Suppression of Lettuce Drop Caused by *Sclerotinia minor* with Composted Sewage Sludge

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ABSTRACT

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Amendment of soil with composted sewage sludge significantly reduced the incidence of lettuce drop caused by *Sclerotinia minor* in the field during a 4-yr period in both fall and spring plantings. Compost was added to the soil in the first 2 yr and residual effects occurred in the final 2 yr of the study. Suppression of disease was correlated with increased soil microbial activity, assessed by a dehydrogenase method, and with total nitrogen, phosphorus, magnesium, calcium, and organic matter content of the soil. Size and weight of lettuce heads were similar from compost-amended and nonamended soils. Survival of sclerotia of *S. minor* was unaffected by the addition of compost to soil, and there were no differences in types or numbers of fungi associated with sclerotia recovered from the soils. Growth of mycelia and formation and germination of sclerotia in vitro were adversely affected by sterilized compost-amended soil and crude soil extracts but not by filter-sterilized extracts of compost-amended soil, compost, or sludge. The suppressive effect of compost on disease of lettuce caused by *S. minor* is complex and may be related to improved physical structure or modified nutrient content of the soil, resulting in increased soil microbial activity.

Compost prepared from municipal sewage sludge is a valuable resource that can provide macronutrients and minor plant nutrients and improve the tilth and productivity of agricultural soils (23). Composted organic matter also reduced diseases caused by several soilborne plant pathogens (12,16,23,27). For example, an undefined "domestic waste" compost reduced infection of *Phaseolus vulgaris* and *Pisum sativum* by *Rhizoctonia solani* and *Pythium ultimum* (27). Composts prepared from tree bark have been used successfully for control of several soilborne pathogens (12). The type of tree from which the bark was obtained and the amount of wood incorporated into the compost influenced the degree of control and the type of disease controlled. For example, compost prepared from hardwood bark suppressed Rhizoctonia damping-off but pinewood bark compost did not (12). Greenhouse studies have shown that percent infection of lettuce seedlings by *Sclerotinia minor* Jagger was reduced consistently 40–50% by adding 10% sewage sludge compost to the potting soil containing the pathogen (16).

The objectives of this study were to assess the effect of soil amendment with sewage sludge compost in the field on the incidence of lettuce drop caused by *S. minor* and to determine the effect on survival of sclerotia of the pathogen and the possible mode of action of the compost in the reduction of disease. Preliminary reports have been published (15,20,23).

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MATERIALS AND METHODS

Soil preparation, compost analysis, and disease assessment. Composted sewage sludge (compost) was produced by the Beltsville forced-aeration method (28), using sludge generated at the Blue Plains Wastewater Treatment Plant, Washington, DC. It was aged for at least 30 days and screened to remove large wood chips (>1 cm) used as a bulking agent. Composts produced from this sludge typically had the following properties: pH 7.3, 35% water, 23% organic carbon, 1.3% total nitrogen, 1.5% phosphorus, 0.2% potassium, 1.4% calcium, 4.5% iron, 340 μg/g zinc, 147 μg/g copper, 5.9 μg/g cadmium, 16 μg/g nickel, 800 μg/g manganese, and 76 μg/g lead. The compost had relatively low levels of macronutrients (slow-release) (26) and heavy metals and was considered safe for use in vegetable production (23).

Compost was added to each of six replicate field plots (2.5 × 4 m, separated by 1-m grass strips) in Beltsville, MD, in two successive years. About 7 t/ha (150 kg/plot) of compost was added on a dry-weight basis and rototilled into each of six replicate plots in the spring of 1980, followed by an additional 10 t/ha (200 kg/plot) in the fall of 1981. Fertilizer (10:10:10) was added to the nonamended plots before each planting at the rate of 1.5 kg of nitrogen per plot. Ground calcitic limestone was added (2.2 kg/plot) to nonamended plots each year to maintain the pH between 6.5 and 7.0. The compost plots were not treated with fertilizer or limestone during the 4-yr study.

Soil fertility was determined before plot establishment and once per year thereafter (Table 1). The soil was a Sassafras sandy loam (56% sand, 28% silt, and 16% clay) with 1.2% organic matter, 592 μg/g dry soil total nitrogen, 107 μg phosphorus, 152 μg magnesium, and 100 μg K_2O. Determinations of three replicate samples were made by standard procedures (7,8).

Plots were artificially infested with sclerotia of *S. minor* (isolate SS-13 from diseased lettuce) in the spring of 1980. Sclerotia were produced in cultures on autoclaved oats (100 g oats and 100 ml water), incubated for 4 mo at room temperature, and harvested by wet sieving-flotation to remove the sclerotia from the oat particles. Sclerotia were added to 100 g of moist sand to enhance distribution over the surfaces of the plots after preparation for planting. The sclerotia were raked into the soil surface to a depth of 5–8 cm. The average count of sclerotia in soil after infestation was about 30/100 g dry weight of soil. Sclerotia in 100-g soil samples were counted after preparation of the field plots before planting lettuce in the spring and fall. Soil was wet-sieved and sclerotia were recovered by wet seive-flotation (3). Samples containing sclerotia were examined under a stereomicroscope, and sclerotia were removed with forceps and counted.

Lettuce (*Lactuca sativa* L. 'Paris White' romaine) seedlings 1–2 mo old, started in Jiffy 7 peat pellets in the greenhouse, were transplanted (60/plot) in May and again in September of each of the 4 yr. Thus disease was assessed in two crops each year for 4 yr. The number of wilted or decayed plants infected with *S. minor* was recorded each week during a 6-wk season and was expressed as percentage of the total planted. Diseased plants were left in the field. The plots were maintained by hand cultivation and rototilled between successive crops. Total harvestable lettuce stand, total plant

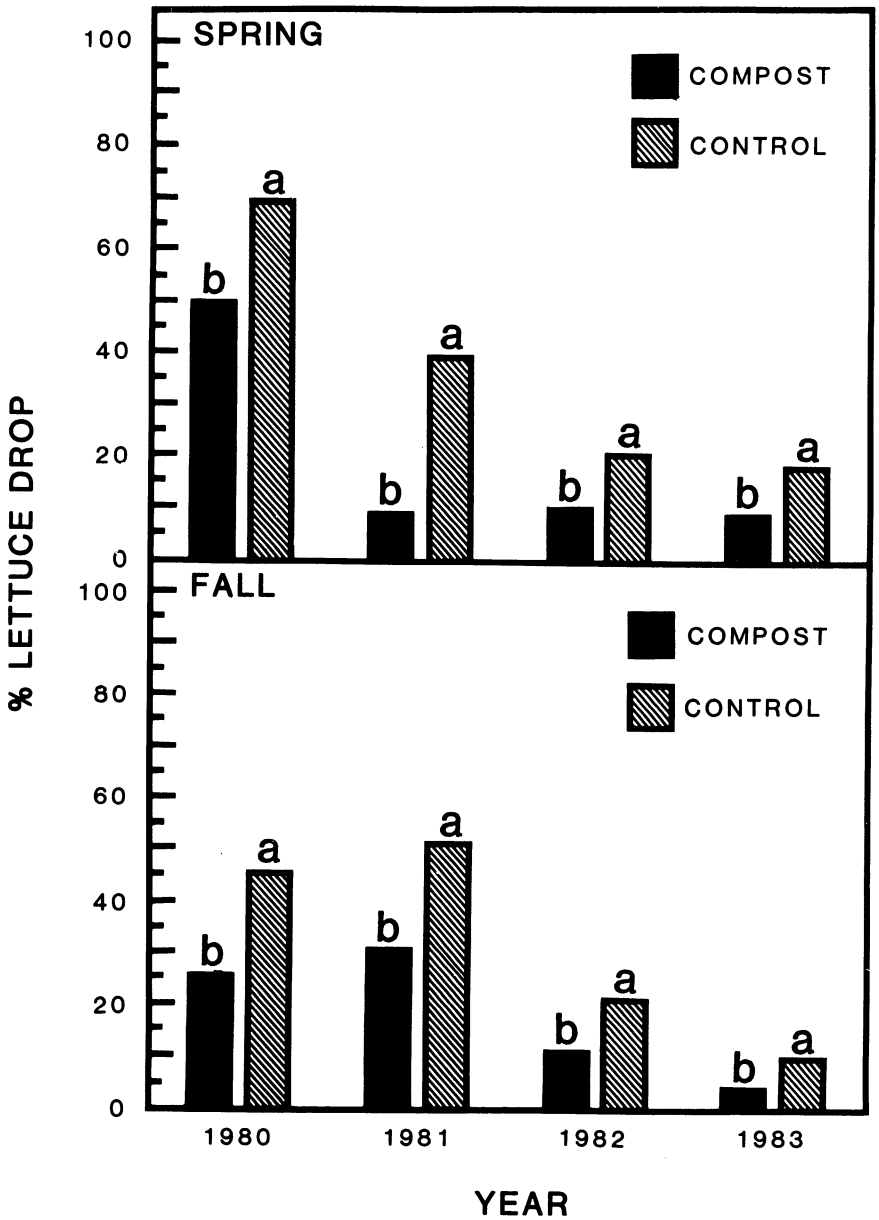

Fig. 1. Percent lettuce drop caused by *Sclerotinia minor* in soil amended with composted sewage sludge or in nonamended soil in spring and fall plantings over a 4-yr period. Pairs of columns with different letters are significantly different according to an analysis of variance ($P = 0.05$).

infection, fresh and dry weights of lettuce heads, and size of heads were recorded at the appropriate harvest dates. During the summer, between successive spring and fall plantings, snap beans (*Phaseolus vulgaris* L. 'Blue Lake') were planted in all the plots. The plots were winter-fallowed, leaving the fall-season lettuce crop debris on the surface for production of natural sclerotial inoculum of *S. minor*. Microelement content of 20 lettuce leaves per plot harvested in the fall of 1980 were determined by atomic absorption spectrophotometry after leaf samples were rinsed, dried, ashed, and acid-digested according to the method of Chaney and Lloyd (11).

Determination of growth and survival of *S. minor*. Survival of *S. minor* in the field was determined by burying sclerotia in plots 1 × 2 m adjacent to the disease-assessment plots. Compost was added to the soil at a rate of 10% (40 kg dry weight), and 300 sclerotia were mixed with 100-g samples of soil from the field and placed in 30 nylon stocking mesh bags and buried in each of three plots (1 × 2 m) per treatment. Samples were withdrawn monthly for 15 mo and sclerotia recovered as indicated previously (3). Sclerotia were counted, surface-disinfested for 30 sec in 50% ethanol, washed in sterile distilled water, and plated on sterile, moist filter paper or on nutrient medium (grams per liter of water: malt extract 6, Soytone 1, yeast extract 0.5, agar 20) to determine germinability. Fungi that grew from sclerotia on the filter paper were isolated and identified.

Mycelial growth and sclerotium production were determined in sterilized compost-amended (compost soil) and nonamended soil with 5% (w/w) cornmeal added to each soil as a nutrient source. The soil-cornmeal mix was sterilized by autoclaving for 1 hr or by irradiation with 3 Mrads from a cobalt-60 source. Agar plugs (5-mm) with mycelium

Table 1. Effects of addition of 10% sludge compost to field soil on lettuce drop, caused by *Sclerotinia minor*, and on selected physical, chemical, and biological parameters

Parameter	Units	Compost-amended soil[v]	Control soil[v]	Correlation with disease incidence[w]
Disease	Percent	15.9	30.1*	...[x]
pH	Log $(H)^{-1}$	7.2*	6.5	−0.273
Soluble salts	μ MHO	4.3	3.3	−0.168
Total N	μg/g Soil (dry wt)	4,299.4*	945.4	−0.368[y]
Nitrate N	μg/g Soil (dry wt)	164.9*	82.4	0.183
Ammonium N	μg/g Soil (dry wt)	6.8	2.8	0.040
Phosphorus	μg/g Soil (dry wt)	371.6*	130.1	−0.350[y]
Potassium	μg/g Soil (dry wt)	101.6	201.0*	0.064
Magnesium	μg/g Soil (dry wt)	87.6*	30.2	−0.497[y]
Calcium	μg/g Soil (dry wt)	2,353.1*	951.6	−0.331[z]
Organic matter	Percent	10.1*	2.7	−0.412[y]
Dehydrogenase	μl H/g soil (dry wt)	0.035*	0.017	−0.280[z]

[v] Values with an asterisk are significantly greater ($P = 0.05$) than the corresponding value according to the analysis of variance procedure (ANOVA).
[w] Correlation coefficient calculated for percent disease in relation to treatment (0 compost or 10% compost).
[x] Coefficient = −0.447, $P = 0.01$. Values are averages for three sampling times.
[y] Correlation coefficient, $P = 0.05$.
[z] Correlation coefficient, $P = 0.01$.

of *S. minor* were placed on these soils (50 g) in 9-cm-diameter petri dishes. The extent of mycelial growth was measured daily, and sclerotia were counted at the end of the experiment. Numbers of sclerotia produced around diseased lettuce plants in the field were determined by collecting soil to a depth of about 5 cm surrounding well-decomposed infected lettuce plants in December 1982, after the fall 1982 planting. Sclerotia were recovered by wet sieving-flotation (3).

Crude and cellfree extracts from compost and nonamended soil were obtained by making 1:2 (w/v) dilutions of soil in sterile distilled water and immediately filtering through Whatman No. 1 filter paper for crude extracts and through No.1 filter paper and 0.45-μm Millipore filters for cellfree extracts. Extracts were added to sterile glass petri dishes containing Whatman No. 1 filter paper. Surface-disinfested sclerotia of *S. minor* (SS-13) were placed on the moistened filter paper, 21 per plate. Plates were checked daily for germination of sclerotia. After 15 days of incubation, the number of germinated sclerotia producing secondary sclerotia was recorded. Filter-sterilized soil extracts were also incorporated into potato-dextrose agar medium to assess the effect on mycelial growth from agar plugs in culture.

Total microbial activity in the soils, collected twice each year as triplicated random samples from each plot, was assessed by a dehydrogenase assay method (24). The results were expressed as microliters of hydrogen transferred and reflected substrate oxidation in the soil.

RESULTS

Disease incidence. For four consecutive years, in both spring and fall plantings of lettuce, disease caused by *S. minor* was significantly less in compost plots than in nonamended control plots (Fig. 1). The values in Figure 1 were obtained 1 wk before harvest and reflect the total harvestable stand. No differences occurred in weight of individual heads of lettuce at harvest (av. 350 g/head) or in the size (av. 60 cm) and dry weight of heads (av. 140 g) between the compost and nonamended plots. Disease incidence was greatest in the nonamended soil in the first planting, possibly because of the large inoculum load, but declined over the 4-yr period of study as the numbers of recoverable sclerotia declined. No infection by *S. minor* occurred in the snap beans planted each summer.

Lettuce leaves harvested in the fall of 1980 were analyzed for microelements that are potential phytotoxins or dietary toxins. All microelements occurred at the same or even lower levels in tissue from lettuce grown in compost soils than in nonamended soils (Table 2).

Survival of sclerotia. Sclerotia of *S. minor* were isolated from the field plots before planting lettuce each spring and fall. The average number of sclerotia recovered over the 4-yr period from the nonamended soil (11.4/100 g soil) was always greater than that from the compost soil (7.0/100 g soil), but the differences were significant ($P = 0.05$) only during the fall and spring of 1982. Even this difference may not be noteworthy since it was often difficult to assess the numbers of sclerotia in the compost soil because of the large amount of organic debris, which made visualization of the sclerotia difficult. The number of sclerotia in both soils decreased dramatically during the first summer, from 25–30 to 5–10/100 g of soil the following fall, and remained relatively constant at this level during the remainder of the study.

Sclerotia buried in subplots in compost or nonamended soil in nylon bags were monitored for survival. At the end of the 14-mo study period, survival in the nonamended soil and in the compost soil was 50 and 35%, respectively, of the original number of sclerotia added. The difference in numbers of viable sclerotia recovered was not significant ($P = 0.05$). Fungi isolated from surface-disinfested sclerotia recovered from the compost and nonamended soils were similar. The species isolated and their frequency of occurrence were about the same. From a total of 450 sclerotia, *Talaromyces trachyspermus* (Shear) Stolk & Samson was isolated 18 and 15, *Fusarium* spp. 14 and 16, and *Penicillium* spp. 10 and 2 times from compost and nonamended soils, respectively. Other miscellaneous fungi commonly associated with soils were infrequently isolated from the sclerotia, but none occurred in high enough numbers to warrant mention.

Physical and biological factors affecting *S. minor*. The values obtained for several soil factors were significantly greater in compost soil than in nonamended soil (Table 1). Total nitrogen and nitrate nitrogen, phosphorus, magnesium, calcium, organic matter, soil reaction, and dehydrogenase activity were greater in the compost than in nonamended soil; potassium was lower in the compost soil, and soluble salts and ammonium nitrogen were the same in the two soils. The percentage of plants infected with *S. minor* was negatively correlated at $P = 0.01$ with total nitrogen, phosphorus, magnesium, and organic matter and at $P = 0.05$ with calcium and dehydrogenase (Table 1).

Effect of compost on growth of mycelium and formation of sclerotia. In compost soil sterilized by autoclaving or by irradiation with cobalt-60 after amendment with 5% cornmeal, mycelial

Table 2. Microelements detected in dried lettuce leaves harvested from sewage sludge compost-amended and nonamended field soils

Micro-element	Amount detected in lettuce tissue[a]	
	Compost-amended soil (μg/g ± SEM)	Nonamended soil (μg/g ± SEM)
Zinc	38.1 ± 1.9	58.0 ± 6.7
Cadmium	0.91 ± 0.04	1.2 ± 0.05
Lead	1.6 ± 0.04	1.9 ± 0.08
Copper	9.5 ± 0.11	8.4 ± 0.10
Nickel	1.8 ± 0.1	2.3 ± 0.16
Iron	78.4 ± 1.1	94.8 ± 1.8
Manganese	27.8 ± 0.59	153.0 ± 9.8

[a] Microelements determined by atomic absorption spectrophotometry after leaves were rinsed, dried, ashed, and acid-digested. Figures represent means of 20 samples; SEM = standard error of the mean.

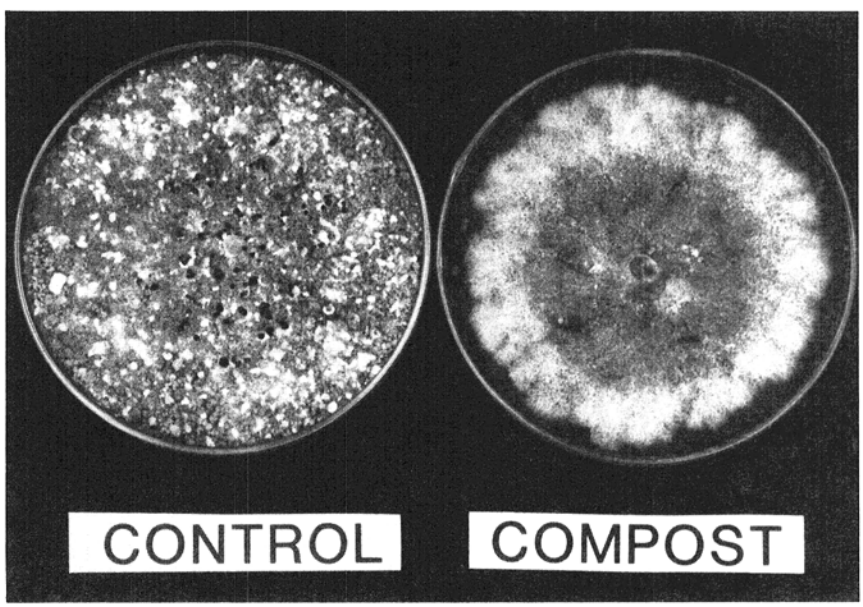

Fig. 2. Effect of amendment of soil with compost on growth and sclerotium formation of *Sclerotinia minor*. Cornmeal was added to the soil at the rate of 5% before autoclaving for 30 min at 15 psi and infesting with a potato-dextrose agar culture of *S. minor* after cooling. Plates were incubated for 1 wk at room temperature.

growth and sclerotium formation of *S. minor* were greatly inhibited (Fig. 2, Table 3). The rate of mycelial growth was slower and the density of surface mycelium was greater in petri dishes containing compost soil than in dishes containing the nonamended control soil (Fig. 2). Additionally, sclerotium formation was retarded in the compost soil. Both the rapidity of sclerotium formation (Fig. 2) and the total number of sclerotia formed (Table 3) were less in the compost soil than in the nonamended soil. Behavior of *S. minor* in soil sterilized by autoclaving or cobalt-60 treatments was the same. Growth of *S. minor* was not tested with compost alone or sewage sludge alone.

Crude aqueous extracts of compost soil reduced mycelial germination of *S. minor* sclerotia placed on filter paper moistened with the extract (Table 3). Extracts from compost alone were less inhibitory to germination than those from compost soil. Extracts from control soil or sewage sludge did not greatly inhibit germination. Sclerotium formation was inhibited by the extract from compost soil, less so by compost extract, and little by the sewage sludge extract. Cellfree extracts were not inhibitory to mycelial growth, sclerotial germination, or sclerotium formation.

Although sclerotium formation was inhibited in vitro, actual numbers of sclerotia formed around diseased lettuce plants in the field were not affected by incorporation of compost into soil. An average of 141,800 sclerotia per plant were recovered from the soil surface around plants in compost soil and 132,965 around infected plants in nonamended soil.

DISCUSSION

Organic matter added to soil may increase, decrease, or not affect diseases caused by soilborne plant pathogens (6,17,22). With lettuce drop caused by *S. minor*, composted sewage sludge reduced the incidence of the disease in the field over a 4-yr period (Fig. 1). This confirms results of previous studies on the effect of compost on *S. minor* infection of lettuce in the greenhouse, where the seedling stage of the disease was also consistently and significantly reduced (15). In addition, composted sewage sludge in greenhouse experiments reduced Aphanomyces root rot of peas; Rhizoctonia root rot of bean, cotton, and radish; Fusarium wilt of cucumber; and Phytophthora crown rot of pepper (15). In another report, an undefined "domestic waste" compost reduced infection of *Phaseolus vulgaris* and *Pisum sativum* by *Rhizoctonia solani* and *Pythium ultimum* (27). Noncomposted sewage sludge added to field soil reduced stalk lodging of corn, but the reduction was not ascribed to a specific etiological agent (19). Diseases caused by other soilborne (15) or airborne pathogens (19) were not affected or, in some cases, were increased by soil amendment (12).

Composted materials suppress some pathogens and not others, thus the mechanism of disease reduction is probably complex. The following three possible mechanisms of disease suppression were suggested for composted tree bark (12): 1) Compost improves the physical structure and thus the aeration of the root substrate. 2) Composts support high levels of antagonists and phagous organisms. 3) Fungicidal or fungistatic compounds from media affect the pathogen. Improving the physical structure of soil by adding organic matter greatly affects soil texture; significantly increases moisture-holding capacity, friability, and drainage; and decreases bulk density, water runoff, and soil leaching (23). Altering the moisture-holding properties of the soil could significantly affect disease caused by *Sclerotinia* spp. (1,4,5). Infection of lettuce by *S. minor* was increased by allowing soil moisture to fluctuate, especially when infested soils became dry before planting (5). Reduced moisture fluctuations in soil may contribute to the decrease in lettuce drop in compost soil.

Biological factors in compost soil may contribute to disease suppression. Dehydrogenase activity was significantly increased in compost soil and was inversely correlated with disease occurrence (Table 1). Previously reported increased microbial activity in soil amended with sewage sludge and compost (18,27) suggests that such activity in compost soil may be related to disease suppression. The added organic matter was inversely correlated with disease incidence. Suppression of soilborne diseases has been correlated before with organic matter in soil (9,14,15,21,27) and is attributed usually to a nonspecific effect related to increased microbial activity of the soil in response to increased nutrient content. Preliminary data (R. D. Lumsden and P. D. Millner, *unpublished*) suggest that significantly greater populations of specific microorganisms such as *Trichoderma harzianum* and *Streptomyces* spp. may be associated with suppression of *S. minor* activity. Populations of these organisms significantly increased in soil after amendment with compost. When representative isolates of these organisms were added to soil, aqueous extracts obtained from the soils were inhibitory to germination and formation of sclerotia. *Trichoderma* spp. have been previously associated with the suppressiveness of bark compost to *R. solani* (12).

Sclerotial germination, mycelial growth, and sclerotium formation were affected by compost soil or crude extracts from amended soil but not by cellfree extracts (Table 3). In addition, extracts from compost alone or from sewage sludge alone were not as inhibitory to germination and formation of sclerota as the extracts from compost soil. Survival of sclerotia of *S. minor* was not affected by adding compost to the soil. There were no notable differences in the types and numbers of fungi isolated from sclerotia buried in the soils, which suggests that mycoparasitism did not contribute significantly to disease suppression. Nutrients and fungistatic compounds may affect pathogenesis by *S. minor*. The nutrient status of the compost soil was significantly greater than that of nonamended soil for total nitrogen, nitrate nitrogen, phosphorus, magnesium, and calcium (Table 1). Of these, all but nitrate nitrogen were positively correlated

Table 3. Effect of compost-amended and nonamended soil and extracts from soils, sewage sludge, and compost on mycelial growth and sclerotial germination of *Sclerotinia minor*

Treatment	Mycelial growth on soil (mm)[a] (days of incubation)			Sclerotia formed on soil (no./dish)	Crude soil extract[b]		Cellfree soil extract[c]	
	2	4	7		Germination[d] (% ± SEM)	Sclerotia[d] (% ± SEM)	Germination[d] (%)	Sclerotia[d] (%)
Control soil	20	62	>90	3,979	83 ± 2.3	26 ± 7.7	99	99
Compost-amended soil	13	35	82	427	33 ± 2.9	0 ± 0.0	97	98
Compost	...	...	...	...	63 ± 2.9	2 ± 0.8	99	84
Sewage sludge	...	...	...	...	86 ± 2.9	13 ± 3.9	99	85

[a] Growth and sclerotium production measured on compost-amended (about 10%) or control soil from field plots amended with 5% cornmeal and autoclaved for 30 min.

[b] Extracts were prepared by filtering a suspension of 1 part nonsterile solid material and 2 parts water through Whatman No. 1 filter paper. SEM = standard error of the mean.

[c] Cellfree extracts of the above were obtained by filtering the suspension through 0.45 Millipore filter.

[d] Percentages are based on numbers of sclerotia germinating or producing secondary sclerotia from a total of 21 sclerotia on each of three replicate plates.

with disease suppression. High levels of available nitrogen encourage disease caused by *Sclerotinia* spp. (J. R. Steadman, *personal communication*). The slow release of nitrogen from organic matter may provide sufficient nutrients for crop production but minimizes secondary effects on the susceptibility of the host to the pathogen. There were no differences in lettuce plant size or weight during the 4-yr study although no supplementary fertilizer was added to the compost soil after the initial treatments.

Calcium in soil, as well as in host tissue, may inhibit pathogen progress in infected tissue to delay pathogenesis and allow time for host disease resistance responses (9,13,27). Similarly, a deficiency of magnesium and manganese is reported to increase susceptibility of seedlings to attack by *S. sclerotiorum* (2). Possibly, an abundance of these elements in soil could slow down or prevent infection by *Sclerotinia* spp. Differences in pH (Table 1) of the two soils would be accounted for by the increased calcium levels in the compost soil. The pH of the soil was not, however, correlated with disease suppression and would not have a significant effect on disease caused by this pathogen over this narrow range of pH differences.

There are no reports of phosphorus affecting susceptibility of lettuce to *Sclerotinia* spp. However, phosphate has been reported to inhibit germination of sclerotia (25). Cadmium also inhibited germination of sclerotia but calcium and magnesium did not (25). Cadmium and other heavy metals are at low levels in the compost (23) and presumably would not affect pathogen activity. In fact, heavy metals are not readily available at the soil pH range of 6.0–7.5 in the field plots (10). In addition, no increased accumulation of heavy metals in the lettuce was present at the rates of composts used (Table 2). The compost used in this study was considered safe in terms of human dietary toxicity and phytotoxity on the basis of its microelemental analysis (10,23).

Fungal inhibitors are released in some bark composts and can account for some observed disease control with certain pathogens (12). These chemical inhibitors are reported to play a role in suppression of Phytophthora root rots in potting media amended with hardwood bark. Although irradiated or autoclaved compost soil slowed growth and sclerotium formation of *S. minor* (Table 3), possibly indicating more than a biological factor responsible for the suppression effect, we could not demonstrate the presence of substances inhibitory to growth and sclerotium production in cellfree aqueous extracts of compost soil, compost, or sludge.

Sewage sludge compost significantly reduced disease incidence over the 4-yr study period. Where available and when economically feasible to use this source of organic matter, compost can serve as a valuable nutrient supplement and a means to improve the suppressiveness of agricultural soil to disease caused by *Sclerotinia* spp.

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