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Comparison of an *in vitro* and a damping-off assay to test soils for suppressiveness to *Pythium aphanidermatum*

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Abstract

Testing of soil samples in greenhouse assays for suppressiveness to soilborne plant pathogens requires a considerable investment in time and effort as well as large numbers of soil samples. To make it possible to process large numbers of samples efficiently, we compared an *in vitro* growth assay with a damping-off assay using *Pythium aphanidermatum* as the test organism on tomato seedlings. The *in vitro* test compares the radial growth or relative growth of the fungus in soil to that in autoclaved soil and reflects suppressiveness of soils to the pathogen. We used soils from a field experiment that had been farmed either organically or conventionally and into which a cover crop (oats and vetch in mixture) had been incorporated 0, 10, 21, and 35 days previously. We obtained a significant, positive correlation between damping-off severities of tomato seedlings in damping-off assays and both relative and radial growth *in vitro*. In addition, radial and relative growth of *P. aphanidermatum* in the *in vitro* assay were positively correlated with several carbon and nitrogen variables measured for soil and incorporated debris. We did not find differences between the two farming systems for either growth measures of *P. aphanidermatum* or disease severities on tomato at different stages of cover crop decomposition. The *in vitro* assay shows potential for use with any fungus that exhibits rapid saprophytic growth, and is most suitable for routine application in suppressiveness testing.

Introduction

Soils in which the development of specific soilborne diseases is impeded are generally called disease suppressive soils (Schroth and Hancock, 1982). A more precise definition is that disease suppression occurs when less disease is caused than would be expected in the presence of a susceptible host and a virulent plant pathogen under conditions normally conducive for infection (Hornby, 1983). Disease suppression can take place at various stages in the life cycle of a pathogen: a reduction in survival of resting structures and mycelium in host debris, inhibition of germination of spores or resting structures, a reduction in growth in the rhizosphere and on the root surface, or inhibition of penetration into the root (Schneider, 1982). Some level of disease suppression is present in all soils which can be demonstrated by comparing disease severity in natural field soil and sterilized soil both artificially infested with a pathogen. However, the level of suppression varies for different soils and management regimes. For instance, root diseases are generally less severe in organically farmed than in conventionally farmed crops (Workneh, 1993; Van Bruggen, 1995). Suppressiveness is a relative quality, in that even in soils in which suppressiveness only occurs to a limited extent (conducive soils), soilborne pathogens are unable to express their full potential as causal organisms of disease (Schroth and Hancock, 1982).

Many approaches have been developed to measure the suppressiveness of a particular soil. Suppressiveness tests can be performed with pathogens and their host plants or with pathogens *in vitro*. Most tests for disease suppression involve addition of a pathogen to a soil, either at different inoculum densities or at one

density added to both sterilized and nonsterilized field soil. In order to compare suppressiveness of different soils, usually a relative measure of suppressiveness of each soil type is calculated by comparing disease severity in natural field soil with that in the same soil after sterilization by autoclaving or gamma-irradiation, both amended with propagules of the pathogen of interest (Alabouvette, 1986). We recently tested soils from organic and conventional farms for suppression of corky root of tomatoes (caused by *Pyrenochaeta lycopersici* Schneider and Gerlach) using gamma-irradiated and nonirradiated soil artificially infested with the pathogen (Workneh and Van Bruggen, 1994). The relative increase in disease severity in sterilized soil compared to natural soil (a measure of disease suppression) was significantly higher for organically managed than in conventionally managed soils, indicating that corky root was more suppressed in soil from organic than in soil from conventional farms. This result was in agreement with field surveys showing that the disease was significantly less severe in organic than in conventional farms.

Several researchers have compared disease incidence or severity in various soils at a range of inoculum levels (Alabouvette, 1986; Mandelbaum and Hadar, 1990; Oyarzun et al., 1994), whereby five or six densities of inoculum of the pathogen under study are mixed with natural field soils in pots, and plants are grown for a predetermined period in the various soils. To allow comparison of disease suppression in different seasons and with different soils, the tests need to be conducted under strictly standardized conditions. Oyarzun et al. (1994) standardized not only soil and air temperature but also water potential by means of an automated watering system. Unfortunately, all these methods of standardization are very labor and time intensive, and in some instances quite costly (Oyarzun et al., 1994).

If the results of simple *in vitro* assays could be correlated with those of plant disease assays, these simpler assays could be used to reduce the costs of suppressiveness testing of soils. Radial growth of a pathogen over a soil surface can be indicative of conduciveness of that soil for the pathogen and the disease it causes. For example, Ko and Kao (1989) developed a simple assay to test soils for suppressiveness to *Pythium splendens* Braun causing damping-off of cucumber, in which sporangia were suspended in cucumber root extract to break the dormancy and then placed on the smooth surface of a small soil block. Spore germination was determined after 24 h of incubation. A similar test was developed by Vujicic and Park (1964) to study the influence of a food base of the fungal inoculum such as malt agar plugs or infected potato tuber tissue on growth of *Phytophthora erythroseptica* Pethybr., where inoculum was placed on the smoothed surface of a soil and radial growth was measured microscopically.

When testing soils for suppressiveness to a soilborne phytopathogenic fungus one usually deals with a large number of soil samples. Performing bioassays with these soils in either growth chambers or greenhouses can use large quantities of soil and be very time intensive. Furthermore, in most cases natural field soil is unsuitable for growing plants in pots and has to be amended with sand or vermiculite to allow for suitable drainage and root conditions. These changes in physico-chemical soil conditions themselves can change the effect of the soil on the pathogen. Moreover, non-autoclaved field soil often already carries high inocula of pathogens, and this will result in misleading control treatments and lower sensitivity of the assays. To avoid changing soil physical and chemical characteristics, relying on test plants, and to remove effects of the native pathogenic microflora, as well as being able to process large numbers of samples efficiently, we modified an *in vitro* suppressiveness test to make it suitable for routine testing in laboratories (Davet, 1976; Davet, 1976; Williams and Willis, 1962). This test exposes the fungus to either natural or autoclaved field soil, while protecting it from direct interactions with the soil community by a cellophane membrane. The objectives of this study were: (i) to compare an *in vitro* suppressiveness test with an *in vivo* test using *Pythium aphanidermatum* (Edson) Fitzp. causing damping-off on tomato, (ii) determine the effect of decomposing cover crop debris on suppression of *P. aphanidermatum* using both of these suppressiveness tests, and (iii) determine whether organically and conventionally managed soils differ in degree of suppressiveness to *P. aphanidermatum*.

Materials and methods

Soils and cover crops. The soils came from a farming systems project started in 1986 that examined the long-term effects of winter cover crops in a semi-arid irrigated system on N availability for subsequent crops and soil structural and biological properties (Stivers and Shennan, 1991; Shennan, 1992). The design of the experiment was a split-plot with four replicate main blocks (farming system: organic and conventional), in

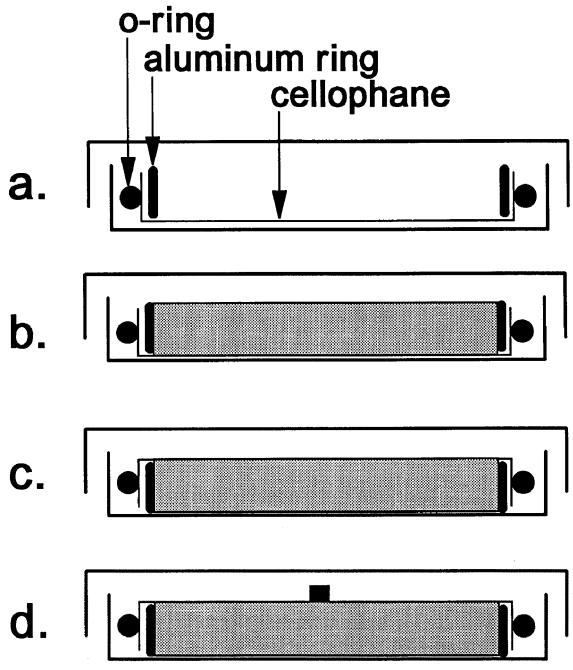

Figure 1. Diagrammatic representation of the *in vitro* agar-ring unit. The assembly consists of a metal ring onto which a moist cellophane membrane is strapped with an o-ring. The ring and cellophane were placed in a glass petri-dish facing downward (a). After autoclaving the unit, 15 ml of soil and approximately 20 ml of cooled, molten water-agar were poured into the ring (b), the assembly left over-night for cooling, the ring turned with the cellophane facing up (c), and a 3-mm agar-plug of a fungal culture was placed on the center of the cellophane (d).

which subplot treatments were either different kinds of winter green manuring (organic system) or winter fallow and use of different levels of ammonium sulfate fertilizer (conventional system). We chose the winter-fallow plots that received 168 kg N/ha in the form of ammonium-sulfate and the green manured plots that were seeded to an oat vetch mixture that supplied a similar amount of nitrogen. In Fall of 1992 the experiment was terminated by planting a cover crop mixture consisting of oats (*Avena sativa* L.) and lana wooly pod vetch (*Vicia dasycarpa* Ten.) in all plots including the plots not previously cover-cropped. The oat-vetch cover crop was mowed and incorporated with a disk on April 14. Soil samples (at least 30, 20 cm deep cores per plot, for a total of 16 liters/plot) were taken 3 days before incorporation, and 7, 20 and 35 days after incorporation of the cover crop and stored in plastic buckets with a loose lid at 5 °C until processed.

In vitro *bioassay*. An *in vitro* bioassay modified from Davet (1976a,b), who used *Pyrenochaeta lycopersici*, *Fusarium solani*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Colletotrichum coccodes*, and Williams and Willis (1962) was used to determine effects of soil on growth and relative growth of *P. aphanidermatum*. For this assay, cellophane (Promega Corporation, Madison, WI) was strapped over an aluminum ring cut from irrigation pipes and held in place with a rubber o-ring (Durometer hardness: 70-A; Material: Buna-N (Nitrile)). This unit was placed with the cellophane facing downwards inside a glass petri dish and autoclaved (Figure 1). Fifteen ml of either autoclaved or nonautoclaved soil were added to the inside of the aluminum ring, followed by 20 ml of 8% molten, but cooled water agar. In another version of this assay we added four germinated tomato seeds (*Lycopersicon esculentum* L. cv. Castlepeel II, Sun Seeds, CA), with a radicle length of 1–2.5 cm on the cellophane equally spaced along the edge of the petri dish before adding soil and water agar, to simulate a presumable effect of an intact rhizosphere. The unit was then left upside down over night to allow for diffusion of nutrients and water into all aggregates and the cellophane. The next morning, the cellophane surface was turned up, and a potato dextrose agarplug from a 48-h-old culture of *P. aphanidermatum*, cut with a sterilized cork-borer (3 mm diameter), was placed on the center of the cellophane surface. After 24 h incubation, the diameter of the fungal colony was measured in two perpendicular directions and averaged (Figure 2). We used five plates each of autoclaved and natural field soil for each replication. After averaging over these five plates a unitless index for the relative reduction in growth on non-sterilized versus sterilized soil was calculated as:

$$\text{Relative growth} = \frac{\text{radial growth (cm) on unsterilized soil}}{\text{radial growth (cm) on sterilized soil}}$$

Growth chamber bioassays. In the growth chamber bioassays we used trays holding 30 3.8-cm-diameter PVC pipes of 15 cm height as pots, with a capacity to hold 200 ml of soil. The PVC tubes stood on a layer of sand with free drainage at a distance of 1.5 cm from each other. One tray was used for each replicate field plot, and 5 PVC tubes each were used for 6 treatments in each tray. Pots and trays were sterilized with 10% bleach before each use. Half of the soil was autoclaved at 120 °C for 60 min and left uncovered in the shade on a balcony for one week to allow for recolonization

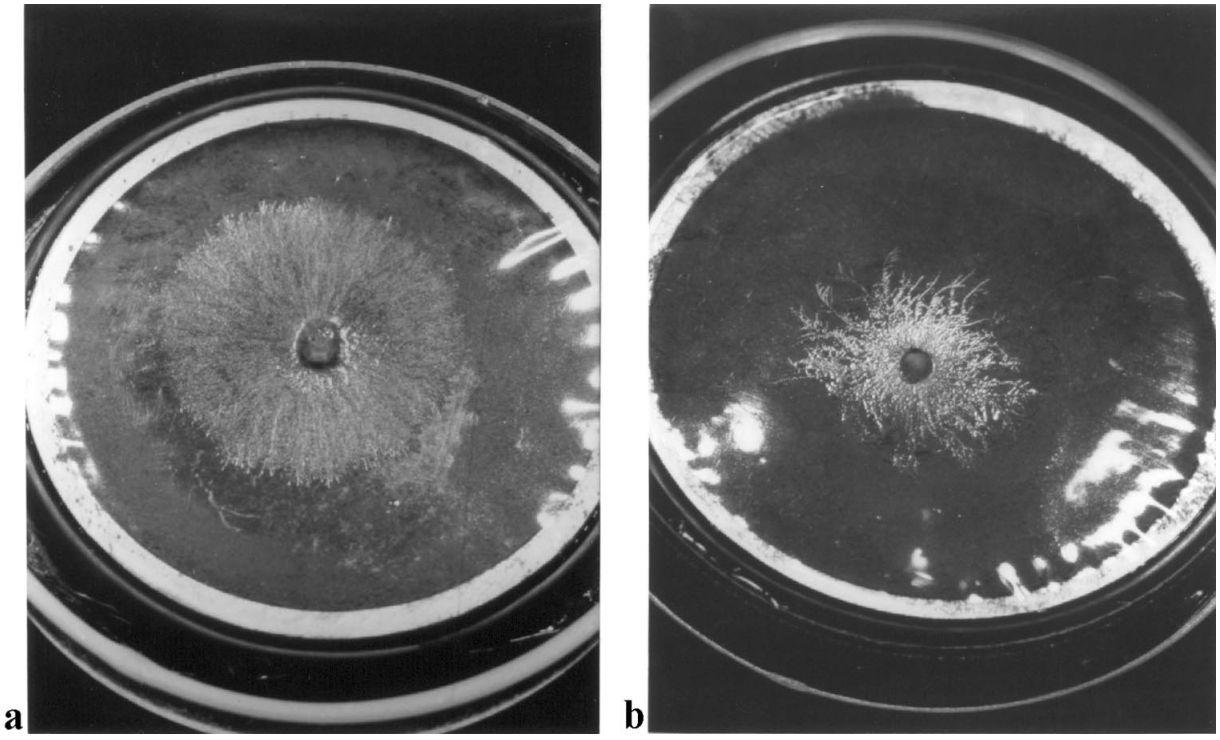

Figure 2. In vitro agar-ring test of a 24-h old colony of *P. aphanidermatum* on a cellophane surface covering a mixture of autoclaved (a) and non-autoclaved (b) soil-water agar.

of the soil by microorganisms and to diminish possible toxic effects from autoclaving. The treatments per tray were unsterilized or sterilized soil with three levels of inoculation with *P. aphanidermatum*, respectively. The three pathogen inoculation levels were a control, where deionized water was applied, and a low and a high level of inoculum (see below). Three uncoated and untreated seeds of tomato cultivar Castlepeel II (Sun Seeds, Hollister, CA) were planted per pot and covered with a thin layer of the same soil. During seedling germination and early emergence, a plastic lid was used on top of the pots to prevent drying of the top layer of soil, and then removed and watered as needed. The inoculum of *Pythium aphanidermatum* was grown in fermentation tanks (4 l erlenmeyer flasks, aerated with filtered air) for 10 days using a medium for tank fermentation containing buffered, nonclarified V-8 juice broth (see chapter by Martin and appendix in (Singleton et al., 1992)). Inoculum was first rinsed through glassfiber wool and then quantified by counting oospores using a haemocytometer. We used an inoculum density of 10^3 oospores per g dw of soil for the low and 10^4 for the high inoculum levels. Oospores were prepared as a suspension in sterile, distilled water, and added to soil in a plastic bag as a fine mist using an aerosol sprayer. The soil was mixed several times to distribute the inoculum. Disease severity was recorded as percentage of seed that died within 21 days after planting (pre- and postemergence damping-off), with or without correction for damping-off in control treatments (natural field soils not inoculated with *P. aphanidermatum*).

Soil microbial and nutrient cycling variables. NO_3, C/N ratio and hot-water soluble carbohydrates of soil, as well as N content of extracted plant debris, were determined as reported in Hu et al. (1996). To determine dry weight of plant debris, debris was extracted from ca. 2000–3000 g soil using a wet-sieving method (Weinhold, 1977). A procedure modified from Robertson and Van Soest (1981) was used to determine % cellulose and lignin of extracted debris as reported in Hu et al. (1996). Fluorescein diacetate (FDA) hydrolytic activity was determined on 5-g subsamples of soil as described previously by Workneh et al. (1993). Microbial biomass C was determined by the chloroform fumigation-extraction method adapted from Vance et al. (1987) using a k_{ec}-factor of 0.33 according

to Sparling and West (1988) as described in Hu et al. (1996).

Statistical analysis. Initial analysis consisted of descriptive statistics for each variable. All variables were tested for normality and homogeneity of variance and transformed if necessary. In only one case were outliers removed, which originated from a field plot that behaved differently from other field plots of the same treatment because it had been flooded by accident. Analyses of variance conducted on samples taken from the field experiment were analyzed as a split-plot design with farming system in the main plot (organic and conventional) and sub-treatments being the different stages of decomposition of cover crop debris using three replications of each treatment combination. All statistics were performed using the SAS system for Windows version 6.10 (SAS Institute Inc., Cary, NC).

Results

Generally, the autoclaved soil yielded significantly higher fungal growth in comparison to the nonautoclaved soil (Figure 2). This is reflected in the relative growth values obtained which ranged between 0.3 and 0.8 (Figure 3). A good correlation (r = 0.53, $P \leq 0.005$) was obtained between relative growth of *P. aphanidermatum* after 24-h of growth in the *in vitro* bioassay and disease severity on tomato seedlings in growth chamber assays (Figure 3). Similarly, there was a significant, positive correlation (r = 0.85, $P \leq 0.001$) between disease severity in the growth chamber and radial growth in the *in vitro* assay (Figure 4). No significant relation was found between % plants that were killed due to damping-off in autoclaved, inoculated soils and growth over autoclaved soil ($P > 0.59$, $r^2 = 0.052$), which supports the premise that the radial growth method does measure effects contributed by a biotic component of the soil rather than nutrient availability in different soils.

When observations before and after cover crop incorporation were separated, positive correlations ($P \leq 0.0001$) were obtained between radial growth (cm) with tomato seedlings and radial growth without tomato seedlings (Figure 5). The slopes of the regression lines were less than 1, but the intercepts were larger than 0. Relatively more growth of *P. aphanidermatum* was observed in the soils with seed. The relationship between relative growth with and without tomato seedlings was not as good ($P \leq 0.05$). Furthermore, no significant relationships could be established between radial growth or relative growth on plates with seeds and growth chamber disease data.

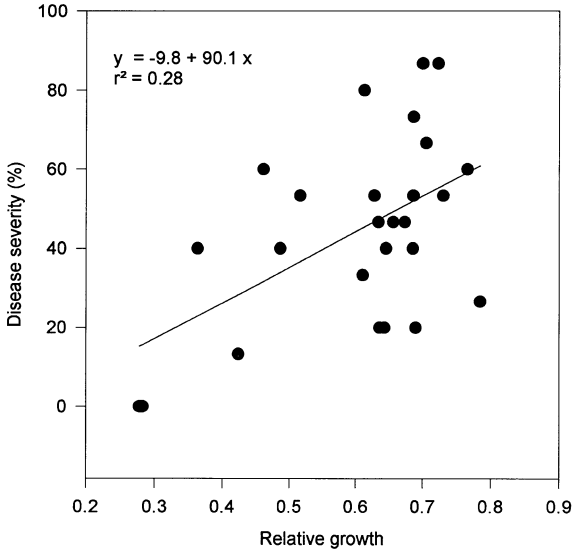

Figure 3. Relationship between disease severity (% of total plants damped off) in growth chamber bioassays and relative growth (unitless; 24-h) of *P. aphanidermatum* determined in the *in vitro* bioassay with soils managed organically and conventionally at different stages of cover crop decomposition.

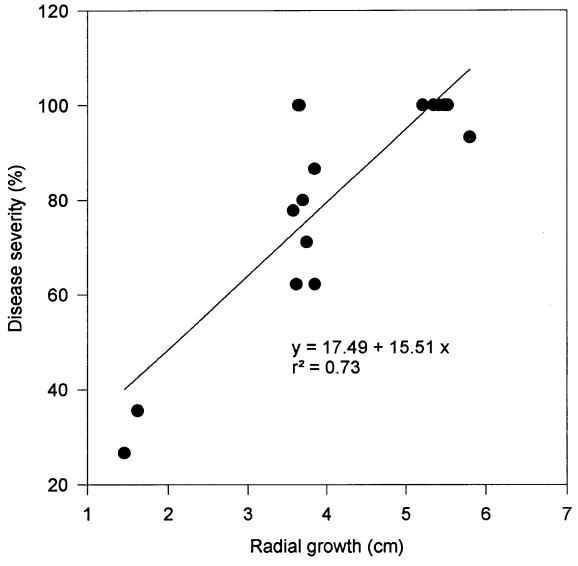

Figure 4. Relationship between disease severity (% of total plants damped off) in growth chamber bioassays and radial growth (24-h) of *P. aphanidermatum* determined in the *in vitro* bioassay with soils managed organically and conventionally at different stages of cover crop decomposition.

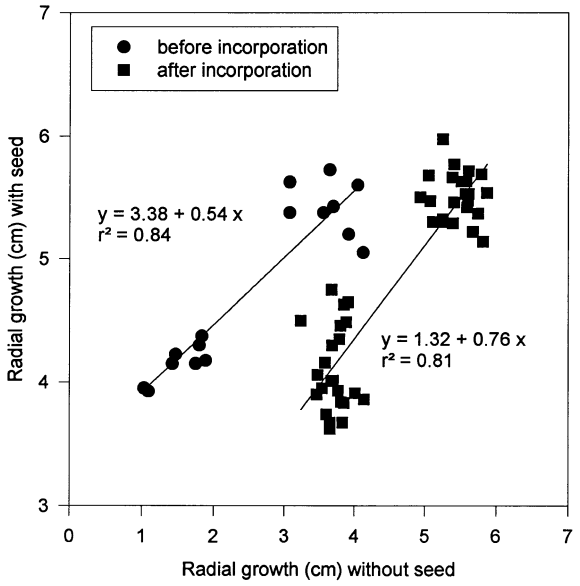

Figure 5. Relationship between radial growth (in 24-h) of *P. aphanidermatum* determined in the *in vitro* bioassay with and without addition of 4 germinated tomato seedlings. Separation of observations receiving no cover crop debris (before incorporation) or receiving cover crop debris (after incorporation) gave the best relationships.

Table 1. Significant Pearson correlation coefficients between relative growth[1] or radial growth[1] (cm) of *Pythium aphanidermatum* after 24-h and soil microbial and nutrient cycling variables (n = 26). Values in brackets are not significant

	Relative growth		Radial growth	
	r	Prob > r	r	Prob > r
Nutrient cycling variables:				
soil NO_3^2	0.49	0.01	0.50	0.009
water-extractable sugars[2]	0.59	0.001	0.58	0.002
C/N ratio of debris	0.45	0.02	0.42	0.03
N of debris[3]	−0.50	0.01	−0.40	0.04
Dry weight of debris[3]	0.54	0.005	0.48	0.01
cellulose[3]	0.76	0.0001	0.61	0.0008
lignin[3]	0.83	0.0001	0.85	0.0001
Soil microbial variables:				
FDA hydrolytic activity[4]	0.49	0.01	0.42	0.03
MBC	0.43	0.02	(0.38)	(0.06)

[1] 'Radial growth' measures growth of *P. aphanidermatum* in units of cm after 24-h incubation using the *in vitro* bioassay. 'Relative growth' is a unitless ratio of radial growth on nonsterilized soil over radial growth on sterilized soil.
[2] in μg g^{-1} soil.
[3] in g kg^{-1} extracted debris.
[4] in μg g^{-1} soil min^{-1}.

The effects of cover crop decomposition over time on disease severity (corrected for disease severity due to native damping-off severity in the control) and relative growth is presented in Figure 6. Prior agricultural management system had no significant effect ($P \geq 0.05$) on any of the disease severities, radial growth or relative growth, when all observations or only observations after incorporation were included in the analysis of variance ($P \geq 0.05$) (Figure 6). Radial growth and relative growth over nonautoclaved soil using all sampling dates were significantly increased after cover crop incorporation compared to before incorporation ($P \leq 0.0001$), but no significant differences could be detected among the three sampling dates after incorporation ($P \geq 0.05$).

The effect of soil C and N pools on fungal growth was assessed by correlating both relative and radial growth of the fungus on the non autoclaved soil. We obtained significant, positive correlations of both growth measures with NO_3 and hot water extractable carbohydrate content of soil, as well as C/N ratio, dry weight, lignin content and cellulose content of extracted debris (Table 1). Among several soil microbial biomass and activity variables we measured, only FDA hydrolytic activity and microbial biomass carbon correlated positively with radial growth (Table 1). A negative correlation of relative/radial growth was obtained with N content of debris (Table 1).

Discussion

Comparison of tests. We obtained a positive, linear relationship between the *in vitro* growth assay and the damping-off assay (Figures 3, 4), indicating that the *in vitro* assay could be used as a measure of suppression of *P. aphanidermatum* by the soil microflora. Variations of this technique have been developed that use cellophane membranes to separate the test organism from plants, soil, or other organisms. For example, soil fungistasis was measured using folded cellulose film containing fungal spores of a range of soil fungi (including *Penicillium* species, *Cladosporium herbarum*, *Trichoderma viride*, *Mucor* species, *Mortierella* species and *Rhizopus nigricans* among others) buried in soil (Dhingra and Sinclair, 1985; Dobbs and Hinson, 1953). A cellophane membrane technique similar to ours (Williams and Willis, 1962) was used for the study of effects of toxic substances produced by fungi on plants (Johnson et al., 1959), and for the effects of root exudates on fungi (Johnson et al., 1959). More recently, Davet (1976a,b) used

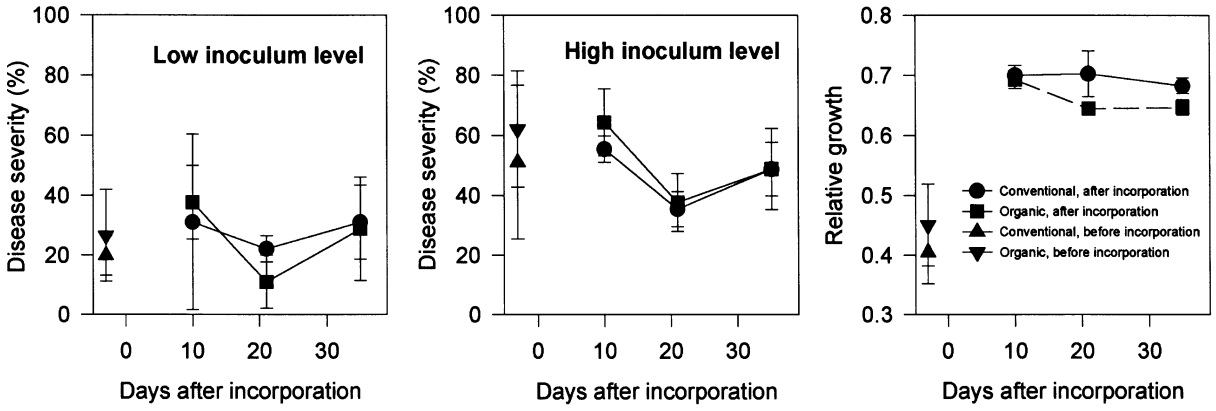

Figure 6. Disease severity in growth chamber experiments at low and high levels of inoculation with *P. aphanidermatum* (% of total plants damped off due to *P. aphanidermatum* after correction for damping-off severity in control soils due to native microflora) contrasted with radial growth of *P. aphanidermatum* at different stages of cover decomposition. The abscissa reflects samples taken 3 days before, and 7, 20 and 35 days after incorporation of the cover crop.

the same *in vitro* assay as we used to test soil for suppressiveness to *Rhizoctonia solani, Pyrenochaeta lycopersici, Fusarium solani, Fusarium oxysporum* and *Colletotrichum coccodes* and to test for competition for nutrients and antibiosis between different combinations of these plant pathogenic fungi. The radial growth of a fungus (on non-autoclaved soil) can be seen as an assay of the ability of the fungus to grow saprophytically in competition with the soil microbial community. Relative growth can be seen as a measure of the degree to which the pathogen can compete with the soil microbial community, since growth on unsterilized soil is scaled by growth in sterilized soil in which no microorganisms compete. Mechanisms that might reduce the performance of the test organism in nonautoclaved field soil as compared to autoclaved field soil include competition for nutrients and antibiosis by the soil microbial community. Direct interactions such as hyperparasitism, parasitism and predation should not occur, since microscopic observation indicated that the cellulose membrane was still intact after the 24 h of incubation.

Several problems with the use of this approach have to be taken into consideration. For instance, an underestimate of the relative growth values of growth of the test fungus may result from stimulation of the growth in the sterile soil due to the nutrients provided by lysed cells. In contrast, toxic elements such as NH_3 released during autoclaving may reduce growth in the autoclaved soil and might thus overestimate the true relative growth value (Alef, 1996). It is impossible to distinguish the relative contribution from either lysed cells or toxic elements to under- or overestimation of the true relative growth. Furthermore, these contributions are expected to be different for different fungi and different soils.

By including seeds in the *in vitro* bioassay we hoped to simulate the effect of an intact rhizosphere and thus expected to obtain a better relationship between relative growth and disease severity. The variability among replicate agar-ring units was increased by including seeds, since more handling of the plates occurred and since seedlings were not always completely submerged in the agar. We thus recommend omission of seedlings, unless the method can be modified to reduce variability.

To increase the precision of the test, one can estimate the density of the hyphae at 2 to 4 fixed points on a petri-dish, for instance 1 cm from the colony center, and transform the radial growth in cm into an estimate of surface area covered by hyphae. Density of the mycelial colony was generally lower in the nonautoclaved agar-ring units (Figure 2). A similar assay developed to test the effect of benomyl on the antagonistic activity of the soil microflora against *Rhizoctonia cerealis* including the estimation of density of mycelial colonies was used by van der Hoeven and Bollen (1980).

Nutrient effects and in vitro *assay*. The correlations between concentrations of different soil C and N pools or soil microbial variables and measurements of growth of a fungus *in vitro* using the agar-ring method, can be used to develop hypotheses regarding the mechanisms that are responsible for reducing fungal growth.

For example, the positive correlation between NO_3-N and relative growth and radial growth, respectively, suggests that NO_3-N is required for abundant growth of *P. aphanidermatum*. Huber et al. (1974) cites a study by Carley (1969) in which root rot on pea and corn caused by *Pythium* spp. was increased by nitrate and decreased by ammonium. However, Kraft and Erwin report that *P. aphanidermatum* does not grow on NH_4-N or NO_3-N as sole nitrogen sources, while *Pythium ultimum* grew relatively well on only mineral N (1967). Little attention has been given to the requirements of different species of Pythium for mineral N. The agar-ring method could be used to establish some of the basic resource relationships for a broad range of fungal species.

The positive correlations between concentrations of all carbon sources (e.g., water-extractable sugars and cellulose) and radial growth/relative growth agree with the fact that *Pythium* spp. are saprophytic sugar fungi (Garrett, 1970). Garrett characterized *Pythium* species as secondary sugar fungi, because they are able to coexist with communities of soil microorganisms that actively degrade cellulose, while they themselves can do so, at best, to a limited extent. Thus high levels of cellulose might support an active microbial community that releases sugars upon which *Pythium* species can thrive, and therefore *Pythium* might be expected to grow better at high than low cellulose levels.

Only one of the variables measuring soil microbial activities and biomass correlated significantly with radial growth and two did so with relative growth. There was a significant positive correlation between FDA hydrolytic activity and both relative and radial growth. This can be explained by the hypothesis that conditions were favorable for heterotrophic growth of soil microorganisms and for growth of *Pythium* species. Preliminary results of counts of copiotrophic and oligotrophic organisms during cover crop decomposition indicate that FDA hydrolytic activity measures primarily the activity of the copiotrophic community (Hu, Grünwald and van Bruggen, unpublished results), which responds primarily to readily available sources of carbon (Williams, 1985). Our experiments lend some support to this mechanism, since we obtained positive and significant correlations between either microbial biomass carbon or FDA hydrolytic activity and cellulose content of debris and hot-water extractable sugars ($P \leq 0.01$). *Pythium*, being an effective saprophyte, responds to the same sources of carbon (Garrett, 1970), which is in agreement with the positive correlation observed between FDA hydrolytic activity and radial growth of *P. aphanidermatum*. FDA hydrolytic activity has been reported to be negatively correlated with damping-off severity caused by *Pythium ultimum* on cucumber or poinsettia (Boehm and Hoitink, 1992; Chen et al., 1988), and our results thus disagree with significant, positive correlations between FDA hydrolytic activity and relative and radial growth. This disagreement could be due to the fact that we used soil samples that were recently amended with fresh organic matter, promoting growth of both *P. aphanidermatum* and the heterotrophic microbial community degrading FDA. Differences may also be attributed to the fact that our system physically separates the test organism from its competitors but still allows it access to the soluble C sources.

Farming systems comparison. We did not observe significant differences for radial/relative growth and disease severity between the organically and conventionally managed soils. Disease severity and growth behaved similarly in the two farming systems at different stages of cover crop decomposition. Since we are relying on soils from one experimental farming systems project, it would be inappropriate to conclude that there are no significant differences between the two farming systems in terms of suppressiveness to *P. aphanidermatum*. Our inability to find differences between farming systems might have been compromised by the fact that we only used 3 to 4 replications for all observations due to the large number of soil nutrient, microbial and disease variables we measured.

The *in vitro* assay of fungal growth has been found to be suitable for large scale testing of soils for suppressiveness to *P. aphanidermatum*. A set of agar-ring tests of up to 240 units can easily be performed twice a week. We believe that this *in vitro* bioassay shows promise for routine use due to the facts that (i) the test can be performed in a more time- and material-efficient way than most other soil suppressiveness tests, and (ii) the test can be used to assess the effect of variables such as soil nutrient status or heterotrophic microbial activity and biomass on the growth and relative growth of the test fungus. We also used this method to look at *Rhizoctonia solani* and observed similar patterns of responses to cover crop decomposition (Grünwald, unpublished results), suggesting that this assay might be useful in studying other fungi that are saprophytes.

Acknowledgements

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