media (1:1 v/v). Peat media is Sunshine Mix #1 (Sun Gro Horticulture, Inc., Vancouver, B.C.) Core compost removed from 1 m deep in pile. Amended compost was core compost mixed with suppressive compost (9:1, v/v) immediately before each assay. Inoculated compost was core compost inoculated with suppressive compost (9:1, v/v) eight hours after put in sterile storage. Edge compost removed from the outer 10 cm of the pile. Suppressive compost was cured yard trimmings compost previously determined to suppress cucumber seedling damping-of caused by *P. ultimum*. All container media infested with *P. ultimum*. Non-infested peat media and core compost controls were symptom free and are not graphed. Within each day, data points with the same letter are not significantly different according to Duncan's multiple range test (*P* value 0.05).

In both experiments, compost sampled from the hot core of curing yard trimmings compost did not suppress seedling damping-off caused by *P. ultimum* when used immediately after sampling. Cooling to ambient temperature and incubating within a sterile environment to avoid microbial colonization did not provide conditions suitable for the development of damping-off suppression over a five or seven-day period (figures 1 and 2). Slowly cooling the core compost in experiment I (core-hot compost) did not induce suppression. The inoculated compost was suppressive after five days incubation in experiment I (figure 1). Suppression was evident after three days incubation in experiment II, with the level of disease suppression becoming statistically equivalent to the non-infested peat-perlite media after four days of incubation (figure 2). All pathogen free control treatments showed no disease symptoms, evidence of reduced germination or phytotoxic reactions. The mean number of healthy seedlings/pot across all control treatments and seedling assays ranged from 6.33 to 7.86 for experiment I and 5.0 to 7.16 for experiment II (data not shown).

Abiotic compost measurements. At the time of sampling for experiment I, the compost pile temperature at 1 m depth was 56 +/- 1 C . For experiment II, the compost temperature was 42 +/- 1 C at 10-cm and 57 +/- 1 C at 1-m depth. Temperatures rapidly declined in the laminar flow hood (Tables 2 and 3). The pH of all compost samples ranged from 6.5 to 7.3 over the duration of experiment I, and from 6.5 to 7.5 for experiment II (data not shown). The EC of all compost samples ranged from 0.42 to 0.73 dS/m in experiment I, and from 0.63 to 0.95 for experiment II (data not shown). Moisture content of all compost samples ranged from 41 to 48% in experiment I, and from 46 to 61% for experiment II (data not shown).

Compost microbial populations and relative microbial activity. In experiment I, culturable populations of bacteria, actinomycetes, fungi, and mucoid yeast were relatively stable from day 1 to day 5 (Table 7.2). In experiment II, different trends in

microbial populations were evident for the core, amended, and inoculated compost (Table 7.3).

Table 7.3. Compost properties for experiment II.. Temperature, relative microbial activity, and microbial populations (cfu/g dry weight) of compost types used to make container media over a seven day period with the compost incubated under sterile conditions.

Compost type[a]	Day	Temper -ature	Microbial activity[b]	Bacteria[c] ($X10^7$)	Actino-mycetes[d] ($X10^7$)	Furigi[e] ($X10^3$)	Mucoid Yeast ($X10^3$)	Filamentous yeasts[f] ($X10^3$)
Core	1	26	4	20	16	16	<2	<1
	3			30	25	98	78	<1
	4	22	4	20	13	43	31	<1
	5			16	14	49	0	<1
	7	22	2.5	39	16	5500	35	<1
Amended	1		4	nd[g]	nd	nd	nd	nd
	3			22	16	10	42	10
	4		4	24	10	77	<2	12
	5			23	10	770	<2	31
	7		2.5	22	4	5200	<2	34
Inoculated	1	25	2	19	15	52	<2	23
	3			144	72	78	nd	nd
	4	22	2	689	295	750	<2	27
	5			361	298	780	<2	35
	7	22	2	662	93	150	<2	53
Edge	1	26	2	480	260	7900	115	<1
	4	23	2	1040	270	6500	107	<1
	7	22	2.5	563	317	4800	58	<1
Suppressive	1	19	8	5	2	132	<2	118
	4	19	8	14	7	167	<2	141
	7	18	8	11	10	206	<2	165

[a]Compost type described in Table 7.1.

[b]Relative microbial activity on a scale of 1 (most active) to 8 (least active) using Solvita test kits (Woods End Research Lab, Mt. Vernon, Maine).

[c]Bacteria enumerated on 5% trypticase soy broth agar with 100 ppm cycloheximide.

[d]Actinomycetes enumerated on water agar pH 10.5 (18 g agar/L) with 50 ppm cycloheximide.

[e]Fungi enumerated on water agar pH 6 (18 g agar/L) with 50 ppm rifampicin.

[f]Yeast enumerated on dilute, selective yeast media (1.5 g yeast extract, 2.5 g peptone, 5 g dextrose, 2.3 g malt agar, 17 g agar per L medium, amended with 100 ppm chlroramphenicol, 50 ppm ampicillin, 500 ppm streptomycin sulfate, and 2 ppm dichloran).

[g]nd indicates no data was collected.

Linear regression analysis indicated that the bacterial population did not increase over the seven day period for the core compost (P value 0.38) or amended compost ($P = 0.97$), but the inoculated compost had a significant exponential curvilinear increase ($P = 0.01$, $R^2 = 0.907$). By day three, the prevalent bacterial colony morphologies became similar to those observed from the suppressive compost used to inoculate (data not shown). The actinomycetes population did not significantly increase in the core compost ($P = 0.65$), decreased in the amended compost ($P = 0.04$), and had a significant exponential curvilinear increase in the inoculated compost over the first five days of incubation ($P = 0.03$, R^2 94.2%). From days four to seven, unidentified fungal cfu increased 100-fold in both the core and amended compost samples; exponential curvilinear regression indicated that there was a trend to significance for the core compost ($P = 0.08$, $R^2 = 0.688$), and significant for the amended compost ($P = 0.02$, $R^2 = 0.985$). The fungal population in the inoculated compost had a relatively weak exponential curvilinear increase over time ($P = 0.10$). Yeast populations varied by compost type, particularly the presence of a filamentous yeast originating from the suppressive compost (Table 7.3).

For both experiments, the relative microbial activity varied over time between compost types (Tables 2 and 3). In experiment I, the microbial activity of the inoculated compost increased from days one to five, whereas no change was recorded in the core of amended compost. In experiment II, 16 hours after inoculation, the inoculated compost had greater microbial activity than the core or amended compost. By day seven both the core and amended compost had the same relative increase in their microbial activity but had statistically different levels of disease suppression.

DISCUSSION

Cooling and incubating the hot compost under sterile conditions did not support the development of suppressive compost. Inoculating the compost with ambient temperature yard trimmings compost followed by an incubation period was necessary to develop suppressive compost. These data indicate that the disease suppression is biological in nature and due to the introduction of microorganisms from

exogenous sources and not from the growth of microflora surviving the heating process.

For both experiments, only the inoculated compost became suppressive within five days of sterile storage. In experiment II, it is unclear why the amended compost became suppressive on day seven while the core compost remained conducive to damping-off (figure 2). The Solvita compost respiration test detected an increase in respiration for both composts from days four to seven, possibly as a result of the increased fungal populations. Suppression developing in the amended compost could possibly be related to the significant fungal population increase and the detectable population of filamentous yeast.

Determining a universal minimum incubation period needed for suppression to develop is difficult because various times have been reported. The data presented here indicates that inoculation was necessary to develop suppression in Rexus yard trimmings compost within a seven day sterile incubation period. Similarly, Craft and Nelson (1996) reported suppression developing the same day that autoclaved brewery sludge or biosolids compost was inoculated with 2% nonautoclaved compost. However, Pythium suppression has been shown to develop when composts temperatures have not declined to the mesophilic range (Kuter et al., 1988; Grebus *et al*, 1994), indicating that either suppression developed very rapidly from mesophilic colonists introduced with peat, or the hot composts were suppressive by a mechanism not explained by the general suppression theory (Hoitink and Fahy, 1986; Hoitink *et al*, 1993).

Based upon the data presented here and previous reports, compost sources that are sufficiently stable for container media but at high temperature should be cooled, inoculated, thoroughly mixed, and incubated at mesophyllic temperatures for at least four days prior to use in container media. Prior recommendations for optimizing disease suppression in container media suggest incubating formulated container media for at least two week at ambient temperatures to stabilize the physiochemical and biological properties (Inbar *et al*, 1993). Further research is needed to understand if

Pythium suppression is differentially affected by using pre-incubated compost in container media compared to blending non-incubated compost and then incubating all components together. However, during peak periods of activity, nurseries often use container media immediately after mixing or delivery and incubating all components together is not practical (personal observation). Incubating hot compost before adding to container media would allow the media to be used immediately for *Pythium* suppression and would help avoid heating the media. Heating formulated container media could eliminate heat sensitive antagonistic microflora and release excessive nutrients from temperature-regulated slow-release fertilizers.

Nursery operations typically manage multiple soil borne pathogens simultaneously and the suppression of pathogens other than *Pythium* spp. needs to be considered (Stephens *et al*, 1983, Jarvis, 1992). The controlled inoculation of compost with specific microbial antagonists can suppress multiple plant pathogens, however, currently available antagonists are heat sensitive and require cooling compost before inoculation (Hoitink, 1987). Compost production can be manipulated to create relative microbial voids with large, rapid decreases in compost temperature; filling the void with microbial antagonists could help the nursery industry consistently use a source of naturally disease suppressive material.

ACKNOWLEDGEMENTS

We thank Beth Hoinacki for the *P. ultimum* isolate. Also Drs. H.A.J. Hoitink and M. Krause for background information on the *P. ultimum* bioassay and discussions on compost mediated disease control. Funding from USDA CRIS # 303-5358-22000-024-00D and the Oregon association of Nurserymen and Oregon Department of Agriculture provided for the research.

Chapter eight: Conclusions

Prior to this research, very little phytopathology data on the use of compost and compost teas that was relative to Oregon existed. In response, multiple projects were undertaken with the unifying theme of assessing compost teas and compost amended container media for plant disease suppression. These efforts aimed to deliver practical information and techniques that growers could utilize to assist their plant disease management efforts. Knowing that a contradiction often exists between the variability observed with biological control and grower's need for consistent control, efforts were focused on identifying methods that would reduce variability of disease suppression.

With compost tea, variability in disease control was addressed by systematically manipulating compost tea production factors to determine if an optimal compost tea production protocol could be determined. Previous research on the influence of compost tea production factors had identified compost source, fermentation nutrients, and adding spray adjuvants as important factors in plant disease suppression (Chapter 2). More recently, compost tea practitioners have explored the uses of aerated compost tea, however, the utility of aerating compost tea for plant disease control had not been assessed.

After working with multiple plant species and pathogens, no one optimal compost tea production protocol was identified. However, based on empirical evidence, practical results for each pathogen were gained regarding the relative importance of various compost tea production factors.

Compost source was the most important compost tea production factor identified in the suppression of gray mold of geranium (Chapter 3), powdery mildew of rose (Chapter 4), and black spot of rose (Chapter 4). The source of compost used to make compost tea was not an important factor for suppressing damping-off of cucumber caused by *Pythium ultimum* (Chapter 5). Interestingly, this is in stark

contrast to suppressing the same disease with compost amended container media, where large differences across compost sources was evident (Chapter 6).

The effect of adding fermentation nutrients was disease specific. Adding fermentation nutrients to compost tea did not significantly increase ($P>0.05$) the suppression of gray mold on geranium (Chapter 3). For black spot of rose, a transient effect of added nutrients was observed, but no significant (P 0.05) effect was evident at the final disease assessment date (Chapter 4). For powdery mildew of rose, the factor of fermentation nutrient was not compared, however, all teas were amended with molasses and all significantly reduced ($P<0.05$) powdery mildew. For *Pythium ultimum* damping-off of cucumber, nutrients were necessary for disease suppression, apparently by increasing bacterial populations capable of interfering with pathogenesis. Differences in nutrient sources was evident. Adding kelp and humic acids effectively stabilized biological control, while a molasses-based nutrient source resulted in erratic control. Evidence indicated that residual nutrients in the molasses-based fermentation nutrient could apparently stimulate *P. ultimum*, thereby counteracting suppression (Chapter 5). Additionally, molasses has been demonstrated to support the growth of *Escherichia coli* and *Salmonella* if inadvertently present in compost tea, posing worker and consumer health concerns (Bess *et al*, 2002; Duffy *et al*, 2002). Therefore, nutrients need to be tested for their effect on both targeted plant pathogens and non-targeted human pathogens.

Spray adjuvants were used to significantly ($P <0.05$) decrease gray mold on geraniums when added to compost tea (Chapter3). Preliminary evidence indicated that spray adjuvants could enhance leaf surface colonization by compost tea microflora. This is a promising area of research for reducing the variability of foliar disease suppression.

In spite of the popular surge in the use of aerated compost tea, aerating compost tea did not significantly ($P < 0.05$) increase the suppression of rose powdery mildew, rust, or black spot (Chapter 4). Likewise, aeration did not increase suppression of gray mold on geraniums (Chapter 3). For the suppression of damping-

off of cucumber caused by *Pythium ultimum*, both aerated and non-aerated compost tea significantly ($P < 0.05$) suppressed the disease, but only when fermentation nutrients were added (Chapter 5). Therefore aeration by itself has not been shown to be a superior method for producing plant disease suppressive compost tea. However, considering that the aerated compost tea produced with kelp, humic acids and rock dust was the most consistent formula for damping-off disease suppression (Chapter 5), the interaction between aeration and fermentation nutrients for optimizing suppression deserves more study.

Regarding compost amended container media, a number of practical findings were recorded for suppressing seedling damping-off caused by *Pythium* spp. and *Rhizoctonia solani* (Chapter 6). Composts varied in their ability to suppress *Pythium* spp. and *R. solani* damping-off disease. Compared to the inoculated peat-perlite media, 66% of the compost amended container media significantly reduced damping-off caused by *P. irregulare*, 64% significantly reduced damping-off caused by *P. ultimum* and 56% of the samples suppressed damping-off of both *Pythium* spp.. Only 17% of the compost samples significantly reduced damping-off caused by *R. solani* when mixed with peat and vermiculite. A large portion (42%) of the compost amended container media had significantly worse damping-off caused by *R. solani* compared to the peat-vermiculite standard. Damping-off caused by all three pathogens was significantly suppressed by 11% of the compost samples. Twenty-two percent of the samples did not significantly suppress damping-off disease caused by any pathogen.

The suppression of *Pythium* damping-off was related to the compost respiration potential and presence of volatizing ammonia (Chapter 6). A minimum respiration rate of 1 mg CO_2-C/g compost C/day was determined to support damping-off suppression. Compost samples that had either very high respiration rates or moderate amounts of ammonia volatilization would not be considered suitable for greenhouse seedling production. Therefore, the actual number of samples suitable for suppressing damping-off is lower than the total numbers indicate. However, from

extensive experimentation and personal observation, it is clear that certain sources of commercially available compost meet the parameters for *Pythium* damping-off suppression and horticultural acceptability. This knowledge could immediately assist the nursery industry to successfully incorporate compost into their soilless media.

The relatively few compost sources capable of suppressing damping-off caused by *Rhizoctonia solani* indicate that, on average, commercial greenhouse producers would not benefit from incorporating compost at this time. One experiment indicated that commercially acceptable control was attained by incubating compost with *Trichoderma hamatum* T 382 for five days before use (Chapter 6). Further work on the inoculation of compost with specific microbial antagonists is needed to increase the potential to suppress *R. solani* with regionally available compost.

Manipulating compost production and handling processes for the consistent production of material capable of suppression *P. ultimum* damping-off was addressed in Chapter seven. Insight was gained into the process needed for the rapid development of compost capable of suppressing *P. ultimum* damping-off. Before this research, it was not clear if suppression could develop simply by cooling curing compost, thereby allowing indigenous microflora to fully develop under mesophyllic conditions. By placing compost removed from the hot core of a compost pile into cool, sterile storage, it became clear that compost needs to be colonized by external sources of mesophyllic microflora for the rapid development of *Pythium* damping-off. This could assist compost producers and users to modify compost handling practices to optimize the biological control potential of the compost.

The challenge remains to integrate these findings into commercial plant production. Unfortunately, this is not a simple matter of technology transfer. In part, the slow rate that conventional agriculture has adopted biological disease control practices has been exasperated by marketing practices and consumer preferences that require food and ornamental plants to be blemish free. Therefore, full-scale implementation of biological control will necessitate both reducing variability of control and changing consumer perceptions of quality agricultural products.

Compost and compost tea are not a panacea for controlling all plant diseases. For some diseases, the level of control would be considered inadequate for conventional agriculture; organic producers with limited control options consider partial disease control to be an important improvement. However, further refinement of composting and compost tea production practices will likely increase the potential for consistently suppressing plant disease with these technologies.

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