INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.
Microbial ecology of Canadian sphagnum peat and compost-amended potting mixes varying in suppressiveness to pythium root rot and damping-off

Boehm, Michael Jon, Ph.D.
The Ohio State University, 1992
MICROBIAL ECOLOGY OF CANADIAN SPHAGNUM PEAT AND
COMPOST-AMENDED POTTING MIXES VARYING IN
SUPPRESSIVENESS TO PYTHIUM ROOT ROT AND DAMPING-OFF

DISSERTATION

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

By

Michael Jon Boehm, B.S., M.S.

* * * * *

The Ohio State University
1992

Dissertation Committee:  
H. A. Hoitink  
A. S. Schmitthenner  
T. L. Graham  
D. L. Coplin  
L. V. Madden

Approved by

Adviser
Department of Plant Pathology
To Connie, my beloved wife
and best friend
ACKNOWLEDGMENTS

My sincere appreciation is expressed to all persons in the Department of Plant Pathology who have contributed to making my graduate work an extremely positive and rewarding experience. Sincere gratitude is expressed to my adviser, Dr. Harry Hoitink, for his guidance, support, seemingly endless patience and very generous contributions to both my education and professional development. In addition, his constructive review of this dissertation is greatly appreciated. Sincere appreciation is also extended to Drs. Fritz Schmitthenner, Terry Graham, Dave Coplin and Larry Madden for serving on my student advisory committee and for their valuable suggestions, insight and guidance throughout the course of this research. Gratitude is expressed to Dr. Ed Wilson, Professor and Chairperson of the Department of Chemistry at the University of Akron, for both the use of the University of Akron's CPMAS $^{13}$C-NMR facilities and for his interpretation of the NMR spectra presented in this dissertation. Special thanks are also extended to Dr. Donna Frost for her assistance with the CPMAS $^{13}$C-NMR analyses and for many helpful discussions concerning NMR spectroscopy. My sincere appreciation is further extended to Ms. Carol Musselman for sharing her knowledge and practical experiences in the area of biological control of soilborne plant pathogens and for her patience, friendship, and assistance throughout my time in the Department.

The technical assistance of the OARDC librarians (Ms. Connie Britton and Ms. Pat Sachariat), photographers (Mr. Ken Chamberlain and Ms. Margaret Latta) and statistics laboratory personnel (Ms. Carolyn Britt) is greatly acknowledged. In addition, special thanks are extended to Drs. Yossi Inbar, Bob Whitmoyer, Randy Rowe, Ravi Bhat, Olli Tuovinen, Roland Seymour, Nilceu Nazareno, and Terry Wheeler for their insight, assistance and valuable discussions dealing with a wide variety of scientific issues. The assistance and Wordperfect expertise of Ms. June Young is also gratefully acknowledged.

Sincere appreciation is extended to Premier Peat Moss Ltd., Rivière-du-Loup, Québec, Canada, for supplying both the sphagnum peat and financial support necessary to complete this research. The efforts of Ms. Dominique Le Quéré, Project Manager at Premier Peat Moss Ltd., are also gratefully acknowledged. Her accommodation of our research needs in Wooster and for making my visit to the bogs of Rivière-du-Loup a reality are greatly appreciated.

Sincere appreciation is also extended to the faculty and staff of Heidelberg College for their guidance and generous contributions to both my education and personal development. I would also like to recognize Ms. Lu Hyde for her dedication to teaching excellence and for serving as an example of what being a "teacher" is really about. Finally, I thank my friends and family for their enduring faith, fellowship and love.
VITA

January 2, 1965 .............. Born-Lakewood, Ohio, U.S.A.

May 1987 .................. B.S. Biology, Heidelberg College
                        Tiffin, Ohio

March 1990 .................. M.S., Plant Pathology,
                           The Ohio State University
                           Columbus, Ohio

March 1990-Present ........ Graduate Research Associate.
                         Department of Plant Pathology,
                         The Ohio State University
                         Columbus, Ohio

PUBLICATIONS

Refereed Journal Articles

activity in potting mixes and its impact on severity of Pythium root

fluorescein diacetate in sphagnum peat container media for
predicting suppressiveness to damping-off caused by Pythium ultimum.
Soil Biology and Biochemistry 23:479-483.

Invited Papers

compost-amended potting mixes naturally suppressive to soilborne

Proceedings of Meetings

production as related to product quality. pp. 318-327. In:
Proceedings of the International Symposium on Compost Production and

organic matter decomposition level and the inoculum potential of
Brazilian Meeting on Biocontrol of Plant Diseases, Campinas, São
Paulo, BRAZIL. October 8-10, 1991.
Non-Refereed Trade Journal Articles


Published Abstracts


FIELDS OF STUDY

Major Field: Plant Pathology

Studies in: Biological Control of Plant Diseases and Microbial Ecology.
Advisor: Dr. Harry A. J. Hoitink
# TABLE OF CONTENTS

DEDICATION ................................................................. ii  
ACKNOWLEDGMENTS .......................................................... iii  
VITA ................................................................. iv  
LIST OF TABLES ........................................................... vii  
LIST OF FIGURES .......................................................... viii  
LIST OF PLATES ............................................................ ix  
INTRODUCTION .............................................................. 1  

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. SUSTENANCE OF MICROBIAL ACTIVITY IN POTTING MIXES AND ITS IMPACT ON SEVERITY OF PYTHIUM ROOT ROT OF POINSETTIA</td>
<td>4</td>
</tr>
<tr>
<td>Introduction</td>
<td>4</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>6</td>
</tr>
<tr>
<td>Results</td>
<td>12</td>
</tr>
<tr>
<td>Discussion</td>
<td>19</td>
</tr>
<tr>
<td>II. IMPACT OF PEAT DECOMPOSITION LEVEL ON MICROBIAL SPECIES DIVERSITY AND SUPPRESSION OF PYTHIUM DAMPING-OFF</td>
<td>22</td>
</tr>
<tr>
<td>Introduction</td>
<td>22</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>24</td>
</tr>
<tr>
<td>Results</td>
<td>30</td>
</tr>
<tr>
<td>Discussion</td>
<td>44</td>
</tr>
<tr>
<td>III. CPMAS $^{13}$C-NMR SPECTROSCOPIC ANALYSIS OF SPHAGNUM PEAT DECOMPOSITION LEVEL IN RELATIONSHIP TO MICROBIAL CARRYING CAPACITY AND PYTHIUM ROOT ROT SUPPRESSION</td>
<td>49</td>
</tr>
<tr>
<td>Introduction</td>
<td>49</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>51</td>
</tr>
<tr>
<td>Results</td>
<td>55</td>
</tr>
<tr>
<td>Discussion</td>
<td>62</td>
</tr>
<tr>
<td>SUMMARY AND CONCLUSIONS</td>
<td>64</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>67</td>
</tr>
</tbody>
</table>
LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Relative population density of rhizosphere bacteria isolated on 0.1 strength TSBA from three cucumber seedling root tips grown in a composted hardwood bark-amended potting mix</td>
<td>37</td>
</tr>
<tr>
<td>2. Relative population density of rhizosphere bacteria isolated on 0.1 strength TSBA from three cucumber seedling root tips grown in a slightly decomposed light (H2) peat mix</td>
<td>38</td>
</tr>
<tr>
<td>3. Relative population density of rhizosphere bacteria isolated on 0.1 strength TSBA from four cucumber seedling root tips grown in a decomposed dark (H4) peat mix</td>
<td>39</td>
</tr>
<tr>
<td>4. Species diversity indices for root tips harvested from cucumber seedlings grown in a composted hardwood bark-amended, a slightly decomposed light (H2) and a decomposed dark (H4) peat mix</td>
<td>40</td>
</tr>
<tr>
<td>5. Sampling unit (SU) comparison matrix of similarity coefficients (resemblance functions) for bacterial taxa isolated from root tips of cucumber seedlings grown in a composted hardwood bark-amended, a slightly decomposed light (H2) and a decomposed dark (H4) peat mix</td>
<td>41</td>
</tr>
<tr>
<td>6. Ability of selected bacterial strains to induce suppression of damping-off of cucumber in sterilized light (H2) and dark (H4) peat mix infested with soil inoculum of <em>Pythium ultimum</em></td>
<td>42</td>
</tr>
<tr>
<td>7. Ability of selected fungal isolates to induce suppression of damping-off of cucumber in sterilized light (H2) and dark (H4) peat mix infested with soil inoculum of <em>Pythium ultimum</em></td>
<td>43</td>
</tr>
</tbody>
</table>
**LIST OF FIGURES**

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Comparison of Pythium root rot severity, population density of <em>Pythium ultimum</em> and microbial activity in a light (H₃) and a dark (H₄) peat mix and a composted pine bark-amended potting mix</td>
<td>16</td>
</tr>
<tr>
<td>2. Comparison of Pythium root rot severity, population density of <em>Pythium ultimum</em> and microbial activity in a light (H₂) and a dark (H₄) peat mix and a composted pine bark-amended potting mix</td>
<td>17</td>
</tr>
<tr>
<td>3. Relationships between root rot severity, population density of <em>Pythium ultimum</em> and microbial activity during the production of a poinsettia crop</td>
<td>18</td>
</tr>
<tr>
<td>4. Rarefaction curves for the expected number of bacterial species isolated on 0.1 strength TSBA from root tips of cucumber seedlings grown in composted hardwood bark-amended, slightly decomposed light (H₂) peat and decomposed dark (H₄) peat mixes</td>
<td>35</td>
</tr>
<tr>
<td>5. Bray-Curtis polar ordination plot showing separation of root tips harvested from cucumber seedlings grown in a composted hardwood bark-amended, a slightly decomposed light (H₃) and a decomposed dark (H₄) peat mix</td>
<td>36</td>
</tr>
<tr>
<td>6. Solid-state cross polarized magic angle spinning (^{13}\text{C}-\text{nuclear magnetic resonance (CPMAS}(^{13}\text{C-NMR)}) spectra revealing differences in carbohydrate content within the fine (105-250 (\mu\text{m}) diameter) particle fraction of a slightly decomposed light (H₂) and a decomposed dark (H₄) Canadian sphagnum peat</td>
<td>58</td>
</tr>
<tr>
<td>7. Solid-state cross polarized magic angle spinning (^{13}\text{C}-\text{nuclear magnetic resonance (CPMAS}(^{13}\text{C NMR)}) spectra revealing differences in carbohydrate content within the fine (105-250 (\mu\text{m}) diameter) particle fraction of a slightly decomposed light (H₃) peat mix harvested 14 and 77 days after planting</td>
<td>59</td>
</tr>
<tr>
<td>8. Comparison of total bacterial and fungal populations and microbial activity in a slightly decomposed light (H₃) and a decomposed dark (H₄) peat mix</td>
<td>60</td>
</tr>
<tr>
<td>9. Microbial biomass based on the concentration of organic C rendered extractable to 0.5 M K₂SO₄ by CHCl₃ fumigation in a slightly decomposed light (H₃) peat mix</td>
<td>61</td>
</tr>
</tbody>
</table>
### LIST OF PLATES

<table>
<thead>
<tr>
<th>PLATE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Differences in the decomposition level of Canadian sphagnum peat used in the preparation of potting mixes.</td>
<td>15</td>
</tr>
<tr>
<td>II. Ability of <em>Trichoderma viride</em> S19 to induce suppression to damping-off of cucumber in sterilized light (H₂) and dark (H₄) peat mix infested with soil inoculum of <em>Pythium ultimum</em></td>
<td>34</td>
</tr>
<tr>
<td>III. Illustration of the scale used to assess <em>Pythium</em> root rot severity of poinsettia</td>
<td>57</td>
</tr>
</tbody>
</table>
INTRODUCTION

Sphagnum peat was the most widely used organic amendment by the horticultural industry until the 1970s. At that time, high quality fibrous sphagnum peat was readily available and concerns about wetland and bog conservation were minimal. The reason for this "preferential" use of sphagnum peat over other organic amendments were its ideal physical properties related to aeration and water retention for use in soilless potting mixes, gardens and landscapes (84). Chemical properties of sphagnum peats, as compared to those of reed sedge peats, for example, also were consistent (30). High quality products, in terms of physical and chemical properties could be formulated consistently with sphagnum peat, therefore.

The stable and consistent chemical and physical properties of sphagnum peat that last for several years in soil or potting mixes, unfortunately may also imply that its biological properties reflect stability. During the early 1970s, the first serious negative reports on such properties of peat were published. The generally conducive nature of peat mixes to Phytophthora (39) and Pythium (31) root rots became known. Dark-colored decomposed sphagnum peat with poor drainage properties caused the worst problems.

During the 1950s, the nursery industry in the United States started to explore the use of tree barks as peat substitutes (108). The first reports on suppressive properties of composted bark-amended potting mixes to Pythium and Phytophthora root rots appeared soon thereafter (31,39,95). Because of the importance of this property in nursery production, and, because bark composts were less costly then sphagnum peat, a world-wide change in the formulation of container media used for the production of woody ornamentals occurred (37). Use of sphagnum peat, which typically
was incorporated in potting mixes at a volumetric amendment rate of 60%, dropped to a maximum of 15%. As a side benefit, peat substitutes have had a significant impact on what today is recognized as a "bog conservation effort".

During the 1980s, another trend developed in the greenhouse industry that resulted again in increased use of high quality fibrous sphagnum peat by the floricultural industry. Although potting mixes amended with various types of mature composts offered superior biological control to plant pathogens, they were more difficult to formulate to precise physical properties than fibrous sphagnum peat specifically harvested from the lesser decomposed surface layer (0-90 cm) of bogs. With increased emphasis on control of fertilizer run-off from greenhouses and automation of production, the use of high quality sphagnum peat in floriculture increased.

Another factor which contributed indirectly to the increased use of high quality peat was burning of peat as a source of fuel in power generating plants. Dark, highly stabilized peat (H_4 to H_10 on the von Post decomposition scale) has the highest energy value (54,55). As a result, slightly decomposed light-colored fibrous sphagnum peat harvested from the surface of bogs became the most widely available source of peat for horticultural applications in Northern Europe, where such power plants prevail. The first reports on the natural suppressiveness of this light peat source to soilborne plant pathogens appeared in the early 1980s from Finland (84,100,101). Some microorganisms capable of inducing suppression to Pythium root rot in this light peat were described (100,101,102,119,120). The microflora involved has not been described in detail, however. These authors also did not consider the possible importance of peat decomposition level in this phenomenon.

The objectives of this Ph.D. program were: 1) to determine the natural suppressiveness of Canadian sphagnum peat to damping-off and root rot caused by *Pythium ultimum*, and, to compare this property with that in
compost-amended mixes, 2) to determine the length of time that the most suppressive sources of peat sustained this activity as compared to the highly suppressive compost-amended mixes, and 3) to characterize the microbiological and chemical factors responsible for suppression of Pythium root rot in these mixes.

Research in this thesis has been divided into three sections. In the first section (Chapter I), the suppressiveness of potting mixes prepared with Canadian sphagnum peats differing in decomposition level is compared with that in a composted pine bark-amended mix which serves as a standard in the floricultural industry. In addition, interactions between peat decomposition level, sustained microbial activity and root rot severity are assessed. In the second section (Chapter II), the microbiology of suppressive and conducive mixes is characterized. Finally, in the last section (Chapter III), carbon reserves in peat which serve as an energy source for sustained microbial activity and Pythium root rot suppression, are characterized.
CHAPTER I
SUSTENANCE OF MICROBIAL ACTIVITY IN POTTING MIXES AND ITS IMPACT ON SEVERITY OF PYTHIUM ROOT ROT OF POINSETTIA

Until recently, potting mixes prepared with sphagnum peat as the sole source of organic matter have been considered conducive to diseases caused by soilborne plant pathogens such as Pythium species (37). Addition of mature composts prepared from tree bark (12,19,36), municipal sewage sludges (38,65,66), separated cattle manure and grape pomace (14,15,43,45,67,68) to peat mixes increases microbial activity and induces microbiostasis and suppression to Pythium root rot and damping-off (11,13,68). A number of microorganisms in compost-amended mixes may play a role in the suppression of Pythium root rot (57,99), as was described also for soil (25). Rhizosphere bacteria able to grow at high rates at both high and low nutrient concentrations (facultative oligotrophs) are most effective as seed inoculants for biological control of Pythium damping-off (99). "General suppression", as described by Cook and Baker (17), best explains this mechanism of biological control of damping-off caused by Pythium spp. in compost-amended substrates (11,13,68).

Slightly decomposed light-colored sphagnum peat, harvested from the surface of bogs, may be suppressive to damping-off and root rot caused by various soilborne plant pathogenic fungi, including Pythium spp. (100,119). The suppressive effect of these peats may last for up to 7 wk after potting.

In this chapter interactions between sphagnum peat decomposition level, changes in microbial activity in the potting mix with time, and suppression of the population of Pythium ultimum Trow and of Pythium root rot during production of poinsettia (Euphorbia pulcherrima Wild. ex
Klotzsch cv 'Rochford') are examined. Suppressiveness of potting mixes prepared with Canadian sphagnum peat differing in decomposition level is compared with that of a composted pine bark-amended mix consistently suppressive to the disease.
MATERIALS AND METHODS

Preparation of potting mixes. Potting mixes that differed in suppressiveness to Pythium damping-off were used. Three mixes were prepared with Canadian sphagnum peat (Premier Peat Moss Ltd., Rivière-du-Loup, Québec, Canada), and perlite. The first of these mixes was prepared with a dark-colored, decomposed peat, classified as H4 on the von Post decomposition scale (84), and the others with light-colored, slightly decomposed (H2 and H3) peats. These peats are depicted pictorially in Plate I. These peats are depicted in Plate I. These mixes were prepared by blending peat and coarse horticultural grade perlite (1:1,v/v) with 7.1 g CaCO3 (<0.15 mm), 1.1 g superphosphate, 1.1 g KNO3 and 1.1 g gypsum per L. Water was added during mixing (3 min) in a cement mixer to raise the moisture level to 50% (w/w). Mixes were stored 14 days at 24 C. Acidity at the time of planting and throughout the growth period ranged from pH 5.5-6.2. The fourth mix, which was a composted pine bark-amended mix, contained composted pine bark (45%,v/v), Canadian sphagnum peat (H3-H4), vermiculite and coarse horticultural grade perlite (Ball Seed Company, West Chicago, IL). The air capacity of all mixes was at least 15% (v/v) in a 10 cm tall pot. Percolation rates were greater than 2 cm/min.

Preparation of inocula. Isolate 211 of P. ultimum, which was originally isolated from poinsettia (97) was used throughout this study. Two procedures were used to prepare inoculum. Inoculum referred to hereafter as "soil inoculum" was prepared as described previously (12) using Ko and Hora's chopped potato soil medium (52). Inoculum referred to as "peat inoculum" was prepared by planting cucumber (Cucumis sativus L. cv 'Straight Eight', 90% germination) seeds in a sterilized sphagnum peat (H4) mix infested with P. ultimum soil inoculum (1.0 g/L). Pots were irrigated daily and incubated 10 days at a constant temperature of 20 C with 16 h of illumination (225 μE m² sec⁻¹) per day. Cucumber seedlings that survived were removed, and a second crop was planted. Cucumber plant residues were removed, and the infested mix was air-dried overnight and
ground with a mortar and pestle. Particles were sieved, and 1-2 mm particles were used as peat inoculum.

Cucumber bioassay. Potting mixes were placed into plastic bags, amended with 21 g slow release fertilizer (Osmocote 14-14-14, Grace-Sierra Chemical Co., Milpitas, CA) and infested with 0.5 g/L *P. ultimum* soil inoculum as described previously (12). Controls included autoclaved (1 h, 121 C) mixes with and without *Pythium* inoculum. Bags were shaken vigorously to ensure uniform distribution of the inoculum and fertilizer. Infested mixes were distributed into five disposable styrofoam pots (400 ml/pot) that had perforated bases to allow adequate drainage. Eight cucumber seeds were planted 1 cm deep in each of the pots. The pots were then placed randomly into a growth chamber set to provide identical conditions as those described above. Disease severity was rated 10 days after planting according to the following scale: 1=symptomless, 2=emerged but wilted, chlorotic or with visible lesions on hypocotyl, 3=post-emergent damping-off, and 4=pre-emergent damping-off. A mean disease severity of eight seedlings per pot was calculated to represent one treatment replication (five replicates per treatment). Diseased seedlings and nongerminated seeds were surface-sterilized in 1% sodium hypochlorite (30 sec), rinsed twice in sterile distilled water and placed on semi-selective sucrose asparagine-pentachloronitrobenzene neomycin-sulfate chloramphenicol (SA-PBNC) agar for re-isolation of the pathogen (88). Randomly selected hyphal tips were transferred to lima bean agar (Difco Laboratories, Detroit, MI) to verify pathogen identity. Middleton's description of *P. ultimum* was used to identify isolates (72).

Poinsettia root rot bioassay. Poinsettia cuttings rooted in Oasis cubes (Smithers Oasis, Kent, OH) were planted in 1.2-L plastic pots. Prior to planting, all potting mixes were infested with 0.15 g/L peat inoculum. Control treatments were not infested. Each pot was filled with potting mix diagonally from the bottom in one side to the top of the other side to approximately 66% of the pot's capacity. Next a double layer of
nylon fabric (0.3-mm mesh) was placed on the surface of the potting mix. This fabric prevented the root system from completely ramifying the potting mix. During planting, a small volume of noninfested mix (50 ml) was added immediately around each rooted cutting. This prevented direct contact of inoculum with root tips damaged during transplanting. The remaining volume of the pot was then filled.

Plants were grown in a greenhouse with naturally occurring photoperiods and were irrigated daily. Fertilizer (200 mg/L Peters 20-20-20, Grace-Sierra Chemical Co., Milpitas, CA) was applied twice weekly. Five plants from each treatment were harvested at various times throughout the growth period. During harvesting, potting mix associated with the root system was dissociated gently with the pot label. Root segments suspected of being infected with P. ultimum were removed for re-isolation of the pathogen. The root ball was then gently submerged into tap water to remove additional potting mix residues, and plants were rated for root rot severity as described below. Pathogen identity was verified as described for the cucumber bioassay.

Three poinsettia root rot bioassays were performed. The first two were similar. The same batches of light peat (H3), dark peat (H4), and composted pine bark-amended potting mix were used in each. Both bioassays were conducted during the late summer and fall when potting mix temperatures ranged from 18-25 C. Poinsettia plants were harvested at 4, 14, 35, 63 and 88 days after planting during bioassay I, and at 4, 14, 28, 35, 42, 63 and 77 days after planting during bioassay II. In both bioassays, root rot severity was rated using a scale in which 1=symptomless, 2=mild root rot, 3=severe root rot, 4=severe root rot and crown infection, and 5=dead plant.

The third poinsettia bioassay was performed between March and June, when potting mix temperatures ranged from 19-27 C. The least-decomposed source of light peat (H3), the dark peat (H4) mix, and a different batch of composted pine bark-amended potting mix were used. Although the peat
potting mixes were prepared as those described in the first two bioassays, the moisture contents of the mixes were not as high (40%, w/w). Plants were harvested at 4, 10, 17, 24, 45, 63 and 77 days after planting. Root rot severity was rated using a revised scale in which 1=symptomless, 2=mild root rot (<1/3 roots rotted), 3=intermediate root rot (between 1/3 and 2/3 roots rotted), 4=severe root rot (>2/3 roots rotted), 5=severe root rot and crown infection, and 6=dead plant. This experiment was repeated once.

**P. ultimum population density.** The population density of *P. ultimum* was determined on SA-PBNC agar using a soil surface dilution plating technique (88). A 2.0-g sample of each of the mixes was added to 20 ml dilute water agar (0.4%) and homogenized for 1 min using a Waring blender. Successive 10-fold dilutions were made, and 0.2-ml aliquots of each dilution were plated in triplicate on SA-PBNC medium. Plates were incubated 48 h at 25 C and rinsed gently with tap water to remove any remaining potting mix residues. The number of colony-forming units (cfu) of *P. ultimum*/g dry weight mix was recorded. This procedure was replicated three times for each potting mix sample.

**Microbial Activity.** Microbial activity was monitored by measuring the rate of hydrolysis of fluorescein diacetate [(FDA), Sigma Chemical Co., St. Louis, MO] (42,89). FDA was dissolved in acetone and stored as a stock solution (2.0 mg/ml) at -20 C. Potting mix samples were collected at each harvest date from just beyond (approximately 2 cm) the growing root systems, below the nylon fabric, or the bottom 5 cm of the pot during the late stages of plant growth. All visible root segments were removed from potting mix samples. Five-gram samples of each potting mix were placed into 250-ml Erlenmeyer flasks, and 20.0 ml of 60 mM potassium phosphate buffer, pH 7.6 (8.7 g KH₂PO₄ and 1.3 g K₂HPO₄ per liter distilled water) were added. The reaction (FDA hydrolysis) was started by adding 0.2 ml of FDA (400μg) from the stock solution to the flask. Each treatment was replicated four times with a control to which FDA had not
been added. Reaction flasks were incubated for 20 min on a rotary shaker (90 rpm) at 25 C. FDA hydrolysis was halted by addition of 20 ml acetone to each of the reaction flasks. Potting mix residues were removed from the mixture by filtration through filter paper (Whatman No. 1). The filtrate was collected in a test tube, covered with Parafilm (American Can Co., Greenwich, CT) and placed into an ice bath to reduce volatilization of the acetone. The concentration of free fluorescein was determined spectrophotometrically with an HP 8452A Diode-Array Spectrophotometer (Hewlett-Packard Co., Palo Alto, CA) and by comparing absorbancies (490 nm) against a standard curve. Background absorbance was corrected for each treatment with the control sample.

Standard curves were prepared in duplicate for each treatment at each harvest date to avoid serious errors caused by adsorption of fluorescein to organic matter, particularly in the more decomposed (H₄) peat mixes (42). Various quantities of FDA (0, 100, 200, 300 and 400 µg in the stock solution) were added to 5.0 ml of phosphate buffer in screw-cap tubes. Tubes were capped tightly and incubated for 60 min in boiling water to hydrolyze the FDA. Cooled fluorescein was then added to 250-ml Erlenmeyer flasks containing 5.0 g samples of the various potting mixes. An additional 15.0 ml of phosphate buffer was used to wash the fluorescein from the tubes into the samples. Flasks were then shaken 20 min on a rotary shaker (90 rpm) at 25 C, after which 20 ml of acetone were added. Finally, the samples were filtered and processed as described above.

Experimental designs and statistical analyses. Completely randomized designs were used in all cucumber bioassays. Each treatment was replicated five times (five pots per treatment). Randomized complete block designs were used in all poinsettia bioassays. Treatments were replicated five times (five pots per treatment) per harvest date. One-way analyses of variance (ANOVA) were preformed with MINITAB statistical software (Minitab Inc., State College, PA). Separations of means were based on the least significant difference (LSD, p=0.05) (21).
Overall relationships between root rot severity, pathogen densities and microbial activity were assessed using ordinary least squares regression. Regression analyses also were performed with MINITAB statistical software. Standardized residuals of data used in regression analyses were plotted to check for normality of the data and ensure that errors were randomly, independently, and normally distributed with a common variance.
RESULTS

Variability in suppressiveness of Canadian sphagnum peat to Pythium damping-off of cucumber. Pythium damping-off was suppressed consistently in three cucumber bioassays that were performed with the least-decomposed light peat (H2) mix. Disease severity values in this mix ranged from 1.4 to 1.7. Disease severity values in the dark peat (H4) mix ranged from 2.7 to 3.2, which is significantly higher (LSD_{0.05}=0.8). The disease severity values in the noninfested and infested controls were 1.1 and 3.7, respectively.

Poinsettia root rot bioassays I and II. Root rot severity: In the first bioassay, symptoms of Pythium root rot were evident 14 days after potting in all infested treatments. Root rot severity (mean=2.0) did not differ significantly (P=0.05) among the potting mixes at this time (Fig. 1A). By 35 days after planting, roots in the dark peat (H4) mix had a higher level of rot (mean=3.4) than those in either the light peat (H3) (mean=2.4) or the composted pine bark-amended mix (mean=2.0), respectively. Plants in the dark peat (H4) mix had higher levels of root rot for the remainder of the growth period. Root rot on plants in the light peat (H3) mix was intermediate with a maximum mean root rot severity of 2.8. Root rot severity in the composted pine bark-amended mix reached a maximum mean value of 2.6 at the final harvest date. The only significant (P=0.05) differences in root rot severity between plants grown in the light peat (H3) and compost-amended mixes occurred 63 days after planting, when mean root rot severities were 2.8 and 2.0, respectively.

P. ultimum population density: During the first bioassay, the population density of P. ultimum in the mixes did not differ significantly (P=0.05) 14 days after planting (Fig. 1B). Thereafter, the densities in all mixes differed significantly. The population density of P. ultimum in the light peat (H3) mix were significantly (P=0.05) lower than those in the dark peat (H4) mix. In the composted pine bark-amended mix it was low throughout the duration of the growth period.
Microbial activity: During the first bioassay, microbial activity in the dark peat (H₄) mix at planting was sufficient to hydrolyze 3.52 \( \mu \)g FDA min⁻¹ g⁻¹ dry weight mix (FDA units) (Fig. 1C). By the fourth day after planting, it had declined significantly \((P=0.05)\) to 1.32 FDA units and remained at this low level for the remainder of the growth period. Microbial activity in the light peat (H₃) and in the composted pine bark-amended mix did not differ initially (4.65 FDA units). By the fourth day after planting, significant differences \((P=0.05)\) were apparent. The relatively high level of microbial activity in the light peat (H₃) mix was sustained during the initial 35 days of the growth period. However, between the thirty-fifth and sixty-third day of the growth period, a significant decline \((P=0.05)\) occurred. Thereafter, microbial activity in this mix remained at this low level.

A significant increase \((P=0.05)\) in microbial activity occurred in the composted pine bark-amended potting mix during the initial 4 days of the growth period. This high level of activity was transitory and had stabilized by day 14 at 4.77 FDA units. Microbial activity in the composted pine bark-amended mix was consistently higher than that in either of the peat mixes used in this bioassay. In the second poinsettia bioassay, trends in root rot severity, population density of \(P. \) ultimum and microbial activity were essentially identical to those observed in the first (Fig. 1).

Poinsettia bioassay III. In the third bioassay, trends in root rot severity (Fig. 2A), population density of \(P. \) ultimum (Fig. 2B) and microbial activity (Fig. 2C) in the light (H₂) peat mix were different from those in poinsettia bioassays I and II. Trends in the dark peat (H₄) and the composted pine bark-amended mixes were similar to those observed in the first two bioassays (Fig. 1). In this batch of light peat (H₂) mix, microbial activity was sustained, root rot severity remained mild and population density of \(P. \) ultimum remained low throughout the growth period. An increase of microbial activity in this peat (H₂) mix was
detected early after planting. This activity was similar to, although significantly lower ($P=0.05$) in intensity, than that observed in the composted pine bark-amended potting mix.

Relationships between root rot severity, population density of *P. ultimum* and microbial activity during the production of a poinsettia crop. Increases in root rot severity and population density of *P. ultimum* observed 28 days after planting and thereafter were negatively correlated with microbial activity (Fig. 3A and B).
Plate I. Differences in the decomposition level of Canadian sphagnum peat used in the preparation of potting mixes. Top, Slightly decomposed light peat, classified as H₂ on the von Post decomposition scale; Middle, Slightly decomposed light peat (H₃); Bottom: Decomposed dark peat (H₄).
Figure 1. Comparison of Pythium root rot severity, population density of *Pythium ultimum* and microbial activity in a light (H3) and a dark (H4) peat mix and a composted pine bark-amended potting mix. A, Root rot severity; 1=symptomless, 2=mild root rot, 3=severe root rot, 4=severe root rot and crown infection, and 5=dead plant. Vertical bars represent standard errors (n=5). B, Population density of *P. ultimum*. Population density data was transformed using Taylor's power law (b=0.76). Vertical bars represent standard errors (n=3). C, Microbial activity based on the rate of hydrolysis of fluorescein diacetate. Vertical bars represent standard errors (n=4).
Figure 2. Comparison of Pythium root rot severity, population density of *Pythium ultimum* and microbial activity in a light (H₂) and a dark (H₄) peat mix and a composted pine bark-amended potting mix. A, Root rot severity; 1=symptomless, 2=mild root rot (<1/3 roots rotted), 3=intermediate root rot (between 1/3 and 2/3 roots rotted), 4=severe root rot (>2/3 roots rotted), 5=severe root rot and crown infection, and 6=dead plant. Vertical bars represent standard errors (n=5). B, Population density of *P. ultimum*. Population density data was transformed using Taylor's power law (b=0.76). Vertical bars represent standard errors (n=3). C, Microbial activity based on the rate of hydrolysis of fluorescein diacetate. Vertical bars represent standard errors (n=4).
Figure 3. Relationships between root rot severity, population density of *Pythium ultimum* and microbial activity during the production of a poinsettia crop. A, Root rot severity versus microbial activity. B, Population density of *P. ultimum* versus microbial activity. Population density data was transformed to natural logarithm.
DISCUSSION

Microbial activity at planting in both compost-amended and sphagnum peat potting mixes have been used to predict damping-off severity of cucumber caused by *P. ultimum* (11,42). A similar relationship has been described for suppression of damping-off of cucumber caused by *Pythium aphanidermatum* (Edson) Fitzp. (67). The results of this work indicate that microbial activity over time also predicts potting mix suppressiveness to *Pythium* root rot of poinsettia. A decrease in microbial activity during the production of the crop was accompanied by an increase in the population density of *P. ultimum* and root rot severity. At low levels of microbial activity, the concentration of water soluble root exudates apparently was sufficient to stimulate germination of *P. ultimum* propagules, infection of roots and development of root rot (13,25,67,96).

The most suppressive mixes were prepared with the least-decomposed sources of organic matter (i.e. composted pine bark-amended or the H2 decomposition level sphagnum peat mixes). The highest populations of *P. ultimum* and root rot developed in the mixes prepared with the most decomposed (H4) peat. In the mix prepared with the H3 decomposition level peat, a shift from suppressiveness to conduciveness was observed. Therefore, organic matter decomposition level was inversely related to microbial activity and directly related to root rot severity. Finally, populations of *P. ultimum* and Pythium root rot were suppressed as long as the rate of hydrolysis of FDA was sustained above a level of 3.2 μg min⁻¹ g⁻¹ dry weight potting mix.

Sphagnum peats harvested from increasing depths in bogs increase in decomposition level and decrease in ability to support growth of heterotrophic microorganisms (109). Kuster concluded in 1963, that "The more decomposed, humified and carbonized the carbon material in peat, the smaller the content of utilizable compounds, and the less available they become for microbes" (54,55). The term "microbial carrying capacity" best
describes this effect. The microbial carrying capacity of a potting mix depends on the decomposition level of the organic components used in the potting mix. During the past two decades, this property of potting mixes and field soils has eluded scientists interested in biological control. For diseases caused by exogenously nutrient-dependent plant pathogens, such as *P. ultimum*, that are suppressed through microbiostasis (13,67), this lack of knowledge is unfortunate. It has not been possible so far, to quantify the energy availability in or the carrying capacity of soil organic matter.

Recent breakthroughs in direct spectroscopy, utilizing cross polarized magic angle spinning $^{13}$C-nuclear magnetic resonance, have permitted nondestructive analysis of the decomposition of carbohydrates in composts (2,44,83), sphagnum peat (24,32,82,116,118), and other sources of soil organic matter (117,118). As a result, direct relationships among organic matter decomposition level, microbial carrying capacity, microbial activity, microbial biomass, the nutritional status of the microflora and disease severity, can now be developed. Potting mixes prepared with slightly decomposed (H$_3$) sphagnum peat offer unique opportunities to explore these fundamental interactions.

The population density of *P. ultimum* did not decline with time in any of the suppressive mixes used in these studies, which is consistent with earlier observations (13,67). Eradication, therefore, may not have played a major role in the suppressive effect in these mixes, although *Pythium* populations may have produced several generations so that rates of regrowth approximately equaled rates of eradication. Disregarding this possibility, we propose that microbiostasis resulting from nutrient competition was the principal mechanism of biological control involved in the suppressive sphagnum peat and composted pine bark-amended potting mixes, as was described previously for other compost-amended mixes (13,67).
Quantitative information on the contributions of specific biocontrol agents in suppression of Pythium root rot in peat mixes is sparse. Suppressiveness was destroyed by heating and restored after the addition of small amounts of light-colored peat which suggests that the effect is biological. Addition of 25% (v/v) suppressive light (H₂) to conducive, dark (H₄) peat does not induce suppression to damping-off caused by P. ultimum (59). The conducive nature of the decomposed dark peat (H₄), therefore, is not due to the absence of biocontrol agents. During harvesting, peat routinely is stored in large piles or windrows, where temperatures within the piles, due to self-heating, are often high enough to kill mesophilic microorganisms. The microflora in processed peat, therefore, is influenced not only by the decomposition level but also by the procedures employed during processing (18,56). Trichoderma viride Pers. ex Fr., Penicillium spp., Chrysosporium spp. and Streptomyces spp. have been proposed as biocontrol agents involved in suppression of Pythium damping-off in Scandinavian sources of light-colored sphagnum peat (100,101,102,119,120). The acidity of sphagnum peat at harvest is too great (pH 3.0-4.7) to support growth of most bacteria. During the formulation of peat mixes, when the acidity is decreased to pH 5.5-6.2, and bacterial growth is no longer inhibited, a mild biological vacuum is created. Changes in microbial populations in peat that occur after formulation have been poorly described. The greatest potential for population development is in mixes prepared with the least-decomposed peats because these peats have the greatest microbial carrying capacity. The short-duration peaks in microbial activity observed in this work within days after planting in the least decomposed light (H₂) peat and the composted pine bark-amended mix support this observation. In conclusion, variability in suppressiveness reported for sphagnum peat in the past probably is attributable to both the composition of the microflora and the carrying capacity (decomposition level) of the peat.
CHAPTER II

IMPACT OF PEAT DECOMPOSITION LEVEL ON MICROBIAL SPECIES DIVERSITY AND SUPPRESSION OF PYTHIUM DAMPING-OFF

Pioneering efforts of Waksman and Stevens (110,111,112) and Waksman and Purvis (109) revealed that high numbers of microorganisms are present in peatlands (lowmoor and highmoor bogs and fens), which, in conjunction with environmental factors, are responsible for the formation of peat from plant remains. Since then, numerous reports on the microbiology of peatlands have been published. In general, these studies have shown that (a) relatively high populations of fungi, bacteria (5,78,87,109,112,115), actinomycetes and occasionally yeasts (3) are found in sphagnum peat deposits; (b) the population density of aerobic microorganisms decreases with increasing depth within the bog profile (6,16,53,54,70,121,122); (c) the population density and activity of cellulolytic microorganisms appears to be confined to the least-decomposed surface (0 to 7.5 cm) layer of the bog (16,53), which is high in cellulose and hemicellulose content (10,23,54,55,84,103,110); (d) the bacterial species composition of the surface layer (0 to 7.5 cm) of sphagnum bogs is characterized by a predominance of Gram-negative bacteria including the genera Pseudomonas, Agrobacterium, Enterobacter, Xanthomonas, Bacillus, Serratia (30,98), Sporocytophaga, Myxococcus (121), Alcaligenes, the Acinetobacter-Moraxella complex (16) and Streptomyces (100); (e) anaerobic (Methanogens), pleomorphic (Nocardia and Arthrobacter) and Bacillus spp. predominate in the subsurface (7.5-30 cm) layers of native sphagnum bogs (16,20,70,91,113,121); (f) Penicillium spp. and Trichoderma viride are commonly isolated from the surface (0 to 7.5 cm) layers of sphagnum bogs and Aleurizum, Cladosporium, Cephalosporium, Chrysosporium, Gelasinospora,
Geotrichium, Gliocladium, Philophora, Verticillium and Rhizophlyctis are isolated less frequently (6,7,30,46,53); and finally (g) that microbial activity and rates of organic matter decomposition in the surface (0 to 7.5 cm) layer of bogs increases after drainage (33,46,48,60,91).

Little information has been published on the microbiology and community ecology of sphagnum peat after it has been harvested from the bog (18,46,54,55,56,69). Even less is known about the microbiology of disease suppression in peat (100,101,105,119,120). The objectives of the research reported in this Chapter are 1) to enumerate, identify and compare microbial communities present in the least-decomposed suppressive light (H₂) peat and compost-amended mixes with that of the decomposed conducive dark (H₄) peat mix described in Chapter I; and 2) to determine whether differences in suppressiveness of these mixes can be attributed to differences in the microflora harbored in these peats.
MATERIALS AND METHODS

Preparation of potting mixes. Three potting mixes differing in natural suppressiveness to Pythium damping-off were used throughout this work. These mixes included the decomposed conducive dark (H₄) and the slightly decomposed suppressive light (H₂) peat mixes described in Chapter I. The third mix, a suppressive composted hardwood bark-amended mix, was prepared by blending mature composted hardwood bark with dark (H₄) sphagnum peat and coarse horticultural grade perlite (5:2:3, v/v), as described by Chen et al. (12). Water was added to all mixes during blending (3 min) in a cement mixer to raise the moisture level to 50% (w/w). Mixes were then stored 14 days at 24 C. Acidity at the time of planting and throughout the growth period ranged from pH 5.5 to 6.2. The air capacity of all mixes was at least 15% (v/v) in a 10-cm tall pot. Percolation rates were greater than 2 cm/min.

Preparation of inocula. Soil inoculum of P. ultimum was prepared as described in Chapter I.

Isolation, enumeration and identification of bacteria. Rhizosphere bacteria were isolated from root tips of cucumber seedlings grown in slightly decomposed light (H₂) and decomposed dark (H₄) peat mixes and from the composted hardwood bark-amended mix, as described previously (58). Cucumber seeds (four per pot) were planted 1-cm deep and incubated for 8 days in a growth chamber set to provide conditions identical to those described in Chapter I. At harvest, seedlings were removed from the pot, shaken gently to remove excess potting mix and then rinsed in 9.0 ml sterile phosphate buffer (7 g K₂HPO₄, 3 g KH₂PO₄ and 0.2 g MgSO₄ 7H₂O per L distilled water). The tip 1 cm of each primary root was removed aseptically and comminuted in 0.1-ml phosphate buffer with a Ten Broeck tissue homogenizer. The resulting suspension was serially diluted in phosphate buffer and 0.1-ml aliquots were plated in triplicate on 0.1 strength typticase soy broth agar [(0.1 TSBA) BBL, Microbiology Systems, Cockeysville, MD]. Plates were incubated 4 days at 25 C and the mean
number of cfu/root tip determined. Next, 70 colonies were non-
discriminately selected and streaked on 0.1 TSBA. All colonies on the
lowest dilution plate were selected for root tips from which extremely low
populations were recovered. Bacterial strains were purified by repetitive
streaking on 0.1 TSBA and identified by gas-chromatographic fatty acid
methyl ester (GC-FAME) analysis with an HP 5898A Microbial Identification
System equipped with version 3.5 of the Aerobic Library, according to
procedures specified by the manufacturer (MIDI, Newark, DE). Strains not
identified by GC-FAME analysis were placed into groups (GC similarity
groups) based on the similarity of their phospholipid fatty acid profiles.
Pure cultures were stored at -70 °C in a 15% (v/v) sterile glycerol-water
solution (93).

The relative population density of bacterial taxa on each root tip was
determined with the following equation: 100n_i/N, in which n_i is the number
of strains assigned to the i-th taxon and N is the total number of strains
for a given root tip. The experiment was done three times for each mix.

Efficacy of bacterial strains as biocontrol agents. Preliminary
screening of all isolated bacterial strains was performed in sterilized
(autoclaved) light (H_2) peat mix infested with 0.5 g/L soil inoculum of P.
ultimum. Bacterial strains were cultured 48 h in full strength TSB (50
ml, 25 °C), washed twice by centrifugation (10 m, 8,000 rpm) and
resuspended in phosphate buffer to a population density of approximately
10^8 cfu per ml (verified by dilution plating). Next, approximately 50
cucumber seeds were added and the suspension was vigorously mixed. Eight
treated seeds were planted 1-cm deep (five pots per treatment) using
sterile forceps. Controls consisted of cucumber seeds soaked in phosphate
buffer and planted in sterilized light (H_2) peat mix with and without soil
inoculum of P. ultimum. The remaining steps of this bioassay were
identical to those described in Chapter I.

Strains most effective at inducing suppression to Pythium damping-off
(mean damping-off severity values ranging from 1.0-2.0 on a scale in which
1=symptomless, 2=emerged but wilted, chlorotic or with visible lesions on hypocotyl, 3=post-emergent damping-off, and 4=pre-emergent damping-off) were re-evaluated at least once in both sterilized light (H2) and dark (H4) peat mixes infested with soil inoculum of P. ultimum using the same procedure as described above.

Isolation and identification of fungal isolates obtained from light (H2) and dark (H4) peat. Aquatic fungi, such as Chytridiomycetes and Oomycetes, were isolated by baiting a saturated peat slurry with various organic substrates. The slurry was prepared by combining peat with sterile distilled water (1:2, v/v). It was incubated 30 min at 20 C and then transferred to five sterile petri dishes (20 ml/dish). The remainder was then filtered through filter paper (Whatman No. 1). Next, 15 ml of the filtrate was added to each petri dish. Gross fungal cultures were isolated by baiting with combinations of sterile organic substrates such as lens paper, roach wings, pine pollen, hemp seeds, and dried human and snake skin. After 2 wk, hemp seeds were transferred to petri dishes containing 25 ml sterile distilled water. The remaining substrates were incubated for an additional 3-14 days at 20 C and examined periodically for evidence of fungal colonization.

Fungal isolates also were isolated by direct and dilution plating of peat samples on acidified potato-dextrose agar [(APDA) Difco Laboratories, Detroit, MI]. The total fungal population density was determined from these dilution plates (triplicate series). Isolates of predominant fungal taxa were purified and stored on potato-dextrose (PDA) agar until their efficacy as biocontrol agents could be tested. Fungal isolates were identified using light and scanning electron microscopy and appropriate taxonomic keys for each taxon (4,50,51,77,85,94). Population densities of individual taxa were not determined.

Efficacy of fungal isolates as biocontrol agents. Efficacy of fungal isolates as biocontrol agents towards Pythium damping-off was determined with a slightly modified version of the cucumber seedling bioassay
described in Chapter I. Light (H₂) and dark (H₄) peat mixes were placed into polyethylene bags (2 L/bag) and autoclaved (1 h, 121 C). Upon cooling, they were infested with spore suspensions of candidate fungal biocontrol agents. Spore suspensions were prepared by placing colonized agar from 7-day (25 C) PDA cultures in 200 ml of sterile distilled water. Suspensions were vigorously stirred and then filtered through double-layered cheesecloth to remove agar residues. The filtrates were collected and spore concentrations determined with a hemacytometer. An appropriate volume of spore suspension was added to each bag of mix to achieve final spore concentrations of approximately 10⁵ cfu/g dry weight mix (verified by dilution plating). The infested mixes were then incubated for 1 wk at 24 C, amended with 21 g slow release fertilizer (Osmocote 14-14-14, Grace-Sierra Chemical Co., Milpitas, CA) and infested with 0.5 g/L soil inoculum of P. ultimum. The remaining steps of this bioassay were identical to those described in Chapter I. Any isolate capable of inducing a mean damping-off severity of 1.0-2.0 was considered effective.

In addition to the fungal isolates obtained from peat, four effective fungal biocontrol agents, originally obtained from various composted hardwood bark-amended potting mixes (75), were included for comparison purposes. These biocontrol agents included: Trichoderma hamatum 382 (Bonord.) Bain, aggr., Trichoderma harzianum 738 Rifai aggr., and Gliocladium virens 700 Miller, Giddens & Foster.

Experimental designs and statistical analyses. Completely randomized designs were used in all cucumber bioassays. Each treatment was replicated five times (five pots per treatment), unless otherwise noted. One way analysis of variance (ANOVA) was performed using Minitab statistical software (Minitab Inc., State College, PA). Separations of means were based on the least significant difference (LSD, P=0.05) (21).

Bacterial species diversity, defined here as a cumulative measure of both the number of species present (species richness) and the distribution of strains among those species (species evenness) was determined on root
tips harvested from cucumber seedlings grown in all three potting mixes (three root tips for the suppressive and four root tips for the conducive mixes).

Species richness was assessed using Hurlbert's (41) rarefaction method. This method was selected over other richness indices because it allows for comparisons to be made between communities based on unequal sample sizes, as was the case among the root tips screened in this work. The expected number of species \( E(S_n) \) was calculated using the following equation:

\[
E(S_n) = \sum (1 - \left( \frac{(N-n_i)}{N/n} \right)^n),
\]

in which \( S_n \) is the number of species at a sample size of \( n \). Rarefaction curves were generated by plotting the \( E(S_n) \) versus \( n \) for each root tip.

Hill's (34) first and second diversity numbers, \( N_1 \) and \( N_2 \), respectively, were used as measures of species diversity. \( N_1 \), a measure of abundant taxa, was calculated with the equation: \( N_1 = e^{H'} \), in which \( H' \) is Shannon's diversity index (90). Shannon's index was calculated using:

\[
H' = -\sum \left[ \frac{(n_i/N)\ln(n_i/N)}{n} \right].
\]

\( N_2 \), a measure of very abundant taxa, was calculated as:

\[
N_2 = 1/\lambda,
\]

in which \( \lambda \) is Simpson's index (92). Simpson's index was calculated with the equation:

\[
\lambda = \sum p_i^2,
\]

in which \( p_i \) is the proportional abundance of the \( i \)th taxon \( (n_i/N) \).

The modified Hill's ratio, \( E_s \), (1) was used to assess species evenness and was calculated as: \( E_s = (N_2 - 1)/(N_1 - 1) \). The computer programs used in these analyses were RAREFRAC.BAS and SPDIVRS.BAS (61).

Similarity in bacterial taxa composition among root tips [sampling units (SUs)] was measured using Dice (22, 62, 63) similarity coefficients (resemblance functions) and polar ordination (29, 64). Dice similarity coefficients were calculated with the equation:

\[
2a/(2a + b + c),
\]

in which \( a \) is the number of common taxa between two SUs (\( X \) and \( Y \)), \( b \) is the number of unique taxa which occur in SU \( X \), but not in SU \( Y \), and \( c \) is the number of unique taxa which occur in SU \( Y \), but not in SU \( X \). This resemblance function is equal to 0 at "no similarity" and approaches 1 when the SUs are identical in bacterial taxa composition (63). The polar ordination
method of Bray and Curtis (9,29,64) was used to position SUs within a coordinate system such that the distances between SUs reflected their similarity in bacterial taxa composition as well as their relation to underlying environmental gradients such as organic matter decomposition level. In this work, SUs were arranged on the basis of percent dissimilarity. Percent dissimilarity between SUs was calculated as:

$$100\left\{1-\frac{(2W)}{(A+B)}\right\},$$

in which \(W\) is the number of common taxa between SUs \(X\) and \(Y\), \(A\) is the total number of taxa in SU \(X\), and \(B\) is the total number of taxa in SU \(Y\). The computer programs used in these analyses were \texttt{SPASSOC.BAS} (62), \texttt{SUDIST.BAS} (63) and \texttt{PO.BAS} (64).
RESULTS

Relative population density of bacterial taxa on cucumber root tips. The number of bacterial taxa isolated on 0.1 TSBA from most root tips increased until about 60 strains had been isolated. Therefore, this number of isolations was selected whenever possible as an end point for recovery of taxa.

The relative population density of bacterial taxa isolated from cucumber root tips in the composted hardwood bark-amended, the light (H₂) and the dark (H₄) peat mixes are presented in Tables 1, 2 and 3. No single taxon predominated on root tips recovered from any of the mixes. The highest relative population density that a given taxon reached on any root tip was 52 percent. The total number of taxa recovered from a single root tip ranged from eight to 21.

*Pseudomonas* spp. predominated on root tips harvested from the suppressive composted hardwood bark-amended (Table 1) and light (H₂) peat (Table 2) mixes. They were not isolated from roots of seedlings grown in the conducive dark (H₄) peat mix (Table 3). *Arthrobacter* spp. were isolated from all root tips harvested from seedlings grown in the conducive dark (H₄) peat mix (Table 3). This genus was absent from root tips harvested from either of the suppressive mixes (Tables 1 and 2). The relative population density of *Bacillus* spp. was significantly (P=0.05) higher on root tips harvested from the conducive dark (H₄) peat mix (4.0–29.4%) compared to either of the suppressive mixes (0–3.7%).

The minimum number of strains isolated per root was 35. This sample size was used, therefore, to directly compare abundance of taxa among roots (47). The expected number of bacterial taxa at this sample size \[E(S_{35})\] from root tips harvested from each of the mixes ranged from nine to fifteen (Fig. 4). Hill’s first and second diversity numbers \((N₁\text{ and } N₂)\) and the modified Hill’s ratio \((E5)\) for each of the mixes are presented in Table 4. In all cases, differences among root tips within each of the mixes were greater than those observed among mixes. This indicated that
potting mix type did not affect bacterial species diversity, as determined by either abundance, evenness, or a combination of these factors.

Dice similarity coefficients indicating the proportional similarity between sampling units are presented in Table 5. Both the Dice similarity coefficients (Table 5) and the Bray-Curtis polar ordination plot (Fig. 5) revealed that the composition of bacterial taxa among root tips harvested from cucumber seedlings grown in the three mixes differed. The highest (0.35-0.71) similarity coefficients were obtained between root tips harvested from the same type of mix. The composition of bacterial taxa among root tips harvested from the light (H2) peat mix, and the composition of bacterial taxa among root tips harvested from the dark (H4) peat mix, were both significantly (P=0.074) more similar than that among root tips harvested from the composted hardwood bark-amended mix. The mean Dice similarity coefficients for root tips harvested from the light (H2) and the dark (H4) peat mixes and from the composted hardwood bark-amended mix were 0.45, 0.47 and 0.24, respectively.

Efficacy of bacterial taxa as biocontrol agents. Strains of 17 bacterial taxa isolated from root tips of seedlings grown in the composted hardwood bark-amended mix induced suppression (mean damping-off severity values <2.0) to Pythium damping-off of cucumber in the slightly decomposed light (H2) peat mix. Sixteen of 157 strains (~10%) isolated from root tips of cucumber seedlings grown in the light (H2) peat mix were effective. In contrast, only two of 154 strains (~1.3%) isolated from root tips of cucumber seedlings grown in the conducive dark (H4) peat mix were effective. The latter strains were isolated from the root tip (Table 3, Root 4) that was most similar in bacterial species composition to that of root tips harvested from seedlings grown in the slightly decomposed light (H2) peat mix (Fig. 5).

Effective strains isolated from the composted hardwood bark-amended mix were identified as Agrobacterium radiobacter, Comamonas acidovorans, C. testosteronii, Corynebacterium flaccumfaciens, Cytophaga aquatilis,
Enterobacter spp., Hydrogenophaga pseudoflava, Micrococcus roseus, Phyllobacterium rubiacearum, Pseudomonas alcaligenes, P. delafieldi, P. facilis, P. fluorescens, P. saccharophilia, P. solanacearum and Xanthomonas maltophilia.

Effective taxa isolated from the light (H2) peat mix included: A. radiobacter, Alcaligenes xylosoxydans, H. pseudoflava, P. rubiacearum, P. fluorescens, P. saccharophilia, P. vesicularis and GC similarity group 1.

A comparison of the most effective bacterial strains in the sterilized light (H2) versus dark (H4) peat mixes infested with soil inoculum of P. ultimum is presented in Table 6. Many strains induced a significantly ($P=0.05$) higher level of suppression in the dark (H4) peat mix than in either of the sterilized infested controls. However, Pythium damping-off severity was significantly ($P=0.05$) lower in the slightly decomposed light (H2) as compared to the decomposed dark (H4) peat mix.

Several isolates of an unidentified actinomycete were isolated from the light (H2) peat. Although these isolates were highly effective (mean damping-off severity values ranging from 1.2-1.5) at inducing suppression to Pythium damping-off, they appeared phytotoxic to cucumber at the concentrations at which they were tested. No further characterization of these isolates was attempted.

Characterization of fungal isolates. Total populations of fungi recovered on APDA from light (H2) and dark (H4) peat samples did not differ significantly ($P=0.05$) and ranged from $10^5$ to $10^6$ cfu/g dry weight peat. Four classes of fungi (Zygomycetes, Chytridiomycetes, Oomycetes and Hyphomycetes) were isolated from light (H2) peat. Species isolated were: Olpidiopsis saproleagniae var saproleagniae (Braun) Cornu, Olpidium allomycetos Karling, Rhizophyllum pollinis-pini (Braun) Zopf, Rhizophylyctis rosea (de Bary and Woronin) Fischer, Rhizopus sp., Saprolegnia sp., Septosperma rhizophydi Wiffen ex Seymour and T. viride. In addition, a previously unknown Siphonaria sp. was isolated. G. virens, Penicillium spp. and several unidentified isolates were recovered from the
dark (H4) peat. The absence of Chytridiomycetes, Oomycetes and Zygomycetes from dark (H4) peat was conspicuous.

Fungal isolates capable of inducing suppression to Pythium damping-off were recovered on APDA from both the suppressive light (H2) and the conducive dark (H4) peat. *T. viride*, *G. virens* and a *Penicillium* sp. were the most effective species isolated. Isolates of Chytridiomycetes and Oomycetes were not tested as biocontrol agents due to their fastidious growth habits.

*Trichoderma* biocontrol agents isolated from peat and compost-amended potting mixes were significantly more efficacious in the light (H2) than in the dark (H4) peat mix (Table 7). Similar results were obtained for *G. virens* and *Penicillium* isolates. This striking difference in efficacy is illustrated in Plate II.
Plate II. Ability of *Trichoderma viride* S19 to induce suppression to damping-off of cucumber in sterilized light ([H₂] left center) and dark ([H₄] right center) peat mix infested with 0.5 g/L soil inoculum of *Pythium ultimum*. Mean damping-off severity values for *T. viride* S19 in the light ([H₂]) and dark ([H₄]) peat mixes were 1.8 and 2.5, respectively (n=10). Mean damping-off severity values in the sterilized light ([H₂]) and dark ([H₄]) controls (outermost rows) and in the sterilized infested controls (adjacent to the noninfested controls) were 1.1 and 3.7, respectively.
Figure 4. Rarefaction curves for the expected number of bacterial species \( E(S_n) \) isolated on 0.1 strength TSBA from root tips of cucumber seedlings grown in a composted hardwood bark-amended (A), a slightly decomposed light (H2) peat (B) and a decomposed dark (H4) peat (C) mix.
Figure 5. Bray-Curtis polar ordination plot showing separation of root tips harvested from cucumber seedlings grown in a composted hardwood bark-amended (CHB), a slightly decomposed light (H2) and a decomposed dark (H4) peat mix. Axes represent percent dissimilarity. Percent dissimilarity between SUs was calculated as: 

$$100\{1-((2W)/(A+B))\},$$

in which $W$ is the number of common taxa between SUs $X$ and $Y$, $A$ is the total number of taxa in SU $X$, and $B$ is the total number of taxa in SU $Y$. 
Table 1. Relative population density of rhizosphere bacteria isolated on 0.1 strength TSBA from three cucumber seedling root tips grown in a composted hardwood bark-amended potting mix.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Relative Population Densitya</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Column headings not provided]</td>
<td>I</td>
</tr>
<tr>
<td>Agrobacterium radiobacter</td>
<td>3.0</td>
</tr>
<tr>
<td>Alcaligenes paradoxus</td>
<td>1.5</td>
</tr>
<tr>
<td>A. xyloxydans</td>
<td>1.5</td>
</tr>
<tr>
<td>Alteromonas haloplanktes</td>
<td>4.5</td>
</tr>
<tr>
<td>Azotobacter chroococcum</td>
<td>2.9</td>
</tr>
<tr>
<td>Bacillus circulans</td>
<td>1.5</td>
</tr>
<tr>
<td>B. pabuli</td>
<td>1.3</td>
</tr>
<tr>
<td>Comamonas acidovorans</td>
<td>2.9</td>
</tr>
<tr>
<td>C. testosterone</td>
<td>4.5</td>
</tr>
<tr>
<td>Corynebacterium flaccumfaciens</td>
<td>3.9</td>
</tr>
<tr>
<td>Cytophaga aquatilis</td>
<td>16.9</td>
</tr>
<tr>
<td>Enterobacter sp.</td>
<td>1.5</td>
</tr>
<tr>
<td>Escherichia fergusonii</td>
<td>2.9</td>
</tr>
<tr>
<td>Hydrogenophaga pseudoflava</td>
<td>7.4</td>
</tr>
<tr>
<td>Klebsiella planticola</td>
<td>1.5</td>
</tr>
<tr>
<td>Micrococcus roseus</td>
<td>1.5</td>
</tr>
<tr>
<td>Phyllobacterium rubiacearum</td>
<td>1.5</td>
</tr>
<tr>
<td>Pseudomonas acaligenes</td>
<td>1.5</td>
</tr>
<tr>
<td>P. aureofaciens</td>
<td>1.5</td>
</tr>
<tr>
<td>P. cepacia</td>
<td>2.9</td>
</tr>
<tr>
<td>P. delafieldii</td>
<td>8.8</td>
</tr>
<tr>
<td>P. facilis</td>
<td>36.4</td>
</tr>
<tr>
<td>P. fluorescens</td>
<td>1.5</td>
</tr>
<tr>
<td>P. gladioli</td>
<td>22.1</td>
</tr>
<tr>
<td>P. saccharophila</td>
<td>30.9</td>
</tr>
<tr>
<td>P. solanacearum</td>
<td>4.5</td>
</tr>
<tr>
<td>Xanthomonas maltophilia</td>
<td>33.3</td>
</tr>
<tr>
<td>GC similarity group 4</td>
<td>-</td>
</tr>
<tr>
<td>GC similarity group 8</td>
<td>-</td>
</tr>
<tr>
<td>GC similarity group 9</td>
<td>-</td>
</tr>
<tr>
<td>GC similarity group 10</td>
<td>-</td>
</tr>
<tr>
<td>GC similarity group 11</td>
<td>-</td>
</tr>
<tr>
<td>GC similarity group 12</td>
<td>-</td>
</tr>
<tr>
<td>GC similarity group 13</td>
<td>-</td>
</tr>
<tr>
<td>GC similarity group 14</td>
<td>-</td>
</tr>
<tr>
<td>GC similarity group 15</td>
<td>-</td>
</tr>
</tbody>
</table>

The relative population density of bacterial taxa on each root tip was calculated as follows: 100n/N, where n is the number of strains assigned to the i<sup>a</sup> taxon and N is the total number of strains for a given root tip.

Strains not identified by GC-FAME analysis were placed into groups (GC similarity groups) based on the similarity of their phospholipid fatty acid profiles.
Table 2. Relative population density of rhizosphere bacteria isolated on 0.1 strength TSBA from three cucumber seedling root tips grown in a slightly decomposed light (H_2) peat mix.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Relative Population Density^a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
</tr>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Agrobacterium radiobacter</td>
<td>2.8</td>
</tr>
<tr>
<td>Alcaligenes paradoxus</td>
<td>4.2</td>
</tr>
<tr>
<td>A. xylosoxidans</td>
<td>1.4</td>
</tr>
<tr>
<td>Bacillus pabuli</td>
<td>1.4</td>
</tr>
<tr>
<td>B. polymyxa</td>
<td>1.4</td>
</tr>
<tr>
<td>Cytophaga aquatilis</td>
<td>2.8</td>
</tr>
<tr>
<td>C. johnsonae</td>
<td>1.4</td>
</tr>
<tr>
<td>Flavobacterium balustinum</td>
<td>-</td>
</tr>
<tr>
<td>Hydrogenophaga pseudoflava</td>
<td>1.4</td>
</tr>
<tr>
<td>Methylobacterium zatmanii</td>
<td>-</td>
</tr>
<tr>
<td>Nocardoides albus</td>
<td>1.4</td>
</tr>
<tr>
<td>Phyllobacterium rubiacearum</td>
<td>14.1</td>
</tr>
<tr>
<td>Pseudomonas aureofaciens</td>
<td>1.4</td>
</tr>
<tr>
<td>P. cepacia</td>
<td>4.2</td>
</tr>
<tr>
<td>P. facilis</td>
<td>1.4</td>
</tr>
<tr>
<td>P. fluorescens</td>
<td>9.9</td>
</tr>
<tr>
<td>P. rubrilineans</td>
<td>-</td>
</tr>
<tr>
<td>P. saccharophila</td>
<td>23.9</td>
</tr>
<tr>
<td>P. syringae pv tabaci</td>
<td>1.4</td>
</tr>
<tr>
<td>P. vesicularis</td>
<td>9.9</td>
</tr>
<tr>
<td>Xanthobacter agilis^b</td>
<td>-</td>
</tr>
<tr>
<td>GC similarity group 1</td>
<td>4.2</td>
</tr>
<tr>
<td>GC similarity group 2</td>
<td>1.4</td>
</tr>
<tr>
<td>GC similarity group 3</td>
<td>1.4</td>
</tr>
<tr>
<td>GC similarity group 4</td>
<td>4.2</td>
</tr>
<tr>
<td>GC similarity group 5</td>
<td>-</td>
</tr>
<tr>
<td>GC similarity group 7</td>
<td>-</td>
</tr>
<tr>
<td>Unidentified taxa</td>
<td>4.2</td>
</tr>
</tbody>
</table>

^aThe relative population density of bacterial taxa on each root tip was calculated as follows: 100n_i/N, where n_i is the number of strains assigned to the i^a taxon and N is the total number of strains for a given root tip.

^bStrains not identified by GC-FAME analysis were placed into groups (GC similarity groups) based on the similarity of their phospholipid fatty acid profiles.
Table 3. Relative population density of rhizosphere bacteria isolated on 0.1 strength TSBA from four cucumber seedling root tips grown in a decomposed dark (H4) peat mix.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Relative Population Density&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Acaligenes xylosoxydans</td>
<td>-</td>
</tr>
<tr>
<td>Arthrobacter citreus</td>
<td>-</td>
</tr>
<tr>
<td>A. globiformis</td>
<td>11.8</td>
</tr>
<tr>
<td>A. mysores</td>
<td>5.9</td>
</tr>
<tr>
<td>A. nicotianae</td>
<td>17.6</td>
</tr>
<tr>
<td>Bacillus pabuli</td>
<td>11.8</td>
</tr>
<tr>
<td>B. polymyx</td>
<td>17.6</td>
</tr>
<tr>
<td>Cellulomonas cellulans</td>
<td>5.9</td>
</tr>
<tr>
<td>C. turbata</td>
<td>-</td>
</tr>
<tr>
<td>Escherichia fergosoni</td>
<td>-</td>
</tr>
<tr>
<td>Klebsiella planticola</td>
<td>-</td>
</tr>
<tr>
<td>Kluvera cryocsescens</td>
<td>-</td>
</tr>
<tr>
<td>Ochrobacterum anthropi</td>
<td>-</td>
</tr>
<tr>
<td>Phyllobacterium rubiacearum</td>
<td>-</td>
</tr>
<tr>
<td>Pseudomonas saccharophilia</td>
<td>-</td>
</tr>
<tr>
<td>Xanthomonas maltophilia</td>
<td>29.4</td>
</tr>
<tr>
<td>GC similarity group&lt;sup&gt;b&lt;/sup&gt; 1</td>
<td>-</td>
</tr>
<tr>
<td>GC similarity group 3</td>
<td>-</td>
</tr>
<tr>
<td>GC similarity group 6</td>
<td>-</td>
</tr>
<tr>
<td>GC similarity group 7</td>
<td>-</td>
</tr>
<tr>
<td>Unidentified taxa</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup>The relative population density of bacterial taxa on each root tip was calculated as follows: 100n/N, in which n<sub>i</sub> is the number of strains assigned to the i<sup>a</sup> taxon and N is the total number of strains for a given root tip.

<sup>b</sup>Strains not identified by GC-FAME analysis were placed into groups (GC similarity groups) based on the similarity of their phospholipid fatty acid profiles.
Table 4. Species diversity indices for root tips harvested from cucumber seedlings grown in a composted hardwood bark-amended (CHB), a slightly decomposed light ($H_2$) and a decomposed dark ($H_4$) peat mix.

<table>
<thead>
<tr>
<th>ROOT TIP</th>
<th>INDICES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diversity</td>
</tr>
<tr>
<td></td>
<td>$N_i^a$</td>
</tr>
<tr>
<td>CHB</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>6.0</td>
</tr>
<tr>
<td>II</td>
<td>9.3</td>
</tr>
<tr>
<td>III</td>
<td>7.2</td>
</tr>
<tr>
<td>Light Peat ($H_2$)</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>12.7</td>
</tr>
<tr>
<td>B</td>
<td>6.1</td>
</tr>
<tr>
<td>C</td>
<td>5.8</td>
</tr>
<tr>
<td>Dark Peat ($H_4$)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6.1</td>
</tr>
<tr>
<td>2</td>
<td>6.5</td>
</tr>
<tr>
<td>3</td>
<td>6.3</td>
</tr>
<tr>
<td>4</td>
<td>3.8</td>
</tr>
</tbody>
</table>

$^a$N$_i$, a measure of abundant taxa, was calculated with the equation: $N_i=e^{H'}$, in which $H'$ is Shannon's diversity index. Shannon's index was calculated with the equation: $H'=-\sum\{n_i/N}\ln(n_i/N)$, in which $n_i$ is the number of strains assigned to the $i^{th}$ taxon and $N$ is the total number of strains for a given root tip.

$^b$N$_2$, a measure of very abundant taxa was calculated with the equation: $N_2=1/\lambda$, in which $\lambda$ is Simpson's diversity index. Simpson's index was calculated as: $\lambda=\Sigma p_i^2$, in which $p_i$ is the proportional abundance of the $i^{th}$ species ($n_i/N$).

$^c$The modified Hill's ratio, $E_i$, was calculated as: $E_i=(N_2-1)/(N_i-1)$. 

Table 5. Sampling unit (SU) comparison matrix of similarity coefficients (resemblance functions) for bacterial taxa isolated from root tips of cucumber seedlings grown in a composted hardwood bark-amended (CHB), a slightly decomposed light (H₂) and a decomposed dark (H₄) peat mix.

<table>
<thead>
<tr>
<th>SU</th>
<th>Light Peat (H₂)</th>
<th>Dark Peat (H₄)</th>
<th>CHB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(2)</td>
<td>(3)</td>
<td>(4)</td>
</tr>
<tr>
<td>(1)</td>
<td>0.49</td>
<td>0.35</td>
<td>0.14</td>
</tr>
<tr>
<td>(2)</td>
<td>0.50</td>
<td>0.11</td>
<td>0.08</td>
</tr>
<tr>
<td>(3)</td>
<td>0.13</td>
<td>0.19</td>
<td>0.11</td>
</tr>
<tr>
<td>(4)</td>
<td>0.50</td>
<td>0.71</td>
<td>0.43</td>
</tr>
<tr>
<td>(5)</td>
<td>0.52</td>
<td>0.30</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Dice similarity coefficients were calculated with the equation: $2a/(2a+b+c)$, in which $a$ is the number of common taxa between two SUs ($X$ and $Y$), $b$ is the number of unique taxa which occur in SU $X$, but not in SU $Y$, and $c$ is the number unique taxa which occur in SU $Y$, but not in SU $X$. This resemblance function is equal to 0 at "no similarity" and approaches 1 when the SUs are identical in bacterial taxa composition.

[^]: Dice similarity coefficients were calculated with the equation: $2a/(2a+b+c)$, in which $a$ is the number of common taxa between two SUs ($X$ and $Y$), $b$ is the number of unique taxa which occur in SU $X$, but not in SU $Y$, and $c$ is the number unique taxa which occur in SU $Y$, but not in SU $X$. This resemblance function is equal to 0 at "no similarity" and approaches 1 when the SUs are identical in bacterial taxa composition.
Table 6. Ability of selected bacterial strains to induce suppression of damping-off of cucumber in sterilized light (H₂) and dark (H₄) peat mix infested with soil inoculum of *Pythium ultimum*.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Damping-off Severity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Light Peat (H₂)</th>
<th>Dark Peat (H₄)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Agrobacterium radiobacter</em></td>
<td>1.4</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td><em>Acaligenes xylosoxydans</em></td>
<td>1.8</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td><em>Hydrogenophaga pseudoflava</em></td>
<td>1.4</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td><em>Phyllobacterium rubiacearum</em></td>
<td>1.8</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em></td>
<td>1.8</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td><em>P. fluorescens</em></td>
<td>1.9</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td><em>P. fluorescens</em></td>
<td>1.9</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td><em>P. saccharophilia</em></td>
<td>1.9</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td><em>P. saccharophilia</em></td>
<td>2.0</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td><em>P. vesicularis</em></td>
<td>1.8</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td><em>P. vesicularis</em></td>
<td>1.9</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td><em>P. vesicularis</em></td>
<td>1.8</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>GC similarity group 1</td>
<td>1.6</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>Non-sterilized Control</td>
<td>1.1</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Non-sterilized Infested</td>
<td>1.0</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Sterilized Control</td>
<td>1.2</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Sterilized Infested</td>
<td>3.7</td>
<td>3.6</td>
<td></td>
</tr>
</tbody>
</table>

LSD<sub>0.05</sub>=0.7

<sup>a</sup> Mean damping-off severity rating of cucumber seedlings grown in sterilized light (H₂) and dark (H₄) peat mix infested with 0.5 g/L soil inoculum of *P. ultimum* (n=40). Cucumber seedlings were rated 10 days after planting according to the following scale: 1=symptomless, 2=emerged but wilted, chlorotic or with visible lesions on hypocotyl, 3=post-emergent damping-off, and 4=pre-emergent damping-off.
Table 7. Ability of selected fungal isolates to induce suppression of damping-off of cucumber in sterilized light (H2) and dark (H4) peat mix infested with soil inoculum of *Pythium ultimum*.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Light Peat (H2)</th>
<th>Dark Peat (H4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gliocladium virens</td>
<td>1.9</td>
<td>2.2</td>
</tr>
<tr>
<td>G. virens</td>
<td>2.3</td>
<td>3.1</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>2.3</td>
<td>3.0</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>2.0</td>
<td>2.9</td>
</tr>
<tr>
<td>Trichoderma viride</td>
<td>1.8</td>
<td>2.4</td>
</tr>
<tr>
<td>Trichoderma hamatum</td>
<td>1.9</td>
<td>2.8</td>
</tr>
<tr>
<td>Non-sterilized Control</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Non-sterilized Infested</td>
<td>1.2</td>
<td>2.6</td>
</tr>
<tr>
<td>Sterilized Control</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Sterilized Infested</td>
<td>3.8</td>
<td>3.7</td>
</tr>
</tbody>
</table>

LSD<sub>0.05</sub>=0.6

*Mean damping-off severity rating of cucumber seedlings grown in sterilized light (H₂) and dark (H₄) peat mix infested with 0.5 g/L soil inoculum of *P. ultimum* (n=40). Cucumber seedlings were rated 10 days after planting according to the following scale: 1=symptomless, 2=emerged but wilted, chlorotic or with visible lesions on hypocotyl, 3=post-emergent damping-off, and 4=pre-emergent damping-off.*
DISCUSSION

Biocontrol of Pythium damping-off in the suppressive mixes, undoubtedly was based on interactions of many different microorganisms. None of the biocontrol agents applied as single treatments induced a level of suppression equal to that of the noninfested control treatments (Tables 6 and 7). A large number of bacterial taxa with activity against Pythium damping-off (17 from the composted hardwood bark-amended and eight from the light (H₂) peat mix) were isolated from the suppressive mixes. Although most of these taxa have been reported previously as biocontrol agents (25, 57, 99), the number isolated from the composted hardwood bark-amended mix far exceeds that previously isolated (six) from a single source (25). The conclusion by Chen et al. (13) and Mandelbaum and Hadar (67), that microbiostasis best explains the mechanism of suppression observed in compost-amended mixes, appears warranted. We propose that this also applied to the light (H₂) peat mix used in this work. In conclusion, the general suppression phenomenon, as postulated in Cook and Baker (17), best describes suppression to Pythium damping-off in these suppressive mixes.

It is of interest that the bacterial species diversity (i.e., species richness and evenness) in the rhizosphere of cucumber seedlings harvested from the three mixes was very similar. Lack of differences in the total but highly variable number of bacteria isolated from a root tip, agrees with previous reports on the microbial carrying capacity of the rhizosphere (66). Differences in organic matter decomposition level among these mixes, therefore, appeared to have little impact on the species diversity of these communities. Rather, the composition of bacterial taxa isolated from root tips of cucumber seedlings was dramatically affected by the mixes. Bacterial taxa isolated from root tips of seedlings grown in the suppressive mixes were similar to those reported in the past as biocontrol agents of soilborne plant pathogens, including *Pythium* spp. (25, 57, 99). Pseudomonads typically comprised 40-50 percent of the total
number of strains isolated from root tips of seedlings in the suppressive mixes. The presence of pleomorphic taxa such as *Arthrobacter* spp. (relative population density ranging from 2.8-35.3%), and the lack of isolation of Pseudomonads from root tips of seedlings grown in the decomposed dark (H4) peat mix, reveals that the composition of bacterial taxa in the conducive habitat resembles that of mineralized soil fractions (40,49,106).

The relatively high population density of *Arthrobacter* spp. on root tips harvested from seedlings grown in the dark (H4) peat mix is similar to the observation of Martin (69), who noted that *Arthrobacter* populations, although low in native dark peat, could account for up to 30% of the total number of strains isolated from such peat after exposure to various chemical amendments.

The bacterial microflora in native dark (H4) peat consists largely of anaerobic bacteria, pleomorphic forms (*Arthrobacter* and *Nocardia*) and *Bacillus* spp. (16,20,23,70,91,113). The predominance of *Bacillus* spp. in the more decomposed dark (H4) peat may be due to the preferential survival of spores under anaerobic low pH conditions that prevail at that depth in bogs (98). In conclusion, the high population density of *Bacillus* spp. isolated from the rhizosphere of cucumber seedlings in the dark (H4) peat mix (4.0-29.4%), as compared to those in the suppressive, higher in microbial carrying capacity light (H2) and composted hardwood bark-amended mixes (0-3.7%), also agrees with past reports on the microbiology of native peatlands.

The predominance of Gram-negative bacteria, such as *Pseudomonas* spp., in the slightly decomposed light (H2) peat (analogous to the surface layer of bogs) and the predominance of *Bacillus* and pleomorphic genera such as *Arthrobacter* in the dark (H4) peat (analogous to the subsurface layers in bogs) is what one would expect to find if minimal changes in microbial composition take place during the harvesting process. Should this be the case, the indigenous microbial community in the peat would be well
positioned to exploit the mild biological vacuum created during mix
preparation.

The Arthrobacter strains tested in this work were not effective as
biocontrol agents. Although Arthrobacter strains with lytic activity
against plant pathogens, including P. aphanidermatum have been reported
(73), their role in biological control in the rhizosphere in this work
appears insignificant.

Attempts were not made to enumerate and characterize oligotrophic
rhizosphere bacteria. Sugimoto et al. (99) showed that obligate
oligotrophs isolated from the rhizosphere of cucumber did not induce
suppression to Pythium damping-off when applied as seed treatments. This
microflora, as well as other taxa that predominate in mineralized soil
fractions (40,49,106) and in the rhizosphere of cucumber seedlings grown
in the compost-amended mixes (57,99), could well play a role in
suppression of the pathogen population. This aspect of disease
suppression was not explored in this work because high rates of Pythium
propagule death have not been encountered in these mixes (13,67,Chapter
I). The oligotrophs could well be most active in the low rate, long-term
eradication of Pythium propagules observed in compost-amended mixes in the
past (12,67).

The significantly (P=0.05) higher efficacy of bacterial (Table 6) and
fungal (Table 7) biocontrol agents in the slightly decomposed light (H2)
compared to the decomposed dark (H4) peat mix suggests that organic matter
decomposition level indeed had an impact on suppressiveness to Pythium
damping-off. The increased efficacy of T. viride perhaps can best be
explained on the basis of the potential for growth of this strongly
cellulolytic fungus in slightly decomposed light (H2) peat, which is
characterized as having a high cellulose and hemicellulose content
(10,23,53,84,103,110). The isolation of actinomycetes and numerous
cellulolytic Chytridiomycetes from the slightly decomposed light (H2) and
the lack thereof from the more decomposed dark (H4) peat supports this
conclusion. Enhanced efficacy of the bacterial biocontrol agents observed in the light (H₂) peat mix may also be attributable to cellulase activity since several of these taxa produce this enzyme (109,121). In the more decomposed and lower cellulose content, dark (H₄) peat (10,23,53,84,103,110), where substrate availability appears to limit microbial activity (Chapter I), growth of these microorganisms would depend more on the assimilation of rhizosphere deposition products (66).

During the formulation of a peat mix, the pH is raised from that of native peat, which ranges from pH 3.1-3.9 to approximately 5.5. This removes the inhibitory effect of peat to colonization by most soil bacteria. During the blending process, considerable fragmentation of peat "fibers" takes place, thus exposing fresh, previously unavailable surface area to the activity of decomposers. Heal et al. (33) offered a similar explanation for the stimulation of microbial activity in the surface layer of bogs as a result of soil fauna activity. In the slightly decomposed light (H₂) peat and compost-amended mixes, the impact of this effect should be much greater than in the decomposed dark (H₄) peat. The short burst in microbial activity observed in Chapter I in the light (H₂) peat mix (Fig. 2C) supports this hypothesis. Activity of the beneficial microflora in the light (H₂) peat mix, therefore, would depend less on rhizosphere deposition products as slow release sources of nutrients for growth and activity.

In conclusion, the isolation of and efficacy of T. viride, G. virens and various Penicillium spp. as biocontrol agents against Pythium damping-off is consistent with the findings of Tahvonen (100,101) and Wolfhechel (119) for Scandinavian sources of light-colored sphagnum peats. The relatively high percent of Pseudomonas strains isolated from root tips harvested from cucumber seedlings grown in the light (H₂) peat mix capable of inducing suppression to Pythium damping-off agrees with the results of Tipping et al. (105). Lastly, the abundance of T. viride and R. rosea, which are strongly cellulolytic, and the increased efficacy of both
bacterial and fungal biocontrol agents in the light (H_2) versus the dark (H_4) peat mix supports our hypothesis that suppression of Pythium damping-off is a function of both the composition of the microflora and the concentration of readily available organic matter (decomposition level) in these mixes.
CHAPTER III
CPMAS $^{13}$C-NMR SPECTROSCOPIC ANALYSIS OF SPHAGNUM PEAT DECOMPOSITION
LEVEL IN RELATIONSHIP TO MICROBIAL CARRYING CAPACITY
AND PYTHIUM ROOT ROT SUPPRESSION

The quality of soil organic matter and its impact on the activity of soil microorganisms, including plant pathogens, has intrigued soil scientists for many years (16, 55, 104, 109, 111). Direct quantification of the potential for soil organic matter to sustain the activity of soil microorganisms in biological control of soilborne plant pathogens has not been possible thus far. Recent breakthroughs in direct spectroscopy, utilizing cross polarized magic angle spinning $^{13}$C-nuclear magnetic resonance (CPMAS $^{13}$C-NMR), have permitted the nondestructive characterization of carbohydrates in soil (2, 76, 79, 117, 118), composts (2, 44, 83), sphagnum peat (24, 32, 81, 82, 116), coal (24) and other sources of organic matter (26, 80). Inbar et al. (44) showed that the concentrations of total carbohydrates declined and that humic substances increased as composts matured and the rate of respiration of the substrate declined (44).

Sphagnum peat appears to be an ideal substrate to evaluate the potential for CPMAS $^{13}$C-NMR to elucidate interactions between organic matter decomposition level, microbiostasis and root rot caused by Pythium ultimum for two reasons. First, the chemical composition of sphagnum peat has been extensively characterized utilizing both wet chemistry (30, 103, 110, 111) and CPMAS $^{13}$C-NMR spectroscopy (24, 32, 81, 82, 116). Secondly, the rate at which microbial activity and suppressiveness decline and the short period in which root rot and Pythium populations increase in
the slightly decomposed light (H₃) peat mix, make it an ideal model system to study these interactions.

In this Chapter the decomposition level of the Canadian sphagnum peat used in the previous Chapters [i.e., the slightly decomposed light (H₂ and H₃) and the decomposed dark (H₄) peat] is characterized utilizing CPMAS ¹³C-NMR spectroscopy. Trends in total bacterial and fungal populations, microbial activity, microbial biomass, Pythium root rot severity and population development of P. ultimum in potting mixes prepared with slightly decomposed light (H₃) and decomposed dark (H₄) peats are reported. Changes in the decomposition level of the light (H₃) and dark (H₄) peat mixes during the production of a poinsettia crop are followed utilizing CPMAS ¹³C-NMR spectroscopy. Finally, relationships between carbohydrate content in the mixes, microbial activity and root rot suppression are discussed.
MATERIALS AND METHODS

Potting mixes, inocula and root rot bioassay. The decomposed dark (H4) and slightly decomposed light (H3) peat mixes described in Chapter I were used in the work reported in this Chapter. Soil inoculum of *P. ultimum* was prepared as described in Chapter I.

A portion (400 cm³) in the bottom of each 1.2 L pot was filled with potting mix infested with soil inoculum of *P. ultimum* (0.38 g soil inoculum/L mix). The remainder (800 cm³) of the pot was then filled with noninfested mix. Thereafter, rooted poinsettia (cv 'Lady') cuttings were planted. Care was taken to prevent direct contact of infested mix in the lower portion of the pot with root tips damaged during transplanting. Control treatments were not infested with *P. ultimum*.

Plants were grown in a greenhouse under natural photoperiods and irrigated daily. Fertilizer (300 mg/L NO₃-N:0 mg/L P:300 mg/L K) was applied every other day. Plants were harvested periodically throughout the growth period. Potting mix associated with the root system was dissociated with the pot label. Root segments suspected of being infected with *P. ultimum* were removed for re-isolation of the pathogen as described in Chapter I. Plants were rated for root rot severity with the following scale in which 1=symptomless, 2=incidental root rot (<ten rotted roots), 3=mild root rot (<1/3 roots rotted), 4=intermediate root rot (between 1/3 and 2/3 roots rotted), 5=severe root rot (>2/3 roots rotted), and 6=dead plant (all roots rotted). This root rot severity scale is illustrated in Plate III. Pathogen identity was verified as described for the cucumber bioassay in Chapter I. This experiment was repeated three times.

Microbial populations. The total population of copiotrophic bacteria was determined on full strength typticase soy broth agar ([TSBA] BBL, Microbiology Systems, Cockeysville, MD), amended with 100 μg/ml pentachloronitrobenzene (PCNB) (35). Total populations of fungi were determined on APDA. The population density of *P. ultimum* was determined.
on SA-PBNC agar using the soil surface dilution plating technique (88) described in Chapter I.

At each harvest, a 10.0-g sample of mix (three replicates) was added to 90 ml dilute water agar (0.4%) and homogenized (1 min) with a Waring blender. The appropriate series of consecutive dilutions (three per dilution series) was plated in triplicate (0.2 ml/plate for APDA and SA-PBNC and 0.1 ml/plate for TSBA) and incubated at 25 C. Colonies on SA-PBNC were counted after 48 h of incubation as described in Chapter I. The number of cfu on APDA and TSBA were determined 4 days after plating. The mean number of cfu on triplicate plates was calculated to represent one replication. Microbial populations were expressed as cfu/g dry weight potting mix.

Microbial activity and biomass. Microbial activity was monitored by measuring the rate of hydrolysis of fluorescein diacetate (FDA) as described in Chapter I.

Microbial biomass, based on the concentration of organic C rendered extractable to 0.5 M K$_2$SO$_4$ by CHCl$_3$ fumigation, was determined with the fumigation-extraction procedure developed by Vance et al. (107). Duplicate samples, each containing approximately 20 ml (55-65% moisture, w/w) potting mix, were placed into 40-ml glass beakers (six replicates per treatment per harvest). One sample was transferred immediately to a 250-ml centrifuge bottle and extracted with 100 ml 0.5 M K$_2$SO$_4$. After 5 h of incubation (25 C) on a reciprocating shaker, potting mix residues were removed from the mixture by filtration through filter paper (Whatman No. 42). The filtrate was collected in a 40-ml screw-cap vial and stored at 4 C until the concentration of organic C was determined (usually 5 days).

The remaining sample was placed in a desiccator (lined with a moist paper towel to maintain humidity) containing approximately 25 ml ethanol-free CHCl$_3$ in a glass beaker with several boiling chips. The desiccator was evacuated until the CHCl$_3$ boiled for 2-3 min and then incubated in the dark at 24 C for 5 days. The beaker of CHCl$_3$ was then removed and the
residual CHCl₃ vapor in the mix was removed by repeated evacuation of the desiccator. The sample was weighed to determine the amount of water lost during fumigation, transferred to a 250-ml centrifuge bottle and extracted with 100 ml 0.5 M K₂SO₄ as described above for the nonfumigated sample.

Microbial biomass, or the concentration of organic C rendered extractable to 0.5 M K₂SO₄ by CHCl₃ fumigation, was determined by subtracting the concentration of organic C in the nonfumigated sample from that of the fumigated sample. The concentration of organic C in both extracts was determined by the dry combustion method (74) using a Coulometrics 5020 CO₂ Coulometer according to procedures specified by the manufacturer (Coulometrics, Inc., Joliet, IL).

Characterization of peat decomposition level. The decomposition level of H₂, H₃ and H₄ peat samples was determined utilizing CPMAS ¹³C-NMR spectroscopy. During a preliminary analysis, it was observed that replicate samples randomly selected from the same source of mix produced different spectra. Further analysis, using samples that were crudely separated into "coarse" and "fine" particles revealed that "particle size" heterogeneity could be explain these differences. Therefore, all subsequent samples collected for CPMAS ¹³C-NMR spectroscopic analysis were fractionated immediately after collection using a wet sieving procedure. The sizes of the sieves were: 4.00, 2.00, 1.00, 0.500, 0.250, 0.105 and 0.053 mm. Sieved potting mix fractions were transferred to petri dishes, lyophilized and stored under vacuum until CPMAS ¹³C-NMR spectroscopic analysis. Two of the fractions, one containing coarse particles (1-2 mm diameter) and the other fines (105-205 μm diameter), were analyzed by CPMAS ¹³C-NMR spectroscopy.

All CPMAS ¹³C-NMR analyses were performed in the Department of Chemistry at The University of Akron (OH) under the direction of Dr. G. E. Wilson. A home-built 200 MHz instrument equipped with a Chemagnetics multinuclear probe and Tecmag (Technology for Magnetic Resonance, Houston, TX) software control was used. All analyses were run after tuning the instrument with
DL-[1-13C]alanine-doped *Bacillus subtilis* whole cell wall sample in a zirconium rotor. The H 90° pulse angle and Hartmann-Hahn match were also established with this standard. The magic angle (54.7° with respect to the magnetic field) and spectrum center were set using hexamethylbenzene as the reference compound.

Optimal contact time was established by running a series of acquisitions where the time was varied between 0.1 and 50 ms. Using a plot of signal intensity versus contact time for several carbons, the apex of the curves yielded an optimal contact time for sphagnum peat of 2 ms. Similarly, the optimal pulse delay of 1 s was determined by first establishing the T1 (relaxation time) for protons through a series of acquisitions where a delay τ was varied. The pulse sequence used was based on one by Zumbulyadis (123). T1 was calculated from the slope of the plot of ln(M0-M)/M0 versus τ, in which M0 is the maximum signal obtained and M is the intensity of a signal at a particular τ (28). A value of three times the T1, or 1 s was used for the pulse delay on peat samples. All peat spectra were acquired in zirconium rotors after 10K scans. A total of 521 data points were collected per scan (total number of data points per sample equal to 5.12 x 10⁶).

Post-processing of data was performed with the software programs NMRI (New Methods Research, Inc., Syracuse, NY) and Tecmag MACNMR (Technology for Magnetic Resonance, Houston, TX).

Experimental designs and statistical analyses. Randomized complete block designs were used in all poinsettia bioassays. One-way analyses of variance (ANOVA) were performed with MINITAB statistical software (Minitab Inc., State College, PA). Separations of means were based on the least significant difference (LSD, P=0.05) (21).
RESULTS

Characterization of peat decomposition level. Both the coarse (1-2 mm diameter) and the fine (105-250 µm diameter) particle fractions of the slightly decomposed light (H₂) and decomposed dark (H₄) peats were distinguished from each other with CPMAS ¹³C-NMR spectroscopy in every test of the mixes (Fig. 6). Residual peaks (signal to noise ratio > 6) in the subtraction spectrum of these samples (H₂ minus H₄) in the 74 and 104 ppm regions revealed that they differed in carbohydrate content (26,27,116).

The subtraction spectra of the fine particles (105-250 µm diameter) recovered by wet sieving from the slightly decomposed suppressive light (H₃) peat mix at 14 and 77 days after potting (day 14 minus day 77 spectra), in each of three experiments revealed a decrease in carbohydrate content, as evidenced by the peak in the 74 ppm region (Fig. 7). Interestingly, the equivalent subtraction spectra of the coarse particles (1-2 mm diameter) recovered from the same potting mix samples did not reveal this difference. Coarse and fine particles recovered on day 14 also did not differ. A slight difference in carbohydrate content was detected in the subtraction spectra of the coarse minus the fine fractions on day 77. Subtraction spectra of the decomposed conducive dark (H₄) peat samples for both particle sizes consistently did not reveal a change in decomposition level, as measured by a decrease in carbohydrate content over time.

Microbial populations. Total populations of bacteria or fungi in the slightly decomposed suppressive light (H₃) and decomposed conducive dark (H₄) peat did not differ significantly (P=0.05) at any time during the growth period. Results presented in Fig. 8A are typical of those obtained in two experiments in which total bacterial and fungal populations were enumerated. The total populations of bacteria in both mixes ranged from 1.12-5.62 x 10⁷ cfu/g dry weight mix. Total populations of fungi ranged from 7.94 x 10⁴ to 6.31 x 10⁵ cfu/g dry weight mix. The slight decrease in fungal populations observed after 56 days was not significant (P=0.05).
Microbial activity. Trends in microbial activity in the slightly decomposed light (H₃) peat mix used in the experiments in this Chapter were consistent with those observed in the slightly decomposed light (H₃) peat mix used in Chapter I (Fig. 1C). Microbial activity at the time of planting was 5.25 μg FDA min⁻¹ g⁻¹ dry weight mix (FDA units). A significant (P=0.05) burst in activity during the initial 14 days, similar to that observed in the light (H₂ and H₃) peat and compost-amended mixes in Chapter I (Figs. 1C and 2C), occurred in this slightly decomposed light (H₃) peat mix as well. A steady and significant (P=0.05) decline was observed between the 14th (6.17 FDA units) and 56th (2.01 FDA units) day of the growth period. Microbial activity remained at this low level until the final harvest date. Microbial activity was consistently low in the decomposed conducive dark (H₄) peat mix as presented in Chapter I.

Microbial biomass. Microbial biomass, based on the concentration of organic C rendered extractable to 0.5 M K₂SO₄ by CHCl₃ fumigation, varied somewhat during the 84 day growth period in the slightly decomposed light (H₃) peat mix (Fig. 9). Differences observed were not significant (P=0.05), however.

Root rot severity and Pythium populations. Trends in Pythium rot severity of poinsettia in the slightly decomposed light (H₃) peat mix were similar to those reported in Chapter I (Fig. 1A). Root rot was evident 28 days after planting. Mild root rot (mean=3.0) was observed after 84 days. The population density of P. ultimum also increased significantly (P=0.05) with time after potting as reported in Chapter I (Fig. 1B). A population of 3.16 x 10^3 cfu/g dry weight potting mix was reached 84 days after planting.
Plate III. Illustration of the scale used to assess Pythium root rot severity of Poinsettia plants in which 1=symptomless, 2=incidental root rot (<ten rotted roots), 3=mild root rot (<1/3 roots rotted), 4=intermediate root rot (between 1/3 and 2/3 roots rotted), 5=severe root rot (>2/3 roots rotted), and 6=dead plant (all roots rotted).
Figure 6. Solid-state cross polarized magic angle spinning $^{13}$C-nuclear magnetic resonance (CPMAS $^{13}$C-NMR) spectra revealing differences (Top) in carbohydrate content within the fine (105-250 μm diameter) particle fraction of a slightly decomposed light (H₂) (Bottom) and a decomposed dark (H₄) (Middle) Canadian sphagnum peat.
Figure 7. Solid-state cross polarized magic angle spinning $^{13}$C-nuclear magnetic resonance (CPMAS $^{13}$C NMR) spectra revealing differences (Bottom) in carbohydrate content within the fine (105-250 μm diameter) particle fraction of a slightly decomposed light (H₃) peat mix harvested 14 (Top) and 77 (Middle) days after planting.
Figure 8. Comparison of total bacterial and fungal populations and microbial activity in a slightly decomposed light (H₃) and a decomposed dark (H₄) peat mix. A, Total bacterial and fungal populations. Population data was transformed to logarithm. Vertical bars represent standard errors (n=9). B, Microbial activity based on the rate of hydrolysis of fluorescein diacetate. Vertical bars represent standard errors (n=4).
Figure 9. Microbial biomass based on the concentration of organic C rendered extractable to 0.5 M K$_2$SO$_4$ by CHCl$_3$ fumigation in a slightly decomposed light (H$_3$) peat mix. Vertical bars represent standard errors (n=6).
DISCUSSION

Differences in carbohydrate content between slightly decomposed light (H2) and decomposed dark (H4) peat samples demonstrated here using CPMAS ¹³C-NMR spectroscopy verifies previous findings by Hammond et al. (32). The slightly decomposed light (H2 and H3) peat samples clearly contained higher carbohydrate concentrations compared to the decomposed dark (H4) peat. It is of interest that only those samples containing elevated carbohydrate concentrations sustained high levels (above 3.2 FDA units) of microbial activity and suppressed both populations of P. ultimum and Pythium root rot. Furthermore, the peat source with an intermediate concentration of carbohydrates (slightly decomposed (H3) peat mixes) lost this activity within 4-8 weeks of crop production.

In the slightly decomposed light (H3) peat mix, where microbial activity and suppressiveness declined with time, the carbohydrate content in the fine (105-250 μm diameter) particles also declined (Fig. 7). Lack of a detectable decline in the coarse particles suggests that these particles at days 14 and 77 were similar in composition. Carbohydrate content in the fines would be more accessible to degradation by soil microorganisms. Although adequate carbohydrate reserves were present in the coarse particles, even after 77 days, the total surface area of these particles relative to that of the fines may have been too low to support a level of microbial activity adequate for effective biological control of Pythium root rot of poinsettia. Alternatively, lack of availability of carbohydrates present in the interior components of the coarse particles with decomposed outer surfaces may also have accounted for the decline in microbial activity. A direct spectroscopic procedure, such as near infrared reflectance spectroscopy can assess surface chemistry, may provide additional information (71).

Trends in microbial biomass and in total bacterial and fungal populations with time in the slightly decomposed light (H3) peat mix were similar. These results are in agreement with previous data in light (H2)
and dark (H₄) peat mixes, in which the extractable lipid phosphate microbial biomass procedure developed by White et al. (114) also revealed that biomass did not differ or change with time (8). This clearly reveals that suppressiveness to Pythium root rot was not a function of the size of the microbial biomass in these mixes, but rather a function of its activity.

Lack of a change in the total microbial biomass as evidenced by three different procedures, and the decline in microbial activity, suggests that the microflora in the slightly decomposed light (H₃) peat mix either declined in nutritional status, underwent a change in species composition, or both. Changes in the species composition and the nutritional status of the microflora with time in the slightly decomposed light (H₃) peat mix will have to be explored to answer that question.

In conclusion, the composition and activity of the biomass, as revealed in Chapters I, II and also in this Chapter, appeared to support suppressiveness, as long as adequate carbohydrate reserves were available in the potting mixes to sustain a level of microbial activity equal to or greater than 3.2 FDA units. Finally, the data presented in this Chapter represents the first direct evidence linking a decrease in available energy in the substrate with a decrease in microbial activity and an increase in the potential for Pythium root rot development.
SUMMARY AND CONCLUSIONS

Interactions between sphagnum peat decomposition level, changes in microbial activity in the potting mix with time, and suppression of the population of *P. ultimum* and of Pythium root rot during the production of poinsettia were examined.

Microbial populations in the least-decomposed suppressive light (H2) peat and compost-amended mixes were compared to those in the conducive dark (H4) peat mix. Bacteria isolated from root tips of cucumber seedlings harvested from each of the mixes were enumerated, identified and tested for their ability to induce suppression to Pythium damping-off of cucumber. The species diversity of these bacterial communities was assessed with various diversity indices. Predominant fungal taxa isolated from the slightly decomposed light (H3) and the decomposed dark (H4) peats were identified. Their efficacy as biocontrol agents to Pythium damping-off was determined in mixes prepared with these peat sources. The impact of peat decomposition level on suppressiveness was assessed by comparing mean damping-off severities of the most efficacious biocontrol agents in mixes prepared with peats varying in decomposition level. The decomposition level of Canadian sphagnum peat mixes differing in suppressiveness was characterized using CPMAS 13C-NMR spectroscopy. Finally, the relationship between carbohydrate content and sustained microbial activity was developed.

The following conclusions were drawn:

1. Potting mixes prepared with Canadian sphagnum peats varied in suppressiveness to root rot and damping-off caused by *P. ultimum*.
2. Suppressiveness was a function of peat decomposition level. The population density of *P. ultimum* and root rot severity were highest in the decomposed dark (H4), intermediate in the slightly decomposed light (H3) and lowest in the least-decomposed light (H2) peat and compost-amended mixes.

3. Microbial activity was a function of peat decomposition level. Microbial activity was highest in the least-decomposed suppressive light (H2) peat and compost-amended mixes and lowest in the decomposed conducive dark (H4) peat mix. In the H3 decomposition level peat mix, microbial activity was sustained for the early portion of the growth period and then collapsed to a level similar to that observed in the conducive dark (H4) peat mix.

4. Suppressiveness was a function of sustained microbial activity. As long as microbial activity was sustained above a threshold level of 3.2 μg fluorescein diacetate hydrolyzed min⁻¹ g⁻¹ g dry weight mix, pathogen populations and root rot were suppressed.

5. Total populations of bacteria and fungi isolated from the slightly decomposed suppressive light (H2) and decomposed conducive dark (H4) peat mix were similar.

6. Peat decomposition level did not have an impact on the bacterial species diversity (i.e., species richness and evenness) of root tips harvested from seedlings in any of the mixes regardless of organic matter decomposition level.

7. The composition of bacterial species isolated from root tips of seedlings in the suppressive and conducive mixes was influenced by the decomposition level of organic matter (i.e. carbohydrate content) in the mix. *Pseudomonas* spp. predominated (relative population density ranging from 40-50%) on root tips harvested from the least-decomposed suppressive mixes. High relative population densities of *Arthrobacter* (2.8-35.5%) and *Bacillus* spp. (4-29.9%) and the lack of *Pseudomonads*, characterized the bacterial species composition of root tips harvested
from the decomposed conducive dark (H₄) peat mix. These differences were clearly revealed utilizing Bray-Curtis polar ordination and the Dice and Czekanowski similarity coefficients.

8. Microorganisms capable of inducing suppression to Pythium damping-off of cucumber were isolated from all mixes. Trichoderma, Gliocladium, and Penicillium spp. were the most effective fungal biocontrol agents. Effective bacterial strains included Agrobacterium radiobacter, Alcaligenes xylosoxydans, Comamonas acidovorans, C. testosteronii, Corynebacterium flaccumfaciens, Cytophaga aquatilis, Enterobacter spp., Hydrogenophaga pseudoflava, Micrococcus roseus, Phyllobacterium rubiacearum, Pseudomonas alcaligenes, P. delafieldii, P. facilis, P. fluorescens, P. saccharophila, P. solanacearum, P. vesicularis, Xanthomonas maltophilia and GC similarity group 1.

9. Eight times as many efficacious strains were isolated from root tips harvested from cucumber seedlings grown in the slightly decomposed light (H₂) as compared to the decomposed conducive dark (H₄) peat mix.

10. Efficacy of bacterial and fungal biocontrol agents was greater in the slightly decomposed light (H₂) as compared to the decomposed dark (H₄) peat mix.

11. A consistent relationship was developed between carbohydrate content in peat, as determined by CPMAS ¹³C-NMR spectroscopic analysis, microbial activity and suppressiveness. Carbohydrate concentrations declined with time in a slightly decomposed light (H₂) peat mix as microbial activity and suppressiveness were reduced below effective levels. Microbial biomass or total populations of fungi and bacteria, did not decline, however, suggesting that the composition of the microflora was affected by peat decomposition level. These factors need to be explored further.


104. Timonin, M. I. 1935. The micro-organisms in profiles of certain

105. Tipping, E. M., Campbell, S. E., Onofriechuk, E. E., Young, S.,
and Munagala, R. 1990. Selection of rhizobacteria for biocontrol of
Pythium ultimum on cucumber for greenhouse application.
Phytopathology 80:1050.


extraction method for measuring soil microbial biomass C. Soil Biol.
Biochem. 19:703-707.

influence of Douglas fir sawdust and certain fertilizer elements on
the incidence of red stele disease of strawberry. Phytopathology
44:601-603.


chemical composition of peat: II. Chemical composition of various

chemical composition of peat: IV. Chemical studies of highmoor peat

chemical composition of peat: V. The role microorganisms in peat

113. Wheatley, R. E., Greaves, M. P., and Inksom, R. H. E. 1976. The
aerobic bacterial flora of a raised bog. Soil Biol. Biochem. 8:453-
460.

114. White, D. C., Davis, W. M., Nickels, J. S., King, J. D., and Bobbies,
R. J. 1979. Determination of the sedimentary microbial biomass by

115. Williams, R. T., and Crawford, R. L. 1983. Microbial diversity of

Applications in Geochemistry and Soil Chemistry. Pergamon Press.

117. Wilson, M. A., Heng, S., Goh, K. M., Pugmire, R. J., and Grant, D.
M. 1983. Studies of litter and acid insoluble soil organic matter
fractions using 13C-cross polarization nuclear magnetic resonance

magnetic resonance spectroscopy of soils and related materials.
Relaxation of 13C nuclei in cross polarization nuclear magnetic
resonance experiments. Org. Geochem. 5:121-130.

119. Wolffhechel, H. 1988. The suppressiveness of Sphagnum peat to

