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Suppression of *Allium* white rot (*Sclerotium cepivorum*) in different soils using vegetable wastes

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Abstract

Mixtures of wet vegetable wastes (*Brassica*, carrot or onion) and dry onion waste were composted at 50 °C for 7 days. The incorporation of the raw or composted vegetable waste mixtures into sandy loam, silt and peat soils reduced the viability of sclerotia of *S. cepivorum* in glasshouse pot bioassays. The reduction in viability was dependent on waste type, rate of incorporation, duration of exposure and soil type. Onion waste was the most effective waste type in reducing sclerotia viability in all three soils. The *Brassica* and carrot wastes were as effective as the onion waste in silt soil but less effective in sandy loam and peat soil. A 50% w/w incorporation rate of the wastes gave the largest reduction in viability, with an increase in reduction over time. Composted onion waste reduced sclerotia viability under glasshouse and field conditions although the effect was smaller in the field. Composted onion waste incorporated into soil at 50% w/w reduced the incidence of *Allium* white rot on onion seedlings in glasshouse pot tests. Incidence and control of the disease differed with soil type. The most consistent control was achieved in peat soil whereas no control was observed in silt soil. Incorporation of the waste 2 months prior to sowing or transplanting reduced seedling emergence in sandy loam soil and growth in all three soil types. The potential for field application of composted vegetable wastes as a sustainable method for control of *Allium* white rot and waste disposal is discussed.

Abbreviations: AWR – *Allium* white rot; GLM – generalised linear model.

Introduction

Allium white rot (AWR) is an economically important disease of onions (*Allium cepa*) caused by the soil-borne fungus *Sclerotium cepivorum* (Coley-Smith, 1987; Entwistle, 1990a). The disease is present in most onion-growing areas of the world where environmental conditions are conducive for the pathogen (Entwistle, 1990b). The pathogen can attack plants from the seedling stage onwards, resulting in death before harvest or post-harvest decay in storage (Entwistle, 1990b). Sclerotia are the only means of survival and may remain dormant in the soil in the absence of host plants for more than 20 years (Coley-Smith, 1990; Entwistle, 1990b). The stimulus for germination is the secretion of alk(en)yl cysteine sulphoxides by the roots of *Allium* species (Coley-Smith and Parfitt, 1986). Soil microflora metabolise these sulphoxides to produce volatile thiols and sulphides which activate sclerotial germination (Coley-Smith and Parfitt, 1986).

Control of AWR has proved difficult due to the longevity of the sclerotia. Various chemical, biological and physical measures have been examined for the control of the disease, including fungicides (Ryley and Obst, 1994), soil fumigants (Entwistle, 1990a), soil solarisation (Melero-Vara et al., 2000) and biological control agents (Clarkson et al., 2002). In addition, a number of studies have attempted to mimic the natural phenomenon of sclerotia germination with chemicals (Coley-Smith and Parfitt, 1986; Coley-Smith, 1990). Whilst these methods have shown promise, there are problems such as enhanced degradation of fungicides in soil (Walker et al., 1986) and inconsistent control (Melero-Vara et al., 2000). With land free of AWR in onion growing areas of the UK and other countries decreasing (Couch and Kohn, 2000), and the loss of methyl bromide as a soil sterilant (De Ceuster and Hoitink, 1999), there is a continued search for alternative methods of controlling AWR.

One possible alternative is to apply organic waste or compost to land as this has been shown to reduce inoculum levels of various soil-borne pathogens due to a suppressive effect of the waste or soil microbiota (Lumsden et al., 1983; De Ceuster and Hoitink, 1999; Smolińska, 2000). Over 100,000 tonnes of waste vegetables are produced in the UK annually (Anonymous, 2001, 2002). In addition, waste peelings and leaf wastes from imported and homegrown vegetables accumulate at packhouses (Noble et al., 2000). Composting under specified conditions of time and temperature ensures most pathogens, including *S. cepivorum*, and pests present in the waste are destroyed and will not subsequently infect the land to which it is applied (Bollen et al., 1989; Coventry et al., 2002). Composted onion waste has previously been shown to reduce viability of sclerotia of *S. cepivorum* in sandy loam soil in glasshouse pot tests (Coventry et al., 2002). Composting temperature and duration were also found to effect the subsequent suppressiveness of onion waste compost to sclerotia of *S. cepivorum* in soil (Coventry et al., 2002).

The objective of this work was to test the hypothesis that incorporation of composted vegetable (*Brassica*, carrot, onion) wastes in different soils reduces the viability of sclerotia of *S. cepivorum* and thereby controls AWR in these soils.

Materials and methods

Flask composting of vegetable wastes

Vegetable wastes (*Brassica*, carrot and onion) were collected from packhouses in eastern England and analysed for moisture, nitrogen (N) and ash contents (Anonymous, 1986). Small-scale flask composting experiments similar to those described in Coventry et al. (2002) were conducted using various mixtures of dry onion waste (shale-skins or onion tops) and wet onion (peelings), *Brassica* (chopped white cabbage leaves, broccoli florets) or carrot (chopped carrots) wastes. The mixtures were prepared to an 80% w/w moisture content (10 parts wet onion waste: 1 part dry onion waste; 6 parts wet *Brassica* or carrot waste: 1 part dry onion waste w/w) previously found to facilitate aerobic composting and to minimise run-off (Coventry et al., 2002). The *Brassica* and carrot wastes were chopped into pieces of c. 30 mm^2 to fit in the flasks and encourage degradation. Three replicates of each mixture were composted in 2-l 'Quickfit' multiadapter flasks immersed in thermostatically controlled waterbaths (Coventry et al., 2002). Urea was added to the waste (4 g kg^{-1} waste) to produce a mixture with c. 1.5% N (dry matter). Each flask was connected to ancillary equipment to aerate and maintain a minimum of 13% v/v O_2 in the waste. The flasks were incubated for 7 days at 50 °C, a time and temperature shown to be suitable for eradicating most pathogens including white rot sclerotia (Bollen et al., 1989; Coventry et al., 2002). A lower composting temperature (42 °C) was previously shown to be unsuitable for producing suppressive composts (Coventry et al., 2002). Compost N, dry matter and ash contents and pH were determined as described previously (Anonymous, 1986).

Effect of vegetable wastes on viability of sclerotia in different soils in pot bioassays

Glasshouse pot bioassays (Coventry et al., 2002) were set up using the composted vegetable wastes (dry onion waste + wet onion or white cabbage or broccoli florets or carrot waste). The composted vegetable wastes were incorporated at three rates (1, 10 and 50% w/w) into sieved (5 mm) sandy loam (Cople, Bedfordshire, UK), silt (Moulton, Lincolnshire, UK) and peat (Ely, Cambridgeshire, UK) soils, with 20% v/v vermiculite to prevent clumping of the soil. Soil containing the same rates of the raw vegetable waste mixtures, as well as untreated soil, were included in the bioassays. Square pots (70 × 70 × 80 [deep] mm Optipots, LBG Ltd., Evesham, UK) were filled with the soil–vegetable waste mixtures together with six polyester mesh bags (20 × 20 mm, 150 μm mesh diameter) containing 2 g of 50:50 (w/w) sand: soil and 100 sclerotia. The isolate of *S. cepivorum* used throughout this study was isolated from infected onions growing in Kirton, Lincs, UK. Sclerotia required for the glasshouse pot bioassays were generated on artificial medium and conditioned prior to use (Coventry et al., 2002).

The matric potentials of the soil and soil–waste mixtures in the pots were monitored with miniature electronic pressure transducer tensiometers (type SWT5, Delta-T Devices Ltd., Cambridge, UK) connected to a logger (type DLZe, Delta-T Devices, Ltd.). The pots were watered from the bottom at regular intervals to maintain a matric potential of −2 to −7 kPa. The mesh bags were retrieved at 1, 2, 3, 6, 9 and 12 months after inoculation (silt soil: December–November; peat soil: April–March). In the sandy loam soil, the mesh bags were only retrieved after 9 and 12 months burial (April–March). Sclerotia were washed from the soil in the bags with water, collected on a 212 μm mesh size sieve, and assessed for degradation (soft or collapsed) by squeezing with forceps. Undegraded sclerotia were surface sterilised in sodium hypochlorite (>5% but <16% available chlorine, Hays Chemical Distribution Ltd., Leeds, UK), rinsed in sterile distilled water and plated on to potato dextrose agar (PDA) containing 20 mg chlortetracycline l^{-1} as previously described (Coventry et al., 2002) to assess viability in terms of germination. The glasshouse heating set points were 14 °C day, 12 °C night, and the ventilation set points were 18 °C day, 16 °C night.

Experimental design and data analysis

Each glasshouse pot bioassay consisted of three replicate pots for each of the 24 vegetable waste treatment combinations (three rates × four waste types × two waste conditions) and 12 replicate pots of untreated soil as a control treatment. For the silt soil and sandy loam soil bioassays these treatments were allocated to pots following a completely randomised design, whilst for the peat soil bioassay they were allocated to pots following a randomised complete block design, with each block containing four untreated control pots. Retrieval time was randomly allocated to bags within pots, as in a split-plot design. However, there was no evidence of within-pot variability differing from between-pot variability, and so the trials were analysed assuming a completely randomised or randomised complete block design, respectively.

Number of soft sclerotia, as a proportion of the number of sclerotia recovered, and of viable (germinating) sclerotia, as a proportion of the number tested (up to 30 where available) were analysed within a generalised linear model (GLM) framework, assuming a binomial distribution and logit link function, using GenStat for Windows (Clarkson et al., 2002). Bags where the total number of recovered sclerotia was significantly less than the mean recovery rate (i.e. less than the overall mean minus two standard deviations) were omitted from the subsequent analysis. The analysis allowed the assessment of differences between the vegetable waste treatments and the untreated control, between the different rates of incorporation, between raw and composted waste, between burial period, and of the interactions between these factors. From the analyses, there was evidence of over-dispersion, i.e. there was more variation in the data than would be expected from a binomial distribution of the numbers of soft or germinating sclerotia. The significance of these effects was therefore assessed using appropriate *F*-tests, and predicted mean proportions for treatment combinations of interest were calculated from the fitted models.

Comparison of effect of composted onion waste on viability of sclerotia under glasshouse and field conditions

On the basis of the results from the glasshouse bioassays, a 50% w/w incorporation rate of the composted onion waste was selected to compare the effect of waste incorporation on sclerotia viability under glasshouse and field conditions. The waste was incorporated into the three soils (sandy loam, silt and peat) and pots (70 × 70 × 80 mm) were filled with the soil–waste mixtures. Polyester mesh bags, each containing 100 sclerotia, were buried in the pots as previously described. Identical pots were placed in the glasshouse and in the field at Horticulture Research International, Wellesbourne, Warwickshire where the pots were sunken into the ground so only their top surface was exposed. The air temperature was recorded in both environments. The glasshouse heating and ventilation set points were as previously described. The polyester mesh bags were retrieved after 1, 2, 3, 6 and 9 months burial and the sclerotia assessed in terms of soft sclerotia retrieved and germination of undegraded sclerotia.

Experimental design and data analysis

There were three replicate pots of each of the six treatments in both the glasshouse and field environments. Retrieval time was randomly allocated to bags within pots, as in a split-plot design. As before, there was no evidence of within-pot variability differing from between-pot variability, and so the trial was analysed assuming a completely randomised design. The number of soft sclerotia, as a proportion of the number recovered, and of germinating sclerotia, as a proportion of the number tested (up to 30 where available), were analysed within a GLM framework, assuming a binomial error distribution and logit link function.

Effect of composted onion waste on Allium *white rot in pot bioassays*

On the basis of the results from the previous sections, a 50% w/w incorporation rate of the composted wet and dry onion waste mixture was selected for AWR glasshouse pot bioassays, similar to those described by Clarkson et al. (2002). Onion waste composted for 7 days at 50 °C was incorporated at 50% w/w into the three soils (sandy loam, silt and peat). The soil–waste mixtures and soils alone were inoculated with conditioned sclerotia (three sclerotia per gram of mixture) and left in a glasshouse for 2 months in bags. This standing period was to simulate the time between compost application and onion crop sowing or set planting in the field. After 2 months, square pots (70 × 70 × 80 mm) were filled with the soil–waste mixtures or soil alone, and two onion seeds (cv. White Lisbon) sown per pot (subsequently thinned to one per pot), or a single 4 week old onion seedling transplanted. Pots with the soil–waste mixtures and soils alone with no sclerotia added were included as controls. The mean shoot length of seedlings at transplanting was 210 mm (mean of 30 seedlings). The matric potential of the soil and soil–waste mixtures was maintained at –2 to –7 kPa as previously described. Due to more rapid drainage and drying out of the peat + 50% compost, the matric potential in these pots occasionally decreased to −40 to −50 kPa before watering. All plants received watering with a nutrient solution weekly (2N:1P:4K). The pots were assessed weekly for up to eight (bioassay from seed) or 15 (bioassay using seedlings) weeks for the presence of AWR, which was scored as dead plants with visible mycelium or sclerotia. Natural plant deaths (non-AWR) were also recorded. These bioassays were repeated using seeds (bioassays from seed I and II) and transplanted onion seedlings (bioassays using seedlings I and II). The pH and moisture content of the soils, and the soils + composted waste, were determined (Anonymous, 1986). The glasshouse heating and ventilation set points were as previously described.

Experimental design and data analysis

The 12 treatment combinations (three soils × ± onion waste × ± sclerotia) in each of the seed and seedling glasshouse pot bioassays were arranged following a completely randomised design. Each plot nominally consisted of 10 pots, though due to lack of material for some of the treatment combinations this had to be reduced in some experiments. In the bioassay from seed I and both bioassays using seedlings there were five replicate plots of the treatment combinations including sclerotia and three replicate plots of those without sclerotia. In the bioassay from seed II, the number of replicates varied from two to six, with generally more replicates for treatments with sclerotia and without waste.

For percentage plant emergence data, and percentage diseased plants relative to number emerged data from the bioassays from seed, two analysis approaches were considered: the analysis of arcsine transformed percentages using ANOVA, and the analysis of proportions within a GLM framework assuming a binomial error distribution and logit link function (Clarkson et al., 2002). The second approach has the benefit of allowing for the variation in the number of emerged seedlings when analysing the percentage disease, whilst the first approach makes the presentation and interpretation of results simpler. For the bioassay from seed II, a residual maximum likelihood estimation (REML) approach (Thompson and Welham, 2000) had to be used instead of ANOVA to allow for the different replication levels of the various treatments. In all GLM analyses there was evidence of over-dispersion, so that overall effects of treatment factors were assessed using approximate F-tests for the deviance ratios. The effect of the inclusion of waste in reducing seed emergence, at various times after sowing, was assessed for each soil both with and without sclerotia using a one-sided t-test on the appropriate estimates from the GLM analysis. Similarly, the effect of the inclusion of waste in reducing the incidence of AWR, at various times after sowing, was assessed for each soil (with sclerotia treatments only) using a one-sided t-test on the appropriate estimates from the GLM analysis. Presented results for percentage disease are based on means obtained from the ANOVA or REML analyses.

For the bioassays using seedlings, the percentage of dead seedlings at 5, 10 and 15 weeks after transplanting were analysed using ANOVA, following arcsine transformation to satisfy the assumption of homogeneity of variance (Clarkson et al., 2002). Three different measures of the percentage of dead seedlings were examined: the total number of dead seedlings as a percentage of the number transplanted; the number of AWR deaths as a percentage of the number transplanted; and the number of AWR deaths as a percentage of the number of seedlings not dead due to natural causes.

Test for phytotoxicity

Similar to the *Allium* white rot bioassays with seedlings described above, 4 week old onion seedlings (cv. White Lisbon) were transplanted into the three soils (sandy loam, silt and peat) alone and with a 50% w/w incorporation of composted onion waste. The seedlings were observed over a 4 week period for any signs of phytotoxicity resulting from the waste incorporation, and the final shoot length and fresh weight of the seedlings recorded.

Experimental design and data analysis

The six treatment combinations (three soils × ± composted onion waste) were arranged following a randomised complete block design. There were three replicate blocks with each plot consisting of 10 pots. The data were analysed using ANOVA.

All differences described in the Results section were significant at $P < 0.05$ unless stated otherwise.

Results

Flask composting of vegetable wastes

Before composting, the dry matter content of the dry onion wastes was 69–80% and those of the onion, *Brassica* and carrot wet wastes were 10–15%, 8–11% and 9–11% respectively. The dry onion waste had a higher ash content (7–15% of dry matter) than the wet wastes (onion 5–8%; *Brassica* 5–8%; carrot 3–4% of dry matter) but lower total N (dry 0.40–0.70%; wet 0.92–4.75% of dry matter). After composting, the dry matter (16–25%) and ash (6–16% of dry matter) contents of the various waste mixtures were not significantly different from each other. Composted *Brassica* waste mixtures had the highest N content (0.79% of dry matter), followed by the composted onion (0.57% of dry matter) and carrot (0.46% of dry matter) waste mixtures. The composted onion and carrot wastes had a lower pH (3.7–4.1) than the composted cabbage (4.7) and broccoli (6.6) wastes.

Effect of vegetable wastes on viability of sclerotia in different soils in pot bioassays

At an incorporation rate of 1% w/w, only onion peelings waste significantly increased the percentage of soft sclerotia in sandy loam and peat soils, where the effect was small (Figure 1). None of the 1% rate treatments were effective in silt soil. Incorporation of all waste types at 10% or 50% w/w in all three soils increased the percentage of soft sclerotia relative to the control ($P < 0.001$) with the exception of carrot and cabbage (10%) wastes in peat soil (Figure 1). The raw and composted wastes were equally effective in the silt and peat soils but the raw broccoli and carrot wastes were slightly more effective than the composted wastes at the 50% rate in the sandy loam soil. At the 10% rate in the sandy loam soil, raw carrot waste was more effective than composted carrot waste, but for the other wastes composting had no significant effect on the percentage of soft sclerotia.

In the sandy loam soil, the percentage of soft sclerotia generally increased with waste incorporation rate, with the largest increase with the 10% and 50% rates of the onion peelings (Figure 1a). In the silt soil, the 10% and 50% rates of all waste types, with the exception of the 10% rate of the carrot waste, significantly increased the percentage

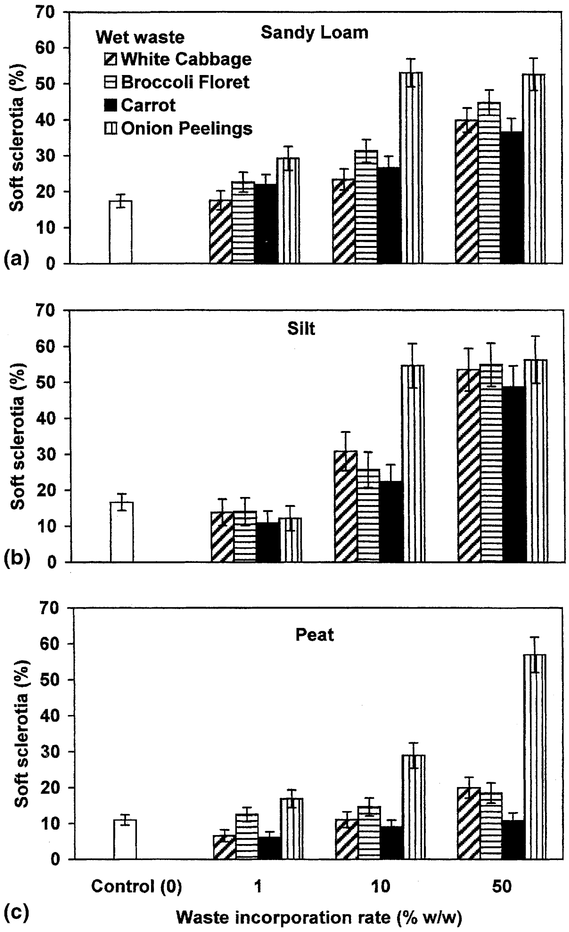

Figure 1. Effect of wet waste type and rate of incorporation in (a) sandy loam, (b) silt and (c) peat soils on the percentage of soft sclerotia retrieved from pot bioassays. Values shown (mean ± SE) were obtained from predicted mean proportions, averaged across condition of the waste (raw and composted), burial period (9 and 12 months) and replicate for the waste treatments, and across burial period and replicate for the untreated control. All wet wastes were mixed with dry onion waste. There were no soft sclerotia in the initial stock of conditioned sclerotia.

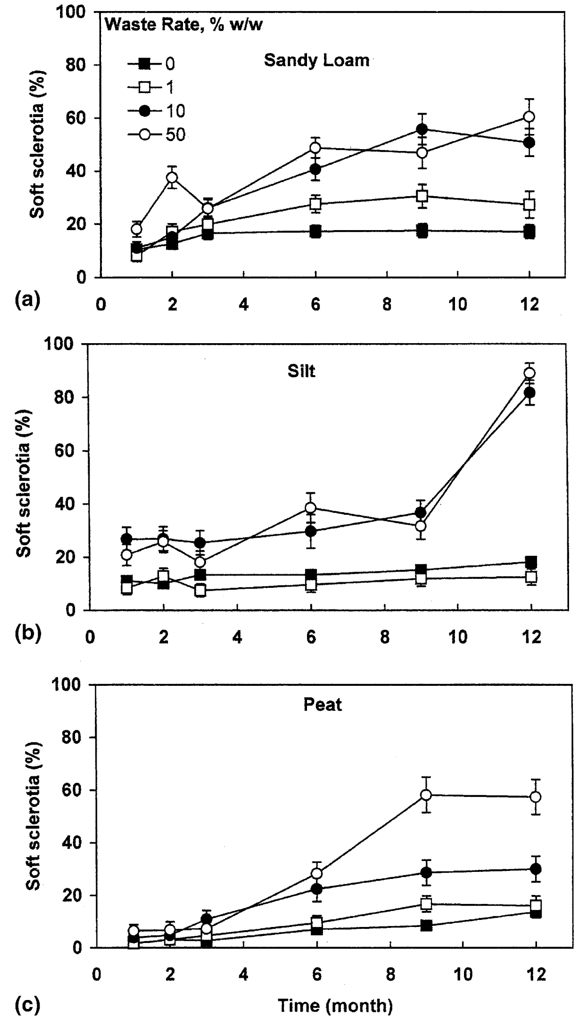

Figure 2. Effect of rate of incorporation of onion peelings and burial period in (a) sandy loam*, (b) silt and (c) peat soils on the percentage of soft sclerotia retrieved from the pot bioassays over time. Values shown (mean ± SE) were obtained from predicted mean proportions, averaged across condition of the waste (raw and composted) and replicate, and for the untreated control, averaged across replicate only. There were no soft sclerotia in the initial stock of conditioned sclerotia. *Sandy loam data for months 1–6 relate to previously published data (Coventry et al., 2002) for onion waste composted at 54 °C.

of soft sclerotia retrieved relative to the control, with the onion peelings again being the most effective (Figure 1b). All three rates of the onion peelings, the 10% and 50% rates of the broccoli waste and the 50% rate of the cabbage waste significantly increased the percentage of soft sclerotia retrieved relative to the control in the peat soil (Figure 1c).

Degradation of sclerotia in the soils was influenced by the duration of exposure to the composted onion waste (Figure 2). Percentage soft sclerotia retrieved increased over time in the presence of the wastes in the three soils. In the sandy loam soil, all three rates of the onion peelings increased the percentage soft sclerotia retrieved over time, with the 10% and 50% rates showing the largest increase relative to the control after 12 months exposure (Figure 2a). In the silt soil, the 1% incorporation rate had no effect on percentage soft sclerotia retrieved (Figure 2b). Similar to the results in the sandy loam soil, the 10% and 50% rates in the silt soil showed the largest increase relative to the control after 12 months (Figure 2b). In the peat soil, the 1% rate increased percentage soft sclerotia retrieved at the 9 month sampling but otherwise had no effect (Figure 2c). The 10% and 50% rates increased soft sclerotia retrieved over time with the 50% rate being the most effective (Figure 2c).

The presence of each of the waste types reduced the percentage germination of undegraded sclerotia relative to the control in the sandy loam ($P < 0.001$) and silt soils (Figure 3a and b). The 50% rate of the broccoli waste was the most effective treatment, particularly in the sandy loam soil (Figure 3a). In the peat soil, the broccoli and carrot wastes slightly reduced germination of undegraded sclerotia retrieved ($P < 0.001$) (Figure 3c). The raw and composted wastes were equally effective in all three soils.

Comparison of effect of composted onion waste on viability of sclerotia under glasshouse and field conditions

Similar to the previous glasshouse bioassay, the percentage soft sclerotia and germination of undegraded sclerotia retrieved from both the glasshouse and field environments varied with soil type, presence and duration of exposure to the waste. The presence of the waste increased the percentage of soft sclerotia retrieved from the silt and peat soils in both environments ($P < 0.001$) (Figure 4) where there was an increase over time ($P < 0.001$). Retrieval of soft sclerotia was significantly higher from the pots in the glasshouse than from those in the field ($P < 0.001$) (Figure 4). Germination of undegraded sclerotia retrieved from each of the soils was reduced in the presence of the waste in both the glasshouse and field environments (Figure 5). For the majority of the treatments, there was no difference in germination between the two environments (Figure 5).

The mean air temperature for months 1–3 (February, March and April) was 16–17 °C in the glasshouse and 5 °C in the field. There was less of a difference in temperature between the two environments over the following 3 month period (May, June, July) with average temperatures of 21 and 15 °C respectively. Similarly, the average

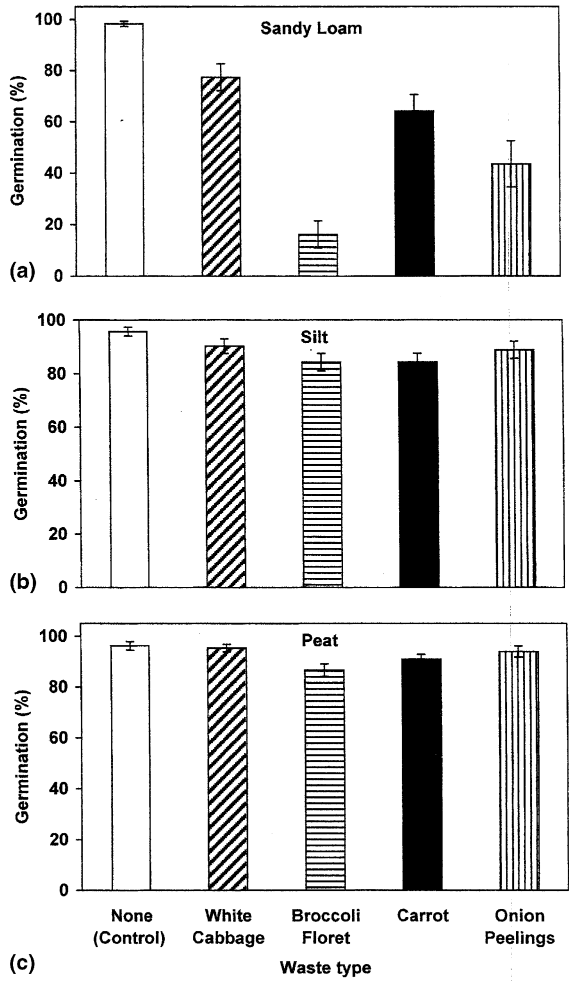

Figure 3. Effect of wet waste type (50% w/w) on percentage germination of undegraded sclerotia retrieved from pot bioassays using (a) sandy loam, (b) silt and (c) peat. Values shown (mean ± SE) were obtained from predicted mean proportions averaged across condition of the waste (raw and composted), burial period (9 and 12 months) and replicate for all waste treatments, and across burial period and replicate for the untreated control.

temperatures recorded in the glasshouse and field for the 3 month period August–October were 18 and 15 °C respectively.

Effect of composted onion waste on Allium *white rot in pot bioassays*

Bioassays from seed

The presence of the composted waste significantly reduced percentage seedling emergence in the silt (soil alone = 82%; soil + waste = 32%) and

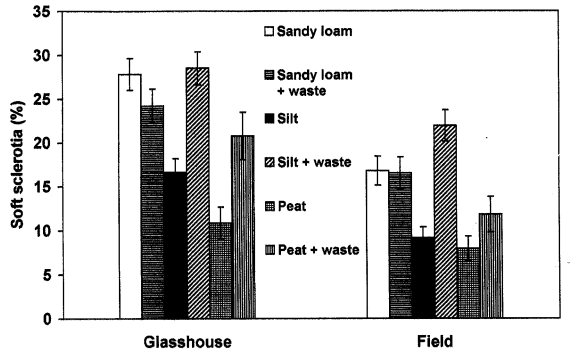

Figure 4. Effect of incorporation of composted onion waste (+ waste, 50% w/w) in three soil types on percentage soft sclerotia retrieved from pots kept in a glasshouse or in a field. Values shown (mean ± SE) were obtained from predicted mean proportions, averaged across burial period and replicate. There were no soft sclerotia in the initial stock of conditioned sclerotia.

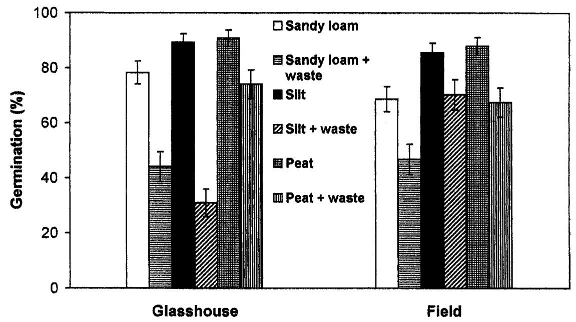

Figure 5. Effect of incorporation of composted onion waste (+ waste, 50% w/w) in three soil types on percentage germination of undegraded sclerotia retrieved from pots kept in a glasshouse or in a field. Values shown (mean ± SE) were obtained from predicted mean proportions, averaged across burial period and replicate.

sandy loam (soil = 94%; soil + waste = 61%) soils in bioassay I and in the sandy loam soil (soil alone = 98%; soil + waste = 23%) in bioassay II. These results indicate that the waste was phytotoxic to seed germination.

In bioassay I, the presence of the composted waste significantly reduced the percentage of plants infected with AWR in the peat soil, whereas there was no significant effect of the composted waste on AWR control in the silt and sandy loam soils (Figure 6a). In bioassay II, the presence of the composted waste in the sandy loam soil significantly reduced the percentage of plants infected with AWR, but had no disease control effect in the peat and silt soils (Figure 6b).

Bioassays using seedlings

In both bioassays using seedlings, the percentage of plants infected with AWR increased over time, in each of the soils alone and in the presence of the composted waste. The presence of the composted waste in the peat soil significantly reduced the percentage of plants infected with AWR compared with peat soil alone in both bioassays, 15 weeks after transplanting (Figure 6c and d). In contrast, the composted waste had no significant effect on AWR control in the silt and sandy loam soils in bioassay I (Figure 6c) and in the sandy loam soil in bioassay II (Figure 6d). Incorporation of the composted waste in the silt soil in bioassay II significantly increased AWR infection (Figure 6d). No AWR was detected in the three soils in the uninoculated pots in any of the bioassays using seeds or seedlings.

The presence of the composted waste had no significant effect on the number of plant deaths not due to AWR in the peat soil in both seedling bioassays and in the sandy loam soil in bioassay I (mean percentages of non-AWR deaths less than 7%). However, there were significantly more non-AWR deaths in the sandy loam soil in bioassay II, and in the silt soil in both bioassays, in the presence of the waste (17% compared with 1% in these soils alone, after 15 weeks). These results suggest the waste may be phytotoxic to onion plants in these soils.

The pH of the soils alone were 7.2 ± 0.15 for sandy loam, 8.4 ± 0.05 for silt and 7.4 ± 0.09 for peat. The addition of composted waste increased these values to 8.4 ± 0.08, 8.9 ± 0.07 and 8.1 ± 0.06 respectively. The average moisture contents (w/w) of the soils were 21% for sandy loam, 16% for silt and 44% for peat. The addition of composted waste increased these values to 33%, 40% and 56% w/w respectively.

Test for phytotoxicity

After 2 weeks growing in the soil-composted onion waste mixtures, there was no obvious visible difference between the seedlings in the soils alone and soil + waste treatments. In week 3 of the bioassay the seedlings in the soil–waste mixtures were visually smaller than in the soils alone. The

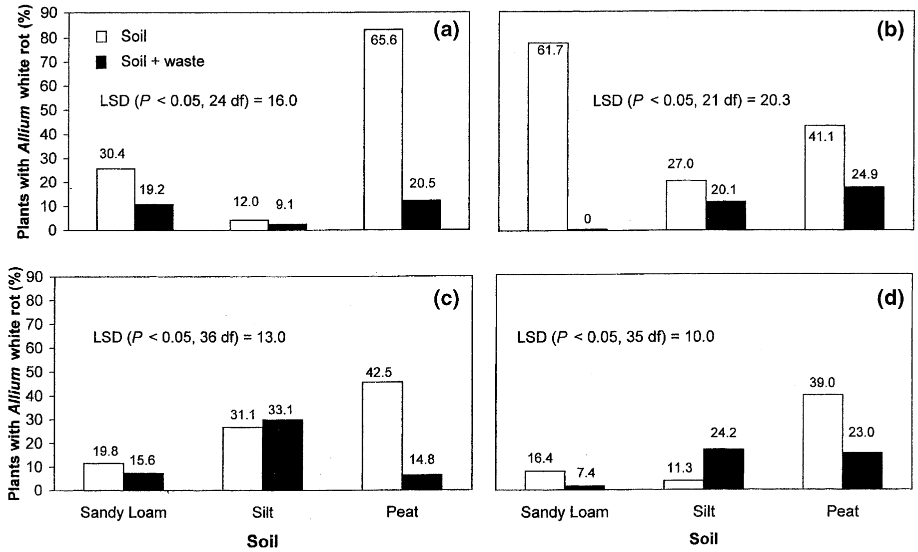

Figure 6. Effect of a 50% w/w rate incorporation of composted onion waste (+ waste) on the percentage of onion plants infected with *Allium* white rot in three soil types inoculated with three sclerotia g^{-1} of mixture in two different pot bioassays using seed (a, b) and seedlings (c, d). Values shown by bars are back-transformed percentages, with arcsine transformed means shown numerically at the top of each bar. LSD values are for arcsine transformed data.

difference in size between the seedlings growing in the soil and soil + waste treatments was still visually apparent at week 4 and evident in the plant lengths and fresh weights recorded at harvest (Table 1). The plants in all three soil types with the onion compost incorporation were significantly smaller than those in the soils alone. Root development in the soil + waste treatments was also poor compared with the seedlings in soil alone. At harvest, the tips of the leaves of the seedlings in all the soil + waste treatments were dead although there were no plant deaths in any of the treatments.

Table 1. Effect of incorporation of composted onion waste (+ waste, 50% w/w) in three soil types on plant length from base of bulb to tip of longest leaf, and fresh weight of eight week old onion seedlings

Soil type	Plant length (mm)	Plant weight (g)
Sandy loam	358	2.8
Sandy loam + waste	286	1.3
Silt	374	2.8
Silt + waste	260	1.0
Peat	337	2.2
Peat + waste	266	0.9
LSD ($P < 0.05$, 10 df)	17.2	0.36

Discussion

Sclerotia control activity of composted vegetable wastes

In this study, the incorporation of raw or composted vegetable waste mixtures to sandy loam, silt and peat soils reduced the viability of sclerotia of *S. cepivorum* in glasshouse pot bioassays. The reduction in viability was dependent on waste type, rate of incorporation, duration of exposure and soil type. Sclerotia of *S. cepivorum* are stimulated to germinate by sulphur-containing volatiles produced from the breakdown of compounds released from *Allium* species (Coley-Smith and Parfitt, 1986). The compost prepared from onion peelings waste was found to be more effective in reducing viability than compost prepared from *Brassica* or carrot wastes. However, composted onion waste was previously shown to have a lower concentration of these sclerotia germination stimulants than raw onion waste, although it was more effective in reducing viability of sclerotia. In these experiments, raw and composted onion waste were equally effective. This suggests that factors other than stimulants released from *Allium* species were also involved in reducing sclerotia viability (Coventry et al., 2002). This is confirmed here by

Broccoli floret waste being more effective than onion peelings waste in reducing germination of undegraded sclerotia. Smolińska (2000) also found that cruciferous plant residues were effective in reducing sclerotia germination of *S. cepivorum*. Fungicidal activity was related to the production of isothiocyanates, produced during enzymatic degradation of glucosinolates in the *Brassica* plant residues (Smolińska and Horbowicz, 1999), although other toxic sulphur-containing volatiles are also produced during the decomposition of cruciferous plant residues (Gamliel and Stapleton, 1993). It has been suggested that these volatiles weaken the sclerotia and allow colonisation by soil micro-organisms (Smolińska et al., 2002). Linderman and Gilbert (1975) showed that sclerotia of *Sclerotium rolfsii* were stimulated by other volatiles from alfalfa hay such as acetaldehyde, with low doses of volatiles being stimulatory and high doses being inhibitory or lethal. Thaning and Gerhardson (2001) found survival of *Sclerotinia sclerotiorum* and *Verticillium dahliae* was reduced by soil incorporation of fresh whole crops of white mustard or oats under polythene sheeting. However, no reduction in survival of *S. cepivorum* was observed although the sclerotia were only exposed to the treatments for 10–13 weeks and the results from this current study suggest a longer period of exposure is required to observe an effect.

The pot experiments showed that compost rates in the range 10–50% w/w reduced sclerotia viability. Lumsden et al. (1983) found that compost rates of 5% and 30% w/w dry matter basis in soil were equally suppressive of *Aphanomyces* root rot of pea and *Rhizoctonia* root rot of bean. However, Ryckeboer (2001) found that the suppression of *Pythium* and *Rhizoctonia* damping-off in peat-based potting mixes was greater and/or less variable when composted organic wastes were incorporated at 20% v/v than at 5% or 10% v/v. For a field application of 50% v/v, there would be sufficient onion waste in the UK to treat an area of 300 ha $year^{-1}$. The proportion of the UK bulb onion production area of 9000 ha (Anonymous, 2002) infested with *S. cepivorum* is unknown. Further work is needed to determine the optimum rate, timing and number of compost applications to reduce sclerotia viability and control AWR under field conditions.

In previous experiments in sandy loam soil (Coventry et al., 2002), composted onion waste was more effective in reducing sclerotia viability than the raw waste. In this study using the same soil, raw and composted onion waste were equally effective. The difference between experiments may have been due to variation in the onion waste (e.g. variety, growing location and conditions). Only composted onion wastes were used in the AWR bioassays due to the potential disease risk of using untreated raw waste in soil.

Disease suppression with composted vegetable wastes

Compost amendment reduced sclerotia viability in all three soils examined, but AWR was only suppressed by compost amendment in the peat and sandy loam soils. This indicates that the soils had an influence on AWR that was independent of their effect on sclerotia viability. A possible factor is soil pH, since the unamended peat and sandy loam soils were more conducive to AWR than the silt soil, which had a higher pH than the other two. Compost amendment, which increased pH of all three soils, only suppressed AWR in the peat and sandy loam soils. This indicates that slightly alkaline conditions (pH 8.1–8.4) were suppressive to AWR, whereas higher or lower pH values were conducive. Coley-Smith (1959) and Rondomanski (1990) however found no relationship between soil pH and suppressiveness to AWR. Although the composted vegetable wastes were acidic, compost amendment slightly increased soil pH, probably due to mineralisation of compost organic nitrogen into ammonium salts (Sikora and Szmidt, 2001).

The presence of the composted waste in the peat soil increased the rate at which the mixture dried out in the pots, probably due to increased drainage. This may explain why AWR control was observed in the peat soil, even though disease levels were generally highest in the unamended peat soil. Sclerotia of *S. cepivorum* which are dried out for short periods and then remoistened in soil, leak nutrients (Smith, 1972). Micro-organisms in close proximity to the sclerotia use these nutrients to colonise and ultimately degrade the sclerotia (Smith, 1972). Adams (1987) also found that reducing the soil moisture level for 7 days and then remoistening the soil reduced the survival of sclerotia of *S. cepivorum*.

Soil organic amendments serve as a source of nutrients that result in an increase in the soil microbial population, which may have an effect on disease suppression (De Ceuster and Hoitink, 1999). The time required for organic wastes to sustain high levels of biocontrol activity varies with soil conditions and type of waste used (Hoitink et al., 2001). There was a decrease in the survival of the sclerotia in the three soils used in this study in the presence of the waste or compost over time. This may have been due to the development of an antagonistic microbial population in these soils. The use of organic soil amendments in combination with known antagonists of *S. cepivorum* (Clarkson et al., 2002) requires further investigation.

The optimum temperature for sclerotial germination of *S. cepivorum* is c. 15 °C (Entwistle, 1990b). The difference in temperature between the glasshouse and field may therefore explain the greater reduction in viability of sclerotia retrieved from the glasshouse pots compared with the field. The low temperatures in the field during the first 3 months of the experiment may have reduced the activity of *S. cepivorum* and of potential antagonists and/or the release of fungicidal volatile compounds. The time of year when compost is applied to land is therefore likely to have an important effect on viability of sclerotia and AWR control.

The presence of the composted onion waste in the soils in the pot experiments reduced onion seed germination, seedling growth and increased the number of non-AWR deaths. Smolińska (2000) also found that the incorporation of *Brassica* plant residues in soil had a detrimental effect on germination of onion seeds and seedling growth. As well as increasing the levels of soluble salts in soils, organic wastes and composted materials often contain substances such as organic acids that have a detrimental effect on seed germination and plant growth (Keeling et al., 1994). Phytotoxic compounds have been shown to increase in soil following compost incorporation but then decline over time (Conklin et al., 2002). A longer delay between the onion waste incorporation and planting may reduce the phytotoxic effects, without adversely affecting AWR control. Growing a tolerant break crop following the incorporation of organic residues has been shown to reduce phytotoxicity of the residues in the succeeding crop (Mazzola and Funnell, 2001).

This study has partly confirmed the original hypothesis that incorporation of composted vegetable wastes in soil can reduce the viability of sclerotia of *S. cepivorum* and provide control of AWR. However, the control varied between soil types in pot bioassays. Levels of AWR control observed in these experiments were only partly explained by loss of viability of *S. cepivorum* sclerotia caused by amendment of soil with composted vegetable waste. Other suppressive effects of compost on AWR may be involved. Compost amendment of soil has been shown to confer resistance of plants to soil-borne pathogens ('induced systemic resistance'), whereby a decrease in disease does not necessarily correspond to a decrease in pathogen inoculum (Hoitink et al., 2001). Future studies should focus on further establishing the exact mechanism(s) whereby composted vegetable wastes antagonise *S. cepivorum* and suppress AWR, and methods for optimising and enhancing AWR control with composted organic wastes in the field.

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