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Chemical properties of compost extracts inhibitory to germination of *Sclerotium rolfsii*

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ABSTRACT

Suppression of *Sclerotium rolfsii* by compost has been previously related to colonization of sclerotia by mycoparasitic fungi. Compost which has undergone prolonged curing was shown to lose its suppressive traits. In this work we sought to decouple the effects of the chemical conditions in the compost from the biological effect of antagonists inhabiting it. This was done by examining the direct effect of sterile compost extracts on the germination of sclerotia, and on the colonization of sclerotia by antagonistic fungal isolates. Sterile extracts of non-cured compost were found to completely inhibit sclerotia germination whereas extracts of cured compost allowed germination. Using buffered solutions which mimic the pH, NH_4^+ concentration and buffering capacity of the extracts, it was shown that germination inhibition was related to effective NH_3 concentration in the extracts. A concentration of 0.5 mM NH_3 in compost extracts was identified as a threshold level above which sclerotia germination was completely inhibited. Sclerotia placed on compost extracts inhibitory to their germination were more vulnerable to attack by mycoparasites. The opportunistic antagonists *Penicillium* and *Petriella* exhibited enhanced colonization of sclerotia on these extracts, while the mycoparasitic fungus *Trichoderma* was found to behave as a primary antagonist, colonizing sclerotia regardless of the environmental conditions.

1. Introduction

Compost and organic soil amendments have been reported to be effective in the control of *Sclerotium rolfsii* (Hadar and Gorodecki, 1991; Danon et al., 2007). The disease control mechanism has been related to suppression of sclerotia germination as a result of antagonistic colonization (Henis et al., 1984). In a recent study (Danon et al., 2007) we have shown that suppression is lost during prolonged compost curing concomitantly with changes in the chemical characteristics of the compost extracts. Specifically, changes in pH, NO_3^-, NH_4^+, dissolved organic carbon (DOC) and UV-VIS absorbance were well correlated to the germination of sclerotia of *S. rolfsii*. Since previous work with *S. rolfsii* has shown that sclerotia in compost are attacked by antagonistic fungi, it has been assumed that the changes in suppression of the pathogen were related to changes in the microbial population which was mitigated by the chemical conditions of the compost. Indeed, changes in ascomycetes and bacterial populations during compost curing have been demonstrated (Danon et al., 2007). On the other hand, changes in suppression may have resulted from a direct effect of the chemical conditions to which the sclerotia in the compost were exposed. These may have been lethal to the sclerotia, or may have made them susceptible to attack by antagonists. The aim of this work was to elucidate the mechanisms of suppression by separating the effects of the chemical factors from the microbial elements. This was done by examining the germination of sclerotia on sterile-filtered compost extracts.

Previous research on compost extracts have mostly dealt with extracts produced by extended extraction times (Scheuerell and Mahaffee, 2002). Such extracts, usually referred to as compost teas, do not represent the chemical conditions in the compost. Where short extraction times have been used, the suppressive effects were related to the microbial activity of the extract, and methods of sterilization (filtration or autoclave) were shown to negate suppressiveness (McQuilken et al., 1994; El-Masry et al., 2002). An exception to this was presented by Cronin et al. (1996) who concluded that a major inhibitory component of the extract was a low molecular weight, heat-stable, non-protein metabolite produced under anaerobic conditions. Elad and Shtienberg (1994) also reported that pasteurization did not negate *Botrytis cinerea* disease reduction in most cases. Hardy and Sivasithamparam (1991) found that sterile extracts had a stimulatory effect on the formation of sporangia of *Phytophthora* spp. whereas non-sterile extracts inhibited sporangial formation and even induced lysis.

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These reports lead to the hypothesis that compost extracts may have a mixed biotic and abiotic effect.

In the current work extraction time was kept short in order to simulate actual compost conditions by avoiding fermentation during extraction. In addition, in order to further identify the dominant factors in the extract which affect disease suppression, sclerotia germination was examined on buffer solutions mimicking the physico-chemical conditions in the extracts. Finally, the effect of the compost extracts on the susceptibility of sclerotia to antagonist attack was examined.

2. Materials and methods

2.1. Compost

Municipal sewage sludge (biosolids, BS) and yard waste (green waste; garden waste) (1:1 v/v) compost was prepared in a commercial composting facility (Dlila facility, Shacham). Approximately 1 m^3 of 3-month-old compost was collected after coarse (10 mm) sieving. The compost at this stage was considered mature and ready for delivery and use as an organic amendment, and was defined as time 0 for the curing process, which began immediately thereafter. Temperature at collection was 72 °C. Curing was performed by piling the compost in a bin and periodically wetting and turning. Compost was turned, rewetted and sampled at the following curing times: 11, 18, 25, 34, 45, 67 and 95 days. Samples of 50 l were collected during turning and stored at 4 °C in plastic barrels. Subsamples were sieved via a 5 mm sieve before further use.

Often, chemical properties of compost extract are determined on 1:10 extracts. In order to more realistically mimic the environment in the compost, a 1:2 extract was used. An extract produced at this ratio would be expected to be about twice as diluted as the actual solution in potting media consisting of compost as a single component, at 60% water holding capacity (assuming approximately 100% w/w water content at 60% water holding capacity). These water content conditions were similar to those prevailing in the compost during the experiments conducted in an earlier study on sclerotia reported by Danon et al. (2007).

Compost extracts were prepared by shaking moist, sieved compost with deionized water at a ratio of 1:2 w/w, dry compost: water, for 2 h. Water contained in the original moist sample was taken into account. Compost suspension was centrifuged at 3000 × *g* for 10 min. Water extract was centrifuged at 25,000 × *g* for 30 min, filtered through a Whatman GFA filter, then through a 0.45 μm sterile filter (Supor, Gelman Laboratory). The extracts were kept at 4 °C until all chemical analyses were completed, within 1 week, or used for bioassays within 1 day.

Bulk compost recovered from the first extraction was incubated moist in the dark for a week in loosely closed nylon bags, at 25 °C. Based on growth rates of microbial populations, we assumed that during this time a new microbial steady state had been achieved. Incubated composts were sampled for water content and extracted a second time in the same manner as the first extract. These samples are representative of the conditions in washed compost, which is a better substrate for potting media. All compost extracts were prepared in triplicates.

2.2. Chemical analyses

pH, electrical conductivity (EC), NO_3^- and NH_4^+ concentration, soluble sugar concentration and Absorbance in the UV-VIS range, were measured on compost extracts as described by Danon et al. (2007). Dissolved organic carbon (DOC) concentration was measured on acidified, diluted samples with a Shimadzu carbon analyzer (TOC-V CPN). Analysis of extracts was conducted in triplicate.

Buffering capacity of the extracts was determined by titration with 0.01 N NaOH over a pH range of 3.5–9.0. In order to distinguish between buffering of dissolved organic matter (DOM) and dissolved bicarbonate, extracts were first acidified then either titrated directly or after 4 min of N_2 pre-titration bubbling followed by continuous N_2 bubbling during titration aiming to eliminate residual CO_2.

2.3. Buffers

A universal buffer, based on Perrin and Dempsey (1983), was prepared as follows: 25 ml stock solution containing 0.0278 M of citric acid, boric acid, K_2HPO_4 and Tris–HCl was brought to the required pH by addition of 2 N NaOH and diluted to 100 ml. This solution had a uniform buffering capacity of 6.8 mM $H^+ l^{-1}$ per pH unit over the pH range of 4–10. Buffers with buffering capacity of 3.9 and 1.3 mM $H^+ l^{-1}$ per pH unit were prepared by using 15 and 5 ml of the stock solution. NH_4Cl was added before dilution to a final concentration of 0–50 mM and pH was corrected to within 0.05 unit with concentrated NaOH or HCl.

2.4. Sclerotia preparation

Sclerotium rolfsii (ATCC 26325) was grown for 3 weeks on potato dextrose agar (PDA) plates until the formation of dark-brown sclerotia. The sclerotia were collected and dried in a desiccator in the presence of moisture free silica for at least 3 weeks until use.

2.5. Germination bioassays

Twenty-five sclerotia were placed on three layers of tissue paper (Kimble, 1 ply) moistened with 4.5 ml of either compost extract or buffer solution in 10 cm Petri dishes. Each treatment was performed in triplicate with sterile deionized water as a control. Sclerotia were incubated at 25 °C in the dark. Germination of sclerotia was noted daily for 6 days. In extract treatments exhibiting no germination, sclerotia were transferred to Petri dishes with water treated tissue paper and examined for 5 additional days, under the same incubation conditions, in order to assess viability.

2.6. Colonization by mycoparasites

Fungi were isolated from attacked sclerotia incubated on suppressive BS compost. Thus, these isolates were suspected to be antagonistic. The isolates' ribosomal internal transcribed spacer (ITS) was sequenced, and the sequences were submitted to the GenBank database and identified as: *Trichoderma* sp. B8, *Penicillium* sp. B35 and *Petriella* sp. P3 (accession numbers EU315010–EU315012).

Spores of *Trichoderma* sp. and *Penicillium* sp. were collected from 2-week-old PDA plates and *Petriella* sp. from 4-week-old PDA plates, into 1 ml of deionized water and diluted to 10^4 spores/ml. Approximately 170 mg of sclerotia were added to 500 μl of a spore suspension and vortexed for 1 min, then blotted on tissue paper and transferred to Petri dishes containing tissue paper moistened with 4.5 ml of either compost extract or water. Controls consisted of sclerotia not mixed with antagonist suspensions. Germination of sclerotia and their colonization by antagonists were evaluated after 3, 4 and 5 days by direct observation under a stereoscope. Sclerotia were considered as germinated where mycelia development was observed and colonized when antagonist mycelia and/or spores were observed.