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

Ernesto Lahoz* • Rosa Caiazzo • Luigi Morra • Angela Carella

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). 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; IC₅₀, 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 crops 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 (Lundsten 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 in vegetable and fruit crops, reported that lettuce yields increased when fertilized with 37 and 74 t ha⁻¹ of a biosolid of S. plus 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.

Received: 4 February, 2009. Accepted: 1 May, 2009.
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% P2O5, 1.8% K2O, 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 repetition was about 30 m². 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 *R. solani* AG1, AG2-1, *P. cactorum*, *C. coccodes*, *S. minor* and *S. sclerotiorum*. The isolates utilized in this study were preserved at 5°C as stock cultures on slants of potato dextrose agar in the Dynamic Soil, Dynamic Plant (Special Issue 1) 99-102 ©2009 Global Science Books. For this purpose 80 sclerotia of *S. sclerotiorum* were put in Petri dishes containing a nutrient medium (Lundsgaard 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 log₁₀ dose concentration–response relationship was linearized using probits and IC₅₀ was evaluated (Lahoz et al. 2008); for each concentration the standard error was calculated. IC₅₀ 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™ 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).

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

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

<table>
<thead>
<tr>
<th>Type of compost</th>
<th>Yield (t/ha)</th>
<th>Disease incidence (%)</th>
<th>Head weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compost</td>
<td>19.1*, 15.8*</td>
<td>12.2, 14.4</td>
<td>253, 336</td>
</tr>
<tr>
<td>Mineral</td>
<td>11.6, 10</td>
<td>50.6*, 33.8*</td>
<td>247, 334</td>
</tr>
</tbody>
</table>

* means are significantly different (p<0.05)
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 log10 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 l1; the curve then showed a sharp increase until 1000 mg l1.

For R. solani AG2-1, 331 mg l1 of COWE resulted in 44% inhibition, after which the percentage of inhibition showed a sharp increase until a concentration of 1000 mg l1 (Fig. 3).

The values of IC50 calculated after probit transformation gave significantly different 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 l1 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; the percent of myceliogenic germination of sclerotia ranged from 95.8% in the control to 12.5% for dishes amended with 1 g l1 of lyophilized COWE.

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.

<table>
<thead>
<tr>
<th>Compost collected from soil</th>
<th>Trial 1</th>
<th>Trial 2</th>
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<tbody>
<tr>
<td>Number/100 g soil*</td>
<td>4.7 ± 0.65</td>
<td>4.9 ± 1.23</td>
</tr>
<tr>
<td>% germination*</td>
<td>53 ± 7.9</td>
<td>56 ± 6.2</td>
</tr>
<tr>
<td>(g L-1)</td>
<td>1000 mg</td>
<td>50 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>4.3 ± 0.67</td>
<td>44 ± 7.2</td>
</tr>
<tr>
<td>* data are not significant different (p=0.05).</td>
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</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Compost water extract Concentration (g L-1)</th>
<th>Sclerotia germinated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>95.8 A</td>
</tr>
<tr>
<td>0.1</td>
<td>87.5 A</td>
</tr>
<tr>
<td>0.5</td>
<td>54.2 B</td>
</tr>
<tr>
<td>1</td>
<td>12.5 C</td>
</tr>
<tr>
<td>Means with the same letter are not significant different (p=0.05).</td>
<td></td>
</tr>
</tbody>
</table>

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.

**ACKNOWLEDGEMENTS**

This work was supported by funds from Campania regional government, project Centro Orticolo Campano and by funds of Italian Ministry for Agricultural Policies, project COL.MI.A. (Collection of Microorganisms of Agricultural Concern).

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