Microbial Activity and Biomass in Container Media for Predicting Suppressiveness to Damping-Off Caused by *Pythium ultimum*

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ABSTRACT

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Predictive guidelines, based on general microbial activity and biomass, were developed for the formulation of container media suppressive to cucumber damping-off caused by *Pythium ultimum*. Disease severity correlated negatively with microbial activity, based on the rate of hydrolysis of fluorescein diacetate, and with microbial biomass, based on extractible phospholipid phosphate content in the container media. A preliminary mathematical model for predicting Pythium damping-off severity, based on both microbial activity and biomass, is proposed, and possible applications and potential limitations are discussed.

Container media used for the production of greenhouse and nursery crops vary in their effects on *Pythium* diseases. Those amended with peat as the sole source of organic matter generally are conducive to seedling disease (3,12,19–21,26). However, a suppressive source of light sphagnum peat from Finland has been described that may remain suppressive to diseases caused by *Pythium* for several weeks after potting (27). Conversely, container media amended with composted tree barks or other types of compost suppress those diseases at potting and up to more than a year thereafter (11).

The mechanisms of suppression of *Pythium* diseases in both the suppressive peat and compost-amended media are biological in nature (3,7,9,27). Seed and root exudates are the principal sources of nutrients affecting activity of *Pythium ultimum* Trow in container media (4). This agrees with similar but earlier findings for field soils (24,25). Chen et al (4) showed that coexistence of large populations of mesophilic microorganisms, high microbial activity and biomass, low concentrations of extractible nutrients, and a high degree of microbiostasis characterized bark compost media suppressive to Pythium damping-off. In this paper, correlations were established between disease severity and microbial activity and biomass in a wide range of container media, with the expectation of developing predictive guidelines for formulation of container media suppressive to Pythium damping-off.

MATERIALS AND METHODS

Container media. Container media used were peat medium 1, consisting of Canadian sphagnum peat, vermiculite, perlite, and granite sand (Metro Mix 200, W. R. Grace Co., Cambridge, MA); peat medium 2, consisting of Canadian sphagnum peat and perlite (Pro Mix, Premier Brands, Inc., New Rochelle, NY); a pine-bark ash medium, consisting of processed pine-bark ash, Canadian sphagnum peat, vermiculite, and granite sand (Metro Mix 350, W. R. Grace Co.); composted pine-bark medium, consisting of composted pine bark, vermiculite, Canadian sphagnum peat, and perlite (Growing Mix 2, Pro-Gro Products, Inc., Elizabeth City, NC); composted hardwood bark medium, consisting of composted hardwood bark, Canadian sphagnum peat, and perlite (4); and composted municipal sludge medium, consisting of composted municipal sludge, Canadian sphagnum peat, and perlite, as described before (3). The composted hardwood bark and sludge media were prepared and stored up to 4 wk before use to ensure that they were fully colonized by mesophilic microorganisms and suppressive to Pythium damping-off (4). The rate of percolation in all container media was $>$ 2.5 cm/min. Air-filled pore space levels were $>$ 15% at container capacity (10-cm-tall column). Physical properties related to drainage in these container media, therefore, were assumed to be similar and not to affect disease development differentially.

Suppressiveness bioassays. Suppressiveness of container media to Pythium damping-off was determined with the cucumber seedling bioassay (3). Container media were placed in plastic bags (2 L/bag). After 10 subsamples were taken for microbial biomass and activity assays (see below), the container media were infested with inoculum of *P. ultimum* (0.75 g/L). The inoculum was prepared in a chopped potato soil mix (3). The infested container media then were distributed into five pots (about 400 ml per pot). Each pot was planted with eight cucumber seeds (1 cm deep). The pots were completely randomized in a temperature-controlled growth chamber (20 C) and watered daily. Disease severity was determined 10 days after planting, according to the following scale: 1 = symptomless; 2 = emerged, but diseased seedling (wilted or with visible lesions on the hypocotyl); 3 = preemergence, and 4 = postemergence damping-off. A mean disease severity of eight seedlings in a pot was calculated to represent one replication (five replicates per treatment). This experiment was performed three times. The sludge medium was used only once due to unavailability.

Microbial biomass and activity. Samples for microbial biomass and activity assays were taken immediately before the container media were infested with inoculum of *P. ultimum* and before suppressiveness bioassays were set up. Four 10-g samples were taken for extraction and determination of microbial biomass. One 10-g sample was taken for dry-weight determination and five 5-g samples were taken for estimation of microbial activity (including one sample as a blank). Thus, four replicates were used in each microbial biomass and activity assay.

Microbial biomass in container media was assessed by extractible phospholipid phosphate, as proposed by White et al (30). This procedure, that also was used previously to determine biomass in container media and in composts (4,18), consists of extraction, separation, and phosphate determination essentially as described before (4). However, the phase separation step was modified to accommodate the large number of samples. Phase

separation was carried out by centrifugation (5,000 *g*, 10 min), rather than in separatory funnels.

Microbial activity in container media was expressed as the rate of hydrolysis of fluorescein diacetate (FDA) (4,23). Because container media varied in their fluorescein adsorption characteristics, standard curves were constructed for each container medium by the procedure described previously (4). Both microbial activity and biomass were expressed on a per cubic centimeter (cc) of container medium at container capacity rather than on a dry-weight basis because the media varied in their bulk density by a factor of two or more. The relation of dry weight to volume at container capacity for each container medium was established previously.

To determine the relationship between disease severity and microbial biomass and microbial activity, as well as the relationship between microbial biomass and activity, regression analyses were performed with the MINITAB computer program. Regression lines, equations, and correlation coefficients are presented with scattergrams.

RESULTS

Relationship between disease severity and microbial biomass and microbial activity. The six container media varied in their suppressiveness to Pythium damping-off. Peat media 1 and 2 and the bark ash medium were conducive, with mean disease severity values of 3.7, 3.3, and 3.7, respectively (Table 1). The composted pine-bark medium, the composted hardwood bark, and the sludge media were suppressive to the disease, with disease severity values of 1.3, 1.5, and 1.1, respectively (Table 1).

Peat medium 1 and the bark ash medium had the lowest amounts of biomass (Table 1). Highest biomass values were found in the bark and sludge compost media. Microbial biomass levels in peat medium 2 and in the composted pine-bark medium were intermediate. The composted hardwood bark medium had the highest microbial activity (Table 1). The bark ash medium had the lowest microbial activity. The results of the trial with six container media are shown in Table 1. The other two trials in which only five container media were used are not included in the table. All data points were used in regression analyses.

Regression analysis showed that disease severity was negatively correlated with microbial biomass ($r = -0.834$, $P < 0.01$) (Fig. 1) and with microbial activity ($r = -0.780$, $P < 0.01$) (Fig. 2). A positive correlation was found between microbial activity and biomass ($r = 0.839$, $P < 0.01$) (not presented). Residual plots after the three regression analyses showed random distributions (not presented).

Regression analyses were performed with data on disease severity, microbial biomass, and microbial activity collected in our laboratory over the past 2 yr, including data on these container media that were published previously (4). Forty-nine samples, ranging from highly conducive to highly suppressive, were used in the analyses. Disease severity for all data was significantly and negatively correlated with microbial biomass ($r = -0.593$, $P < 0.001$) (Fig. 3). Disease severity also was significantly and negatively correlated with microbial activity ($r = -0.770$, $P < 0.001$) (Fig. 4). An alternative analysis of the relationship between disease severity and microbial activity, which excludes the four data points with the highest activity, is indicated by the broken line in Figure 4. Multiple regression of disease severity on microbial activity and biomass for all data points yielded the following equation:

$$Y = 4.20 - 2.44\ X_1 - 7.76\ X_2$$

where Y is disease severity in the 1 to 4 rating scale; X_1 is microbial activity in microgram hydrolyzed FDA min^{-1} cc^{-1}; and X_2 is microbial biomass in micromoles phospholipid phosphate per cubic centimeter container medium. The residual plot after the regression analysis showed a random distribution (Fig. 5). The multiple correlation coefficient equaled 0.81.

TABLE 1. Pythium damping-off severity of cucumber and microbial biomass and activity in container media

Container medium[a]	Disease severity[b]	Microbial biomass[c]	Microbial activity[d]
Peat 1	3.7 ± 0.4	0.018 ± 0.007	0.48 ± 0.06
Peat 2	3.3 ± 0.4	0.043 ± 0 003	0.25 ± 0.01
PBA	3.7 ± 0.2	0.014 ± 0.003	0.15 ± 0.02
CPB	1.3 ± 0.3	0.051 ± 0.008	0.51 ± 0.05
CHB	1.5 ± 0.3	0.124 ± 0.004	1.23 ± 0.04
CMS	1.1 ± 0.2	0.108 ± 0.003	0.74 ± 0.03

[a] Container media were peat medium 1 (Peat 1), peat medium 2 (Peat 2), processed bark ash (PBA), composted pine bark (CPB), composted hardwood bark (CHB), and composted municipal sludge (CMS).

[b] Disease severity based on the following scale: 1 = symptomless; 2 = emerged, but diseased; 3 = preemergence; and 4 = postemergence damping-off. Means ± standard error ($N = 5$).

[c] Microbial biomass, based on total extractible phospholipid phosphate (micromole per cubic centimeter [cc] container medium). Means ± standard error ($N = 4$).

[d] Microbial activity, based on hydrolysis of fluorescein diacetate (microgram hydrolyzed FDA min^{-1} cc^{-1} container medium). Means ± standard error ($N = 4$).

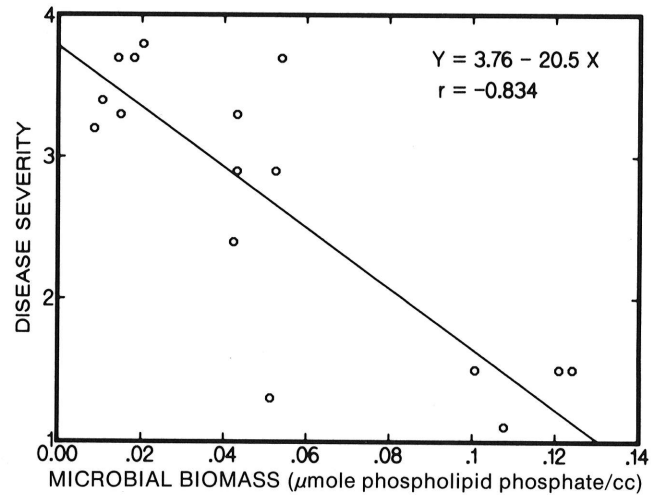

Fig. 1. Relationship of disease severity to microbial biomass based on extractible phospholipid phosphate in six container media. Each data point represents the mean of five replications of disease severity and the mean of four replications for microbial biomass for each treatment. cc = cubic centimeter.

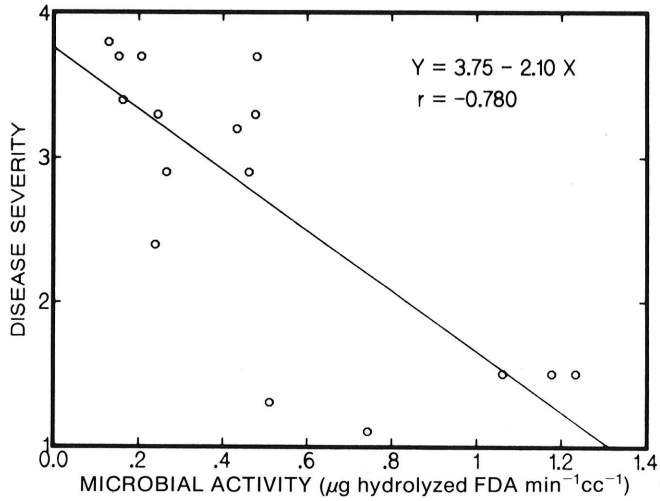

Fig. 2. Relationship of disease severity to microbial activity based on the rate of hydrolysis of fluorescein diacetate in six container media. Each data point represents the mean of five replications of disease severity and the mean of four replications for microbial activity for each treatment. cc = cubic centimeter.

DISCUSSION

The relationship between microbial activity and disease suppressiveness in the six container media used (Figs. 1 and 2) further supports our previous conclusion (4) that microbial activity and biomass in container media, based on the rate of hydrolysis of FDA and extractible phospholipid phosphate, play an important role in suppression of Pythium damping-off. These findings substantiate earlier speculations on the role of microbial activity and biomass in *Pythium* disease severity. For example, Watson (29) examined effects of lettuce residues on disease severity caused by *P. ultimum*. Although populations of *P. ultimum* and disease incidence increased initially, disease severity caused by *P. ultimum* eventually decreased with time. Because populations of *P. ultimum* remained unchanged, Watson (29) proposed that the reduction in inoculum potential was due to enhanced competition from other soil microbes stimulated by the lettuce residues. Lumsden et al (15) found that *Pythium* diseases were suppressed 1 yr after compost application in the field. Because survival of the *Pythium* pathogens was not affected, they attributed suppression to enhanced soil microbial activity resulting from the compost amendment. Recently, Lumsden et al (16) related suppressiveness of lettuce drop caused by *Sclerotinia minor* in soil amended with the same compost to increased dehydrogease activity and other soil factors. Our work represents the first quantitative effort that relates suppressiveness to Pythium damping-off with microbial activity and biomass. It should be possible to use this methodology to evaluate such interactions in field soil. Procedures used to assess microbial activity and biomass in these container media were used previously in soil samples (23,30), but not in relation to a plant disease.

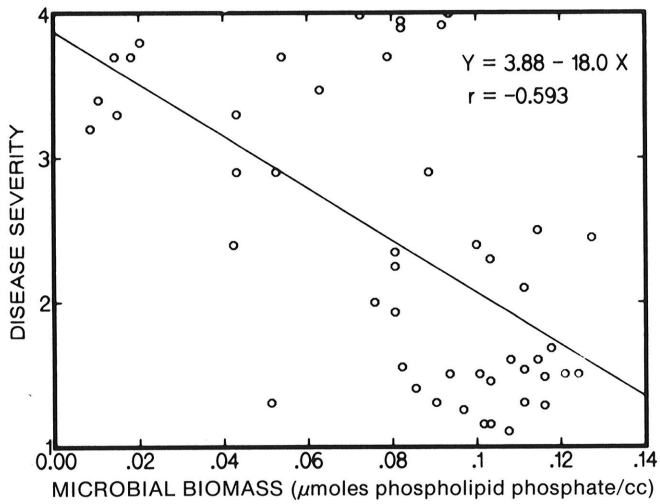

Fig. 3. Relationship of disease severity to microbial biomass based on extractible phospholipid phosphate in 49 container-media samples. Each data point represents the mean of five replications of disease severity and the mean of four replications for microbial biomass for each treatment. cc = cubic centimeter.

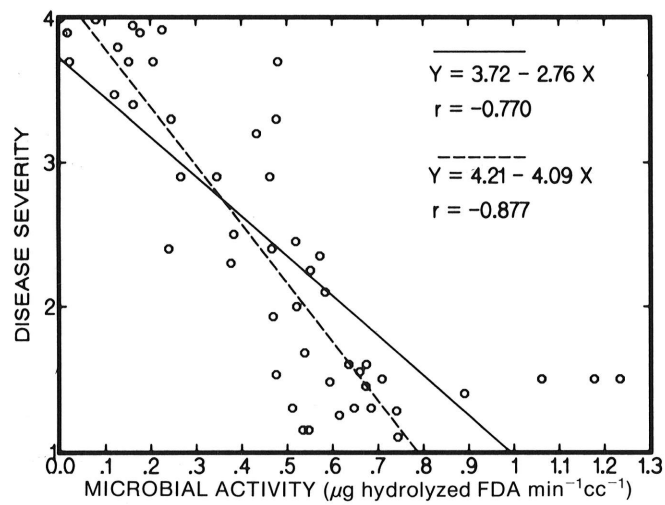

Fig. 4. Relationship of disease severity to microbial activity based on the rate of hydrolysis of fluorescein diacetate in 49 container-media samples. Each data point represents the mean of five replications of disease severity and the mean of four replications for microbial activity for each treatment. Solid line is based on all data points. Broken line excludes four data points with the highest microbial activity. cc = cubic centimeter.

A number of microorganisms have been reported to be biocontrol agents for suppression of Pythium damping-off (8). Possible mechanisms proposed for this activity include mycoparasitism (13,28), antibiosis (10), and nutrient competition (5,17). The principal mechanism responsible for suppression varies in different situations. In our studies, data suggest (Figs. 3–5) that the general microflora in container media played a predominant role in suppression of Pythium damping-off. We propose, therefore, that general microbial activity and biomass, as determined by FDA hydrolysis and phospholipid phosphate, respectively, are main predictors of suppressiveness of container media to damping-off of cucumber caused by *P. ultimum*. We also propose that the preliminary models developed here be tested further for container media in field settings, and that they be explored for soil systems.

In this study, neither microbial activity nor microbial biomass alone can be used to successfully predict suppressiveness of individual batches of container media. For example, microbial biomass in peat medium 2 and the composted pine bark (CPB) medium, which are conducive and suppressive media, respectively, did not differ. Likewise, microbial activity in peat medium 1 is not different from that in composted pine bark (Table 1). However, the combination of microbial activity and biomass would improve accuracy of prediction. The low values of coefficient of correlation between disease severity and microbial biomass in Figure 3 is largely due to the presence of thermophilic microorganisms in the center of compost piles, as discussed in a previous publication (4).

All mathematical models have limitations. This applies to our work as well. Microbial activity is indicative of suppressiveness only when the container medium itself is not stimulatory to population development of *Pythium* spp. For example, high nutrient concentrations that stimulate populations of *Pythium* spp. can overcome the suppressive effect (2,4,17,22,25,29). Water relationships also play an important role in development of *Pythium* diseases (6). For example, high moisture conditions, such as flooding, possibly may overcome suppressiveness despite high microbial activity and biomass. Procedures predicting Pythium damping-off severity may be limited to greenhouse and nursery

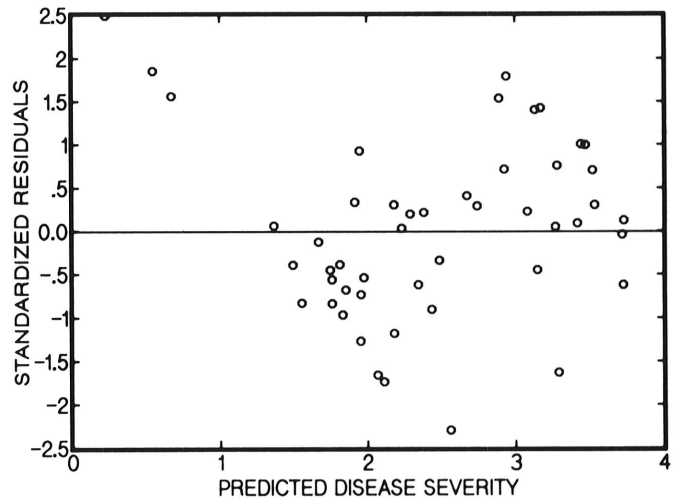

Fig. 5. Plot of standardized residuals versus predicted disease severity after multiple regression analysis of disease severity on microbial activity, and biomass.

crop conditions for plants produced in container media where such factors can be controlled.

The proposed methodology may be particularly useful for *Fusarium* spp. and other pathogens sensitive to fungistasis (14). Alabouvette et al (1) related changes in soil microbial activity (based on the rate of respiration following glucose amendments) to suppression of Fusarium wilt. Procedures used in our work that determine microbial biomass and activity, as well as activity per unit biomass (4), should allow detection of quantitative changes in the "general suppressive" nature of such soils. These procedures also may reveal quantitative differences within soils with time or among soil types and, thus, elucidate the environment in which biocontrol agents must compete.

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