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Project Summary

Effects of Composted Municipal Sludge on Soilborne Plant Pathogens

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Efficacy of composted municipal sludge (CMS) for suppression of Phytophthora root rot of soybean in field soil and for suppression of Rhizoctonia and Pythium diseases of ornamental plants produced in container media was investigated over a 3-year period.

CMS increased yields of soybean by improving soil fertility and/or by partial control of Phytophthora root rot (PRR). Disease-enhancing effects of salt in the CMS were controlled with a selective fungicide. In the absence of the fungicide, beneficial effects were obtained only by application of CMS at least 3 months prior to planting with cultivars tolerant to PRR. Increased yields of soybeans were observed when high rates of CMS were applied to corn crops and soybeans were grown in the next year without further CMS application. No residual effects were observed from high rates of CMS applied to soybeans.

All container media prepared with CMS cured 4 months or more and stored 4 weeks after formulation became consistently suppressive to Rhizoctonia and Pythium diseases of ornamentals at levels adequate to avoid losses under commercial conditions. An unknown beneficial microflora involved in suppression, if present, survived in the outer low-temperature layer of curing piles only.

Several bacterial and fungal isolates were identified that effectively induced suppression to Rhizoctonia and Pythium "damping-off" in CMS media. The relative contributions of these microbes to the overall suppressive effects remain to be determined. Most bacterial strains were more efficacious in combination with an isolate of *Trichoderma*. A synergistic interaction was found among an isolate of *Trichoderma hamatum* and of *Flavobacterium balustinum* for suppression of Rhizoctonia damping-off. These isolates, if added to conducive CMS media, consistently rendered them suppressive in the absence of the 4-week incubation period required for development of natural suppression.

This Project Summary was developed by EPA's Water Engineering Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

A significant number of municipalities in the United States are using the composting process for further treatment of municipal sewage sludge. Composted municipal sludge (CMS) has been utilized effectively as a source of plant nutrients, and the organic matter in CMS may also improve physical and biological properties of soils.

Severity of a number of plant diseases can be affected by CMS. Some are decreased in severity, whereas others are increased as a result of field soil amendments. The mechanism of this variability is not understood. However, suppression of disease has been associated with increased microbial activity of treated soil. Several microorganisms have been isolated from composts that are involved in the suppression of soilborne diseases in soils or container media amended with composts. Their fate during composting of municipal sludges is unknown.

Phytophthora Root Rot of Soybeans

Phytophthora root rot of soybean is one of the most severe diseases of this crop in the northern soybean growing region. Approximately 10 million acres are infected and can be severely damaged in wet years. Presently, control of root rot is based on resistance of cultivars which has been overcome by new *Phytophthora* races; application of the fungicide metalaxyl (a potential ground-water pollutant) to soil; or a combination of good drainage, complete soil tillage, rotation, and metalaxyl seed treatment of highly tolerant (least susceptible) cultivars. The latter integrated control package is not available to farmers who wish to reduce soil erosion through conservation tillage.

Additional durable and nonpolluting options are needed for control of this important disease. Phytophthora diseases can be controlled by adding certain organic amendments to the soil. One way to rapidly increase soil organic matter is with manures and CMS. Manure is not readily available to farmers that focus on cash cropping. On the other hand, CMS, derived from municipal sludge, is available in many areas of the United States where soybeans are produced. CMS can be spread on fields with a conventional manure spreader, thus making widespread utilization possible. However, CMS may have a high salt content, which can create adverse effects. Both high fertilizer application rates and high chloride concentrations increase PRR of soybean.

The experiments reported here were designed to investigate three areas. First, effects of various CMS rates on root rot severity and yield of soybean in Phytophthora-infested soil were examined. Second, the effect of application timing was determined. Finally, the residual effects of CMS applied during the previous season were evaluated.

Suppression of Rhizoctonia and Pythium Diseases of Ornamentals Produced in Container Media

Composts prepared from a variety of tree barks are utilized successfully for suppression of soilborne diseases of ornamental plants produced in container media. These composts are particularly effective for control of Rhizoctonia and Pythium diseases when microbial antagonists have recolonized the compost after peak heating has occurred. The mechanisms of suppression involved vary with the pathogen, the source or type of compost used, and the level of decomposition achieved during the composting process. This portion of the study shows that container media amended with CMS can be used effectively for suppression of Thizoctonia and Phthium diseases of greenhouse and nursery crops if certain precautions are taken to overcome the detrimental effects imposed on microbial antagonists by the long-term high temperature treatment (>55°C) necessary to ensure fecal pathogen and parasite destruction. In addition, beneficial microorganisms involved in the suppression of Rhizoctonia and Pythium diseases were identified.

Objectives

The objectives of research reported here were: 1) to determine the effects of CMS on soybean PRR severity in the field; 2) to develop procedures for the formulation of container media with CMS that consistently suppress diseases caused by soilborne plant pathogens of ornamental plants; and 3) to determine the nature of the beneficial microflora involved in suppression of these diseases.

Methods

Characteristics of the Compost

Composted municipal sludge was obtained from the Columbus Southerly Composting Facility where the aerated static pile composting process has been used on a daily basis since 1980. Raw sludge cake, consisting of a 1:1 ratio (dry wt basis) of primary sludge and waste activated sludge, was dewatered with centrifuges aided by polymer as a coagulant. Sludge cake, averaging 16% to 17% total solids, was mixed with hardwood chips (1 in. to 2 in. chips) at a ratio of 5 volumes of chips to 1 volume of sludge. Mixing was accomplished with front-end loaders in a covered mixing area. The composting process generally was complete after 21 days. Piles were then processed and stacked with or without aeration for a 60-day curing period. Thereafter, the mixture was dried by passive solar drying on a concrete pad. Wood chips were recovered by screening with a 3/8-in. rotary screen. Screened compost was then stored in bays in a covered shed. A weekly composited compost analysis was associated with each bay of compost. Compost thus prepared was used throughout this work. A summary of the chemical properties of the CMS is presented in Table 1.

Soybean Phytophthora Root Rot

Field experiments were conducted for 3 years at the Northwest Branch of Ohio Agricultural Research and Development Center (OARDC) of The Ohio State University located at Custar, OH, in a Hoytville clay loam containing 50% clay, 40% silt, and 10% sand. The plot area used during the first 2 years was tiled, but gates located at the tile exits were closed to reduce drainage. In the third year, the location was changed so that the plots could be irrigated 3 days after planting to provide optimum conditions for Phytophthora infection. This field was tiled, but the tiles were old and nonfunctional.

Plots consisted of 8 rows, each 26 m long and spaced 76 mm. Each plot was split into two treatments of four rows each. In the first year the split treatments were the cultivars Sloan (some tolerance to PRR) and OX 20-1 (no tolerance to PRR). In the second and third years the split treatments consisted of the cultivar Sloan, with and without 280 g metalaxyl/ha (5.6 kg/ha of Ridomil 5G*). This systemic soil fungicide provides good control of Phytophthora when applied in the seed furrow at planting time.

CMS application rates were 0, 10, 20, and 40 tonne/ha. Application times were preplant, applied within 1 week before planting to maximize salinity effects; and early, applied at least 3 months before planting, either in February or November, to minimize salinity. To determine the fertility effects

*Mention of trade names or commercial products does not constitute endorsement or recommendation for use

Table 1 *Characteristics of Municipal Sludge and Composted Municipal Sludge from the Columbus Southwesterly Compost Facility*

	Sludge*		Composted Sludge	
	Mean	Range	Mean	Range
Volatile Solids %	71	57-79	59	43-74
Conductivity (mmhos/cm)†			10.5	7.5-15.0
Cl (µg/mL			310	245-360
pH	5.8	5.4-6.3	7.5	6.2-8.4
N (%, total Kjeldahl)	6.5	3.2-8.4	4.5	3.2-5.3
P (%)‡	3.2	1.6-5.8	3.3	2.5-4.7
K (%)	1.5	0.2-2.2	1.6	0.9-1.9
Ca (%)			3.6	3.4-3.8
Mg (%)			0.86	0.84-0.87
Cd (mg/kg)	8.8	2.7-26	20	13-24
Cr (mg/kg)	131	72-290	262	150-325
Cu (mg/kg)	218	130-270	255	190-640
Pb (mg/kg)	170	92-560	274	160-320
Ni (mg/kg)	45	18-74	85	54-111
Zn (mg/kg)	1,057	550-1,900	1,664	1,000-1,971
Na (mg/kg)			776	699-827

*The sludge was a 1:1 mixture of raw and waste activated sludges. Means are based on 64 samples. Analyses performed by City of Columbus Central Surveillance Laboratory.
†Determined in a saturated paste extract.
‡All metal concentrations were determined after nitric perchloric acid digestion.

of CMS, plot soil was brought into the laboratory, mixed with the equivalent of 0, 10, 20, and 40 tonne dry wt CMS/ha, planted with soybeans under conditions that promote maximum Phytophthora losses, and, after 2 weeks, submitted to the Research Extension Analytical Laboratory (REAL), OSU, for fertility analysis (Table 2). Based on this analysis, a fertility control preplant treatment was applied consisting of superphosphate (200 kg P_2O_5/ha) and NaCl (327 kg/ha) which was the soluble salt equivalent of 20 tonne/ha CMS.

Disease analysis consisted of counts of healthy and diseased plants 5 weeks after planting. In late October, the weights and percentage moisture of the seed from the two center rows of each plot were taken to determine effects of treatments on yield. All treatments were replicated four times. Data were subjected to an analysis of variance with application times and CMS rates as whole plots and cultivars or metalaxyl soil fungicide treatment as split plots.

Preparation of Container Media

A CMS container medium was prepared by mixing CMS with Canadian sphagnum peat and perlite (1:2:1,v/v, pH 5.5). This medium was not amended with fertilizer since sludge compost media release adequate amounts of plant nutrients for at least 6 weeks after potting. A peat container medium was prepared from Canadian sphagnum peat and perlite (1:1, v/v) and adjusted to pH 5.5 with a 2:1 (w/w) mixture of dolomitic and hydrated lime. It was fertilized to adjust fertility to levels identical to that in the CMS medium. The peat medium is conducive to Rhizoctonia and Phthium damping-off and, therefore, served as a control in this work.

Suppression of Pythium and Rhizoctonia damping-off was determined with cucumber and radish bioassays, respectively. Inocula of *Rhizoctonia solani* and of *Pythium ultimum* were added at rates of 0.5 g/L container medium. This amount of inoculum killed 50% of the plants in a suppressive medium and all plants in a totally conducive peat medium. Mean disease severity ratings were determined for various sludge compost and peat media to compare efficacy of CMS for suppression of these diseases.

Table 2. *Fertility Factors in Hoytville Silty Clay Affected by Applications of Composted Municipal Sludge*

Rate (dry tonne/ha)	kg/ha					
	P	K	Mn	Zn	B	NO_3
0	134	498	19	96	1.8	340
10	214	501	20	105	2.1	370
20	353	545	28	139	2.4	532
40	575	642	39	222	3.1	578
LSD (0.05)	93	55	8	58	0.26	120

Table 3. *Effect of Composted Municipal Sludge on Yield (kg/ha) of Soybeans Under Mild Phytophthora Root Rot Pressure in 1984*

Rate (dry tonne/ha)	Application Time	Variety: Sloan (susc)	Variety: OX 20-8 (very susc)	Means
		Yield (kg/ha)		
0	Feb 84	2651	1959	2305
10	Feb 84	2590	1953	2272
20	Feb 84	2849	1863	2356
40	Feb 84	3345	2157	2751
0	May 84	2779	2235	2507
10	May 84	2657	2076	2366
20	May 84	2923	1581	2252
40	May 84	3087	1558	2322
Salt Control*	May 84	2563	1612	2087
LSD = 0.05		499		256

*200 kg of P_2O_5 and 327 kg NaCl/ha, the soluble salt equivalent of 20 tonne CMS/ha

Interactions Among Beneficial Microorganisms in Container Media

Bacteria and fungi were isolated by baiting with radish and cucumber roots produced in CMS media or with Rhizoctonia and Pythium soil propagules incubated in sandwiches in the suppressive media. Pure cultures of 650 bacterial isolates and 30 fungal isolates were then tested for their abilities to induce suppression to Rhizoctonia and Pythium damping-off using the bioassays described above. Spontaneous rifampicin-resistant mutants were isolated from the most effective bacterial strains. The root colonizing potential of these mutants was then tested. Finally, various mixtures of bacterial antagonists with strain 382 of *Trichoderma hamatum* were examined to reveal synergistic interactions among antagonists in CMS media.

Results

Soybean PRR

In 1984, the 40 tonne dry wt CMS/ha treatment applied in early February significantly increased the yield of the moderately susceptible cultivar Sloan (Table 3). Yield differences were not found in the highly susceptible cultivar, OX 20-8. There were no differences in stand or plant death among any of the variety-treatment combinations. The lack of yield response in the highly susceptible OX 20-8 could be explained by greater hidden root rot damage offsetting a potential fertility response.

High rates of CMS applied preplant (May) did not increase yield of the moderately susceptible cultivar Sloan and also significantly reduced the yield of highly susceptible OX 20-8. These results were not accompanied by significant differences in stand or plant death from PRR. The salt-phosphate treatment significantly reduced the yields of both cultivars. This agrees with previous findings for effects of chloride on soybean PRR severity. The salt content of CMS applied preplant probably increased hidden root rot, which prevented a yield response in Sloan and decreased yield in OX 20-8. The yield of Sloan in the high CMS treatment was significantly better than that in the salt-phosphate treatment, indicating a response to organic matter even in the presence of high salts. Cultivar OX 20-8 did not respond to organic matter in the presence of salt. Thus, the response of cultivars to organic matter may have been associated with their level of susceptibility to Phytophthora.

In conclusion, the 1984 season demonstrated that the yield response of soybeans may depend on susceptibility to PRR and application time. Possible negative effects of high salt in CMS could be eliminated by early application. To separate the fertility and disease severity effects, experiments for 1985 and 1986 were changed to incorporate control of Phytophthora with Ridomil, a specific fungicide. Ridomil applied in the furrow with the seed at planting time controls PRR damage in all but the most susceptible cultivars.

In 1985 there were no differences between Ridomil-treated and untreated plots. The salt-phosphate treatment had no effect on yield. Thus, in 1985, PRR did not occur. Both the 20 tonne/ha and 40 tonne/ha rates of CMS significantly increased yield (Table 4). The beneficial fertility effects of CMS were clearly evident in this year in the absence of Phytophthora damage.

Residual effects of CMS applied in 1984 were evaluated in 1985. There were no differences among any of the CMS rates in soybean-soybean plots (Table 4). All yield levels were low. In the corn-soybean sequence, the high rate of CMS (40 tonne/ha) applied to corn in 1984 significantly increased the yield of soybean in 1985. Thus, the residual effects of CMS were only evident if applied to corn, which is the logical time to apply CMS in a cropping system.

In 1986, only two rates of CMS, 0 and 40 tonne dry wt/ha, were compared. Ridomil, application time, and salt-phosphate treatments also were included.

Table 4. *Effect of Composted Municipal Sludge on Yield (kg/ha) of Soybean Under Phytophthora-Free Conditions in 1985*

Sludge (tonne/ha)	Crop and Time of Application: Feb 84 Soybean	May 84 Soybean	May 84 Corn	Nov 84 Soybean	May 85 Soybean
0	3073	2898	3370	3208	3208
10	3086	2965	3423	3235	3316
20	3060	2979	3423	3504	3693
40	3154	3113	3558	3693	3706
Salt Control*	-	2979	3370	-	3383
LSD (0.05) = 270 Kg					

*200 kg of P_2O_5 and 327 kg NaCl/ha, the soluble salt equivalent of 20 tonne CMS/ha.

The crop was irrigated with 10 cm water over a 2-day period approximately 3 days after planting when the seedling tap roots were about 5 cm long. Flooding induced conditions favoring PRR. Yield levels were not high in this experiment because of a 9-week drought between July and September and fertility benefits were not evident. However, a significant disease response to treatments was evident.

Preemergence and postemergence damping-off occurred in plots not treated with Ridomil (Table 5). Stands were poorest in plots with CMS applied preplant and best in plots treated with CMS early. Ridomil controlled damping-off and resulted in good stands and improved yields in all sludge treatments. In plots not treated with Ridomil, only the early CMS application significantly increased yields compared to the preplant application and the controls. Under this severe disease pressure, early application of CMS increased yields over the control as much as treatment with Ridomil. Response from CMS and Ridomil was not additive, possibly because the late season drought prevented a fertility response. The 1986 data confirm the importance of time of application of CMS.

The following conclusions on PRR in soybeans can be drawn from these data.

1. CMS applied just prior to planting may increase Phytophthora damage. Yields will be increased or decreased depending upon severity of Phytophthora.
2. A salt-phosphate mixture equivalent to fertility in CMS, has the same effect as CMS applied immediately before planting.
3. CMS applied 3 to 6 months before planting reduced Phytophthora and increased yield.
4. CMS applied in the absence of Phytophthora increases yields even in very susceptible cultivars.
5. Response to 40 tonne dry wt CMS/ha is better than to 20 or 10 tonne dry wt/ha.
6. Ridomil applied at recommended rates reverses the negative effects of CMS applied immediately before planting.

Effect of Compost Curing Time on Suppressiveness of CMS

All batches of CMS stored in curing piles with temperatures in the center of the pile higher than 60°C and formulated into a CMS medium, were consistently suppressive to Pythium damping-off. However, these CMS media varied in their effects on Rhizoctonia damping-off. CMS cured 4 months or more in piles and formulated into media, became consistently suppressive to Rhizoctonia damping-off within an additional 4 weeks of storage.

The environment in which the compost was cured affected suppressiveness. CMS stored next to piles of bark compost suppressive to Rhizoctonia damping-off, became suppressive earlier than CMS stored in isolated locations. Microorganisms isolated from the suppressive CMS that could cause this effect were *Trichoderma* spp. and a number of bacterial antagonists. Without this recolonization of CMS with antagonists of *R. solani*, suppressiveness did not develop.

Table 5. *Effect of Composted Municipal Sludge on Stands (plants/26 m of Row Spaced 76 cm) and Yields (kg/ha) of Sloan Soybean Under Severe Phytophthora Root Rot Pressure in 1986*

Sludge (tonne/ha)	Time of Application	Stand (plants/26 m rows): No Ridomil	Ridomil	Mean	Yield (kg/ha): No Ridomil	Ridomil	Mean
0	Feb 86	211	352	281	984	2420	1705
40	Feb 86	226	318	272	2056	2615	2332
0	May 86	176	346	261	627	2251	1442
40	May 86	105	312	209	512	2736	1624
Salt Control*	May 86	207	354	280	815	2440	1624
LSD (0.05)			89	52		607	337

*200 kg of P_2O_5 and 327 kg NaCl/ha, the soluble salt equivalent of 20 tonne CMS/ha.

Table 6 *Suppressiveness of a Nursery Sludge Compost and a Conducive Peat Container Medium to Pythium and Thizoctonia Damping-Off Over a 2-year Period*

Container Medium	Pathogen* Treatment	Disease Severity Rating† Pythium May	July	Nov	Oct	Rhizoctonia May	July	Nov	Oct
Sludge	Check	1.1	1.0	1.0	1.1	1.5	1.2	1.2	1.1
Compost	Infested	1.8	2.6	2.3	2.1	3.7	2.6	2.8	2.9
Peat	Check	1.2	1.1	1.7	1.2	1.3	1.3	1.7	1.1
Control	Infested	3.9	4.0	3.5	3.8	3.9	3.9	3.6	3.8
LSD (P = 0.05)				0.8			0.3		

*Infested with 0.5 g Rhizoctonia solani or Pythium ultimum soil inoculum per L container medium.
†Disease severity ratings: 1 = symptomless; 2 = diseased but not damped-off. 3 = postemergence damping-off; 4 = preemergence damping-off, based on five pots each planted with 32 radish seed. May, July and November represent 1984. October represents 1985 values.

Suppression on Diseases During the Production of Potted Crops

Pythium diseases caused by *P. ultimum* or *P. aphanidermatum* in floricultural pot crops were suppressed in CMS media throughout a 5-month production period. Similarly, Pythium root rot caused by *P. irregulare* in nursery crops was suppressed over a 2-year production period (Table 6). Diseases caused by *R. solani* were also suppressed in both floricultural (Figure 1) and nursery crops (Table 6), but only consistently if the formulated container medium was stored 4 weeks before utilization.

It was concluded that CMS becomes naturally suppressive to Pythium damping-off but that 4 months of curing is required followed by an additional 4-week storage period of the formulated medium required to ensure suppressiveness to Rhizoctonia damping-off.

Efficacy of Microbial Antagonists

T. hamatum 382 added as an antagonist by itself to a conducive CMS medium did not induce a significant level of suppression to Rhizoctonia damping-off (Table 7). However, a combination of

Table 7. *Suppression of Rhizoctonia Damping-Off Induced by Bacterial Antagonists Alone and in Combination with* Trichoderma hamatum *in a Sludge Compost Container Medium*

Bacterial Antagonist*	Disease Severity Rating† Bacterial Antagonist Alone	Bacterial Antagonist with T. hamatum
None	2.9	2.6
Bacillus cereus *106*	3.0	2.0
Enterobacter cloacae *127*	2.6	2.6
D. cloacae *313*	2.1	2.1
Flavobacterium balustinum *299 (ATCC53198)*	3.1	2.1
Janthinobacterium lividum *275*	2.7	2.5
Pseudomonas fluorescens *biovar III, A91*	2.9	2.4
P. fluorescens *biovar V, A1*	2.4	2.6
P. fluorescens *biovar V, A498*	2.7	2.3
P. putida *305*	2.2	1.8
P. putida *315*	2.3	2.1
P. putida *371*	2.3	2.5
P. stutzeri *280*	2.4	2.1
Xanthomonas maltophilia *76 (ATC53199)*	3.0	2.4
LSD 0.05	0.4	

*Bacterial antagonists were added to the heated (60°C, 5 days) sludge compost container medium at initial population levels of 10^6 CFU·g dry wt T. hamatum was added at 10^4 CFU g dry wt.
†Infested with 0.5 g Rhizoctonia solani soil inoculum·L container medium. Mean disease severity rating determined 7 days after incubation at 26°C from five pots planted with 32 radish seeds each. 1 = symptomless; 2 = diseased but not damped-off; 3 = postemergence damping-off; and 4 = preemergence damping-off. Mean disease severity in the container medium not infested with R. solani was 1.2.

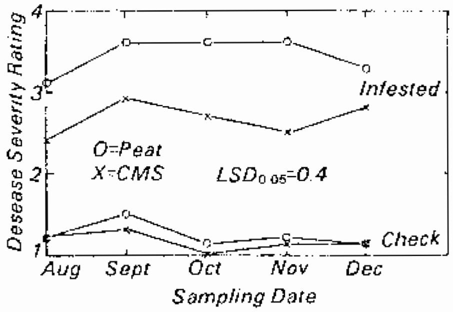

Figure 1. *Suppressiveness of a peat and a sludge compost medium (CMS) to* Rhizoctonia *damping-off during production of poinsettia plants. Disease severity rating: 1=symptomless; 2=diseased but not damped-off; 3=post-emergence damping-off; and 4=preemergence damping-off. Infested with 0.5 g* Rizoctonia inoculum *per liter container medium. $LSD_{0.05}$ for all sampling times combined=0.4.*

Flavobacterium balustinum strain 299 with *T. hamatum* 382 significantly (P = 0.05) reduced damping-off, even though each bacterial antagonist by itself was not effective. This bacterial antagonist significantly enhanced population development of the *T. hamatum* strain early after its addition to the compost-amended medium (Figure 2). A synergistic effect was established, therefore. This effect occurred in compost cured 4 months that was conducive if not infested with the antagonists. Several other combinations of bacterial antagonists and *T. hamatum* 382 were identified that had similar synergistic interactions in suppression of Pythium damping-off in the CMS medium.

It was concluded that microbial antagonists can be utilized to develop predictable levels of suppression to Rhizoctonia damping-off in container media amended with conducive CMS. This process eliminates the need for storage of formulated CMS media.

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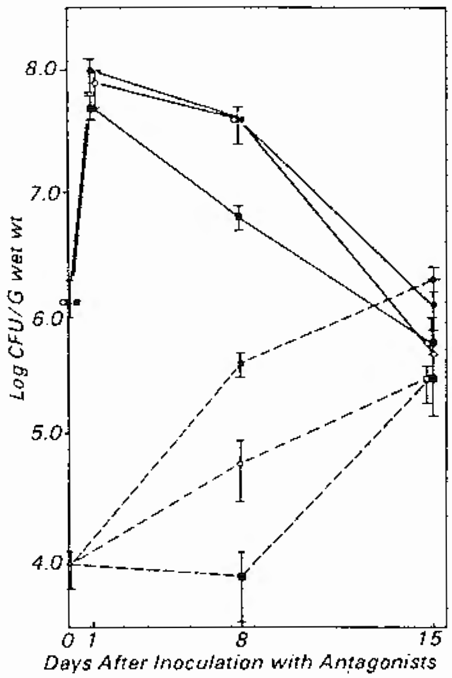

Figure 2. *Population development of* Flavobacterium balustinum *299R (———) and* T. hamatum *382 (- - - -) in a container medium planted with cucumber and amended with compost* F. balustinum *and* T. hamatum *were inoculated individually in a container medium amended with (1) conducive compost from the high temperature center of the curing pile (O); (2) suppressive edge compost (■); or (3) were introduced as a combination treatment in the conducive center compost medium (●). Vertical bars indicate standard error.*

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The complete report, entitled "Effects of Composted Municipal Sludge on Soilborne Plant Pathogens," (Order No. PB 88-195 714/AS; Cost: $14.95, subject to change) will be available only from:
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