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Suppression of Lettuce Drop caused by *Sclerotinia sclerotiorum* in the Field using Municipal Solid Waste Compost and Fungistatic Effect of Water Extract

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ABSTRACT

Sclerotinia sclerotiorum (Lib.) De Bary is the principal etiological agent of lettuce drop in Italy and worldwide. The objectives of this study were: i) to evaluate the efficacy of source-separated municipal waste compost when used as fertilizer at a rate of 25 t ha^{-1} on a dry matter basis in suppressing *S. sclerotiorum* on lettuce; ii) to investigate the effect of compost water extract on myceliogenic germination of sclerotia; iii) to determine the effect of water extract on 6 pathogens of lettuce. In order to achieve these objectives, two lettuce crop cycles in open fields, two fungal bioassays and soil assays of sclerotial content in the laboratory were carried out in 2008. Results demonstrated that the incidence of *S. sclerotiorum* in open fields was significantly lower in compost than in mineral fertilized plots (-31% in average). Marketable yield was significantly higher for compost amended plots than those mineral fertilized (+ 6.5 t ha^{-1} in average). No variation in soil population of the pathogen was observed. Sterile water extract from mature compost was able to inhibit growth of *S. sclerotiorum* and *Rhizoctonia solani* AG 2-1 with IC_{50} values of 308 and 438 mg l^{-1} respectively, while it was not effective toward four other pathogens tested, including *R. solani* AG 1. *S. sclerotiorum* myceliogenic germination of sclerotia was also affected by water extract. Fungistasis could be one of the possible mechanisms of action, but this hypothesis does not imply that biological rather than physical mechanisms should act contemporary. Water extract of mature compost demonstrated the potential for extraction of active molecules. In addition, the extract was not phytotoxic, as reported for certain immature compost teas.

Keywords: compost amendment, fungal bioassays, lettuce yield, *Rhizoctonia solani* AG 2-1

Abbreviations: AG, anastomosis group; **ANOVA**, analysis of variance; **COWE**, compost water extract; **IC_{50}**, concentration at which fungal growth is inhibited by 50% relative to controls

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) De Bary is the principal etiological agent of lettuce drop in Italy (Gullino *et al*. 2007) and worldwide (Subbarao 1998; Wu and Subbarao 2006). Lettuce drop is the most important disease of lettuce both in the field and greenhouse, causing epidemics mainly in spring and autumn. *S. sclerotiorum* has a wide host range that can include broccoli, cabbage, cauliflower, carrot, celery, beans, tomato, pepper, potato, sunflower, eggplant, squash, asparagus, beet, broadbean, and several flower crops. It is able to infect lettuce at all stages. On developed plants the initial symptoms are wilting of leaves and crown necrosis, followed by plant necrosis and death. After the onset of initial symptoms, a mass of white mycelia is apparent on plant stems at the soil surface. Large black, irregularly shaped sclerotia are normally present on necrotic stem tissue. Resistant varieties are not available and fungicide applications are necessary to avoid yield losses; nevertheless, the reduction of agrochemical use is one of the major challenges in lettuce production.

Biological control of soil-borne pathogens using microorganisms has been investigated and proposed, such that commercial products containing one (*Trichoderma harzianum* or *Coniothyrium minitans*) or more biological control agents (*Trichoderma harzianum* and *Trichoderma viride*) have been tested in many environments (Chitrampalam *et al*. 2008) and registered (Lorito *et al*. 1998; Koch 1999; Saharan and Metha 2008). In addition, integrated control of *S. sclerotiorum* using *Coniothyrium minitans* combined with a reduced rate of fungicide has been proposed (Budge and Whipps 2001). In all these cases results seem to vary with environment and control in many cases is not really comparable with that of fungicide applications. The use of organic amendments to control soil-borne pathogens has also been investigated since the 1970s; moreover, the use of organic matter has also been proposed to improve soil structure and fertility (Magid *et al*. 2001; Conklin *et al*. 2002). Reviews on the use of compost and organic matter in conventional and organic agricultural systems have recently been published (Noble and Coventry 2004; Bonanomi *et al*. 2007). Less work has been done on the suppression of soil-borne pathogens in the field than in controlled chambers and containers. Furthermore a few papers investigated the suppression of both *Sclerotinia minor* (Lumdsen *et al*. 1983, 1986) and *S. sclerotiorum* (Asifiri *et al*. 1994) by means of compost amendments in the field on lettuce. The rate of compost application that is effective for pathogen suppression is important and different rates of application are reported in the literature (Noble and Coventry 2004). On the other hand, the rate of compost application also influences crop yield. Roe (1998) in a review about compost effects for vegetable and fruit crops, reported that lettuce yields increased when fertilized with 37 and 74 t ha^{-1} of a biosolids + yard trimmings compost. Few other examples are available in literature on lettuce response to compost fertilization.

Several mechanisms of disease control have been demonstrated and reported (Noble and Coventry 2004) for compost application. In this respect, water extract of mature compost could play a role in suppressing disease (El-Masry *et al*. 2002) even if the suppressive effect of compost is predominantly biological.

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The objectives of this study were: i) to evaluate the efficacy of compost in suppressing *S. sclerotiorum* in the field on lettuce when used as fertilizer; ii) to investigate the effect of compost water extract at four different concentration levels on myceliogenic germination of sclerotia; iii) to determine the effect of compost water extract at four different concentration levels on 6 pathogens of lettuce.

MATERIALS AND METHODS

Type of compost

Municipal solid wastes compost was produced by GESENU S.p.A. at Perugia using an organic fraction source separated from municipal solid wastes and mixed (1:1; v/v) with yard trimmings. The resulting compost had the following properties: 26% water, 74% dry matter, 28% organic C, 2.1% total N, 0.8% P_2O_5, 1.8% K_2O, 13.3 C/N ratio, 67.2 mg/kg Cu, 146 mg/kg Zn, 0.2 meq/100 g electrical conductivity.

Field experiments

Experiments were conducted at the Agricultural Research and Experimentation Council, Unit of Scafati in 2008 to evaluate the efficacy of compost application against lettuce drop caused by *S. sclerotiorum*. Two fertilization strategies were compared: Compost amendment vs. mineral N fertilizer. A randomized complete block design with four replicates was adopted; each replication was about 30 m^2. A space of 4 m was left between compost and mineral fertilized plots. Two crop cycles were carried out in a field with a history of *S. sclerotiorum* losses in order to work with natural infection. *Lactuca sativa* L. cv. 'Mirella' (SAIS seeds S.p.A. Italy) was planted in double rows on raised beds not mulched. The plant population was equivalent to 70,000 plants ha^{-1}. The rate at which compost was applied to the soil was 25 t ha^{-1} of dry matter according to the results obtained in previous research by Pagano *et al*. (2008). Mineral N fertilization was with ammonium nitrate sulphate at the rate of 100 kg ha^{-1} distributed before transplantation. Weeds were controlled by one hand weeding. At harvest the number of diseased plants per plot was determined and the incidence of lettuce drop was assessed. All marketable lettuce plants for each plot were collected and weighed; yield was expressed as ton ha^{-1}.

Sclerotia counts

To count sclerotia in soil, four subsamples in each plot were randomly collected drawing from the 0-15 cm layer. The final sample of 2 kg was wet sieved, sclerotia were then isolated using the method reported by Adams (1979) and expressed as number of sclerotia 100 g of $soil^{-1}$.

Fungal bioassay

1. Fungal isolates

Six fungi were isolated from lettuce: *Rhizoctonia solani* AG1, *Rhizoctonia solani* AG 2-1, *Phytophthora cactorum*, *Colletotrichum* sp., *Sclerotinia minor* and *Sclerotinia sclerotiorum* were selected for this study. The isolates utilized in this study were preserved at 5°C as stock cultures on slants of potato dextrose agar in the mycological collection of the Agricultural Research and Experimentation Council at Scafati.

2. Bioassays

Compost water extract (COWE) was obtained by shaking 1 kg of compost with 1 l of distilled water for 4 h at 25°C. The suspension was filtered through Whatman paper n° 1, centrifuged at 4000 rpm for 15 min and liquid was collected, lyophilised (Edwards High Vacuum, Minifast 1700) and stored at -20°C.

Bioassays were designed in order to evaluate the effects of COWE on the 6 above-mentioned fungi. The amended agar method was employed (Lahoz *et al.* 2008) in which potato dextrose agar was sterilized and then mixed with appropriate amounts of filter-sterilized (Whatman 0.45 µm) COWE dissolved in water to obtain five different final COWE concentrations (1000, 500, 100, 10, and 0 mgL^{-1}). The amended medium was allowed to solidify in 9-cm Petri dishes and then 5-mm mycelial plugs, which were taken from the growing margins of the 6 fungal cultures at day 10, were placed on the medium with the mycelial side down. Plates were incubated at 24-25°C. Each concentration was replicated four times with four controls (no COWE) for each fungus. The bioassay was repeated twice. Radial growth was recorded after 48 h and then every 24 h by measuring two perpendicular diameters of fungal colonies until the control reached the edge of Petri dishes, in order to calculate IC_{50} values (the concentration at which the fungal growth was inhibited by 50% relative to controls).

The same method was used to evaluate the effect of COWE on myceliogenic germination of sclerotia; for this purpose 80 sclerotia of *S. sclerotiorum* were put in Petri dishes containing a nutrient medium (Lumdsen *et al*. 1986) and data of COWE at five different concentration levels (1000, 500, 100, 10, and 0 mg L^{-1}) were collected when most sclerotia in the control dishes had germinated.

Statistical analysis of data

The test for homogeneity of variance applied to the data of the two bioassays, allowed the results to be combined into one experiment. The effect of COWE was expressed as the percentage inhibition. The $\log_{10}$ dose concentration–response relationship was linearized using probits and IC_{50} was evaluated (Lahoz *et al*. 2008); for each concentration the standard error was calculated. IC_{50} data were analysed by ANOVA, means were separated by least significant difference (LSD) ($p=0.05$). Productive data were also analysed by ANOVA and means separated as above mentioned. All statistical calculations were made using the STATISTICA^{TM} software package (StatSoft Inc., Tulsa, OK, USA).

RESULTS

Field experiment

Disease incidence (**Table 1, Fig. 1**) was greater in the first trial than in the second one. In both trials incidence of *S. sclerotiorum* was significantly lower in compost than in mineral fertilized plots (-38.4 and -24.4% in the first and second trials, respectively). In both trials, commercial yields were significantly higher in compost amended plots than mineral fertilized ones with an increase of 7.5 and 5.8 t ha^{-1}, respectively for the two trials. At harvest the surviving lettuce heads showed no difference in average weight related to the type of soil fertilization (**Table 1**).

Table 1 Data of lettuce yield and *S. sclerotiorum* incidence of two field experiments.

	Yield (t/ha)		Disease incidence (%)		Head weight (g)	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Mineral	11.6	10	50.6*	33.8*	247	334
Compost	19.1*	15.8*	12.2	14.4	253	336

* means are significantly different (p=0.05)

Fig. 1 Lettuce drop caused by *Sclerotinia sclerotiorum* as influenced by compost amendment (right) and mineral N fertilization (left).

Sclerotia counts

No significant differences were recorded for either the number of sclerotia or their percentage germination after sampling from compost-amended and -unamended soils (**Table 2**).

Fungal bioassay

At the tested concentrations, COWE showed different effects related to each of the fungal species. **Fig. 1** shows the $\log_{10}$ concentrations vs. percentage inhibition curve, where each point is the mean of the two experiments with 4 replicates.

Regarding *Phytophthora cactorum*, *Pythium* sp., *Rhizoctonia solani* AG 1 and *Colletotrichum acutatum* no decrease in radial growth was observed at any of the tested concentrations (**Fig. 2**).

S. sclerotiorum was shown to be sensitive to COWE. The logarithm concentrations *vs* percentage inhibition curve (**Fig. 3**) reached a percentage inhibition above 50% at about 331 mg l^{-1}; the curve then showed a sharp increase until 1000 mg l^{-1}.

For *R. solani* AG2-1, 331 mg l^{-1} of COWE resulted in 44% inhibition, after which the percentage of inhibition showed a sharp increase until a concentration of 1000 mg l^{-1} (**Fig. 3**).

The values of IC_{50} calculated after probit transformation gave significant differently results related to the two fungal species inhibited by COWE and were 308 and 438 mg l^{-1} for *S. sclerotiorum* and *R. solani* AG 2-1, respectively.

S. sclerotiorum and *R. solani* AG 2-1 isolates began to show a slow recovery in growth 1 week after the last growth assessment. **Table 3** reports the data of myceliogenic germination of sclerotia at different COWE concentrations; the percent of germination ranged from 95.8% in the control to 12.5% for dishes amended with 1 g l^{-1} of lyophilized COWE.

DISCUSSION

Organic matter amendments with different materials may increase, decrease or have no effect on soil-borne pathogens (Bonanomi *et al*. 2007). A few studies report the suppression of *S. sclerotiorum* on lettuce in the field after compost amendments, however no studies report the use of source-separated municipal solid waste compost for this purpose (Noble and Coventry 2004). In the present report source-separated municipal solid waste compost amendments at the rate of 25 t ha^{-1} controlled lettuce drop in two field experiments to a large extent. An important point is the rate of application, which can vary from 10 t ha^{-1} (Fuchs 1995) to 121 t ha^{-1} (Maynard and Hill 2000). However the maximum rates of compost application to land according to the EU nitrate directive should be limited to the equivalent of 170 or 340 kg N ha^{-1} for soils with or without nitrogen leaching risk, respectively. The rate applied was calculated taking into account that compost amendment has to fulfil a double objective: to maintain or restore the soil organic carbon pool and to sustain crop yield with adequate supply of nutrients. Under the conditions in this study, on the basis of preceding research (Pagano *et al.* 2008), these objectives seem to be achieved applying compost rates between 15 and 30 t ha^{-1}. The rate of 25 t ha^{-1} was confirmed to be effective because it suppressed disease, sustained yields and did not diminish the weight of healthy head lettuce in comparison to mineral fertilization.

Many mechanisms involved in soil-borne disease suppression by compost have been reported and in a few cases it was due to the eradication of the pathogen. The activity of toxic compounds such as ammonia, volatile fatty acids, glucosinolates, thiocyanates has been investigated and reported to be one of the most important mechanisms in suppression of diseases due to *Verticillium dahliae* and *Phytophthora* and plant parasitic nematodes (Tsao and Oster 1981; Hawk and Lazarovitis 1994; Akhtar and Malik 2000; Shetty *et al.* 2000). In this study the numbers of viable sclerotia recovered from compost and mineral fertilized plots were not statistically different, indicating no variations in fungal population. On the other hand, data of growth inhibition obtained in bioassays using water extract showed that fungal

Table 2 Results of number of *S. sclerotiorum* sclerotia collected in compost and mineral fertilized soils and percent of myceliogenic germination in two field experiments.

	Sclerotia collected from soil			
	Trial 1		Trial 2	
	Compost	Mineral	Compost	Mineral
Number/100 g soil*	4.7 ± 0.65	4.9 ± 1.23	4.6 ± 0.9	4.3 ± 0.67
% germination*	53 ± 7.9	56 ± 6.2	50 ± 2.7	44 ± 7.2

* data are not significant different (p=0.05).

Table 3 Results of compost water extract effect on *S. sclerotiorum* sclerotia germination.

Compost water extract Concentration (g L^{-1})	Sclerotia germinated (%)
0	95.8 A
0.1	87.5 A
0.5	54.2 B
1	12.5 C

Means with the same letter are not significant different (p=0.05).

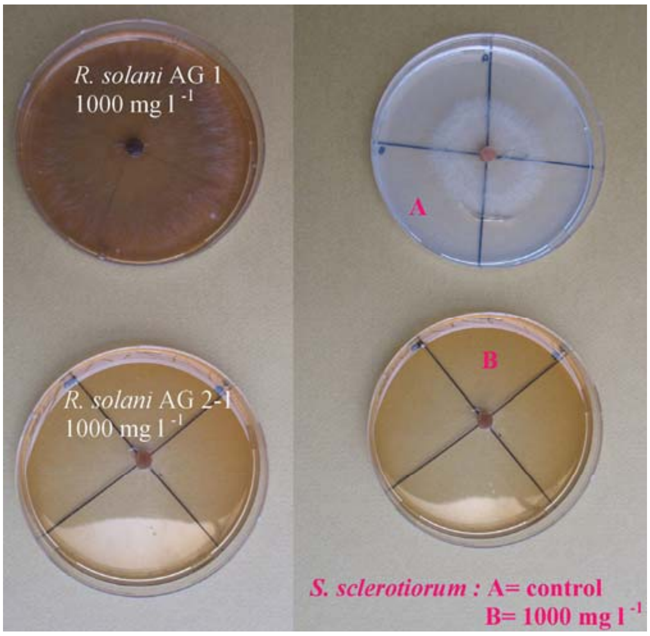

Fig. 2 Compost water extract effect at the highest concentration on *Sclerotinia sclerotiorum, Rhizoctonia solani* AG 2-1 and AG 1.

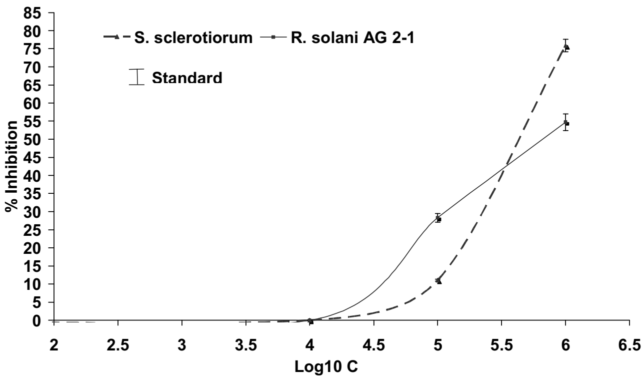

Fig. 3 Effect of increasing compost water extract concentrations on the inhibition of radial growth of two sensitive fungal species. Each point represents the mean of two different experiments with four replicates.

species tested were differently affected by the addition of sterilized extract to the media. Of the 6 species tested only *S. sclerotiorum* and *R. solani* AG 2-1 showed reduced growth rates, indicating that the water extract contains specific inhibitory substances that could play a role in suppression. Regarding *R. solani*, it is well known that different anastomosis groups have different responses both to toxins (Van den Boogert 1996) and fungicides (Kataria and Gisi 1996); this evidence was also observed in our experiments. Regarding *Sclerotinia* species no specificity has previously been reported for any mature compost water extract. The growth of *S. sclerotiorum* and *R. solani* AG 2-1 isolates slowly recovered after 1 week from growth assessment, demonstrating that COWE has a fungistatic rather than fungicidal effect. Moreover higher concentrations of filter sterilized water extract were able to inhibit myceliogenic germination of sclerotia. These two findings indicate that fungistasis could be one of possible mechanisms of action (El Masry *et al*. 2002), but this hypothesis does not exclude the action of other biological rather than physical mechanisms. This study has indicated that water extract of mature compost may contain potentially active molecules, while at the same time is not phytotoxic, as has indeed been reported for certain immature compost teas (Bonanomi *et al*. 2007).

Work is in progress to identify what type of substances municipal waste compost contains and the specific mechanisms involved in fungal growth inhibition.

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REFERENCES

Adams PB (1979) A rapid method for quantitative isolation of sclerotia of *Sclerotinia minor* and *Sclerotinia cepivorum* from soil. *Plant Disease Reporter* **63**, 349-351

Akhtar M, Malik A (2000) Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: a review. *Bioresource Technology* **74**, 5-47

Asifiri K, Morgan W, Parbery DG (1994) Suppression of *Sclerotinia* soft rot of lettuce with organic soil amendments. *Australian Journal of Experimental Agriculture* **34**, 131-136

Bonanomi G, Antignani V, Pane C, Scala F (2007) Suppression of soilborne fungal diseases with organic amendments. *Journal of Plant Pathology* **89**, 311-324

Budge SP, Whipps JM (2001) Potential for integrated control of *Sclerotinia sclerotiorum* in glasshouse lettuce using *Coniothyrium minitans* and reduced fungicide application. *Phytopathology* **91**, 221-227

Chitrampalam P, Figuli PJ, Matheron ME, Subbarao KV, Pryor BM (2008) Biocontrol of lettuce drop caused by *Sclerotinia Sclerotiorum* and *S. minor* in desert agroecosystems. *Plant Disease* **92**, 1625-1634

Conklin AE, Erich MS, Liebman M, Lambert D, Gallandt ER, Halteman WA (2002) Effect of red clover (*Trifolium pratense*) green manure and compost soil amendments on wild mustard (*Brassica kaber)* growth and incidence of disease. *Plant and Soil* **238**, 245-256

El-Masry MH, Khalil AI, Hassouna MS, Ibrahim HAH (2002) *In situ* and *in vivo* suppressive effect of agricultural composts and their water extracts on some phytopathogenic fungi. *World Journal of Microbiology and Biotechnology* **18**, 551-558

Fuchs JG (1995) Practical use of quality compost for plant health and vitality improvement. In: Insam H, Riddech N, Klammer S (Eds) *Microbiology of Composting*, Springer-Verlag, Berlin, pp 435-444

Hawke MA, Lazarovitis G (1994) Production and manipulation of individual microsclerotia of *Verticillium dahliae* for use in studies of survival. *Phytopathology* **84**, 883-890

Gullino M, Gilardi G, Garibaldi A (2007) Malattie fungine emergenti su lattuga, rucola e valerianella. *L'Informatore Agrario* **16**, 52-60

Kataria HR, Gisi U (1996) Chemical control of *Rhizoctonia* species. In: Sneh B, Jabaji-Hare S, Neate S, Dijst G (Eds) *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control*, Kluwer Academic Publishers, pp 537-547

Koch E (1999) Evaluation of commercial products for microbial control of soil-borne plant diseases. *Crop Protection* **18**, 119-125

Lahoz E, Pisacane A, Iannaccone M, Palumbo D, Capparelli R (2008) Fungistatic activity of iron-free bovin lactoferrin against several fungal plant pathogens and antagonists. *Natural Product Research* **22**, 955-961

Lorito M, Woo SL, Duffy B, Rosenberger U, Defago G (1998) Advances in understanding the antifungal mechanisms of *Thricoderma* and new applications for biological control. *Bulletin-OILB SROP* **21**, 73-80

Lumdsen RD, Lewis JA, Millner PD (1983) Effect of composted sewage sludge on several soilborne pathogens and diseases. *Phytopathology* **73**, 1545-154

Lumdsen RD, Millner PD, Lewis JA (1986) Suppression of lettuce drop caused by *Scletotinia minor* with composted sewage sludge. *Plant Disease* **70**, 197-201

Magid J, Henriksen O, Thorup-Kristensen K, Mueller T (2001) Disproportionately high N-mineralisation rates from green manures at low temperatures – implications for modeling and management in cool temperate agro-ecosystems. *Plant and Soil* **228**, 73-82

Maynard AA, Hill DE (2000) Leaf compost suppresses disease, improves onion yields. *BioCycle* **41**, 69-71

Noble R, Coventry E (2004) Suppression of soil-borne plant diseases with Composts: a review. *Biocontrol Science and Technology* **15**, 3-20

Pagano L, Iovieno P, Zaccardelli M, Morra L (2008) Soil organic matter dynamic as affected by municipal food waste compost fertilization in southern Italy. *Advances in GeoEcology* **39**, 357-370

Roe NE (1998) Compost utilization for vegetable and fruit crops. *HortScience* **33**, 934-937

Saharan GS, Metha N (2008) *Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management*, Springer Science + Business Media B.V., The Netherlands, pp 485

Shetty KG, Subbarao KV, Huisman OC, Hubbard JC (2000) Mechanism of broccoli-mediated *Verticillium* wilt reduction in cauliflower. *Phytopathology* **90**, 305-310

Subbarao KV (1998) Progress toward integrated management of lettuce drop. *Plant Disease* **82**, 1068-1078

Tsao PH, Oster JJ (1981) Relation of ammonia and nitrous acid to suppression of *Phytophthora* in soils amended with nitrogenous organic substances. *Phytopathology* **71**, 53-59

Van Den Boogert PHJF (2006) Mycoparasitism and biocontrol. In: Sneh B, Jabaji-Hare S, Neate S, Dijst G (Eds) *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control*, Kluwer Academic Publishers, Doordrecht, The Netherlands, pp 485-493

Wu BM, Subbarao KV (2006) Analyses of lettuce drop incidence and population structure of *Sclerotinia sclerotiorum* and *S. minor*. *Phytopathology* **96**, 1322-1329