Factors Affecting Suppression of Pythium Damping-Off in Container Media Amended with Composts

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ABSTRACT

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A cucumber seedling bioassay was developed to determine suppressiveness of container media to damping-off caused by *Pythium ultimum*. Container media amended with samples of composted hardwood bark or composted municipal sludge, removed from the low-temperature edge of 4-mo or older compost piles, were suppressive to Pythium damping-off. Those amended with samples from the high temperature (>60 C) center of the same piles were conducive. The suppression was biological in nature because heating (60 C, 5 days) destroyed the suppression, incorporation of small volumes (10% v/v) of suppressive compost into conducive container media restored suppression, and addition of antagonists to conducive container media induced suppression. Conducive and suppressive container media responded differentially to increasing inoculum densities of *Pythium*. Estimated ED_{50} values based on logarithm-probability transformation were 268, 30, 880, and 855 mg of inoculum of *Pythium* per liter of conducive composted municipal sludge, heat-treated composted hardwood bark and suppressive composted municipal sludge, and composted hardwood bark container media, respectively. In the absence of cucumber plants, the population of *P. ultimum* declined at a similar rate in conducive and suppressive container media over a period of 40 days. However, in the presence of cucumber plants, buildup of populations of *P. ultimum* was prevented in suppressive but not in conducive container media.

Pythium ultimum Trow is a ubiquitous soilborne plant pathogen. It is the primary damping-off *Pythium* sp. in Ohio bedding plant greenhouses (25). Fungicide drenches are usually recommended for its control, particularly for conducive peat container media (26).

Composts have been used with various levels of success for suppression of Pythium damping-off. Lumsden et al (12) reported that application of composted municipal sludge to soil did not reduce Pythium damping-off immediately after its application. However, the treated soil became suppressive after 1 yr. Another compost, prepared from a mixture of wastes, also has been used to suppress Pythium damping-off on cucumber (4). Container media amended with composted licorice roots or composted grape marc suppressed cucumber damping-off caused by *P. aphanidermatum* (Edson) Fitzp. (13). Container media amended with composted hardwood bark suppressed Pythium and Rhizoctonia damping-off, and addition of fungicides did not have adverse effects on the suppressive characteristics of the container media (26).

Container media amended with composts prepared from tree bark suppress a variety of soilborne plant diseases including those caused by *Pythium* spp. (7,9,10,19). Container media that contain bark have largely replaced peat container media for production of nursery stock and some floral crops in the United States, Australia, and European countries (9,10,23). Recycling of organic wastes into composts is chosen increasingly as a least objectionable procedure in dealing with waste problems (10). An increasing number of compost types, therefore, is becoming available to the ornamentals industry. The impact of composts on the soil microflora, plant pathogens, and plant diseases is not well understood (7,9,10). This study was undertaken to examine the effect of composted hardwood bark and composted municipal sludge-amended container media on Pythium damping-off, the relationship of compost temperature to disease suppression, the relationship of disease severity to increasing inoculum density, and the effects of composts on population development of *P. ultimum* in conducive and suppressive container media. A preliminary report was published earlier (5).

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MATERIALS AND METHODS

Preparation of container media. Container media used were mixtures of Canadian sphagnum peat and perlite (1:1, v/v); composted hardwood bark, Canadian sphagnum peat, and perlite (5:2:3, v/v); and composted municipal sludge, Canadian sphagnum peat, and perlite (1:2:1, v/v). These three container media hereafter are referred to as the peat, bark compost, and sludge compost media, respectively. Details of the container media formulations, their physical properties, and chemical amendments have been described elsewhere (9,19). The sludge compost medium was not fertilized because it releases adequate concentrations of major and minor nutrients for growth of ornamentals in containers (23). Unless specified otherwise, composts were taken from the high temperature (>60 C) center or low temperature (<40 C) edge of compost piles. Both types of composts were prepared in 2.2-m-tall piles, which were turned and mixed every 2 wk for 4 mo. Therefore, samples taken from the center and edge of compost piles were considered to be similar in physical and chemical properties. Temperatures in the edge of piles ranged from 20 to 40 C and those in the center ranged from 55 to 70 C for at least 5 days before sampling. Under some conditions, temperatures in the center of bark compost piles did not reach 60 C. Therefore, edge bark compost medium was heat-treated in an oven at 60 C for 5 days to simulate compost pile conditions.

Preparation of inocula. Isolate 211 of *P. ultimum*, originally isolated from a diseased poinsettia plant at the Plant Disease Clinic, Department of Plant Pathology, The Ohio State University, Columbus, was used throughout this study. Inoculum

was prepared in Ko and Hora's chopped potato soil medium, which contained 50 g of chopped potato and 500 ml of soil in a 1-L flask (19). It was sterilized for 1 hr on 2 consecutive days and seeded with two 5-mm-diameter agar disks from a 24-hr culture of *P. ultimum* on lima bean agar (LBA; 10 g of frozen lima bean, 20 g of Difco agar per liter of distilled water). The chopped potato soil medium was incubated 14 days at 25 C, removed from the flask, air-dried overnight, ground with a mortar and pestle, sieved through a 2-mm-mesh sieve, and collected on a 1-mm-mesh sieve. The 1- to 2-mm-diameter particles were used as inoculum of *Pythium*.

Antagonists used in this study were *Trichoderma hamatum* (Bon) Bain #382 (ATCC 20765), *Flavobacterium balustinum* #299 (ATCC 53198), and *Xanthomonas maltophilia* #76 (ATCC 53199) (G. A. Kuter, P. C. Fahy, and H. A. J. Hoitink, *unpublished*). They were isolated from composted hardwood bark and suppressed Rhizoctonia damping-off in bark compost media. Bacterial isolates were grown 48 hr (25 C) in Difco nutrient broth in a shake culture (50 ml per 250-ml flask) and harvested by centrifugation (3,000 *g*, 5 min). The pellet was resuspended in a phosphate buffer (7 g of K_2HPO_4, 3 g of KH_2PO_4, and 0.2 g of $MgSO_4$ per liter of distilled water, pH 6.8). Final cell concentrations were determined with a bacterial counting chamber (Hausser Scientific, Blue Bell, PA). Isolate #382 of *T. hamatum* was grown on potato-dextrose agar in petri dishes under continuous illumination for 7 days. Conidia were harvested by flooding agar cultures with sterile distilled water and a drop of Tween 20. Conidial suspensions were filtered through two layers of cheesecloth and spore concentrations were determined with a hemacytometer. Bacterial and fungal spore preparations were added to center compost container media (either sludge or bark compost) to give inoculum densities of 10^7 and 10^5 colony-forming units (cfu) per gram dry weight, respectively. Final population densities were verified by dilution plating on a semi-selective medium for *Trichoderma* (15) and on Difco nutrient agar. Sometimes 10% (v/v) edge composts were incorporated into center compost media. Bioassays were performed to determine efficacy of these antagonist inocula in restoring suppression.

Bioassay. Cucumber seeds (*Cucumis sativus* L. cultivar Straight Eight, 90% germination, eight per pot) were planted 1 cm deep in disposable styrofoam pots (10 cm deep, 10 cm top diameter) with a perforated base containing approximately 400 ml of container medium. Unless specified otherwise, 1.5 g of inoculum of *Pythium* was added per 2 L of container medium in polyethylene bags. Bags were shaken vigorously to ensure uniform distribution of inoculum and contents were then distributed into five pots. Plants were grown at a constant temperature of 20 C and under continuous illumination (225 μEm^{-2} sec^{-1}) and were watered daily. A disease severity rating was made 10 days after planting according to the following scale: 1 = symptomless; 2 = emerged, but diseased (either wilted, yellowed, or with visible lesions on hypocotyl); 3 = post-, and 4 = preemergence damping-off. A mean of eight seedlings in a pot was computed to represent one replication. Diseased seedlings and ungerminated seeds were surface sterilized in 1% sodium hypochlorite (30 sec), rinsed in sterile distilled water three times, and placed on a semi-selective medium SA-PBNC (22) to reisolate the pathogen. The SA-PBNC medium contained the following ingredients per liter of distilled water: 2.5 g of sucrose, 0.27 g of asparagine, 0.15 g of KH_2PO_4, 0.15 g of K_2HPO_4, 0.1 g of $MgSO_4 \cdot 7H_2O$, 80 mg of $CaCl_2 \cdot 2H_2O$, 2 mg of thiamine HCl, 10 mg of ascorbic acid, 27 mg of Terraclor (75% pentachloronitrobenzene), 20 mg of Benlate (50% benomyl), 100 mg of neomycin sulfate, 10 mg of chloromycetin, 1 ml of $ZnSO_4 \cdot 7H_2O$ (44 mg per 10 ml of water), 1 ml of $FeSO_4 \cdot 7H_2O$ (10 mg per 10 ml of water), 1 ml of $MnCl_2 \cdot 4H_2O$ (7 mg per 100 ml of water), 2 ml of cholesterol (5 mg/ml of N,N-dimethyl formamide), and 20 g of Difco agar. The ingredients were added and dissolved one at a time. Isolations also were made from seeds that had not germinated in noninfested container media.

Compost temperatures and disease suppression. A typical temperature gradient existed in compost piles with increasing temperatures from the edge to the center of a pile ranging from ambient to 70 C (10). Compost samples were removed from zones in compost piles with temperatures $<$ 40 C, 40–50 C, 50–60 C, and $>$ 60 C (mean temperatures of 35, 45, 55, and 65 C). Care was taken to prevent contamination as much as possible. Container media were then prepared with the compost samples and assayed for disease suppression within 4 hr after removing samples from compost piles.

Inoculum density and disease severity. Container media were infested with several concentrations of inoculum of *Pythium* (0, 125, 250, 500, 750, and 1,000 mg/L of heat-treated bark compost medium and 0, 250, 500, 750, 1,000, and 1,250 mg/L of the center and edge sludge compost media and edge bark compost medium) to determine the relationship between inoculum density and disease severity. Bioassays were performed as described above. Disease severity values were converted to a disease index per unit basis by using $y = (x - 1)/3$, where x is the disease severity rating and three equals the total number of increments between one and four in the disease severity rating scale. It was assumed that the individual susceptibility of cucumber plants to infection by *P. ultimum* in our container media was distributed normally. The disease index in terms of probits was regressed on log of inoculum density (3) for estimation of ED_{50} values (inoculum densities that cause a mean disease severity of 2.5).

Population dynamics of *Pythium ultimum*. The peat medium, the heat-treated, and edge bark compost media were infested with *P. ultimum* (about 600 cfu per gram dry weight) and then distributed into pots. Cucumber seeds were disinfected with 1% sodium hypochlorite (30 sec) and rinsed three times (30 sec each) with sterile distilled water before planting to reduce chances of contamination of the composts with seedborne microorganisms. Both seeded and unseeded pots were placed in sealed polyethylene bags after watering. A millipore filter (0.45-μm pore size) was taped over a hole in each bag to improve air exchange and prevent contamination with airborne microorganisms. Infested container media were planted and replanted to cucumber or not planted at all. Noninfested container media served as controls for all treatments. Population levels of *P. ultimum* were determined at 10-day intervals over a 40-day period with a soil surface dilution plating technique (18) on the SA-PBNC medium. In an initial trial, a soil plug method (21) and the dilution plating technique were compared for efficiency of isolation. The dilution plating gave consistently higher counts than the soil plug method. A 5-g sample was taken from thoroughly mixed container medium from each pot, added to 50 ml of dilute water agar (0.3%), and homogenized 1 min in a Waring blender. Additional 10-fold dilutions were then made in dilute water agar. One-milliliter subsamples of various dilutions were plated in triplicate on the SA-PBNC medium and incubated 40 hr at 25 C. Dilution plates were next rinsed with tap water to remove debris. Colonies of *P. ultimum* were counted and recorded as colony-forming units per gram dry weight of container medium (dry weight based on a second 5-g sample). Regression analyses on semilogarithmic transformations were performed with populations of *Pythium* in the nonplanted container media. Comparisons of slope values and positions of regression lines were based on the standard Student's t test. To estimate population development in container media planted with cucumber, an equation presented by Yarwood and Sylvester (27) was used to calculate population doubling time.

To verify that colonies counted were *P. ultimum*, hyphal tip isolations were made ramdomly from 10% of all colonies and transferred to LBA. Cultures were incubated in the dark at 25 C for 6–7 days and then examined with a microscope. Middleton's (17) description of *P. ultimum* was used for identification.

To observe sporulation of *P. ultimum* in diseased cucumber seedlings, diseased seedlings collected at harvest were washed carefully with tap water to remove container medium particles and stained by boiling for 10 min in a chlorohydrate-acid fuchsin stain, washed in lactophenol 3–5 min (6), crushed between two microscopic slides, and examined microscopically.

Experimental design and statistical analyses. Completely randomonized designs were used in all experiments. Each treatment was replicated five times (five pots per treatment) and each experiment was performed at least twice. One-way analysis of

variance was performed by using a MINITAB computer program. Data transformation and regression analyses were employed when appropriate. Separations of means were based on least significant difference (LSD, $P = 0.05$).

RESULTS

Edge and center composts and disease suppression. The peat medium was highly conducive to Pythium damping-off with a disease severity value of 3.7 (Fig. 1). The container media amended with center sludge or bark composts (prepared with composts exposed to 60 C for at least 5 days before sampling) were also conducive (disease severity values of 2.8 and 3.0, respectively). These values were not significantly different ($LSD_{0.05} = 0.85$) from that in the peat medium. Disease severity values in the container media amended with edge sludge and bark composts were only 1.8 and 1.4, respectively, and were not significantly different from each other. They were significantly ($P = 0.05$) lower than those in the peat and center compost container media (Fig. 1). Pythiaceous fungi were not isolated from seeds that had not yet germinated in noninfested controls. *P. ultimum* was isolated consistently from diseased seedlings and from ungerminated seeds in infested container media. Sporangia and oospores were observed in stained preparations of diseased seedlings from both conducive and suppressive container media. The number of sporangia and oospores per lesion ranged from 5 to 200. Differences did not appear to be related to container media from which the seedlings had been removed.

A clear relationship existed between disease severity and temperature of the zones in the compost piles from which samples were taken for preparation of container media. Disease severity increased with an increase in temperature (Fig. 2). Disease severity values in the container medium amended with bark compost taken from the edge (35 C) and center (65 C) of the compost pile were 1.2 and 3.3, respectively. Container media amended with bark compost taken from other temperature zones (45 and 55 C) were moderately suppressive. Similar results were obtained with samples of bark compost removed from a second pile.

Inoculum density and disease severity. The conducive and suppressive bark and sludge compost media responded differentially to increasing inoculum densities of *P. ultimum* (Fig. 3A). The heat-treated bark compost medium, again, was highly conducive. The disease index reached 0.97 (3.9 in the disease severity rating scale) with an inoculum density of 125 mg/L (Fig.

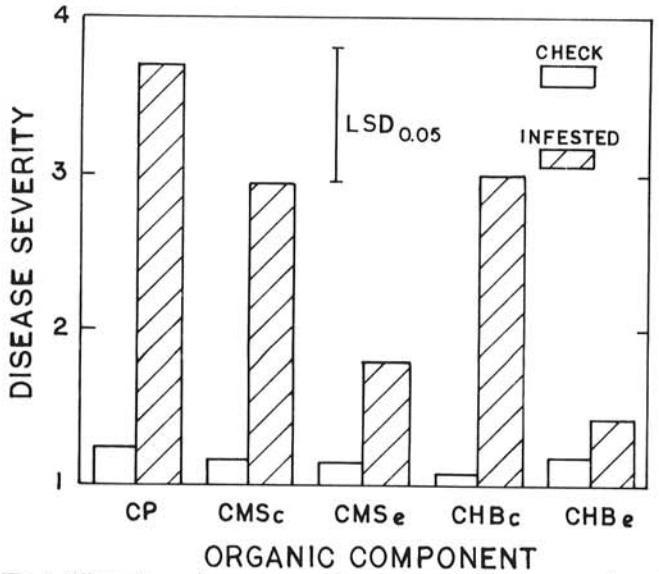

Fig. 1. Effect of organic components in container media on suppression of Pythium damping-off. CP = Canadian sphagnum peat, CMS = composted municipal sludge, and CHB = composted hardwood bark; c and e indicate samples removed from the center and edge of compost piles, respectively. Disease severity rating scale: 1 = symptomless, 2 = emerged but diseased seedlings, 3 = post-, and 4 = preemergence damping-off.

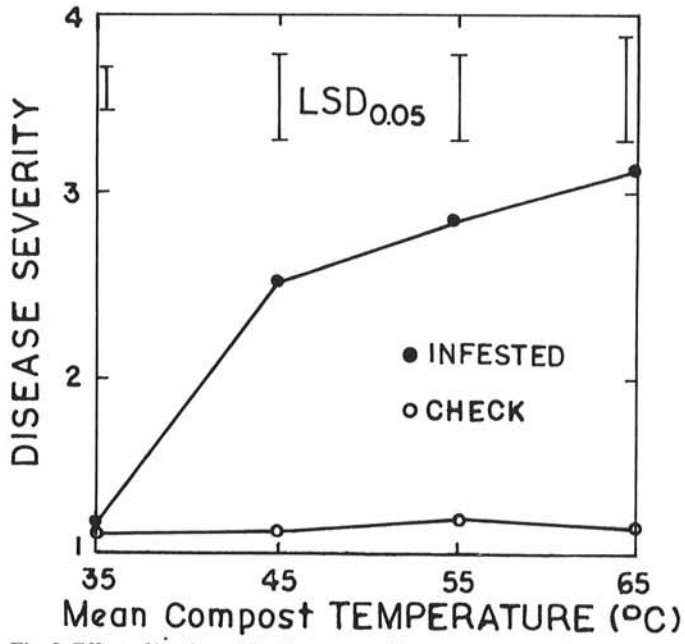

Fig. 2. Effect of hardwood bark compost pile temperature on induction of suppression to Pythium damping-off. ● = infested and o = noninfested. Disease severity rating scale: 1 = symptomless, 2 = emerged but diseased seedlings, 3 = post-, and 4 = preemergence damping-off.

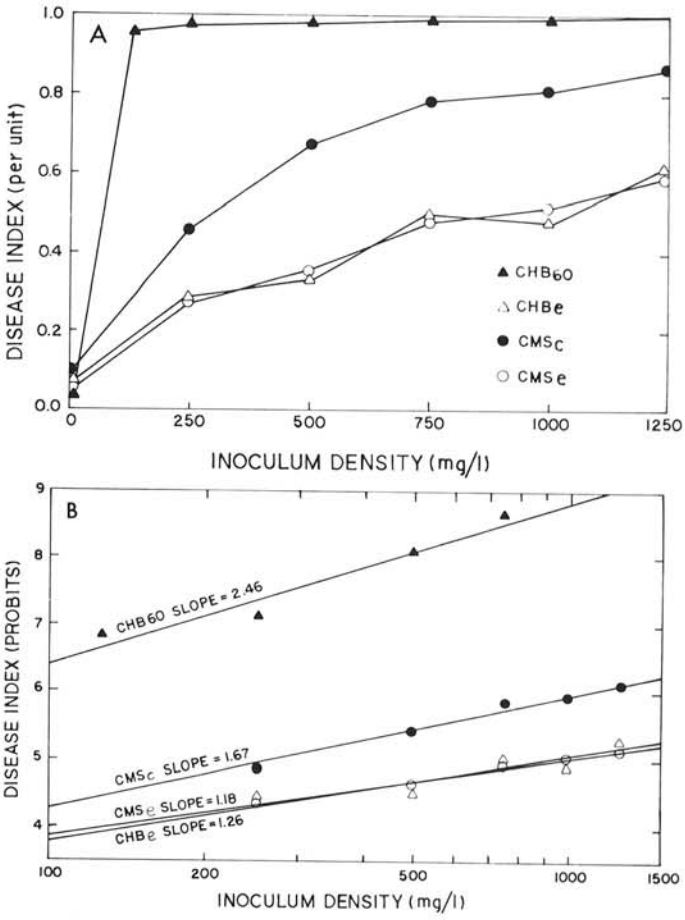

Fig. 3. Relation of inoculum density to disease severity in conducive and suppressive container media. **A,** Arithmetic plot, disease index per unit was calculated from a disease severity rating scale: 1 = symptomless, 2 = emerged but diseased seedlings, 3 = post-, and 4 = preemergence damping-off. **B,** Logarithm-probability transformation. Δ = composted hardwood bark from low temperature edge (CHBe); o = composted municipal sludge from low temperature edge; and ● = composted municipal sludge from high temperature center of compost piles and incorporated into container media, and ▲ = heat-treated (60 C, 5 days) CHBe container medium.

3A). The container medium prepared with sludge compost removed from the center of the pile was less conducive. Both container media amended with edge samples of sludge and bark composts were suppressive, but disease indices increased with increasing inoculum densities. Regression analyses after logarithm-probability transformation are presented in Figure 3B. The estimated ED_{50} values were 268, 880, 30, and 855 mg of inoculum of *Pythium* per liter for the center and edge sludge compost media and heat-treated and edge bark compost media, respectively.

Specific antagonists and disease suppression. Addition of effective antagonists to a conducive bark compost medium rendered it suppressive to the disease (Table 1). Several types of antagonists were effective. For example, the disease severity value in a center bark compost medium fortified with isolate #299 of *F. balustinum* was 1.5, which is not significantly different ($P = 0.05$) from that of the suppressive bark compost medium. Addition of isolate #382 of *T. hamatum* to a center bark compost medium reduced the disease severity value from 3.0 to 1.8 (Table 1). Incorporation of 10% (v/v) edge compost into center compost container medium also significantly reduced disease severity. Similar data were also obtained with sludge compost media (Table 1).

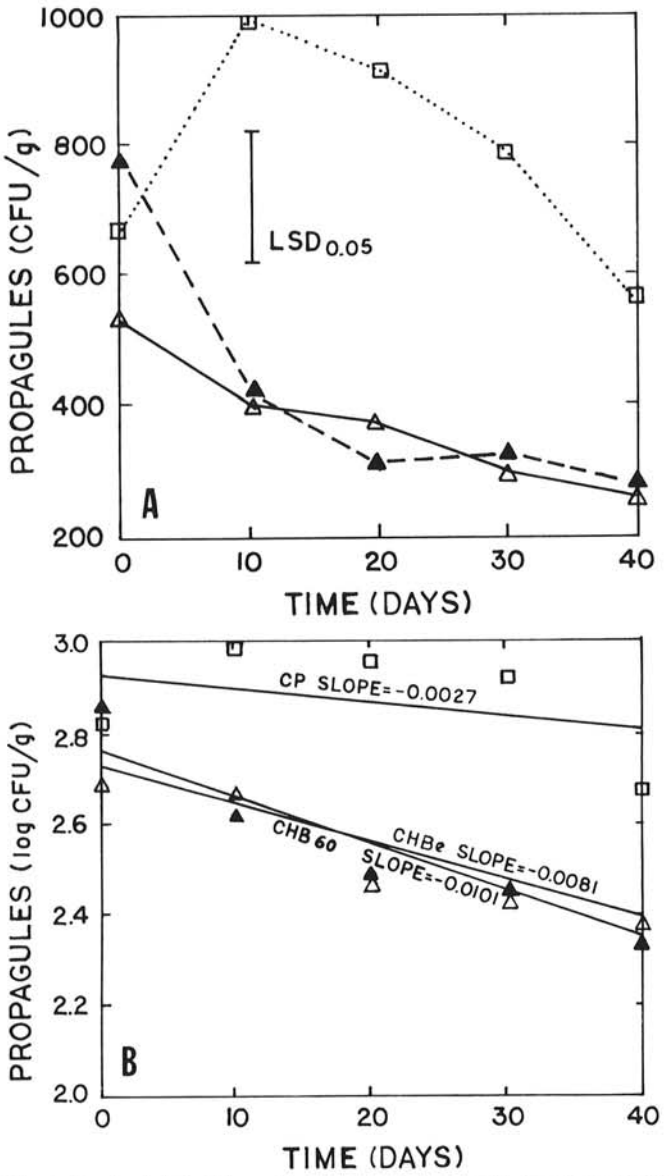

Fig. 4. Survival of *Pythium ultimum* in container media not planted with cucumber. **A,** Arithmetic plot. **B,** Semilogarithmic transformation. □ = Canadian sphagnum peat, Δ = composted hardwood bark from low temperature edge of a compost pile (CHBe). ▲ = heat-treated (60 C, 5 days) CHBe container medium. Slope values in B for CHBe and CHB60 container media were significantly ($P = 0.05$) negative.

Population dynamics of *P. ultimum*. Ninety-three percent of the colonies of *Pythium* (158 out of 170 colonies) isolated from dilution plates were identified as *P. ultimum*. The remaining 7% produced *P. ultimum*-like sporangia, but no sexual organs were observed. They were considered to be *P. ultimum*, however. Pythiaceous fungi were not detected in noninfested container media. Population density of *P. ultimum* in the peat medium (in nonplanted pots) increased from 640 to 990 cfu per gram during the first 10 days. Thereafter, the population density declined to about 580 cfu per gram. An increase in inoculum density was not observed in either bark compost medium (Fig. 4A). Regression of log colony-forming units per gram on time for the heat-treated and edge bark compost media yielded slopes of −0.0101 and −0.0081, respectively. They were significantly different ($P = 0.05$) from zero, but not from each other. The slope for the peat medium (−0.0027) was not different ($P = 0.05$) from zero. The intercept value for the peat medium (2.926) was significantly ($P = 0.05$) higher than that for the bark compost media (Fig. 4B).

In container media planted with cucumber, population development of *P. ultimum* was quite different. With time, population densities of *P. ultimum* increased in the heat-treated bark compost medium from 780 cfu per gram to 4.8×10^3, 2.2×10^4, 2.8×10^4, and 2.9×10^4 cfu per gram dry weight of container medium after the first, second, third, and fourth plantings, respectively. A similar trend of population development of *P. ultimum* was also observed in the peat medium, but it developed to much higher levels (Fig. 5). Population development of *P. ultimum* was suppressed significantly in the edge bark compost medium, even in pots planted and replanted to cucumber. Population densities here increased from an initial level of 550 cfu per gram to a final level of 2.4×10^3 cfu per gram in 40 days. The estimated doubling time for the population of *P. ultimum* in the peat medium and in the conducive and suppressive bark compost media were 6, 7, and 12.7 days, respectively. After each planting, disease severity values in the edge bark compost medium were significantly lower than those in the peat and heat-treated bark compost media (data not shown).

DISCUSSION

Container media amended with high temperature (>60 C) composts, removed from compost piles that had cured for 4 mo, were conducive to Pythium damping-off of cucumber. On the other hand, container media amended with low temperature edge composts were suppressive. This applied to composted hardwood bark as well as to composted municipal sludge. Our data (center vs.

TABLE 1. Effect of antagonists on suppression of Pythium damping-off of cucumber in container media amended with composted hardwood bark (CHB) or composted municipal sludge (CMS)

Compost sample[a]	Antagonist inoculum[b]	*Pythium* inoculum[c]	Disease severity[d] CHB	CMS
Edge	···	−	1.2	1.1
Edge	···	+	1.5	1.8
Center	···	−	1.1	1.2
Center	···	+	3.0	2.9
Center	*Trichoderma hamatum* 382	+	1.8	2.4
Center	*Flavobacterium balustinum* 299	+	1.5	1.7
Center	*Xanthomonas maltophilia* 76	+	1.9	2.0
Center	10% (v/v) edge compost	+	1.7	2.1
LSD ($P = 0.05$)			0.6	0.7

[a] Compost samples taken from the edge (35 C) or center (60 C) of compost piles, respectively, and incorporated into container media.
[b] Initial inoculum levels were 10^5 and 10^7 CFU per gram dry weight container medium for fungi and bacteria, respectively.
[c] Container media infested (+) with 0.75 g of inoculum of *Pythium* per liter.
[d] Mean disease severity rating determined 10 days after planting, 1 = symptomless, 2 = emerged but diseased seedlings; 3 = post-, and 4 = preemergence damping-off. Based on 40 seedlings in five replicates.

edge effects) agree with those of such composts on Rhizoctonia damping-off (19). The peat medium was consistently conducive, thus verifying previous reports on conduciveness of sphagnum peat media to *Pythium* diseases (20). The suppressiveness in container media amended with either type of compost (bark or sludge) to Pythium damping-off was considered to be biotic in nature because: heating of suppressive container media at 60 C for 5 days eliminated suppression (Fig. 3A, CHB60), addition of antagonists to conducive container media reestablished suppression (Table 1), and addition of small amounts of suppressive composts to conducive container media restored suppression (Table 1).

Toward the end of the composting process, physical and chemical differences between edge and center compost samples are minimal due to frequent turning and mixing of windrows. However, differences in composition of the microbial community of edge and center samples are substantial (16). Edge composts (low temperatures) are colonized by mesophilic microorganisms, whereas center composts (high temperatures) are colonized by thermophilic microorganisms. McKinley and Vestal (16) determined activity of compost microbiota in various temperature zones of compost piles. They expressed microbial activity as the rate of acetate incorporation into microbial lipids and found highest activities in samples from low temperature zones (25–45 C). Samples from high temperature zones (55–74 C) had a relatively low activity. When they incubated compost samples from high temperature zones at low temperature, the thermophilic microflora was not active (16). Preparation of our container media with high temperature (60 C) composts, followed by incubation at low temperature (20 C), therefore, created a biological vacuum. The biological vacuum can be exploited by opportunistic microorganisms, e.g., *P. ultimum*. An increasing biological vacuum may explain the increasing conduciveness of container media prepared with compost samples removed from increasing depths (higher temperatures) of compost piles (Fig. 2).

Inoculum density and disease severity curves represent a type of dosage-mortality curve (2,3). Logarithm-probability transformation of data in this work generated straight lines and facilitated interpretation. The ED_{50} value and the slope of the curve obtained from the linear dosage-response curve provide parameters for comparison of suppressiveness of the container media. Ideally, a suppressive container medium should have a high ED_{50} value and a flat curve as in the suppressive sludge and bark compost media (Fig. 3B). Analyses of the data in Figure 3A by Baker's model (2) suggest that in the suppressive bark and sludge compost media only inoculum on the rhizoplane infected cucumber because the slope values on a log-log basis did not differ ($P = 0.05$) from 0.67. In the conducive container medium of municipal sludge, inoculum in the rhizosphere infected cucumber because the slope did not differ ($P = 0.05$) from 1 (data on the heat-treated bark compost medium were not analyzed due to over-dosage).

P. ultimum persists in soil for a long period of time (24), and environmental conditions influence its passive survival (11). The peat medium apparently provided nutrients for growth of *Pythium* in the first 10 days (Fig. 4A). Container media amended with bark compost, conducive or suppressive to Pythium damping-off, were less suitable for survival of *P. ultimum* than the peat medium. As was reported before by Hancock (8) for California soils, artificially high inoculum densities were not stable in either the conducive or the suppressive bark compost media and declined at similar rates. Thus, the observed decrease in population levels of *Pythium* could not account for the suppressive effect.

Our data on population dynamics of *P. ultimum* in container media were in agreement with previous findings for soils. Martin et al (14) found that addition of *Laetisaria arvalis* Burdsall to pasteurized and untreated soil reduced damping-off of table beet without altering populations of *Pythium*. In the absence of *L. arvalis*, significant population buildup of *P. ultimum* was observed in soils planted with table beet (14). Bouhot (4) found that suppression of Pythium damping-off, induced by addition of composted organic wastes, was due to saprophytic competition

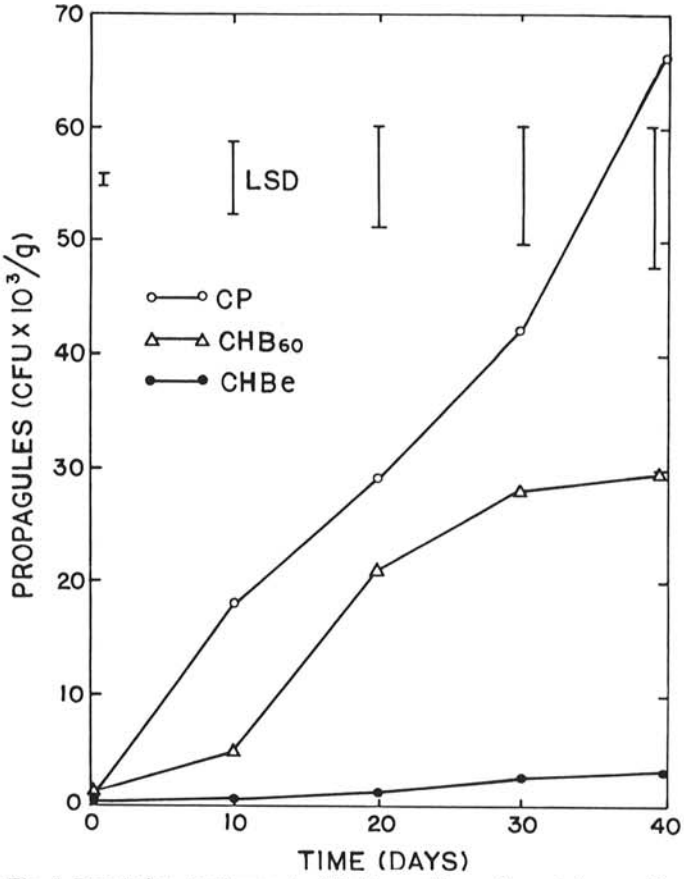

Fig. 5. Population development of *Pythium ultimum* in container media planted and replanted with cucumber. o = Canadian sphagnum peat (CP); • = composted hardwood bark from low temperature edge of a compost pile (CHBe); Δ = heat-treated (60 C, 5 days) CHBe container medium (CHB60).

and not to elimination of *Pythium* spp. Lumsden et al (12) reported that survival of *P. ultimum* in soil was not affected by amendments of composted sewage sludge. Pathogen populations should decrease during the period when biological control occurs, if the suppressive effect is due to antibiosis or lysis (1). Our results (Fig. 5) suggest that antibiosis or exploitation was not the principal mechanism responsible for suppression, at least in these batches of compost-amended container media. In the conducive container media planted with cucumber, the seedlings provided nutrients and stimulated germination of *Pythium*, followed by infection, colonization of, and sporulation in plant tissue. In the suppressive container medium, nutrients provided by cucumber plants apparently were not available to, or available in insufficient quantities either for reproduction of *P. ultimum* or for it to cause damping-off. Undoubtedly the microflora in the suppressive container medium was actively taking up nutrients and creating a nutrient sink.

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