Effects of Available Carbon Source on Microbial Activity and Suppression of *Pythium aphanidermatum* in Compost and Peat Container Media

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ABSTRACT

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Incorporation of a glucose/asparagine mixture of 10 carbon units to each nitrogen unit (C:N ratio = 10:1) into container media resulted in a rapid increase in microbial respiration rate and enzymatic activity in composted, separated, cattle-manure medium, but not in peat or limed peat media. Respiration was increased with an increase in ambient temperature. Glucose was depleted much faster in compost medium than in peat media. Compost medium amended with glucose/asparagine was more conducive to bacterial growth than peat media and less conducive to fungal growth. Hyphae of *Pythium aphanidermatum* grown on nylon fabric and buried in container media were rapidly lysed in compost medium as compared with peat-based media. Glucose/asparagine amendment delayed hyphal lysis of *Pythium*. Light and scanning electron microscopy of hyphae of *Pythium* retrieved from container media showed no evidence of direct parasitism on hyphae by other soil fungi; however, bacteria were associated with the lysing hyphae. Oospores of *Pythium* supported on nylon fabric and buried in container media were not lysed, nor did their viability differ in the different media. Amendment of glucose/asparagine to container media resulted in an increase in disease incidence; however, only after several consecutive amendments of glucose/asparagine to compost medium was suppression of Pythium damping-off negated.

Suppression of soilborne plant pathogenic fungi in compost-amended container media has received much attention in the last decade (16). Suppression of Pythium diseases has been reported for media amended with composts prepared from different organic wastes, such as tree barks, licorice roots, municipal sewage sludge, and separated cattle manure (4,7,14,25,26). Suppression of Pythium diseases in these media has been attributed mainly to biological factors (6,7,14,16), and competition for available nutrients has been proposed as the principal mechanism (5,6,11). Other proposed mechanisms include hyperparasitism (13,15,40), iron competition (2,37), and production of soluble (21) and volatile (17) inhibitors or hydrolytic enzymes (33) by soil microflora.

Seed and root exudates, including volatile substances, act as recognition signals that trigger germination of dormant propagules (5,30). Exudates are also the principle sources of organic nutrients required for germination and growth of resting structures of *Pythium* spp. (3,29). High levels of active microbial populations reduce the amplitude and the duration of the signal pulse provided by roots and seeds in soils by depletion of that energy source required for germination of propagules of *Pythium* (19).

After wastes have been decomposed through composting to a point where the waste itself no longer can serve as a food base for propagules of *Pythium*, and after the compost has been recolonized by a mesophilic microflora so that microbiostasis prevails, concentrations of free organic nutrients are low enough so that propagules of *Pythium* remain dormant (5). Changes in total biomass and soluble nutrient status in container media have been correlated with suppressiveness to *P. ultimum* and have been used to predict suppressiveness of container media to *P. ultimum* (5).

Because competition for nutrients is considered an important mechanism of suppression of seedling damping-off caused by *Pythium aphanidermatum* (Edson) Fitzp., we studied the impact of nutrient amendment on microbial activity and on the fate of the pathogen in suppressive compost and conducive peat container media.

MATERIALS AND METHODS

Container media. A medium containing composted, separated cattle manure and vermiculite (2:1, v/v) was prepared. This medium hereafter is referred to as compost medium. Two media prepared with German sphagnum peat and vermiculite (2:1, v/v) were used. The first peat medium, referred to hereafter as limed peat medium, was adjusted to pH 7.4 with calcium carbonate (10 g/L), measured in a 1:10 (solid/water) extract. The second peat medium, not amended with lime, had a pH of 3.8, and is referred to as peat medium. Physical and chemical properties of the compost and peat used in the container media were reported previously (9). All container media (except those used in greenhouse experiments) were adjusted to a water matric potential of −1.0 kPa with a constant water matric potential apparatus (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) and incubated at 30 C (if not stated otherwise) for at least 2 wk before use. Adjusted water contents of container media were 88.4, 87.5, and 65% (w/w) for peat, limed peat, and compost media, respectively. Media used in greenhouse experiments were stored in a refrigerator at 4 C followed by incubation under moist conditions at 30 C at least 2 wk before use.

Greenhouse experiments. *P. aphanidermatum* was isolated from field-grown diseased cucumber seedlings on a glucose-asparagine medium selective for *Pythium* spp. (38) and identified according to Middleton (28) and Waterhouse (42).

Cucumber seeds (*Cucumis sativus* L. 'Dlila'; 95% germination, 10 per pot) were planted 1 cm deep in polypropylene pots (19 × 14 × 7 cm) containing 500 cm^3 infested container medium. Infested peat medium was used as inoculum. Three repeated plantings with cucumbers were used to increase inoculum density, when 90–100% of the plants had died from damping-off. The population density of *Pythium* on selective medium (38) ranged from 5×10^3 to 5×10^4 colony-forming units (cfu)/g of peat medium. Inoculum was suspended in 250 ml of water and added to the growth medium at 2,000 cfu/L immediately before seeding. The percentage of surviving seedlings was calculated at 14 days postplanting.

In treatments where glucose/asparagine was added during the first 3 days of growth, the amendment (0.5 g per container per day) was dissolved in 50 ml tap water and applied to the medium

every 12 hr (0.25 g per application). Control treatments received the same volume of water.

After the first bioassay, percent damping-off was recorded, cucumber plants were harvested, container media of all replicates of the same treatment were mixed and returned to six pots and replanted. In the third bioassay, the entire procedure was repeated.

Preparation of glucose/asparagine amendment. Anhydrous D(+)-glucose, 3.363 weight units (Merck Co., Darmstadt, West Germany), and a 1.0 weight unit of anhydrous L-asparagine (Sigma Chemical Co., St. Louis, MO) were mixed thoroughly to yield a mixture of 10 carbon units to each nitrogen unit (C:N = 10:1). In all experiments where glucose/asparagine mixture was added to container media, it was mixed into wet medium in powder form (5 mg/g medium, unless stated otherwise), except for the greenhouse experiments, in which the amendment first was dissolved in water (10 mg ml^{-1}). Residual glucose in container media after amendment with glucose/asparagine mixture was monitored in a water extract of the media by the glucose oxidase method (20).

Determination of CO_2 evolution. Triplicate 3-g samples of wet medium (water potential −1 kPa) were placed in 25-ml glass bottles equipped with a gas-tight rubber cap. The bottles were flushed with fresh air (approximately 0.035% CO_2) before sealing, and they were placed in an incubator for either 1 or 24 hr. A 1-ml air sample was taken with a syringe to determine CO_2 concentration by gas chromatography (GC model 580, Gow-Mac Instrument Co., Bound Brook, NJ). The GC was equipped with a poropak Q column and a TCD detector. Results were expressed as milliliters CO_2 100 g^{-1} wet weight container medium per hour. In experiments where the glass bottles were sampled several times, the bottles were flushed with fresh air after each sampling and resealed for further incubation.

Hydrolysis of fluorescein diacetate. Hydrolysis of fluorescein diacetate (FDA) was performed with a slightly modified method of Schnürer and Rosswall (39). FDA (Sigma Chemical Co.) was dissolved in acetone (analytical grade) and stored as a stock solution (2 mg ml^{-1}) at −20 C. A 1-g medium sample (wet weight) was dispersed in 100 ml of sterile 60 mM potassium phosphate buffer (pH 7.6) and then amended with FDA to a final concentration of 10 μg FDA ml^{-1}. The suspension was incubated in 250-ml Erlenmeyer flasks at 30 C in a rotary shaker (120 rpm). One-milliliter samples were removed from the suspension at 9-min intervals and centrifuged for 1 min in a microliter centrifuge (No. 2021, Heittich Zentrefugen, West Germany) to remove particles of medium. The amount of fluorescein was measured as absorbance at 494 nm with an HP 8451A diode array spectrophotometer (Hewlett Packard, Palo Alto, CA). FDA also was added to a sample blank before centrifugation. A standard curve of fluorescein was prepared from a stock solution containing 200 mg of fluorescein dissolved in 20 ml of hot ethanol. The stock solution was diluted in KH_2PO_4 buffer (pH 7.6) with 1 g of medium sample and in buffer (pH 7.6) with and without the medium sample to yield final concentrations of 0.625, 1.25, 2.5, 5, and 10 μg fluorescein ml^{-1}. Because there was less than 1% reduction in absorbance in the standard curve with the container medium sample, adsorption of fluorescein to the container medium thereafter was disregarded. FDA hydrolysis rate was linear for at least 90 min after the addition of FDA to the suspension. Unless stated otherwise, FDA hydrolysis was measured as fluorescein units released from FDA within 60 min after addition of FDA to the container medium suspension.

Lysis of hyphae of *Pythium* in container media. A modification of the nylon fabric technique developed for *Pythium* by Lumsden (23) was used to support and recover hyphae of *P. aphanidermatum*. Three-milliliter subsamples of sterile liquid growth medium (2.4 g of sucrose, 0.37 g of asparagine, 0.15 g of KH_2PO_4, 0.15 g of K_2HPO_4, 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of neomycin sulfate, and 0.01 g of chloramphenicol in 1 L of deionized water) were poured into 9-cm-diameter disposable petri dishes. The medium in the dishes was touched with a sterile needle wetted in Tween 80 (Sigma Chemical, Co.) to spread the medium as a thin layer over the entire bottom of the dish. Five agar disks of *P. aphanidermatum*, taken from the growing edge of a culture on sucrose-asparagine selective solid medium (38), were placed in each dish. Nylon monofilament fabric, with a pore size of 80–100 μm (Swiss Silk Bolting Co., Ltd., Zurich, Switzerland), was cut into 5-mm^2 pieces and autoclaved (121 C, 30 min), and five nylon pieces were placed beside each agar disk (25 nylon pieces per dish). After 24 hr at 30 C, nylon squares containing sparse hyphae of *Pythium* (Fig. 13B) were removed with a surgical blade. Five nylon squares, with the fungus interwined among the filaments (5–10 hyphal strands in each pore), were placed in a Millipore apparatus (Millipore Corporation, Bedford, MA) in which a new nylon fabric (100–150 μm pore size) circle was mounted. The nylon squares with hyphae were washed with 250 ml of sterile deionized water to remove adherent nutrients. Next, the nylon circle with the five nylon squares was removed from the Millipore apparatus and covered with a second circle of nylon fabric to form a "sandwich." This "sandwich" was buried 2-cm deep in a 100-ml plastic cylinder (4.7 cm diameter) containing 50 g of container medium (previously adjusted to a water potential of −1.0 kPa); cylinders were incubated vertically at 30 C. After incubation, "sandwiches" were retrieved, and nylon squares were mounted on slides, stained with trypan blue, and microscopically examined.

Oospore production and isolation. Oospores were harvested from 3-mo-old cultures grown in 1,000-ml Roux bottles containing 100 ml of carrot-broth medium (50 g of fresh carrot was homogenized in 1,000 ml of tap water and the coarse particles were removed by filtration through cheesecloth). Mycelial mats were collected from the growth medium, submerged for 5 min in 250 ml of sterile cold water, retrieved, and then homogenized in a sterile stainless steel blender in 100 ml of sterile water (4 min at low speed and 1 min at high speed); temperature during blending did not exceed 25 C. Homogenate was filtered successively through two layers of sterilized nylon cloth (125-μm and 75-μm pore size) and centrifuged at 10,000 g for 10 min. The oospore pellet was washed by centrifugation at 1,000 g for 30 sec in 100 ml of sterile water, and the supernatant was discarded; the pellet was resuspended in 100 ml of sterile water and dispersed with a Vortex-Genie mixer (Scientific Instruments, Inc., Bohemia, NY). Washing and resuspension were repeated three times. The isolated oospores were collected on sterile nylon cloth (10-μm pore size), resuspended in 250 ml of sterile water at 10 C, and filtered again through a nylon mesh (48-μm pore size). Concentration of oospores was estimated with the aid of a hemocytometer. Then the oospore suspension was centrifuged at 5,000 g for 10 min, resuspended in sterile water to a final concentration of 10^4 oospores per milliliter, and stored at 10 C for no longer than 10 days before use.

Germination of oospores after their retrieval from container media. The method described by Sneh (41) was modified to accommodate a large number of samples. One-half milliliter of oospore suspension containing 5×10^3 oospores was applied with vacuum to a 13-mm-diameter sterile nylon cloth (10-μm pore size). Pieces of nylon containing the oospores were buried horizontally in container media in "sandwiches" as described previously, taking care that the side of the disks containing the oospores faced up. After incubation, disks containing the oospores were retrieved from the "sandwiches" (maintaining an upright position at all times) and placed on double-sided adhesive tape pieces attached to a precooled (5 C) glass plate (100 × 150 mm) on crushed ice. Tape pieces were arranged on the glass plate to fit the wells of a 24-well plastic plate (model 3524, Costar Europe Ltd., Solterweg 305a, 1171 VC Badhoevedorp, The Netherlands); 6-mm-diameter holes previously were drilled in the bottom of each well. The multiwell plate was attached securely to the glass with rubber bands so that each well covered one nylon circle containing oospores. Melted selective medium (38), cooled to 45 C, was poured into the wells through the 6-mm-diameter holes. After the medium had solidified, the plate was separated from the glass, leaving the nylon fabric attached to the glass plate. With this procedure, most of the oospores were peeled from the nylon fabric and remained attached to the agar.

A 2-mm section of agar cylinder containing the adhered oospores was cut with a surgical blade and placed on a glass slide. Germinability of the oospores was determined after a 16-hr incubation at 30 C.

Germination of oospores in container media. Oospores produced on carrot broth medium as described above were activated according to Ruben et al (36), except that the desiccation stage was performed for 24 hr on mycelial mats containing oospores rather than in solid growth medium. After activation, 5×10^3 oospores were applied to nylon fabric (13-mm-diameter, 10-μm pore size) by suction. The fabric then was buried in container media as described for hyphae. After 16 hr, oospores were retrieved and the germination was determined.

Bacterial and fungal counts. Viable bacteria were counted by a dilution plating technique (three replicates) on compost or peat extract media (500 g of compost or peat autoclaved in 1 L of deionized water for 30 min, then sieved through cheesecloth and centrifuged 5 min at 6,000 g; 100 ml of supernatant was amended with 1 g of glucose, 0.5 g of K_2HPO_4, 20 g of agar, brought up to a final volume of 1 L with deionized water, and autoclaved). Total fungal cfu's were determined by dilution plating on Martin's rose bengal medium (27). Population density of *P. aphanidermatum* was determined by dilution plating on sucrose-asparagine selective medium (38).

Scanning electron microscopy. Nylon fabric bearing oospores or hyphae of *Pythium* retrieved from container media was fixed for 48 hr in a closed vessel containing two petri dishes, one with 5% OsO_4 in 0.1 M pH 7.4 phosphate buffer, and the other with 25% glutaraldehyde. Samples were air-dried for 24 hr, coated with gold palladium in a Polaron E150 (Polaron Equipment Ltd., Watford, England), and observed in a scanning electron microscope (JEOL JSM 35C, Tokyo, Japan).

Statistical analysis. In microbial activity assays (e.g., FDA hydrolysis and respiration response), each container medium treatment was replicated three times, and each experiment was performed three times. Data points presented are the means of the nine resultant determinations. Regression analyses were performed with the PLOT-IT computer program (Scientific Programming Enterprises, Haselett, MI) by nonlinear or polynomial regression analysis of the amount of released fluorescein or CO_2 as a function of time, or of increasing concentrations of glucose/asparagine. Goodness of fit was assessed by analysis of residuals and approximate confidence limits for the nonlinear model parameters.

In hyphal survival assays, treatments consisted of three replicates, each containing five squares of nylon fabric with hyphae of *P. aphanidermatum*. In each nylon square, 20-mm-long hyphal strands (200, 100-μm segments) were examined to determine the length of hyphae lysed. The total hyphal length examined in each treatment was 3 cm. Each experiment was performed three times, and data points presented are the means of percent lysis in 15 samples.

In greenhouse bioassays, each replicate consisted of a pot containing 10 cucumber seeds, six pots per treatment. Each experiment was performed twice, and data points presented are means of percent seedling survival in 12 pots. Data were transformed to arc sine square roots and statistically analyzed by analysis of variance (ANOVA); means were separated by Duncan's multiple range test. In greenhouse experiments with increasing inoculum densities, data were analyzed by nonlinear regression of the percent survival of seedlings at the end of a growth cycle as a function of the inoculum density at the beginning of the first growth cycle with the PLOT-IT computer program. Goodness of fit was assessed by analysis of residuals and approximate confidence limits for the nonlinear model parameters. In all experiments, data from repeated experiments were pooled only when error variances of the separate ANOVAs were homogeneous. Regression lines, an equation model, and correlation coefficients are presented in all relevant figures. All significant differences were at $P = 0.05$.

RESULTS

Pythium damping-off incidence in container media. *Pythium* at 2,000 cfu/L or more caused significant damping-off in the first planting in peat medium (Fig. 1C), and replantings increased damping-off occurrence. In the limed peat medium (Fig. 1B), significant disease occurred only after infestation with 5,000 cfu/L. However, replantings increased disease incidence at all inoculum densities. No significant disease occurred in the compost medium (Fig. 1A), although some disease increase occurred during the second growth cycle in this medium initially infested with 5,000 cfu/L.

Container media were infested with 2,000 cfu/L, and the change in inoculum density was monitored at the end of each growth cycle (Fig. 2). The inoculum density did not increase significantly in the suppressive compost medium, but did increase in both conducive peat media. In the limed peat, inoculum density in-

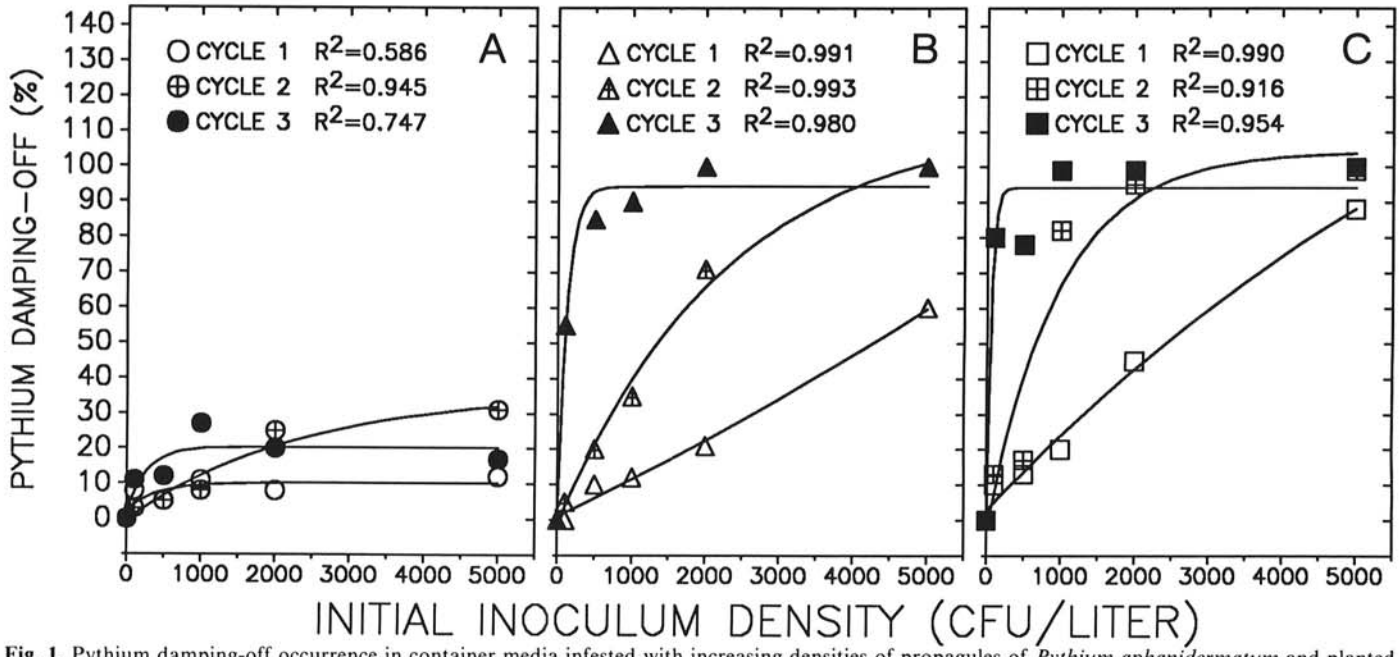

Fig. 1. Pythium damping-off occurrence in container media infested with increasing densities of propagules of *Pythium aphanidermatum* and planted with cucumber. **A,** Compost medium; **B,** limed peat medium; and **C,** peat medium. The regression analysis equation was exponential with position constant: $Y = B_1^{(B_2 X)} + B_3$.

creased less than in the peat medium. Similar results were consistently obtained in other experiments.

Effect of glucose/asparagine amendment on suppression of cucumber damping-off. Amendment of the compost or peat media with glucose/asparagine did not affect germination of cucumber seeds in any plantings (Fig. 3). Infested compost medium (unamended control) was suppressive to damping-off in both plantings. A single amendment of glucose/asparagine added to the infested compost medium at planting did not reduce the suppressive effect. However, disease development in the second planting in this medium was significantly different from that in the uninfested control. Amendment of glucose/asparagine for 3 consecutive days (six amendments at 12-hr intervals) after planting slightly increased disease in the first growth cycle (30% diseased seedlings) but not significantly from the uninfested control (5%). However, disease incidence was similar to that of conducive peat medium (40%). In the second growth cycle, a significant reduction was observed in the suppressiveness of the compost medium amended with glucose/asparagine for 3 days, but not in the compost medium amended only once.

Effect of amendment with glucose/asparagine on microbial counts. Bacterial population densities were higher in the compost medium than in the peat medium (Fig. 4). An increase of more than one order of magnitude in bacteria was observed in the limed peat medium as compared with the peat medium. Amendment with glucose/asparagine resulted in increased bacterial densities in the first 24 hr in all media. The fastest and greatest increase occurred in the compost medium (more than a tenfold increase within 12 hr after the amendment, and almost two orders of magnitude after 24 hr) as compared with the unamended control. Bacterial densities remained high 1 wk after amendment. In the peat medium (Fig. 4B), the increase in bacterial density at 1 day after amendment was much lower than in the compost medium and somewhat lower than in the limed peat medium. At the end of 1 day of incubation, bacterial density increased by more than one order of magnitude. In contrast to the compost medium, bacteria continued to proliferate in the peat medium for 2 days after amendment. However, after 1 wk of incubation at 30 C, the density decreased to a level slightly higher than that of the unamended control. In the limed peat medium, the bacterial population density was higher than in the peat medium, but only a slight multiplication was observed after amendment.

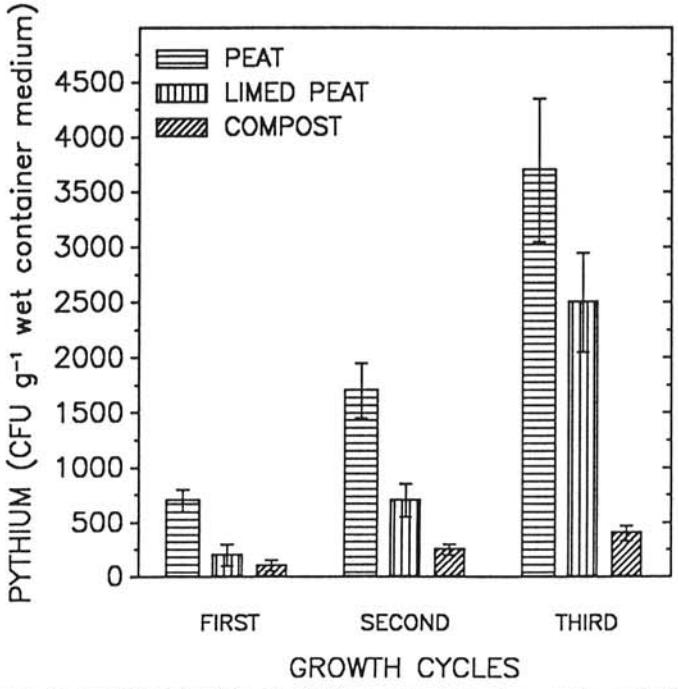

Fig. 2. Inoculum densities of *Pythium aphanidermatum* at the end of each of three consecutive 14-day growth cycles. Container media were infested with 2,000 cfu/L before the first cycle. Bars = standard errors.

The population density of total fungi was lower in the compost medium as compared with the peat container media (Fig. 4). Fungal population density in all media increased with incubation time, even in unamended media. Amendment further increased total fungi in all media, but the fastest increase was recorded in the compost medium 1 day after amendment. The highest density of fungal propagules was recorded in the limed peat medium 1 wk after amendment.

Effect of glucose/asparagine amendment on microbial activity. At 18 hr after amendment, respiration rate in all media incubated at 22 C was low but increased with the amendment (Fig. 5). Respiration increase was positively correlated with increase in the amount of glucose/asparagine added to the container media. The highest increases in respiration rate were recorded in the compost medium. In the peat medium, increases were slightly higher than in the limed peat medium. The rate of respiration in the unamended compost control was 2.8-fold higher than that in the peat medium, and 1.5-fold higher than that in the limed peat medium. In some experiments with compost media prepared with mature suppressive compost that had been stored outdoors for 1 yr or more in open containers, the respiration rate was lower and not significantly different from those of peat-based media; however, amendment with glucose/asparagine resulted in an increase in respiration similar to that observed in a less mature compost (data not shown).

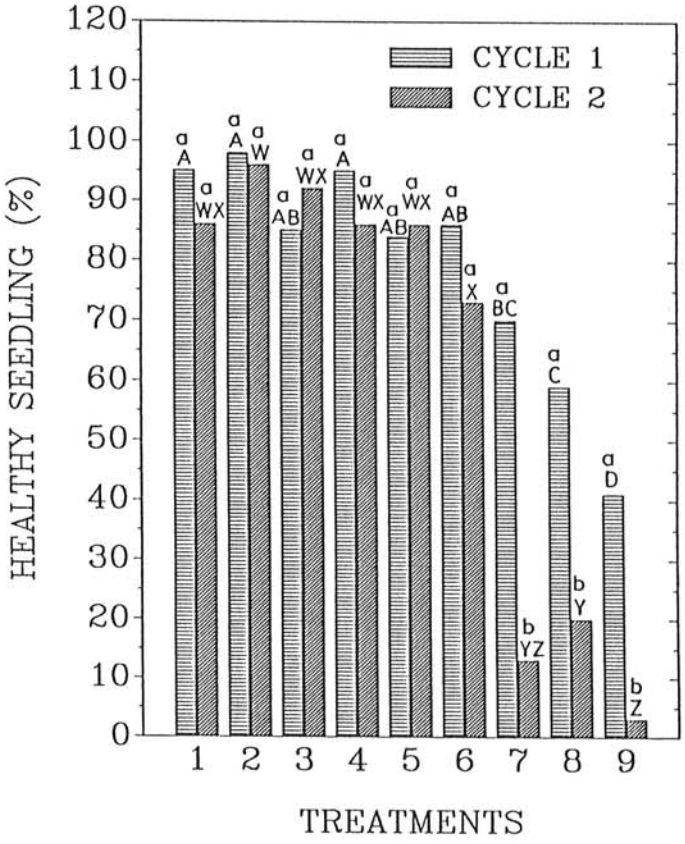

Fig. 3. Effect of a glucose/asparagine amendment on suppression of Pythium damping-off of cucumber seedlings in two 14-day growth cycles. **1,** Compost container medium; **2,** compost container medium amended with glucose/asparagine 1 day after sowing; **3,** peat container medium; **4,** peat medium amended with glucose/asparagine 1 day after sowing; **5,** compost container medium infested with *Pythium aphanidermatum*; **6,** compost container medium infested with *Pythium* and amended with glucose/asparagine 1 day after infestation; **7,** compost container medium infested with *Pythium* and amended with glucose/asparagine 3 consecutive days after sowing; **8,** infested peat container medium; **9,** same as for 7 but with peat medium. Each growth cycle was analyzed separately. Data followed by the same letter are not significantly different at $P = 0.05$. Uppercase letters A–D are for differences among treatments (1–9) in the first growth cycle; uppercase letters W–Z are for differences among treatments (1–9) in the second growth cycle. Lowercase letters are for differences between growth cycles within each treatment (1–9).

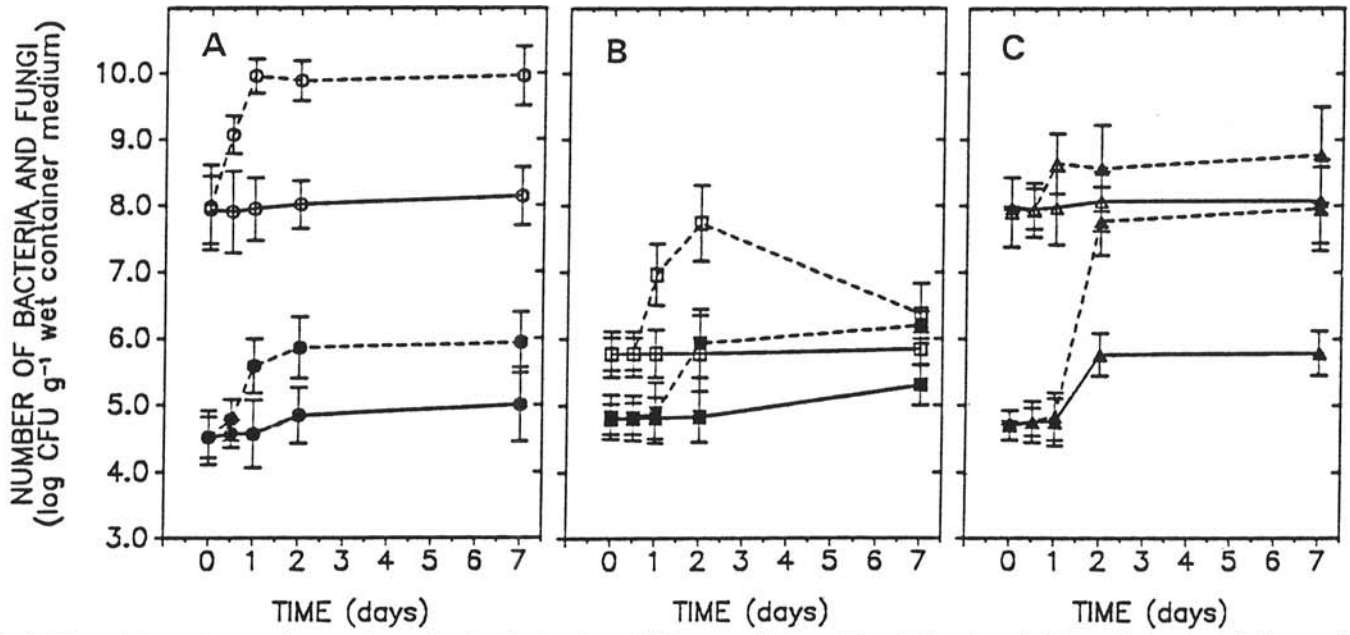

Fig. 4. Effect of glucose/asparagine amendment (broken line) on bacterial (open symbols) and fungal (closed symbols) population densities in container media; solid line = unamended control. **A,** Compost container medium; **B,** peat container medium; **C,** limed peat container medium. Bars = standard errors.

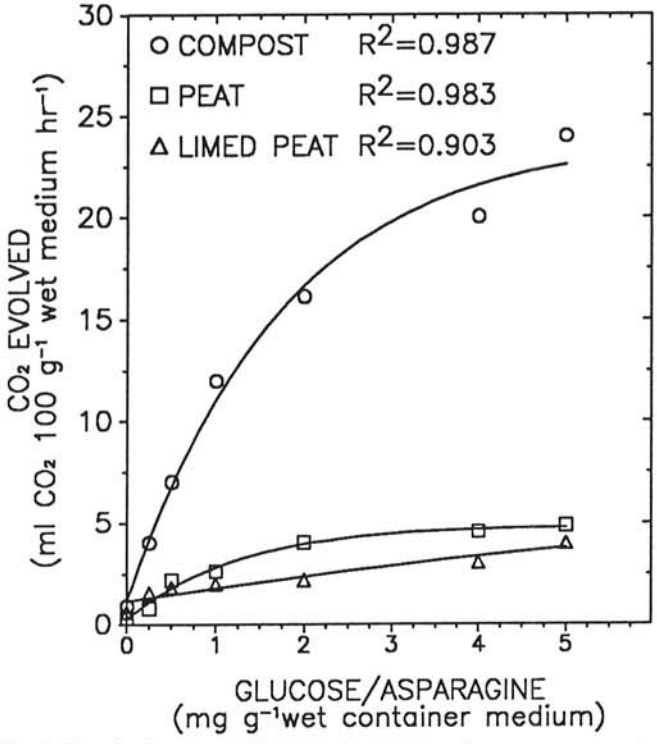

Fig. 5. Respiration rate (CO_2 evolution) 18 hr after amendment with increasing amounts of glucose/asparagine. Incubation temperature was 22 C. The nonlinear regression equation was an asymptotic exponential function with position constant: $Y = B_1(1.0^{-(-B_2X)}) + B_3$.

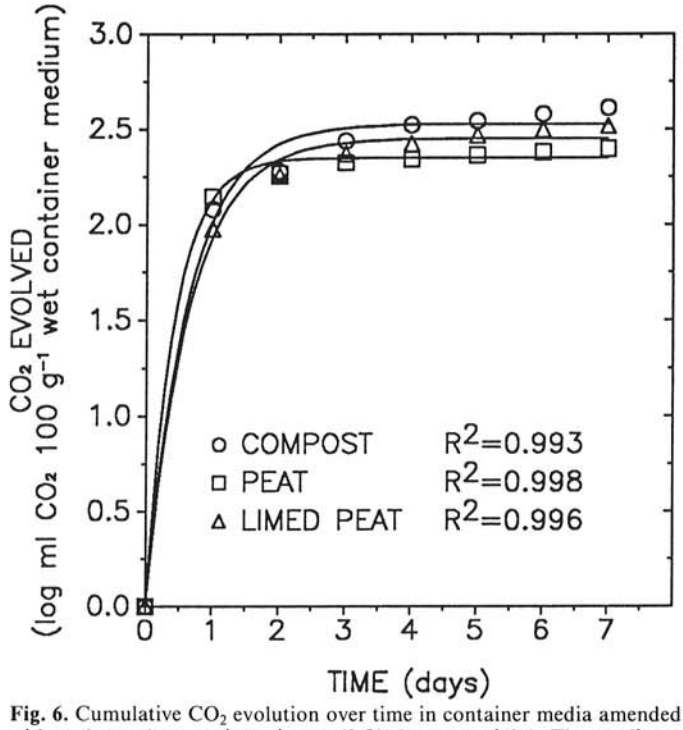

Fig. 6. Cumulative CO_2 evolution over time in container media amended with a glucose/asparagine mixture (0.5% by wet weight). The nonlinear regression equation was an exponential function with position constant: $Y = B_1^{(B_2X)+B_3}$.

The accumulation of CO_2 after amendment with glucose/asparagine was monitored daily for 1 wk after amendment (Fig. 6). Every 24 hr, the headspace in the incubation flasks was sampled, flushed with fresh air (~0.035% CO_2, v/v), sealed, and further incubated at 30 C. The highest accumulation of CO_2 during the first 24 hr was measured in the peat medium and the lowest in the limed peat medium. However, the accumulation during the second day of incubation was lower in both peat-based media than in the compost medium. This higher rate of accumulation of CO_2 in compost medium samples was observed consistently throughout the rest of the incubation times.

The container media differed considerably in the rate of increase of respiration during the first day following amendment (Fig. 7). At 22 C, there was a lag period of about 10 hr in the compost medium before the respiration rate increased. After 18 hr, the respiration rate reached a peak of more than 20 ml of CO_2 100 g^{-1} wet weight hr^{-1} and then decreased rapidly. After 22 hr, the respiration level in the peat medium was similar to that in the compost medium. At 30 C, the lag time was shortened to 4 hr in the compost medium, reaching a peak after 11 hr (Fig. 8). Thereafter, it descended to a level lower than that in peat medium in less than 14 hr from amendment. In both experiments, the respiration rates during the lag phase in the limed peat medium were significantly higher than in the peat medium, but the lag

period in the peat medium was shorter when compared to that in the limed peat medium.

Depletion of glucose in amended container media was negatively correlated with an increase in respiration (Fig. 9). When glucose was depleted from the compost medium, glucose levels in peat media still were high. At 24 and 36 hr after amendment, the level in the peat and in the limed peat media, respectively, had decreased to below 500 μg glucose g^{-1} wet weight of medium (data not shown). The respiration rate (per hour) was highest in the peat medium in the first 2 days following amendment (Fig. 10). After 2.5 days, and throughout the following 3 days of incubation, the rate in the compost medium was higher than that in the peat media. Four days after nutrient amendment, respiration increased in the peat medium to a level similar to that in the compost medium.

The rate of FDA hydrolysis in container media was highest in the compost medium (Fig. 11) and lowest in the peat medium, with or without amendment. $CaCO_3$ amendment (limed peat medium) increased the rate of FDA hydrolysis over unlimed peat. Glucose/asparagine amendment significantly increased the rate of FDA hydrolysis in all media. In compost and peat media, the effect of glucose/asparagine on FDA hydrolysis increased in 1 day following amendment (Fig. 12); peak activity was recorded 24 hr after amendment. The activity in the compost medium was three times greater than that in the peat medium and more than 10 times that of the limed peat medium. Further incubation resulted in activity decline in both compost and peat media until the fourth day from nutrient amendment. Fourteen days after amendment, the effect on FDA hydrolysis rate still was pronounced in the compost and limed peat media but not in the peat medium (data not shown).

Effect of glucose/asparagine amendment on survival of hyphae of *P. aphanidermatum*. Lysis of hyphae of *P. aphanidermatum* attached to nylon fabric "sandwiches" buried in container media and incubated at 30 C was readily observed after staining with trypan blue (Fig. 13B). Hyphae lysed very rapidly in the compost medium (Fig. 14). After 24 hr, 80% of the hyphae were lysed. Oospores or digitate lobate sporangia were not observed. Further incubation reduced surviving hyphae to less then 10 and 1% after 48 and 72 hr, respectively. After 96 hr, cell walls (as in Fig. 13B, arrow) of lysed hyphae no longer could be seen easily. Only dense colonies of bacteria indicated the original path of hyphal growth over the nylon filaments. After 120 hr, most of the bacteria no longer were clustered in areas of degraded hyphae. In the conducive peat medium, the rate of lysis was lower. After 96 hr of incubation, 50% lysis was observed, and digitate lobate sporangia were abundant. In the limed peat medium, lysis of hyphae of *Pythium* was faster than in the peat medium but not as fast as in the compost medium. Sporangia were produced 48 hr after the hyphae had been introduced into the medium.

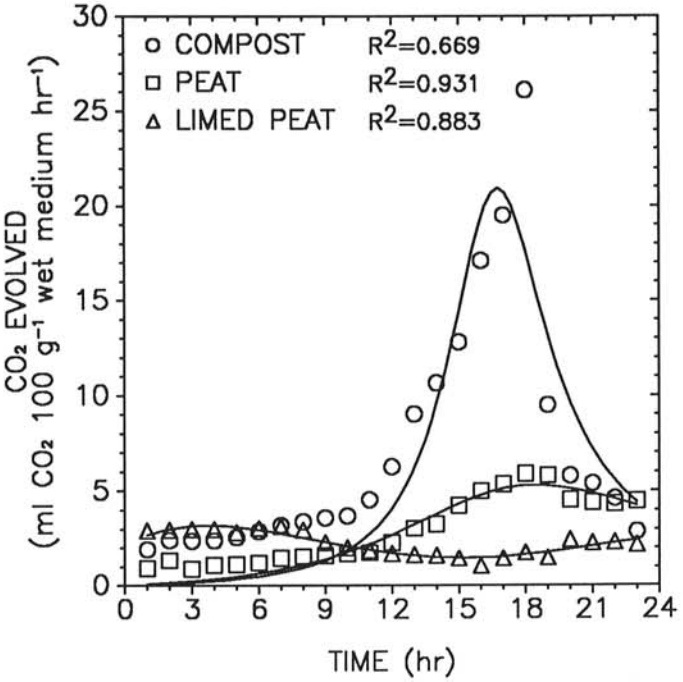

Fig. 7. Effect of a glucose/asparagine amendment (0.5% by wet weight) on respiration rate in container media at 22 C. The nonlinear regression equation was a second order logistic function: $Y = B_1/(1.0 + B_2^{(-(B_3X + B_4X))})$.

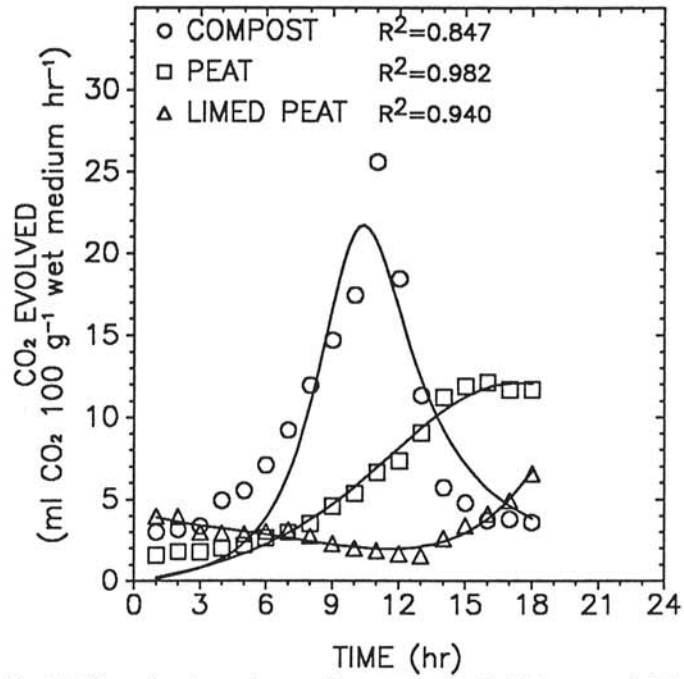

Fig. 8. Effect of a glucose/asparagine amendment (0.5% by wet weight) on respiration rate in container media at 30 C. Equation model used for nonlinear regression analysis was a hoop function: $Y = X/(B_1X^2 + B_2X + B_3)$.

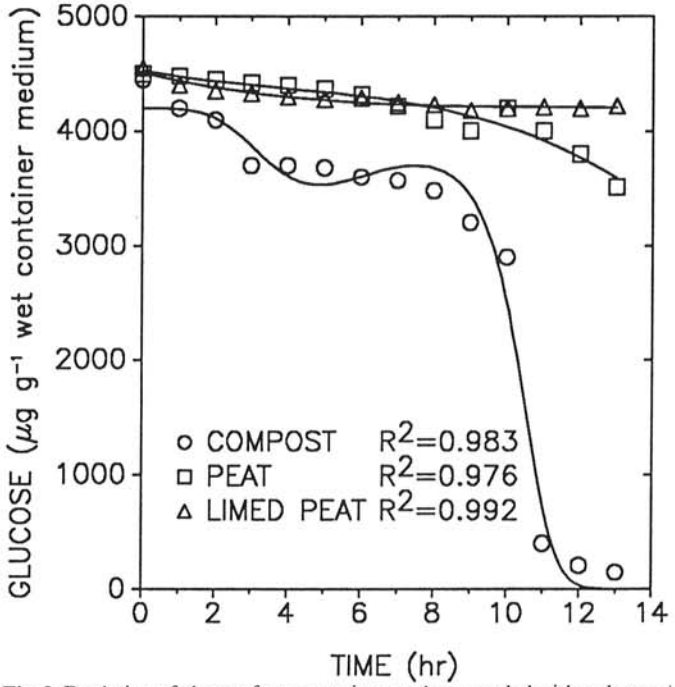

Fig. 9. Depletion of glucose from container media amended with a glucose/asparagine mixture (0.5% by wet weight). Equation model used for the nonlinear regression analysis of the compost treatment was a logistic saddle function: $Y = B_1/(1.0^{(B_2X + B_3X + B_4X2 + B_5X3)})$. The equation model used for the regression analysis of the peat and limed peat container media was: $Y = B_0 + B_1X + B_2X^2$.

Addition of glucose/asparagine mixture to container media resulted in a decrease in lysis of hyphae of *Pythium* in all media (Fig. 14). Only 3, 7, and 18% lysis was observed in the peat, limed peat, and compost media, respectively, after 24 hr. Further incubation in the compost medium resulted in a rapid lysis of hyphae. In the peat medium, less than 17% lysis was observed after 48 hr, and no digitate sporangia were formed. Amendment of the limed peat medium with glucose/asparagine delayed the lysis of hyphae. At the end of 96 hr, lysis was not significantly different from that in the compost medium; however, more sporangia were produced and oospores were observed.

Hyphal lysis in compost medium was delayed by additional amendments with glucose/asparagine (Fig. 15). After 48 hr, only 40% lysis occurred as compared with more than 90% in the unamended control, and sporangia were observed (Fig. 13B). Examination of these sporangia with a scanning electron microscope (SEM) 4 days after the amendment revealed bacteria associated with lysing sporangia (Fig. 13C). Most sporangia already were beginning to lyse after 96 hr. After 72 hr of incubation in the amended compost, some oospores were produced. These oospores were not colonized by bacteria or lysed during the 48 hr following their formation. This experiment was duplicated at 22 C, resulting in a 25% decrease in the lysis rate (data not shown).

Germination of oospores in container media amended with glucose/asparagine. Activated oospores introduced into compost or peat media did not germinate spontaneously without glucose/asparagine amendment (0.5% w/w) added 12 hr before introduction. Oospore germination 16 hr after introduction into container media (28 hr after glucose amendment) was 60, 40, and 5% in peat, limed peat, and compost media, respectively. Introduction of oospores into compost medium simultaneously with the glucose/asparagine increased the germination rate to 30%. The germination rate of oospores plated on selective medium was 65% after a 16-hr incubation at 30 C.

Germinability of oospores of *Pythium* after incubation in container media. Oospores (2 mo old) isolated from carrot medium germinated poorly on a selective medium. Without the activation treatment, only 15.3 and 22.0% germinated after 6 and 16 hr, respectively. Nonactivated oospores were introduced into the container media without glucose/asparagine amendment and to media amended 3 days before introduction of oospores, then extracted from the media and tested for germinability (Table 1). Incubation of nonactivated oospores in limed peat container medium for 24 hr increased germination over the control, but germination was not increased after incubation in the same medium amended with glucose/asparagine. Oospore germination

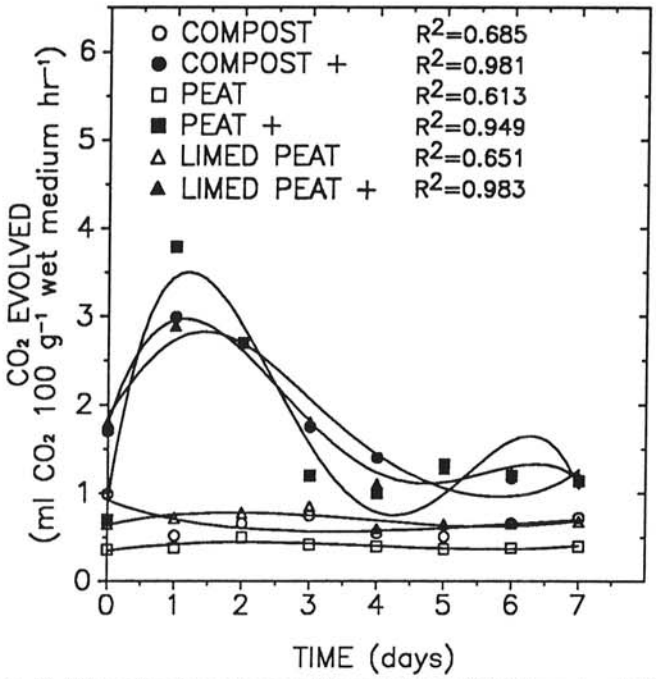

Fig. 10. Effect of a glucose/asparagine amendment (0.5 % by wet weight) on respiration rate in the week following amendment. Amended container media are indicated with solid symbols. Equation model used for the polynomial regression analysis was: $Y = B_0 + B_1X + B_2X^2 + B_3X^3 + B_4X^4$.

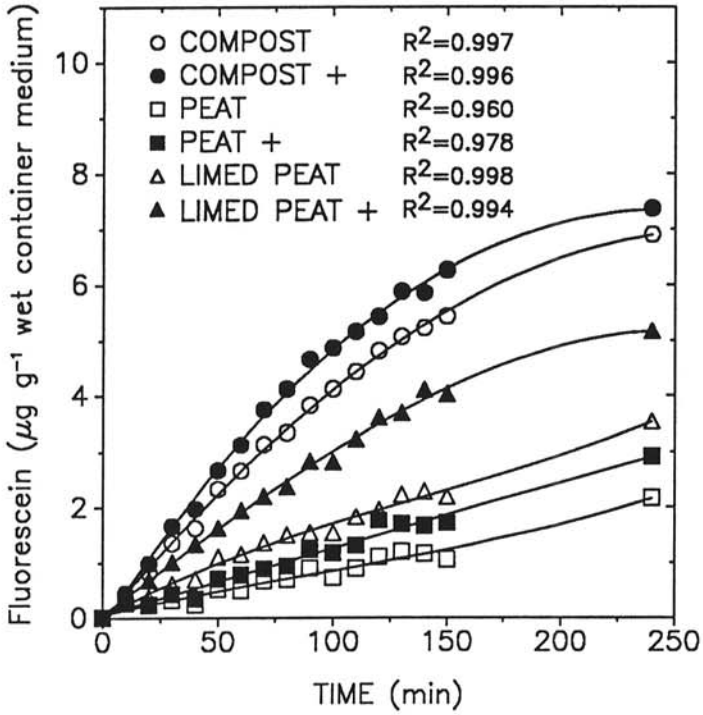

Fig. 11. Effect of incubation time and glucose/asparagine amendment (0.5% by wet weight) on increase in fluorescein diacetate (FDA) hydrolysis. FDA was added simultaneously with the nutrient amendment. Amended container media are indicated with solid symbols. Equation model used for the polynomial regression analysis was: $Y = B_0 + B_1X + B_2X^2$.

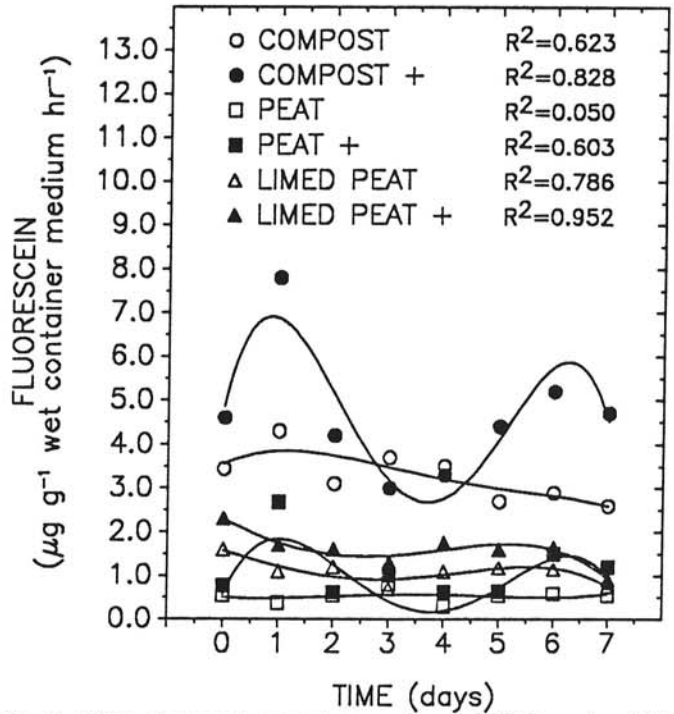

Fig. 12. Effect of glucose/asparagine amendment (0.5% by wet weight) on fluorescein diacetate (FDA) hydrolysis in the week following amendment. Amended container media are indicated with solid symbols. Equation model used for the polynomial regression analysis was: $Y = B_0 + B_1X + B_2X^2 + B_3X^3$.

was increased after a 24-hr incubation in compost and peat medium. Amendment with glucose/asparagine further increased germination in compost medium over the control, but not in the peat medium. Incubation in container media for an additional 48 hr further increased the germination rate in all media except the amended limed peat medium. However, incubating oospores for 6 days (9 days after the glucose/asparagine amendment) increased germination in the amended limed peat medium to a level similar to other media. Oospores incubated in container media for 1 mo maintained high germinability, except those in conducive peat medium in which germination was lower. This reduction apparently resulted from the activation and germination of many oospores during the incubation period, since the recovery of oospores from the nylon "sandwiches" was reduced.

Destruction of oospores incubated for 30 days in container media not amended with glucose/asparagine was similar in all container media (25% estimated via SEM). Most oospores were released from the oogonial wall but were not invaded by soil microorganisms (Fig. 13D).

DISCUSSION

Container media containing composted, separated cattle manure consistently suppressed damping-off caused by *P. aphanidermatum*, which agrees with previous reports (16,26). The suppressive effect was consistent at high inoculum levels even during replanting. Conversely, sphagnum peat was conducive to the disease, and high concentrations of inoculum of *Pythium* caused significant losses even in the first planting. Griffin (12) reported that soil pH influenced damping-off incidence caused by *Pythium*. Indeed, liming reduced disease incidence and inoculum buildup in first and second growth cycles when initial inoculum density was equal to or lower than 2,000 cfu/L of container medium. However, both peat-based media were conducive compared with the compost medium.

One of the greatest difficulties encountered in a study of soil-microorganism activity involves overcoming errors resulting from sampling and sample handling (35). Microbial activity generally is influenced by carbonaceous nutrient availability and fluctuation during sampling and storage. Thus, significant differences in microbial populations and their activities are found in stored samples, especially if air-dried (34). These problems are more prominent when comparing widely divergent ecosystems, such as peat- and compost-amended container media. To minimize sampling errors, container media were incubated at a constant water potential of −1 kPa (−10 mbar) and a constant 30 C for at least 2 wk before data were collected. In most investigations concerning incubation of soil samples under constant water content, water

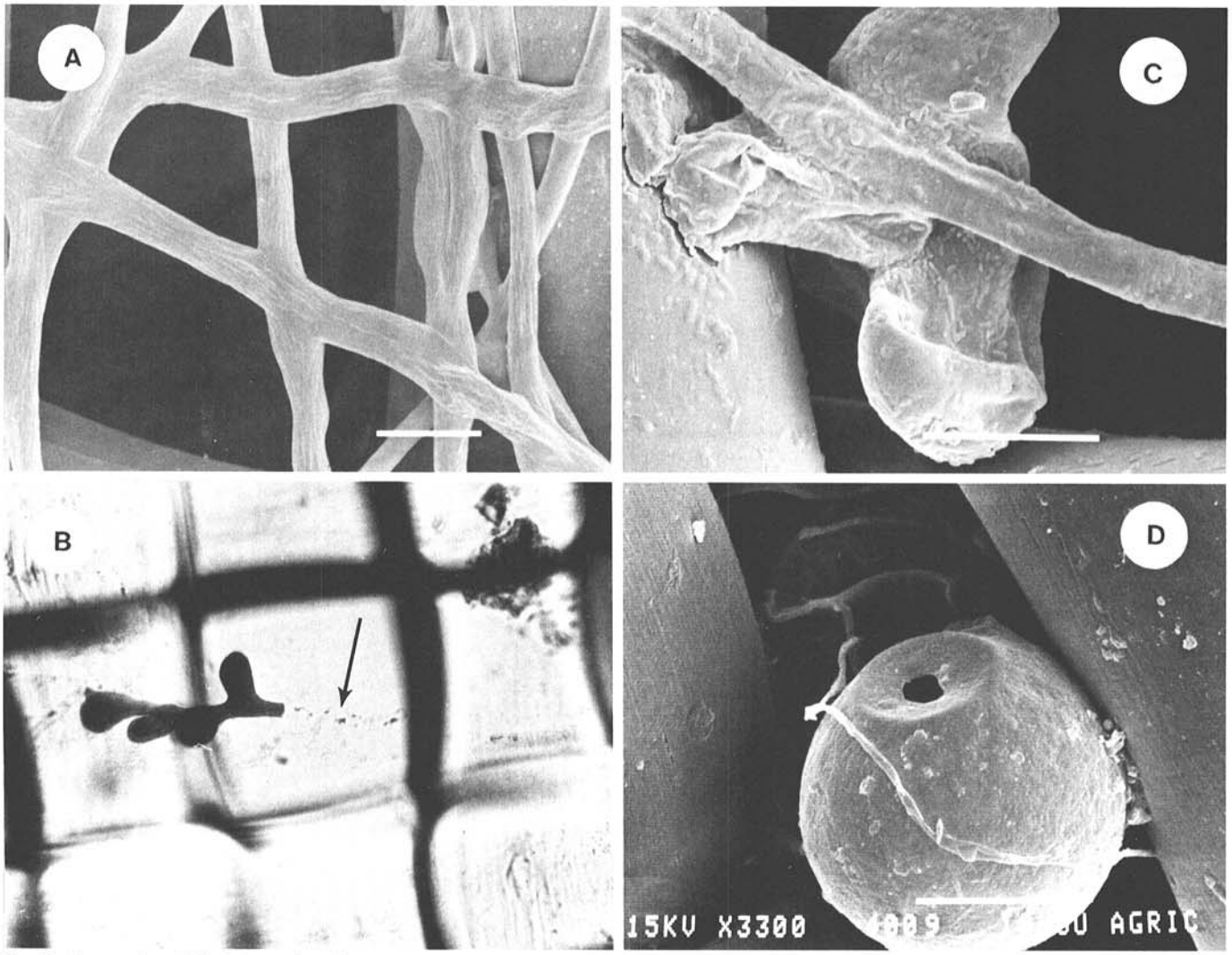

Fig. 13. Propagules of *Pythium aphanidermatum* on nylon fabric before and after incubation in container media. **A,** SEM micrograph of 24-hr-old hyphae of *P. aphanidermatum* contained on a fabric having a 100-μm pore size; bar = 10 μm. **B,** Hyphae of *Pythium* retrieved from compost medium amended with a glucose/asparagine (0.5% by wet weight) 2 days after burial. Most hyphae were lysed (arrow) and sporangia are formed (×400); nylon fabric pore size = 80 μm. **C,** SEM micrograph of the same treatment as B. Hyphae, sporangium, and associated bacteria are seen; the sporangium is in the early stages of lysis; bar = 10 μm. **D,** SEM micrograph of oospores of *P. aphanidermatum* retrieved from compost container medium 30 days after burial. The hole in the oospore is the result of sexual fertilization by an antheridium and not a perforation made by some soil parasite; bar = 10 μm.

content was adjusted daily by weighing and adding water to compensate for evaporative loss (35), or incubation was performed in sealed containers (3,11). Incubation under constant water potential provided conditions that improved our ability to compare samples.

Direct observation of hyphae of *Pythium* on nylon fabric first was suggested by Lumsden (23) as a technique for studying the ecology of this pathogen. This technique provided a rapid and convenient means for assessing lysis or fungistasis (as measured by oospore germination) in container media.

Nutrient stress causes lysis (22) and autolysis (18,21) of fungal hyphae. Autolysis under conditions of nutrient deprivation apparently stimulates the formation of asexual resting structures (31). Indeed, in the peat and compost container media amended with glucose/asparagine, the formation of many sporangia was associated with depletion of the nutrient source. The fact that amendment of glucose/asparagine into compost medium resulted in the formation of sporangia but did not prevent lysis of hyphae of *Pythium* further supports the hypothesis of a stronger level of nutrient stress in the compost medium compared with peat media. Because cell walls of hyphae were completely degraded in compost medium in 4 days, and bacteria were associated with the lysing hyphae, we assume that lysis more than autolysis was involved (at least at the final stage). The formation of water-soluble or volatile secondary metabolites that may cause lysis of hyphae of *Pythium* also was suspected, but such products were not found in mature compost (data not shown).

Total microbial activity or microbial densities alone were not reliable predictors of suppression of *Pythium*. The highest respiration rate and density of bacteria were observed in the conducive limed peat medium. Total fungal population densities were slightly higher in the peat-based media than in the compost medium. Alabouvette et al (1) positively correlated initial respiratory response (biomass) and suppression of *Fusarium oxysporum* Schlecht. in a soil and indicated that not only biomass but also rapid consumption of amended glucose are characteristic of suppressive soils. Chen et al (5) correlated biomass and suppression of *P. ultimum* in compost-amended media. However,

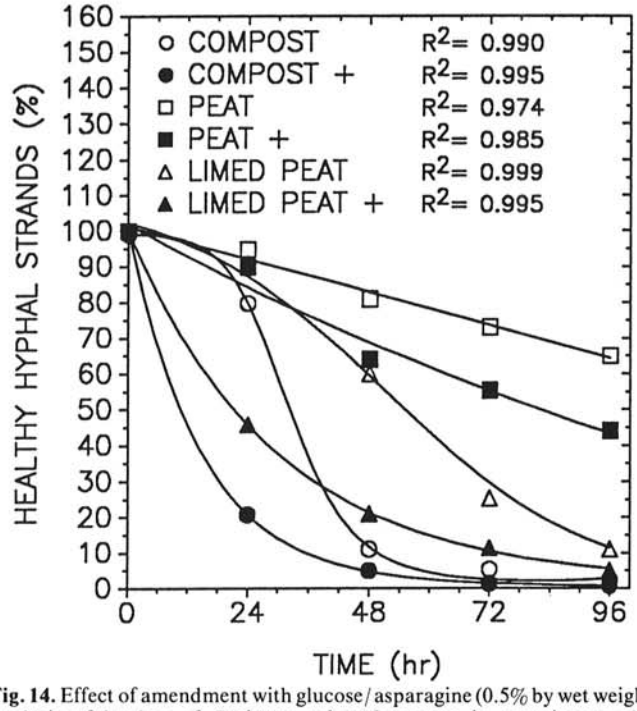

Fig. 14. Effect of amendment with glucose/asparagine (0.5% by wet weight) on lysis of hyphae of *Pythium aphanidermatum* in container media. Amended container media are indicated with solid symbols. Model equations used for the regression analyses were: $Y = B_1^{(B_2X)} + B_3$ in the regression analysis of the peat container medium, peat container medium amended with glucose/asparagine, and compost container media; $Y = B_1/(1.0 + B_2^{(-B_3X)})$ for the analysis of glucose/asparagine-amended limed peat and compost container media.

FDA hydrolysis in the compost medium positively correlated with disease suppression. Recently, Chen et al (8) proposed that a high rate of hydrolysis of FDA combined with high biomass may be a predictive guideline for suppression of Pythium damping-off in container media.

Suppression of soilborne pathogens due to competition for available carbonaceous nutrient is suspected if applications of amendments with nutrients negate the suppressive effect (21). Glucose/asparagine added to container media delayed the lysis of hyphae of *Pythium* in all container media. However, the rapid consumption of glucose in the compost medium resulted only in a short delay of hyphal lysis; and further applications of the amendment delayed hyphal lysis but did not negate it. Similarly, in greenhouse experiments, suppression of damping-off in the compost medium was not negated by a single amendment of nutrients; however, six consecutive applications for 3 days at 12-hr intervals resulted in a significant increase in disease incidence.

Apparently the rate of consumption of available nutrients is a key factor in the suppression of *Pythium* in compost medium. This type of suppression is a general type of competition not related to any one, specific, known microorganism (10).

In contrast to small or nonexistant differences between the compost and the conducive peat media with regard to respiration

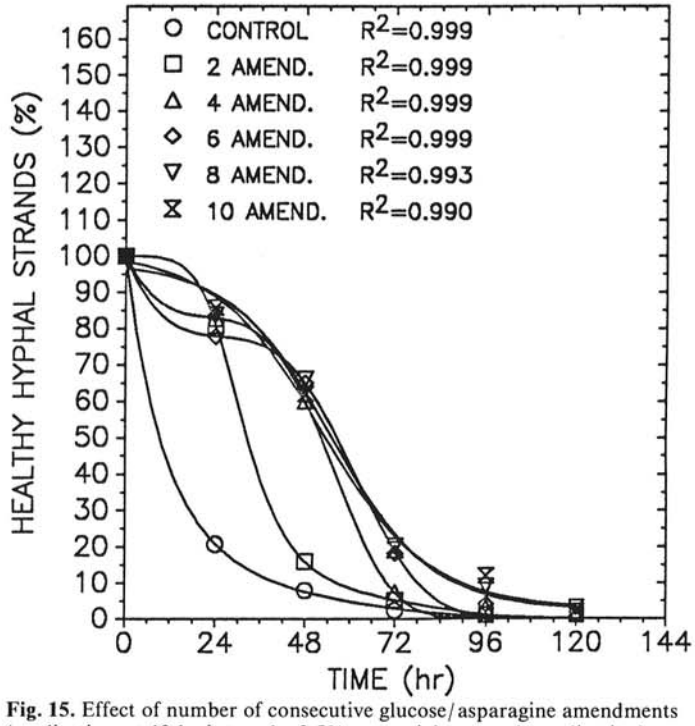

Fig. 15. Effect of number of consecutive glucose/asparagine amendments (application at 12-hr intervals, 2.5% wet weight at each application) on lysis of hyphae of *Pythium apahanidermatum* in compost container medium. Equation model used for the nonlinear regression analysis was an exponential function with position constant: $Y = B_1^{(B_2X)} + B_3$.

TABLE 1. Effect of preincubation in container media on percent germination of oospores of *Pythium aphanidermatum* after 16 hr on selective medium

Container medium	Percent germination of oospores			
	Day 1[a]	Day 3	Day 6	Day 30
Limed peat	66.5 ± 0.8[b]	90.4 ± 3.5	66.5 ± 4.9	95.2 ± 5.2
Limed peat +[c]	12.4 ± 0.4	19.9 ± 2.1	74.4 ± 7.2	83.2 ± 4.9
Peat	48.2 ± 0.8	61.6 ± 7.1	60.0 ± 5.7	18.0 ± 1.2
Peat +	21.0 ± 1.2	40.0 ± 4.1	57.0 ± 4.2	50.0 ± 4.2
Compost	51.0 ± 1.6	75.1 ± 5.2	70.0 ± 4.3	80.5 ± 8.0
Compost +	65.2 ± 5.2	84.9 ± 7.2	62.5 ± 3.5	88.2 ± 7.2

[a] Incubation time (days) in container medium, before plating oospores on Schmitthenner's selective medium (38).
[b] ± = Standard error.
[c] + = Amended with glucose/asparagine.

rate and microbial densities, differences following glucose/asparagine amendment were large. Bacterial densities in compost medium increased within 12 hr but not in the conducive peat media. The kinetics of respiration increase varied with incubation temperature; however, at both 22 and 30 C, the respiration rate increased rapidly and reached a higher maximum in the compost medium than in the conducive peat media. This indicates a longer lag period and a longer doubling time in the peat media. In fact, it seemed that the high level of $CaCO_3$ added to the limed peat medium inhibited the respiration increase. However, these differences in respiration rate were detectable only for a short period after the glucose/asparagine amendment. As soon as glucose was depleted, respiration in the compost medium decreased sharply to levels that prevailed in the system before amendment. Similar phenomena were reported by Alabouvette et al (1) in suppressive soil from the Chateaurenared region. Monitoring the amount of CO_2 evolved after glucose/asparagine amendment on the basis of 24-hr total respiration may lead to misinterpretation of data. Differences in respiration were exhibited mostly in the kinetics of the rate of increase and not in total respiration. Total respiration in the first 24 hr in the conducive peat medium was even greater than in the suppressive compost medium, probably as a result of a more efficient assimilation of carbon (higher yield coefficient) into microbial biomass in the compost medium, which is supported by the increase in bacterial populations in compost medium 12 hr after amendment as compared with populations in the peat media. Enzymatic activity, measured as FDA hydrolysis, was increased to a higher level in compost medium than in peat medium 24 hr post glucose/asparagine amendment. However, FDA hydrolytic activity was much lower in the second day following the amendment, although high microbial densities still were recorded. Four days after amendment, there was an increase in FDA hydrolysis activity in peat and compost media, probably due to an increase in fungal population density, but a similar increase was not recorded in the limed peat medium although fungal densities were high.

Glucose/asparagine amendment stimulated germination of oospores of *Pythium* in peat-based media more than in the suppressive compost medium; more germination occurred in the compost medium amended with glucose/asparagine simultaneously with the oospores than in the same medium amended 12 hr before oospores were added. It seems, therefore, that the high rate of glucose depletion in the compost medium shortened the time needed for oospore stimulation by the available nutrient, thus preventing oospore germination.

The compost medium was not suppressive to oospores. In fact, their incubation in the suppressive medium activated dormant oospores to a higher germination percentage than did the conducive peat medium. Lumsden and Ayers (24) activated dormant oospores of *P. ultimum* by incubation in nonsterile soil. Amendment of glucose/asparagine in our study increased the stimulatory effect, whereas addition of $CaCO_3$ to peat medium reduced suppression of *Pythium* as well as microbial populations and activities. Liming and the resultant neutral environment may have induced a greater variety of microorganisms to intensify their activity than in the acidic peat medium environment. As expected, $CaCO_3$ increased the initial respiration rate, FDA hydrolysis, and suppression of cucumber damping-off as compared with the peat medium. However, the stimulatory effects of a glucose/asparagine amendment on respiration rate and bacterial population were reduced by lime amendment.

Apparently, only the combination of lime with the glucose/asparagine amendment resulted in an inhibitory effect, because liming alone increased bacterial population densities and activities. Zelles et al (43) observed that lime either stimulated bioactivity or inhibited it in forest soil. Because combining liming and glucose/aspagragine amendment reduced germinability of oospores of *Pythium*, increased lysis of hyphae, and improved suppression of *Pythium* damping-off, we assumed that the production of inhibitory substances may be involved. Ammonia is a mycostatic agent in alkaline soils and in soils made alkaline by the addition of lime (32). Possibly, the amendment of glucose/asparagine induced the formation of ammonia in the limed peat medium, thereby restricting microbial activity and reducing disease as a result of inhibition of *Pythium*. The production of ammonia in low concentrations is a possible mechanism of suppression of *P. ultimum* by *Enterobacter cloacae* (17); however, we have not explored this avenue of research.

We propose that the kinetics of increase in microbial activity in the first 24 hr after glucose/asparagine amendment in combination with assessment of hyphal lysis of *Pythium* in container media by the nylon-fabric method can give a fast and accurate indication of potential suppressiveness to *P. aphanidermatum* in container media.

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