Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Crop Protection 22 (2003) 1093–1097
Crop Protection
www.elsevier.com/locate/cropro

Effect of sewage sludge on the rot and seedling damping-off of bean plants caused by *Sclerotium rolfsii*

I. dos Santos[a], W. Bettiol[b,*]

[a] *Centro Federal de Educação Tecnológica do Paraná, CP 571, Pato Branco PR 85503-390, Brazil*
[b] *Embrapa Meio Ambiente, CP 69, Jaguariúna SP 13.820-000, Brazil*

Received 23 April 2002; received in revised form 1 May 2003; accepted 27 May 2003

Abstract

The effect of sewage sludge at concentrations of 12.4, 24.8, 37.2, and 49.6 t/ha on the severity of diseases caused by *Sclerotium rolfsii* upon the emergence, final stand and plant dry matter weight, the soil's electric conductivity and microbial activity was studied in beans grown under field conditions on a dystrophic Red Yellow Ultisol. Soil was infested with 100 g/m^2 of unhulled rice containing the pathogen. Sewage sludge reduced the incidence and the severity of the diseases caused by *S. rolfsii* and increased emergence and the final stand of the bean plants in three bean cropping seasons. Microbial activity, measured through the hydrolysis of fluorescein diacetate and the release of CO_2, and the electric conductivity were directly proportional to the concentration of sewage sludge. For all cropping seasons, emergence and final stand of plants were positively correlated with the microbial activity in the soil and with the soil's electric conductivity. Sludge concentrations did not influence survival of the sclerotia for a 120-day period. No problem was detected with regard to plant development.

Keywords: *Phaseolus vulgaris*; Biosolids; Organic matter; Microbial activity; Suppressive soil

1. Introduction

Application of sewage sludge interferes with the soil's physical, chemical and biological properties, and among the latter are those related to plant diseases. In this regard, incorporation of sewage sludge into the soils reduced the incidence or the severity of Sclerotinia drop on lettuce, caused by *Sclerotinia minor* (Lib.) de Bary (Millner et al., 1982; Lumsden et al., 1986); root rot on sorghum and sugarcane, caused by *Pythium arrhenomanes* Drechsler (Bettiol and Krügner, 1984; Dissanayake and Hoy, 1999); root rot on peppers, caused by *Phytophthora capsici* Leonian (Lumsden et al., 1983); Fusarium wilt on cucumber and basil, caused by *Fusarium oxysporum* Schlecht (Lumsden et al., 1983; Ferrara et al., 1996); damping-off caused by *Rhizoctonia solani* Kühn and *Pythium ultimum* Trow on peas and cotton (Lewis et al., 1992); dollar spot on creeping bentgrass and annual bluegrass turf, caused by *Sclerotinia homoeocarpa* F.T. Bennett (Nelson and Craft, 1992); root rot caused by *Pythium graminicola* Subram on creeping bentgrass (Craft and Nelson, 1996); root rot of beans, cotton and radish, caused by *R. solani* (Lumsden et al., 1983); damping-off in cucumber seedlings, caused by *Pythium aphanidermatum* (Edson) Fitzp. (Santos et al., 2000); and damping-off and rot in the collar of bean plants, caused by *Sclerotium rolfsii* Sacc. (Santos and Bettiol, 2001). On the other hand, Kim et al. (1997) reported that sewage sludge did not interfere with the incidence of root and crown rot, caused by *P. capsici* on bell pepper, nor did it reduce the pathogen population in the soil. However, there are reports also of increased diseases as a consequence of sewage sludge incorporation, for example the corn stalk rot caused by *Fusarium* (Bettiol, 2000), and root rots caused by *P. ultimum* and *Thielaviopsis basicola* (Berk & Broome) Ferraris in beans, peas, and cotton (Millner et al., 1982).

The way by which sewage sludge reduces the severity of diseases, according to reports found in most papers, seems to be related to an increase in microbial activity in the soil and to the microbiota itself comprised in the

*Corresponding author. Tel.: +55-019-867-8700, fax: +55-019-867-8740.
E-mail address: bettiol@cnpma.embrapa.br (W. Bettiol).

0261-2194/03/$ - see front matter
doi:10.1016/S0261-2194(03)00140-6

organic material (Chen et al., 1987; Craft and Nelson, 1996; Dissanayake and Hoy, 1999).

Since no literature information was found relating to sewage sludge effects on *S. rolfsii* for the soil and climate conditions in Brazil, this study was aimed at evaluating the effect of sewage sludge on the diseases caused by *S. rolfsii* on bean plants (*Phaseolus vulgaris* L.) and its possible mode of action.

2. Material and methods

The trials were carried out at Embrapa Meio Ambiente, in Jaguariúna, SP, Brazil, latitude 22°41′ south, longitude 47°W.Gr. and altitude 570 m, on a dystrophic Red Yellow Ultisol (pH = $CaCl_2$ 5.4; OM = 22 g/dm^3; P = 4 mg/dm^3 K = 1.54, Ca = 44, Mg = 11, Al + H = 28 and CEC = 84.5 mmol/dm^3; V% = 66.9). The sewage sludge was obtained from the wastewater treatment plant, Franca, SP, which treats predominantly home sewage. The composition of the sewage sludge (Table 1) utilized in the trials complied with the guidelines for sludge use in agriculture adopted by CETESB (1999).

The effect of sewage sludge on *S. rolfsii* was evaluated in 1 m^2 plots delimited by clay roof tiles placed side by side, containing infested soil, 2 months prior to sludge application, with 100 g of the substrate per plot. For that purpose, a *S. rolfsii* isolate was grown 10 days at 25 ± 2°C in sterilized culture medium containing 100 g unhulled rice and 150 ml distilled water, in 1-l containers. Before trial installation, two bean crops were cultivated to ensure that the disease occurred and was homogeneous in the plots. The sewage sludge was incorporated into the soil at concentrations of 12.4, 24.8, 37.2, and 49.6 t/ha. These concentrations were calculated to supply the nitrogen necessary for the crop, and two, three, and four times the necessary amount, respectively. These concentrations were compared to the unfertilized soil and the soil fertilized with N 50 kg/ha, P 60 kg/ha, K 50 kg/ha, according to recommendations by Raij et al. (1996) for the bean crop. Sowing, with 80 seeds of the Carioquinha variety per plot, was done 1 week after sludge application, in two successive cropping periods. A second sludge application was executed 9 months after the first, at the same concentrations previously utilized, and a crop was grown. The emergence, final stand, and severity of the disease, as well as the pH, electric conductivity, and plant dry matter weight were evaluated in all cropping periods. The microbial activity, by means of fluorescein diacetate hydrolysis (FDA) and CO_2 release, was evaluated only in the third cropping period. The severity of the disease, considering the percentage of plant tissue showing lesion injury at the plant collar, was evaluated for the three cropping periods, using the following rating scale: 1 = plants with no visible symptoms; 3 = plants with approximately 10% of their hypocotyls and/or roots covered by lesions; 5 = approximately 25% of the hypocotyl and/or roots covered by lesions, however the tissues remain firm, with root system deterioration and evident leaf discoloration; 7 = approximately 50% of the hypocotyl and/or roots covered by lesions and severe rot of the root system; and, 9 = approximately 75% or more of the tissues covered by lesions, in a highly developed state of decomposition, with severe reduction of the root system (Schoonhoven and Pastor-Corrales, 1987). Isolation of the pathogen from diseased plants was performed after the disease evaluation to confirm the results. The determination of FDA hydrolysis was based upon the methodology described by Boehmn and Hoitink (1992) and Ghini et al. (1998), and the CO_2 release determination was based on Grisi's (1978) methodology.

Table 1
Chemical characteristics of sewage sludge utilized in the experiments

pH (water)	6.4
Humidity (65°C)	52.1
C (g kg^{-1})	374.4
N Kjeldahl (g kg^{-1})	50.8
N-ammoniacal (mg kg^{-1})	119.5
N-nitrate-nitrite (mg kg^{-1})	54.8
P (g kg^{-1})	21.3
K (g kg^{-1})	0.99
Ca (g kg^{-1})	16.8
Mg (g kg^{-1})	2.5
S (g kg^{-1})	13.3
Mo (mg kg^{-1})	<1
B (mg kg^{-1})	7.1
Na (g kg^{-1})	0.6
Cr (mg kg^{-1})	1325
Mn (mg kg^{-1})	267.4
Fe (mg kg^{-1})	31,706
Ni (mg kg^{-1})	74.7
Cu (mg kg^{-1})	359.2
Zn (mg kg^{-1})	1590
Al (mg kg^{-1})	33,550
Cd (mg kg^{-1})	2
Pb (mg kg^{-1})	118.8
Ar (mg kg^{-1})	<1
Se (mg kg^{-1})	0
Hg (mg kg^{-1})	<1

Concentration values given are based on dry matter.
Concentration values for nitrogen in the ammoniacal and nitrate forms were determined in the sample in the original conditions.

Another factor evaluated in the field trial was the survival of pathogen sclerotia in the first and the third cultivation. In order to assess this factor, a sample of soil was collected from six points in each plot, 10 cm deep, totaling 500 g. After homogenizing, a 50 g aliquot was extracted from each sample and sifted through a set of five sieves with 4.75, 2.36, 1.00, 0.053, and 0.037 mm mesh, under water pressure from a faucet. The three sieves with the smaller mesh were dipped into a white

Table 2
Effect of sewage sludge rates on the emergence (EM), stand (ST), and severity of the disease (SEV) caused by *S. rolfsii* on bean plants (variety Carioquinha); and on the pH, electric conductivity (EC) and microbial activity (FDA and release of CO_2) in the soil

Treatment	First cultivation					Second cultivation					Third cultivation						
	EM	ST	SEV	pH	EC	EM	ST	SEV	pH	EC	EM	ST	SEV	pH	EC	FDA	CO_2
Control	20.0	9	5.3	7	48	48.7	15	7	6.9	110	57.5	36	5.7	6.7	103	10.6	30.7
12.4 t/ha	26.2	14	5.1	6.5	59	48.7	18	5.7	6.6	140	73.7	51	5.5	6.5	231	15.3	55.3
24.8 t/ha	37.5	26	5.8	6.3	60	61.2	25	5.5	6.4	142	67.5	45	5.3	6.2	381	19.3	88.3
37.2 t/ha	42.5	27	5.1	6.3	82	63.7	32	5.4	6.4	188	78.7	54	4.3	6.1	462	22.8	106
49.6 t/ha	43.7	28	5	6.1	84	70.0	36	5.1	6.1	224	82.5	56	3.6	5.8	482	28.3	115
NPK	31.2	11	5.1	6.4	80	48.7	15	6.3	6.4	176	61.2	39	6.2	6.4	190	13.6	36.7
R^2	0.91	0.86	NS	0.92	0.92	0.93	0.99	NS	0.94	0.90	0.74	0.74	0.89	0.95	0.99	0.96	0.93

Treatments with sewage sludge rates are determined on the basis of the nitrogen content provided by the sludge, considering 1, 2, 3, and 4 times the amount needed by the crop. NPK mineral fertilization with 50, 60, and 50 Kg/ha, respectively. Emergence and final stand are based on seeding 80 seeds per plot. Severity of the disease grades from 1 to 9. CE = μs/cm; FDA = μg FDA/g dry soil; CO_2 = mg CO_2/100 g dry soil. Data from treatments 12.4 and 49.6 mg/ha are means of four replicates and the others are means of three replicates. NS = no significant differences.

basin containing water for sclerotia removal with the aid of tweezers. The sclerotia were submitted to surface disinfestation in 2% sodium hypochlorite for 3 min, and plated onto Petri dishes containing water–agar medium. After incubation for 24 h in a growth room at 25 ± 2°C, the sclerotia viability was evaluated based on their germination.

The data obtained were submitted to analysis of variance, polynomial regression analysis (SAS, 1999).

3. Results and discussion

Seedling emergence, in the three cropping periods under field conditions, was directly proportional to the sewage sludge concentration applied to the soil, with $R^2 = 0.91$, 0.93, and 0.74, for the first, second and third cropping periods, respectively (Table 2). The increase in emergence in the plots treated with sludge was more conspicuous in the first cultivation period, when the pathogen had a more drastic effect, with a 34% increase for the lowest dosage, up to a 118% increase for the highest.

The final stand was directly correlated with sewage sludge rates in the three cultivations ($R^2 = 0.86$, 0.99, and 0.74, respectively) (Table 2). The results indicate that, the more intense the damping-off in pre and post-emergence, reducing the number of plants per plot, the more evident the effect of the sewage sludge in controlling the disease. For the first cultivation, the increase in number of plants varied from 50.5% for the lowest sludge rate up to 201% for the highest. Even for the highest sludge rate, where the most protection of plants against the plant pathogen is observed, the low emergence percentage and low number of plants per plot can be explained by the existing inoculum potential in the experiment, which was extremely high, a situation that is not found under normal cropping conditions in the field. This indicates that the results obtained with sewage sludge application in the soil to protect the plants against the pathogen are promising.

The severity of the disease, different from the emergence and the final stand, showed more evident results in the third cropping period. In the first cropping period, when the attack was more drastic, the sludge promoted a significant increase in emergence and final stand, but did not prevent the formation of lesions in the plants. Since in the third cultivation the attack of the pathogen was lower than that in the first, it contributed toward making the increase in sludge rate reduce the severity of the disease ($R^2 = 0.89$). These results evidence the sewage sludge performance in controlling the diseases caused by *S. rolfsii.* The isolation of the pathogen from diseased plants confirmed that the casual agent was *S. rolfsii*.

There are several reports on the effect of sewage sludge in controlling diseases, such as *Sclerotinia* on lettuce (Lumsden et al., 1983), *R. solani* on beans, cotton, and radish, *Aphanomyces euteiches* on peas (Millner et al., 1982), and *R. solani* and *P. ultimum* on peas (Lewis et al., 1992). However, our research seems to be the first report on the control of diseases caused by *S. rolfsii* on beans, with the incorporation of sewage sludge into the soil.

In most investigations where sewage sludge reduced the incidence and/or the severity of plant diseases, the authors indicated that the increases in microbial activity caused by the application of sewage sludge to the soil, and the microbiota itself comprised in the organic material, were responsible for controlling the diseases (Chen et al., 1987; Craft and Nelson, 1996; Dissanayake and Hoy, 1999). It was verified in this paper as well that

Table 3
Correlation coefficient between emergence (EM), stand (ST), and severity (SEV) of the diseases caused by *S. rolfsii* in bean plants, and the pH, electric conductivity (EC) and microbial activity (FDA and release of CO_2) in soil treated with sewage sludge, in three cultivations

	First cultivation		Second cultivation		Third cultivation			
	pH	EC	pH	EC	pH	EC	FDA	CO_2
EM	−0.93	0.90	−0.92	0.90	−0.82	0.82	0.87	0.82
ST	−0.92	0.84	−0.93	0.96	−0.79	0.81	0.84	0.82
SEV	0.10	−0.50	0.93	−0.81	0.91	−0.85	−0.95	−0.88

Table 4
Effect of sewage sludge on the survival of sclerotia of *S. rolfsii* and on the dry matter weight of bean plants[a]

Treatment[b]	1° cultivation			2° cultivation	Between 2° and 3° cultivation		3° cultivation
	NS	VS	DW (g)	DW (g)	NS	VS	DW (g)
Control	15.6	14.3	2.48	3.1	7.6	6.9	3.0
12.4 t/ha	9.5	9.5	2.48	3.4	5.75	4.5	3.3
24.8 t/ha	12.6	12.3	2.89	3.39	6.3	5.1	3.1
37.2 t/ha	19.6	17.3	2.9	3.2	7.6	7.2	3.25
49.6 t/ha	12.5	11.75	2.67	3.6	8.3	7.25	3.5
NPK	10.6	9.7	2.14	3.48	6.6	4.3	3.4

[a] The statistical analysis did not show differences between treatments for any of the evaluations.
[b] Treatments with sewage sludge rates are determined on the basis of the nitrogen content provided by the sludge, considering 1, 2, 3, and 4 times the amount needed by the crop; and NPK mineral fertilization with 50, 60, and 50 kg/ha. NS = number of sclerotia found in plot in 50 g of soil; VS = viable sclerodia; DW = dry matter weight of plants in grams.

the microbial activity, determined by means of FDA hydrolysis and release of CO_2, was directly proportional to the sewage sludge rate incorporated into the soil (Table 2). The microbial activity in the soil is increased during organic matter decomposition, present in many composts, and this microbial activity is expressed as an antagonistic action among the microorganisms (Millner et al., 1982; Chen et al., 1987). Therefore, activating the microbiota in the soil is a means to potentialize natural biological control. According to Ghini et al. (1998), biological control projects that restrict their scope to the use of microorganisms that are able to grow in culture medium are constrained to a limited number of organisms; under natural conditions of the soil, what is important is the sum of effects. The same authors conclude that determining the total microbial activity is more important than determining the activity of a few groups, because the higher this activity, the higher the competition between the microorganisms. In this research, the increase in microbial activity is positively correlated with the reduction of diseases on bean plants (Table 2). The FDA hydrolysis was positively correlated with emergence ($r = 0.87$) and stand ($r = 0.84$), but negatively correlated with severity of the disease ($r = 0.95$). The release of CO_2 showed similar results, being positively correlated with emergence ($r = 0.82$) and stand ($r = 0.82$), but negatively correlated with severity of the disease ($r = 0.88$) (Table 3).

The release of CO_2, besides indicating higher microbial activity, could be involved in the inhibition of germination of the pathogen's sclerotia. Barreto (1997) affirm that the fungus *S. rolfsii* requires high oxygen, and this fact limits sclerotia germination in the interior of heavy soils. Thus, the development of the pathogen only occurs near the surface. However, its inability in reaching the plants is related to its sensitivity to CO_2, which is a product of microbial growth and becomes an antagonistic mechanism (Griffin, 1977). It was verified by Smith (1973), that in soils submitted to air containing 0.1 ppm ethylene, *S. rolfsii* sclerotia remained dormant. Thus, it is possible that the high rates of sludge, and especially the nitrogen it contains, could have contributed to increased ethylene compared to the control.

Another factor that could be related to the sludge action in reducing the severity of disease is EC, since it was negatively correlated with sewage sludge concentration in the three cropping periods (Table 2). It is possible that the increase of EC and consequent salinity of the soil have harmed in a direct way and/or reduced the competitive potential of *S. rolfsii* with the microbiota of the soil. And the increase of the osmotic pressure can inhibit the development of some microorganisms, mainly the fungi.

There was no influence of sewage sludge rates on the number of recovered and viable sclerotia (Table 4). The results obtained in this experiment indicate that the sludge, though reducing the intensity of the diseases caused by *S. rolfsii*, did not interfere with the survival of the pathogen's resistance structures, during the period in which evaluations took place. As was verified, the incorporation of sewage sludge increases microbial activity and the release of CO_2. These factors could have inhibited the germination of sclerotia in the soil. Notwithstanding, it is possible that in laboratory conditions, the sclerotia were not under the competition pressure from the soil's microbiota and subject to high CO_2 concentrations, and thus were able to germinate completely.

The sclerotia survival results for *S. rolfsii* indicate that the sewage sludge does not directly interfere with the viability of sclerotia (Table 4). However, it was verified that, somehow, the sludge reduces the pathogen's action with respect to its ability to produce disease. This fact could suggest that the sewage sludge induces the soil's capacity to suppress the disease, and not the pathogen.

One of the concerns in this experiment was related to the possible plant toxicity of sewage sludge to bean plants. Nevertheless, the plant dry matter weight was not different between treatments for the three cultivations (Table 4), and in general, the sewage sludge yielded the highest plant weights.

References

Barreto, M., 1997. Doenças do amendoim. In: Kimati, H., Amorin, L., Bergamin Filho, A., Camargo, L.A., Rezende, J.M. (Eds.), Manual de Fitopatologia. Doenças das Plantas Cultivadas. Ceres, São Paulo, pp. 65–77.

Bettiol, W., 2000. Efeito do lodo de esgoto na incidência da podridão do colmo do milho causada por *Fusarium*. Fitopatol. Bras. 25 (suppl.), 359 (Abstract).

Bettiol, W., Krügner, T.L., 1984. Influência do lodo de esgoto na severidade da podridão de raiz do sorgo causada por *Pythium arrhenomanes*. Summa Phytopathol. 10 (2), 243–251.

Boehmn, M.J., Hoitink, H.A.J., 1992. Sustenance of microbial activity in potting mixes and its impact on severity of *Pythium* root rot of *Poinsettia*. Phytopathology 82 (3), 259–264.

CETESB, 1999. Aplicação de biossólidos de sistemas de tratamento biológico em áreas agrícolas—Critérios para projeto e operação (P4 230). Cetesb, São Paulo.

Chen, W., Hoitink, H.A.J., Schmitthenner, A.F., 1987. Factors affecting suppression of *Pythium* damping-off in container media amended with composts. Phytopathology 77 (5), 755–760.

Craft, C.M., Nelson, E.B., 1996. Microbial properties of composts that suppress damping-off and root rot of creeping bentgrass caused by *Pythium graminicola*. Appl. Environ. Microbiol. 62, 1550–1557.

Dissanayake, N., Hoy, J.W., 1999. Organic material soil amendment effects on root rot and sugarcane growth and characterization of the materials. Plant Dis. 83 (11), 1039–1046.

Ferrara, A.M., Avataneo, M., Nappi, P., 1996. First experiments of compost suppressiveness to some phytopathogens. In: Bertoldi, M., Sequi, P., Lemmes, B. (Eds.), The Science of Composting. Glasgow: Blackie Academic Part 2, pp. 1157–1160.

Ghini, R., Mendes, M.D.L., Bettiol, W., 1998. Método de hidrólise de diacetato de fluoresceina (FDA) como indicador de atividade microbiana no solo e supressividade a *Rhzoctonia solani*. Summa Phytopathol. 24 (3–4), 239–242.

Griffin, D.M., 1977. Water potential and wood-decay fungi. Annu. Rev. Phytopathol. 15, 319–329.

Grisi, B.M., 1978. Método químico de medição da resperação edáfica: alguns aspectos técnicos. Ciênc. Cult. (São Paulo) 30 (1), 82–88.

Kim, K.D., Nemec, S., Musson, G., 1997. Effects of composts and soil amendments on soil microflora and *Phytophthora* root and crown root of bell pepper. Crop Prot. 16 (2), 165–172.

Lewis, J.A., Lumsden, R.D., Millner, P.D., Keinath, A.P., 1992. Suppression of damping-off of peas and cotton in the field with composted sewage sludge. Crop Prot. 11 (6), 260–266.

Lumsden, R.D., Lewis, J.A., Millner, P.D., 1983. Effect of composted sewage sludge on several soilborne pathogens and diseases. Phytopathology 73 (11), 1543–1548.

Lumsden, R.D., Millner, P.D., Lewis, J.A., 1986. Suppression of lettuce drop caused by *Sclerotinia minor* with composted sewage sludge. Plant Dis. 70 (3), 197–201.

Millner, P.D., Lumsden, R.D., Lewis, J.A., 1982. Controlling plant disease with sludge compost. Biocycle 23 (1), 50–52.

Nelson, E.B., Craft, C.M., 1992. Suppression of dollar spot on creeping bentgrass and annual bluegrass turf with compost-amended topdressings. Plant Dis. 76 (9), 954–958.

Raij, B., Cantarella, H., Quaggio, J.A., Furlani, A.M.C., 1996. Recomendações de adubação e calagem para o Estado de São Paulo. Instituto Agronômico & Fundação IAC, Campinas.

Santos, I., Bettiol, W., 2001. Efeito do lodo de esgoto na podridão do colo e tombamento de plântulas de feijoeiro induzidas por *Sclerotium rolfsii*. Summa Phytopathol. 27 (1), 113.

Santos, I., Mazzeo, A.N., Bettiol, W., 2000. Efeito do lodo de esgoto no tombamento de plântulas de pepino induzido *por Pythium aphanidermatum*. Summa Phytopathol. 26 (1), 141 (Abstract).

SAS, 1999. Statistical Analysis System (Software). SAS/STAT User's Guide, Version 8. SAS Institute, Cary, NC, USA.

Schoonhoven, A.van., Pastor-Corrales, M.A., 1987. Standard System for the Evaluation of Bean Germplasm. CIAT, Cali.

Smith, A.M., 1973. Ethylene as a cause of soil fungistasis. Nature 246, 311–313.