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SPECIFIC SUPPRESSION OF PLANT DISEASES PROVIDED BY COMPOST-AMENDED SUBSTRATES

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in the Graduate School of The Ohio State University

By

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2001

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ABSTRACT

Natural potting mixes prepared with Sphagnum peat and/or composted pine bark may induce suppression to Rhizoctonia diseases and induce systemic resistance (SR) in plants. Unfortunately, these effects are variable in nature and the SR-active microflora remains unknown. The objectives of this work were to 1) determine the frequency by which natural potting mixes control these diseases, 2) determine the impacts of organic components used in potting mixes on the efficacy of biocontrol agents against Rhizoctonia diseases and 3) identify the most SR-active microorganisms for preparation of potting mixes inducing SR consistently in plants. The results show that < 20% of natural composted pine bark mixes suppressed Rhizoctonia damping-off of radish and that only 12.5% reduced the severity of bacterial spot of radish caused by *Xanthomonas campestris* pv. *armoraceae* (*Xca*). The biocontrol agents *Trichoderma hamatum* 382 (*T*$_{382}$) and *Chryseobacterium gleum* 299R$_2$ (*C*$_{299}$R$_2$) consistently induced suppression to Rhizoctonia damping-off in composted pine bark mixes. They were less effective in light, slightly decomposed peat and ineffective in more decomposed dark peat mixes. Similar results were obtained for control of Rhizoctonia crown and root rot of poinsettia.
Several bacterial strains were isolated from the rhizosphere of radish seedlings grown in SR-active compost-amended potting mixes that suppressed the severity of bacterial spot. None of these strains provided consistent control. $T_382$ was the most SR-active biocontrol agent recovered from compost-amended mixes. Bacterial spot severity was significantly ($P \leq 0.05$) reduced on radish seedlings grown in the composted pine bark mix fortified with $T_382$ compared to seedlings grown in the control mix. $T_382$ fortified-composted pine bark mix also reduced the population of $Xca$ in infected plants relative to the control mix. However, it was not as effective as acibenzolar-s-methyl which induces SAR in radish. Spatial separation of $T_382$ and the pathogen on the host plant was maintained suggesting that a systemic response was induced.
DEDICATION

To my grandfathers, Herbert Meinhart Kinney and Ralph Frederick Krause,
and my dear friend, John Robert Hall
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INTRODUCTION

Compost-amended potting mixes may reduce the severity of diseases caused by soilborne plant pathogens (Hoitink and Fahy, 1986). These potting mixes have been used extensively in the ornamentals industry since the early 1970’s. Potting mixes prepared with composted pine bark are most widely accepted by this industry (Hoitink et al., 1991). Root rots caused by Phytophthora and Pythium spp. are suppressed most effectively in compost-amended media (Chen et al., 1988b; Mandelbaum and Hadar, 1990; Hardy and Sivasithamparam, 1991; Boehm and Hoitink, 1992; You and Sivasithamparam, 1994). Suppression of these diseases is due to the activities of numerous biological control agents which naturally colonize composts after peak heating (Hardy and Sivasithamparam, 1991; Boehm et al., 1993, 1997; You and Sivasithamparam, 1994). The microbial carrying capacity of the mix (Boehm et al., 1993) determines the longevity of this effect. This mechanism of suppression often is referred to as the “general suppression” phenomenon (Baker and Cook, 1974).

Boehm et al. (1997), utilizing Sphagnum peat in potting mixes, demonstrated that the decomposition level of the peat determines the potential for natural biological control of Pythium root rots. They showed that potting mixes prepared with H₂ to H₃ on the von...
Post decomposition scale light Sphagnum peat (Puustjärvi and Robertson, 1975) supports active populations of numerous bacterial biocontrol agents. In contrast, the much more decomposed H₄ Sphagnum peat, which was harvested from much greater depths in peat bogs, does not support biocontrol. *Pseudomonas* spp. widely recognized as biocontrol agents (Weller, 1988) predominate in the suppressive light peat mixes. On the other hand, Gram-positive and pleomorphic bacterial taxa not recognized as biocontrol agents predominate in the conducive H₄ on the von Post scale Sphagnum peat mix. A critical finding by Boehm et al. (1993), was that inoculation of H₄ Sphagnum peat potting mixes with biocontrol agents does not provide biological control of Pythium damping-off or root rot of cucumber as effectively as it does in lesser decomposed Sphagnum peat mixes. It was concluded, therefore, that organic matter decomposition level is critical to sustained biological control of Pythium root rot (Boehm et al., 1997). These findings were verified recently for composted cow manure by Stone (1997) who showed that the concentration of lignin and lignin-protected cellulose in sawdust-bedded composted cow manure is critical to biological control of Pythium root rot of cucumber. In conclusion, soil organic matter decomposition level has a major effect on biological control of Pythium root rots.

Even though composts have been used effectively for suppression of Phytophthora and Pythium root rots, the severity of damping-off caused by *Rhizoctonia solani* typically is increased when composts are first applied (Nelson and Hoitink, 1982,
Parasitism plays a major role in suppression of diseases caused by *R. solani*. *Trichoderma* spp. have been identified as important biological control agents in the eradication of sclerotia of *R. solani* buried in composted bark-amended substrates (Kuter et al., 1983; Nelson et al., 1983; Kok et al., 1996; Lewis et al., 1998; Tuitert et al., 1998). These and most other biocontrol agents are sensitive to temperatures above 40°C and survive only in the outer layers of compost piles (Nelson and Hoitink, 1982; Chen et al., 1988a). Unfortunately, this specific microflora does not consistently colonize composts after peak heating to induce suppression of diseases caused by *R. solani* (Nelson et al., 1983, Kuter et al., 1988). In contrast, composts consistently become colonized after peak heating by the great diversity of biocontrol agents that can suppress Phytophthora and Pythium root rots (Chen et al., 1998b; Hardy and Sivasithamparam, 1991; Boehm et al., 1993; You and Sivasithamparam, 1994). One way to solve the lack of suppression of Rhizoctonia damping-off is to cure composts for at least four months before utilization (Kuter et al., 1988). Another approach is to apply composts in field soil several months before planting (Lumsden et al., 1983).

Several reports show that composts can be inoculated after peak heating with specific biocontrol agents to consistently induce suppression to Rhizoctonia damping-off and solve the variability problem of colonization with appropriate biocontrol agents after peat heating and, thus, avert the need for long-term curing (Kwok et al., 1987; Phae et al., 1988).
Yet other reports show that *Trichoderma* isolates can be inoculated into peat mixes to induce suppression to Rhizoctonia damping-off (Chet, 1987; Harman, 1992). It is not known whether organic matter decomposition level plays a role in efficacy here as it does for control of Pythium damping-off and root rot. One of the objectives of this research is to test this concept.

Commercial scale inoculation of potting mixes with biocontrol agents is a costly procedure. For growers to accept such a practice, it must be consistently effective and similar in cost to treatment of crops with fungicides. One of the goals of this research, therefore, was to determine the incidence of natural suppression of Rhizoctonia damping-off in two peat mixes differing in decomposition level versus a composted pine bark-amended potting mix widely available to the ornamentals industry, and, thus, determine the need for controlled inoculation of mixes with biocontrol agents. A second goal was to determine the impact of the type of organic matter used in potting mixes on the efficacy of the introduced biocontrol agents against Rhizoctonia damping-off and crown and root rot diseases.

Recently, it was discovered that composts may induce systemic resistance (SR) in plants to diseases caused by foliar as well as soilborne plant pathogens (Zhang et al., 1996, 1998). Unfortunately, not all compost-amended potting mixes induced SR in the foliage of plants (Zhang et al., 1998). Although rhizobacteria capable of inducing SR in
plants have been identified (reviewed in van Loon et al., 1998), the microorganisms in naturally SR-active compost-amended potting mixes inducing this systemic effect remain unknown. The remaining goal of this research, therefore, was to determine the frequency by which composts amended into potting mixes induce SR in plants against foliar plant pathogens and then to identify microorganisms causing this effect in plants.
CHAPTER 1

EFFECT OF POTTING MIX ORGANIC MATTER TYPE ON BIOLOGICAL CONTROL OF RHIZOCTONIA DAMPING-OFF OF RADISH AND OF RHIZOCTONIA CROWN AND ROOT ROT OF POINSETTIA

Introduction

Potting mixes may naturally suppress damping-off caused by *Rhizoctonia solani* Kühn (Nelson and Hoitink, 1982; Tahvonen, 1982; Gorodecki and Hadar, 1990; Grebus et al., 1994). Unfortunately, suppressiveness of potting mixes to these diseases is highly variable in nature, whether they are prepared with Sphagnum peat or composts (Nelson and Hoitink, 1982; Tahvonen, 1982). Differences in populations of specific biocontrol agents with activity against *R. solani* naturally supported in organic materials from which potting mixes are prepared contribute to this variability (Garrett, 1962; Elad et al., 1980; Tahvonen, 1982; Kuter et al., 1983; Nelson et al., 1983; Baker, 1987; Lewis et al., 1998). The decomposition level of the organic matter used in the mix also seems to play a role, but this factor has not been examined in detail (Nelson and Hoitink, 1983; Chung et al., 1988a; Tuitert et al., 1998).
Several biocontrol agents have been proposed as specific inoculants for use in potting mixes to consistently induce suppression to Rhizoctonia damping-off and reduce the variability problem. Proposed fungal biocontrol agents include isolates of *Trichoderma* spp. (Elad et al., 1980; Nelson and Hoitink, 1982; Tahvonen, 1982; Kuter et al., 1983; Lewis and Papavisas, 1985; Baker, 1987; Harman and Björkman, 1998; Lewis et al., 1998), *Penicillium* spp. (Gorodecki and Hadar, 1990), as well as binucleate isolates of *R. solani* (Cartwright and Benson, 1996; Harris and Adkins, 1998). Proposed bacterial biocontrol agents for control of Rhizoctonia diseases include *Burkholderia cepacia* (formerly known as *Pseudomonas cepacia*) (Cartwright and Benson, 1993, 1994), *Pseudomonas fluorescens* (Davey and Papavisas, 1963; Bagnasco et al., 1998), *Bacillus* spp. (Phae et al., 1990) and *Streptomyces* spp. (Tahvonen, 1982). Binary inoculants consisting of fungal isolates and bacterial strains, which are more effective than single inoculants by themselves, also have been proposed. For example, Kwok et al. (1987) demonstrated that *Chryseobacterium gleum* strain 299 (formerly *Flavobacterium balustinum* strain 299) and *Trichoderma hamatum* isolate 382 interact in biological control of Rhizoctonia damping-off of radish. Another approach developed for induction of suppression to Rhizoctonia damping-off in compost-amended potting mixes is to cure composts after peak heating until effective biocontrol agents have naturally colonized the composts (Kuter et al., 1988; Tuitert et al., 1998). The disadvantage of this approach is that up to 4 mo may be required to induce natural suppression consistently (Kuter et al., 1988).
Very little is known about the impact of the decomposition level of the organic fraction in potting mixes on the activity of biocontrol agents against Rhizoctonia diseases. Organic matter in soils or potting mixes must be fully colonized by soil microorganisms to induce microbiostasis so that the pathogen, *R. solani*, cannot utilize it directly as a food base (Garrett, 1962; Davey and Papavisas, 1963; Nelson and Hoitink, 1982; Lumsden et al., 1983; Chung et al., 1988b). Low concentrations of cellulose added to soil improve biocontrol of Rhizoctonia damping-off (Rouse and Baker, 1978). However, the high concentrations of glucose present in some sources of organic matter before composting, when the concentration of cellulose also is high, not only stimulate growth of the pathogen (Garrett, 1962; Davey and Papavisas, 1963; Nelson and Hoitink, 1982; Nelson et al., 1983; Lumsden et al., 1983; Chung et al., 1988b), but also repress parasitism of *R. solani* by *Trichoderma* spp. (Chung et al., 1988a, b; Cruz et al., 1993). *Trichoderma* spp. reach much higher populations as saprophytes in potting mixes prepared with fresh organic matter before composting when cellulose concentrations are high than in mixes prepared with mature compost in which the cellulose concentration is low and suppression is induced (Nelson et al., 1983; Chung et al., 1988b). During composting, the readily available sources of carbon (glucose, cellulose, etc.) are destroyed (Chen and Inbar, 1993). As a result, specific isolates of *Trichoderma* spp. parasitize sclerotia of *R. solani* there leading to eradication of the pathogen and biological control. This beneficial effect occurs even though the population of *Trichoderma* can be two orders lower in compost than in fresh materials where damping-off is not suppressed (Chung et al., 1988b).
The length of time that various sources of organic matter utilized in container media sustain these suppressive effects to diseases caused by *R. solani* remains unknown. This impact of the chemical composition of substrates in potting mixes on microbial consortia active in biological control of Pythium damping-off and root rots was reviewed recently (Hoitink and Boehm, 1999). Utilizing $^{13}$C-NMR and DRIFT spectroscopy, it was established that the concentrations of carbohydrates in Sphagnum peat, present mostly as protected cellulose (Boehm et al., 1997), and of both lignins and protected cellulose in composted sawdust-bedded cow manure (Stone, 1997), determine the longevity of the suppressive effect against Pythium damping-off and root rot. The rate of hydrolysis of fluorescein diacetate (FDA) (Schnürer and Rosswall, 1982) somehow directly relates suppressiveness of potting mixes to Pythium damping-off and root rots (Chen et al., 1988b; Mandelbaum and Hadar, 1990; Inbar et al., 1991; Boehm and Hoitink, 1992; Boehm et al., 1993, 1997). Potting mixes prepared with less decomposed organic matter, which are high in FDA activity, also are high in microbial biomass (Chen et al., 1988b; Boehm et al., 1993, 1997) and have been identified as “high in microbial carrying capacity” (Boehm et al., 1993). Within days after their formulation, such mixes become colonized naturally by bacterial taxa such as *Pseudomonas* spp. and *Pantoea* spp. capable of providing biological control of Pythium damping-off and root rots (Chen et al., 1988b; Boehm et al., 1993). Even so, these same mixes may not become colonized naturally by biocontrol agents capable of inducing suppression of Rhizoctonia damping-off until 4 mo later (Kuter et al., 1988; Boehm et al., 1993; Tuitert et al., 1998). In contrast,
*Arthrobacter* spp., *Micrococcus* spp. and oligotrophs are the predominant bacterial taxa naturally colonizing low-in-FDA-activity and low-in-microbial-carrying-capacity more decomposed Sphagnum peat mixes which are conducive to Pythium damping-off (Boehm et al., 1993, 1997). This microflora is less active or inactive in biocontrol of Pythium damping-off (Boehm et al., 1993). Similar correlations between suppressiveness and FDA activity have been established for the effects of organic amendments in field soils on the severity of Pythium damping-off of pea (Brüns, 1996; Brüns et al., 1996), for Phytophthora root rot of tomato (Workneh et al., 1993) and for some nematode diseases (Marull et al., 1997).

The objectives of this research were to determine whether the decomposition level of sources of organic matter commonly used in potting mixes affect the efficacy of the binary biocontrol agent inoculum consisting of *C. gleum* 299 (*C*299*R*2) and *T. hamatum* 382 (*T*382) in control of 1) Rhizoctonia damping-off of radish in a short-term bioassay, and 2) Rhizoctonia crown and root rot of poinsettia in a more long-term bioassay to evaluate the longevity of the suppressive effect. An integral part of this project was the characterization of the range in suppressiveness to these diseases occurring naturally in sources of Sphagnum peat and composted pine bark widely used in potting mixes.
Materials and Methods

Potting mixes. Three potting mixes differing in organic matter decomposition level, FDA activity and natural suppressiveness to Pythium damping-off and root rots were formulated as described previously (Boehm and Hoitink, 1992). A "light peat mix" was prepared by blending light, slightly decomposed H₃ [on the von Post decomposition scale (Puustjärvi and Robertson, 1975)] Sphagnum peat received from Canada (Premier Peat Moss Ltd., Quebec, Canada), Ireland (Bord na Móna, Newbridge Co., Kildare, Ireland) or several Baltic countries (Klasmann Deilmann GmbH, Geeste-Gross Hesepe, Germany), with coarse horticultural grade perlite at a volumetric ratio of 3:2. A "dark peat mix" was prepared with more-decomposed H₄ (on the von Post decomposition scale) dark Sphagnum peat from Canada (received from Premier Peat Moss Ltd., Quebec, Canada) and coarse horticultural grade perlite as described above for the light peat mix. Both peat mixes were amended with 1.1 g superphosphate, 1.1 g KNO₃, 1.1 g gypsum, and a 3:2 ratio of dolomitic lime and calcium carbonate (<100 mesh) per liter mix to adjust the pH to within the range of 5.5 to 5.7. A third mix, referred hereafter as "composted pine bark mix", was prepared with dark H₄ Sphagnum peat (Premier Peat Moss Ltd., Quebec, Canada), composted pine bark, medium horticultural grade vermiculite, and coarse horticultural grade perlite at volumetric ratios of 7:7:5:1. Some of the batches of composted pine bark mix were prepared at Earthgro, Inc., Glastonbury, CT, according to a procedure described in Hoitink et al. (Hoitink et al., 1991). Other batches were
prepared at OSU with the same type of compost and other raw materials. The following chemical additives were incorporated per liter of composted pine bark mix: 0.73 g nitroform slow release fertilizer (38-0-0), 0.55 g superphosphate (0-45-0), 0.82 g FeSO₄ (anhydrous), 0.57 g MgSO₄ (anhydrous), 0.02 g ZnSO₄, 0.01 g CuSO₄, and 2.77 g gypsum. The pH of the mix ranged from 5.5 to 5.8. The moisture content of all mixes was adjusted to approximately 50% on a total weight basis. The air capacity of all mixes after saturation with water followed by drainage in 10-cm-tall pots was > 20% (v/v).

FDA activity in the dark peat mixes, determined as described in Boehm and Hoitink (1992), was used to verify potting mix decomposition level (Boehm et al., 1997). In the dark peat mixes used in this work, FDA activity was consistently < 3.2 μg min⁻¹ g⁻¹ dry weight potting mix. In the light peat and composted pine bark mixes, it remained > 3.2 μg min⁻¹ g⁻¹ dry weight potting mix throughout each long-term poinsettia bioassay. The highest values consistently occurred in the composted pine bark mix as described previously (Boehm and Hoitink, 1992; Chen et al., 1988a).

**Inoculation of potting mixes with biocontrol agents.** Eight batches of biocontrol agent-fortified mixes were prepared for each type of mix. A commercial preparation of C₂₉⁹R₂ was received as frozen paste inoculum (1.0 x 10¹¹ CFU g⁻¹) from Chr Hansen Biosystems Inc., Milwaukee, WI. Inoculum of T₃₈₂ was received as a granular preparation (2.0 x 10⁸ CFU g⁻¹) from Sylvan Bioproducts, Inc., Cabot, PA. The biocontrol agents were blended separately into potting mixes during the mixing process to
achieve initial inoculum densities of $2.0 \times 10^6$ CFU g$^{-1}$ dry wt mix for $C_{299}R_2$ and of $1.0 \times 10^5$ CFU g$^{-1}$ dry wt mix for $T_{382}$. These fortified batches of composted pine bark mix were prepared over a two-year-period at Earthgro Inc. by blending the biocontrol agents during potting mix formulation into the mixes as part of the full-scale production process. Controls also prepared at Earthgro Inc. represented the same batches of materials but were not fortified with the biocontrol agents and are referred hereafter as natural batches of composted pine bark mix.

Light and dark peat mixes were fortified at OSU during mix preparation as described above with the same commercial batches of biocontrol inocula as those used at Earthgro, Inc. Control peat mixes were not fortified with $C_{299}R_2$ or $T_{382}$, and are referred hereafter as natural peat mixes.

The population of $C_{299}R_2$ in potting mixes was determined by tracking a natural rifampicin-resistant mutant ($C_{299}R_2^*$) on a selective medium containing 0.1 g rifampicin L$^{-1}$ medium (Kwok et al., 1987). The minimal detectable population of $C_{299}R_2$ in the potting mixes on this medium was $10^3$ CFU g$^{-1}$ dry wt mix. The unique colony morphology of $C_{299}R_2$ allowed it to be easily separated from colonies of other microorganisms that grew on this medium. Verification of $C_{299}R_2$ identity by GC-FAME analysis (MIDI Library version 3.6, Microbial ID, Inc., Newark, DE) confirmed that the procedure was effective.
The population of $T_{382}$ was determined on a selective medium for *Trichoderma* (Chung and Hoitink, 1990) by examining asexual fruiting structures under a dissecting microscope at $\times 200$. *T. hamatum* produces phialides which allow it to be distinguished readily from other *Trichoderma* spp. (Bissett, 1991). A specific DNA primer, based on a random amplified polymorphic DNA (RAPD) marker developed by Abbasi et al. (1999), was used to confirm the population of $T_{382}$ among other isolates of *T. hamatum* recovered on the selective medium.

Immediately after formulation of the biocontrol agent-fortified and natural (control) potting mixes, 10 g wet wt potting mix was suspended in 90 ml 0.1% sterilized water agar (three replicates per sample). Dilution plates were then made in triplicate on the selective media. Colonies of $C_{299} R_2$ (or $C_{299}$) and $T_{382}$ were counted after four days and ten days of incubation at 25°C, respectively. The mean population density of each organism in the potting mixes was monitored throughout the cropping cycle. Populations of both organisms were not always monitored on the same day because of the large amount of time involved in verification of biocontrol agent identity for each procedure.

**Rhizoctonia damping-off bioassay.** Suppression of Rhizoctonia damping-off in the three potting mixes was determined with a radish (*Raphanus sativus* L.) bioassay, utilizing the cultivar Early Scarlet Globe (85% germination) according to Kwok et al. (1987). Isolate 19 of *Rhizoctonia solani* Kühn, an AG-4 mating type originally isolated from poinsettia in the OSU Plant and Pest Diagnostic Clinic, was used in this bioassay.
Soil inoculum of *R. solani* was prepared in chopped potato soil as described by Ko and Hora (1971). Air-dried soil inoculum was ground with a mortar and pestle and then screened to collect 1 to 2 mm particles as described by Nelson and Hoitink (1982). Prior to planting, potting mixes were infested with 0.5 g of this screened soil inoculum per liter mix, an inoculum density that consistently separates mixes strongly suppressive from those conducive to Rhizoctonia damping-off (Nelson and Hoitink, 1982). Slow release fertilizer (Osmocote 17-6-12 Plus Minors, The Scotts Company, Marysville, OH) was incorporated into the mix at 13.5 g per liter mix.

The following treatments were used for each potting mix in each damping-off bioassay: pathogen-infested and non-infested controls; biocontrol agent-fortified and natural mixes (not fortified with the biocontrol agents); and autoclaved (autoclaved twice for 1 h at 24-h intervals) infested controls. Radish seeds were planted with a pneumatic seeder at a mean distance of 1.4 cm from one another in 10-cm-diameter polystyrene foam pots containing approximately 400 cm³ potting mix (32 seeds per 78.5 cm² area) as described earlier (Nelson and Hoitink, 1983). Pots were watered and incubated in growth chambers at 24-25 C under 24 h illumination (225 microeinsteins m⁻² s⁻¹). After 7 days, seedlings were rated for damping-off severity based on a disease severity scale in which: 1 = symptomless; 2 = small root or stem lesion, but not damped-off; 3 = large root or stem lesion, but not damped-off; 4 = postemergence damping-off; and 5 = preemergence damping-off. Identity of the pathogen was confirmed by its isolation on acidified Difco potato dextrose agar (APDA) followed by examination with light microscopy. All radish
bioassays were configured according to a randomized complete block design. Each
treatment was replicated five times (five pots per treatment). Effects of potting mixes on
damping-off severity were determined by performing Kruskal-Wallis analysis (a non-
parametric statistical procedure for ranking and analyzing rating and ordinal data) of
damping-off severity data using Minitab statistical software (Release 12, Minitab, Inc.,
State College, PA). If a significant chi-square test \( P = 0.05 \) was obtained for treatment
effect, significant differences between pairs of mean ranks were determined by calculating
a least significant difference (LSD) for ranks.

To obtain the range of suppressiveness to Rhizoctonia damping-off in the natural
mixes (not fortified with biocontrol agents), 81 batches of \( H_3 \), 30 batches of \( H_4 \)
Sphagnum peat, and 71 batches of composted pine bark mixes were tested with this
radish bioassay. The overall range for mean damping-off severity values for the control
(non-infested), the pathogen-infested, and the autoclaved and pathogen-infested
treatments were calculated for each type of natural mix, and the percentages of means
with values \( \leq 2.5 \) and of means with values \( \geq 4.0 \) were then determined from mean
damping-off severity values recorded for each batch. Kruskal-Wallis analysis was used to
determine if batches varied in suppression. The rank-based LSD test was performed on
Kruskal-Wallis mean ranks using a harmonic mean of the number of batches per mix (\( \bar{n} \)),
because the number of batches varied per bioassay.
To determine the consistency of the efficacy of the biocontrol agents against Rhizoctonia damping-off, damping-off bioassays were performed on 8 different batches of natural and fortified composted pine bark mix (prepared at Earthgro Inc. as described above) and 8 natural and fortified batches of each type of peat mix (prepared at OSU). Although several different commercial batches of biocontrol agent inocula were used, the same batches of $C_{299}$ and $T_{382}$ were used for each of the 8 batches of the three mixes over the 2-yr period. Data were analyzed as described above.

**Rhizoctonia crown and root rot bioassay.** Suppression of Rhizoctonia crown and root rot of poinsettia (*Euphorbia pulcherrima*) by the biocontrol agents $C_{299}$ and $T_{382}$ in the three types of potting mixes was evaluated utilizing the natural as well as the biocontrol agent-fortified mixes as described above. Rooted cuttings of poinsettia (*E. pulcherrima* Willd. ‘Freedom Red’), received from Paul Ecke Ranch, Encinitas, CA, rooted in Oasis blocks (Smithers-Oasis, Kent, OH), were planted in 1.2-L, 15-cm-diameter plastic pots (one per pot) filled with 1200 ml potting mix. Soil inoculum of *R. solani* isolate 3, an AG-4 mating type (received from D. M. Benson, North Carolina State University, Raleigh, NC), was prepared as described above for isolate 19 of *R. solani*. The inoculum was applied to the surface of the mix in each pot (40 mg at two opposing locations, at a 5-cm distance from the stem) immediately after planting of the crop according to Benson (1991). This inoculum was then covered with approximately 1 cm of the same potting mix. Unless specified otherwise, the following treatments were used...
in this bioassay for each potting mix type: natural (not fortified) and biocontrol agent-fortified mixes, pathogen-infested and non-infested control mixes, autoclaved pathogen-infested mix, and a fungicide-drenched pathogen-infested control. A fungicide drench recommended for poinsettia (Nameth, 1997) containing thiophanate methyl [0.68 mg a.i. ml\(^{-1}\) water (Cleary's 3336F, W.A. Cleary Chemical, Dayton, NJ)] and metalaxyl [0.02 mg a.i. ml\(^{-1}\) (Subdue 2E, Syngenta Crop Protection Inc., Greensboro, NC)] was applied immediately after potting and monthly thereafter (125 ml solution per 1200 ml pot).

Plants were grown and maintained in a greenhouse at a temperature range of 17-25 C with naturally occurring photoperiods. Fertilizer (200 mg/L of Peters 20-20-20 Poinsettia Fertilizer, The Scotts Company, Marysville, OH) was applied twice per week. Plants were harvested after 6–8 weeks (due to seasonal impacts on crop development among experiments) and rated for crown and root rot severity based on a scale in which: 1 = symptomless; 2 = mild root rot; 3 = mild crown rot; 4 = severe crown rot; and 5 = dead plant. Identity of the pathogen was verified by dilution plating on APDA.

A randomized complete block design was used in each poinsettia bioassay. Three replicates of three plants each were used per treatment in the first bioassay. Because of differences in availability of rooted cuttings, the number of plants per replicate as well as the number of replicates per treatment were adjusted accordingly in the second and third bioassays. Kruskal-Wallis analysis was used to determine whether mix and biocontrol agent-fortification affected results. Significance of differences among mean ranks was determined with the rank-based LSD test. Mean crown and root rot severity values
calculated for each test for each treatment were then pooled to determine: a) overall mean crown and root rot severity, and b) mean ranks for each treatment. Significance of differences in mean ranks was determined using the rank-based LSD test.

**Results**

**Rhizoctonia damping-off of radish in natural and biocontrol agent-fortified potting mixes.** The mean damping-off severity value for all natural (not fortified with biocontrol agents) batches of dark peat mix tested was 3.8, revealing that damping-off in these mixes containing the most decomposed organic matter was very severe (Table 1.1). Even so, one of 30 batches tested was highly suppressive, with a mean damping-off severity value of 1.6. Two of 81 batches of the less decomposed natural light peat mix tested also were highly suppressive, but the mean damping-off severity value for all batches of this mix tested was 4.1 and not significantly (P = 0.05) different from that observed in natural dark peat mixes. The overall mean damping-off severity value for the natural composted pine bark mixes was also high (3.4) and did not differ significantly (P = 0.05) from mean disease severity of either type of natural peat mix (Table 1.1). Fourteen of the 71 (19.7%) different batches of the least decomposed natural composted pine bark mix tested had a disease severity value ≤ 2.5 and suppressed Rhizoctonia damping-off of radish. Thus, most (80.3%) of the natural composted pine bark mixes were also conducive to Rhizoctonia damping-off. In many of these batches (34% with a disease
severity value > 4.0), the disease was as severe as in either type of natural peat mix. *R. solani* was recovered on APDA from lesions on radish seedlings harvested from the infested mixes. Control plants remained free of symptoms.

Autoclaving increased damping-off severity in all of the naturally suppressive mixes, as shown in Table 1.2. Damping-off severity values in these autoclaved natural mixes (without biocontrol inoculum) ranged from 3.9 in the composted pine bark mix to 4.3 in the light peat mix to 4.6 in the dark peat mix. Inoculation of the same batch of composted pine bark mix with the biocontrol agents C\textsubscript{299}R\textsubscript{2} and T\textsubscript{382} (fortified mix) significantly (*P* ≤ 0.05) reduced Rhizoctonia damping-off severity and to a level below that observed in the autoclaved mix but not that of the natural mix (Table 1.2). In addition, inoculation of light peat mix with the biocontrol agents did significantly (*P* ≤ 0.05) reduce the mean severity of the disease below that observed in either the natural or autoclaved light peat mix. In this instance, disease severity was significantly (*P* ≤ 0.05) lower in the natural batch of composted pine bark mix than in either the natural dark peat or the light peat mix. In this bioassay, the biocontrol agents were significantly (*P* ≤ 0.05) less effective in only the dark peat mix (mean severity value of 3.6) than in the composted pine bark mix (mean severity value of 1.6).

A summary of the efficacy of the biocontrol agents in eight batches of all three types of mixes fortified with C\textsubscript{299} and T\textsubscript{382} is presented in Table 1.3. None of the eight batches of natural dark peat or light peat mixes (controls not fortified with C\textsubscript{299}R\textsubscript{2} and T\textsubscript{382}), and only 25% of the natural batches of composted pine bark mix (not fortified with C\textsubscript{299}
Overall mean Rhizoctonia damping-off severity values for the biocontrol agent-fortified dark peat, light peat and composted pine bark mixes were 3.7, 3.2 and 2.2, respectively. The mean damping-off severity values in the fortified composted pine bark did not exceed 2.6 (Table 1.3). In contrast, damping-off severity values in 50% of the batches of the biocontrol agent-fortified dark peat mix were ≥ 4.0 and values in 25% of the fortified light peat batches were ≥ 4.0. Thus, the collective comparison of these overall means revealed that the biocontrol agents were consistently more effective (P ≤ 0.05) in reducing the severity of Rhizoctonia damping-off in the composted pine bark mix than in either peat mix. Furthermore, the biocontrol agents varied in efficacy in the peat mixes and lack of control was most prevalent in the dark peat mix.

**Rhizoctonia crown and root rot of poinsettia.** Data for a poinsettia bioassay presented in Table 1.4 reveal that the disease in the natural composted pine bark mix infested with *R. solani* was significantly (P ≤ 0.05) more severe (crown and root rot severity value of 4.1) than in either of the infested natural dark peat or natural light peat mixes used in that experiment (disease severity values of 2.4- and 2.9, respectively). The crown and root rot severity values observed in these natural batches of natural dark peat and light peat mixes did not differ significantly (P ≤ 0.05). Most plants in the natural mixes infested with *R. solani* developed stem rot lesions and died within 18 days after planting. Severe crown and root rot developed later on the remaining plants during
production of the crop and none remained symptomless. Severe crown and root rot developed on poinsettia plants produced in all three types of autoclaved potting mixes infested with *R. solani* and most plants died within 20 days after potting. The plants produced in mixes not infested with *R. solani* did not develop symptoms of the disease. *R. solani* was recovered on APDA from diseased plants produced in infested mixes.

Inoculation of the composted pine bark mix with the biocontrol agents C<sub>299R</sub> and T<sub>382</sub> (fortified mixes) significantly (*P* ≤ 0.05) reduced Rhizoctonia crown and root rot severity over that in the natural mix, from a high mean crown and root rot severity value of 4.1 in this batch of natural mix down to a value of 1.2 in the fortified mix (Table 1.4). In the batch of light peat mix, the biocontrol agents reduced crown and root rot severity from a mean value of 2.9 down to 1.7. In the dark peat mix, the biocontrol agents did not significantly (*P* ≤ 0.05) reduce crown and root rot severity, but this particular batch of mix (control mix not fortified with the biocontrol agents) was naturally suppressive to the disease (crown and root rot severity value of 2.4). Efficacy of the fungicide drench (metalaxyl and thiophanate-methyl) did not differ significantly (*P* ≤ 0.05) from the biocontrol agents in any of these mixes. Finally, the biocontrol agents did not noticeably alter growth or the physical appearance of poinsettia plants in control mixes not infested with *R. solani*. In conclusion, all three types of biocontrol agent-fortified mixes used in this experiment (Table 1.4) suppressed (disease severity values < 2.0) Rhizoctonia crown and root rot, whereas the natural mixes ranged from conducive to suppressive.
The overall mean crown and root rot severity values determined from three different poinsettia bioassays for natural (not fortified with the biocontrol agents) dark peat, light peat and composted pine bark mixes infested with *R. solani* were 3.2, 3.1 and 3.0, respectively. These values did not differ significantly from each other (*P* ≤ 0.05; Table 1.5). In the fortified mixes, overall mean crown and root rot severity values for these three experiments were significantly (*P* ≤ 0.05) lower, down to mean values of 1.9 and 2.3 for the light peat and the composted pine bark mixes, respectively. The dark peat mix fortified with the biocontrol agents did not significantly (*P* ≤ 0.05) reduce overall crown and root rot severity below that observed in the natural dark peat mix infested with the pathogen. Drenching of the infested natural mixes (not fortified with the biocontrol agents) with the fungicides metalaxyl and thiophanate-methyl also significantly (*P* ≤ 0.05) reduced crown and root rot severity in all three mixes (Table 1.5). The fungicide drench in the infested natural mixes was slightly more effective in reducing disease severity than the biocontrol agents in both the light peat and the composted pine bark mixes. In the dark peat mix, however, the fungicide drench was less effective and did not significantly (*P* ≤ 0.05) reduce *Rhizoctonia* crown and root rot severity below that value observed in the biocontrol agent-fortified dark peat mix. Control plants not infested with *R. solani* remained symptomless. *R. solani* was recovered on APDA from diseased plants produced in infested mixes.
Populations of *Chryseobacterium gleum* 299 and *Trichoderma hamatum* 382 in potting mixes. The initial population of *C*$_{299}$R$_{2}$ in the light and dark peat mixes rapidly declined from $5.0 \times 10^6$ and $2.5 \times 10^6$ CFU g$^{-1}$ dry wt mix, respectively, down to $2.5 \times 10^3$ and $1.2 \times 10^4$ CFU g$^{-1}$ dry wt mix at 70 days after potting (Fig. 1.1). The population of *C*$_{299}$R$_{2}$ in the composted pine bark mix was not different from that in either type of peat mix during the first 30 days but it remained above $2.5 \times 10^5$ CFU g$^{-1}$ dry wt thereafter. It consistently was not recovered from control mixes (data not shown). These trends were consistent among all experiments.

The population of *T*$_{382}$, introduced into all mixes to an initial inoculum density of $\sim 10^5$ CFU g$^{-1}$ dry wt mix, consistently remained at $10^5$ CFU g$^{-1}$ dry wt or above this level in the composted pine bark mix through 90 days in all batches tested. In both peat mixes, a significant decline in populations was consistently observed soon after planting. An example of the trends for one experiment is presented in Fig. 1.1 where a decline in populations in both peat mixes was observed even after 2 weeks. The population of *T*$_{382}$ increased with time after the initial decline in both types of peat mixes and after 70 days its population was not significantly ($P \leq 0.05$) different from that in the composted pine bark mix. The identity of *T*$_{382}$ recovered on the selective medium was confirmed with RAPD analysis. Although *Trichoderma* was recovered from control mixes (not fortified with *T*$_{382}$) on the selective medium, none of the isolates was identified as *T*$_{382}$. 

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Discussion

This work revealed that numerous batches of potting mixes prepared with the slightly less decomposed natural light Sphagnum peat as well as the natural composted pine bark mixes (not fortified with the biocontrol agents C_{299}R_2 or T_{382}) did not consistently suppress Rhizoctonia damping-off of radish (Table 1.1), even though these high-in-microbial-carrying-capacity mixes consistently provide natural control of Pythium damping-off of cucumber and of Pythium root rot of poinsettia (Chen et al., 1988b; Le Quéré et al., 1990; Boehm and Hoitink, 1992; Boehm et al., 1993; Grebus et al., 1994; Ryckeboer et al., 1999). Only 14 of 71 different batches of the natural composted pine bark potting mix strongly suppressed this disease and only two of 81 different natural batches of light peat potting mix were effective. On the other hand, all but one of the 30 different batches of the low-microbial-carrying-capacity natural dark peat mix, which is consistently conducive to both Pythium damping-off (Le Quéré et al., 1990; Mandelbaum and Hadar, 1990; Boehm and Hoitink, 1992; Boehm et al., 1993) and Pythium root rot of poinsettia (Boehm and Hoitink, 1992), failed to naturally suppress Rhizoctonia damping-off (Table 1.1). Thus, the organic matter decomposition level of the Sphagnum peat (dark versus light peat) used in these potting mixes did not fully determine the potential for the development of natural suppressiveness to Rhizoctonia damping-off. As mentioned
above, the microbial carrying capacity of Sphagnum peat has a predictive impact in
potting mixes on natural suppression of Pythium damping-off (Le Quéré et al., 1990;

Amendment of the more decomposed dark Sphagnum peat with composted pine bark
improved the percentage of potting mixes naturally suppressive to Rhizoctonia damping-
off (up to 19.7%; Table 1.1). This probably was due to introduction of biocontrol agents
with the compost and the less decomposed nature of organic matter in composted pine
bark, as reported previously for mixes amended with composted hardwood bark,
composted cow manure or composted yard wastes (Nelson and Hoitink, 1982; Hadar and
Gorodecki, 1991; Grebus et al., 1994; Tuitert et al., 1998). Even so, less than 20% of
these natural batches of composted pine bark mix were suppressive to Rhizoctonia
damping-off, though such compost-amended mixes naturally suppress Pythium damping-
off consistently (Chen et al., 1988b; Mandelbaum and Hadar, 1990; Boehm and Hoitink,
1992). Because autoclaving destroyed suppressiveness in all of the mixes tested in this
work (Tables 1.2, 1.3 and 1.4), and inoculation with biocontrol agents at least in part
consistently restored suppressiveness (see example test in Table 1.2), we may conclude
that natural suppression of Rhizoctonia damping-off observed in this work was due to
biocontrol agents naturally present in these mixes. Therefore, lack of suppression in more
than 80% of the natural batches of the composted pine bark mix was most likely due to lack of colonization by an appropriate microflora capable of providing biocontrol of Rhizoctonia damping-off, as concluded previously for other compost-amended mixes (Nelson et al., 1983; Hadar and Gorodecki, 1991).

The foregoing reveals that the predominant bacterial biocontrol agents naturally colonizing the high in microbial carrying capacity light Sphagnum peat and composted pine bark mixes, which induce suppression of Pythium damping-off consistently and include numerous strains of fluorescent *Pseudomonas* spp. and *Pantoea* spp. (Boehm et al., 1993, 1997), did not effectively control Rhizoctonia damping-off. This confirms the specific nature of suppression of Rhizoctonia damping-off in potting mixes (Elad et al., 1980; Nelson et al., 1983; Gorodecki and Hadar, 1990; Hadar and Gorodecki, 1991; Grebus et al., 1994; Harman and Björkman, 1998), as opposed to the general suppression phenomenon which best describes suppression of Pythium damping-off (Chen et al., 1988b; Mandelbaum and Hadar, 1990; Boehm et al., 1993, 1997; Hoitink and Boehm, 1998).

The biocontrol agents $C_{299}R_2$ and $T_{382}$ effectively induced suppression to Rhizoctonia damping-off in each of the batches of fortified composted pine bark mix (Tables 1.2 and 1.3). This is in agreement with earlier reports on this ability of these biocontrol agents in fortified potting mixes prepared with composted hardwood bark (Kwok et al., 1987) and composted yard waste in the US (Grebus et al., 1994) and in Europe (Ryckeboer et al., 1999). Their significantly ($P \leq 0.05$) lower efficacy against
Rhizoctonia damping-off observed in both types of Sphagnum peat mixes appears to be due to the microbial carrying capacity or the decomposition level of the Sphagnum peat, because efficacy of $C_{299}R_2$ and $T_{382}$ in the dark, more decomposed Sphagnum peat mix (Boehm et al., 1997) was significantly worse ($P \leq 0.05$) than that in the less decomposed, higher carbohydrate (cellulose) light Sphagnum peat mix (Tables 1.2 and 1.3). We conclude, therefore, that the decomposition level or the microbial carrying capacity of the potting mix, indeed had a significant impact on the efficacy of the biocontrol agents $C_{299}R_2$ and $T_{382}$ against Rhizoctonia damping-off. This impact of carbohydrate content in the form of protected cellulose (Boehm et al., 1993) in the more conducive dark versus the more suppressive light Sphagnum peat mix and the compost-amended mix on Rhizoctonia damping-off is reminiscent of the work by Rouse and Baker (1978). They showed that low rates of cellulose amendment in soil increased natural suppressiveness to this disease. We discussed above how high cellulose concentrations (10–20% on a dry wt basis) repress biocontrol of Rhizoctonia damping-off induced by *Trichoderma*, whether present as added purified cellulose (Chung et al., 1988b) or as cellulose in fresh bark (Nelson et al., 1983). Both the concentration and the form of this food base seems critical to sustained biological control (Hoitink and Boehm, 1999). Unfortunately, these properties of soil organic matter relative to biocontrol remain poorly defined.

Interestingly, the binary inoculum of $C_{299}R_2$ and $T_{382}$ significantly ($P \leq 0.05$) reduced Rhizoctonia crown and root rot severity of poinsettia in both the light peat and composted pine bark mixes (Table 1.5), and this efficacy was equivalent to that achieved
by the fungicide drench. The biocontrol agents were not effective against Rhizoctonia
crown and root rot in the dark peat mix, though crown and root rot severity values
observed in the biocontrol agent-fortified dark peat, light peat and composted pine bark
mixes did not differ significantly ($P \leq 0.05$) from each other. Though the biocontrol
agents were effective in the light peat and composted pine bark mixes in both bioassays
(Tables 1.3 and 1.5), the differences in mean disease severity of biocontrol treatments
across potting mix types did not follow the same trend for both bioassays. This
difference in trends may possibly be explained as follows. *Rhizoctonia solani* is a very
aggressive pathogen (Garrett, 1962; Benson, 1991). In the damping-off bioassay used in
this work, the inoculum of the pathogen was distributed evenly throughout the potting
mixes among seeds planted at uniform distances (Nelson and Hoitink, 1983). Most of the
visible disease symptoms developed within the first 4 days after potting. In the longer-
term poinsettia bioassay, care was taken to place the inoculum at a 5-cm distance from
the stem to avoid damping-off and stem rot. Since root rot did not develop until several
weeks after potting in this bioassay, ample opportunity existed here for interactions
between the biocontrol agents and *R. solani* in the potting mix. In contrast, much less
opportunity for such activity prevailed in the radish bioassay where most infections
occur within the first few days (Nelson and Hoitink, 1982).

$C_{299}R_2$ probably contributed to biocontrol of both diseases in the fortified composted
pine bark-amended mix because it maintained high populations throughout the 90 day test
period. This agrees with earlier observations for composted hardwood bark mixes (Kwok
et al., 1987). In the peat mixes, however, its population declined rapidly, which in part
can account for the higher damping-off severity observed in these mixes. $C_{299}R_{2}$
probably contributed very little to the control of Rhizoctonia crown and root rot of
poinsettia because its population was very low ($<10^4$ CFU g$^{-1}$ dry wt. mix) late in the
production cycle of this crop when this disease was most severe.

Trends in populations of *T. hamatum* 382 (confirmed by RAPD analysis) perhaps
best explain the degree of control observed for Rhizoctonia crown and root rot of
poinsettia across the three potting mixes. Its population was sustained between $10^5$ and
$10^6$ CFU g$^{-1}$ dry wt. mix, which is within the range published earlier for composted
hardwood bark-amended potting mixes suppressive to Rhizoctonia damping-off (Nelson
et al., 1983; Kwok et al., 1987; Chung and Hoitink, 1990). In both peat mixes, however,
where Rhizoctonia damping-off was not controlled effectively (Tables 1.2 and 1.3), its
population declined soon after potting. This may have been due to lysis of the conidial
inoculum of *T. hamatum* 382 introduced as commercial inoculum into these lower-microbial-carrying-
capacity peat mixes. The population of *T. hamatum* 382 increased later (Fig. 1.1) in the peat mixes
when symptoms of Rhizoctonia crown and root rot were most severe in control mixes
infested with *R. solani* that were not fortified with the biocontrol agents (Table 1.5). In
summary, lack of substrate availability for growth of conidial inoculum of *T. hamatum* 382
introduced into the peat mixes may have contributed to failure in biocontrol of
Rhizoctonia damping-off in those mixes. In conclusion, only the fortified, highest-

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microbial-carrying-capacity composted pine bark mix consistently supported populations of both biocontrol agents and also suppressed both types of Rhizoctonia diseases.

It could be argued that the selection of $C_{299}R_2$ and $T_{382}$ as candidate biocontrol agents for testing the ability of Sphagnum peat mixes to support biocontrol activity against Rhizoctonia damping-off represented a poor choice of microorganisms for these three different types of potting mixes. This binary inoculum was chosen for several reasons. First, *Trichoderma* spp. have been identified several times as effective naturally occurring biocontrol agents in potting mixes prepared with light Sphagnum peat (Tahvonen, 1982; Boehm et al., 1993). Furthermore, several reports show that *Trichoderma* isolates can induce suppression of Rhizoctonia damping-off in peat mixes (Chet and Baker, 1981; Lewis et al., 1998). Finally, an isolate of *Trichoderma viride* capable of providing biological control of Rhizoctonia damping-off was isolated from the most strongly suppressive batch of light peat used in this work (Boehm, 1993). We have determined that this isolate was not more effective against Rhizoctonia damping-off than $T_{382}$ (Hoitink, unpublished results). $C_{299}$ was isolated originally from the rhizosphere of a radish seedling produced in a Sphagnum peat mix amended with composted hardwood bark (Kwok et al., 1987). It was selected from 6200 other bacterial strains as the most effective co-inoculant for $T_{382}$ (Kwok et al., 1987). It readily colonizes the rhizosphere of radish seedlings and interacts with $T_{382}$ to improve biological control of Rhizoctonia damping-off (Kwok et al., 1987).

The strong naturally suppressive effect against Rhizoctonia damping-off observed in
the one out of 30 batches of natural dark Sphagnum peat mix tested seems to contradict our findings but possibly can be explained as follows. During the harvesting process of Sphagnum peat from bogs, fine particles in light peat harvested from the surface of bogs often remain behind in the field and accumulate as harvesting proceeds. Some sources of dark Sphagnum peat, therefore, are mixtures of dark and less decomposed light peats. Dark Sphagnum peat contains high concentrations of highly humified peat fractions which strongly adsorb fluorescein and this, in turn, yields low FDA activity (Inbar et al., 1991). The suppressive batch of dark Sphagnum peat used in this work, therefore, may have contained a considerable quantity of undetected light Sphagnum peat which would account for part of the suppressive effect even though it was low in FDA activity (< 3.2 μg FDA hydrolyzed min⁻¹ g⁻¹ dry wt potting mix) (Boehm and Hoitink, 1992). Another explanation is that the inoculum of *T. viride* naturally present in the suppressive batch of light peat had fully colonized the mix and produced chlamydospores able to resist perturbations occurring after potting. We attempted to check for this possibility in our work but were unable to definitively identify propagules of *T₃₈₂* by light and scanning electron microscopy in these highly organic mixes. This process of formation of chlamydospores may also account for suppression of crown and root rot of poinsettia observed in the fortified peat mixes late during the crop when the population of *T₃₈₂* had
recovered. It is also possible, however, that specific and as of yet unidentified biocontrol agents capable of providing biological control of Rhizoctonia damping-off in dark more decomposed Sphagnum peat mixes were present naturally in this sample.

In summary, efficacy of the biocontrol agents C$_{299}$ and T$_{382}$ may well be representative of that which could be obtained with other biocontrol agents. Further testing will have to be performed to substantiate this postulate. It is clear, however, that the microbial carrying capacity of potting mixes must be considered in biological control of Rhizoctonia damping-off induced by introduced biocontrol agents, as was established earlier for control of Pythium damping-off (Boehm et al., 1993). We conclude that organic matter decomposition level of potting mixes can have a major impact on efficacy of biocontrol agents for suppression of Rhizoctonia damping-off of radish and this should be considered in biocontrol recommendations.
Table 1.1. Severity of Rhizoctonia damping-off of radish in different batches of natural dark peat, light peat, and composted pine bark-amended potting mixes.

<table>
<thead>
<tr>
<th>Potting Mix&lt;sup&gt;b&lt;/sup&gt;</th>
<th>R. solani Inoculum&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Mean&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Mean Rank&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Range&lt;sup&gt;f&lt;/sup&gt;</th>
<th>% Means with Value ≤ 2.5&lt;sup&gt;g&lt;/sup&gt;</th>
<th>Number of Batches&lt;sup&gt;h&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark Peat</td>
<td>−</td>
<td>1.2</td>
<td>38.1</td>
<td>1.0 - 1.6</td>
<td>100</td>
<td>30</td>
</tr>
<tr>
<td>Dark Peat</td>
<td>+</td>
<td>3.8</td>
<td>184.2</td>
<td>1.6 - 4.6</td>
<td>3.3</td>
<td>30</td>
</tr>
<tr>
<td>Autoclaved Dark Peat</td>
<td>+</td>
<td>3.3</td>
<td>139.5</td>
<td>2.2 - 4.1</td>
<td>7.7</td>
<td>13</td>
</tr>
<tr>
<td>Light Peat</td>
<td>−</td>
<td>1.2</td>
<td>38.5</td>
<td>1.1 - 1.4</td>
<td>100</td>
<td>31</td>
</tr>
<tr>
<td>Light Peat</td>
<td>+</td>
<td>4.1</td>
<td>217.7</td>
<td>2.3 - 4.8</td>
<td>2.5</td>
<td>81</td>
</tr>
<tr>
<td>Autoclaved Light Peat</td>
<td>+</td>
<td>3.3</td>
<td>175.8</td>
<td>3.1 - 4.5</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Composted Pine Bark</td>
<td>−</td>
<td>1.2</td>
<td>37.4</td>
<td>1.1 - 1.5</td>
<td>100</td>
<td>13</td>
</tr>
<tr>
<td>Composted Pine Bark</td>
<td>+</td>
<td>3.4</td>
<td>162.4</td>
<td>1.3 - 4.7</td>
<td>100</td>
<td>71</td>
</tr>
<tr>
<td>Autoclaved Composted Pine Bark</td>
<td>+</td>
<td>3.3</td>
<td>139.1</td>
<td>2.2 - 4.1</td>
<td>19.7</td>
<td>13</td>
</tr>
</tbody>
</table>

LSD<sub>0.05</sub> (Ranks) 61.6

<sup>a</sup> Mean damping-off severity determined 7 days after planting. Rating based on 5 pots of 32 plants each and damping-off severity scale in which: 1 = symptomless; 2 = small root or stem lesion; 3 = large root or stem lesion; 4 = postemergence damping-off; and 5 = preemergence damping-off.

<sup>b</sup> Autoclaved mixes were autoclaved twice for 1 h at 24 h intervals prior to planting.

<sup>c</sup> Potting mixes infested with 0.5 g soil inoculum of R. solani isolate 19 per liter mix; − = not infested.

<sup>d</sup> Mean damping-off severity values for all batches of a mix.

<sup>e</sup> Mean rank of Rhizoctonia damping-off severity; Kruskal-Wallis chi-square = 188.2, df = 8, P < 0.001.

<sup>f</sup> Range of mean damping-off severity values based on all replicates for each mix.

<sup>g</sup> Percent of batches of each mix with mean damping-off severity values ≤ 2.5.

<sup>h</sup> Number of batches analyzed.
### Table 1.2. Ability of the biocontrol agents *Chryseobacterium gleum* 299 and *Trichoderma hamatum* 382 to induce suppression of *Rhizoctonia* damping-off of radish in a dark peat, a light peat, and a composted pine bark-amended potting mix.

<table>
<thead>
<tr>
<th>Potting Mix</th>
<th>Biocontrol Inoculum</th>
<th><em>R. solani</em> Inoculum</th>
<th>Damping-off Severity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mean</th>
<th>Mean Rank&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Mean</th>
<th>Mean Rank</th>
<th>Mean</th>
<th>Mean Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dark Peat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural</td>
<td>−</td>
<td>−</td>
<td></td>
<td>1.4</td>
<td>11.8</td>
<td>1.2</td>
<td>8.7</td>
<td>1.1</td>
<td>4.5</td>
</tr>
<tr>
<td>Natural</td>
<td>−</td>
<td>+</td>
<td></td>
<td>4.1</td>
<td>44.7</td>
<td>4.1</td>
<td>44.3</td>
<td>2.8</td>
<td>25.4</td>
</tr>
<tr>
<td>Natural</td>
<td>+</td>
<td>+</td>
<td></td>
<td>3.6</td>
<td>35.7</td>
<td>3.1</td>
<td>28.5</td>
<td>1.6</td>
<td>17.0</td>
</tr>
<tr>
<td>Autoclaved</td>
<td>−</td>
<td>+</td>
<td></td>
<td>4.6</td>
<td>54.9</td>
<td>4.3</td>
<td>48.6</td>
<td>3.9</td>
<td>41.9</td>
</tr>
<tr>
<td>LSD&lt;sub&gt;0.05&lt;/sub&gt; (Ranks)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean damping-off severity based on 5 pots of 32 plants each determined 7 days after planting with a damping-off severity scale in which: 1 = symptomless; 2 = small root or stem lesion; 3 = large root or stem lesion; 4 = postemergence damping-off; and 5 = preemergence damping-off.

<sup>b</sup> *C. gleum* 299 and *T. hamatum* 382 were inoculated during mix formulation into potting mixes at initial inoculum densities of $2.0 \times 10^6$ and $1.0 \times 10^5$ CFU g$^{-1}$ dry wt mix, respectively; − = no inoculum.

<sup>c</sup> Potting mixes infested with 0.5 g soil inoculum of *R. solani* isolate 19 per liter potting mix; − = not infested.

<sup>d</sup> Mean rank of Rhizoctonia damping-off severity; Kruskal-Wallis chi square = 52.22, df = 11, $P < 0.001$.)
Table 1.3. Variability in efficacy of the biocontrol agents *Chryseobacterium gleum* 299 and *Trichoderma hamatum* 382 in control of Rhizoctonia damping-off of radish in fortified and natural dark peat, light peat, and composted pine bark-amended potting mixes.

<table>
<thead>
<tr>
<th>Potting Mix</th>
<th>R. solani Inoculum</th>
<th>Mean Biocontrol Fortified Mix</th>
<th>Mean Rank</th>
<th>Range</th>
<th>% Means with Value ≤ 2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark Peat</td>
<td>-</td>
<td>1.2</td>
<td>26.1</td>
<td>1.0 - 1.4</td>
<td>100</td>
</tr>
<tr>
<td>Dark Peat</td>
<td>+</td>
<td>3.6</td>
<td>106.2</td>
<td>3.0 - 4.1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.3 - 4.6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Autoclaved Dark Peat</td>
<td>+</td>
<td>3.5</td>
<td>96.6</td>
<td>2.7 - 4.1</td>
<td>0</td>
</tr>
<tr>
<td>Light Peat</td>
<td>-</td>
<td>1.2</td>
<td>27.6</td>
<td>1.1 - 1.4</td>
<td>100</td>
</tr>
<tr>
<td>Light Peat</td>
<td>+</td>
<td>3.8</td>
<td>114.0</td>
<td>2.9 - 4.1</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.4 - 4.1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Autoclaved Light Peat</td>
<td>+</td>
<td>3.7</td>
<td>108.6</td>
<td>3.1 - 4.1</td>
<td>0</td>
</tr>
<tr>
<td>Composted Pine Bark</td>
<td>-</td>
<td>1.1</td>
<td>20.3</td>
<td>1.1 - 1.3</td>
<td>100</td>
</tr>
<tr>
<td>Composted Pine Bark</td>
<td>+</td>
<td>3.4</td>
<td>95.9</td>
<td>2.3 - 4.4</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.6 - 2.6</td>
<td>87.5</td>
<td></td>
</tr>
<tr>
<td>Autoclaved Composted Pine Bark</td>
<td>+</td>
<td>3.5</td>
<td>96.6</td>
<td>2.7 - 4.1</td>
<td>0</td>
</tr>
</tbody>
</table>

| LSD<sub>0.05</sub> (Ranks) | 27.3 |

* Mean damping-off severity determined 7 days after planting. Rating based on 5 pots of 32 plants each and damping-off severity scale in which: 1 = symptomless; 2 = small root or stem lesion; 3 = large root or stem lesion; 4 = postemergence damping-off; and 5 = preemergence damping-off.

b Mean damping-off severity values for all batches of a mix.

c Mean rank of Rhizoctonia damping-off severity; Kruskal-Wallis chi square = 107.8, df = 17, P < 0.001.

d Range of mean damping-off severity values based on all replicates for each mix.

e Percent of batches of each mix with mean damping-off severity values ≤ 2.5.

f Autoclaved mixes were autoclaved twice for 1 h at 24 h intervals prior to planting.

g Potting mixes infested with 0.5 g soil inoculum of *R. solani* isolate 19 per liter mix; - = not infested.
Table 1.4. Efficacy of the biocontrol agents *Chrysobacterium gleum* 299 and *Trichoderma hamatum* 382, and the fungicides metalaxyl and thiophanate-methyl in providing control of *Rhizoctonia* crown and root rot of poinsettia (*Euphorbia pulcherrima* Willd. 'Freedom Red') in a dark peat, a light peat, and a composted pine bark-amended potting mix.

<table>
<thead>
<tr>
<th>Potting Mix</th>
<th>Biocontrol Inoculum</th>
<th><em>R. solani</em> Inoculum</th>
<th>Fungicide Drench</th>
<th>Crown and Root Rot Severitya</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Mean Rankc</td>
<td>Mean</td>
<td>Mean Rank</td>
</tr>
<tr>
<td>Natural</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Natural</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Natural</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Natural</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Natural</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Autoclaved</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

LSD$_{0.05}$ (Ranks) 30.6

---

a Mean crown and root rot severity determined 42 days after planting based on the following scale in which: 1 = symptomless; 2 = mild root rot; 3 = mild root and crown rot; 4 = severe root and crown rot; and 5 = dead plant.
b C. gleum 299 and T. hamatum 382 were inoculated during mix formulation into potting mixes at initial inoculum densities of $2.0 \times 10^6$ and $1.0 \times 10^5$ CFU g$^{-1}$ dry wt mix, respectively.
c Soil inoculum of *R. solani* isolate 3 was applied to the surface of the mix at opposite points 4 cm from the stem in each pot immediately after planting of the crop; – = not infested.
d Fungicide drench (thiophanate-methyl and metalaxyl) was applied monthly at the recommended rate to each pot.
e Mean rank of *Rhizoctonia* crown and root rot severity; Kruskal-Wallis chi-square = 71.5, df = 17, $P = 0.001$. 

---

Crown and Root Rot Severitya:

<table>
<thead>
<tr>
<th>Potting Mix</th>
<th>Crown and Root Rot Severitya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark Peat</td>
<td>Light Peat</td>
</tr>
<tr>
<td>Mean</td>
<td>Mean Rank</td>
</tr>
<tr>
<td>1.0</td>
<td>52.0</td>
</tr>
<tr>
<td>1.0</td>
<td>52.0</td>
</tr>
<tr>
<td>2.4</td>
<td>92.1</td>
</tr>
<tr>
<td>1.9</td>
<td>83.6</td>
</tr>
<tr>
<td>2.0</td>
<td>84.6</td>
</tr>
<tr>
<td>3.8</td>
<td>130.0</td>
</tr>
</tbody>
</table>

---

LSD$_{0.05}$ (Ranks) 30.6
Table 1.5. Variability in efficacy of the biocontrol agents *Chryseobacterium gleum* 299 and *Trichoderma hamatum* 382, and the fungicides metalaxyl and thiophanate-methyl in control of Rhizoctonia crown and root rot of poinsettia (*Euphorbia pulcherrima* Willd. 'Freedom Red') in three batches of dark peat, light peat, and composted pine bark-amended potting mixes.

<table>
<thead>
<tr>
<th>Potting Mix</th>
<th>Biocontrol Inoculum $^b$</th>
<th>$R. solani$ Inoculum $^c$</th>
<th>Fungicide Drench $^d$</th>
<th>Crown and Root Rot Severity $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Range of Means $^e$ Overall Mean Mean Rank $^f$</td>
</tr>
<tr>
<td>Dark Peat</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.0 1.0 4.0</td>
</tr>
<tr>
<td>Dark Peat</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>2.4 - 3.8 3.2 30.3</td>
</tr>
<tr>
<td>Dark Peat</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>1.6 - 2.9 2.1 22.0</td>
</tr>
<tr>
<td>Dark Peat</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>1.6 - 2.5 2.0 20.7</td>
</tr>
<tr>
<td>Light Peat</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.0 1.0 4.0</td>
</tr>
<tr>
<td>Light Peat</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>2.9 - 3.4 3.1 32.5</td>
</tr>
<tr>
<td>Light Peat</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>1.6 - 2.5 1.9 20.2</td>
</tr>
<tr>
<td>Light Peat</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>1.0 - 1.8 1.3 13.8</td>
</tr>
<tr>
<td>Composted Pine Bark</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.1 - 1.4 1.2 7.2</td>
</tr>
<tr>
<td>Composted Pine Bark</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>2.8 - 4.1 3.0 30.5</td>
</tr>
<tr>
<td>Composted Pine Bark</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>1.2 - 2.9 2.3 21.3</td>
</tr>
<tr>
<td>Composted Pine Bark</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>1.5 - 1.7 1.6 15.5</td>
</tr>
<tr>
<td>LSD $q_{0.05}$</td>
<td></td>
<td></td>
<td></td>
<td>8.7</td>
</tr>
</tbody>
</table>

$^a$ Mean crown and root rot severity determined 42–60 days after planting based on a scale in which: 1 = symptomless; 2 = mild root rot; 3 = mild root and crown rot; 4 = severe root and crown rot; and 5 = dead plant.

$^b$ *C. gleum* 299 and *T. hamatum* 382 were inoculated during mix formulation into potting mixes at initial inoculum densities of $2.0 \times 10^6$ and $1.0 \times 10^5$ CFU g$^{-1}$ dry wt mix, respectively; - = no inoculum.

$^c$ Soil inoculum of *R. solani* isolate 3 was applied to the surface of the mix at two opposite locations 4 cm from the stem in each pot immediately after planting of the crop; - = not infested.

$^d$ Fungicide drench (thiophanate-methyl and metalaxyl) was applied monthly at the recommended rate.

$^e$ Range of mean crown and root rot severity values based on all replicates for each mix.

$^f$ Mean rank of Rhizoctonia crown and root rot severity; Kruskal-Wallis chi-square = 29.4, df = 11, $P = 0.002$. 

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Figure 1.1. Mean population densities (± standard errors) of *Chryseobacterium gleum* 299R2 and *Trichoderma hamatum* 382 during production of a poinsettia crop in dark peat, light peat, and composted pine bark potting mixes. — = composted pine bark mix, --- = light peat mix, and — = dark peat mix.
CHAPTER 2

SPECIFICITY OF SYSTEMIC RESISTANCE INDUCED IN RADISH AGAINST BACTERIAL SPOT BY COMPOST-AMENDED SUBSTRATES

Introduction

It is well established that incorporation of composts into soil may affect the severity of diseases caused by soilborne plant pathogens. Recently, it was shown that such amendments may affect the severity of diseases of aerial plant parts. For example, Tränkner (1992) reported that composted cow manure incorporated into soil reduced the severity of powdery mildew of wheat. Clulow et al. (1995) reported that potatoes were protected from late blight by coating tubers with composts. More recently, Abbasi et al. (2000) showed that incorporation of composted yard wastes into field soil reduced the incidence of bacterial spot and anthracnose in conventional tomato production. The concentrations of essential plant nutrients in the foliage of plants produced on compost-amended versus control plots did not differ. Therefore, effects of the compost on foliar disease severity could not be explained on the basis of fertility. The incidence of bacterial speck and anthracnose on tomato fruit was also reduced significantly ($P \leq 0.05$) in an
organic production trial by amendment of the soil with composted vegetable cannery wastes. Although the mechanism by which these compost treatments reduced the severity of these foliar and fruit diseases was not established, the fertility data suggest that induced resistance may have played a role.

Zhang et al. (1996) first demonstrated that composts indeed can induce systemic responses in plants leading to reduced disease severity. Utilizing a split-root system, they showed that Pythium root rot of cucumber was suppressed relative to the control if one half of the root system was grown in a dark, conducive-to-root-rot Sphagnum peat mix, infested with *Pythium ultimum* with the other half in a composted pine bark- or spruce bark-amended potting mix suppressive to the disease. This systemic effect against *P. ultimum* was consistent among two different batches of two different types of compost-amended mixes; a composted pine bark- and a composted spruce bark-amended potting mix. In contrast, the severity of anthracnose of cucumber was suppressed on plants grown in only two of these four batches of compost-amended mixes. Thus, the systemic effect induced in cucumber roots against Pythium root rot was not expressed consistently in the foliage against anthracnose. Autoclaving destroyed the effect in all of the mixes whereas inoculation of a sterilized mix with a small quantity of suppressive mix restored the effect. This suggested that beneficial microorganisms played a role in the systemic effect. Utilizing a growth pouch bioassay, Han et al. (2000) showed that *Pantoea agglomerans* strain E278A, isolated from a composted hardwood bark mix suppressive to Rhizoctonia damping-off (Kwok et al., 1987), induced systemic resistance
(SR) in radish against bacterial leaf spot caused by *Xanthomonas campestris* pv. *armoraciae* (*Xca*). They also reported that **T**<sub>382</sub>, also isolated from composted hardwood bark suppressive to Rhizoctonia damping-off (Nelson et al., 1983), could induce SR in radish against bacterial spot. One of the goals of this research was to determine the frequency by which natural composts induce this effect and thus determine whether specific microbial consortia play a role in compost-mediated SR.

Since 1990, numerous reports have shown that rhizosphere microorganisms may induce SR in plants (van Loon et al., 1998). These microorganisms may induce resistance via any of several pathways leading to localized acquired resistance (LAR) (Ross, 1961a), systemic acquired resistance (SAR) (Ross, 1961b), induced systemic resistance (ISR) (van Loon et al., 1998; Pieterse and van Loon, 1999), and possibly other induced resistance mechanisms yet to be characterized. *Induced resistance* refers to the development of a condition of enhanced defensive capacity established in a plant after appropriate stimulation (Kúc et al., 1982, 1985; van Loon et al., 1998).

In nature, plants are continuously exposed to invading microorganisms and, thus, are likely to be primed for resistance against further attacks (Heil, 1999). Induced resistance, in addition to preformed infection barriers, contributes to overall resistance in plants and may provide a selective advantage for survival of plants (van Loon et al., 1998). Induced resistance can be activated by avirulent or incompatible races or forms of necrotizing pathogens, nonpathogens, chemicals, or virulent pathogens applied under certain environmental conditions (van Loon et al., 1998). Localized infection of a plant by a
necrotizing pathogen can induce quantitative protection against a broad spectrum of microorganisms in host tissues (Hammerschmidt and Kuc, 1995; Ross, 1961a). LAR occurs in plants when resistance to diseases, including expression of pathogenesis-related (PR) proteins (van Loon and Antoniw, 1982), is induced but limited to the tissue exposed to chemical or biological activators (Ross, 1961a; van Loon et al, 1998).

SAR can be induced in plants by mild or low doses of necrotizing pathogens (Sticher et al., 1997; van Loon et al, 1998), by chemical bioactivators such as salicylic acid (SA), or by any of its functional analogues such as dichloroisonicotinic acid (Schweizer et al., 1997) or acibenzolar-s-methyl (Lawton et al., 1996). Rhizosphere microorganisms also can induce SAR (Maurhofer et al., 1994; De Meyer and Hofte, 1997). SAR depends on the accumulation of SA, both locally and systemically. SA causes systemic activation of SAR genes whose products include PR-1, β-1,3-glucanase (PR-2), and chitinase (PR-3). These genes, therefore, are used as molecular markers for SAR. Examples of rhizosphere microorganisms that have been shown to induce SAR include *Pythium oligandrum* (Benhamou et al., 1997) and *Pseudomonas fluorescens* P3 (Maurhofer et al., 1994).

ISR is induced in plants by nonpathogenic microorganisms including plant growth-promoting rhizobacteria (Wei et al., 1991; Liu et al., 1995a,b; van Loon et al., 1998). ISR occurs without accumulation of and response to SA in the host, but does depend on response to ethylene and jasmonic acid. These microorganisms do not induce SAR gene expression above constitutive levels and molecular markers for ISR currently are not available though an ISR locus was identified recently in Arabidopsis (Ton et al., 1999).
Rhizosphere microorganisms reported to induce ISR include *P. fluorescens* WCS417r (Pieterse et al., 1996, 1998; van Wees et al., 1997; van Loon et al., 1998), *Serratia marcescens* 90–166 (Press et al., 1997), and *Trichoderma harzianum* T39 (De Meyer et al., 1998).

The foregoing reveals that several different microorganisms might contribute via any of several mechanisms to systemic resistance induced in plants by compost-amended potting mixes. Zhang et al. (1998) showed that SR induced by a composted pine bark mix fortified with *T.382* did not result in peroxidase accumulation nor in *PR-2* induction in arabidopsis plants expressing SR against bacterial speck caused by *Pseudomonas syringae* pv. *maculicola*. This seemed to demonstrate that the active batches of compost-amended potting mixes did not induce SAR but rather a response similar to that described for ISR (van Loon, 1998; Pieterse and van Loon, 1999). Another goal of this work, therefore, was to identify the microorganisms in compost-amended potting mixes that induce systemic resistance in plants.

Bioassays used for screening of biocontrol agents for SR activity typically require approximately four weeks or more to complete (Wei et al., 1991; Leeman et al., 1995a,b; Raupach et al., 1996). To reduce the potential for contamination among treatments in such long bioassays, Han et al. (2000) developed a more rapid bacterial leaf spot of radish seedling growth pouch bioassay that required only 14–18 days utilizing *Xanthomonas campestris* pv. *armoraciae* as the bacterial leaf spot pathogen (Sahin and Miller, 1996). The disadvantage of this method was that plants could not be maintained free of nutrient
deficiency symptoms beyond the short test period. A third goal of this research was, therefore, to adapt this bioassay to one utilizing sterilized composts that allowed testing of biocontrol agents for efficacy but avoided problems of earlier work.

The specific objectives of this work were to: 1) identify composts and compost-amended potting mixes that suppress bacterial leaf spot of radish using a rapid seedling bioassay; 2) isolate rhizosphere microorganisms from radish seedlings grown in compost-amended potting mixes that induce suppression of bacterial spot and to identify those strains that consistently induce suppression of bacterial spot; 3) compare the efficacy of these SR-active microorganisms to those reported to induce SR, including $T_{382}$; and 4) determine whether possible synergistic interactions occur among these microorganisms in the systemic suppression of bacterial leaf spot of radish.

Materials and Methods

Potting mixes. Two types of potting mixes were used throughout this work. A potting mix referred hereafter as “peat mix” was prepared by blending dark Sphagnum peat, $H_4$ on the von Post decomposition scale (Puustjärvi and Robertson, 1975), with coarse horticultural grade perlite (7:3 v/v) in a portable cement mixer. This mix was amended with 1.1 g gypsum, 1.1 g KNO$_3$, 1.1 g super phosphate (0-46-0) and a 3:2 ratio of dolomitic lime and calcium carbonate (< 100 mesh) to adjust pH within the range of 5.5–5.7 (Boehm et al., 1997). A mix referred hereafter as “composted pine bark mix” was
prepared either at Earthgro Inc. (Glastonbury, CT) or at OSU. It was formulated with composted pine bark prepared according to Hoitink et al. (1991), dark H₂ sphagnum peat, medium horticultural grade vermiculite, and coarse horticultural grade perlite at volumetric ratios of 7:7:5:1. This potting mix contained the following additives per liter of mix: 0.73 g nitroform slow release fertilizer (38-0-0), 0.55 g superphosphate (0-46-0), 0.82 g FeSO₄, 0.57 g MgSO₄, 0.02 g ZnSO₄, 0.01 g CuSO₄, and 2.8 g gypsum. The pH of this mix ranged from 5.5–5.8. Some of the batches of composted pine bark mix received from Earthgro Inc. had been fortified during mix formulation with commercial inoculants of Chryseobacterium gleum 299 (C₂₉₉; Chr Hansen Biosystems Inc., Milwaukee, WI) and Trichoderma hamatum 382 (T₃₈₂; Sylvan Bioproducts, Cabot, PA) to establish initial populations of $2.0 \times 10^6$ cfu and $1.0 \times 10^5$ cfu g⁻¹ dry weight mix, respectively. Other batches, either produced with composted pine bark from the same windrow or from an entirely different lot were not fortified and are referred hereafter as the “natural” composted pine bark mix. The percent air-filled pore space after saturation and drainage in both types of potting mixes was >20%.

To determine the frequency by which natural composts might reduce the severity of bacterial spot, several sources and types of composts listed in Appendix A were amended into various batches of composted pine bark mix at volumetric rates of 7.5% and 15%. A composted organic cannery and vegetable farm waste obtained from Hirzel Farms, Lucky, OH, was amended into the composted pine bark mix at volumetric rates of 5.0% and 10%. A composted yard waste obtained from KB Compost (Groveport, OH) was
amended into the composted pine bark mix at a volumetric ratio of 10%. All of these composts were collected from the outer low temperature sections of piles after peak heating (internal process temperature < 40 C). Field soil suppressive to bacterial spot of tomato (OSU/OARDC, Wooster, OH) was collected and amended into composted pine bark mix at volumetric rates of 2, 7.5 and 15%.

Immediately before planting of radish seeds, 15-9-12 plus minors slow release fertilizer (The Scotts Company, Marysville, OH) was incorporated into the peat and the composted pine bark mixes at a rate of 10.5 g per liter mix. The foliage of 15-day-old radish seedlings was analyzed for concentrations of major and minor essential plant nutrients at the STAR soil and plant analysis laboratory at OSU/OARDC, Wooster, OH. Results of preliminary experiments revealed that the concentrations of foliar nutrients in plants grown in the peat and composted pine bark mixes did not differ significantly and were within the recommended range for radish (Sonneveld and van den Bos, 1995). The quantities and types of slow release fertilizers added per liter mix to the composted manure-, biosolids- and soil-amended potting mixes was adjusted based on feedback from potting mix fertility analyses received from the STAR laboratory to avoid overloading of the mixes with essential plant nutrients.
**Bacterial leaf spot of radish bioassay.** The procedure used to test the ability of the various potting mixes to reduce the severity of bacterial spot of radish was based on a seedling bioassay originally developed by Miller et al. (1998) and modified by Han et al. (2000). In this new bioassay, radish (*Raphanus sativus* L. cv. ‘Cabernet’; Syngenta Seeds, Inc., Boise, ID) seeds were planted in each of five, 10-cm-diameter polystyrene foam pots (10 seeds/pot) containing approximately 400 cm³ potting mix for each treatment. Pots were irrigated with tap water as needed and incubated 10 d in a greenhouse at 22-25 °C under a combination of sunlight and supplemental lighting (225 microeinsteins m⁻² s⁻¹; 12 h d⁻¹). The number of emerged seedlings per pot was reduced to five by thinning plants of similar growth stage (two first true leaves > 1 cm in length) and size at seven days after planting. At ten days after planting, the pots were transferred to growth chambers providing 23 °C, 85–95% relative humidity (RH), and 12 h illumination (225 microeinsteins m⁻² s⁻¹) d⁻¹. Radish seedlings were then challenged with *Xanthomonas campestris* pv. *armoraciae* strain 704b (*Xca* 704b). This strain, isolated from radish in Ohio, is naturally-resistant to streptomycin sulfate (Sahin and Miller, 1996). Inoculum of *Xca* 704b was grown for 48 h at 25 °C on a rotary shaker (90 rpm) in sterile sucrose-peptone broth (SPB) (20 g sucrose and 5 g Bacto peptone [Becton Dickinson, Sparks, MD] per liter). Cultures were then centrifuged (3840 × g, 5 min), the supernatant was discarded, and the cells were resuspended to an inoculum density of 3.3 × 10⁶ CFU ml⁻¹ in sterile tap water containing 0.02% (v/v) Silwet® L-77 (OSi Specialties, Danbury, CT), a wetting agent. This suspension was then sprayed onto the two first
true leaves of radish seedlings until run off. Non-inoculated control plants were sprayed with autoclaved tap water containing 0.02% Silwet® L-77. In preliminary experiments, Silwet® L-77 at this concentration did not induce SR or cause symptoms of phytotoxicity. During the first 48 h after inoculation, the RH in the growth chambers was maintained within the range of 85–90%. Thereafter, it was lowered to 55–60%. Five days after inoculation when symptoms of bacterial spot had developed fully, the first two true leaves of each radish seedling were rated for disease severity based on a scale where: 1 = symptomless, 2 = few lesions to 10% leaf area affected, 3 = 10–25% leaf area affected, 4 = 25–50% leaf area affected, 5 = 50–75% leaf area affected, and 6 = >75% leaf area affected by lesions or dead leaf. Control potting mixes in each bioassay were the peat mix which did not affect the severity of bacterial spot (Zhang et al., 1996, 1998) and the heated (5 d, 60 C) composted pine bark mix as in Zhang et al. (1996, 1998)

**Isolation and identification of SR-active rhizobacteria.** “SR-active” rhizobacteria were isolated from roots of radish seedlings grown in two types of compost-amended potting mixes that significantly ($P \leq 0.05$) reduced the severity of bacterial leaf spot. They were a composted biosolids-amended mix and a batch of composted pine bark mix prepared at Earthgro Inc. in July 1996 that had also been fortified with $C_{299}$ and $T_{382}$. The control batch of this same composted pine bark mix that had not been fortified with $C_{299}$ and $T_{382}$ at Earthgro Inc. did not reduce the severity of bacterial spot. This mix hereafter is referred as the “natural” composted pine bark mix.
Only one procedure was used to isolate SR-active rhizobacteria from roots of radish plants grown in the composted pine bark mix that suppressed bacterial spot of radish. A 3 cm root tip section was excised from each of three, seven-day-old radish seedlings grown in this mix. Potting mix particles visible to the naked eye were rinsed gently from each root section with autoclaved tap water. The root sections then were comminuted separately in Ten Broek homogenizers containing 0.45 ml sterilized bacterial dilution buffer (BDB; 7 g K$_2$HPO$_4$ and 3 g KH$_2$PO$_4$ per L glass distilled water) containing 0.15% (v/v) agar (Becton Dickinson). The suspensions were serially diluted in BDB and plated in triplicate on 0.1× trypticase soy broth agar (TSBA) (Becton Dickinson).

Three procedures were used to isolate rhizobacteria from the roots of radish seedlings grown in the composted biosolids-amended mix that suppressed the severity of bacterial spot. The “rinsed root” method as described above was used. In the second procedure, the same comminuted root sample dilutions were heated 20 min to 80 C to destroy vegetative cells and facilitate recovery of heat-resistant spore-forming bacteria (“heat-tolerant” cells). Heated dilution suspensions were then plated in triplicate on 0.1× TSBA as described above. In the third procedure, individual root tips were surface sterilized by their immersion for 30 s in a 10 ml H$_2$O$_2$ solution, rinsed twice in 10 ml autoclaved tap water, immersed for 30 s in a 10 ml aqueous NaClO$_3$ solution (1.05%; v/v) containing 0.05% (v/v) Triton X-100 (Aldrich, Milwaukee, WI), and rinsed twice in 10 ml autoclaved tap water, all according to Musson et al. (1995) to recover endophytic SR-active rhizobacteria. Individual surface-sterilized root tips were comminuted into
suspensions and serially diluted and plated in triplicate on 0.1x TSBA as described above. Plates were incubated at 25 C for 48 h. Twenty discrete bacterial colonies (≥ 1 mm in size) were picked non-discriminantly from each of three plates for each root tip in a pattern according to Boehm et al. (1993) from plates containing between 20 and 200 colonies. They were streaked onto 0.1x TSBA to obtain pure cultures of each bacterial strain. Strains were assigned numbers in sequence according to the order in which they were picked, and were grouped according to their root tip and isolation method. This procedure assures a collection of a high diversity of bacterial strains from this medium (Boehm et al., 1993). Once pure rhizobacterial cultures were obtained, strains were cultured on 1.0x TSBA (25 C, 48 h) and stored in 15% (v/v) sterile glycerol-water solution at -75 C (Sleesman and Leben, 1978).

To prepare potting mix inoculum for the radish bacterial leaf spot bioassays, individual rhizobacterial strains were cultured 48 h at 24 C in autoclaved 250 ml erlenmeyer flasks containing 50 ml of autoclaved trypticase soy broth (TSB) (Becton Dickinson) on a rotary shaker (90 rpm). The cultures were centrifuged once (3840 x g, 5–10 min, depending on ease of separation of cells from supernatant), and resuspended in autoclaved tap water. Each washed culture was inoculated into 2 L heated (60 C, 5 d) composted pine bark mix to establish an initial inoculum density ranging from 1 x 10^6 – 3 x 10^7 CFU g^-1 dry wt mix. T_{382} utilized as an SR-positive control (Han et al., 2000) fortified into the composted pine bark mix to establish 1 x 10^6 CFU T_{382} conidial inoculum g^-1 dry wt mix. All potting mixes were incubated at 25 C for 5 d. The heated
composted pine bark mix served as a control. Radish seeds were planted and seedlings were maintained as described above. During planting and irrigation, care was taken to avoid contamination among treatments. Disease severity was determined as described above.

The bacterial strains that significantly \( (P \leq 0.05) \) reduced the mean severity of bacterial spot in the foliage of radish seedlings below that value obtained for radish seedlings grown in the heated composted pine bark mix were tested in three additional bioassays. Those strains that significantly \( (P \leq 0.05) \) reduced the severity of bacterial spot in at least three of the four bioassays are referred to hereafter as SR-active. These strains were identified using gas chromatography-fatty acid methyl ester (GC-FAME) analysis with a model HP6890 Microbial Identification System equipped with the Microbial Identification Anaerobic Library (v4.0; Microbial Identification Inc., Newark, DE) according to procedures specified by the manufacturer. Strains with a similarity index \( \geq 0.5 \) were classified at the species level, strains with a similarity index \( < 0.5 \) and \( \geq 0.1 \) were classified at the genus level, and strains with a similarity index \( < 0.1 \) were assigned a group (GC similarity group) based on the similarity of fatty acid profiles archived in the system.
Consistency of SR induced by $T_{382}$ and ability of SR-active rhizobacteria to increase efficacy of $T_{382}$ against bacterial leaf spot of radish. Several attempts were made to examine the ability of $T_{382}$ to consistently reduce the severity of bacterial leaf spot of radish and of the most SR active strains to improve the efficacy of $T_{382}$ against this disease. In these bioassays, inocula of the test bacterial strains and $T_{382}$ were added separately to the heated composted pine bark mix to establish initial inoculum densities for each as described above. Fortified mixes were then incubated 5 d before the bacterial leaf spot of radish bioassay was performed, also as described above. The ability of $T_{382}$ to significantly ($P < 0.05$) suppress bacterial spot of radish was evaluated in five bioassays. In each of three additional radish bacterial spot bioassays, combinations of $T_{382}$ with each SR-active rhizobacterial strain were then compared to $T_{382}$ used alone for their abilities to suppress bacterial spot severity. The heated composted pine bark mix and plants not inoculated with $Xca$ 704b served as controls. Acibenzolar-s-methyl (Actigard™ 50 WG, Syngenta Crop Protection, Greensboro, NC), referred hereafter as acibenzolar, was used as a positive SAR control (Lawton et al., 1996). Initially, it was applied as a topical spray 48 h before inoculation of radish seedlings with $Xca$ 704b. It stunted plants and caused necrosis on some seedlings as described by Louws et al. (2001) for tomato. However, it was discovered in preliminary tests that this compound applied as a drench (heated mix saturated with 50 μg mL$^{-1}$ solution) 48 h before inoculation with the pathogen could induce SAR consistently without causing phytotoxic effects. This treatment was incorporated as a positive SR control in all bioassays in which the efficacy
of $T_{382}$ was evaluated in combination with the SR-active rhizobacterial strains. Other control treatments included a non-heated control mix and plants not inoculated with $Xca$ 704b.

Several bacterial strains reported to induce SR in plants were tested in the composted pine bark mix. These strains included: *Bacillus amyloliquefaciens* IN937a, *B. cereus* C10 and *Paenibacillus macerans* 3PI-8 from J. W. Kloepper, Auburn University, Auburn, AL; *Pseudomonas chlororaphis* 63-28, *Burkholderia cepacia* Ral-3, and *Serratia proteamaculans* 1-102 from G. Brown, Agrium Biologicals, Saskatoon, Canada; and *Pseudomonas fluorescens* WCS417r from C. M. J. Pieterse, Utrecht University, Utrecht, The Netherlands. These and other strains tested are listed in Appendix B. Each of the strains was incorporated into the heated composted pine bark mix and tested for efficacy in three bioassays as described above for other rhizobacteria.

**Populations of $Xca$ 704b in radish leaves and *Trichoderma* populations in potting mixes or on radish leaves.** To determine foliar populations of $Xca$ 704b and of $T_{382}$ or other *Trichoderma* spp., two samples each containing one leaf from each of five pots were collected at random from each treatment after rating of plants for disease severity. Each sample was macerated with a ball-bearing tissue grinder (Agdia Inc., Elkhart, IN) in a sterilized Ziplock resealable freezer pouch containing a 1:4 ratio (w/w) of fresh radish foliage to sterilized BDB. This suspension was then diluted serially (1/10) in BDB and plated onto SPB agar medium (SPB with 15 g agar [Becton Dickinson] per L) containing
150 mg streptomycin sulfate (Sigma, St. Louis, MO). To detect populations of \textit{Xca} 704b, each dilution was either spotted (10 \mu L drop applied to the surface of the medium, 3 replicates per sample; Han et al., 2000) onto or poured (100 \mu L spread across the surface of the medium, 3 replications per sample) on SPB agar. Foliar dilutions were also plated (1 mL or 100 \mu L) onto a selective medium for \textit{Trichoderma} (TSM) developed by Chung and Hoitink (1990). The population of \textit{T}_{382} in potting media was also determined on TSM. Immediately after formulation of the fortified and control potting mixes, at planting and at 15 d after planting, 10 g wet wt potting mix was suspended in 100 ml 0.1\% sterilized water agar (three replicates per sample). Dilution plates were then poured in triplicate on the selective medium. A PCR-based technique developed by Abbasi et al. (1999) was used to verify propagule densities of \textit{T}_{382} on TSM plates.

\textbf{Experimental designs and statistical analyses.} In all bioassays, each treatment was replicated five times (five pots) with five seedlings per pot (after thinning). Pots were arranged in a randomized complete block design. Each compost batch was tested at least once for its ability to suppress bacterial spot of radish. Bacterial strains that induced suppression of bacterial spot in an initial bioassay were each tested in three additional bioassays for their abilities to the severity of bacterial spot significantly reduce below that observed on control plants inoculated with \textit{Xca} 704b. \textit{T}_{382} was tested in five bioassays for its ability to induce suppression of bacterial spot of radish. It was also tested in three additional bioassays singly and in combination with the bacterial strains
that significantly \((P \leq 0.05)\) reduced the severity of bacterial spot below that of control plants in at least three of four bioassays. Mean bacterial spot severity values were determined by performing one-way analysis of variance using Minitab statistical software (Release 12, Minitab, Inc., State College, PA) based on five replicates of ten true leaves per pot. If a significant \(F\)-test was obtained among treatments, significance of difference among means was determined using Fishers Least Significant Difference (LSD) test (Fisher, 1949).

Results

**Initial screening of compost-amended media for suppression of bacterial spot of radish.** A total of 80 different batches of compost-amended potting mixes were screened on radish seedlings for their ability to suppress bacterial spot. The types of compost amendments tested included composts prepared from pine bark, yard waste, municipal biosolids, cannery biosolids waste, and dairy manures, as well as different types of vermicomposts. A maximum of 15 batches of compost-amended potting mixes and controls could be screened in each bioassay. Therefore, several bioassays had to be performed to screen all 80 batches. The dark peat mix was used as a control in each bioassay.
Ten of the 80 different batches of compost-amended potting mixes significantly ($P \leq 0.05$) suppressed the severity of bacterial spot over that observed on inoculated plants in the dark peat mix. Two of the ten batches of compost-amended mixes that most effectively suppressed bacterial spot of radish were: 1) a batch of composted biosolids-amended potting mix, and 2) a batch of composted pine bark mix which had been fortified during mix formulation at Earthgro Inc., Lebanon, CT, with $C_{299}$ and $T_{382}$. This biocontrol agent-fortified batch of composted pine bark mix was the only batch of compost-amended mix that consistently suppressed the severity of bacterial spot diseases in repeated bioassays. The results for one of these bioassays are presented in Table 2.1. Mean disease severity values observed on inoculated plants in the dark peat mix and the natural CPB mix (7/96) were 3.1 and 3.2, respectively. Fortification of this batch of CPB mix with $C_{299}$ and $T_{382}$ during mix formulation significantly ($P \leq 0.05$) reduced the severity of bacterial spot to a value of 2.4. A different batch (11/97) of natural CPB mix, also received from Earthgro Inc., did not significantly ($P \leq 0.05$) reduce the severity of bacterial spot in this bioassay. Amendment of this natural batch of CPB mix with a SR-active tomato soil (2%; v/v) collected from plots at OARDC, Wooster, also did not significantly ($P \leq 0.05$) reduce the severity of bacterial spot. Finally, amendment of this batch with composted yard waste (10%; v/v) also did not have a significant effect. Control plants not inoculated with $Xca$ 704b remained free of symptoms of bacterial spot.
Isolation and identification of SR-active rhizobacteria. Rhizosphere microorganisms screened for their ability to reduce the severity of bacterial spot in the heated composted pine bark-amended mix differed widely in efficacy among bioassays. Therefore, only those microorganisms that significantly \((P \leq 0.05)\) reduced the severity of bacterial spot in at least three of four bioassays performed with each isolate were considered as positive for "SR activity".

Only three of 168 rhizobacterial strains (1.8%) isolated from roots of radish seedlings grown in the biocontrol agent-fortified composted pine bark mix significantly \((P \leq 0.05)\) reduced the severity of bacterial spot below that observed on inoculated seedlings grown in the heated composted pine bark control mix (Table 2.2). The most active isolates included strains identified by GC-FAME analysis as \textit{Pantoea} and \textit{Klebsiella} spp.

Only 3 of 122 bacterial strains (2.5%) isolated from washed radish roots grown in the composted biosolids mix reduced the severity of bacterial spot (Table 2.2). They were identified as \textit{Microbacterium} spp. and \textit{Bacillus} spp. Furthermore, seven of a total of 159 bacterial strains (4.4%) recovered from surface sterilized radish roots (putative endophytes) harvested from the same mix also significantly \((P \leq 0.05)\) reduced the severity of bacterial spot. This group included strains identified as \textit{Arthrobacter globiformis}, \textit{Bacillus} spp., \textit{Paenibacillus} spp., and \textit{Pseudomonas putida}. Finally, 7 of 95
bacterial strains (7.4%) recovered after heating (80 C, 20 min) of macerated root tips from the same composted biosolids mix significantly ($P \leq 0.05$) reduced the severity of bacterial spot. These active rhizobacteria included strains identified as *Bacillus* spp. and *Exiguobacterium* spp.

Several bacterial strains reported to induce SR in other pathosystems were evaluated for their ability to suppress bacterial spot of radish (Table 2.3). Only one strain, *Bacillus cereus* strain C10, significantly ($P \leq 0.05$) decreased the severity of bacterial spot in two of three bioassays. Only one additional strain, *Bacillus pumilus* strain SE-34, decreased the severity of bacterial spot in one of the three bioassays. Thus, none consistently reduced the severity of bacterial spot in all bioassays. Five additional strains that consistently inhibited germination and growth of seedlings or caused malformations of the foliage were not included in the data analysis. In all of these experiments, severe disease was observed on inoculated control plants grown in the heated composted pine bark mix. Control plants that were not inoculated with *Xca* 704b remained symptomless.

**Suppression of bacterial spot of radish by *Trichoderma hamatum* 382.** Efficacy of a heated composted pine bark mix fortified during mix formulation with $T_{382}$ (introduced population of $1 \times 10^6$ cfu g$^{-1}$ dry wt mix) against bacterial spot of radish was compared with that of acibenzolar in each of five radish bioassays (Table 2.4). In four of these bioassays, inoculation of the mix with $T_{382}$ significantly ($P \leq 0.05$) reduced the severity of bacterial spot below the value observed on plants in the heated control mix. $T_{382}$
reduced the severity of bacterial spot to mean values ranging from 2.5–3.3. In contrast, acibenzolar consistently reduced the severity of the disease in all five bioassays, and mean disease severity values ranged from 2.4–3.1. In each bioassay, radish seedlings inoculated with \textit{Xca} 704b grown in the heat-treated composted pine bark-amended mix developed severe symptoms of bacterial spot with mean severity values between 3.5 and 3.8. Seedlings not inoculated with \textit{Xca} 704b remained free of disease symptoms.

**Interactions between SR-active bacterial strains and \textit{Trichoderma hamatum} 382.**

The activity of mixtures of \textit{T} \textsubscript{382} with any of several bacterial strains that significantly ($P \leq 0.05$) reduced the severity of bacterial spot in at least three of four initial screening bioassays was examined in each of three additional bioassays. \textit{Xca} 704b caused severe disease in all three bioassays on plants grown in the heated composted pine bark-amended mix (control mix not fortified with \textit{T} \textsubscript{382}). In each of these bioassays, \textit{T} \textsubscript{382} again significantly ($P \leq 0.05$) reduced the severity of bacterial spot below those values observed on the inoculated plants grown in the heated composted pine bark mix (Table 2.5). Only one bacterial strain, \textit{Bacillus} spp. TH218, used in combination with \textit{T} \textsubscript{382} significantly ($P \leq 0.05$) reduced the severity of bacterial spot (mean disease severity value of 2.8) below that value observed for \textit{T} \textsubscript{382} by itself (mean disease severity value of 3.1). This result was obtained in only one of the three bioassays. In another bioassay, this strain significantly ($P \leq 0.05$) decreased the efficacy of \textit{T} \textsubscript{382} (mean disease severity value of 2.7). The mean disease severity (value of 3.3) for this treatment was significantly ($P \leq$
0.05) below the value observed on the inoculated plants grown in the heated control mix (mean disease severity value of 3.6). In the remaining bioassay, this strain did not significantly \((P \leq 0.05)\) affect the efficacy of \(T_{382}\). None of the bacterial strains consistently improved the efficacy of \(T_{382}\) and in most cases, efficacy of \(T_{382}\) was not reduced significantly \((P \leq 0.05)\) either. In these experiments, acibenzolar was significantly \((P \leq 0.05)\) more effective than \(T_{382}\) in only one of the three bioassays.

Acibenzolar consistently reduced the severity of bacterial spot below the values observed on control plants inoculated with \(Xca\) 704b. Plants not inoculated with \(Xca\) 704b consistently remained symptomless.

**Populations of \(Xca\) 704b and of \(T_{382}\).** Five days after inoculation of radish seedlings, the population of \(Xca\) 704b in infected radish leaves of plants grown in the composted pine bark mix that had been fortified with \(T_{382}\) (Table 2.5) was significantly \((P \leq 0.05)\) lower than that recovered by dilution plating from inoculated plants grown in the heated control mix not fortified with \(T_{382}\) (Table 2.6). In two of these bioassays (I and III), differences in populations of \(Xca\) 704b were less than one log order of magnitude. In the second bioassay (II), \(T_{382}\) reduced the population of \(Xca\) 704b by one log order. Acibenzolar significantly \((P \leq 0.05)\) reduced the foliar population of \(Xca\) 704b by one log order below the control in two of the three bioassays (II and III). In the remaining bioassay (I), acibenzolar did not significantly \((P \leq 0.05)\) reduce the population of \(Xca\) 704b below that observed for \(T_{382}\), although the population of \(Xca\) 704b was
significantly lower than that on the control inoculated plants.

The population of $T_{382}$ recovered on the selective *Trichoderma* from the heated composted pine bark mix immediately after inoculation of the mix was $5 \times 10^5$ g$^{-1}$ dry wt mix. It declined slightly, thereafter, until a population of $2 \times 10^5$ was recovered 15 d after planting when the severity of bacterial spot on radish foliage was also determined. The identity of $T_{382}$ colonies on the selective *Trichoderma* medium was confirmed using the PCR procedure developed by Abbasi et al. (1999). Three attempts were made to recover $T_{382}$ from the foliage of radish plants and it was not recovered on the selective *Trichoderma* medium in any of these tests. It was also not detected on dilution plates from control plants (plants from a potting mix not fortified with $T_{382}$) either. These results were consistent among three experiments.


Discussion

This work clearly established that suppression of bacterial spot of radish induced by compost-amended potting mixes is a rare phenomenon. Only ten of 80 batches (12.5%) of compost-amended potting mixes significantly ($P \leq 0.05$) decreased the severity of the disease. Only two of these (2.5%) strongly suppressed the disease (Table 2.2). This finding is reminiscent of the data in Chapter 1 which showed that 19.7% of a total of 81 batches of natural compost-amended potting mixes suppressed Rhizoctonia damping-off (Table 1.1, Chapter 1). In contrast, all potting mixes formulated with adequately stabilized composts, that also establish optimum physical properties relative to drainage and aeration as well as nutrient concentrations in potting mixes, consistently suppress Pythium damping-off (Chen et al., 1988b; Mandelbaum and Hadar, 1990; Hoitink et al., 1991; Boehm et al., 1992; Hoitink and Boehm, 1999). Thus, the systemic response induced in radish against bacterial spot by natural potting mixes seems to be a highly specific phenomenon.

The types of SR-active microorganisms recovered from the rhizosphere of radish seedlings grown in the two potting mixes that suppressed bacterial spot in general are in agreement with those isolated previously from field soils (van Loon et al., 1998). It is surprising that only one SR-active fluorescent *Pseudomonas* strain (*P. putida* TE314) was recovered from radish roots. Recovery of SR-active *Enterobacter* strains (Table 2.2) is not surprising since this taxon is abundant in the rhizosphere (Mahaffee and Kloeppe,
Many of the active strains recovered in this work were *Bacillus* strains (10 of 20), which agrees with data on tomato and cucumber roots from field soil (Mahaffee and Kloeppe, 1997; Benhamou et al., 1998). Unfortunately, very few of the rhizobacterial strains recovered from radish seedlings grown in the two potting mixes suppressive to bacterial spot consistently suppressed this disease in bioassays. Heat treatment of comminuted root tips yielded the highest percentage (7.3%) of SR-active bacterial strains followed by the endophyte recovery method (4.4%).

The SR-active rhizosphere bacterial strains recovered in this work (Table 2.2) were more effective than those received from other laboratories (Table 2.3). *Pseudomonas fluorescens* WCS417r (Pieterse et al., 1996; van Wees et al., 1997), which effectively suppressed the incidence of Fusarium wilt of radish, consistently failed to suppress bacterial spot in this work. In preliminary experiments performed by Sahin (not reported in this thesis), it was determined that WCS417r improved growth of the radish cultivar ‘Fuego’ which was also used by Han et al. (2000). In the work reported here, the cultivar ‘Cabernet’ was used because ‘Fuego’ was no longer available. *Bacillus cereus* C10 was the most consistent strain of this group in suppressing bacterial spot of radish. Even so, none of the bacterial strains in this work were as effective as Tg,82.

Several reports suggest that isolates of *Trichoderma* spp. may induce SR in (Meera et al., 1994; Chang et al., 1997; De Meyer et al., 1998; Yedidia et al., 1999; Han et al., 2000). De Meyer et al (1998) demonstrated that application of *T. harzianum* conidia induced SR against grey mold caused by *Botrytis cinerea* on common bean, lettuce, pepper, tobacco
and tomato. Yedidia et al. (1999) showed that *T. harzianum* T-203 applied to roots of cucumber initiated increased peroxidase and chitinase activities in both the roots and leaves of treated seedlings. Using a growth pouch bioassay, Han et al. (2000) showed that T382 applied to radish roots induced SR against bacterial spot caused by *Xca*. The results in this work clearly support the research of Han et al. (2000). T382 was not recovered from the foliage of treated plants, whereas it maintained a population ≥ 2.5 x 10^5 CFU g\(^{-1}\) dry wt. potting mix in the fortified composted pine bark mix. Thus, spatial separation was maintained on the plant while both the disease severity and the population of the pathogen were reduced significantly (*P* = 0.05). In conclusion, SR seems the most plausible mechanism underlying suppression of bacterial spot in the composted pine bark mixes.
Table 2.1. Severity of Xanthomonas bacterial leaf spot of radish seedlings grown in various compost-amended potting mixes compared to a dark Sphagnum peat mix.

<table>
<thead>
<tr>
<th>Potting Mix1</th>
<th>Xca 704b Inoculum2</th>
<th>Mean Bacterial Spot Severity3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark Peat Mix</td>
<td>-</td>
<td>1.0</td>
</tr>
<tr>
<td>Dark Peat Mix</td>
<td>+</td>
<td>3.1</td>
</tr>
<tr>
<td>CPB Mix (7/96)</td>
<td>+</td>
<td>3.2</td>
</tr>
<tr>
<td>CPB Mix + C299 + T382 (7/96)</td>
<td>+</td>
<td>2.4</td>
</tr>
<tr>
<td>CPB Mix (11/97)</td>
<td>+</td>
<td>2.6</td>
</tr>
<tr>
<td>CPB Mix (11/97) + 2% (v/v) Compost-Amended Field Plot Soil</td>
<td>+</td>
<td>2.6</td>
</tr>
<tr>
<td>CPB Mix (11/97) + 10% (v/v) Composted Yard Waste</td>
<td>+</td>
<td>3.0</td>
</tr>
<tr>
<td>LSD0.05</td>
<td></td>
<td>0.7</td>
</tr>
</tbody>
</table>

1 Radish seeds were planted in either: a batch of dark Sphagnum peat-amended potting mix (Dark Peat Mix); batches of composted pine bark-amended potting mix (CPB Mix); batches of CPB Mix fortified with the biocontrol agents *Chryseobacterium gleum* 299 and *Trichoderma hamatum* 382 (CPB Mix + C299 + T382); a batch of CPB Mix amended with 2% (v/v) field soil collected from plots suppressive to bacterial spot of tomato; or CPB Mix amended with composted yard waste (10%; v/v). Dates of CPB Mix batch formulation are indicated in parentheses.

2 "+" = radish foliage sprayed 10 d after seeding with a 3.3 x 10⁶ cfu ml⁻¹ suspension of *Xanthomonas campestris* pv. *armoraciae* strain 704b containing 0.02% SilWet L-77 until run-off. "−" = radish foliage sprayed with autoclaved tap water control containing 0.02% SilWet L-77.

3 Mean bacterial spot severity of 10 true leaves per pot, 5 pots per treatment, based on a rating scale where: 1 = symptomless, 2 = few lesions to 10% leaf area affected by lesions, 3 = 10–25% leaf area affected, 4 = 25–50% leaf area affected, 5 = 50–75% leaf area affected, and 6 = >75% leaf area affected or dead leaf.
Table 2.2. Incidence of SR activity among rhizobacteria isolated from an SR-active batch of composted pine bark-amended potting mix fortified with C<sub>299</sub> and T<sub>382</sub> and an SR-active batch of composted biosolids-amended potting mix.

<table>
<thead>
<tr>
<th>Potting Mix Type&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Isolation Procedure&lt;sup&gt;2&lt;/sup&gt;</th>
<th># Strains Tested&lt;sup&gt;3&lt;/sup&gt;</th>
<th>SR-Active Strains (%)&lt;sup&gt;4&lt;/sup&gt;</th>
<th>Strain Identity&lt;sup&gt;5&lt;/sup&gt;</th>
<th>Number&lt;sup&gt;6&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPB</td>
<td>Rinsed Roots</td>
<td>168</td>
<td>1.8</td>
<td><em>Enterobacter cloacae</em></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Enterobacter hormaechei</em></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Klebsiella planticola</em></td>
<td>1</td>
</tr>
<tr>
<td>CBS</td>
<td>Rinsed Roots</td>
<td>122</td>
<td>2.5</td>
<td><em>Bacillus</em> spp.</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Microbacterium</em> spp.</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GC similarity group 148</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Heat-tolerant</td>
<td>95</td>
<td>7.3</td>
<td><em>Bacillus</em> spp.</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Exiguobacterium</em> spp.</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Endophyte recovery</td>
<td>159</td>
<td>4.4</td>
<td><em>Arthrobacter globiformis</em></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Bacillus</em> spp.</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Corynebacterium</em> bovis</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Paenibacillus</em> spp.</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Pseudomonas putida</em></td>
<td>1</td>
</tr>
<tr>
<td>Total CBS</td>
<td></td>
<td>376</td>
<td>4.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Potting mixes that most strongly suppressed bacterial spot of radish were a batch of composted pine bark-amended (CPB) potting mix fortified with *Chryseobacterium gleum* 299 and *Trichoderma hamatum* 382 and a batch of composted biosolids-amended (CBS) potting mix.

2 Strains were isolated on 0.1 TSA from: a) rinsed macerated roots, b) same as a) but heated to 80°C for 20 min (heat-tolerant), and c) surface sterilized macerated roots (endophyte recovery).

3 Number of rhizobacterial strains recovered with each procedure from radish seedlings grown in either the suppressive composted pine bark-amended (CPB) mix or the suppressive composted biosolids-amended mix (CBS).

4 Incidence of rhizobacterial strains that consistently induced suppression of bacterial spot in radish.

5 SR-active bacterial strains identified by GC-FAME analysis.

6 Number of strains recovered from radish root tips corresponding to the adjacent strain identity.
Table 2.3. Efficacy of various SR-active rhizobacterial strains inoculated into composted pine bark-amended potting mix in the suppression of bacterial spot of radish.

<table>
<thead>
<tr>
<th>Potting Mix Treatment</th>
<th>( Xca , 704b ) Inoculum</th>
<th>Mean Bacterial Spot Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heated CPB Mix</td>
<td>-</td>
<td>1.0</td>
</tr>
<tr>
<td>Heated CPB Mix</td>
<td>+</td>
<td>2.8</td>
</tr>
<tr>
<td>CPB Mix</td>
<td>+</td>
<td>2.7</td>
</tr>
<tr>
<td>( Bacillus , amyloliquefaciens , IN937a )</td>
<td>+</td>
<td>2.4</td>
</tr>
<tr>
<td>( B. , cereus , C1 )</td>
<td>+</td>
<td>2.9</td>
</tr>
<tr>
<td>( B. , cereus , C10 )</td>
<td>+</td>
<td>2.2</td>
</tr>
<tr>
<td>( B. , laterosporus , 1PL-2 )</td>
<td>+</td>
<td>2.7</td>
</tr>
<tr>
<td>( B. , pasteurii , C9 )</td>
<td>+</td>
<td>2.5</td>
</tr>
<tr>
<td>( B. , pumilus , INR-7 )</td>
<td>+</td>
<td>2.5</td>
</tr>
<tr>
<td>( B. , pumilus , SE-49 )</td>
<td>+</td>
<td>3.0</td>
</tr>
<tr>
<td>( B. , pumilus , SE-76 )</td>
<td>+</td>
<td>3.2</td>
</tr>
<tr>
<td>( B. , sphaericus , SE-56 )</td>
<td>+</td>
<td>2.9</td>
</tr>
<tr>
<td>( B. , subtilis , IN937b )</td>
<td>+</td>
<td>3.2</td>
</tr>
<tr>
<td>( B. , subtilis , 1PN-19 )</td>
<td>+</td>
<td>2.4</td>
</tr>
<tr>
<td>( Paenibacillus , macerans , 3PI-8 )</td>
<td>+</td>
<td>3.0</td>
</tr>
<tr>
<td>( Pseudomonas , chlororaphis , 63-28 )</td>
<td>+</td>
<td>3.5</td>
</tr>
<tr>
<td>( Ps. , fluorescens , 31-12 )</td>
<td>+</td>
<td>2.6</td>
</tr>
<tr>
<td>( Ps. , fluorescens , 63-49 )</td>
<td>+</td>
<td>2.3</td>
</tr>
</tbody>
</table>

\[ \text{LSD}_{0.05} = 0.6 \]

1 Radish (\textit{Raphanus sativus} L. cv. 'Cabernet') seeds planted in: a heated composted pine bark-amended potting mix (Heated CPB Mix), a natural composted pine bark-amended mix (CPB Mix), and Heated CPB Mix inoculated with individually with washed 48 h cultures of rhizobacterial strains previously reported to induce SR (\(10^7\)–\(10^8\) cfu g\(^{-1}\) dry wt. potting mix).

2 "+" = radish foliage sprayed 10 d after seeding with a \(3.3 \times 10^6\) cfu ml\(^{-1}\) suspension of \textit{Xanthomonas campestris pv. armoraciae} strain 704b containing 0.02% SilWet L-77 until run-off. "-" = radish foliage sprayed with autoclaved tap water control containing 0.02% SilWet L-77.

3 Mean bacterial spot severity of 10 true leaves per pot, 5 pots per treatment, based on a rating scale where: 1 = symptomless, 2 = few lesions to 10% leaf area affected by lesions, 3 = 10–25% leaf area affected, 4 = 25–50% leaf area affected, 5 = 50–75% leaf area affected, and 6 = >75% leaf area affected or dead leaf.
Table 2.4. Efficacy of *Trichoderma hamatum* 382-fortified composted pine bark mix and acibenzolar drench in the suppression of Xanthomonas bacterial spot of radish.

<table>
<thead>
<tr>
<th>Potting Mix Treatment</th>
<th>Xca 704b Inoculum</th>
<th>Bioassay</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPB Mix</td>
<td>-</td>
<td></td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Heated CPB Mix</td>
<td>-</td>
<td></td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>CPB Mix (non-heated negative SR control)</td>
<td>+</td>
<td></td>
<td>3.5</td>
<td>3.7</td>
<td>3.8</td>
<td>3.6</td>
<td>3.5</td>
</tr>
<tr>
<td>Heated Mix (heated negative SR control)</td>
<td>+</td>
<td></td>
<td>3.8</td>
<td>4.2</td>
<td>3.9</td>
<td>3.4</td>
<td>3.5</td>
</tr>
<tr>
<td>T_{382} Fortified CPB Mix</td>
<td>+</td>
<td></td>
<td>3.2</td>
<td>3.3</td>
<td>3.2</td>
<td>2.5</td>
<td>3.2</td>
</tr>
<tr>
<td>Heated control mix + acibenzolar</td>
<td>+</td>
<td></td>
<td>2.7</td>
<td>3.1</td>
<td>2.7</td>
<td>2.4</td>
<td>2.9</td>
</tr>
<tr>
<td>LSD_{0.05}</td>
<td>0.4</td>
<td>0.5</td>
<td>0.4</td>
<td>0.3</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Mean bacterial spot severity of 10 true leaves per pot, 5 pots per treatment for five different bioassays (I–V), based on a rating scale where: 1 = symptomless, 2 = few lesions to 10% leaf area affected by lesions, 3 = 10–25% leaf area affected, 4 = 25–50% leaf area affected, 5 = 50–75% leaf area affected, and 6 = >75% leaf area affected or dead leaf.

2 Radish (*Raphanus sativus* L. cv. 'Cabernet') seeds planted in: a heated composted pine bark-amended potting mix (Heated CPB Mix), a natural composted pine bark-amended mix (CPB Mix), Heated CPB Mix fortified with 1 x 10^6 CFU T_{382} g^{-1} dry wt mix, and Heated CPB Mix drenched with 50 μg mL^{-1} acibenzolar solution 48 h prior to inoculation with the pathogen.

3 "+" = radish foliage sprayed 10 d after seeding with a 3.3 x 10^6 cfu ml^{-1} suspension of *Xanthomonas campestris pv. armoraciae* strain 704b containing 0.02% SilWet L-77 until run-off. "-" = radish foliage sprayed with autoclaved tap water control containing 0.02% SilWet L-77.
Table 2.5. Ability of *Trichoderma hamatum* 382 used singly or in combination with selected SR-active rhizobacteria to control bacterial leaf spot caused by *Xanthomonas campestris* pv. *armoraciae* 704b in three bioassays.

<table>
<thead>
<tr>
<th>Potting Mix Treatment²</th>
<th>Xca 704b Inoculum³</th>
<th>Bioassay</th>
<th>Bioassay</th>
<th>Bioassay</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPB Mix</td>
<td>-</td>
<td>I 1.0</td>
<td>II 1.0</td>
<td>III 1.0</td>
</tr>
<tr>
<td>Heated CPB Mix</td>
<td>-</td>
<td>I 1.0</td>
<td>II 1.0</td>
<td>III 1.0</td>
</tr>
<tr>
<td>CPB Mix (non-heated negative SR control)</td>
<td>+</td>
<td>3.3</td>
<td>3.9</td>
<td>3.3</td>
</tr>
<tr>
<td>Heated CPB Mix (heated negative SR control)</td>
<td>+</td>
<td>3.6</td>
<td>3.7</td>
<td>3.4</td>
</tr>
<tr>
<td>T₃₈₂ fortified CPB Mix</td>
<td>+</td>
<td>2.7</td>
<td>3.0</td>
<td>3.1</td>
</tr>
<tr>
<td>T₃₈₂ + Bacillus spp. TH202</td>
<td>+</td>
<td>3.2</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>T₃₈₂ + B. spp. TH218</td>
<td>+</td>
<td>3.3</td>
<td>3.3</td>
<td>2.8</td>
</tr>
<tr>
<td>T₃₈₂ + B. spp. TR160</td>
<td>+</td>
<td>2.7</td>
<td>2.9</td>
<td>3.1</td>
</tr>
<tr>
<td>T₃₈₂ + Paenibacillus spp. TE312</td>
<td>+</td>
<td>3.2</td>
<td>3.2</td>
<td>3.1</td>
</tr>
<tr>
<td>T₃₈₂ + Pseudomonas putida TE314</td>
<td>+</td>
<td>3.1</td>
<td>3.5</td>
<td>3.1</td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em> strain WCS 417r</td>
<td>+</td>
<td>3.4</td>
<td>3.7</td>
<td>3.5</td>
</tr>
<tr>
<td>Acibenzolar drench (positive SAR control)</td>
<td>+</td>
<td>2.3</td>
<td>2.8</td>
<td>2.5</td>
</tr>
</tbody>
</table>

LSD₀.₀₅ 0.3 0.4 0.3

1 Mean bacterial spot severity of 10 true leaves per pot, 5 pots per treatment, based on a rating scale where: 1 = symptomless, 2 = few lesions to 10% leaf area affected by lesions, 3 = 10–25% leaf area affected, 4 = 25–50% leaf area affected, 5 = 50–75% leaf area affected, and 6 = >75% leaf area affected or dead leaf.

2 Radish (*Raphanus sativus* L. cv. 'Cabernet') seeds planted in: a heated composted pine bark-amended potting mix (Heated CPB Mix), a natural composted pine bark-amended mix (CPB Mix), and Heated CPB Mix fortified with individually with washed 48 h cultures of SR-active rhizobacterial strains (10⁷–10⁸ CFU g⁻¹ dry wt. potting mix) and/ or 1 x 10⁶ CFU T₃₈₂ g⁻¹ dry wt mix, and Heated CPB Mix drenched with 50 μg mL⁻¹ acibenzolar solution 48 h prior to inoculation with the pathogen.

3 "+" = radish foliage sprayed 10 d after seeding with a 3.3 x 10⁶ CFU ml⁻¹ suspension of *Xanthomonas campestris* pv. *armoraciae* strain 704b containing 0.02% SilWet L-77 until run-off. "-" = radish foliage sprayed with autoclaved tap water control containing 0.02% SilWet L-77.
Table 2.6. Effect of *Trichoderma hamatum* 382 inoculated into a composted pine bark mix on the population of *Xanthomonas campestris* pv. *armoraciae* strain 704b in radish foliage in three bioassays.

<table>
<thead>
<tr>
<th>Potting Mix Treatment</th>
<th>Xca 704b Inoculum</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heated control mix</td>
<td>-</td>
<td>&lt;1.5</td>
<td>&lt;1.5</td>
<td>&lt;1.5</td>
</tr>
<tr>
<td>Control mix</td>
<td>+</td>
<td>8.1</td>
<td>9.3</td>
<td>7.4</td>
</tr>
<tr>
<td>Heated control mix</td>
<td>+</td>
<td>7.8</td>
<td>9.4</td>
<td>7.4</td>
</tr>
<tr>
<td>Heated mix inoculated with T&lt;sub&gt;382&lt;/sub&gt;</td>
<td>+</td>
<td>7.5</td>
<td>8.3</td>
<td>6.9</td>
</tr>
<tr>
<td>Heated mix drenched with acibenzolar</td>
<td>+</td>
<td>7.2</td>
<td>8.3</td>
<td>6.4</td>
</tr>
<tr>
<td>LSD&lt;sub&gt;0.05&lt;/sub&gt;</td>
<td>0.5</td>
<td>0.4</td>
<td>0.4</td>
<td></td>
</tr>
</tbody>
</table>

1 Foliar population of Xca 704b was determined by macerating the five first true leaves randomly collected from each pot in each treatment in bacterial and dilution plating on sucrose peptone agar containing streptomycin sulfate (150 μg ml<sup>-1</sup>). Data shown are mean population values for two samples collected from each treatment for each of three bioassays. The detection limit for Xca 704b was 30 CFU g<sup>-1</sup> fresh wt foliage.

2 Radish (*Raphanus sativus* L. cv. 'Cabernet') seeds planted in: a heated composted pine bark-amended potting mix (Heated CPB Mix), a natural composted pine bark-amended mix (CPB Mix), Heated CPB Mix inoculated with 1 x 10<sup>6</sup> CFU T<sub>382</sub> g<sup>-1</sup> dry wt mix, and Heated CPB Mix drenched with 50 μg mL<sup>-1</sup> acibenzolar solution 48 h prior to inoculation with the pathogen.

3 "+" = radish foliage sprayed 10 d after seeding with a 3.3 x 10<sup>6</sup> cfu ml<sup>-1</sup> suspension of *Xanthomonas campestris* pv. *armoraciae* strain 704b containing 0.02% SilWet L-77 until run-off. "-" = radish foliage sprayed with autoclaved tap water control containing 0.02% SilWet L-77.
SUMMARY AND CONCLUSIONS

Less than 20% of the batches of the natural composted pine bark mix tested in this work and less than 5% of the batches of the natural dark peat and light peat mixes examined were suppressive to Rhizoctonia damping-off of radish. Thus, significant opportunities for utilization of biocontrol agents with activity against this disease exist in the greenhouse industry.

The biocontrol agents $C_{299}$ and $T_{382}$ significantly reduced the severity of Rhizoctonia damping-off of radish in composted pine bark mixes fortified with these agents as compared to the non-fortified control mixes. The biocontrol agents $C_{299}$ and $T_{382}$ were significantly ($P \leq 0.05$) less effective in the light peat mix. They had no significant effect against Rhizoctonia damping-off in the dark peat mix. In conclusion, the decomposition level of the organic components used in these potting mixes significantly affected the efficacy of these biocontrol agents against Rhizoctonia damping-off.

The severity of Rhizoctonia crown and root rot observed on poinsettia plants in all three types of potting mixes fortified with the biocontrol agents $C_{299}$ and $T_{382}$ did not differ significantly ($P \leq 0.05$). The degree of control provided by the the biocontrol agents also did not differ significantly ($P \leq 0.05$) from that provided by drenching with...
fungicides. In conclusion, no relationship between organic matter decomposition level of potting mixes and efficacy in biological control obtained with $C_{299}$ and $T_{382}$ was observed for this disease.

The biocontrol agents $C_{299}R_2$ and $T_{382}$ maintained high populations in the composted pine bark mix, which is highest in microbial carrying capacity among the mixes tested (Boehm et al., 1997). The population of $C_{299}R_2$ declined within days after incorporation into either type of peat mix. Although $T_{382}$ initially declined in population in both types of peat mixes, its population recovered after 60–75 days when Rhizoctonia crown and root rot disease pressures are most severe. It was postulated, therefore, that control of Rhizoctonia crown and root rot of poinsettia observed in both types of peat mixes was largely due to the activity of $T_{382}$.

A low percentage (12.5%) of the compost-amended potting mixes tested in this work suppressed bacterial leaf spot of radish caused by $Xca$ and only two of 80 tested were strongly suppressive to the disease. Therefore, this natural suppressive effect seemed even more rare than the naturally suppressive effect against Rhizoctonia damping-off.

Twenty of 544 rhizobacterial strains recovered from root tips of radish seedlings grown in two SR-active mixes decreased the severity of bacterial spot when inoculated into a heated composted pine bark mix. None of these provided consistent control, however. $T_{382}$ was identified as the most SR-active biocontrol agent. Acibenzolar applied as a drench to radish seedlings suppressed the severity of bacterial spot more effectively than
$T_{382}$ in some but not all of eight comparative experiments. The population of $Xca$ 704b in the foliage of radish seedlings grown in the composted pine bark mix fortified with $T_{382}$ was significantly ($P \leq 0.05$) below that on inoculated plants grown in the heated control mix in two of three bioassays, while acibenzolar consistently suppressed the pathogen. $T_{382}$ was not recovered from the foliage of seedlings in which bacterial spot was suppressed. It remained spatially separated from the pathogen on the host. It may be concluded, therefore, that $T_{382}$ induced SR in radish against bacterial spot of radish *sensu* van Loon et al., 1998.
LITERATURE CITED


APPENDIX A

Compost-amended potting mixes tested for their ability to suppress bacterial leaf spot of radish caused by *Xanthomonas campestris pv. armoraciae*.

<table>
<thead>
<tr>
<th>Compost-Amended Mixes Tested</th>
<th>Source of Compost Amendment</th>
<th>Amendment Rate (%;v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composted Pine Bark</td>
<td>Earthgro Inc., Glastonbury, CT</td>
<td>35, 50</td>
</tr>
<tr>
<td>Composted Pine Bark</td>
<td>Pro-Gro Products Inc., McCormick, SC</td>
<td>50</td>
</tr>
<tr>
<td>Composted Pine Bark</td>
<td>Paygro Inc., S. Charleston, OH</td>
<td>35, 50</td>
</tr>
<tr>
<td>Composted Pine Bark</td>
<td>Willoway Nurseries, Avon, OH</td>
<td>50</td>
</tr>
<tr>
<td>Municipal Biosolids Compost (Com-Til)</td>
<td>City of Columbus, OH</td>
<td>7.5, 15.0</td>
</tr>
<tr>
<td>Municipal Biosolids Compost (Technagro)</td>
<td>City of Akron, OH and KB Compost, Akron, OH</td>
<td>7.5, 15.0</td>
</tr>
<tr>
<td>Yard Waste Compost</td>
<td>KB Compost, Groveport, OH</td>
<td>7.5, 15.0</td>
</tr>
<tr>
<td>Food Waste Vermicompost (Oregon Soil)</td>
<td>OSU Dept. of Entomology, Columbus, OH</td>
<td>7.5, 15.0</td>
</tr>
<tr>
<td>Swine Biosolids Vermicompost (Vermicycle)</td>
<td>OSU Dept. of Entomology, Columbus, OH</td>
<td>7.5, 15.0</td>
</tr>
<tr>
<td>Compost-Amended Field Soil</td>
<td>OARDC, Wooster, OH</td>
<td>2.0, 7.5, 15.0</td>
</tr>
<tr>
<td>Dairy, Equine and Poultry Biosolids Blend Compost</td>
<td>Young Farms, Union City, OH</td>
<td>5.0, 10.0</td>
</tr>
<tr>
<td>Vegetable Cannery Waste Compost</td>
<td>Hirzel Farms, Lucky, OH</td>
<td>5.0, 10.0</td>
</tr>
</tbody>
</table>
APPENDIX B

Sources of rhizobacterial strains tested for their ability to induce systemic resistance against bacterial leaf spot of radish caused by *Xanthomonas campestris pv. armoraciae*.

<table>
<thead>
<tr>
<th>Bacterial Strain Tested</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus amyloliquefaciens</em> IN937a</td>
<td>J. W. Kloepper, Auburn University, AL</td>
</tr>
<tr>
<td><em>B. cereus</em> C1</td>
<td>J. W. Kloepper, Auburn University, AL</td>
</tr>
<tr>
<td><em>B. cereus</em> C10</td>
<td>J. W. Kloepper, Auburn University, AL</td>
</tr>
<tr>
<td><em>B. laterosporus</em> 1PL-2</td>
<td>J. W. Kloepper, Auburn University, AL</td>
</tr>
<tr>
<td><em>B. pasteurii</em> C9</td>
<td>J. W. Kloepper, Auburn University, AL</td>
</tr>
<tr>
<td><em>B. pumilus</em> INR-7</td>
<td>J. W. Kloepper, Auburn University, AL</td>
</tr>
<tr>
<td><em>B. pumilus</em> SE-49</td>
<td>J. W. Kloepper, Auburn University, AL</td>
</tr>
<tr>
<td><em>B. pumilus</em> SE-76</td>
<td>J. W. Kloepper, Auburn University, AL</td>
</tr>
<tr>
<td><em>B. sphaericus</em> SE-56</td>
<td>J. W. Kloepper, Auburn University, AL</td>
</tr>
<tr>
<td><em>B. subtilis</em> IN937b</td>
<td>J. W. Kloepper, Auburn University, AL</td>
</tr>
<tr>
<td><em>B. subtilis</em> 1PN-19</td>
<td>J. W. Kloepper, Auburn University, AL</td>
</tr>
<tr>
<td><em>Burkholderia cepacia</em> Ral-3†</td>
<td>G. Brown, Agrium Biologicals, Saskatoon, Canada</td>
</tr>
<tr>
<td><em>Chryseobacterium gleum</em> 299</td>
<td>H. A. J. Hoitink, OSU/OARDC, Wooster,</td>
</tr>
<tr>
<td><em>Paenibacillus macerans</em> 3PI-8</td>
<td>J. W. Kloepper, Auburn University, AL</td>
</tr>
<tr>
<td><em>Paenibacillus macerans</em> 3PI-14†</td>
<td>J. W. Kloepper, Auburn University, AL</td>
</tr>
<tr>
<td><em>Pseudomonas chlororaphis</em> 63-28</td>
<td>G. Brown, Agrium Biologicals, Saskatoon, Canada</td>
</tr>
<tr>
<td><em>Ps. fluorescens</em> 31-12</td>
<td>G. Brown, Agrium Biologicals, Saskatoon, Canada</td>
</tr>
<tr>
<td><em>Ps. fluorescens</em> 63-49</td>
<td>G. Brown, Agrium Biologicals, Saskatoon, Canada</td>
</tr>
<tr>
<td><em>Ps. fluorescens</em> WCS417r</td>
<td>C. M. J. Pieterse, Utrecht University, The Netherlands</td>
</tr>
<tr>
<td><em>Serratia proteamaculans</em> 1-102†</td>
<td>G. Brown, Agrium Biologicals, Saskatoon, Canada</td>
</tr>
</tbody>
</table>

† Strains that inhibited germination or caused detrimental growth effects in radish seedlings.