Project Title:	Integrated *Allium* white rot control using composts and *Trichoderma viride*
Project Number:	HortLINK CSA 6958/HL 0176 LFV
Project Leaders:	John Whipps, Ralph Noble and John Clarkson, Warwick HRI
Report:	Final Report, March 2009
Previous reports:	Progress report for the period June-November 2008 Second year report, May 2008 Progress report for the period April-October 2007 First year report, March 2007 Progress report for the period April-September 2006
Key Workers:	Emma Coventry, Nelum Deshappriya, Geoff Clark, Warwick HRI
Location of Project:	Warwick HRI, University of Warwick, Wellesbourne, Warwickshire, CV35 9EF. Warwick HRI, University of Warwick, Willington Road, Kirton, Boston, Lincolnshire, PE20 1NN
Project Co-ordinator:	Robert Oldershaw Goldwood (Moulton) Limited Long Lane, Moulton, Spalding, Lincs, PE12 6PP
Date Project Commenced:	01 April 2006
Date Project Completed:	31 March 2009
Key Words:	*Allium* white rot (AWR), *Sclerotium cepivorum*, *Trichoderma viride*, Tebuconazole, Folicur, compost

Signed on behalf of: **Warwick HRI**

Brian Thomas ***31 March 2009***

Signature:…… ***Date***: …….

Name Professor Brian Thomas
Deputy Director

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Grower Summary

Headlines

- Allium white rot (AWR) disease results in 15% onion yield loss in the UK. This can be reduced by Windrow Onion Waste Compost (OWC) treatments or T. viride S17A- colonised green waste compost.

- Windrow treatments applied 18 months prior to planting reduce AWR disease and give an onion yield of at least three times higher than Folicur-treated sets. Folicur is under threat from the new EU legislation (Directive 91/414)

- T. viride S17A-colonised green waste compost applied 2-4 weeks before set planting controls AWR disease for up to 2 onion growing seasons, and gives comparable or higher yields to Folicur-treated plots

Background and expected deliverables

Allium white rot (AWR) caused by the sclerotium-forming fungus *Sclerotium cepivorum* is a major soil-borne disease affecting *Allium* crops. A loss of 15% of the UK onion crop due to this disease equates to approximately £7M per annum. In addition, in some heavily infested areas in the UK, land has been taken out of production. The efficacy of AWR control with fungicides can be variable and tebuconazole (Folicur) is under threat from new EU legislation (Directive 91/414). Alternative control measures for AWR are therefore required.

Previous research at Warwick HRI has shown that application of composted onion waste or the fungus *Trichoderma viride* to soil control AWR reproducibly in both glasshouse and field tests. Onion waste for composting and control of AWR is, however, limited in supply and composts from other more plentiful waste streams may provide effective alternatives. Certain sulphur-containing compounds from onion waste have been implicated in suppressing AWR by stimulating sclerotia of *S. cepivorum* to germinate which subsequently die in the absence of a host. Other sulphur containing composts such as Brassica waste and poultry manure seem toxic to S. cepivorum after sclerotia germination. In addition to sulphur- containing composts, there are large amounts of green waste compost that require disposal. Preliminary pot results from an EU project (RECOVEG) have indicated that composted green wastes may be suitable for supporting the growth and proliferation of *T. viride* resulting in an enhanced compost for controlling AWR.

This project aimed to identify composts which suppress AWR either alone or when enhanced with *T. viride* and to establish *T. viride* on onion sets to provide integrated and sustainable disease control for the industry.

Summary of the project and main conclusions

Objective 1: Determine the ability of various green waste composts to support the growth and proliferation of *T. viride* S17A and assess the enhanced composts for their ability to destroy *S. cepivorum* sclerotia and control AWR.

Objective 2: Develop a method to apply *T. viride* S17A to onion sets and assess the ability of this treatment to control AWR.

Green waste compost of different ages, produced from various feedstocks (fruit, vegetables, parks and gardens waste), was sourced to provide a range of growing conditions for *T. viride* S17A. *T. viride* S17A grew well in all the composts tested and maintained a high level of colonisation over a 70 day period. On the basis of these results, a single source of one year old green waste compost was selected to be used further in the project in glasshouse and field trials.

Glasshouse tests were initially used to determine the effect of *T. viride* S17A-colonised green waste compost on the control of AWR. Green waste compost was inoculated with *T. viride* S17A and the colonised compost incorporated into soil. The soil-green waste compost mixtures were inoculated with sclerotia of *S. cepivorum*, filled into pots and a single onion set, variety Hercules, planted in each pot.

Figure 1 shows the progression of AWR in the different treatments over time. At the end of the tests, the level of AWR in all the green waste compost alone and *T. viride* S17A-colonised green waste compost treatments was less than in the control (soil alone). The 40% green waste + *T. viride* S17A treatment was the most effective in controlling AWR. A *T. viride* S17A set treatment and Folicur-treated sets showed no disease control.

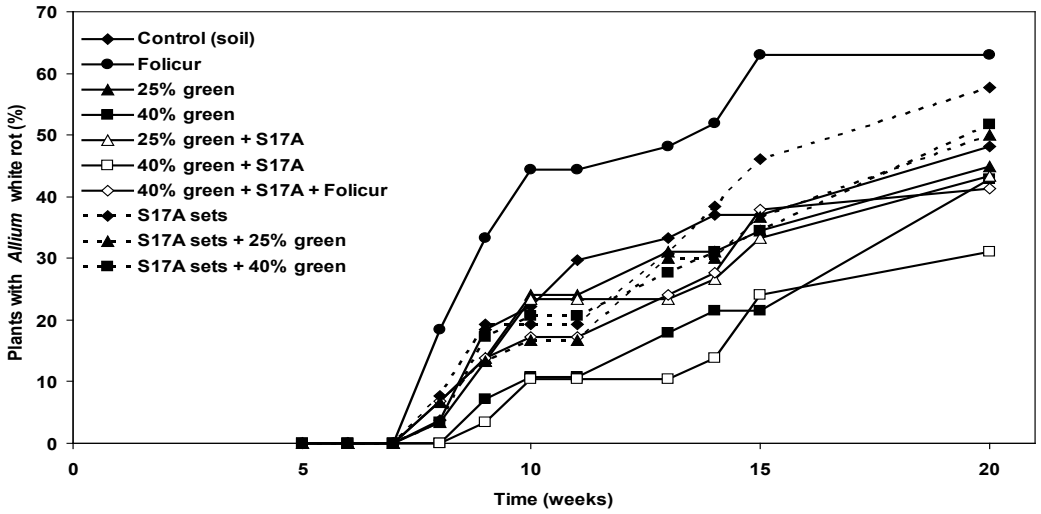

Figure 1: Plants with *Allium* white rot in the various green waste compost-*T. viride* S17A treatments in the glasshouse

T. viride S17A-colonised green waste compost incorporated at a 25% (v/v) rate 2-4 weeks before set planting effectively controlled AWR in four field trials with very high disease pressure. Figure 2 shows the AWR recorded in the assessment areas within the treatment plots of the trials at Wellesbourne and Kirton during the 2007 and 2008 growing seasons. No disease was observed in the *T. viride* S17A-colonised green waste compost treatment throughout the 2007 growing season at Wellesbourne or Kirton. At Wellesbourne, both the *T. viride* S17A set treatment and green waste treatments also reduced the level of AWR compared with the control. In contrast, at Kirton, there was no difference in the AWR recorded in these two treatments and the control at the end of the 2007 trial. The disease pressure at Kirton was very high and this may explain the difference in the efficacy of these treatments on the two sites. In the 2008 growing season, the *T. viride* S17A-colonised green waste compost treatment was the only treatment that showed any disease control (Figure 2). The disease levels in this treatment were at least 50% less than in the controls on both sites. No disease control was observed with the Folicur-treated sets.

Figure 2: Onion plants (%), in the assessment areas within the treatment plots, infected with *Allium* white rot throughout the 2007 and 2008 growing seasons at Wellesbourne and Kirton. * Folicur-treated sets not assessed in 2007

Figure 3 shows the total yield of onions harvested from each of the treatments from the 2007 and 2008 trials at Wellesbourne and Kirton. The *T. viride* S17A-colonised green waste compost gave a comparable or higher onion yield to Folicur-treated sets.

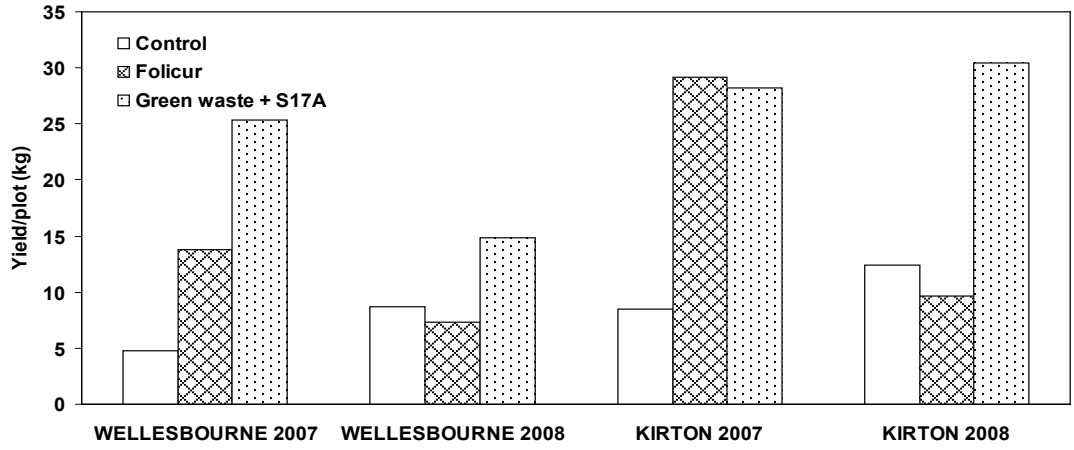

Figure 3: Onion yield (kg) from various treatments in the 2007 and 2008 trials at Wellesbourne and Kirton

To determine whether any effects of the treatments were carried over from one season to the next, the 2007 green waste and *T. viride* S17A trials were replanted in 2008 with no further treatments applied. At Wellesbourne, *T. viride* S17A was recovered from the *T. viride* S17A-colonised green waste compost treatment plots in

2008 at a high level, 18 months after application. Figure 4 shows the AWR and onion yield recorded in the treatment plots of the replanted trial at Wellesbourne during the 2008 growing season. The *T. viride* S17A-colonised green waste compost was the most effective in controlling disease and gave the highest healthy yield. The yield from this treatment was twice as high as any of the other treatments, including the Folicur-treated sets. The Kirton site was severely flooded in August 2007 and there was no detectable survival of *T. viride* S17A in the following season. No disease control was observed at Kirton in this replant trial in the 2008 growing season.

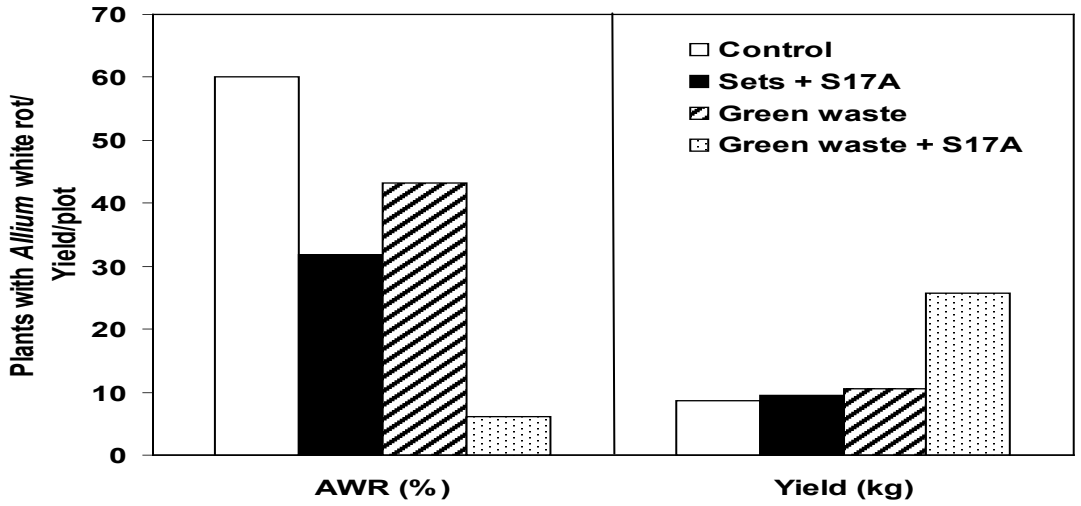

Figure 4: Onion plants infected with *Allium* white rot (%) and onion yield (kg) recorded in the replanted treatment plots in the 2008 growing season following compost application in 2007 at Wellesbourne

Objective 3: Determine the ability of sulphur-containing composts produced from different waste streams to destroy *S. cepivorum* sclerotia and reduce AWR and identify any physical and chemical properties including sulphur content and volatile sulphur-containing compounds as factors influencing the efficacy of the composts.

A number of sulphur-containing composts were sourced and tested for their effect on sclerotia viability and control of AWR in glasshouse tests. The onion waste composts (OWC) stimulated germination of the sclerotia over time and the known sclerotia germination stimulant, dipropyl disulphide, was detected in these composts. This is recognised as the mechanism through which at least part of the AWR control observed with OWC is attributed. In contrast, no stimulation of germination was observed with the other treatments. A number of other volatile compounds were

detected in the various composts. The poultry manure compost released high levels of ammonia, dimethyl sulphide and tert-butyl mercaptan. Although this compost did not stimulate sclerotia germination it reduced sclerotia viability suggesting that the volatiles released from this compost were toxic to the sclerotia.

Figure 5 shows the progression of AWR in the different sulphur-containing composts over time in the second of two pot bioassays. The level of disease in the control and low N poultry manure treatments increased rapidly over time. At the end of the glasshouse tests, the lowest disease levels were recorded in the OWC and high N poultry manure treatments.

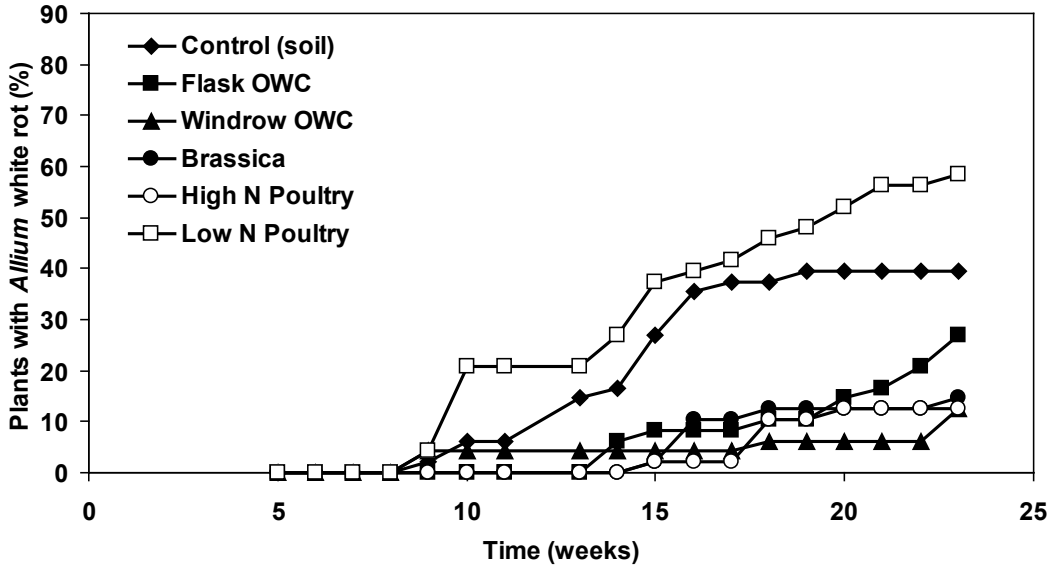

Figure 5: Plants with *Allium* white rot in the various sulphur-containing composted waste treatments in the glasshouse

Windrow OWC and poultry manure compost were selected to be used in field trials. Figure 6 shows the AWR recorded in the assessment areas within the treatment plots at Wellesbourne and Kirton throughout the 2008 growing season. Windrow OWC applied at a 25% (v/v) incorporation rate 18 months before onion planting (OWC 2006) effectively controlled AWR at Wellesbourne and Kirton. Windrow OWC applied six months prior to planting (OWC 2007) effectively controlled AWR at Wellesbourne but not at Kirton. Effective OWC treatments gave an onion yield at least three times higher than the yield from Folicur-treated sets. In contrast to the glasshouse test results, no control of AWR was observed with the poultry manure compost in the field.

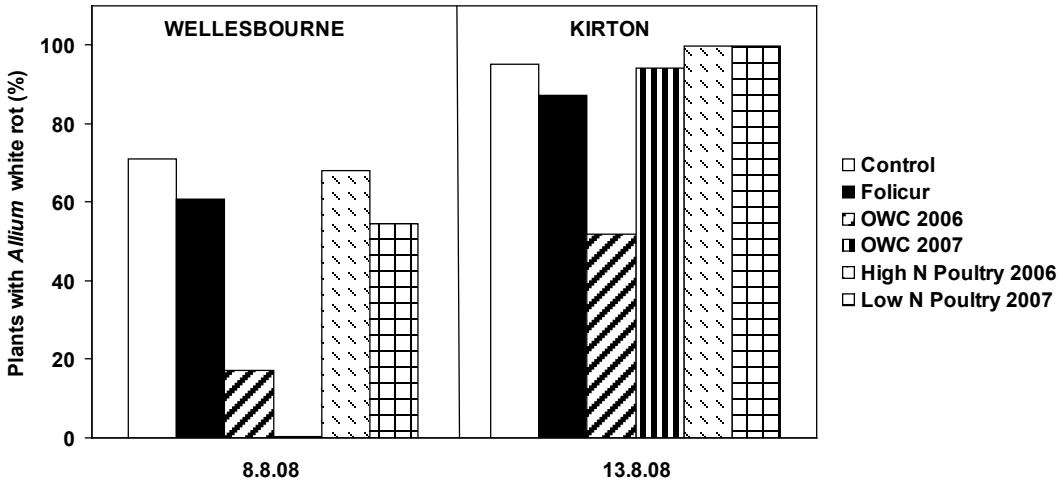

Figure 6: Onion plants (%), in the assessment areas within the treatment plots, infected with *Allium* white rot throughout the 2008 growing season at Wellesbourne and Kirton

Financial benefits

Financial gains from increased onion yield are to be made through:

- Control of AWR using composts and *T. viride*. *T. viride* survives in soil and continues to control AWR in more than one season following compost application.
- Reduction in costs currently incurred through disposal of onion wastes
- Reduction in fungicide usage and the length of crop rotations currently needed to avoid build-up of the white rot pathogen
- The potential for inorganic fertiliser substitution through composting.
- Technology suitable for both conventional and organic production.

A cost/benefit analysis of applying *Trichoderma*-colonised compost was compared with a Folicur spray programme. This took into account the nutrient content and inorganic fertiliser substitution value of the compost. The analysis showed that if compost were applied within the planting row at 5 t/ha, the treatment would break-even with a Folicur spray programme after a single season. Alternatively, if compost was broadcast at 30 t/ha and gave AWR control for two seasons, the treatment would be £34/ha cheaper than a Folicur spray programme.

Action points for growers

- Semi-commercial scale production and application of *T. viride* S17A-colonised compost is currently being conducted. If successful, the treatment should be available to growers from 2010.
- Windrow composting is an effective method of onion waste disposal and the resulting compost can be used to suppress AWR on infested land.
- Growers should reduce organic fertiliser applications to land where composts are applied.

SCIENCE SECTION

MILESTONES

Year 1

Primary Milestones

1.1 Produce replicated batches of green waste composts and sulphur-containing composts with standardised feedstocks under controlled conditions in flask-scale bench composting equipment; determine physico-chemical properties of composts, and volatile sulphur compounds (month 6).

1.2 Inoculate green waste composts produced under controlled conditions and onion sets with *Trichoderma viride* and monitor colonisation and survival (month 6).

2.1 Determine the ability of *T. viride*-enhanced compost and *T. viride*-treated onion sets to destroy *Sclerotium cepivorum* sclerotia and reduce *Allium* white rot (AWR) in pot bioassays (month 12).

3.1 Test sulphur-containing composts for their ability to destroy *S. cepivorum* sclerotia and reduce AWR in pot bioassays; determine activity (sclerotia germination stimulation or toxicity of sulphur compounds) (month 12).

Year 2

Primary Milestones

2.2 Determine the ability of *T. viride*-enhanced compost and *T. viride*-treated onion sets to destroy *S. cepivorum* sclerotia and reduce AWR in laboratory and glasshouse pot bioassays (month 15).

3.2 Produce specified sulphur-containing composts under controlled conditions and test for their ability to destroy *S. cepivorum* sclerotia and reduce AWR in laboratory and glasshouse pot bioassays (month 21).

4.1 Determine the most effective sulphur-containing composts, *T. viride*-enhanced composts, fungicides and combinations for control of AWR in glasshouse pot bioassays (month 24).

5.1 Produce large-scale composts under specified conditions; test the most effective individual and combination treatments for control of AWR in the field and growth of treated onion sets (month 22).

Year 3

Primary Milestones

3.3 Identify sulphur containing-compounds from onion waste compost and other sulphur-containing composts that relate to stimulation of sclerotia germination or sclerotia toxicity (month 27).

4.2 Determine the most effective sulphur-containing composts, *T. viride*-enhanced composts, fungicides and combinations for control of AWR in glasshouse pot tests (month 27).

5.2 Test the most effective individual and combination treatments for control of AWR in the field and growth of treated onion sets (month 33).

6.1 Complete and submit final reports to HDC and HortLINK co-ordinator (month 36).

Progress

All the Milestones have been completed.

INTRODUCTION

Allium white rot (AWR) caused by the sclerotium-forming fungus *Sclerotium cepivorum* is a major soil-borne disease affecting *Allium* crops. A loss of 15% of the UK onion crop due to this disease equates to approximately £7M per annum. In addition, in some heavily infested areas in the UK, land has been taken out of production. The efficacy of AWR control with fungicides can be variable and tebuconazole (Folicur) is under threat from new EU legislation (Directive 91/414). Alternative control measures for AWR are therefore required.

Currently, over 50 million tonnes of organic waste is disposed of in landfill in the EU annually. With the decrease in the availability of landfill sites, and the need to reduce the quantity of biodegradable waste disposed in this way in accordance with the EU Landfill Directive, an alternative to landfill disposal is required. One alternative is to compost these wastes and then incorporate them into soil or growing media for crop production. Composted wastes have been shown to suppress various soil-borne pathogens and so disposal of wastes in this way offers the possibility of sustainable disease control.

Previous research at Warwick HRI has shown that application of composted onion waste or the fungus *Trichoderma viride* to soil can control AWR reproducibly in both glasshouse and field tests. Onion waste for composting and control of AWR is, however, limited in supply and composts from other more plentiful waste streams

may provide effective alternatives. Certain sulphur-containing compounds from onion waste have been implicated in suppressing AWR by stimulating sclerotia of *S. cepivorum* to germinate which subsequently die in the absence of a host. However, different sulphur compounds derived from other sulphur-containing composts, such as poultry manure, sewage sludge and *Brassica* waste, may be toxic to the pathogen. *Brassica* waste has already been demonstrated to have some effect against *S. cepivorum*. In addition to sulphur-containing composts, there are large amounts of green waste compost that require disposal. Preliminary pot results from an EU project (RECOVEG) have indicated that composted green wastes may be suitable for supporting the growth and proliferation of *T. viride* resulting in an enhanced compost for controlling AWR.

More recently, *T. viride* applied with either onion compost or tebuconazole was shown to result in improved AWR control in glasshouse pot tests compared to any single treatment alone. This suggests that combination treatments of *T. viride*, compost and tebuconazole can provide a more effective, sustainable control strategy for AWR in the future.

The aims of this project were to identify composts which suppress AWR either alone or when enhanced with *T. viride* and to establish *T. viride* on onion sets to provide integrated and sustainable disease control for the industry.

This report details work carried out to address the following:

1. Production of composts from different feedstocks.
2. Assess the ability of *T. viride* to colonise various green waste composts.
3. Determine the effect of *T. viride*-colonised composts on sclerotia viability and *T. viride*-colonised composts and onion sets on the control of AWR in glasshouse pot bioassays.
4. Determine the effect of volatiles from various sulphur-containing composts on the viability of sclerotia of *S. cepivorum* and control of AWR in glasshouse pot bioassays.
5. Determine the effect of field-scale application of green waste compost, *T. viride* and sulphur-containing composts on the viability of sclerotia of *S. cepivorum* and control of AWR.

MATERIALS AND METHODS

General procedures

Organisms

Sclerotium cepivorum – Sclerotia from two 14-day-old potato dextrose agar (PDA) plate cultures (Kirton strain) were removed using a sterile spatula and added to 10 ml sterile distilled water (SDW). The suspension of sclerotia was homogenised for 30 seconds and then added to 500 ml SDW. This suspension (100 ml) was used to inoculate mushroom spawn bags (Van Leer Packaging Systems Limited, Poole, UK). Each bag contained 1920 g sand (Dried Silica Sand, Hepworth Minerals and Chemicals Cheshire, UK), 80 g flaked maize (Midland Shires Farmers Limited, Worcester, UK) which had been ground in a blender to <1 mm diameter particle size, and 175 ml water, which had been autoclaved at 121 °C for 15 minutes. The bags were heat sealed after inoculation and incubated for six weeks at 20 °C. To harvest the sclerotia, water was added to the sand-maize-sclerotia mix and the sclerotia decanted into a 212 μm mesh size sieve. The sclerotia were left to dry in a laminar air flow cabinet, then mixed to 50% (v/v) with sand, enclosed within fine polyester mesh bags (150 μm pore size) (Lockertex Limited, Warrington, UK) and buried outside in soil *c.* 150 mm deep for 12 weeks. This "conditioning" period in soil was necessary to break dormancy and ensure sclerotia would germinate in the presence of onions. After this period the sclerotia were recovered as described above. This provided a stock of conditioned sclerotia for future glasshouse and field experiments.

***Trichoderma viride* S17A** – This organism was originally isolated by John Whipps from AWR infected material. A spore suspension provided by Anita Scruby, Warwick HRI was used to inoculate PDA slopes. The slopes were incubated at 20 °C and then cold stored (4 °C) to serve as the stock of this organism. Spore suspensions were obtained by adding 20 ml SDW to three-week-old PDA plate cultures; a sterile spatula was used to dislodge the spores. These were used to inoculate two different substrates for subsequent introduction into green waste composts:

(i) Wheat bran – 5 ml of spore suspension were added to 250 ml conical flasks containing sterile wheat bran (12 g) and water (30 ml) and incubated for three days at 20 °C prior to use.

(ii) Rye grain – 5 ml of spore suspension were added to honey jars (*c.* 320 ml) containing 150 g sterile rye grain (Sylvan Spawn Limited, Peterborough, UK) and incubated for 14 days at 20 ^{o}C prior to use.

To generate spores of *T. viride* S17A to apply to onion sets, the organism was grown on malt agar as sporulation was more profuse on this medium. Spores from three-week-old malt agar plate cultures were suspended in SDW and filtered through double layer polyester mesh to remove any mycelial fragments. The spore concentration in the suspensions was determined by serial plate dilutions on to PDA amended with 0.02 g l^{-1} chlortetracycline and 2 ml l^{-1} Triton X-100, and use of a haemocytometer.

Effect of pH, conductivity and Folicur on the growth of *Trichoderma viride* S17A *in vitro* (Milestone S1.3)

Green waste compost is known to vary in its pH and conductivity, both of which can have an impact on fungal growth. To determine the effect of pH and conductivity on the growth of *T. viride* S17A, PDA was amended with either 10% phosphoric acid or various concentrations of NaOH to reduce or increase the pH respectively, or NaCl to increase the conductivity. A 6 mm disk cut from the edge of an actively growing *T. viride* S17A culture was used to inoculate Petri dishes with the amended PDA. The diameter of the growing colony was measured in two directions at right angles to each other daily.

To assess the feasibility of an integrated AWR control strategy using Folicur and *T. viride* S17A, the fungicide was added to PDA to give a range of concentrations (0.01-1% Folicur). This range of concentrations was selected as previous work has shown a 0.5% Folicur dip treatment of sets to effectively control AWR. The Folicur-amended PDA was inoculated with *T. viride* S17A and colony growth measured as described above.

Composted wastes (Milestone 1.1)

Green waste compost prepared from different feedstocks and of different maturity was collected from two of the industrial partners (Organic Recycling Limited and Engine Farm). Three green waste composts were collected from Organic Recycling Limited, two marketed for use as top soil conditioners and one as a multipurpose compost. The top soil conditioners were prepared from fruit and vegetable waste while the multipurpose compost was prepared from prunings blended with top soil conditioner. Three different ages of green waste compost (young – three weeks old;

middle – two months old; old – three-four months old) consisting of lettuce, celery, onion, straw and wood chips were collected from Engine Farm. Two other sources of green waste compost were obtained from Tunnel Tech North Limited, Doncaster, UK and J. Moody Limited, Wolverhampton, UK. The green waste compost from J. Moody Limited consisted of a mixture of parks and gardens waste composted in windrows for five months. The green waste compost from Tunnel Tech North Limited was prepared from parks and gardens waste and was *c.* one year old. The pH, conductivity, moisture, ash and nitrogen content of the various green waste composts were determined.

In order to make the composts more conducive to *T. viride* S17A colonisation, based on the results from the effect of pH on the growth of *T. viride* S17 *in vitro* (see Figure 1a), two rates (20% and 25% w/w) of horticultural grade peat or onion waste were added to the composts to reduce their pH.

Flask-scale preparation of composted sulphur-containing wastes

Sulphur-containing wastes were collected from two of the industrial partners, Moulton Bulb Company Limited (onion waste) and Anglian Water (sewage). Two other sulphur-containing wastes, poultry manure (Warwick HRI) and Brassica waste (predominantly cabbage with a small amount of cauliflower purchased from a supermarket) were also obtained.

All the sulphur-containing wastes required composting prior to use. The onion waste mixture consisted of 10 parts wet (peelings) to 1 part dry (shale) (w/w) onion waste. To reduce the moisture content and subsequent run-off of the Brassica, poultry manure and sewage, these wastes were mixed with prunings (*c.* 3 cm long twigs) prior to composting. The poultry manure and Brassica waste were individually mixed with 25% (v/v) prunings. The sewage was heated in a compost tunnel at 50 ^{o}C for 20 hours to destroy any pathogens prior to handling and dewatered by decanting the solids that floated to the surface. The treated sewage was mixed with 50% (v/v) prunings. The waste mixtures were composted in 10 litre “Quickfit” multiadapter flasks immersed in thermostatically controlled waterbaths. The waste mixtures were placed on a perforated stainless steel platform within each flask and the flasks immersed in the waterbaths such that the water level was above the level of the enclosed waste. Each flask was connected to ancillary equipment providing independent aeration of the waste. The waste was aerated for two minutes in every 30 minutes at a flow rate of 250 ml min^{-1}. The waste was incubated at 50 ^{o}C for

seven days after which the pH, conductivity, moisture, ash, total nitrogen and total sulphur content were determined.

Colonisation of green waste compost by *Trichoderma viride* S17A (Milestone 1.2)

On the basis of the pH and conductivity analyses of the various green waste composts and results from preliminary experiments, three green waste composts and one soil were selected to examine *T. viride* S17A colonisation. The composts selected were Top soil conditioner (one year old) from Organic Recycling Limited, Moody green waste and Tunnel Tech green waste. The soil used was a silty clay (Big Cherry Field, Wellesbourne, Warwickshire, UK). Each waste (110g) and 1% (w/w) of either a three-day-old wheat bran or 14-day-old rye grain culture of *T. viride* S17A were added to a honey jar with a screw-top lid. Treatments with peat (25% w/w), added to reduce the pH of the green waste compost and potentially facilitate better colonisation, were also examined. Some of the treatments felt dry to touch and water was added prior to addition of the inocula to attempt to standardise the moisture content of all the treatments. The jars were incubated at 20 ^{o}C in a growth room with a 12 hour photoperiod. The green waste compost and green waste compost + peat mixtures were sampled prior to adding the inoculum to determine the background level of *Trichoderma* present, and thereafter at time zero (just after addition of the inocula), 3, 7, 10, 14, 21, 28, 35, 42, 56 and 70 days post-inoculation to monitor colonisation and survival of *T. viride* S17A. Samples were serial diluted in SDW, plated on to PDA amended with 0.02 g l^{-1} chlortetracycline and 2 ml l^{-1} Triton X-100, and incubated for three days at 20 ^{o}C before colonies of *T. viride* S17A were counted. There were three replicate jars per treatment and two samples (**A** and **B**) were removed from each jar at sampling.

Application of *Trichoderma viride* S17A to onion sets (Milestone 1.2)

Onion sets (variety Forum QI supplied by Elsoms Seeds Limited) were placed in a plastic box and partially submerged in an aqueous spore suspension (*c.* 2 x 10^6 spores/cfu ml^{-1}) of *T. viride* S17A. The box was agitated continuously by hand for two minutes to ensure the entire surface area of the sets was coated with the suspension. Any liquid remaining in the box was then drained off and the sets allowed to air dry. The sets were then either cold stored (4 ^{o}C) or held at room temperature (*c.* 20 ^{o}C). At various intervals post-inoculation the sets were agitated in SDW using a whirlimixer and any spores dislodged were plated out and counted as described above to determine survival.

In addition to the effect of temperature on the survival of the spores on the sets, various stickers/foaming agents were individually incorporated into the spore suspension to improve adhesion and retention of the spores on the surface of the sets. The additions to the spore suspensions included 1% foam concentrate (Expyrol F-4, 3%, supplied by English Set Company), 1% guar gum and 1% polyvinyl acetate (PVA). The sets were treated and sampled to determine spore survival as described above. There were three replicate sets per treatment at each sampling time.

Effect of *Trichoderma viride* S17A-colonised green waste compost on sclerotia viability (Milestone 2.1)

On the basis of the results from the green waste compost colonisation experiments, the top soil conditioner – one year old (hereafter referred to as green waste compost) and the rye grain substrate were selected for use in glasshouse bioassays.

Green waste compost was inoculated with 1% (w/w) 14-day-old *T. viride* S17A-colonised rye grain (3.5×10^7 cfu g^{-1}). The inoculum was allowed to colonise the green waste compost for 14 days at 20 ^oC and then the compost was incorporated at two rates (10% and 25%, v/v) into sieved (5 mm) sandy silt loam soil (Kirton, Lincolnshire, UK). *T. viride* S17A-colonised soil was similarly incorporated at a 25% rate. Soil containing the same two rates of uninoculated green waste compost was also included in the bioassay. The soil and green waste compost were sampled prior to adding the inoculum, to determine the background level of *Trichoderma* present, and thereafter on addition of the rye grain inoculum, 14 days post-inoculation and on mixing the colonised green waste compost and soil with uninoculated soil to complete the treatments, to monitor colonisation and survival of *T. viride* S17A.

Square pots (70 x 70 x 80 [deep] mm Optipots, LBG Ltd., Evesham, UK) were filled with the soil-green waste compost mixtures together with 4 polyester mesh bags (20 x 20 mm, 150 μm mesh diameter) containing 2 g of 50:50 (v/v) sand: soil and 100 sclerotia. There were five replicate pots of each treatment, including the control (uninoculated soil). The glasshouse heating and ventilation set points were 15 ^oC and 17 ^oC respectively. The pots were watered from the bottom at regular intervals and the mesh bags retrieved after two, four and six weeks and three months burial. The sclerotia were washed from the bags with water, collected on a 212 μm mesh size sieve, and assessed for degradation (soft or collapsed) by squeezing with forceps. The viability of the hard sclerotia was assessed in terms of germination as follows: sclerotia were surface sterilised in sodium hypochlorite (>5% but <16%

available chlorine, Hays Chemical Distribution Limited, Leeds, UK), rinsed in SDW 4 times and plated on to PDA containing 0.02 g l^{-1} chlortetracycline. The sclerotia were incubated at 20 ºC for 14 days after which colony growth was seen as evidence of viability.

Effect of *Trichoderma viride* S17A-colonised green waste compost and onion sets on the control of *Allium* white rot (Milestones 2.1, 2.2 and 4.1)

Bioassay 1

Green waste compost (top soil conditioner – one year old from Organic Recycling Limited) was inoculated with 1% (w/w) 14-day-old *T. viride* S17A-colonised rye grain (3.5×10^7 cfu g^{-1}). The inoculum was allowed to colonise the green waste compost for 14 days at 20 ºC and then the compost was incorporated at two rates (10% and 25%, v/v) into sieved (5 mm) sandy silt loam soil (Kirton, Lincolnshire, UK). *T. viride* S17A-colonised soil was similarly incorporated at a 25% rate. Following incorporation of the *T. viride* S17A-colonised materials into soil, the mixtures were inoculated with sclerotia (eight sclerotia/g mixture) and left for 14 days to allow further colonisation by *T. viride* S17A and the opportunity for it to act on the sclerotia prior to planting untreated, Folicur-treated or *T. viride* S17A-treated onion sets. Soil containing the same two rates of uninoculated green waste compost was also included in the bioassay. The soil and green waste compost were sampled prior to adding the inoculum, to determine the background level of *Trichoderma* spp. present, and thereafter on addition of the rye grain inoculum, 14 days post-inoculation, on mixing the colonised green waste compost and soil with uninoculated soil to complete the treatments, just prior to set planting, and at the end of the bioassay (week 23), to monitor colonisation and survival of *T. viride* S17A.

Square pots (70 x 70 x 80 [deep] mm Optipots, LBG Ltd., Evesham, UK) were filled with the soil-green waste compost mixtures and a single onion set, variety Forum QI (Elsoms Seeds Limited), planted in each pot. Pots with the soil-compost mixtures and soil alone with no sclerotia added were included as controls. There were three replicate plots with 10 pots per treatment per plot. All plants received watering with a nutrient solution (2N:1P:4K) every 14 days. The pots were assessed weekly for the presence of AWR, which was scored as dead plants with visible mycelium or sclerotia. Natural plant deaths (non-AWR) were also recorded. The glasshouse heating and ventilation set points were 15 ºC and 17 ºC respectively.

Bioassay 2

A similar bioassay to Bioassay 1 was used to examine the effect of incorporation of a higher rate of compost, and a combination treatment of *T. viride* S17A-colonised green waste compost and Folicur-treated sets, on the control of AWR. Green waste compost (top soil conditioner – one year old) was inoculated with 1% (w/w) five-week-old *T. viride* S17A-colonised rye grain. The same batch of *T. viride* S17A-colonised green waste compost was used in the Warwick HRI Trial 3: 2008 (see below). Similar to Bioassay 1, the compost was incorporated at two rates (25% and 40%, v/v) into sieved (5 mm) sandy silt loam soil, inoculated with sclerotia (eight sclerotia/g mixture) and left for 14 days to allow further colonisation by *T. viride* S17A prior to planting untreated, Folicur-treated or *T. viride* S17A-treated onion sets. Soil containing the same two rates of uninoculated green waste compost was also included in the bioassay. The soil and green waste compost were sampled prior to adding the inoculum, and thereafter on addition of the rye grain inoculum, 25 days post-inoculation, just prior to set planting and at the end of the bioassay (week 20), to monitor colonisation and survival of *T. viride* S17A. The experimental protocol and glasshouse settings are as described for Bioassay 1 with the exception of the onion variety used (variety Hercules, Elsoms Seeds Limited).

Effect of sulphur-containing composted wastes on the viability of sclerotia of *Sclerotium cepivorum* and the control of *Allium* white rot (Milestones 3.1 and 3.2)

Bioassay 1

Composted sulphur-containing wastes [flask produced onion waste compost (OWC), windrow produced OWC – Moulton Bulb Company, Brassica, poultry manure and sewage sludge composts] were incorporated (25%, v/v) into sieved (5 mm) sandy silt loam soil and inoculated with sclerotia (eight sclerotia/g of mixture) and *c.* 250 ml added to Kilner jars. Two polyester mesh bags (20 x 20 mm, 150 μm mesh diameter), containing 2 g of 50:50 (v/v) sand: soil and 100 sclerotia, were buried in half the Kilner jars in each treatment. In each Kilner jar, a porcelain crucible (22 mm deep, 30 mm diameter), containing 12 g of wet sand supporting a 25 mm diameter, 0.2 μm pore size cellulose nitrate membrane filter (Whatman) with 15 sclerotia, was placed on top of the soil-compost mixtures. These sclerotia allowed the effect of the volatiles released from the composts to be determined. The Kilner jars were kept in a cooled glasshouse (15 ^{o}C). The lids of half the Kilner jars were modified to allow gas samples to be removed for gas chromatography-mass spectrometry (GCMS) analysis and detection with Dräger tubes. The air in these jars was analysed by GCMS (IGER, Okehampton, UK), and Dräger tubes were also used to detect the levels of O_2, CO_2 and various sulphur-containing compounds, three days after set up. Thereafter, the air in the jars was sampled weekly using Dräger gas detection tubes. The sclerotia in the crucibles were examined weekly for any germination resulting from stimulation by volatiles released from the composts, and the compost mixtures sprayed with water. Germinated sclerotia were removed from the membranes. Three months after set up, the viability of sclerotia remaining in the crucibles was assessed in terms of germination as described earlier for the *T. viride* S17A-green waste sclerotia viability bioassay. One bag of sclerotia was also removed from the Kilner jars after three months burial to determine their viability after direct contact with the composts. The second bag of sclerotia was recovered from each Kilner jar after 10 months burial and the compost mixtures used to set up an onion seedling bioassay (see below). There were 12 replicate jars of each treatment, including the control (soil alone). Control Kilner jars containing uninoculated treatments (six replicate jars per treatment) were used to check for any phytotoxicity of the composts to onion seedlings.

The soil-compost mixtures in the Kilner jars were transferred to square pots (70 x 70 x 80 [deep] mm) and a four-week-old onion seedling (cv. Hercules F1,

supplied by Elsoms Seeds Limited) transplanted to determine the effect of the composts on the control of AWR. In addition to the Kilner jars, three replicate polythene bags of each treatment (each containing *c.* 3 l of material) were prepared to provide treatments for further pots in a fully replicated bioassay. These polythene bags, similar to the Kilner jars, contained bags of sclerotia that were recovered after three and 10 months burial to assess the effect of the treatments on sclerotia viability. Control bags (each containing *c.* 1.5 l of material) contained uninoculated composts. Following recovery of the sclerotia, the soil-compost mixtures in the bags were transferred to pots and an onion seedling transplanted. There were four replicate plots (Kilner jars and three polythene bags) with 18 pots per treatment per plot. The pots were watered and assessed weekly as described for the green waste bioassays. The glasshouse heating and ventilation set points were as previously described.

Bioassay 2

A similar bioassay to Bioassay 1 was used to examine the effect of a low nitrogen poultry manure compost on sclerotia viability and control of AWR. All experimental details are as described above for Bioassay 1 with the exception that the sewage sludge compost was replaced by a low nitrogen poultry manure compost. This new treatment was included as ammonia was detected in the poultry manure compost during the set up of the plant bioassay described above (Bioassay 1), some 10 months after preparation of the soil-compost mixtures. To avoid any phytotoxicity as a result of any ammonia released, less poultry manure and more green waste were used in the preparation of the low nitrogen poultry manure compost. The low nitrogen poultry manure compost was prepared with 33% (w/w) poultry manure compared with 80% (w/w) used in Bioassay 1. Both rates were included in this bioassay to allow comparison of efficacy and phytotoxicity.

Field trials (Milestones 5.1 and 5.2)

(a) Warwick HRI – Wellesbourne and Kirton

Sclerotia (0.2 g m^{-2}), mixed with sand, were spread uniformly over two experimental sites at both Wellesbourne (soil type = silty clay) and Kirton (soil type = sandy silt loam) in November 2006. The experimental sites were arranged in plots 1.8 m wide consisting of four 6-m-long rows, with a guard plot between each experimental plot. The two trials at each site used the same treatments and replication but with a different randomisation of treatments.

Trial 1: 2007

On the basis of the results from the *T. viride* S17A green waste compost colonisation bioassay (Milestone 1.2), the top soil conditioner – one year old (Organic Recycling Limited) was the green waste compost selected for use in this trial. The treatments tested were green waste compost, *T. viride* S17A-colonised green waste compost, *T. viride* S17A-treated sets, Folicur-treated sets and a control which received no compost, *T. viride* S17A or fungicide treatment, with three replicate plots per treatment.

Green waste compost was inoculated with 1% (w/w) three-week-old *T. viride* S17A-colonised rye grain and incubated at 16 ^{o}C for five weeks to allow colonisation of the compost. The compost treatments were applied as a 3.75 cm layer to plots in March 2007 and power harrowed into 15 cm to give a 25% (v/v) incorporation rate. Similar to the pot bioassays, the green waste compost was sampled prior to adding the rye grain *T. viride* S17A inoculum, to determine the background level of *Trichoderma* spp. present, and thereafter on addition of the rye grain inoculum and just prior to soil incorporation. The field plots were also sampled throughout the growing season to monitor the *Trichoderma* spp. level during the trial. One polyester mesh bag containing 100 sclerotia, as described previously, was buried in each of the plots following application of the compost treatments. Just prior to set planting (cv. Hercules, supplied by Elsoms Seeds Limited) in April 2007, the bags of sclerotia were removed and the effect of the treatments on sclerotia viability assessed. At the same time, a sample of soil was taken from the different treatment plots for general loam and mineral nitrogen analysis. Emergence of the sets in 2 x 1 m lengths within each plot was recorded over a six week period following planting. The plants were assessed for AWR in these 1 m lengths throughout the growing season. Onion bulbs were harvested in September 2007 and assessed after one month storage for the presence of AWR and other rots. The bulbs were graded according to the size categories <40 mm, 40-60 mm and >60 mm diameter and yield per treatment determined.

Trial 2: 2006-2008

On the basis of the results from the sulphur-containing composts Bioassay 1 (Milestone 3.1), windrow OWC and high nitrogen poultry manure + prunings compost were selected for use in this field trial. Either of these composts were applied as a 3.75 cm layer to each of five plots in November 2006 and power harrowed into 15 cm to give a 25% (v/v) incorporation rate. Winter wheat (cv. Hereward) was sown on the field sites in November 2006 and 2 polyester bags of sclerotia buried in plots, to be

retrieved and assessed for viability after 10 and 16 months burial as previously described. The winter wheat was mown off in August 2007 and the bags of sclerotia removed temporarily from the plots to allow the trial to be sub-soiled. One bag from each plot was retained to assess sclerotia viability. In October 2007, windrow OWC and low nitrogen poultry manure + green waste compost (prepared as described for the sulphur-containing wastes Bioassay 2, Milestone 3.2) were applied to each of a further five or ten plots. The composts were applied and incorporated as previously described. The bags of sclerotia removed temporarily from the plots were reburied along with additional bags to determine the residual effects of the composts applied in 2006 and the effect of the 2007 treatments. The bags of sclerotia were then removed from the treatment plots in March 2008 to determine the effect of the treatments on sclerotia viability. At the same time, a sample of soil was taken from the different treatment plots for general loam and mineral nitrogen analysis and to determine the level of *Trichoderma* spp. present. The trial was planted with sets (cv. Hercules) in April 2008 with five replicate plots per treatment and recorded as described for Trial 1. There were 15 untreated plots; ten were planted with untreated sets and five were planted with Folicur-treated sets. Some of the treated plots were planted with Folicur-treated sets to provide combination treatments. The combination treatment at Wellesbourne consisted of the 2007 applied low N poultry manure compost with Folicur-treated sets, whereas the combination treatment at Kirton combined the 2007 applied OWC with Folicur-treated sets.

A sample of the onion crop from the 2007 applied treatments was analysed for pyruvate content by Cranfield University and tested for flavour and pungency by taste panels at Anglian Water and Warwick HRI.

Trial 3: 2008

This trial was a repeat of Trial 1. All experimental details are as described above with the exception that five-week-old *T. viride* S17A-colonised rye grain was used and allowed to colonise the green waste compost for 25 days prior to field application. In addition, as no effect of the treatments on sclerotia viability was observed in either the glasshouse bioassay (Milestone 2.1) or in Trial 1, no bags of sclerotia were buried in the field plots. Onion sets (cv. Hercules) were planted in April 2008. The treatment plots were sampled and crop emergence, AWR and yield assessed as described for Trial 1.

Trial 4: 2008

Onion sets (cv. Hercules) were planted in April 2008 in the trial areas at Wellesbourne and Kirton planted in Trial 1 in 2007 to determine whether there was any carry over of treatment effects from one year to the next. The trial was recorded as described for Trial 1.

(b) Grower trials

Trial 1 - Bedfordshire Growers Limited (2007)

Green waste compost and *T. viride* S17A-colonised green waste compost were applied to land at Bedfordshire Growers Limited in March 2007 as described for Warwick HRI Trial 1. Polyester mesh bags of sclerotia, as previously described, were buried in March 2007 in each of the treated and untreated (control) plots where no waste was applied. There were two replicate plots of each treatment. The sclerotia were recovered from the experimental site in August 2007 after six months burial to determine the effect of the treatments on sclerotia viability. At the time of recovery, the plots were sampled for the presence of *Trichoderma* spp.

Trial 2 – Bedfordshire Growers Limited (2008)

Green waste compost (Organic Recycling Limited) was applied to naturally infested AWR land at Bedfordshire Growers Limited in April 2008 as described for Warwick HRI Trial 1. Onion sets (cv. Hercules - untreated, *T. viride* S17A-treated and Folicur-treated) were planted in the treated and untreated plots in April 2008. In each of the six plots, emergence of sets and presence of AWR were recorded by Bedfordshire Growers Limited.

Trial 3 – Moulton Bulb Company Limited (2008)

Green waste compost and *T. viride* S17A-colonised green waste compost were applied to land at Moulton Bulb Company Limited in March 2008 as described for Warwick HRI Trial 1. Onion sets (cv. Hercules – untreated) were planted in the treated and untreated plots in April 2008. There were three replicate plots of each treatment. In addition, three plots were planted with Folicur-treated sets to provide a chemical control comparison. The trial was recorded as for Warwick HRI Trial 1.

RESULTS

Effect of pH, conductivity and Folicur on the growth of *Trichoderma viride* S17A *in vitro* (Milestone S1.3)

The effect of pH and conductivity on the growth of *T. viride* S17A on PDA is shown in Figure 1. Growth was fastest at the lowest pH (4.78) and conductivity (6.1 mS cm^{-1}) tested and gradually decreased with increasing pH and conductivity, with no growth recorded on PDA with a pH of ≥ 6.68 (Figure 1a). Growth was however still recorded on PDA with a conductivity >30 mS cm^{-1} although the rate was reduced (Figure 1b).

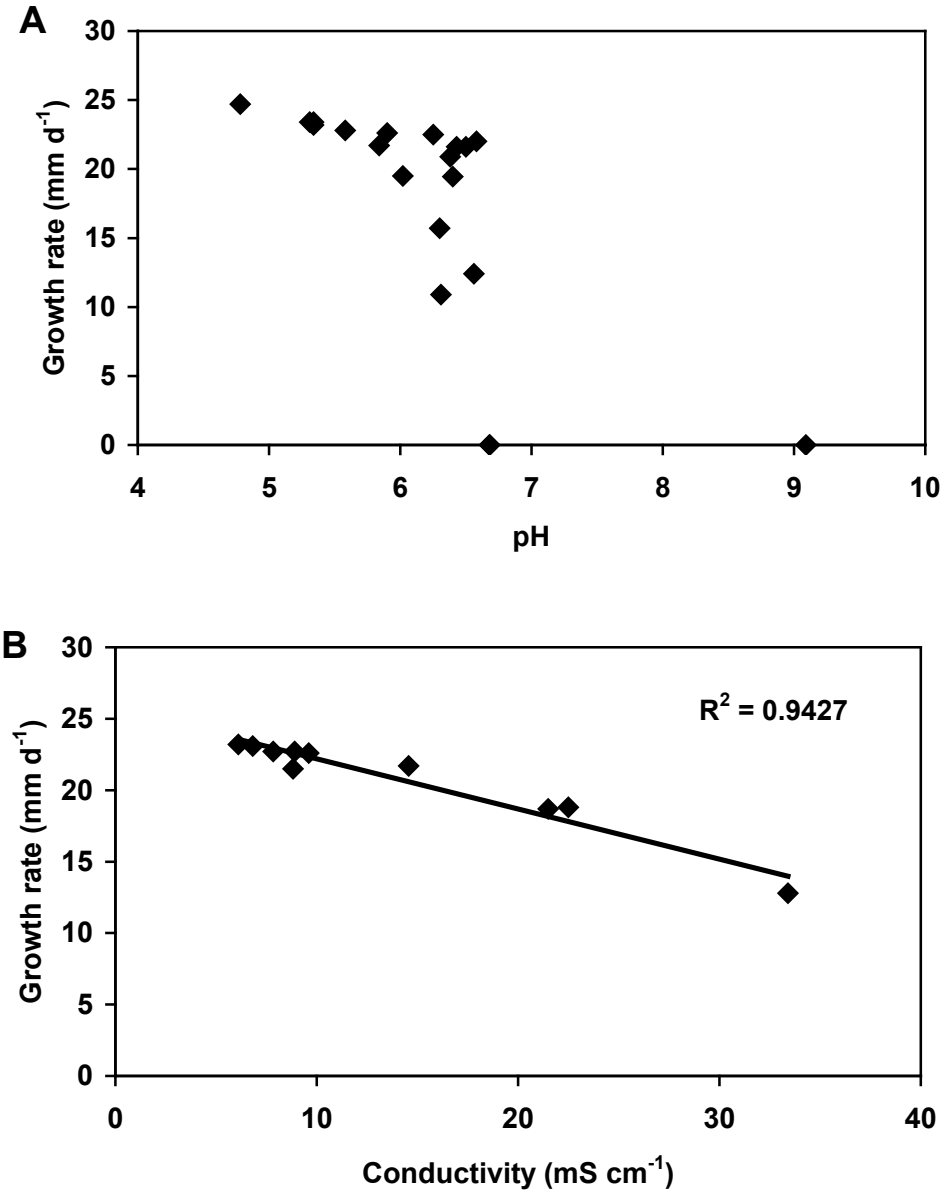

Figure 1: Effect of (a) pH and (b) conductivity on the growth of *T. viride* S17A on PDA

The growth rate of *T. viride* S17A on unamended PDA was 22 mm d^{-1}. The presence of Folicur in the PDA inhibited the growth of *T. viride* S17A at all concentrations tested with the exception of 0.01% Folicur which reduced growth rate to 5 mm d^{-1}. From these results it would appear that the growth of *T. viride* S17A is incompatible with Folicur treatment.

Composted wastes (Milestone 1.1)

The pH, conductivity, moisture and ash content of the various green waste composts collected are detailed in Table 1. Based on the effect of pH on the growth of *T. viride* S17A *in vitro* (Figure 1a), the pH of all the green waste composts sampled appeared to be too high to support its growth. The conductivity of the green waste composts was well below the conductivity shown to have a negative effect on growth *in vitro* (Figure 1b).

The addition of horticultural grade peat (pH 3.38, conductivity 0.25 mS cm^{-1}) or onion waste (pH 3.84, conductivity 0.78 mS cm^{-1}) reduced the pH of the green waste composts (Table 2).

Table 1: pH, conductivity, moisture and ash content of various green waste composts. DM = Dry matter. n.d. = not determined

Green Waste Compost	pH	Conductivity (mS cm^{-1})	Moisture (%)	Ash (% DM)
Top soil conditioner – 1 year old	7.78	2.23	32	78
Top soil conditioner – 7 months old	8.21	0.91	37	n.d.
Blended green waste – 1 year old	8.71	0.88	24	n.d.
Moody green waste	7.90	1.29	31	60
Tunnel Tech green waste	7.57	2.96	42	71
Engine Farm – young	7.26	1.65	76	38
Engine Farm – middle	7.74	1.18	81	27
Engine Farm – old	8.41	1.54	68	49

Table 2: pH and conductivity measurements of various green waste composts amended with horticultural grade peat or onion waste (% w/w)

Green waste compost mixture	pH	Conductivity (mS cm^{-1})
Top soil conditioner – 1 year old + 20% peat	5.76	1.64
Top soil conditioner – 1 year old + 25% peat	5.79	1.89
Top soil conditioner – 1 year old + 20% onion	6.59	2.07
Top soil conditioner – 1 year old + 25% onion	6.59	2.08
Moody green waste + 20% peat	6.74	1.00
Moody green waste + 25% peat	6.38	1.08
Moody green waste + 20% onion	7.47	1.12
Moody green waste + 25% onion	6.70	1.29
Tunnel Tech green waste + 20% peat	6.06	2.30
Tunnel Tech green waste + 25% peat	5.64	2.23
Tunnel Tech green waste + 20% onion	6.61	2.53
Tunnel Tech green waste + 25% onion	6.38	2.68
Engine Farm – young + 20% peat	6.43	1.32
Engine Farm – young + 25% peat	6.22	1.38
Engine Farm – young + 20% onion	6.94	1.71
Engine Farm – young + 25% onion	6.85	1.64

Flask-scale preparation of composted sulphur-containing wastes

The pH of the sulphur-containing wastes ranged from 4.37 (flask OWC) to 8.79 (poultry manure + prunings) (Table 3). The conductivity of the wastes was relatively low with the exception of the poultry manure (5.22 mS cm^{-1}). With the exception of the green waste from Engine Farm (Table 1), the moisture content of the sulphur-containing wastes was much higher than the green wastes (Table 3). The ash contents of the sulphur-containing composts were very similar with the exception of the windrow OWC. The ash content of the windrow OWC was very high in comparison to the flask OWC and this may be due to the presence of some soil observed in the windrow OWC whereas the flask OWC only consisted of onion waste.

Table 3: pH, conductivity, moisture and ash content of the sulphur-containing composts. DM = dry matter. n.d. = not determined

Compost mixture	pH	Conductivity (mS cm^{-1})	Moisture content (%)		Ash (% DM)
			Before composting	After composting	
Flask OWC	4.37	0.60	85	86	11
Windrow OWC	7.91	0.80	n.d.	60	61
Poultry manure + prunings	8.79	5.22	62	66	15
Brassica + prunings	8.43	1.82	79	72	12
Sewage sludge + prunings	5.67	0.45	89 (no prunings)	68	12

The nitrogen content of the different soil types, sulphur-containing and green waste composts is shown in Table 4. The poultry manure compost contained the most nitrogen, with the windrow OWC being the compost with the lowest nitrogen content.

Table 4: Nitrogen content of soil, sulphur-containing and green waste composts

Compost/soil	Ammonium-N (mg kg^{-1})	Total Nitrogen (Kjeldahl g kg^{-1})
Kirton soil	48	2.2
Wellesbourne soil	32	1.0
Flask OWC	325	23.8
Windrow OWC	169	6.6
Brassica + prunings	4470	25.2
Poultry manure + prunings	16300	59.4
Sewage sludge + prunings	781	21.3
Tunnel Tech green waste	129	10.3
Moody green waste	86.0	17.7
Top soil conditioner – 1 year old	96.0	13.2
Engine Farm – young	4490	24.8

Colonisation of green waste compost by *Trichoderma viride* S17A (Milestone 1.2)

The pH, conductivity and moisture content of the composts used to set up the *T. viride* S17A colonisation experiment are shown in Table 5. The moisture content of the compost treatments varied from 41 to 69%, with the treatments with the addition of peat having a higher moisture content than the compost on its own. The addition of 25% peat to the composts reduced their pH to within the range where growth was recorded *in vitro* (Figure 1a). The conductivity of all the treatments was much lower than that which had been found to have an effect on growth *in vitro* (Figure 1b).

The background count of *Trichoderma* spp. was similar in all treatments (*c.* 10^3 cfu g^{-1}) prior to the addition of the *T. viride* S17A inocula (Table 6). Addition of the inocula to the treatments increased the count of *T. viride* S17A (Figures 2 and 3). The rye grain inoculum had a much higher count (1.25 x 10^9 cfu g^{-1}) than the wheat bran inoculum (1.61 x 10^6 cfu g^{-1}). As a consequence, the level of *T. viride* S17A recovered from the treatments at time zero, i.e. just after inoculation, was much lower in the treatments inoculated with the wheat bran inoculum (*c.* 10^5 cfu g^{-1}) compared with those inoculated with the rye grain inoculum (*c.* 10^7 cfu g^{-1}) (Figures 2 and 3). The level of *T. viride* S17A peaked in most of the treatments by 10 days post-inoculation. Despite the difference between the two inocula at time zero, the maximum level of *T. viride* S17A was higher in the treatments inoculated with the wheat bran inoculum compared with the rye grain inoculum. The top soil conditioner maintained the highest level of colonisation of the green waste composts inoculated with either the wheat bran or rye grain inocula. The level of *T. viride* S17A in the top soil conditioner inoculated with the rye grain inoculum was significantly higher than in the soil alone. There was no significant difference between the level of *T. viride* S17A in the other rye grain inoculated green waste composts and the soil alone. The green waste composts inoculated with the wheat bran inoculum were no different in their *T. viride* S17A level compared with the soil alone, with the exception of the Moody green waste compost where the level was significantly lower. The inoculated soil/green waste compost mixtures were relatively homogenous in that there was little variation between the two samples taken from each jar. The results from the effect of pH on the growth of *T. viride* S17A *in vitro* suggested that the soil and green waste compost treatments on their own would not support *T. viride* S17A growth. Peat was added to the various treatments to render the soil and green waste composts more conducive to growth but *T. viride* S17A colonised and maintained high levels in all treatments, with the addition of peat not being obviously advantageous.

Table 5: pH, conductivity and moisture content of soil/compost mixtures used to set up *T. viride* S17A compost colonisation experiment. n.d. = not determined

Treatment	pH	Conductivity (mS cm^{-1})	Moisture (%)
Wellesbourne soil	6.81	0.13	15
Wellesbourne soil + 25% peat	n.d.	n.d.	41
Moody green waste	7.65	2.62	60
Moody + 25% peat	6.38	1.08	69
Top soil conditioner – 1 year old	7.62	3.50	40
Top soil conditioner + 25% peat	5.79	1.89	58
Tunnel Tech green waste	7.40	3.63	50
Tunnel Tech + 25% peat	5.64	2.23	61

Table 6: *Trichoderma* spp. present in the various treatments prior to inoculation

Treatment	Count (cfu g^{-1})
Wellesbourne soil	1.2×10^3 ($\pm$ 1.67×10^2)
Wellesbourne soil + 25% peat	3.3×10^3 ($\pm$ 8.43×10^2)
Top soil conditioner – 1 year old	$<3.0 \times 10^2$ ($\pm$ 0)
Top soil conditioner + 25% peat	5.3×10^3 ($\pm$ 1.28×10^3)
Moody green waste	$<3.0 \times 10^2$ ($\pm$ 0)
Moody + 25% peat	1.0×10^3 ($\pm$ 0)
Tunnel Tech green waste	$<3.0 \times 10^2$ ($\pm$ 0)
Tunnel Tech + 25% peat	4.2×10^3 ($\pm$ 9.46×10^2)

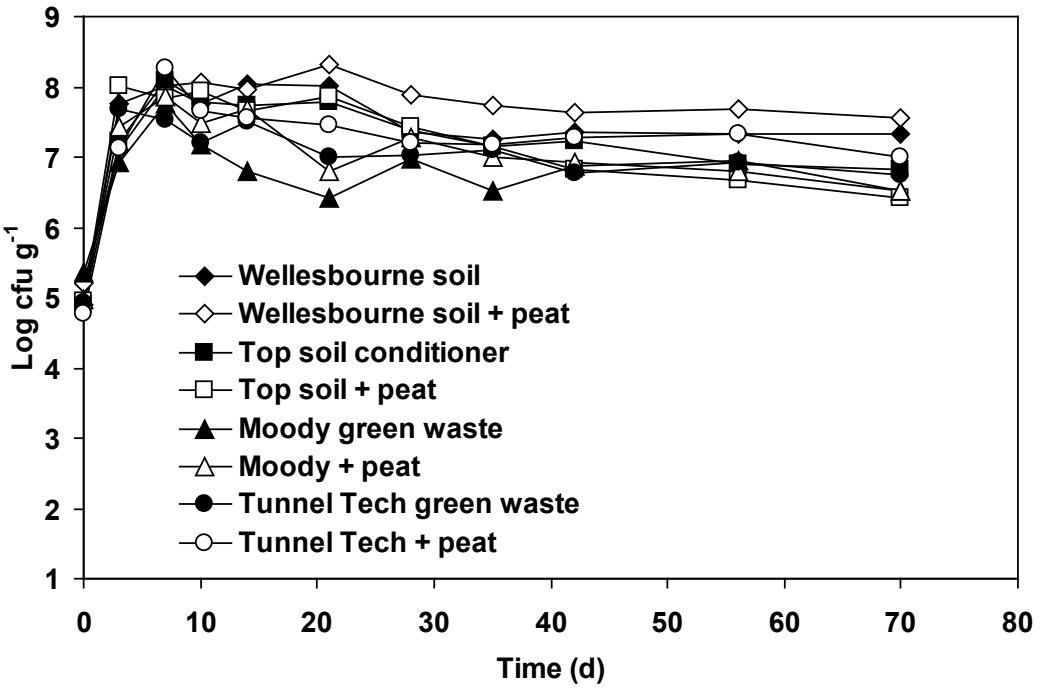

Figure 2: Colonisation and survival of *T. viride* S17A from a wheat bran culture in various green waste compost/soil treatments over time. Values are the mean of six replicates from two samples (**A** and **B**)

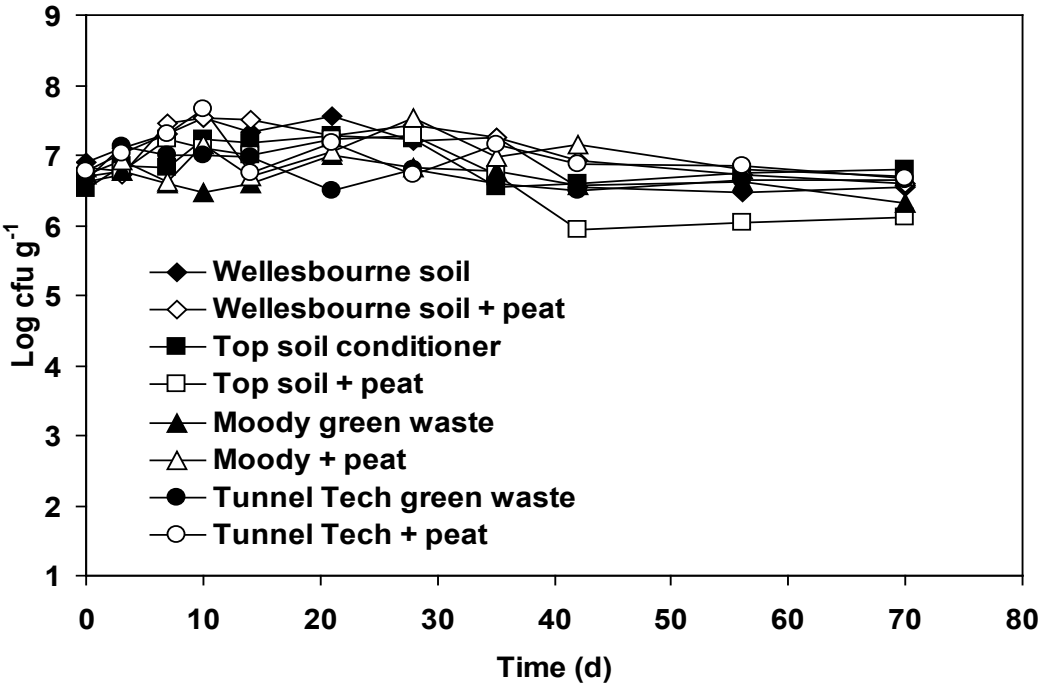

Figure 3: Colonisation and survival of *T. viride* S17A from a rye grain culture in various green waste compost/soil treatments over time. Values are the mean of six replicates from two samples (**A** and **B**)

Application of *Trichoderma viride* S17A to onion sets (Milestone 1.2)

Figure 4 shows the level of *T. viride* S17A recovered from the treated sets over time. Generally, the level of *T. viride* S17A recovered from the sets remained relatively constant within the treatments over the sampling time. The addition of 1% foam concentrate, 1% guar gum or 1% PVA to an aqueous suspension of *T. viride* S17A did not enhance retention/survival of the organism on the surface of the sets, evident from the trend of a lower recovery of *T. viride* S17A in their presence (Figure 4). The storage temperature (4 or 20 °C) of the treated sets between sampling intervals did not influence recovery. No *Trichoderma* spp. were recovered from the sets treated with 1% foam concentrate, 1% guar gum or 1% PVA alone.

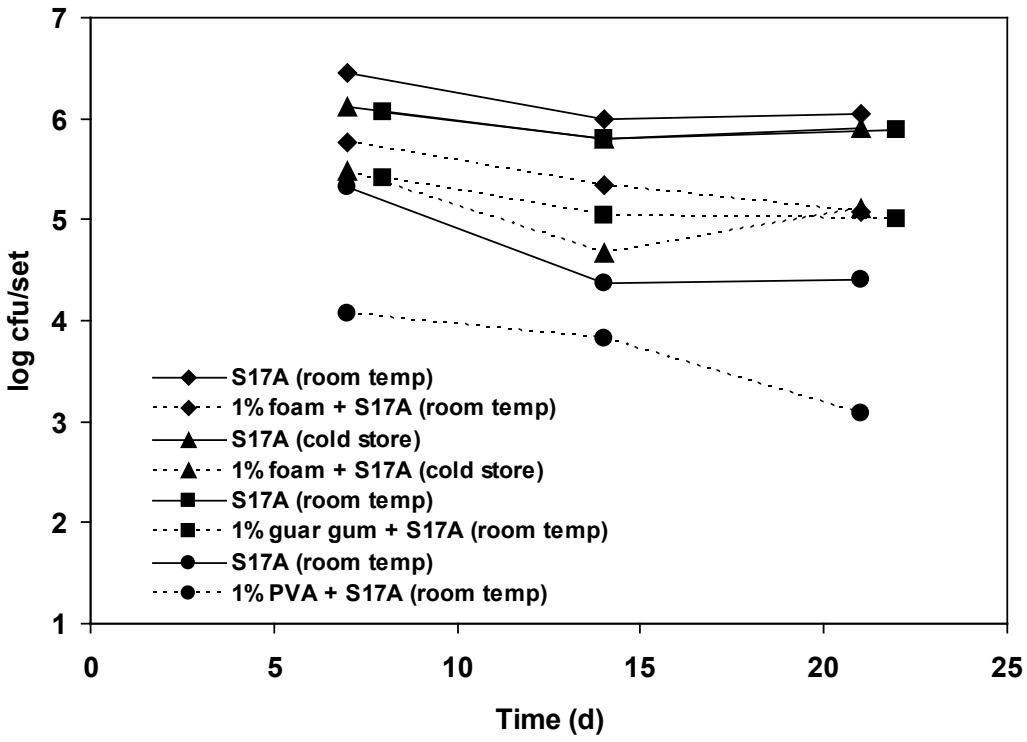

Figure 4: *T. viride* S17A recovered from onion sets over time. Values are the mean of three replicate sets. The symbols represent four different experiments. S17A = *T. viride* S17A

Effect of *Trichoderma viride* S17A-colonised green waste compost on sclerotia viability (Milestone 2.1)

The background count of *Trichoderma* spp. in the soil and green waste compost was $<10^3$ cfu g^{-1} prior to the addition of the rye grain inoculum. Addition of the rye grain inoculum (3.5×10^7 cfu g^{-1}) to the soil and green waste compost increased the count

of *T. viride* S17A to *c.* 2.0 x 10^4 cfu g^{-1}. Fourteen days post-inoculation the level of *T. viride* S17A had increased to *c.* 2.0 x 10^7 cfu g^{-1} in both the soil and green waste compost. On mixing the colonised soil and green waste compost with uncolonised soil for the glasshouse tests the level of *T. viride* S17A in all the inoculated treatments was *c.* 3.6 x 10^6 cfu g^{-1}.

Figure 5 shows the viability of the sclerotia recovered from the treatments after the various burial periods. The treatments had no significant effect on the viability of the sclerotia with the exception of the 10% green waste + *T. viride* S17A treatment which slightly reduced the viability of sclerotia compared with the control (Kirton soil) after three months burial (Figure 5). The presence of the green waste compost in the treatments had no effect on the moisture content, with the moisture content of all the treatments at the end of the bioassay being very similar (17-21%).

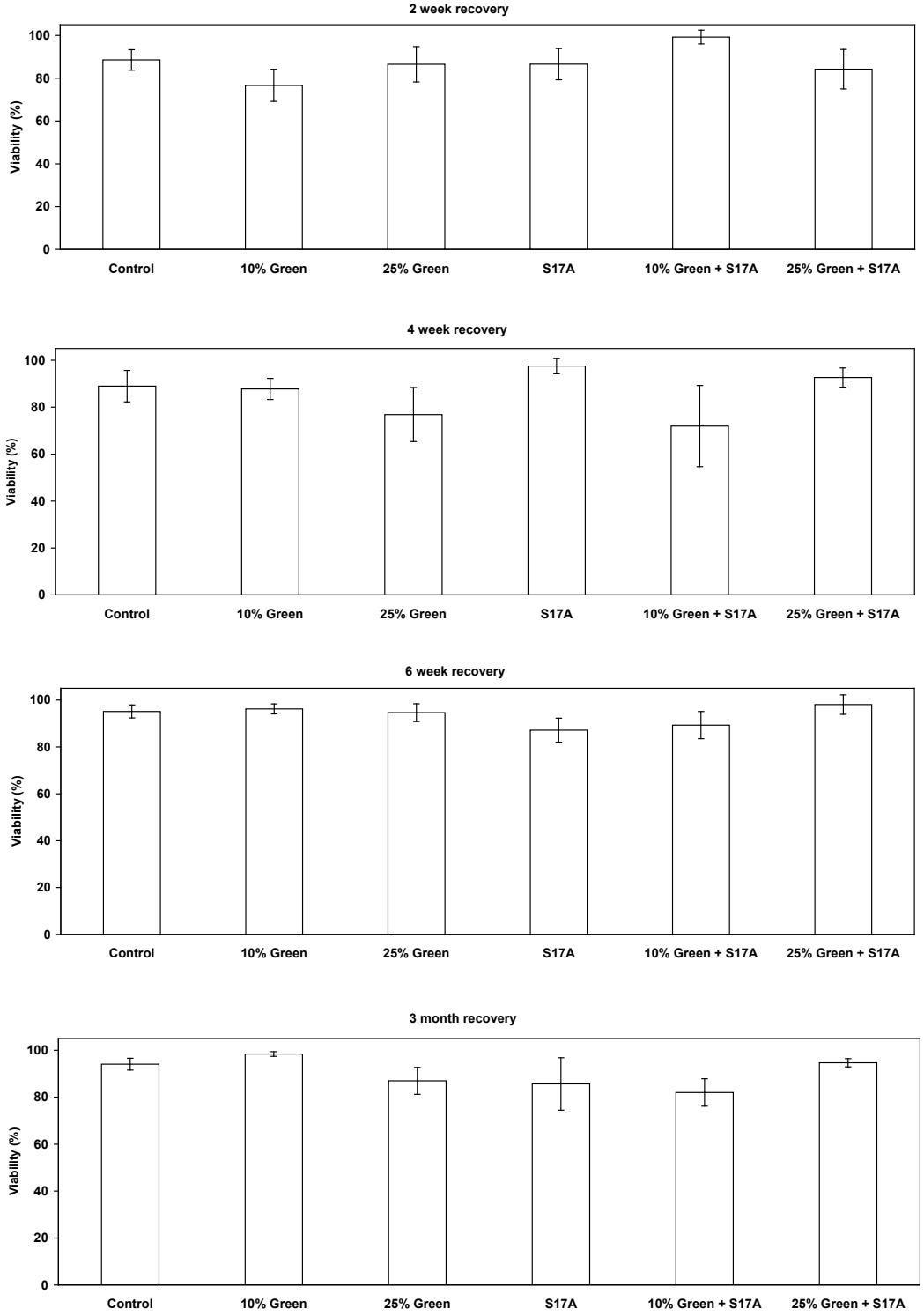

Figure 5: Viability of sclerotia recovered from pots after various periods buried in the treatments. Values are the mean of five replicate mesh bags, each containing 100 sclerotia, ± 1 standard error. Control = Kirton soil. S17A = soil colonised with *T. viride* S17A

Effect of *T. viride* S17A-colonised green waste compost and onion sets on the control of *Allium* white rot (Milestones 2.1, 2.2 and 4.1)

Bioassay 1

(i) Recovery of Trichoderma *spp. from treatments*

The background count of *Trichoderma* spp. in the soil and green waste compost was $<10^3$ cfu g^{-1} prior to the addition of the *T. viride* S17A inoculum. Addition of the *T. viride* S17A inoculum (3.5×10^7 cfu g^{-1}) to the soil and green waste compost increased the count of *Trichoderma* spp. to *c.* 2.0×10^4 cfu g^{-1}. Fourteen days post-inoculation, the level of *Trichoderma* spp. had increased to *c.* 4.5×10^7 cfu g^{-1} in both the soil and green waste compost. On mixing the colonised soil and green waste compost with uncolonised soil to complete the treatments for the glasshouse tests, the level of *Trichoderma* spp. in all the inoculated treatments was *c.* 3.6×10^6 cfu g^{-1}. At set planting, 14 days post-mixing of the colonised materials with uncolonised soil, the level of *Trichoderma* spp. in all the *T. viride* S17A inoculated soil/compost treatments was *c.* 3.5×10^6 cfu g^{-1}. The level of *T. viride* S17A on the sets treated with this organism was 1.2×10^6 cfu per set at planting.

Table 7 shows the level of *Trichoderma* spp. recovered from the various treatments at the end of the bioassay (week 23). A similar level (*c.* 10^3 cfu g^{-1}) of *Trichoderma* spp. to the initial background count was recovered from all the treatments with no *T. viride* S17A added. The level recovered from the *T. viride* S17A set treatment was also similar to the soil and green waste compost background count. In contrast, the level recovered from the treatments where *T. viride* S17A had been added to soil alone or green waste compost and allowed to colonise prior to set planting was much higher at *c.* 10^5-10^6 cfu g^{-1}. This level of recovery indicates good survival of *T. viride* S17A throughout the course of the bioassay and is comparable to the level of *Trichoderma* spp. in these treatments at set planting (*c.* 3.5×10^6 cfu g^{-1}).

Table 7: *Trichoderma* spp. recovered from the various treatments at the end of the glasshouse bioassay (week 23). Values are the mean of three replicate samples ± 1 standard error

Treatment	***Trichoderma* spp. (cfu g^{-1})**
Control	1.0×10^3 (± 0)
Folicur	1.0×10^3 (± 0)
10% green waste	1.0×10^3 (± 0)
25% green waste	1.4×10^3 (± 4.44×10^2)
T. viride S17A in soil	3.2×10^5 (± 7.22×10^4)
T. viride S17A sets	1.0×10^3 (± 0)
10% green waste + *T. viride* S17A	2.6×10^5 ± (5.43×10^4)
25% green waste + *T. viride* S17A	3.4×10^6 (± 9.20×10^5)
T. viride S17A sets + 10% green waste	1.1×10^4 (± 5.60×10^3)
T. viride S17A sets + 25% green waste	1.3×10^3 (± 2.36×10^2)

(ii) Allium *white rot assessment*

Figure 6 shows the progression of AWR in the different treatments over time. Disease was first detected after five weeks, in the 10% green waste treatment, and observed in all treatments, including the Folicur-treated sets, after 12 weeks, with the *T. viride* S17A set treatment being the last to show disease symptoms. At 15 weeks the level of AWR in all the *T. viride* S17A set treatments was less than in the control (soil alone) and Folicur treatment. By the end of the bioassay there was no difference between the level of disease in the control and any of the green waste compost-*T. viride* S17A or Folicur treatments. No disease was detected in the uninoculated (no sclerotia added) pots.

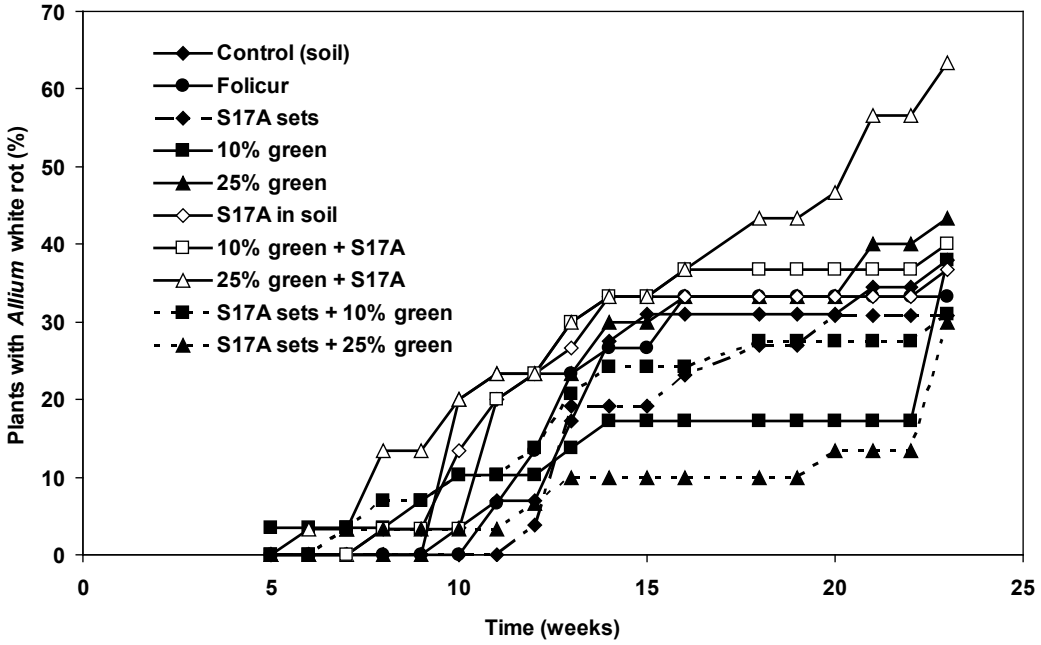

Figure 6: Plants with *Allium* white rot in the various green waste compost-*T. viride* S17A treatments. Values are the mean of 30 replicate pots

Figure 7 shows the weight of the plants in the different treatments at the end of the bioassay (week 23). In the uninoculated treatments (no sclerotia added), the plants grown in the green waste compost treatments were significantly heavier than those grown in soil with no green waste added (control, *T. viride* S17A and *T. viride* S17A sets treatments) (Figure 7a). In the inoculated treatments (sclerotia added), the plants grown in the 25% green waste treatments with and without the addition of *T. viride* S17A and the 10% green waste + *T. viride* S17A treatment were also significantly heavier than those grown in soil with no green waste added (control and *T. viride* S17A treatments (Figure 7b). No obvious symptoms of AWR (white mycelium, black sclerotia) were detected in the plants that remained at harvest. However, the presence of the pathogen did have an effect on plant growth in that the final plants in the inoculated treatments had a significantly lower plant weight than those in the uninoculated treatments (Figure 7a and 7b).

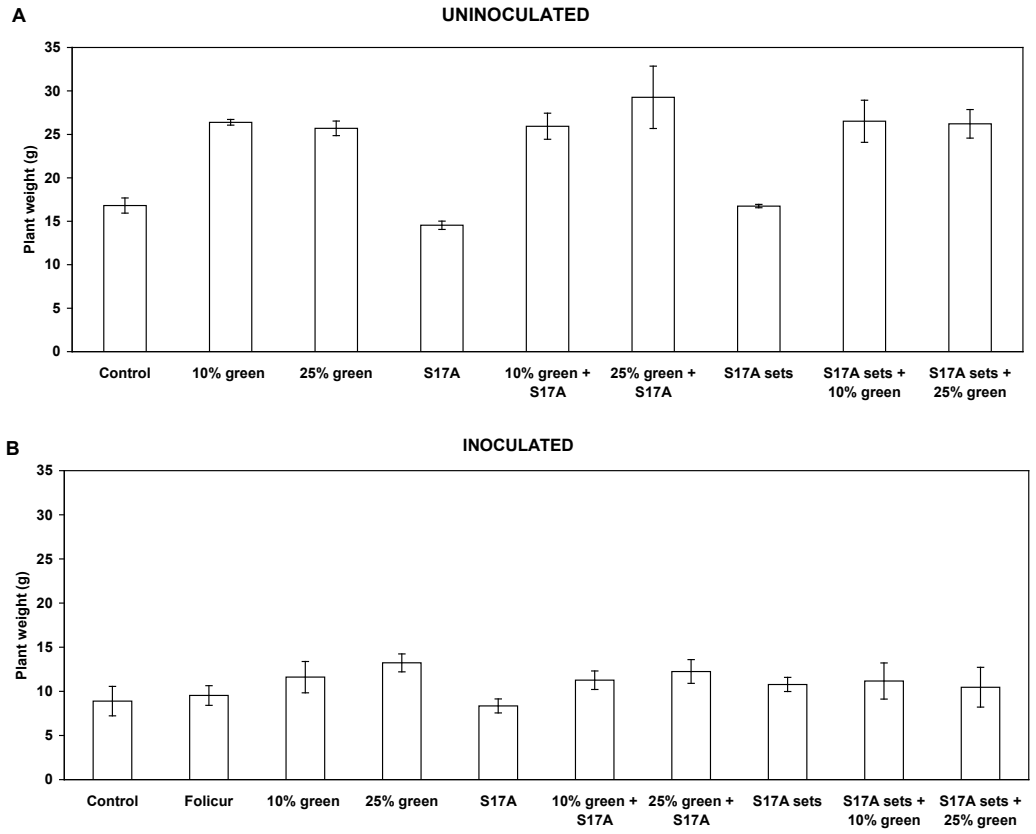

Figure 7: Weight of plants grown in the (a) uninoculated and (b) inoculated (*S. cepivorum*) treatments at harvest. Values are the mean of 7-30 replicate pots ± 1 standard error

Bioassay 2

(i) Recovery of Trichoderma *spp. from treatments*

Similar to Bioassay 1, the background count of *Trichoderma* spp. in the soil and green waste compost was *c.* 10^3 cfu g^{-1} prior to the addition of the *T. viride* S17A inoculum. Addition of the *T. viride* S17A inoculum (7.6 x 10^8 cfu g^{-1}) to the green waste compost increased the count of *Trichoderma* spp. to 1.5 x 10^6 cfu g^{-1}. Twenty five days post-inoculation, the level of *Trichoderma* spp. in the green waste compost had increased to 3.7 x 10^7 cfu g^{-1}. On mixing the colonised green waste compost with uncolonised soil to complete the treatments for the glasshouse tests, the level of *Trichoderma* spp. in all the inoculated treatments was *c.* 8.6 x 10^5. At set planting, 14 days post-mixing of the colonised green waste compost with uncolonised soil, the level of *Trichoderma* spp. in all the *T. viride* S17A inoculated green waste compost treatments was *c.* 9.0 x 10^6 cfu g^{-1}. The level of *T. viride* S17A on the sets treated with this organism was 6.8 x 10^4 per set at planting.

Table 8 shows the level of *Trichoderma* spp. recovered from the various treatments at the end of the bioassay (week 20). A similar level (*c.* 10^3 cfu g^{-1}) of *Trichoderma* spp. to the initial background count was recovered from all the treatments with no *T. viride* S17A added. The level recovered from the *T. viride* S17A set treatments was also similar to the soil (control) and green waste compost background count. In contrast, the level recovered from the treatments where *T. viride* S17A had been added to green waste compost and allowed to colonise prior to set planting was much higher at *c.* 2.5 x 10^6 cfu g^{-1}. This level of recovery indicates good survival of *T. viride* S17A throughout the course of the bioassay and is comparable to the level of *Trichoderma* spp. in these treatments at set planting (*c.* 9.0 x 10^6 cfu g^{-1}).

Table 8: *Trichoderma* spp. recovered from the various treatments at the end of the glasshouse bioassay (week 20). Values are the mean of three replicate samples ± 1 standard error

Treatment	***Trichoderma* spp. (cfu g^{-1})**
Control	1.0 x 10^3 (± 0)
Folicur	1.2 x 10^3 (± 1.72 x 10^2)
25% green waste	1.0 x 10^3 (± 0)
40% green waste	1.1 x 10^3 (± 7.78 x 10^1)
25% green waste + *T. viride* S17A	1.7 x 10^6 (± 1.63 x 10^5)
40% green waste + *T. viride* S17A	3.3 x 10^6 (± 3.15 x 10^5)
40% green waste + *T. viride* S17A + Folicur	3.4 x 10^6 (± 2.04 x 10^5)
T. viride S17A sets	1.4 x 10^3 (± 3.36 x 10^2)
T. viride S17A sets + 25% green waste	1.9 x 10^3 (± 8.52 x 10^2)
T. viride S17A sets + 40% green waste	1.0 x 10^3 (± 0)

(ii) Allium *white rot assessment*

Figure 8 shows the progression of AWR in the different treatments over time. Disease was first detected after eight weeks in most of the treatments, including the Folicur-treated sets, and observed in all treatments after nine weeks. At the end of the bioassay, the level of AWR in all the green waste only and *T. viride* S17A-colonised green waste treatments was less than in the control (soil alone) and Folicur alone treatment. The 40% green waste + *T. viride* S17A treatment was the most

effective in controlling AWR. The *T. viride* S17A set treatments and Folicur-treated sets alone treatment showed no disease control. No disease was detected in the uninoculated (no sclerotia added) pots.

Figure 9 shows the weight of the plants in the different treatments at the end of the bioassay (week 20). In both the uninoculated (no sclerotia added) (Figure 9a) and inoculated (Figure 9b) treatments, the plants grown in the green waste compost treatments were significantly heavier than those grown in soil with no green waste compost added (control, Folicur and *T. viride* S17A sets treatments) (Figure 9a). In the uninoculated treatments, composts with *T. viride* S17A produced a slightly higher plant weight than composts without *T. viride* S17A. No obvious symptoms of AWR (white mycelium, black sclerotia) were detected in the remaining inoculated plants at harvest. The presence of the pathogen had a negative effect on plant growth, even in plants that showed no visible symptoms of AWR at harvest. The plants grown in the inoculated treatments (with sclerotia) had a significantly lower plant weight than those in the uninoculated treatments (Figure 9a and 9b). In the inoculated treatments, plant weight in composts with *T. viride* S17A was significantly higher than plant weight in composts without *T. viride* S17A or in the control treatment.

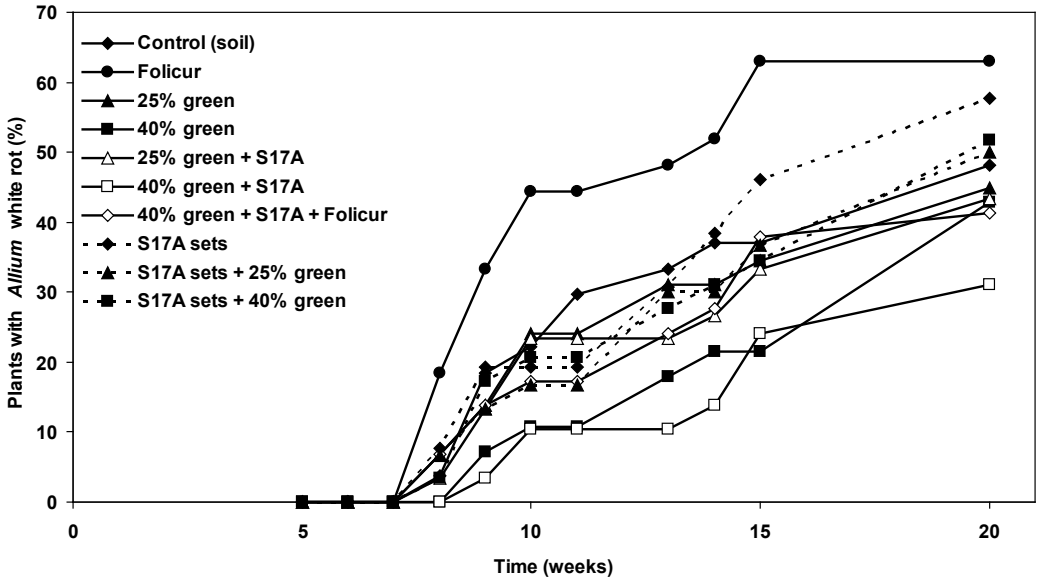

Figure 8: Plants with *Allium* white rot in the various green waste compost-*T. viride* S17A treatments. Values are the mean of 30 replicate pots

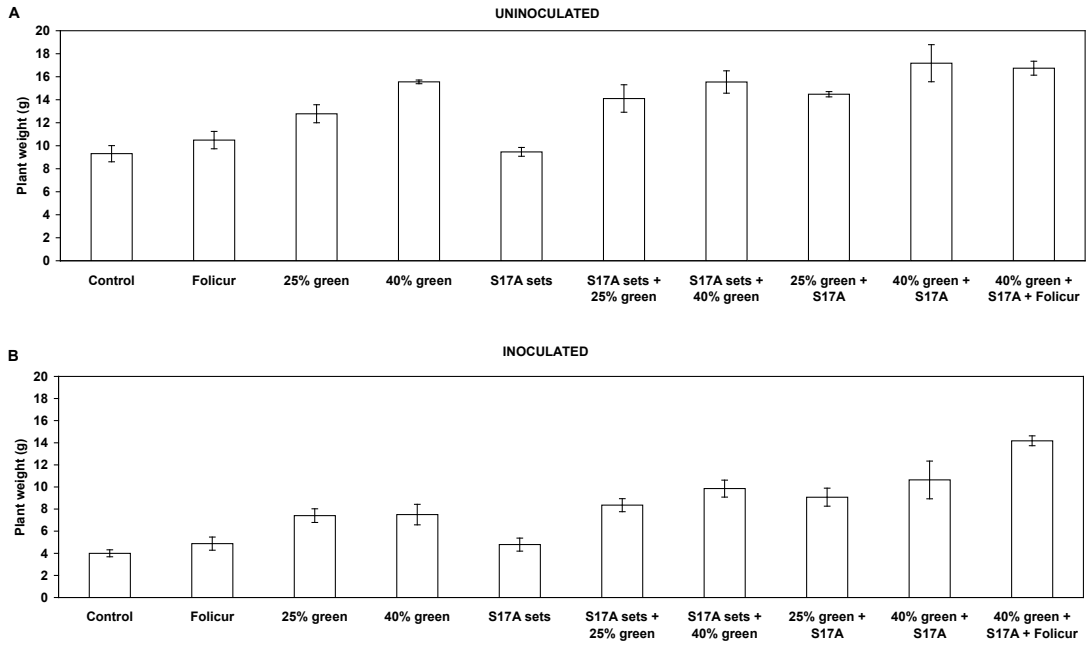

Figure 9: Weight of plants grown in the (a) uninoculated and (b) inoculated (*S. cepivorum*) treatments which did not show symptoms of AWR at harvest. Values are the mean of 14-30 replicate pots ± 1 standard error

Effect of sulphur-containing composted wastes on the viability of sclerotia of *Sclerotium cepivorum* and the control of *Allium* white rot (Milestones 3.1 and 3.2)

Bioassay 1

(i) Analysis of sulphur-containing composted wastes and Kilner jar environments

The total sulphur and nitrogen contents of the soil and composted wastes are shown in Table 9. The windrow OWC had the lowest sulphur content of the composted wastes. The poultry manure compost had the highest sulphur content which was more than twice as high as the waste with the next highest content. The poultry manure compost also contained the most nitrogen, with the windrow OWC being the compost with the lowest nitrogen content.

Table 9: Total sulphur and nitrogen contents of soil and composted wastes

Compost/soil	Total Sulphur (mg kg^{-1})	Ammonium-N (mg kg^{-1})	Total Nitrogen (Kjeldahl g kg^{-1})
Kirton soil	358	48	2.2
Flask OWC	3030	325	23.8
Windrow OWC	1280	169	6.6
Brassica + prunings	2860	4470	25.2
High N poultry manure + prunings	6850	16300	59.4
Sewage sludge + prunings	2260	781	21.3

A number of compounds (sulphur-containing and others) were detected in the Kilner jars 3 days after set up using GCMS (see Appendix 1). The concentrations detected varied both between and within composts. Only one compound ([1,1':3',1"-Terphenyl]-2'-ol) was specific to one compost (Brassica) and it was only detected in one of the Kilner jars. The known sclerotia germination stimulant, dipropyl disulphide, was detected in both the flask and windrow OWC with the level in the windrow OWC (8 ppm) much higher than in the flask OWC (0.5 ppm).

There are few sulphur-containing volatiles that are produced by composts in high enough concentrations to be detected and measured using Dräger tubes. Dräger tubes detected dimethyl sulphide (DMS) in all the composted waste treatments three days after set up, with the highest level detected in the sewage sludge treatment (Figure 10). Dimethyl sulphide has previously been shown to exhibit slight sclerotia germination stimulatory activity (Coley-Smith & King, 1969). The level of this volatile in the flask OWC, windrow OWC and sewage sludge treatments decreased over time to below the level of detection at 25 days. The level of DMS in the Brassica treatment increased up to eight days and thereafter declined to below the detection level at 25 days. In contrast, the level of DMS in the poultry manure compost increased over time up to 67 days (Figure 11) and was still detected after three months. In addition to DMS, tert-butyl mercaptan (TBM) was consistently detected in the poultry manure compost (Figure 11).

The levels of CO_2 and O_2 detected in the Kilner jars using Dräger tubes are shown in Figure 12. The measurements confirmed the treatments were not anaerobic with at least 11% O_2 detected in all treatments (Figure 12a). The levels of CO_2 cycled throughout the monitoring period, reflecting when the microcosms were aired when the crucibles were removed weekly for assessment (Figure 12b). The

CO_2 levels were higher in the compost amended treatments than in the unamended soil.

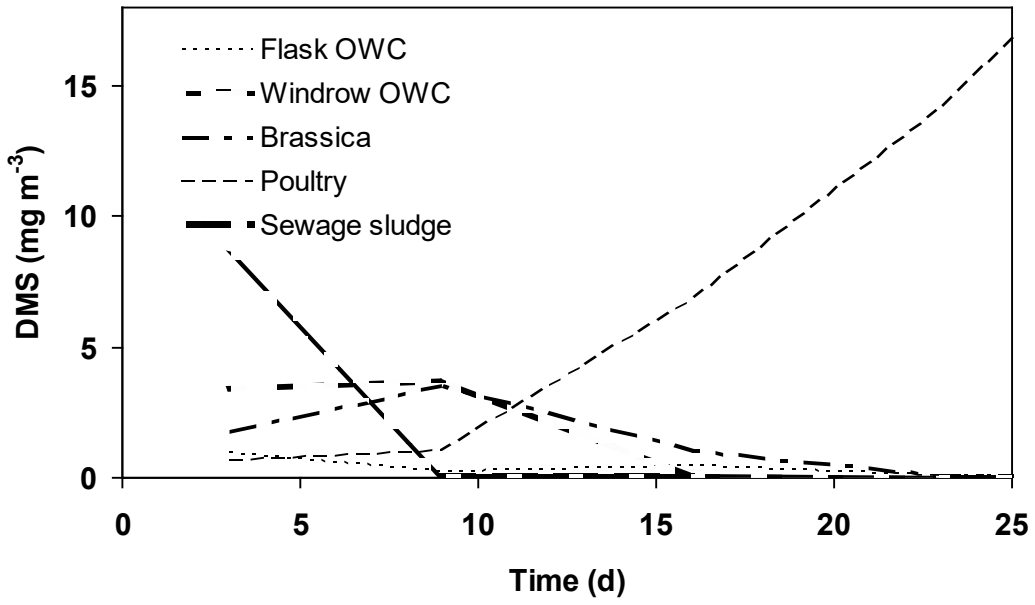

Figure 10: Dimethyl sulphide (DMS) detected in Kilner jar microcosms using Dräger tubes

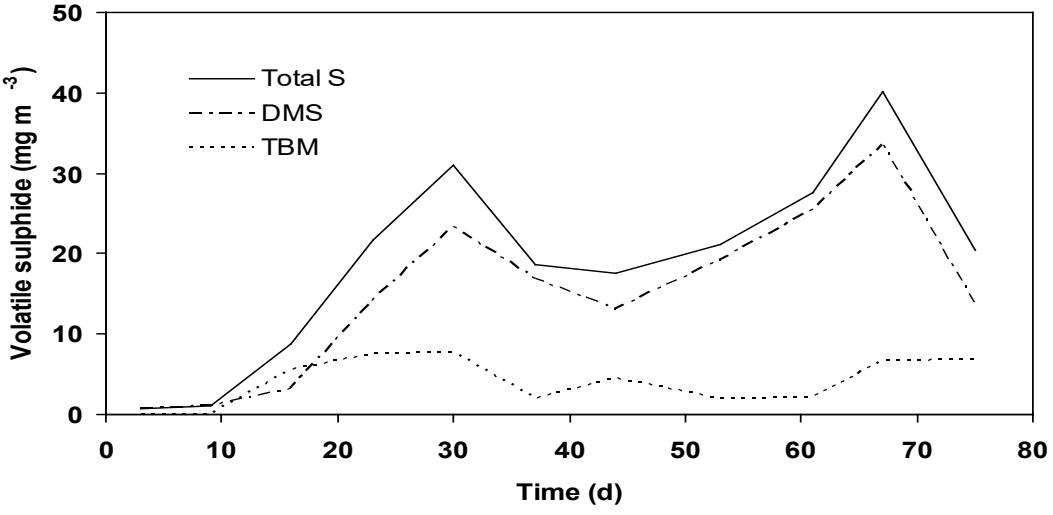

Figure 11: Dimethyl sulphide (DMS) and tert-butyl mercaptan (TBM) detected in the poultry manure treatment in Kilner jar microcosms using Dräger tubes

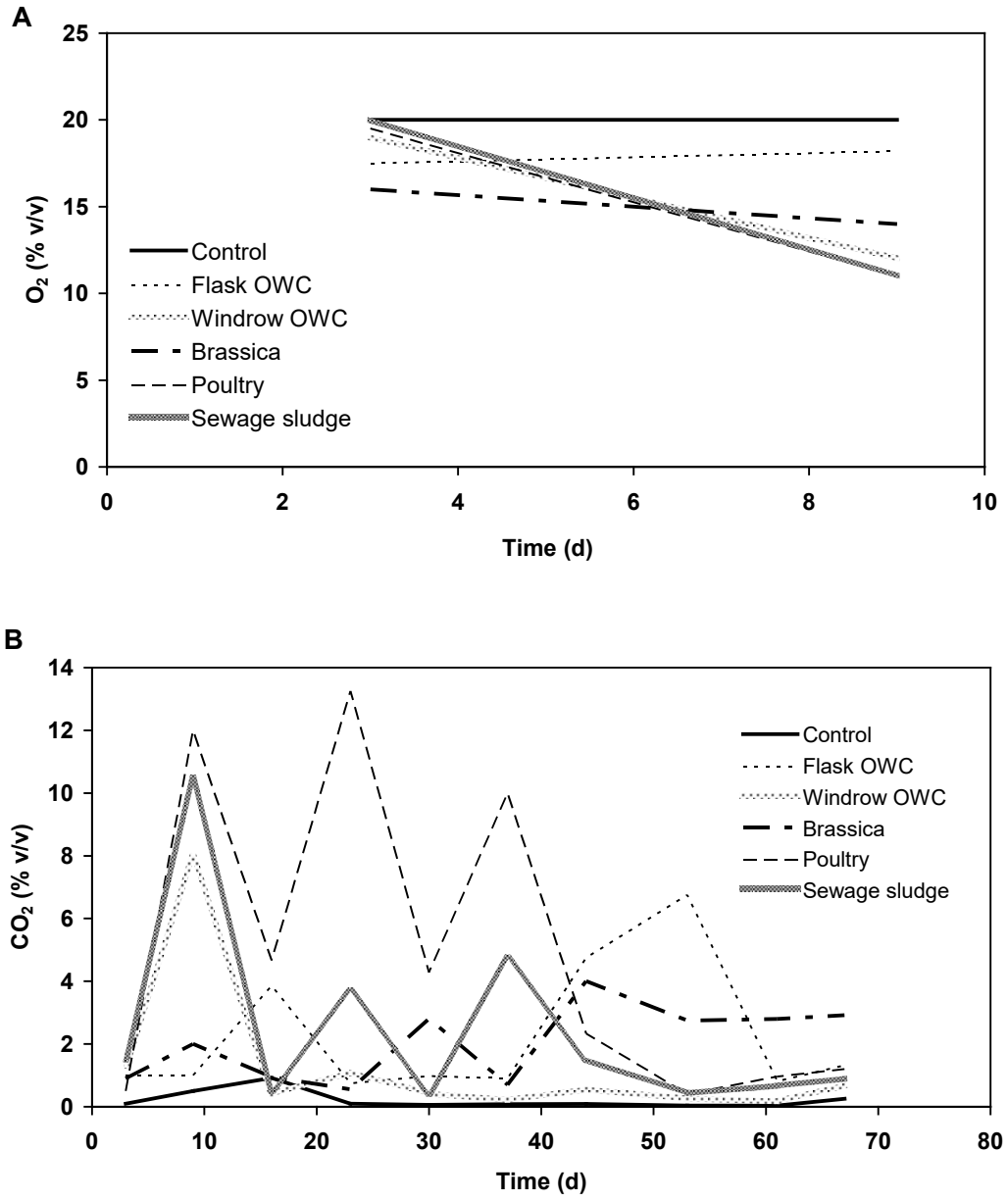

Figure 12: Levels of (a) O_2 and (b) CO_2 detected in Kilner jar microcosms using Dräger tubes. Control = Kirton soil

(ii) Effect of compost volatiles on sclerotia in crucibles

The effect of volatiles released from the composted wastes on the sclerotia in the crucibles is shown in Figure 13. The presence of the OWC stimulated germination of sclerotia over time, with the flask OWC being more stimulatory than the windrow OWC. This is interesting as a much higher level of the germination stimulant dipropyl disulphide was detected in the windrow OWC than in the flask OWC. This result may be due to a dose-response effect in that the level in the windrow OWC is initially stimulatory but as it reaches a higher level it becomes inhibitory. Other compounds were however detected by GCMS that may play a role in germination stimulation. In addition, the measurements were made over time and therefore the length of exposure of the sclerotia to the various volatiles may be a factor. No significant stimulation of germination was observed with the other treatments.

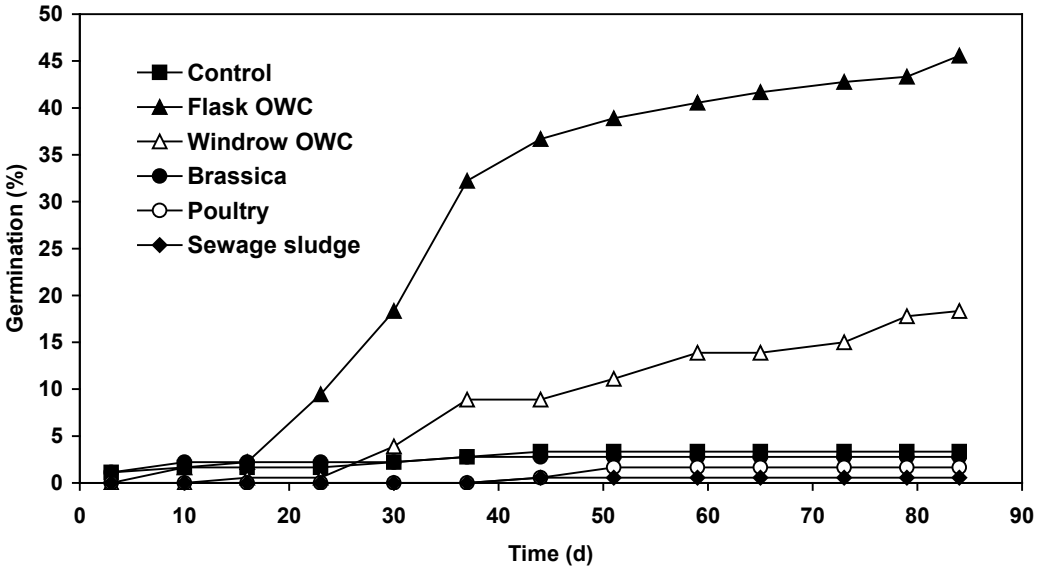

Figure 13: Germination of sclerotia on cellulose nitrate membrane in crucibles in Kilner jar microcosms. Values are the mean of 12 replicate crucibles, each containing 15 sclerotia. Control = Kirton soil

The viability of sclerotia recovered from the crucibles after three months in the Kilner jar microcosms is shown in Figure 14. Viability of sclerotia in the compost treatments was very similar to the control (>90%) with the exception of the poultry manure treatment where viability was reduced to <50%. This result suggests that the volatiles released from the poultry manure treatment were toxic to the sclerotia.

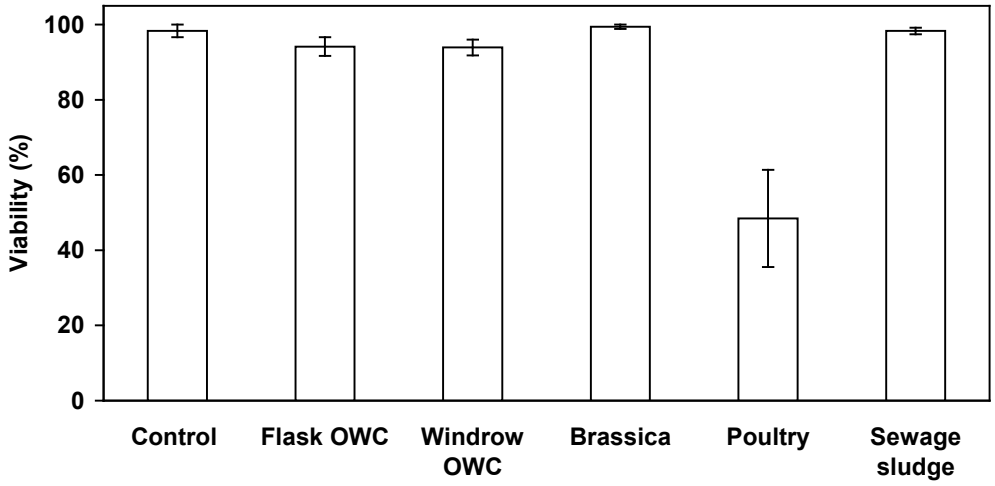

Figure 14: Viability of sclerotia recovered from the cellulose nitrate membrane in the crucibles after three months in the Kilner jar microcosms. Values are the mean of 12 replicates ± 1 standard error. Control = Kirton soil

(iii) Recovery of sclerotia from soil-compost mixtures

The moisture contents of the soil and soil-compost mixtures in which bags of sclerotia were buried were very similar, ranging from 20-28%. The viability of the sclerotia recovered from the bags after three months burial in the soil-compost mixtures in the Kilner jar microcosms and polythene bags is shown in Figure 15. In the Kilner jar microcosms, both the flask and windrow OWC reduced the viability of sclerotia recovered, with the poultry manure treatment being the most effective in reducing sclerotia viability (Figure 15a). The Brassica and sewage sludge treatments had no effect on sclerotia viability in this environment (Figure 15a). In the polythene bags (Figure 15b), a greater reduction in sclerotia viability was observed compared with the Kilner jar microcosms. In contrast to the results from the Kilner jar microcosms, all the treatments reduced sclerotia viability in the polythene bags, although the OWCs and poultry manure treatments were consistently the most effective treatments in the two environments.

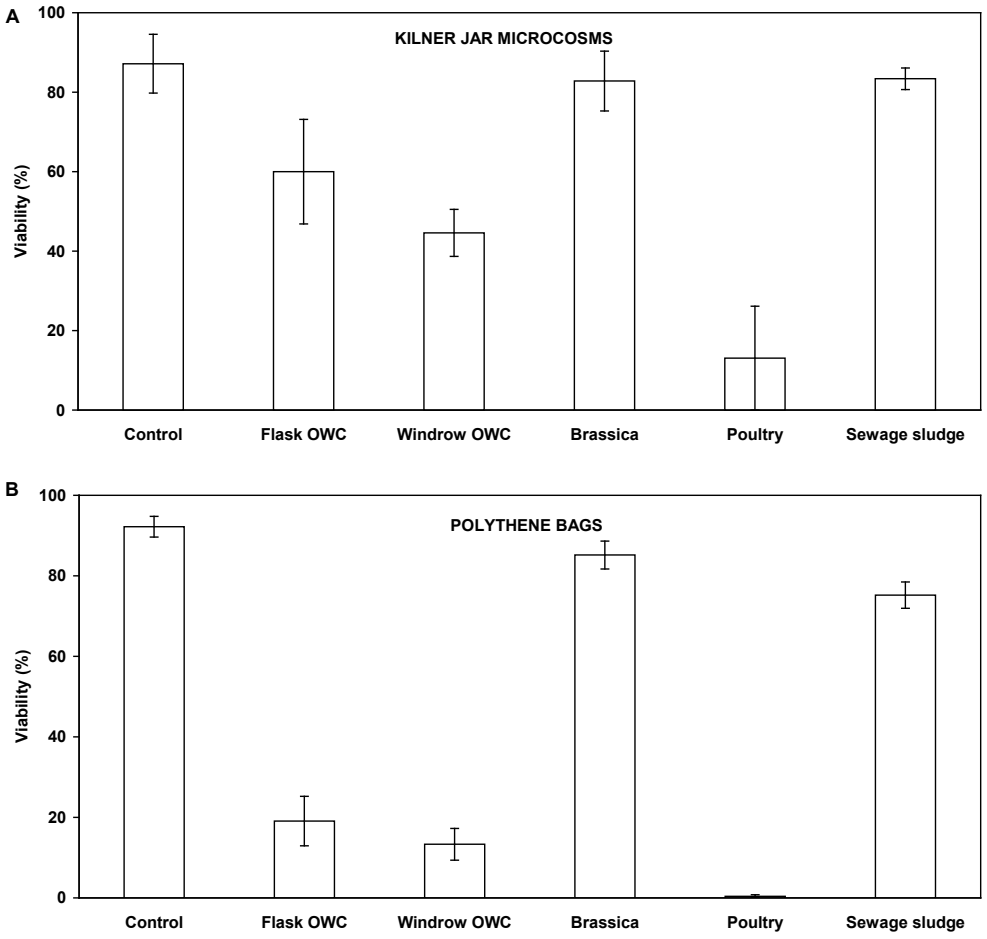

Figure 15: Viability of sclerotia recovered from the (a) Kilner jar microcosms and (b) polythene bags after three months burial in the treatments. Values are the mean of six replicate mesh bags, each containing 100 sclerotia ± 1 standard error. Control = Kirton soil

The viability of the sclerotia recovered from the mesh bags after 10 months burial in the soil-compost mixtures in the Kilner jar microcosms and polythene bags is shown in Figure 16. The viability of the sclerotia recovered from the two environments was comparable within treatments. Similar to the results after three months burial, both the flask and windrow OWC and poultry manure compost reduced the viability of sclerotia recovered (Figure 16). A greater reduction in viability was observed with the OWC treatments in the Kilner jar microcosms after 10 months burial (Figure 16a) compared with the three month burial (Figure 15a). These treatments were the most effective in reducing viability after 10 months burial in both environments (Figure 16a and 16b). Similar to the results after three months burial, the Brassica and sewage sludge treatments had very little effect on sclerotia viability (Figure 16).

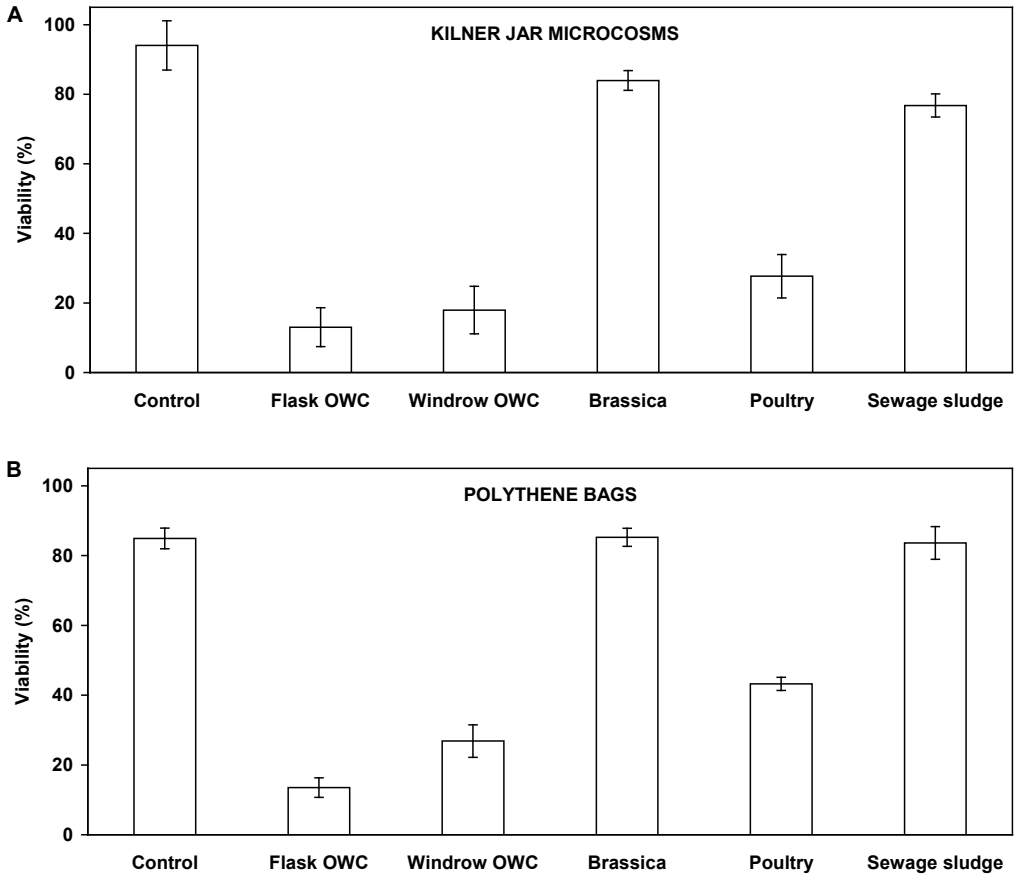

Figure 16: Viability of sclerotia recovered from the (a) Kilner jar microcosms and (b) polythene bags after 10 months burial in the treatments. Values are the mean of six replicate mesh bags, each containing 100 sclerotia ± 1 standard error. Control = Kirton soil

(iv) Allium *white rot assessment*

Figure 17 shows the progression of AWR in the different treatments over time. Disease was first detected in the Brassica treatment at nine weeks. The level of disease in this treatment increased rapidly over time, peaking at 77% in week 15, with a further increase to 85% recorded at harvest (23 weeks). The level of disease in the other treatments, including the control, was comparatively low suggesting that the Brassica treatment stimulated the pathogen. The poultry manure compost effectively reduced AWR compared with the control. There was no effect of the two onion waste composts on the control of AWR during the course of the bioassay. No disease was detected in the uninoculated (no sclerotia added) pots.

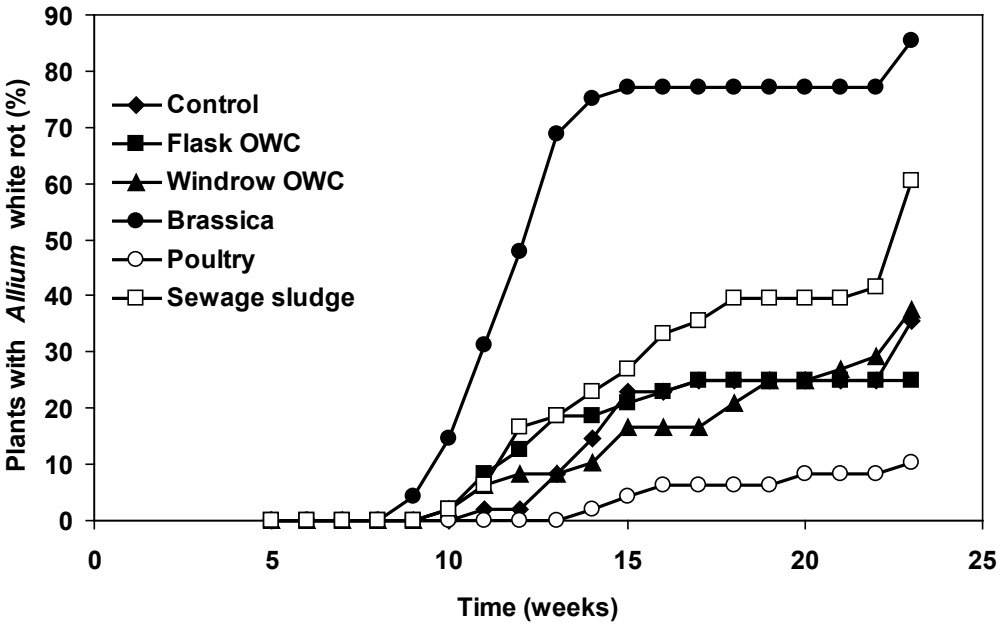

Figure 17: Plants with *Allium* white rot in the various sulphur-containing composted waste treatments. Values are the mean of 48 replicate pots

Figure 18 shows the weight of the plants in the different treatments at the end of the bioassay (week 23). The plants grown in the onion waste and poultry manure composts had a similar weight to those grown in soil alone (control). In contrast, the plants grown in the Brassica and sewage sludge composts had a lower plant weight than those grown in soil alone. Similar to the results from the green waste bioassay, there were no obvious symptoms of AWR (white mycelium, black sclerotia) detected in the plants in the inoculated treatments (Figure 18b) remaining at harvest. However, with the exception of the poultry manure compost treatment, the presence of the pathogen in the inoculated treatments did have an effect on growth. Similar to the results from the green waste compost bioassays (Figures 7 and 9), the plants in the inoculated treatments had a lower plant weight than those in the uninoculated treatments (Figure 18a and 18b).

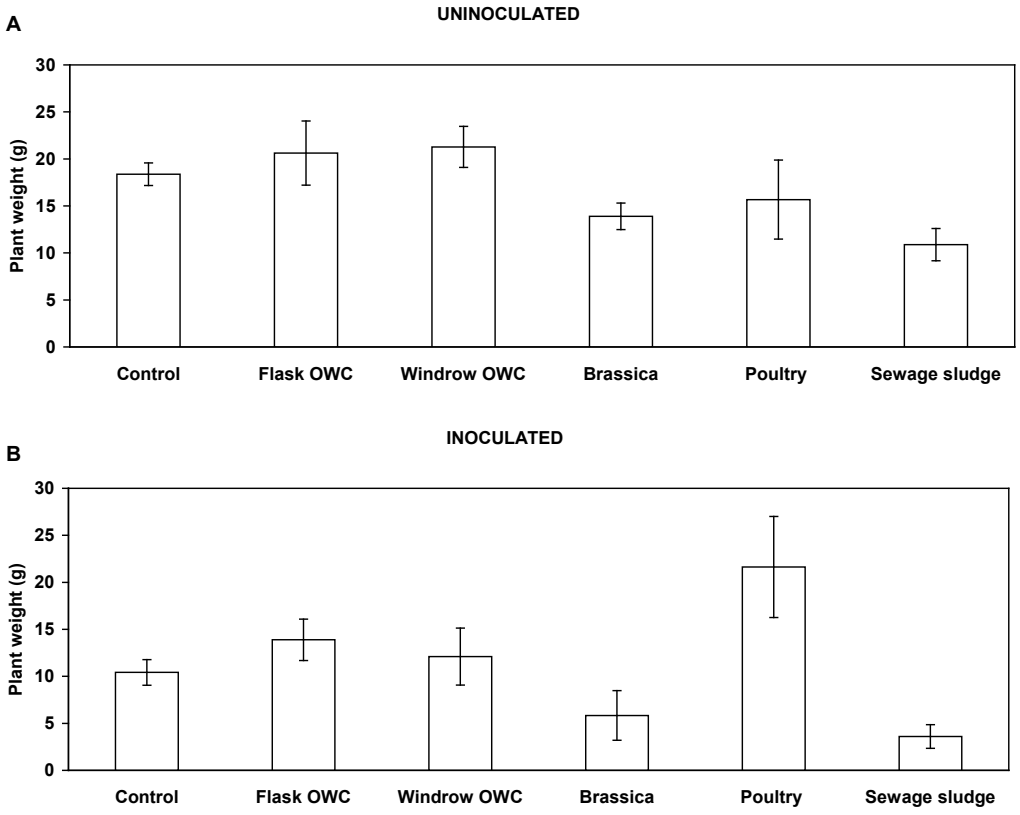

Figure 18: Weight of plants grown in the (a) uninoculated and (b) inoculated (*S. cepivorum*) treatments at harvest. Values are the mean of 18–24 (uninoculated) and 5–51 (inoculated) replicate pots ± 1 standard error

Bioassay 2

(i) Analysis of sulphur-containing composted wastes and Kilner jar environments

The total sulphur and nitrogen contents of the soil and composted wastes are shown in Table 10. The flask OWC had the lowest sulphur content of the composted wastes. The high N poultry manure compost had the highest sulphur and nitrogen content. The low N poultry manure compost contained almost 50% less total nitrogen then the high N poultry manure compost.

Table 10: Total sulphur and nitrogen contents of soil and composted wastes

Compost/soil	Total Sulphur (mg kg^{-1})	Ammonium-N (mg kg^{-1})	Total Nitrogen (Kjeldahl g kg^{-1})
Kirton soil	384	3	2
Flask OWC	3630	94	28
Windrow OWC	3940	60	25
Brassica + prunings	4940	5160	24
Low N poultry manure + prunings	4160	4810	34
High N poultry manure + prunings	6840	18500	64

Similar to Bioassay 1, a number of compounds (sulphur-containing and others) were detected in the Kilner jars three days after set up using GCMS (see Appendix 2). The concentrations varied both between and within composts. A number of sulphur-containing volatiles were detected in only the OWCs (allyl methyl sulphide, dipropyl disulfide, propane 1-(methylthio)-, 1-propene 1-(methylthio)-, 1-propene 3-(methylthio)-, and thiophene 2,5-dimethyl). The known sclerotia germination stimulant, dipropyl disulphide, was detected in the flask OWC (0.04 ppm). This level was 10 times lower than that detected in Bioassay 1. In contrast to Bioassay 1, no dipropyl disulphide was detected in the windrow OWC.

With the exception of the flask OWC, Dräger tubes detected DMS in all the composted waste treatments three days after set up, with the highest level detected in the Brassica treatment (Figure 19). The level of this volatile in the windrow OWC, Brassica and low N poultry manure treatments decreased over time to below the level of detection at 17 days. In contrast, the level of DMS in the high N poultry manure treatment increased over time up to 24 days (Figure 19) and was still detected after 58 days (Figure 20). The level of DMS detected in this treatment was lower than that detected in Bioassay 1. Similar to Bioassay 1, in addition to DMS, TBM was detected in the poultry manure treatments over time (Figure 20). The level of TBM detected in the high N poultry manure treatment was higher than that detected in Bioassay 1.

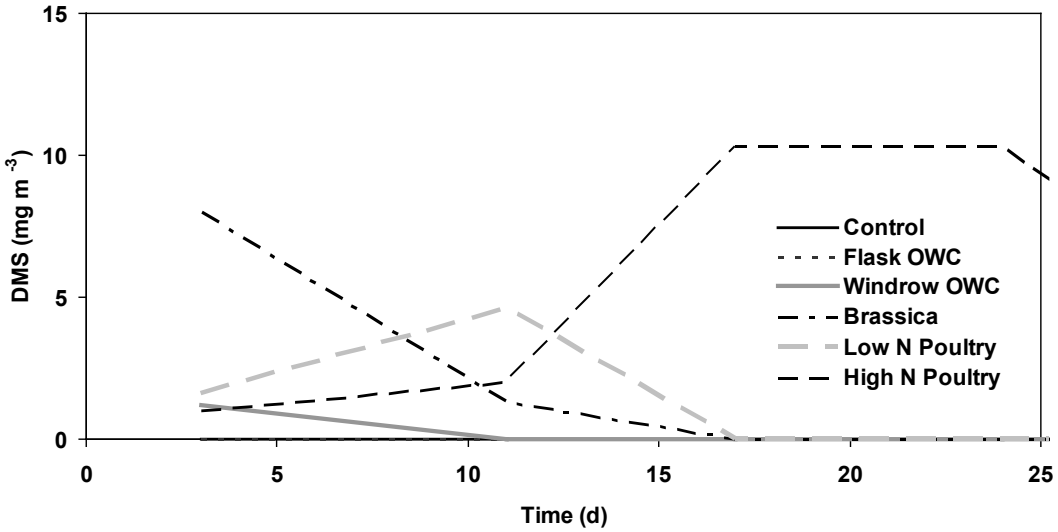

Figure 19: Dimethyl sulphide (DMS) detected in Kilner jar microcosms using Dräger tubes. Control = Kirton soil

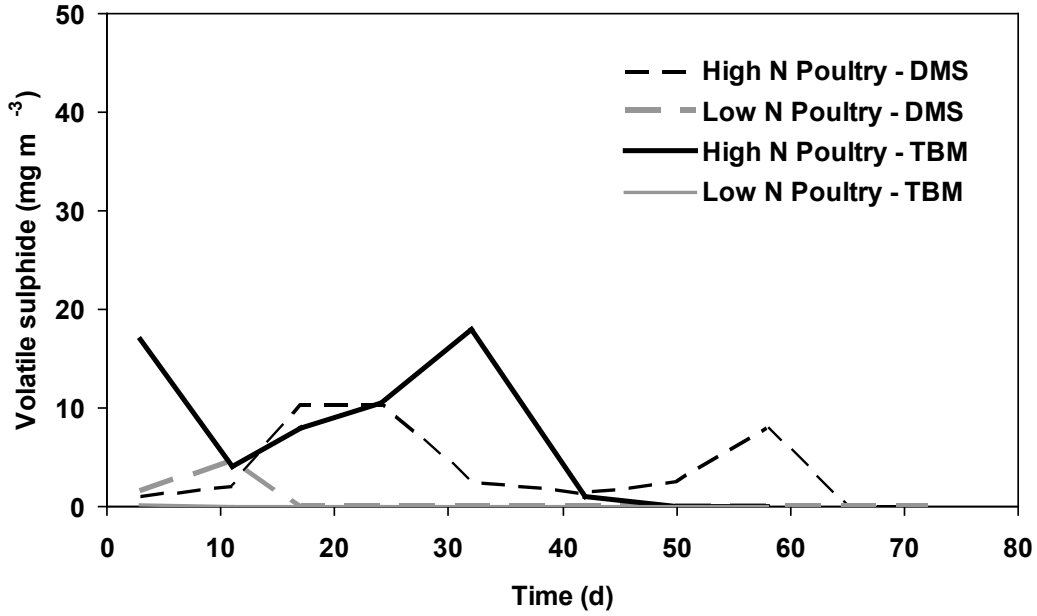

Figure 20: Dimethyl sulphide (DMS) and tert-butyl mercaptan (TBM) detected in the poultry manure treatments in Kilner jar microcosms using Dräger tubes

Ammonia (NH_3) was detected in the Brassica and poultry manure treatments (Figure 21) using Dräger tubes. The highest levels were detected in the high N poultry manure treatment. These levels were considerably higher than those detected in the low N poultry manure treatment.

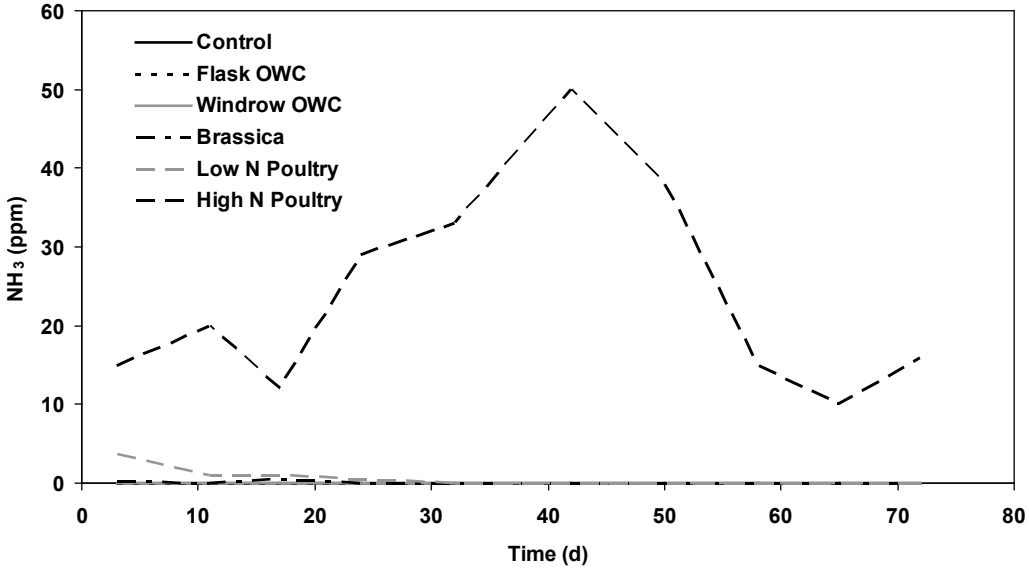

Figure 21: Ammonia (NH_3) detected in Kilner jar microcosms using Dräger tubes. Control = Kirton soil

The levels of CO_2 detected in the Kilner jars using Dräger tubes are shown in Figure 22. Similar to Bioassay 1, the CO_2 levels cycled throughout the monitoring period, reflecting when the microcosms were aired when the crucibles were removed weekly for assessment, and possibly a change in the microbial community. The CO_2 levels of the compost amended treatments were higher than in the non-amended soil.

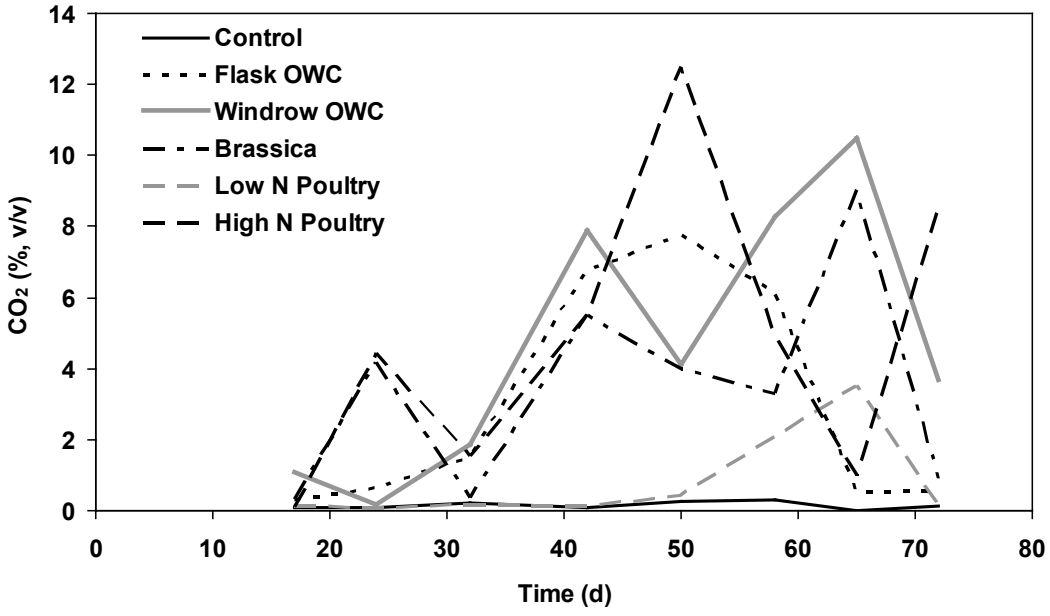

Figure 22: Carbon dioxide (CO_2) detected in Kilner jar microcosms using Dräger tubes. Control = Kirton soil

(ii) Effect of compost volatiles on sclerotia in crucibles

The effect of volatiles released from the composted wastes on the sclerotia in the crucibles is shown in Figure 23. Similar to Bioassay 1, the presence of the OWC stimulated germination of sclerotia over time, with the flask OWC being more stimulatory than the windrow OWC. A number of sulphur-containing volatiles were only detected in the OWCs and of these, most were unique to the flask OWC (see Appendix 2). This may explain the difference in result obtained with the two OWCs. In addition, of the sulphur-containing volatiles detected in both the OWCs, the concentrations were higher in the flask OWC compared with the windrow OWC. No significant stimulation of germination was observed with the other treatments.

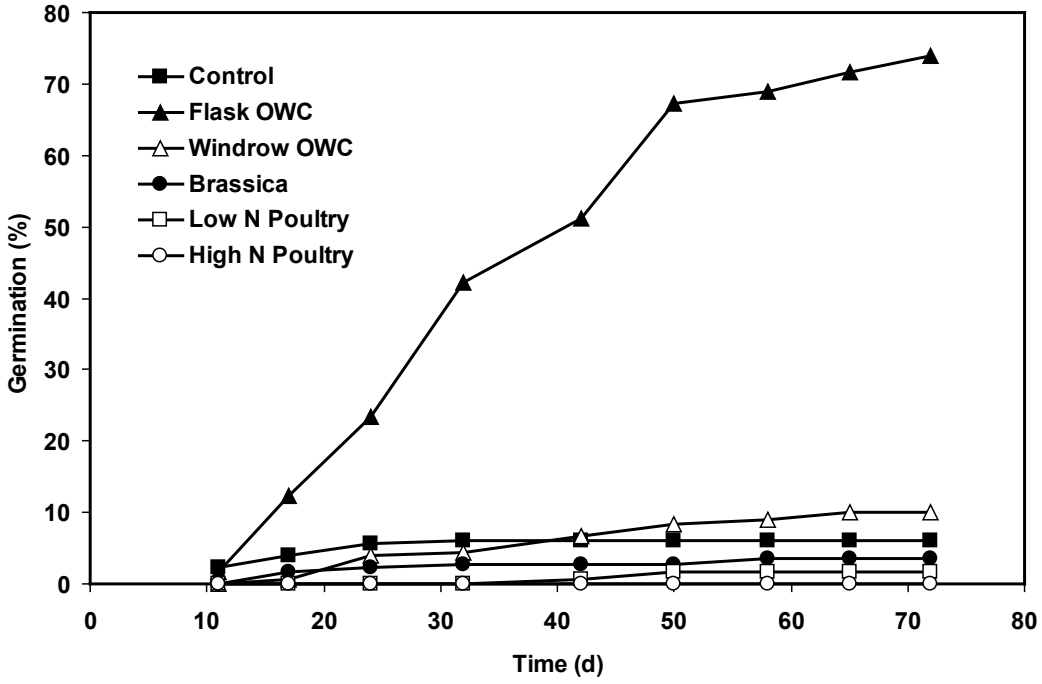

Figure 23: Germination of sclerotia on cellulose nitrate membrane in crucibles in Kilner jar microcosms. Values are the mean of 12 replicate crucibles, each containing 15 sclerotia. Control = Kirton soil

The viability of sclerotia recovered from the crucibles after three months in the Kilner jar microcosms is shown in Figure 24. Similar to Bioassay 1, the high N poultry manure treatment was the most effective in reducing sclerotia viability. In contrast, the low N poultry manure treatment had no effect on sclerotia viability. The flask OWC reduced sclerotia viability (Figure 24) although no effect on sclerotia viability at this stage was observed in Bioassay 1 with this treatment. The viability presented for the flask OWC in Bioassay 2 (Figure 24) may be lower than the actual viability as most of the sclerotia in this treatment were stimulated to germinate (Figure 23), and of those remaining that were assessed for viability a number were contaminated

during incubation. The windrow OWC and Brassica treatments had no effect on viability compared with the control.

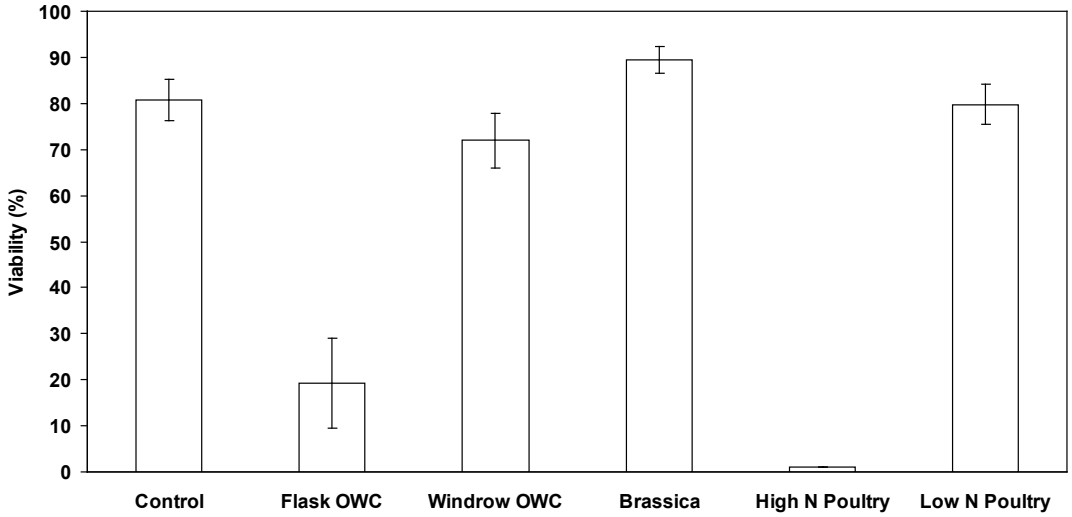

Figure 24: Viability of sclerotia recovered from the cellulose nitrate membrane in the crucibles after three months in the Kilner jar microcosms. Values are the mean of 12 replicates ± 1 standard error. Control = Kirton soil

(iii) Recovery of sclerotia from soil-compost mixtures

The viability of the sclerotia recovered from the mesh bags after 10 months burial in the soil-compost mixtures in the Kilner jar microcosms and polythene bags is shown in Figure 25. The viability of the sclerotia recovered from the two environments was comparable within treatments. Similar to the results from Bioassay 1, both the flask and windrow OWC and high N poultry manure compost reduced the viability of sclerotia recovered (Figure 25), with the high N poultry manure compost being the most effective in reducing sclerotia viability. In contrast, the low N poultry manure compost had no effect on sclerotia viability. Similar to the results from Bioassay 1, the Brassica treatment had little effect on sclerotia viability.

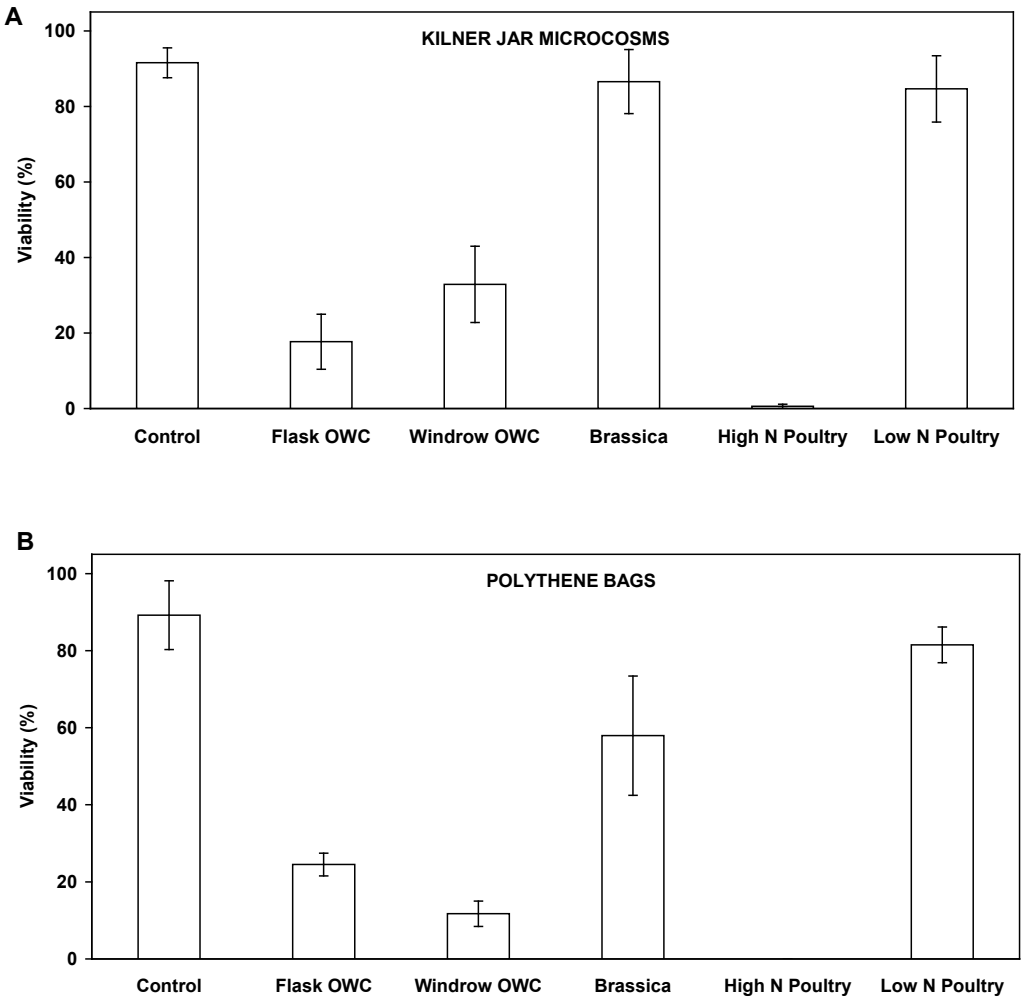

Figure 25: Viability of sclerotia recovered from the (a) Kilner jar microcosms and (b) polythene bags after 10 months burial in the treatments. Values are the mean of six replicate mesh bags, each containing 100 sclerotia ± 1 standard error

(iv) Allium *white rot assessment*

Figure 26 shows the progression of AWR in the different treatments over time. Disease was first detected at nine weeks, in the control, windrow OWC and low N poultry manure treatments, and observed in all treatments at 15 weeks, with the Brassica and high N poultry manure treatments being the last to show disease symptoms. The level of disease in the control and low N poultry manure treatments increased rapidly over time. In contrast, the level of AWR in the windrow OWC treatment remained at a very low level during the course of the bioassay. At the end of the bioassay, the lowest disease levels were recorded in the windrow OWC and high N poultry manure treatments (Figure 26). In Bioassay 1, the highest level of

disease was recorded in the Brassica treatment. In contrast, in this bioassay (Bioassay 2), the Brassica treatment controlled AWR as effectively as the windrow OWC and high N poultry manure treatments (Figure 26). No disease was detected in the uninoculated pots.

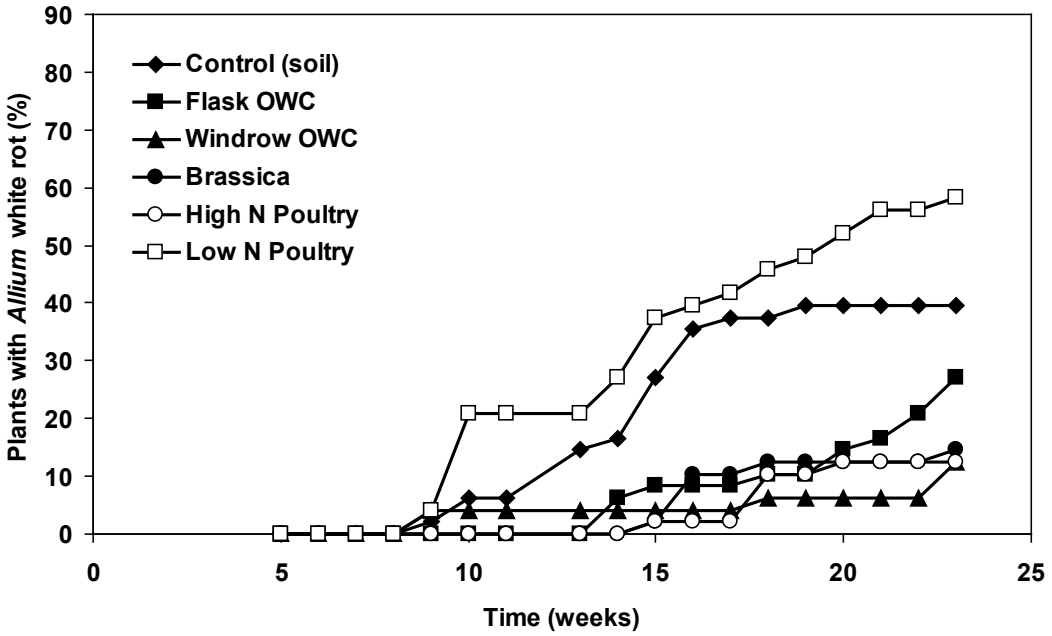

Figure 26: Plants with *Allium* white rot in the various sulphur-containing composted waste treatments. Values are the mean of 48 replicate pots

Figure 27 shows the weight of the plants in the different treatments at the end of the bioassay (23 weeks). The plants grown in the OWC and high N poultry manure treatments had a similar weight to those grown in soil alone (control). In contrast, the plants grown in the Brassica and low N poultry manure composts had a higher plant weight than those grown in soil alone. Similar to Bioassay 1, there were no obvious symptoms of AWR (white mycelium, black sclerotia) detected in the plants in the inoculated treatments (Figure 27b) remaining at harvest. However, the presence of the pathogen in the inoculated treatments did have an effect on growth. The plants grown in the Brassica and low N poultry manure composts had a lower plant weight in the inoculated treatments compared with growth in the uninoculated treatments (Figure 27a and 27b).

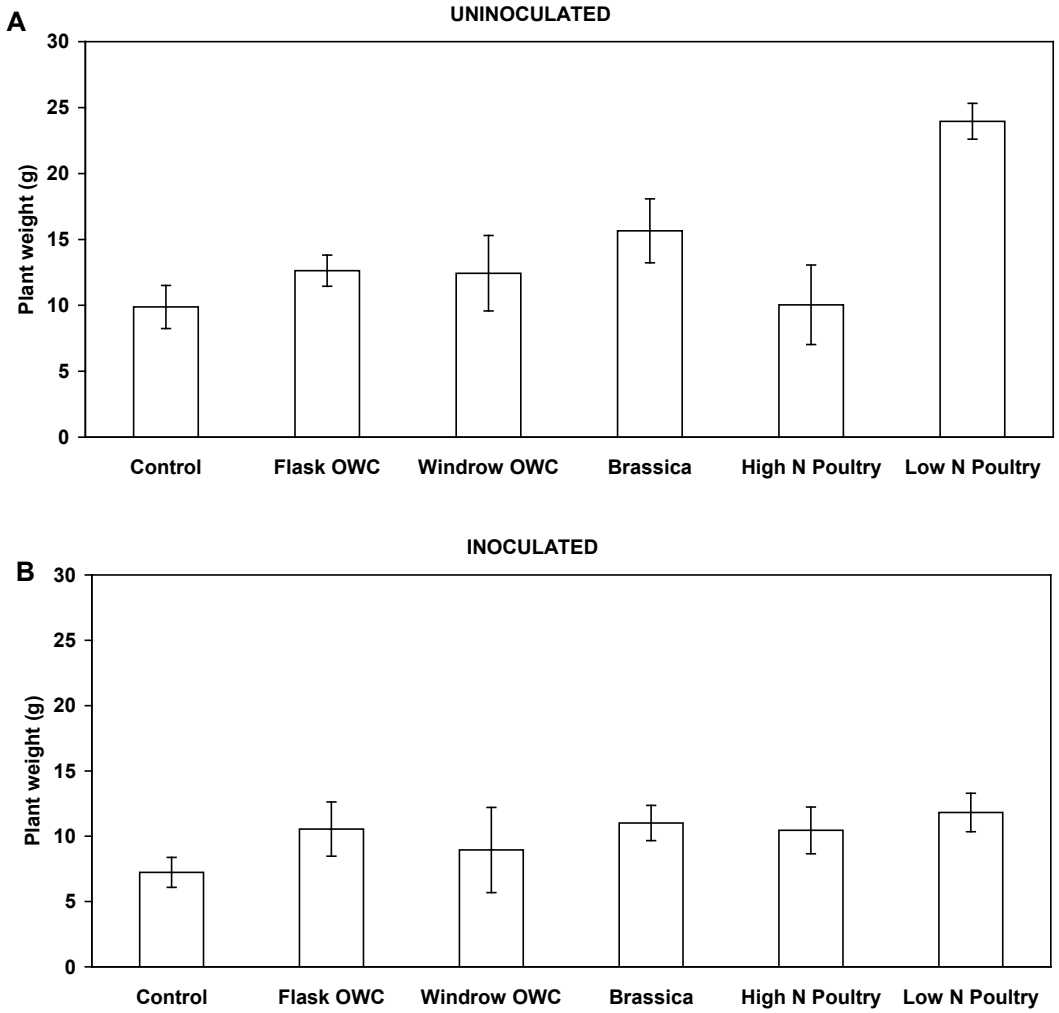

Figure 27: Weight of plants grown in the (a) uninoculated and (b) inoculated (*S. cepivorum*) treatments at harvest. Values are the mean of 16–24 (uninoculated) and 20–41 (inoculated) replicate pots ± 1 standard error

Field trials (Milestone 5.1)

(a) Warwick HRI – Wellesbourne and Kirton

Trial 1: 2007

(i) Analysis of soil samples from treatment plots

Table 11 details the general loam and mineral nitrogen analyses of the treatment plots at Wellesbourne prior to set planting in April 2007. The *T. viride* S17A set plots had a higher level of magnesium and ammonium nitrogen than the other plots, and the green waste only plots had a higher potassium level than the other treatments.

There was however no clear trend in these parameters with respect to the treatments and this difference was presumably due to natural variability over the trial site.

Table 12 details the general loam and mineral nitrogen analyses of the treatment plots at Kirton prior to set planting in April 2007. The green waste and green waste + *T. viride* S17A treatment plots had a much higher nitrate nitrogen content than the other treatments. Similar to the analyses at Wellesbourne (Table 11), no obvious trend was apparent in the other parameters with respect to treatment.

(ii) Recovery of Trichoderma *spp. from field plots*

The level of *T. viride* S17A in the rye grain used to inoculate the green waste compost was 7.3 x 10^7 cfu g^{-1}. The background level of *Trichoderma* spp. in the green waste compost prior to inoculation was 1.6 x 10^3 cfu g^{-1}. The level of *T. viride* S17A in the green waste compost on addition of the inoculum was 8.4 x 10^5 cfu g^{-1}, increasing to 9.1 x 10^7 cfu g^{-1} five weeks post-inoculation, just prior to field application. Table 13 details the level of *Trichoderma spp.* recovered from the various treatment plots at Wellesbourne throughout the growing season. A consistently high level of *Trichoderma* spp. was recovered from the green waste + *T. viride* S17A treatment plots throughout the growing season. In contrast, the level of *Trichoderma* spp. in the other treatments remained consistently low at a background level. Similar results were obtained from the Kirton field trial (Table 14). The difference in the level of *Trichoderma* spp. recovered from the green waste + *T. viride* S17A treatment and the other treatments suggests that the *T. viride* S17A inoculum survived well in the green waste. In contrast, the *T. viride* S17A applied to the sets (level of *T. viride* S17A on treated sets at planting = 4.2 x 10^5 cfu per set) did not proliferate in the soil in the field plots (Table 13 and 14).

Table 11: Analysis of samples taken from the treatment plots at Wellesbourne prior to planting onion sets in April 2007

Treatment	pH	Phosphorus (mg l^{-1})	Potassium (mg l^{-1})	Magnesium (mg l^{-1})	Ammonium Nitrogen (mg l^{-1})	Nitrate Nitrogen (mg l^{-1})	Moisture (%)
Control	6.60	86	339	132	3.24	9.72	13.6
T. viride S17A sets	5.80	65	250	190	11.50	16.43	14.8
Green waste	6.75	91	500	131	4.93	16.43	14.8
Green waste + *T. viride* S17A	5.90	67	322	158	5.01	10.01	16.1

Table 12: Analysis of samples taken from the treatment plots at Kirton prior to planting onion sets in April 2007

Treatment	pH	Phosphorus (mg l^{-1})	Potassium (mg l^{-1})	Magnesium (mg l^{-1})	Ammonium Nitrogen (mg l^{-1})	Nitrate Nitrogen (mg l^{-1})	Moisture (%)
Control	7.30	65	209	137	2.00	5.67	16.0
T. viride S17A sets	7.30	75	264	129	1.67	10.86	16.2
Green waste	7.30	118	350	129	2.00	23.30	16.0
Green waste + *T. viride* S17A	7.50	75	293	122	2.42	33.08	16.3

Table 13: *Trichoderma* spp. recovered from the various treatment plots throughout the growing season (2007) at Wellesbourne. March = green waste incorporation. April = set planting. Values are the mean of three replicate plots ± 1 standard error

Treatment	***Trichoderma* spp. (cfu g^{-1})**			
	March	**April**	**July**	**August**
Control	2.2×10^3 ($\pm 3.22 \times 10^2$)	1.0×10^3 (± 0)	1.1×10^3 ($\pm 8.15 \times 10^1$)	9.7×10^2 ($\pm 2.59 \times 10^1$)
T. viride S17A sets	1.9×10^3 ($\pm 2.24 \times 10^2$)	1.1×10^3 ($\pm 6.16 \times 10^1$)	1.1×10^3 ($\pm 1.03 \times 10^2$)	1.0×10^3 (± 0)
Green waste	1.2×10^3 ($\pm 8.15 \times 10^1$)	1.6×10^3 ($\pm 1.87 \times 10^2$)	1.4×10^3 ($\pm 1.79 \times 10^2$)	1.0×10^3 (± 0)
Green waste + *T. viride* S17A	5.2×10^6 ($\pm 3.91 \times 10^5$)	6.7×10^6 ($\pm 1.39 \times 10^6$)	5.0×10^6 ($\pm 3.68 \times 10^5$)	3.8×10^6 ($\pm 3.41 \times 10^5$)

Table 14: *Trichoderma* spp. recovered from the various treatment plots throughout the growing season (2007) at Kirton. Values are the mean of three replicate plots ± 1 standard error

Treatment	***Trichoderma* spp. (cfu g^{-1})**	
	July	**August**
Control	1.4×10^4 ($\pm 4.25 \times 10^3$)	3.8×10^4 ($\pm 1.00 \times 10^4$)
T. viride S17A sets	1.3×10^4 ($\pm 4.18 \times 10^3$)	3.8×10^4 ($\pm 5.97 \times 10^3$)
Green waste	1.9×10^3 ($\pm 3.23 \times 10^2$)	1.6×10^4 ($\pm 5.14 \times 10^3$)
Green waste + *T. viride* S17A	5.0×10^6 ($\pm 6.92 \times 10^5$)	2.5×10^6 ($\pm 4.19 \times 10^5$)

(iii) Retrieval of sclerotia buried in plots

Figure 28 shows the viability of sclerotia recovered from the field plots just prior to set planting. Due to the timing of the compost application and set planting, the sclerotia were only in the field plots for two weeks. Similar to the results from the glasshouse green waste sclerotia viability bioassay, the treatments had no effect on viability after this burial period.

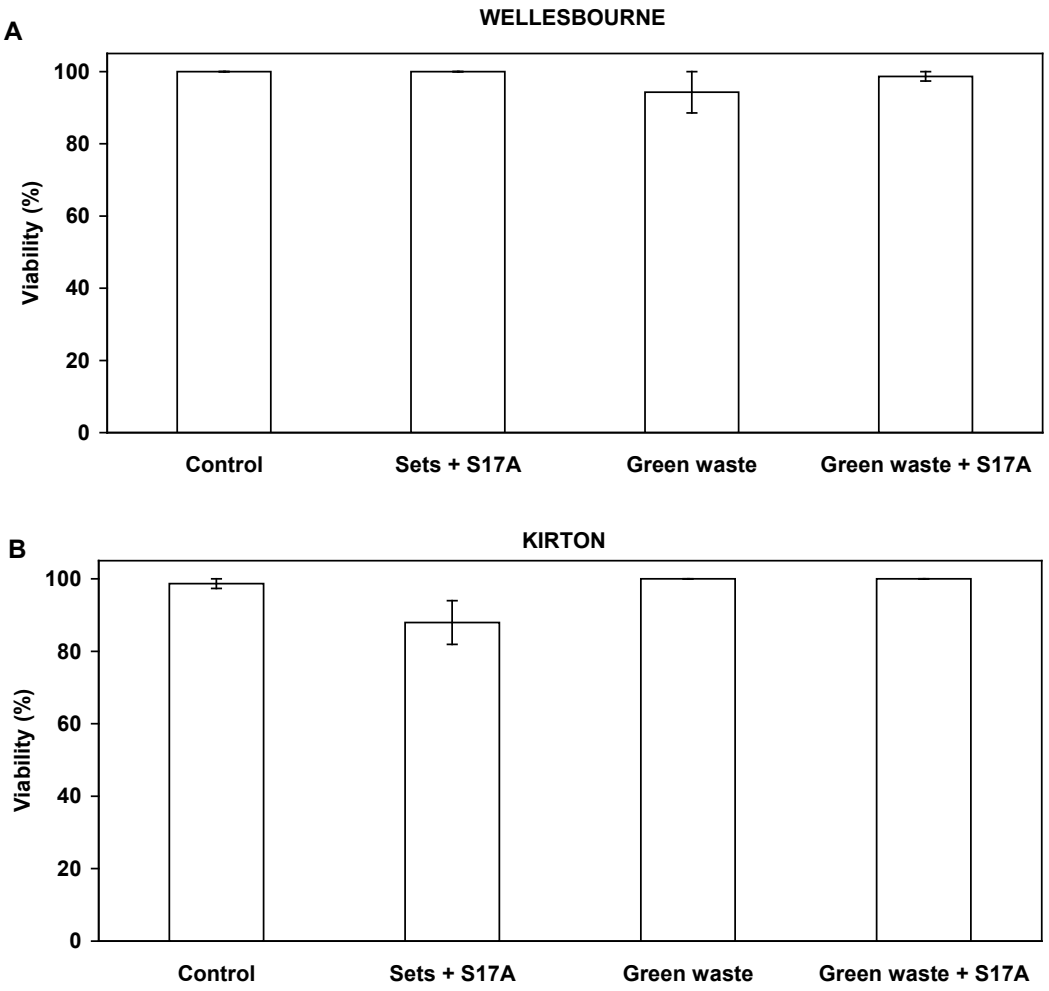

Figure 28: Viability of sclerotia recovered from the treatment plots at (a) Wellesbourne and (b) Kirton after two weeks burial in 2007. Values are the mean of three replicate mesh bags, each containing 100 sclerotia ± 1 standard error

(iv) Emergence of sets

The emergence of sets, planted in April 2007, in 2 x 1 m lengths within the treatment plots at Wellesbourne and Kirton is shown in Figure 29. The sets treated with *T. viride* S17A were the slowest to emerge in both trials. However, by the final assessment the emergence in the four different treatments within the trials at

Wellesbourne and Kirton was very similar (Figure 29a and 29b). With the exception of the *T. viride* S17A set treatment, emergence was higher at Kirton than at Wellesbourne.

(v) Allium *white rot assessment*

The AWR recorded in the 2 x 1 m lengths within the treatment plots at Wellesbourne and Kirton throughout the growing season is shown in Figure 30. Disease was recorded on both sites with a particularly high level at Kirton (Figure 30b). No disease was observed in the green waste + *T. viride* S17A treatment throughout the growing season at either site (Figure 30a and 30b). In contrast, disease was observed in all the other treatments at both sites. At Wellesbourne, both the *T. viride* S17A set and green waste treatments reduced the level of AWR compared with the control, with the *T. viride* S17A set treatment being the most effective of these treatments (Figure 30a). In contrast, at Kirton, there was no difference in the AWR recorded in the *T. viride* S17A set and green waste treatments, and the control at the end of the trial (Figure 30b). The apparent difference in efficacy of these treatments observed at the two sites may have been due to the high disease pressure at Kirton.

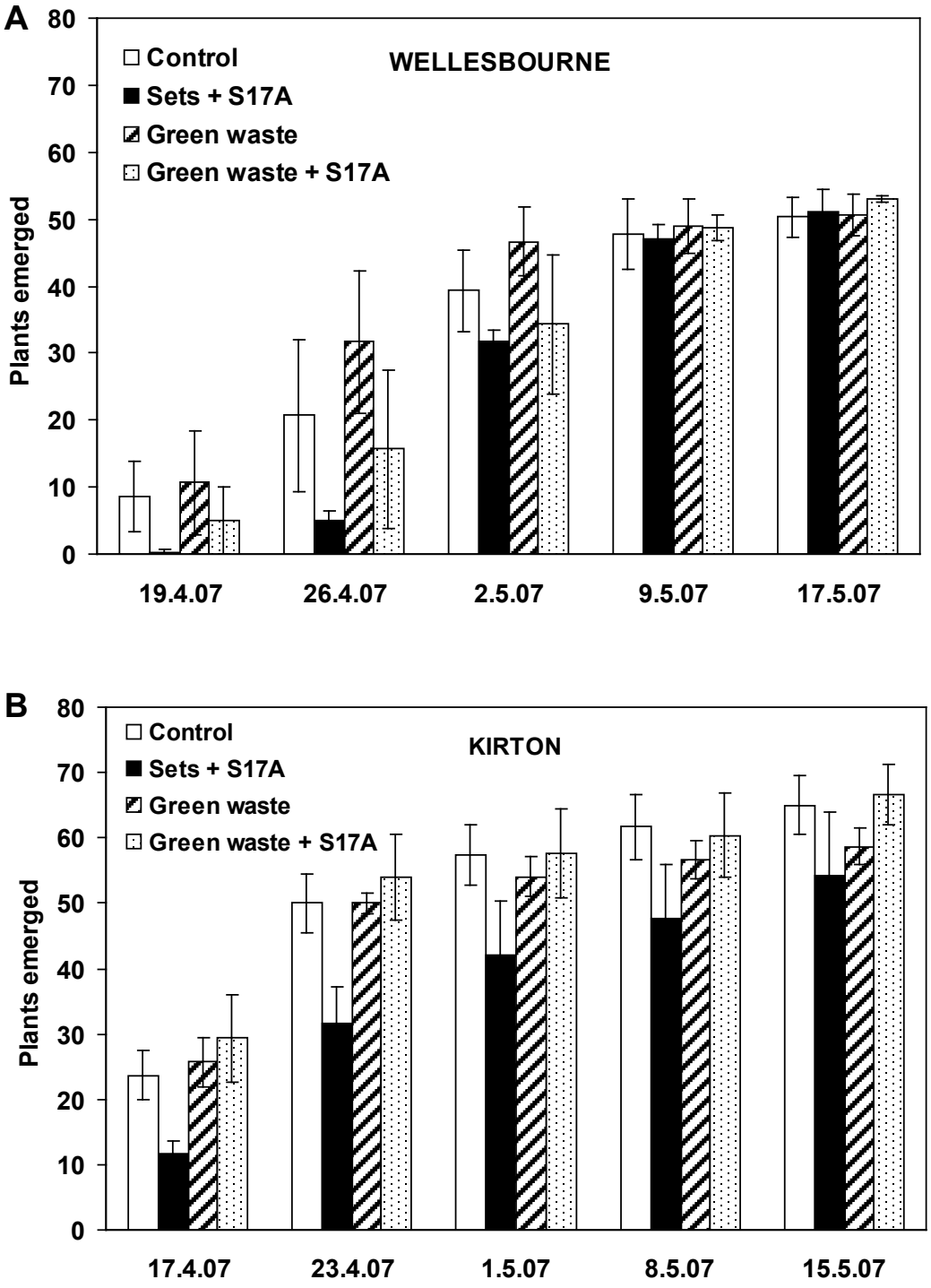

Figure 29: Emergence of sets (cv. Hercules), planted in April 2007, in 2 x 1 m lengths within the various treatment plots at (a) Wellesbourne and (b) Kirton. Values are the mean of three replicate plots ± 1 standard error

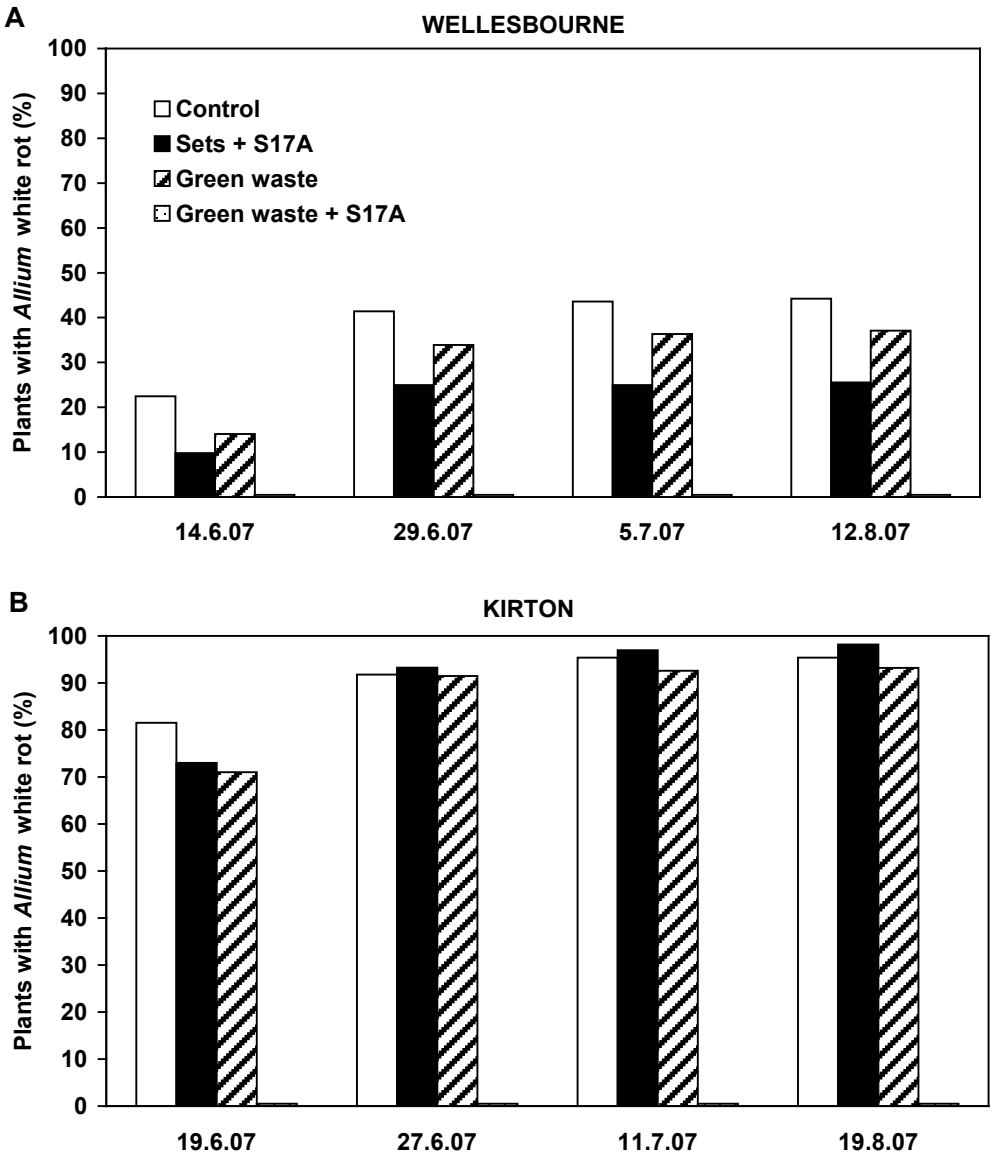

Figure 30: Onion plants (%), in the 2 x 1 m lengths within the treatment plots, infected with *Allium* white rot throughout the growing season (2007) at (a) Wellesbourne and (b) Kirton. Values are the mean of three replicate plots

(vi) Onion yield

The total yield of onions harvested from each of the treatments, as well as Folicur-treated sets planted, is shown in Figure 31. At Wellesbourne, the green waste + *T. viride* S17A treatment gave the highest healthy yield. This yield was more than twice as high as any of the other treatments and almost double the yield from the Folicur-treated sets (Figure 31a). The *T. viride* S17A set treatment gave a comparable

healthy yield to the control. The green waste only treatment increased the healthy yield compared with the control.

At Kirton, the green waste + *T. viride* S17A treatment gave an almost identical healthy yield to the plots with Folicur-treated sets (Figure 31b). This yield was more than twice as high as any of the other treatments. The *T. viride* S17A set and green waste only treatments gave a similar healthy yield to the control.

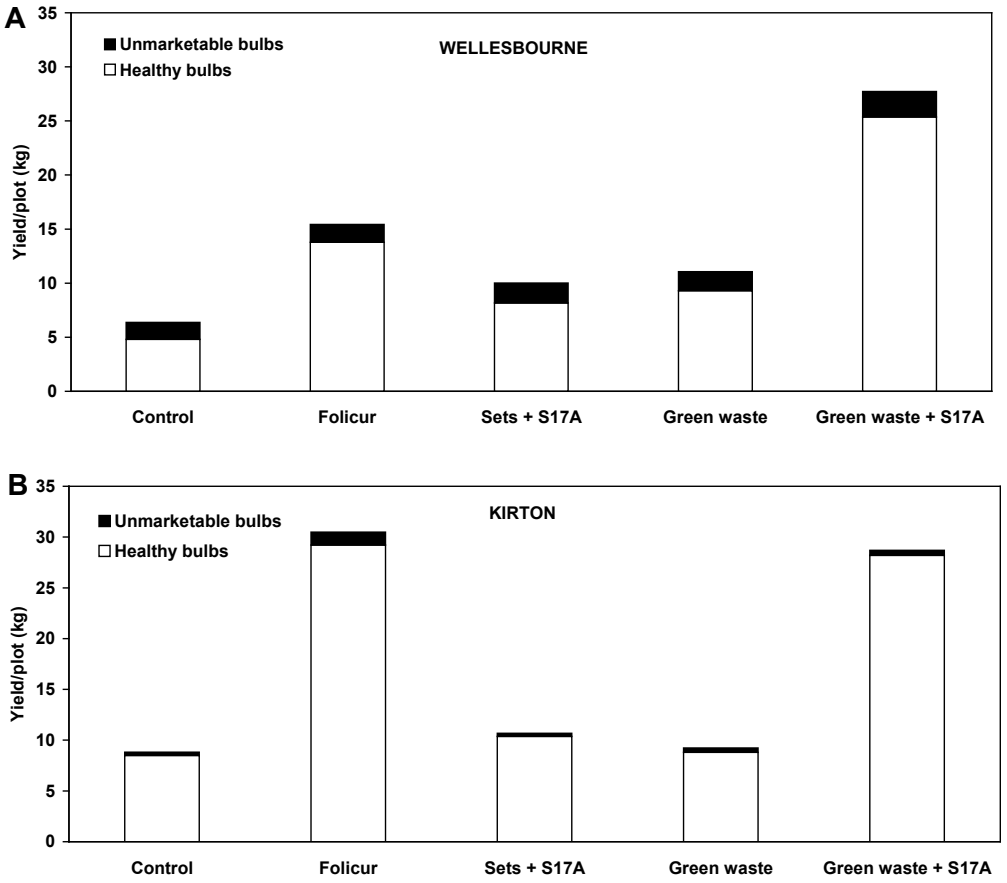

Figure 31: Onion yield (kg) from the various treatments at (a) Wellesbourne and (b) Kirton in 2007. Values are the mean of three replicate plots. Unmarketable bulbs = diseased, damaged, poor growth or shrivelled

Figure 32 shows the yield of bulbs in the three different size categories from the Wellesbourne trial. All treatments increased the yield of the <40 mm diameter bulbs compared with the control. The green waste alone, green waste + *T. viride* S17A and Folicur treatments increased the yield of the 40-60 mm diameter bulbs compared with the control. Only the green waste treatment significantly increased the yield from the largest bulb size category. Due to plot-plot variability, the increase in yield of the >60 mm diameter bulbs resulting from the green waste + *T. viride* S17A treatment was not significant.

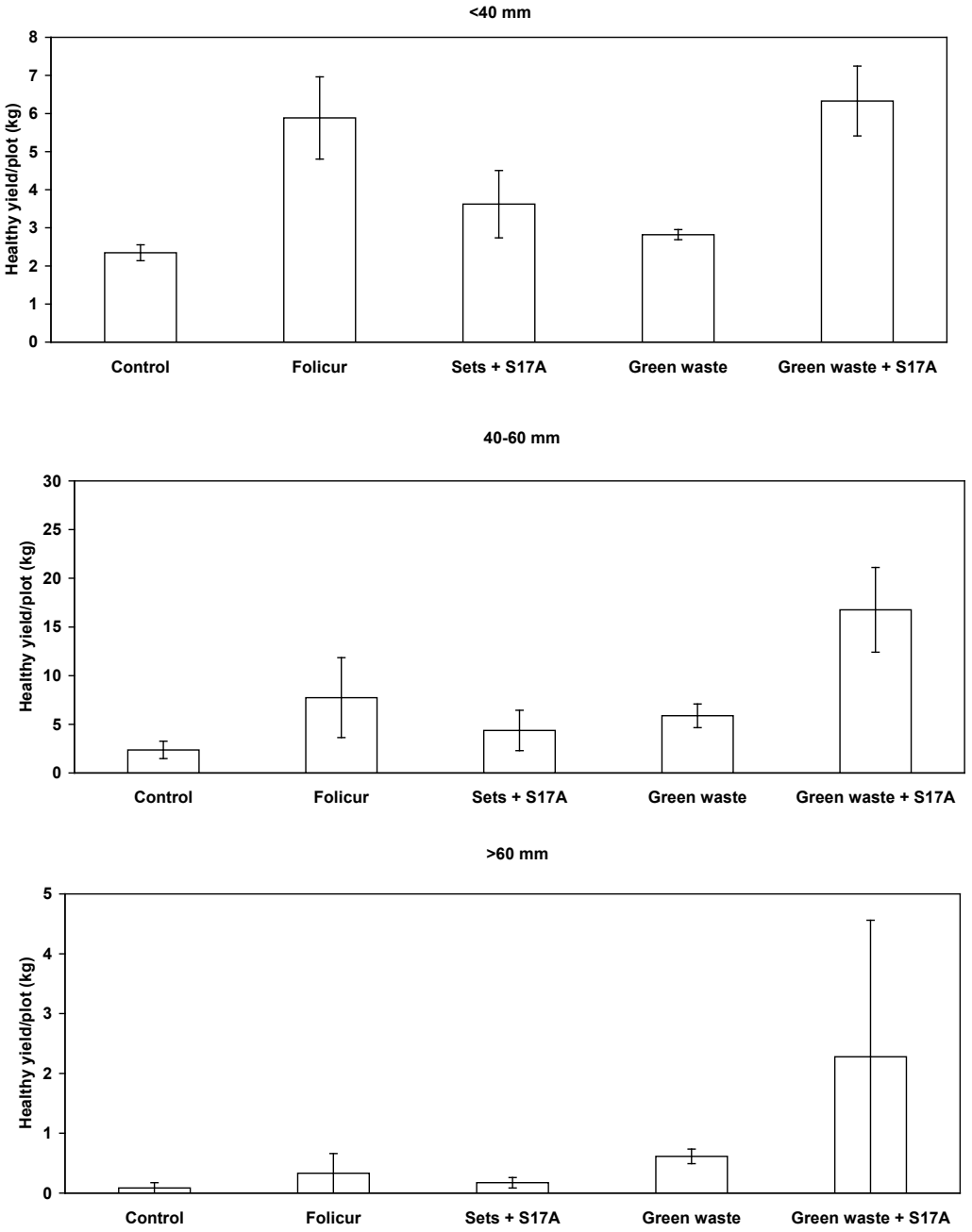

Figure 32: Healthy yield of onions from the various treatments in the three size categories, <40 mm, 40-60 mm and >60 mm diameter, from the Wellesbourne trial in 2007. Values are the mean of three replicate plots ± 1 standard error

Figure 33 shows the yield of bulbs in the three different size categories from the Kirton trial. The green waste + *T. viride* S17A treatment increased the yield from each size category compared with the control. The Folicur treatment also showed a similar trend. All treatments increased the yield of the >60 mm diameter bulbs above the control.

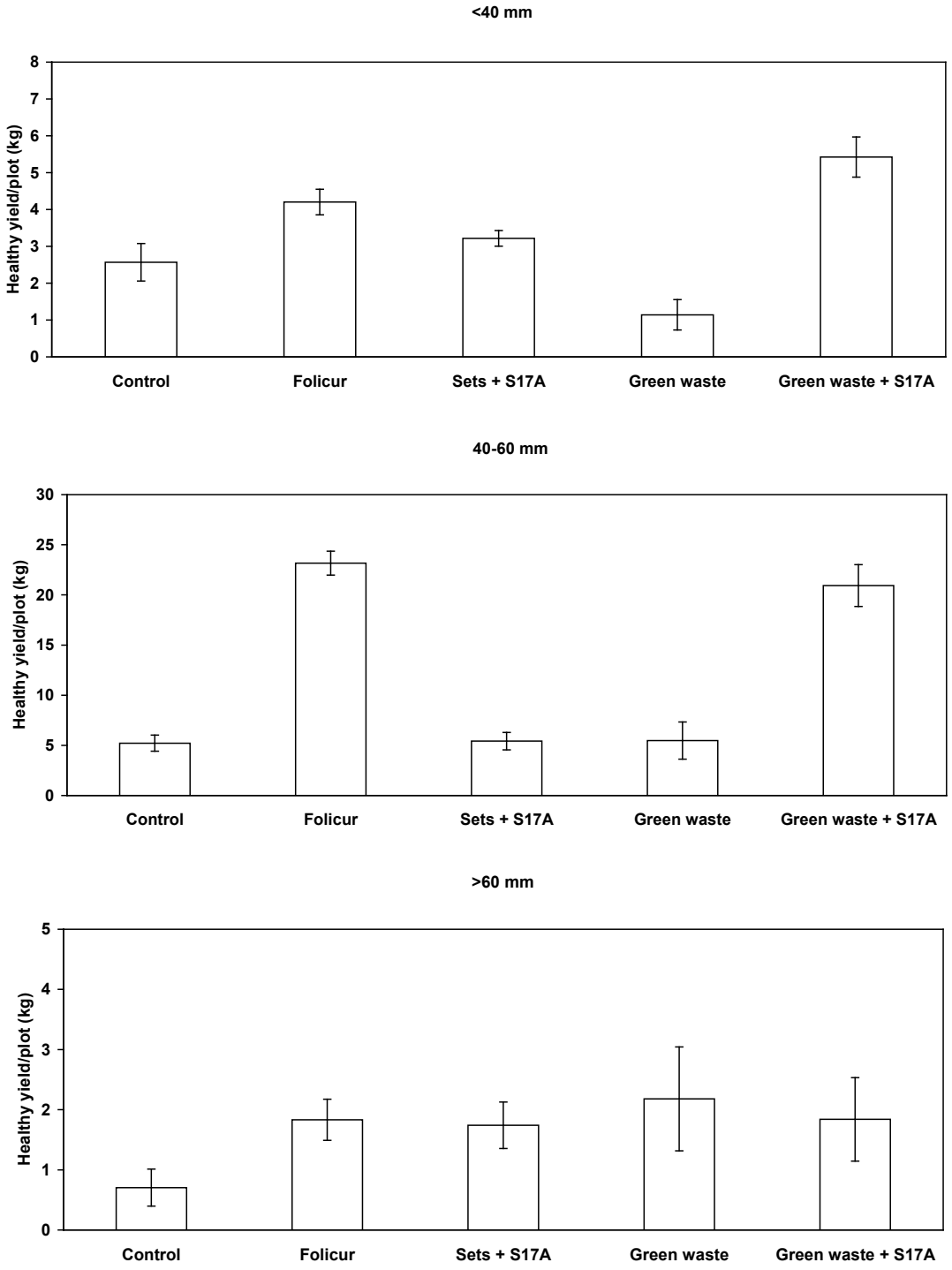

Figure 33: Healthy yield of onions from the various treatments in the three size categories, <40 mm, 40-60 mm and >60 mm diameter, from the Kirton trial in 2007. Values are the mean of three replicate plots ± 1 standard error

Trial 2: 2006-2008

(i) Analysis of soil samples from treatment plots

Table 15 details the general loam and mineral nitrogen analyses of the treatment plots at Wellesbourne prior to set planting in April 2008. All the compost treatments increased the pH, phosphorus, potassium and magnesium concentrations of the soil compared with the control (untreated soil). Application of the windrow OWC resulted in the largest increase in pH, whereas the poultry manure treatments had the highest levels of phosphorus, potassium and magnesium. There was no clear trend in the mineral nitrogen analysis with respect to treatment.

Table 16 details the general loam and mineral nitrogen analyses of the treatment plots at Kirton prior to set planting in April 2008. In general, addition of the various composts to the plots increased the phosphorus, potassium, magnesium and mineral nitrogen concentrations of the soil compared with the control (untreated soil), with the poultry manure treatments showing the largest increase.

Soil moisture content at the time of sampling was increased by compost application at both sites (Tables 15 and 16).

Application of the windrow OWC and poultry manure composts had no effect on the *Trichoderma* spp. population with respect to the untreated soil, with a similar level (*c.* 10^3 cfu g^{-1} soil) recovered from the treated and untreated plots at both Wellesbourne and Kirton.

(ii) Retrieval of sclerotia buried in plots

Figure 34 shows the viability of sclerotia recovered from the field plots after 10 and 16 months burial. The results were very similar on the two sites, Wellesbourne and Kirton. Both the windrow OWC and high N poultry manure compost applied in 2006 reduced the viability of sclerotia recovered, with the windrow OWC treatment showing the greatest reduction in viability. With the exception of the OWC applied in October 2007 at Wellesbourne (Wellesbourne sclerotia viability: Control = 92.1 $\pm$ 4.16; OWC 2007 = 72.1 $\pm$ 10.70), the composts applied to the plots at Wellesbourne and Kirton in November 2006 and October 2007 had no effect on the sclerotia buried in October 2007 after five months burial (results not shown).

(iii) Emergence of sets

The emergence of sets, planted in April 2008, in 2 x 1 m lengths within the treatment plots at Wellesbourne and Kirton is shown in Figure 35. At Wellesbourne,

emergence in all the treatments was comparable to the control (Figure 35a). At Kirton, emergence in most treatments was comparable to the control (Figure 35b). The exceptions to this trend were the Folicur and low N poultry manure compost 2007 treatments, where emergence was respectively lower and higher compared with the control.

(iv) Allium *white rot assessment*

The AWR recorded in the 2 x 1 m lengths within the treatment plots at Wellesbourne and Kirton throughout the growing season is shown in Figure 36. A high level of disease was recorded on both sites. At Wellesbourne, the OWC applied in 2006 and 2007, the low N poultry manure compost applied in 2007 and the combination (Low N poultry 2007 + Folicur) treatment reduced the level of disease compared with the control (Figure 36a). The OWC 2007 treatment was the most effective in controlling AWR, with no disease observed in this treatment (Figure 36a). The Folicur-treated sets alone showed no disease control (Figure 36a). In contrast, at Kirton, the OWC applied in 2006 was the only compost treatment that reduced the level of disease compared with the control (Figure 36b). The Folicur-treated sets reduced the level of disease compared with the control although not to the extent as observed with the OWC 2006 treatment (Figure 36b).

Table 15: Analysis of samples taken from the treatment plots at Wellesbourne prior to planting onion sets in April 2008

Treatment	pH	Phosphorus (mg l^{-1})	Potassium (mg l^{-1})	Magnesium (mg l^{-1})	Ammonium Nitrogen (mg kg^{-1})	Nitrate Nitrogen (mg kg^{-1})	Moisture (%)
Control	6.10	56	206	96	6.98	11.63	15.7
Windrow OWC 2006	6.65	77	339	111	3.66	8.32	15.9
Windrow OWC 2007	6.85	85	394	108	3.69	11.73	16.5
High N poultry manure 2006	6.30	113	485	142	5.39	5.05	16.9
Low N poultry manure 2007	6.50	100	590	154	3.69	11.76	16.6

Table 16: Analysis of samples taken from the treatment plots at Kirton prior to planting onion sets in April 2008

Treatment	pH	Phosphorus (mg l^{-1})	Potassium (mg l^{-1})	Magnesium (mg l^{-1})	Ammonium Nitrogen (mg kg^{-1})	Nitrate Nitrogen (mg kg^{-1})	Moisture (%)
Control	7.15	72	156	116	3.68	10.56	12.5
Windrow OWC 2006	7.25	82	259	118	2.99	11.45	15.6
Windrow OWC 2007	7.20	95	367	116	4.61	13.84	15.1
High N poultry manure 2006	6.85	137	510	198	5.78	19.81	15.2
Low N poultry manure 2007	7.30	121	500	214	5.76	16.95	14.9

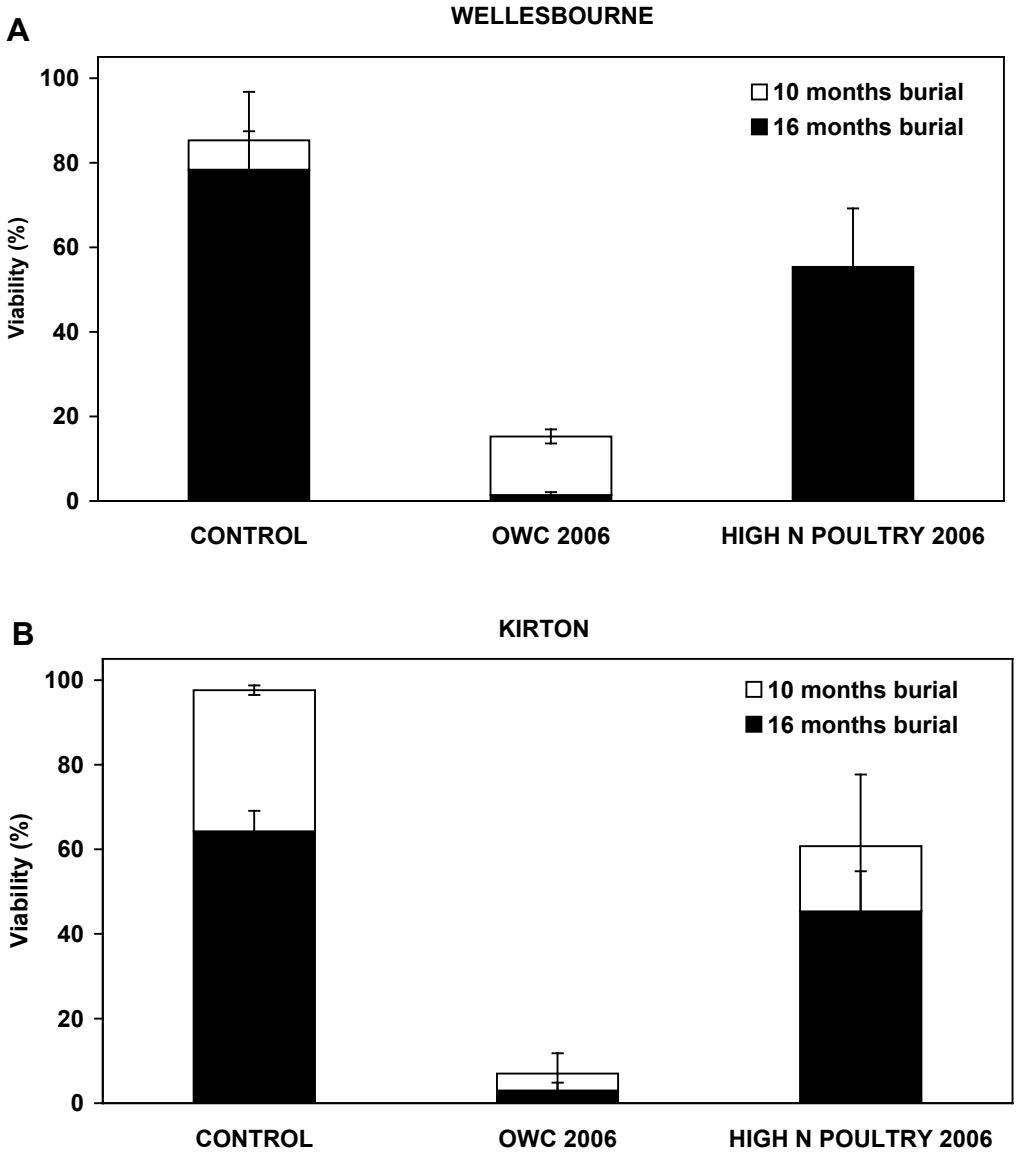

Figure 34: Viability of sclerotia recovered from the treatment plots, with compost applied in 2006, at (a) Wellesbourne and (b) Kirton after 10 and 16 months burial. Values are the mean of five replicate mesh bags (10 for the control), each containing 100 sclerotia ± 1 standard error

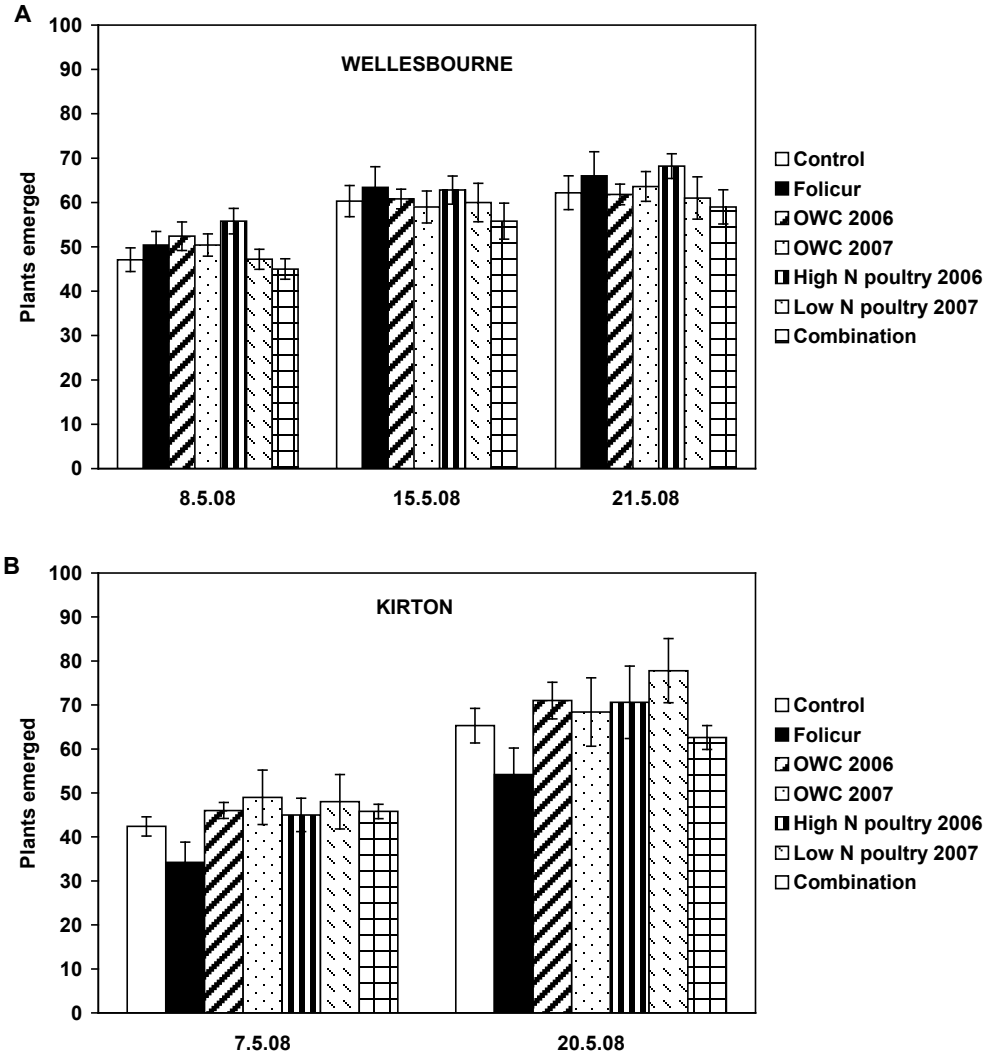

Figure 35: Emergence of sets (cv. Hercules), planted in April 2008, in 2 x 1 m lengths within the various treatment plots at (a) Wellesbourne and (b) Kirton. Values are the mean of five replicate plots (10 for the control) ± 1 standard error

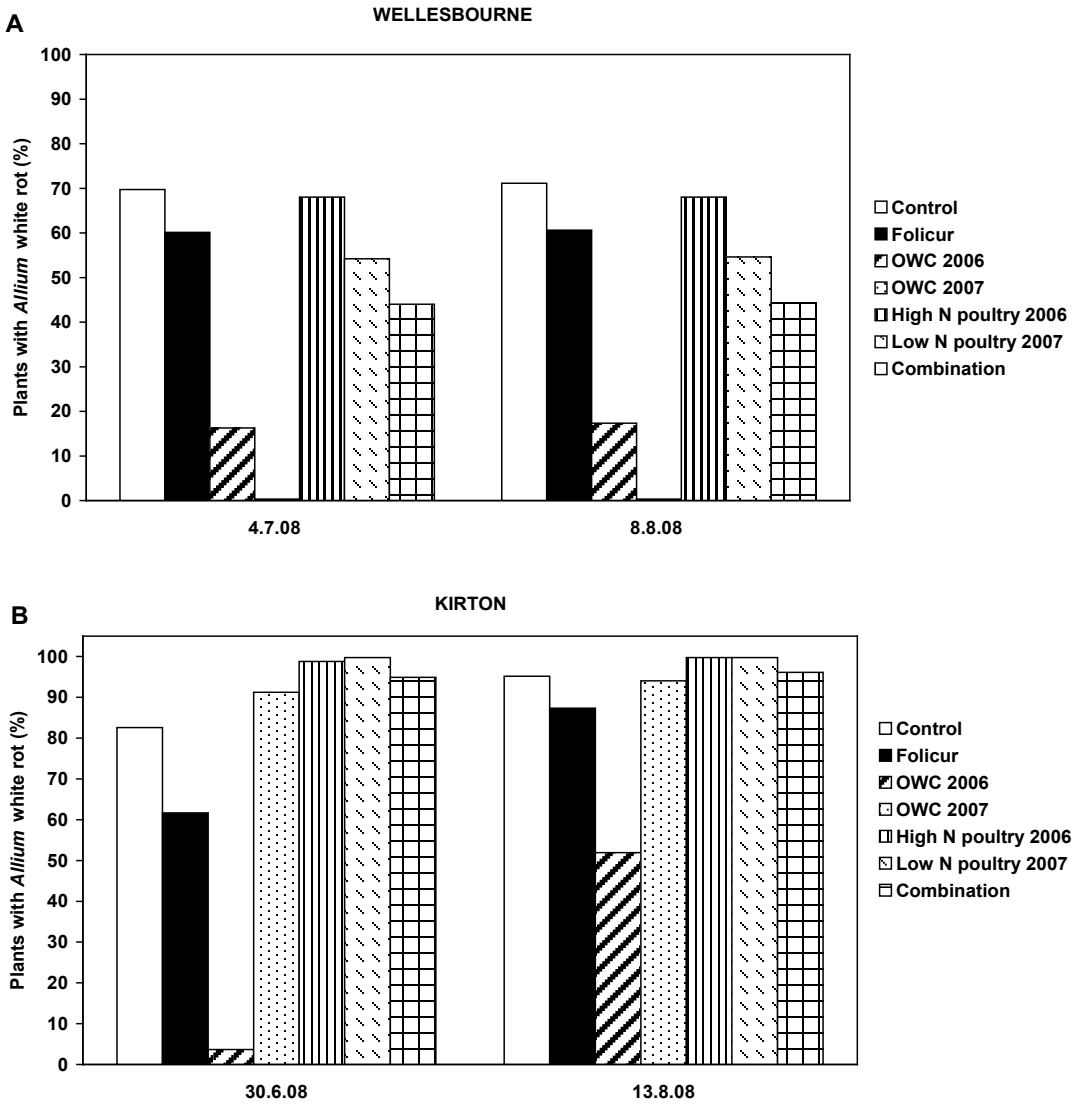

Figure 36: Onion plants (%), in the 2 x 1 m lengths within the treatment plots, infected with *Allium* white rot throughout the 2008 growing season at (a) Wellesbourne and (b) Kirton. Values are the mean of five replicate plots (10 for the control)

(v) Onion yield

The total yield of onions harvested from each of the treatments at Wellesbourne and Kirton is shown in Figure 37. At Wellesbourne, the OWC treatments gave the highest healthy yields, with the OWC 2007 treatment giving the highest yield (Figure 37a). The yields obtained from the OWC treatments were more than twice as high as any of the other treatments and three times higher than the yield from the Folicur-treated sets (Figure 37a). The poultry manure compost treatments, including the combination treatment (low N poultry 2007 + Folicur), gave similar yields to the control.

At Kirton, the OWC 2006 treatment gave the highest healthy yield (Figure 37b). The yield obtained from this treatment was seven times as high as any of the other treatments and five times higher than the yield from the Folicur-treated sets (Figure 37b).

Figure 38 shows the yield of bulbs in the three different size categories from the Wellesbourne trial. The low N poultry manure compost treatment and combination treatment increased the yield of the <40 mm diameter bulbs compared with the control. Both the OWC treatments increased the yield of the 40-60 mm and >60 mm diameter bulbs compared with the control. The yield of bulbs from all the other treatments in these two size categories was comparable to the control.

Figure 39 shows the yield of bulbs in the three different size categories from the Kirton trial. The OWC 2006 treatment increased the yield of the 40-60 mm and >60 mm diameter bulbs compared with the control. The yield of bulbs from all the other treatments in these two size categories was comparable to the control.

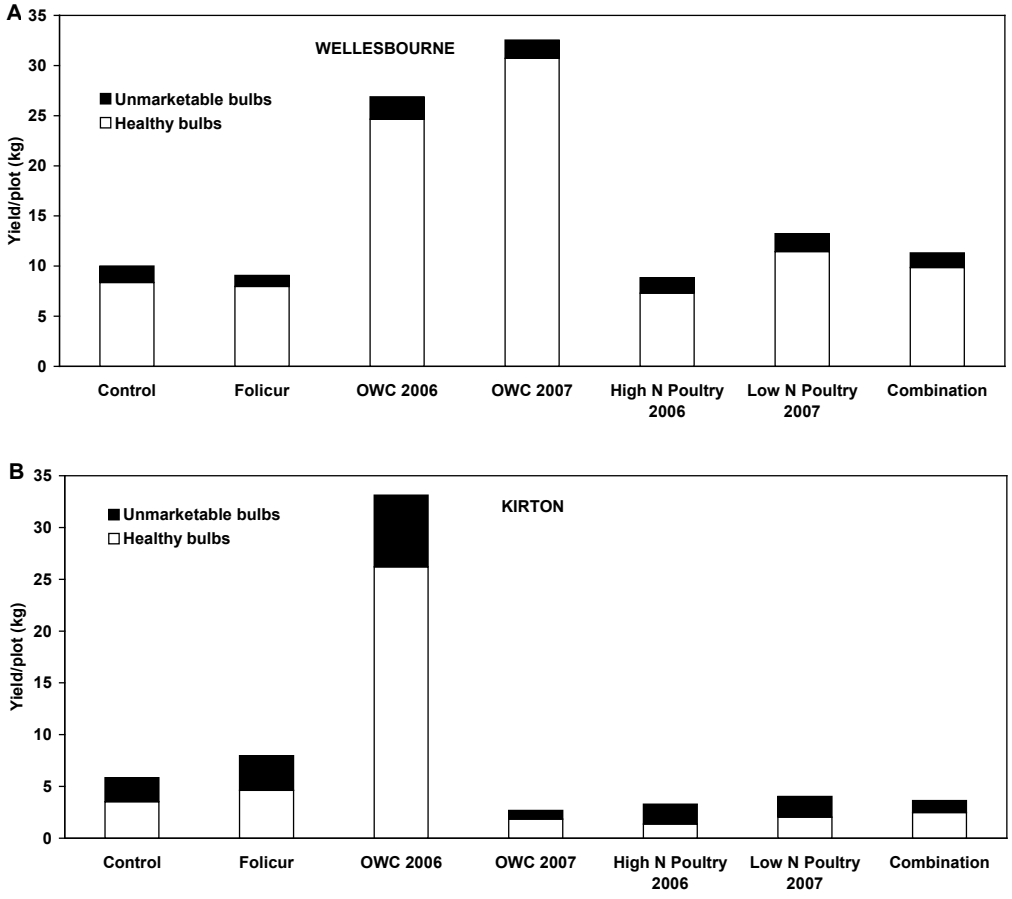

Figure 37: Onion yield (kg) from the various treatments at (a) Wellesbourne and (b) Kirton in 2008. Values are the mean of five replicate plots. Unmarketable bulbs = diseased, damaged, poor growth or shrivelled

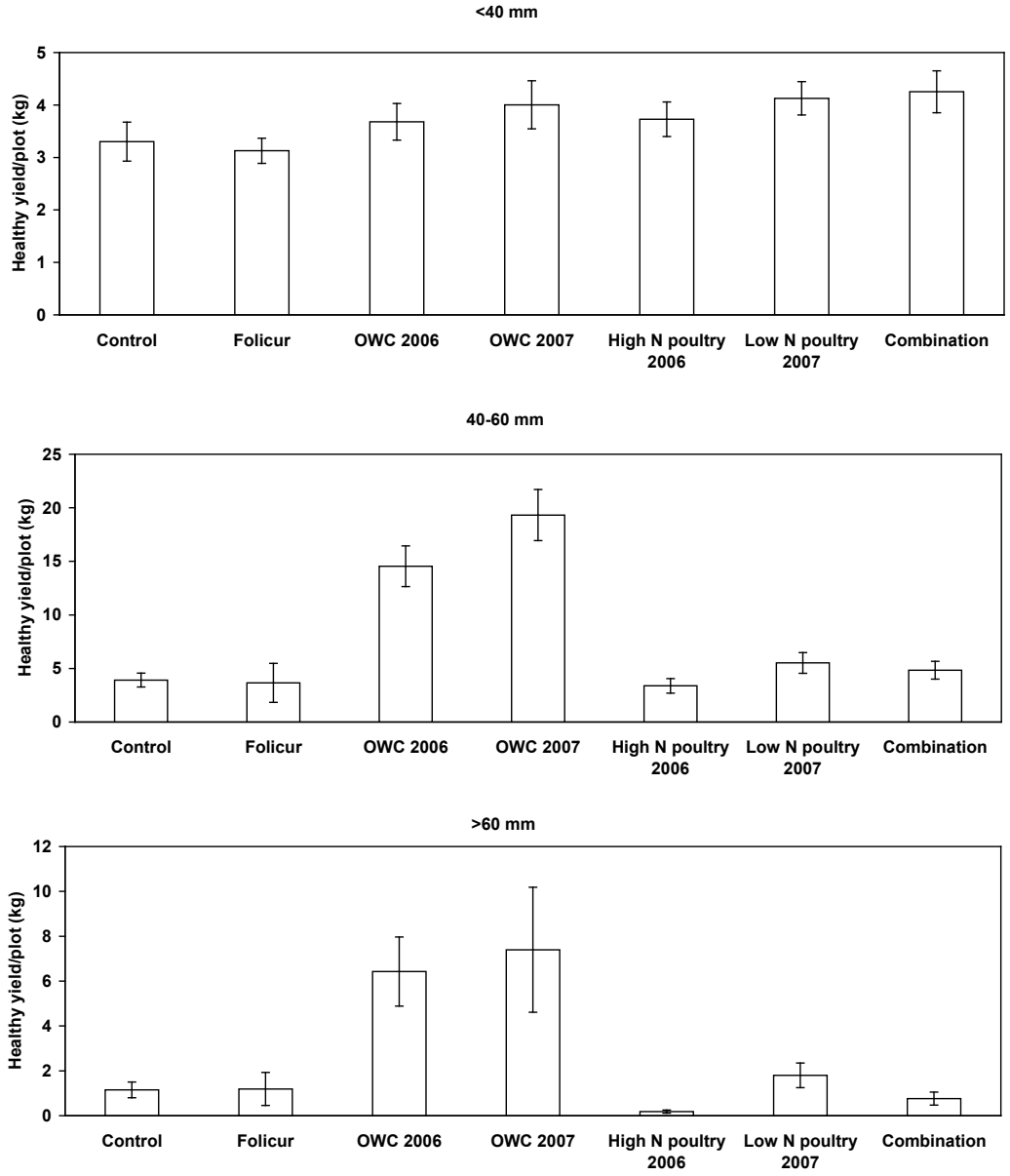

Figure 38: Healthy yield of onions from the various treatments in the three size categories, <40 mm, 40-60 mm and >60 mm diameter, from the Wellesbourne trial in 2008. Values are the mean of five replicate plots ± 1 standard error

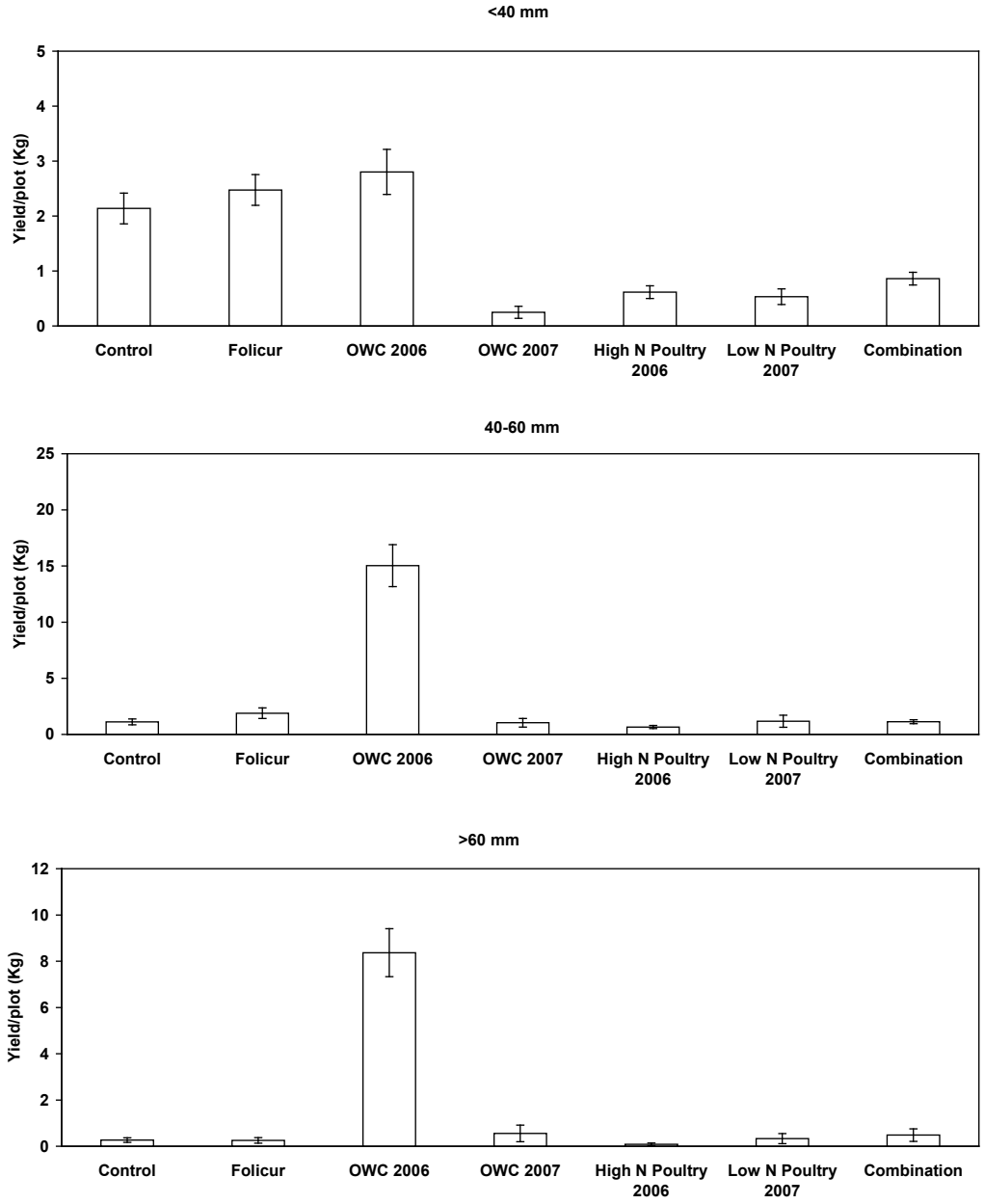

Figure 39: Healthy yield of onions from the various treatments in the three size categories, <40 mm, 40-60 mm and >60 mm diameter, from the Kirton trial in 2008. Values are the mean of five replicate plots ± 1 standard error

(vi) Pyruvate analysis and taste panel results

Table 17 shows the pyruvate content and taste panels results of onions grown on control, OWC 2007 and Poultry 2007 treatment plots at Wellesbourne and Kirton. The onions grown on the compost-treated plots had a higher pyruvate content than those grown on untreated (control) plots at both sites. In addition, the pyruvate content of the onions grown on the various treatment plots at Kirton was higher than

that of those grown at Wellesbourne (Table 17), presumably a reflection of the influence of the different soil types (Tables 15 and 16) on the crop.

The onions were scored by the taste panels using the following flavour and pungency scale:

1. Bland, no pungent onion taste
2. Bland with a hint of pungency
3. Bland but definite pungency
4. Noticeable pungent onion taste
5. Definite pungency but no tear jerking
6. Very pungent, noticeable tear jerking
7. Very pungent, definite tear jerking

The taste panel at Warwick HRI found the onions grown on the compost-treated plots at Wellesbourne and the OWC 2007 plots at Kirton to be more pungent than the onions grown on the untreated (control) plots (Table 17). In addition, the onions grown at Kirton were generally considered more pungent by the Warwick HRI taste panel than those grown at Wellesbourne.

In contrast to the Warwick HRI taste panel, the Anglian Water taste panel found the onions grown at Wellesbourne more pungent than those grown at Kirton (Table 17). They did not find a noticeable difference in pungency between the treatments at Wellesbourne but found onions grown with OWC at Kirton to be less pungent than the control or poultry manure compost treatments.

Table 17: Pyruvate content and taste panels flavour and pungency results of onions grown on control, OWC 2007 and Poultry 2007 treatment plots at Wellesbourne and Kirton. Taste panel results are the mean scores of 7 persons

Treatment	Wellesbourne			Kirton		
	Pyruvate ($\mu mol\ g^{-1}$ FW)	Taste Panel		Pyruvate ($\mu mol\ g^{-1}$ FW)	Taste Panel	
		HRI	Anglian		HRI	Anglian
Control	4.93	2.57	3.71	6.47	4.42	3.00
OWC 2007	5.62	4.00	3.57	7.73	5.71	2.00
Poultry 2007	5.58	4.85	3.57	7.00	4.00	2.86

Trial 3: 2008

(i) Analysis of soil samples from treatment plots

Table 18 details the general loam and mineral nitrogen analyses of the treatment plots at Wellesbourne prior to set planting in April 2008. The green waste only plots had a higher nitrogen content than the other treatments although the green waste + *T. viride* S17A plots had a similar level to the control. Similar to Trial 1, there was little difference or no clear trend within parameters with respect to the treatments.

Table 19 details the general loam and mineral nitrogen analyses of the treatment plots at Kirton prior to set planting in April 2008. The green waste and green waste + *T. viride* S17A plots had the highest potassium content. Similar to the results at Wellesbourne (Table 18), the green waste only plots had a higher nitrogen content than the other treatments although the green waste + *T. viride* S17A plots had a level much closer to the control treatment.

(ii) Recovery of Trichoderma *spp. from field plots*

The level of *T. viride* S17A in the rye grain used to inoculate the green waste compost was 7.6 x 10^8 cfu g^{-1}. The background level of *Trichoderma* spp. in the green waste compost prior to inoculation was 1.0 x 10^3 cfu g^{-1}. The level of *T. viride* S17A in the green waste compost on addition of the inoculum was 1.5 x 10^6 cfu g^{-1}, increasing to 3.7 x 10^7 cfu g^{-1} 25 days post-inoculation, just prior to field application. Table 20 details the level of *Trichoderma* spp. recovered from the various treatment plots at Wellesbourne throughout the growing season. Similar to Trial 1, a consistently high level of *Trichoderma* spp. was recovered from the green waste + *T. viride* S17A treatment plots throughout the growing season (Table 20). In contrast, the level of *Trichoderma* spp. in the other treatments remained consistently low at a background level. Similar results were obtained from the Kirton field trial (Table 21). The difference in the level of *Trichoderma* spp. recovered from the green waste + *T. viride* S17A treatment and the other treatments suggests that the *T. viride* S17A inoculum survived well in the green waste. In contrast, similar to Trial 1, the *T. viride* S17A applied to the sets (1.6 x 10^5 cfu per set at planting) did not proliferate in the soil in the field plots (Table 20 and 21).

(iii) Emergence of sets

The emergence of sets, planted in April 2008, in 2 x 1 m lengths within the treatment plots at Wellesbourne and Kirton is shown in Figure 40. Similar to the final emergence assessment in Trial 1, the emergence in the four different treatments at

Wellesbourne (Figure 40a) was very similar. In contrast, at Kirton, there was a higher emergence of sets in the green waste + *T. viride* S17A treatment compared with the control (Figure 40b). Emergence in the other treatments was comparable to the control.

(iv) Allium *white rot assessment*

The AWR recorded in the 2 x 1 m lengths within the treatment plots at Wellesbourne and Kirton throughout the growing season is shown in Figure 41. A high level of disease was recorded on both sites. Similar to Trial 1, the green waste + *T. viride* S17A treatment was the only treatment that showed any disease control. Disease levels in this treatment at the end of the trial were at least 50% less than in the controls on both sites. No disease control was observed with the Folicur-treated sets.

Table 18: Analysis of samples taken from the treatment plots at Wellesbourne prior to planting onion sets in April 2008

Treatment	pH	Phosphorus (mg l^{-1})	Potassium (mg l^{-1})	Magnesium (mg l^{-1})	Ammonium Nitrogen (mg l^{-1})	Nitrate Nitrogen (mg l^{-1})	Moisture (%)
Control	6.40	85	327	100	3.64	11.58	15.3
T. viride S17A sets	6.75	105	354	94	2.96	8.22	14.9
Green waste	6.65	90	361	113	5.32	18.29	15.8
Green waste + *T. viride* S17A	6.70	83	327	103	3.63	11.53	15.0

Table 19: Analysis of samples taken from the treatment plots at Kirton prior to planting onion sets in April 2008

Treatment	pH	Phosphorus (mg l^{-1})	Potassium (mg l^{-1})	Magnesium (mg l^{-1})	Ammonium Nitrogen (mg l^{-1})	Nitrate Nitrogen (mg l^{-1})	Moisture (%)
Control	7.60	81	203	119	2.99	1.83	15.8
T. viride S17A sets	7.15	82	243	122	3.28	4.10	14.7
Green waste	7.30	93	500	149	4.87	12.09	16.6
Green waste + *T. viride* S17A	7.00	95	500	161	1.51	4.70	16.6

Table 20: *Trichoderma* spp. recovered from the various treatment plots throughout the growing season (2008) at Wellesbourne. March = green waste incorporation. Values are the mean of three replicate plots ± 1 standard error.

Treatment	***Trichoderma* spp. (cfu g^{-1})**		
	March	**June**	**August**
Control	1.0×10^3 (± 0)	1.0×10^3 (± 0)	1.0×10^3 (± 0)
T. viride S17A sets	1.0×10^3 (± 0)	1.0×10^3 (± 3.70×10^1)	1.0×10^3 (± 0)
Green waste	1.0×10^3 (± 0)	1.0×10^3 (± 0)	1.0×10^3 (± 3.70×10^1)
Green waste + *T. viride* S17A	8.6×10^5 (± 7.55×10^4)	4.3×10^5 (± 4.86×10^4)	7.5×10^5 (± 9.39×10^4)

Table 21: *Trichoderma* spp. recovered from the various treatment plots throughout the growing season (2008) at Kirton. Values are the mean of three replicate plots ± 1 standard error

Treatment	***Trichoderma* spp. (cfu g^{-1})**	
	June	**August**
Control	1.0×10^3 (± 0)	1.0×10^3 (± 0)
T. viride S17A sets	1.0×10^3 (± 0)	1.0×10^3 (± 0)
Green waste	1.0×10^3 (± 0)	1.0×10^3 (± 0)
Green waste + *T. viride* S17A	1.0×10^6 (± 8.46×10^4)	9.3×10^5 (± 8.28×10^4)

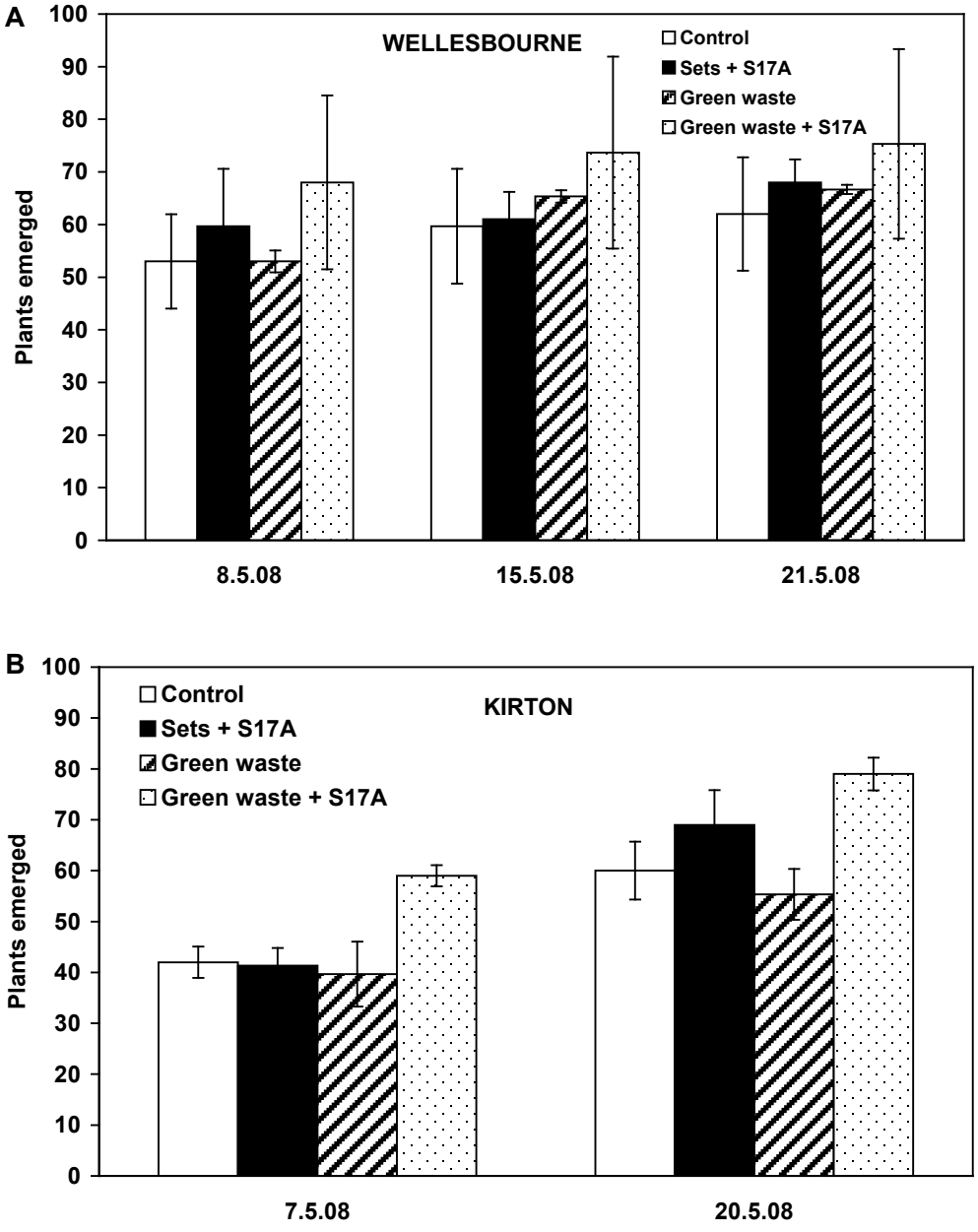

Figure 40: Emergence of sets (cv. Hercules), planted in April 2008, in 2 x 1 m lengths within the various treatment plots at (a) Wellesbourne and (b) Kirton. Values are the mean of three replicate plots ± 1 standard error

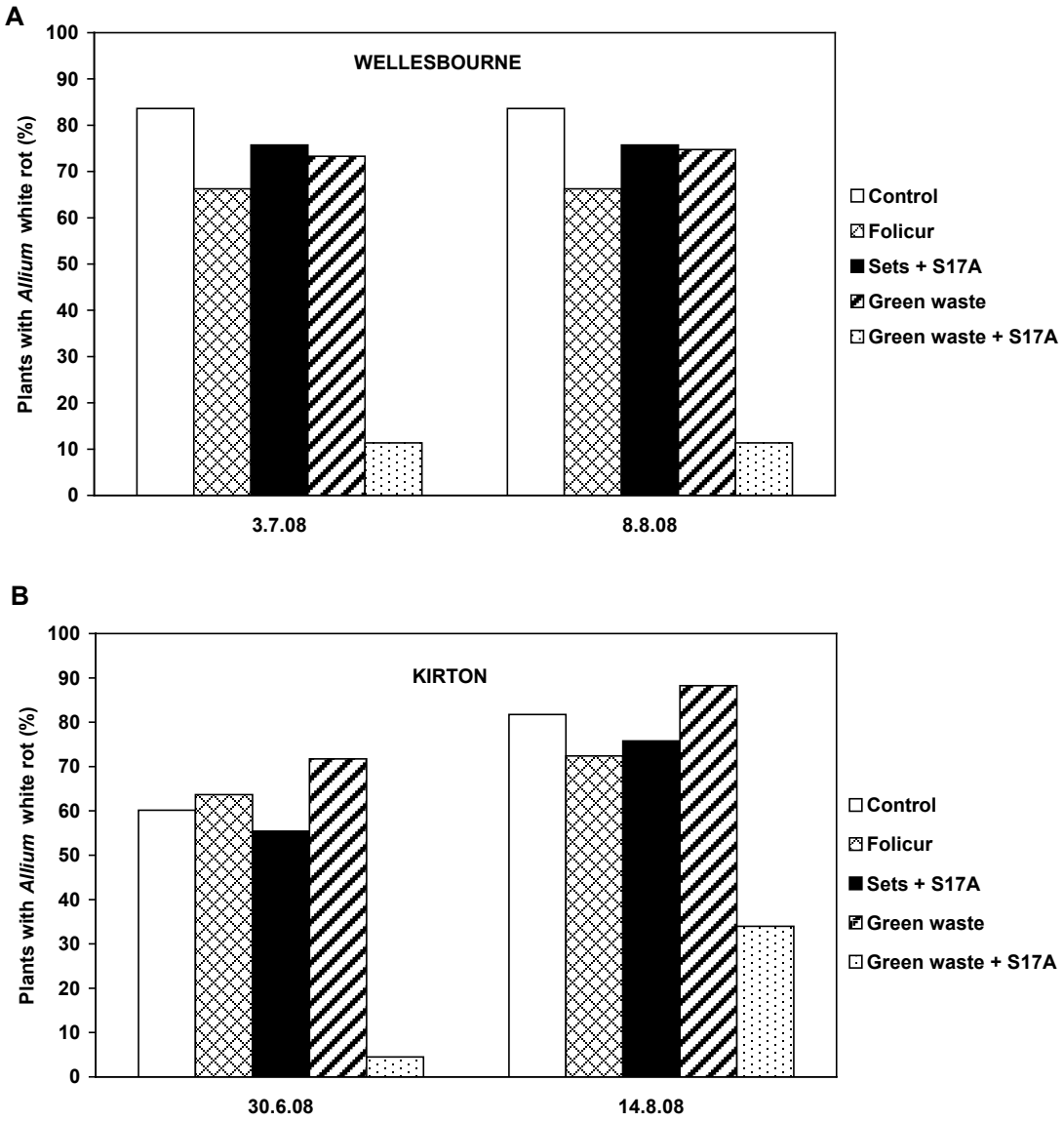

Figure 41: Onion plants (%), in the 2 x 1 m lengths within the treatment plots, infected with *Allium* white rot throughout the growing season (2008) at (a) Wellesbourne and (b) Kirton. Values are the mean of three replicate plots

(v) Onion yield

The total yield of onions harvested from each of the treatments at Wellesbourne and Kirton is shown in Figure 42. At Wellesbourne, similar to Trial 1, the green waste + *T. viride* S17A treatment gave the highest healthy yield (Figure 42a). The yield from this treatment was twice as high as any of the other treatments, including the Folicur-treated sets. None of the other treatments increased the yield above that obtained from the control plots.

At Kirton, similar to Wellesbourne, the green waste + *T. viride* S17A treatment gave the highest healthy yield (Figure 42b). The yield from this treatment was more than twice as high as any of the other treatments and three times higher than the yield from the Folicur-treated sets. None of the other treatments increased the yield above that obtained from the control plots.

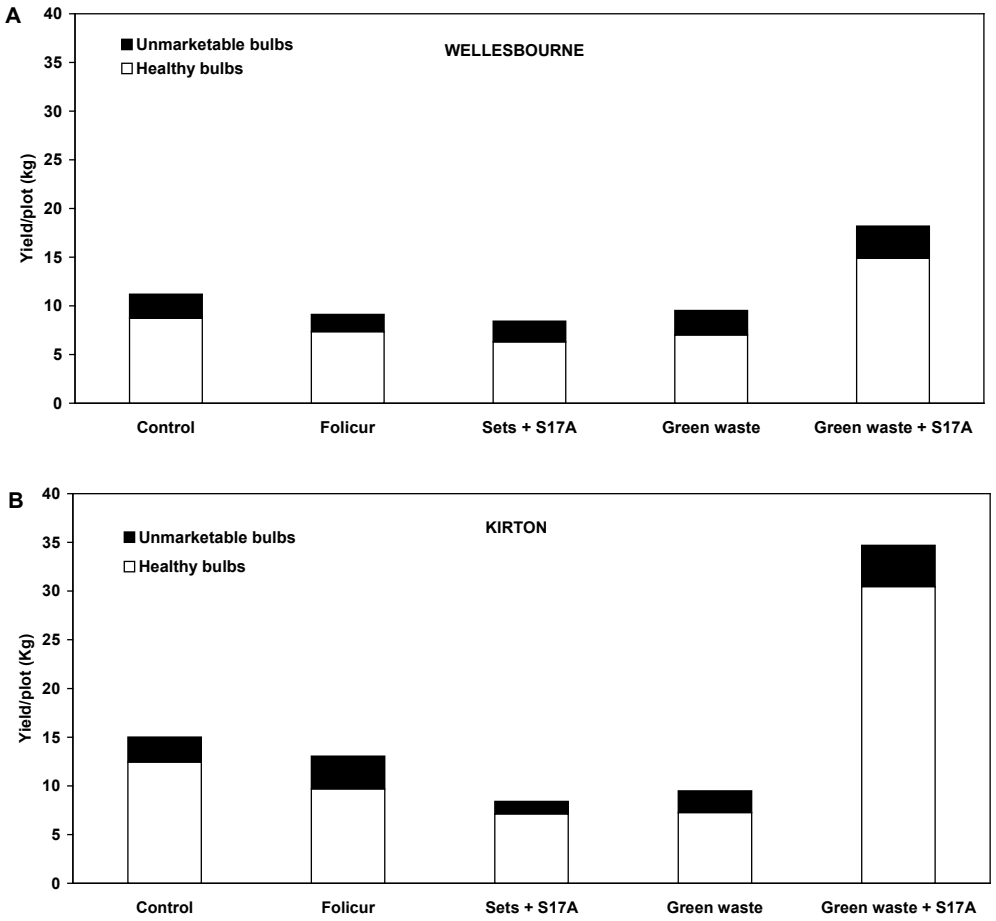

Figure 42: Onion yield (kg) from the various treatments at (a) Wellesbourne and (b) Kirton in 2008. Values are the mean of three replicate plots. Unmarketable bulbs = diseased, damaged, poor growth or shrivelled

Figure 43 shows the yield of bulbs in the three different size categories from the Wellesbourne trial. The green waste + *T. viride* S17A treatment increased the yield of the <40mm and 40-60 mm diameter bulbs compared with the control. The yield of bulbs from all the other treatments in the three different size categories was comparable to the control.

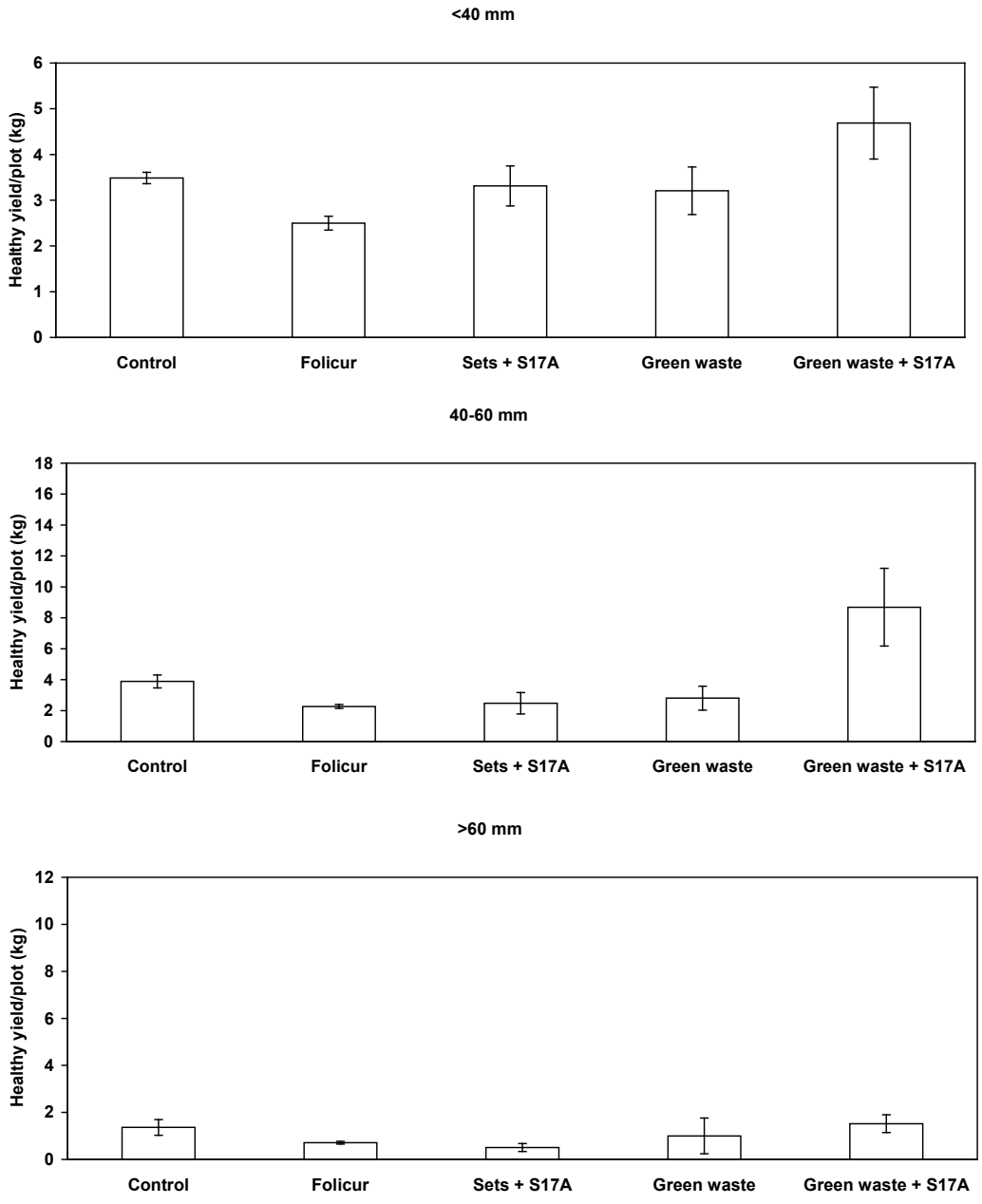

Figure 43: Healthy yield of onions from the various treatments in the three size categories, <40 mm, 40-60 mm and >60 mm diameter, from the Wellesbourne trial in 2008. Values are the mean of three replicate plots ± 1 standard error

Figure 44 shows the yield of bulbs in the three different size categories from the Kirton trial. The green waste + *T. viride* S17A treatment increased the yield of the 40-60 mm and >60 mm diameter bulbs compared with the control. The yield of bulbs from all the other treatments in these two size categories was comparable to the control.

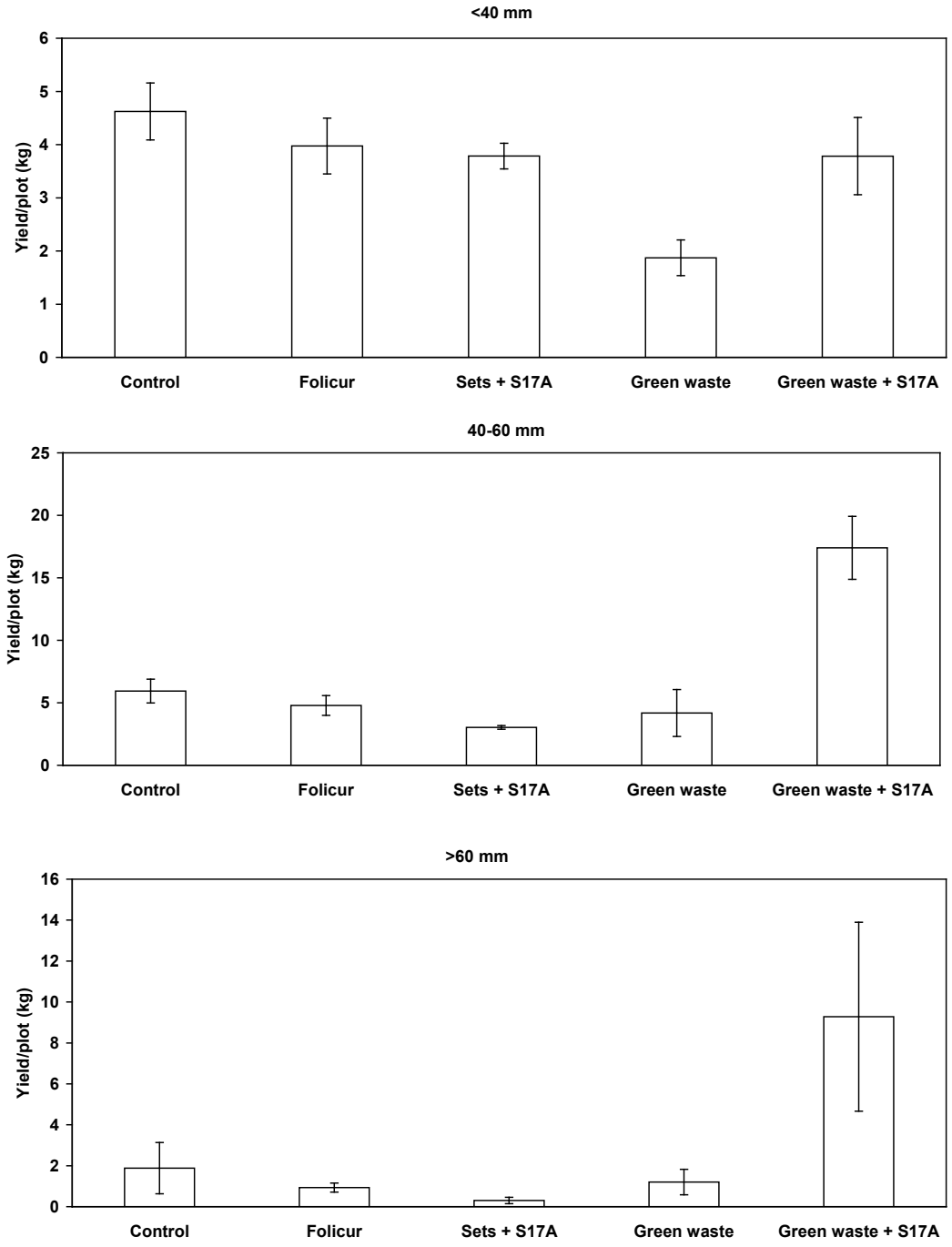

Figure 44: Healthy yield of onions from the various treatments in the three size categories, <40 mm, 40-60 mm and >60 mm diameter, from the Kirton trial in 2008. Values are the mean of three replicate plots ± 1 standard error

Trial 4: 2008

(i) Recovery of Trichoderma *spp. from field plots*

Table 22 details the level of *Trichoderma* spp. recovered from the various treatment plots at Wellesbourne throughout the growing season. Similar to Trial 1, a high level of *Trichoderma* spp. was recovered from the green waste + *T. viride* S17A treatment plots, indicating survival of the *T. viride* S17A applied to the plots in the green waste compost in 2007. The level of *Trichoderma* recovered from the other treatments was similar to the control (no compost or *T. viride* S17A applied).

Table 23 details the level of *Trichoderma* spp. recovered from the various treatment plots at Kirton throughout the growing season. In contrast to the results from the Wellesbourne trial (Table 22), recovery of *Trichoderma* spp. from the various treatments was similar to the control (Table 23). This may have been due to the severe flooding of the site during August 2007 which could have had an adverse effect on the survival of *T. viride* S17A in the soil.

Table 22: *Trichoderma* spp. recovered from the various treatment plots throughout the growing season (2008) at Wellesbourne (compost applied in 2007). Values are the mean of three replicate plots ± 1 standard error

Treatment	***Trichoderma* spp. (cfu g^{-1})**	
	June	**August**
Control	1.0×10^3 (± 0)	1.1×10^3 (± 1.11×10^2)
T. viride S17A sets	1.0×10^3 (± 0)	1.0×10^3 (± 0)
Green waste	1.0×10^3 (± 0)	1.0×10^3 (± 0)
Green waste + *T. viride* S17A	5.1×10^5 (± 6.56×10^4)	6.9×10^5 (± 7.08×10^4)

Table 23: *Trichoderma* spp. recovered from the various treatment plots throughout the growing season (2008) at Kirton (compost applied in 2007). Values are the mean of three replicate plots ± 1 standard error

Treatment	***Trichoderma* spp. (cfu g^{-1})**	
	June	**August**
Control	1.0×10^3 (± 0)	1.0×10^3 (± 0)
T. viride S17A sets	1.0×10^3 (± 0)	1.0×10^3 (± 0)
Green waste	1.0×10^3 (± 0)	1.0×10^3 (± 0)
Green waste + *T. viride* S17A	1.0×10^3 (± 0)	1.0×10^3 (± 0)

(ii) Emergence of sets in plots treated in 2007

The emergence of sets, planted in April 2008, in 2 x 1 m lengths within the treatment plots at Wellesbourne and Kirton is shown in Figure 45. At Wellesbourne, emergence in the different treatment plots used in Trial 1 in 2007 was very similar (Figure 45a). At Kirton, there was a higher emergence of sets in the plots that had received green waste in 2007 compared with the control (Figure 45b). Emergence in the other treatments was comparable to the control.

(iii) Allium *white rot assessment*

The AWR recorded in the 2 x 1 m lengths within the treatment plots at Wellesbourne and Kirton throughout the growing season is shown in Figure 46. Disease was recorded in all treatments on both sites. At Wellesbourne, similar to Trial 1, the green waste + *T. viride* S17A treatment was the most effective in controlling disease, indicating a carry over of effect of the 2007 applied treatment from one growing season to the next (Figure 46a). This is consistent with the high level of *Trichoderma* spp. recovered from this treatment (Table 22). No disease control was observed with the other treatments. At Kirton, in contrast to the results at Wellesbourne, there was no carry over of effect of the 2007 applied treatments, with no disease control observed in the green waste + *T. viride* S17A treatment (Figure 46b). Recovery of *Trichoderma* spp. from the various treatments in 2008 at Kirton was similar to the background level in the soil (Table 23), and this may explain the lack of disease control observed in this trial.

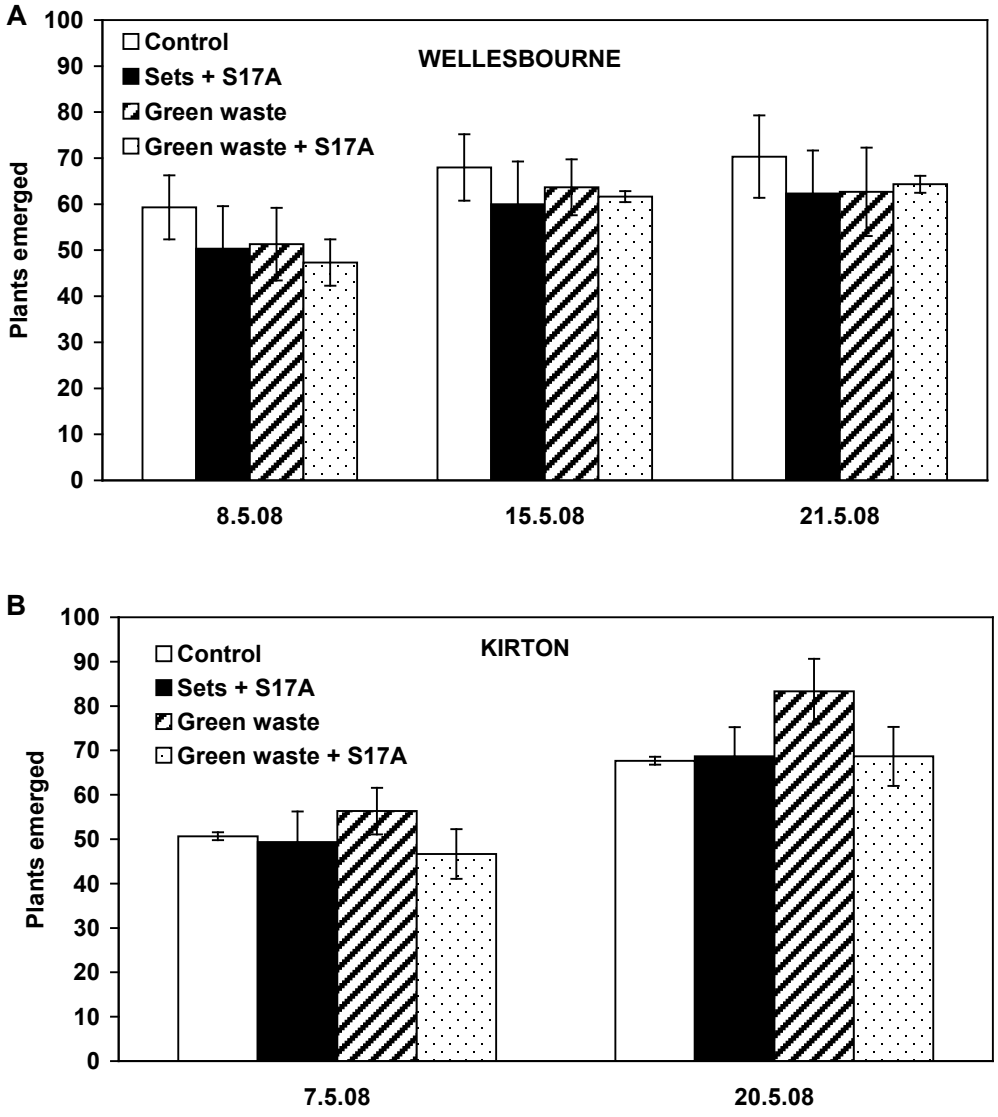

Figure 45: Emergence of sets (cv. Hercules), planted in April 2008, in 2 x 1 m lengths within the various treatment plots at (a) Wellesbourne and (b) Kirton, applied in 2007. Values are the mean of three replicate plots ± 1 standard error

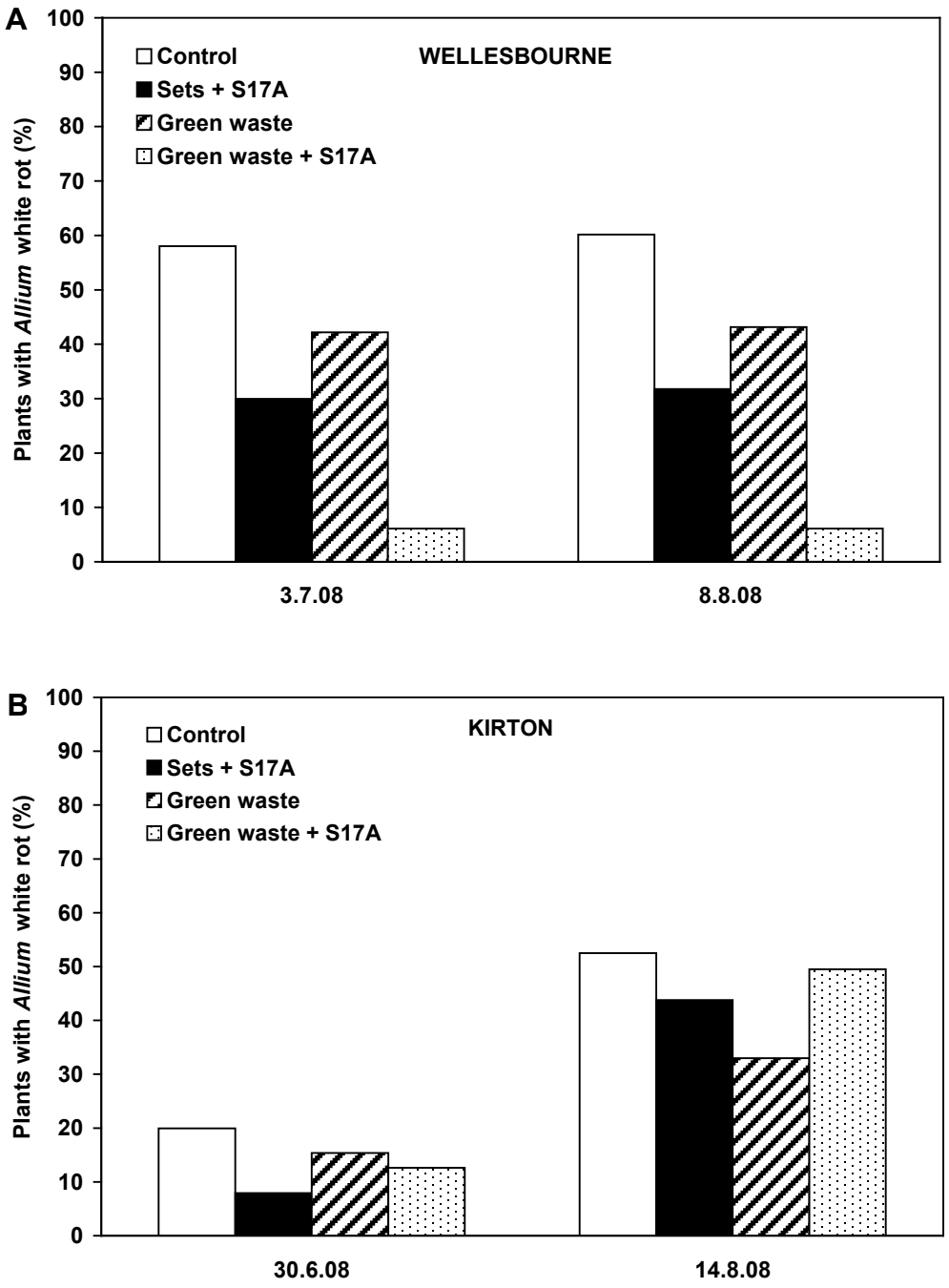

Figure 46: Onion plants (%), in the 2 x 1 m lengths within the treatment plots (compost applied in 2007), infected with *Allium* white rot throughout the growing season (2008) at (a) Wellesbourne and (b) Kirton. Values are the mean of three replicate plots

(iv) Onion yield

The total yield of onions harvested from each of the treatments at Wellesbourne and Kirton is shown in Figure 47. At Wellesbourne, similar to Trial 1 and Trial 3, the green waste + *T. viride* S17A treatment gave the highest healthy yield (Figure 47a). The yield from this treatment was more than twice as high as any of the other treatments. The yields from the *T. viride* S17A set treatment and green waste alone treatment were comparable to the control.

At Kirton, the yield from each of the treatments was higher than the respective treatments at Wellesbourne (Figure 47b). The green waste + *T. viride* S17A treatment gave the highest healthy yield, although the yield from each of the treatments at Kirton was very similar to the control.

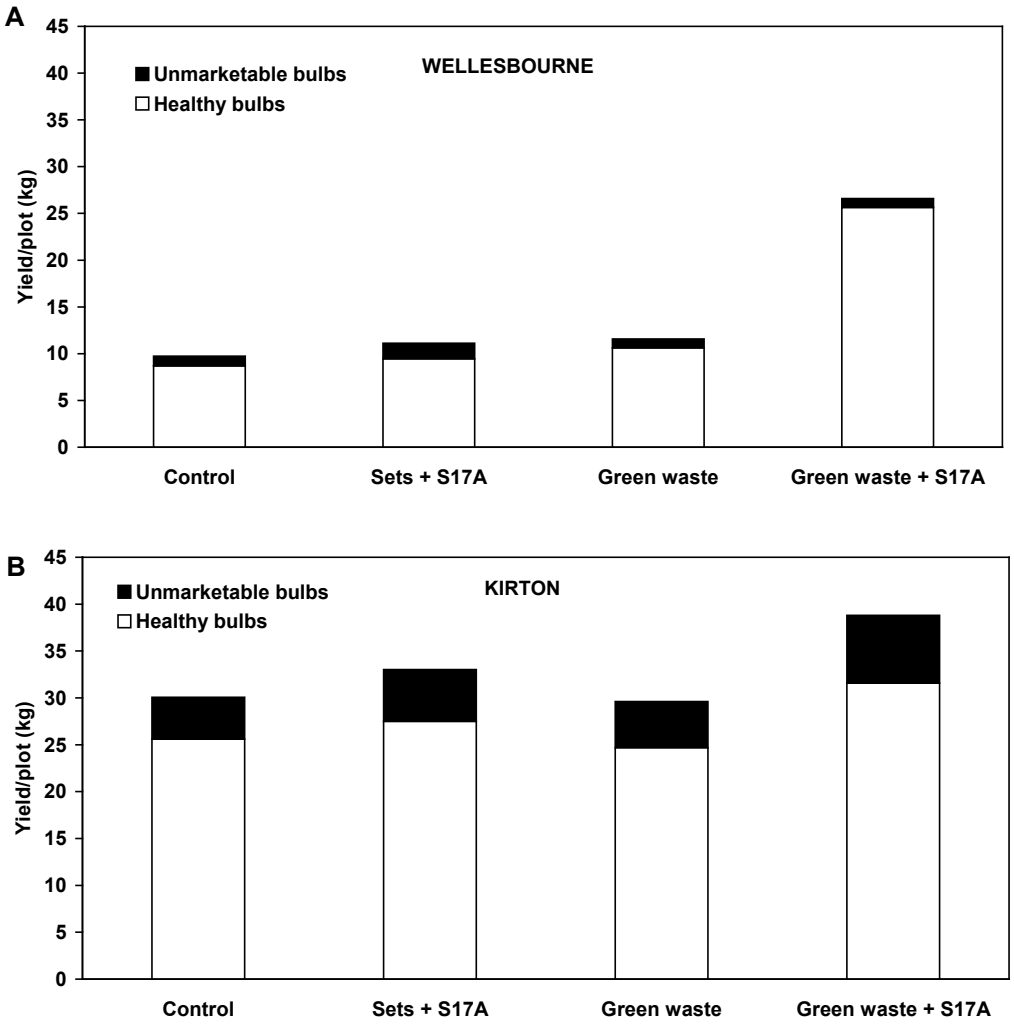

Figure 47: Onion yield (kg) from the various treatments (compost applied in 2007) at (a) Wellesbourne and (b) Kirton in 2008. Values are the mean of three replicate plots. Unmarketable bulbs = diseased, damaged, poor growth or shrivelled

Figure 48 shows the yield of bulbs in the three different size categories from the Wellesbourne trial. The green waste + *T. viride* S17A treatment increased the yield of bulbs from all three size categories compared with the control. The yield of bulbs from all the other treatments in the three different size categories was comparable to the control.

Figure 49 shows the yield of bulbs in the three different size categories from the Kirton trial. The green waste + *T. viride* S1A treatment increased the yield of the 40-60 mm and >60 mm diameter bulbs compared with the control. The yield of bulbs from all the other treatments in these two size categories was comparable to the control.

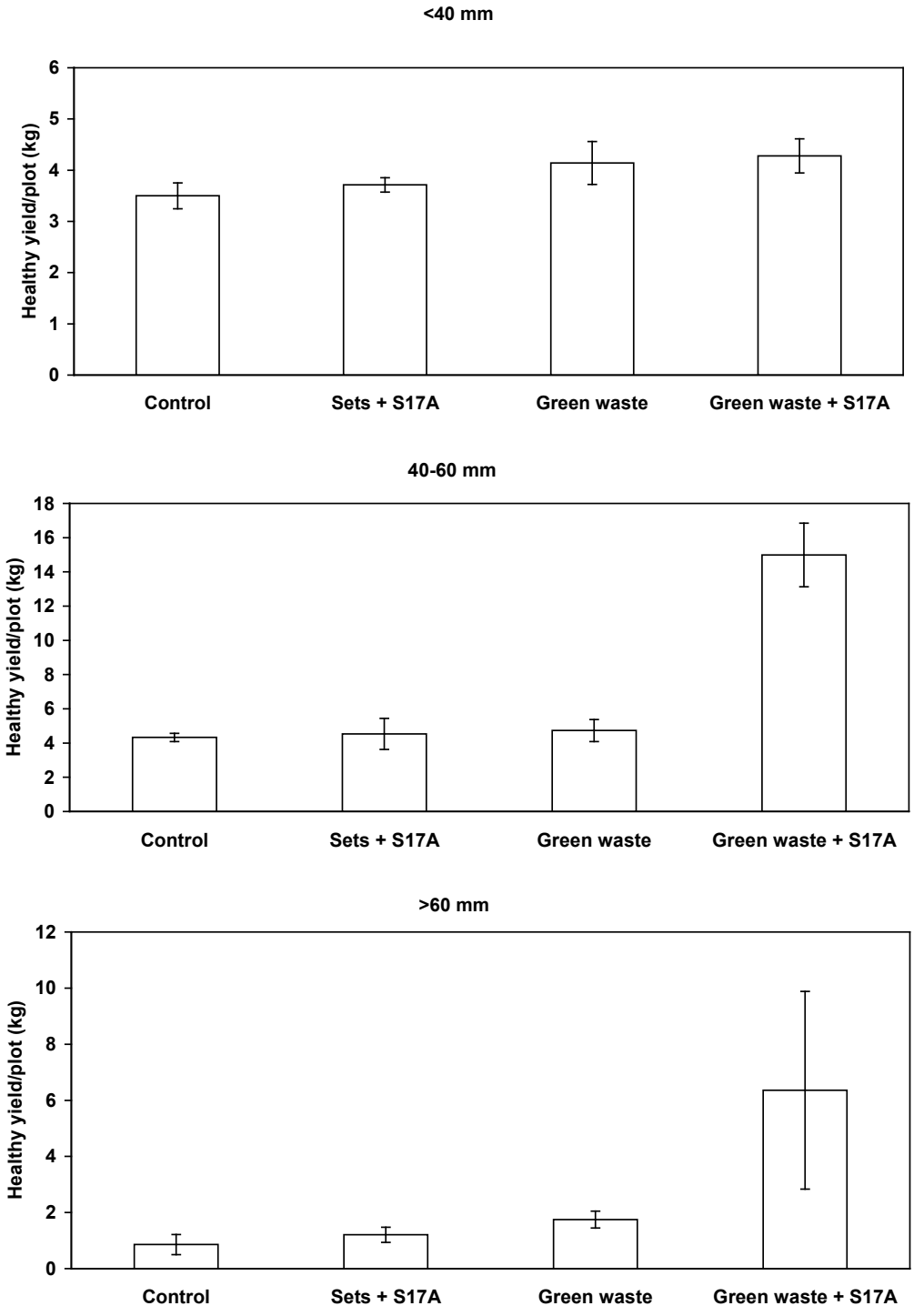

Figure 48: Healthy yield of onions from the various treatments (compost applied in 2007) in the three size categories, <40 mm, 40-60 mm and >60 mm diameter, from the replanted Wellesbourne 2008 trial. Values are the mean of three replicate plots ± 1 standard error

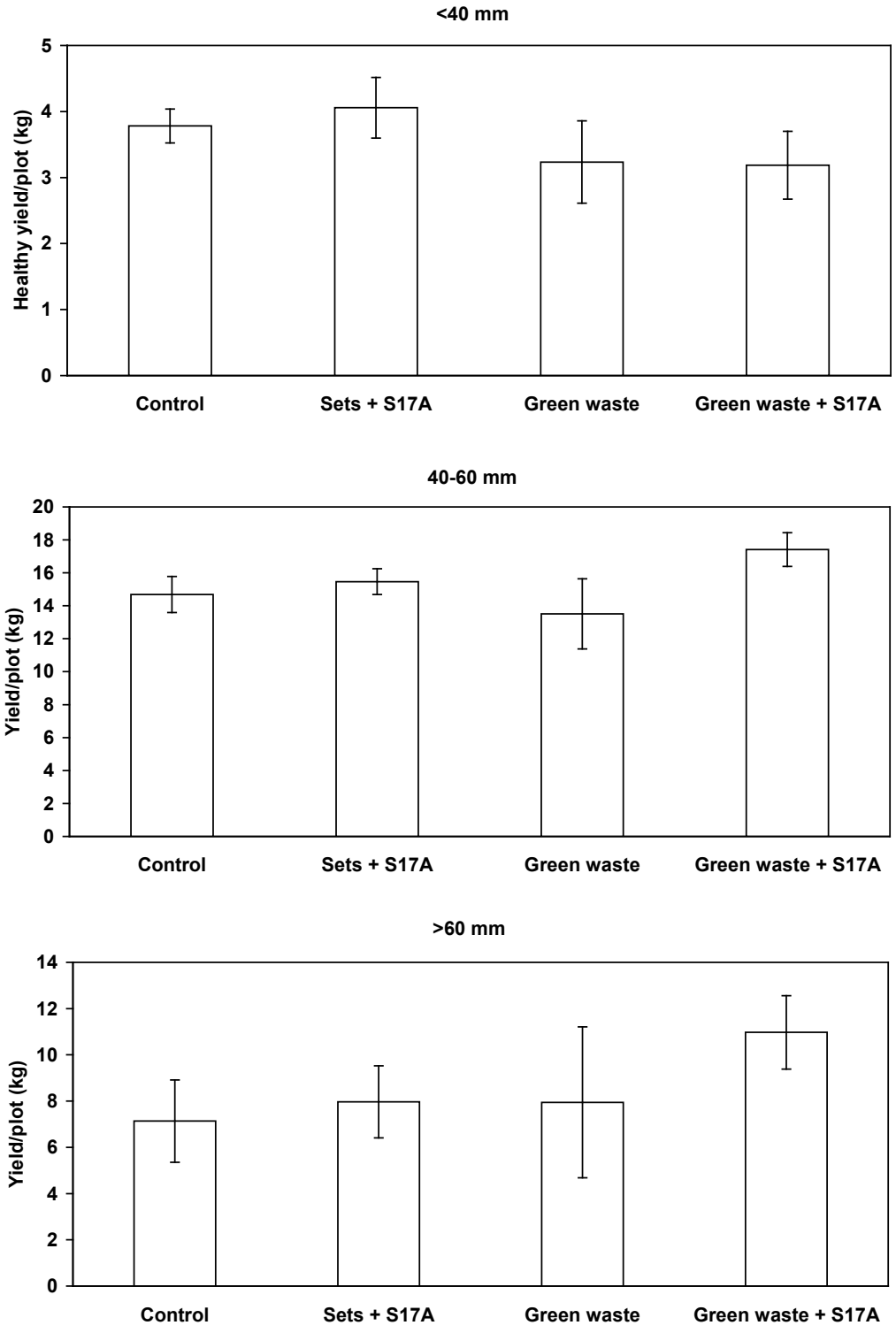

Figure 49: Healthy yield of onions from the various treatments (compost applied in 2007) in the three size categories, <40 mm, 40-60 mm and >60 mm diameter, from the replanted Kirton 2008 trial. Values are the mean of three replicate plots ± 1 standard error

(b) Growers trials

Trial 1 - Bedfordshire Growers Limited (2007)

(i) Recovery of Trichoderma *spp. from plots*

Similar to the field trials at Wellebourne and Kirton, a high level of *Trichoderma* spp. was recovered from the plots with green waste + *T. viride* S17A applied (4.5 x 10^6 cfu g^{-1}) and a low level (*c.* 10^3 cfu g^{-1}) from the green waste only and untreated plots.

(ii) Retrieval of sclerotia buried in plots

The viability of the sclerotia recovered from the plots after six months burial is shown in Figure 50. Similar to the results from the glasshouse green waste sclerotia viability bioassay, the treatments had no effect in reducing sclerotia viability.

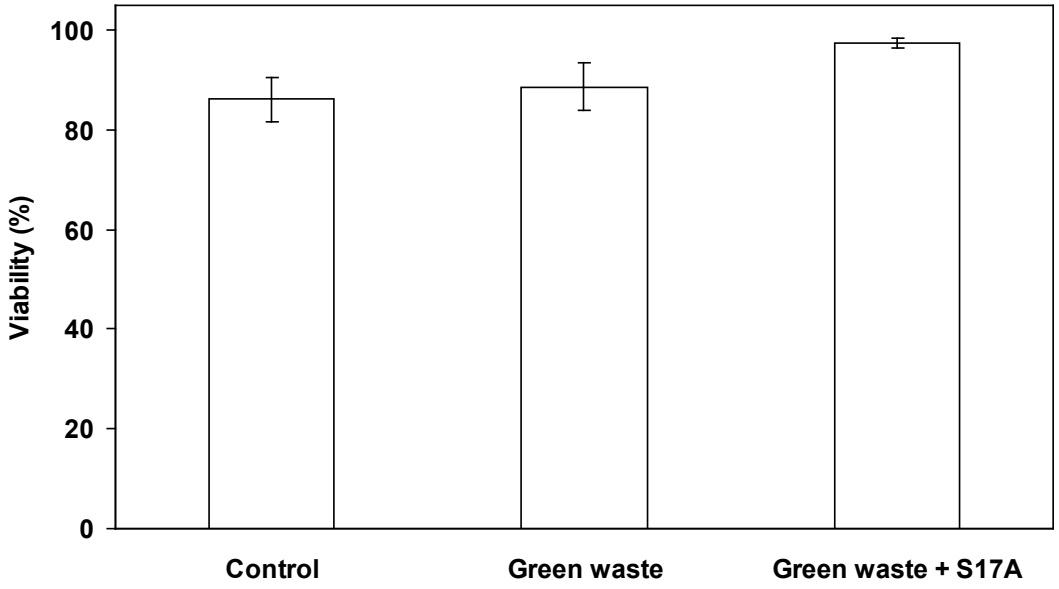

Figure 50: Viability of sclerotia recovered from the treatment plots after six months burial. Values are the mean of three replicate mesh bags, each containing 100 sclerotia ± 1 standard error

Trial 2 – Bedfordshire Growers Limited (2008)

(i) Emergence of sets

The emergence of sets, planted in April 2008, per row within the treated and untreated plots is shown in Figure 51. Emergence was similar in the treated and untreated plots with the three different set treatments.

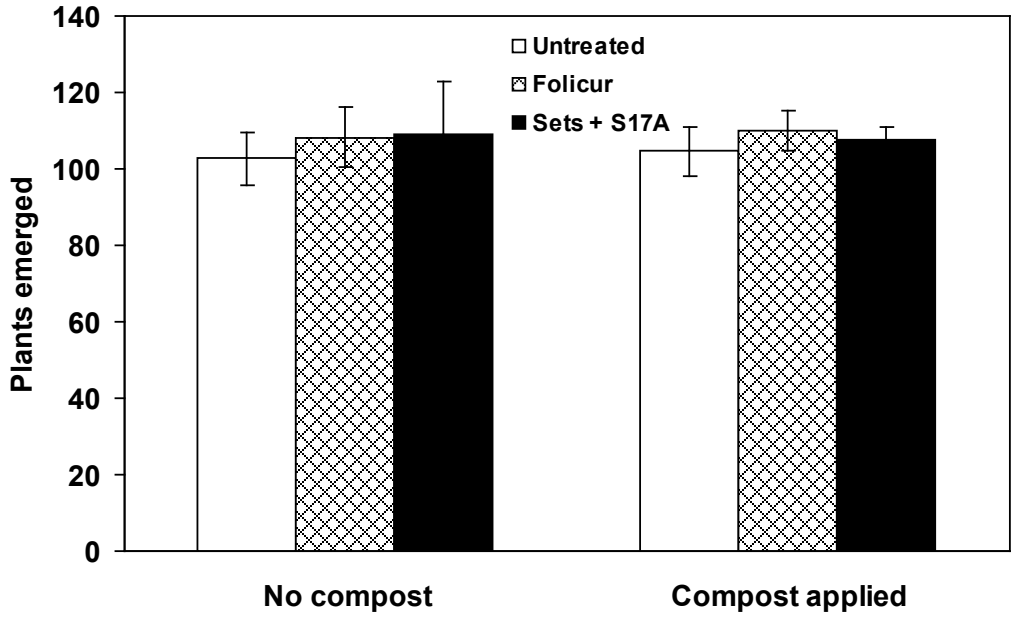

Figure 51: Emergence of sets (cv. Hercules - untreated, *T. viride* S17A-treated and Folicur-treated), planted in April 2008, per row within the treated (green waste compost applied) and untreated areas in the Bedfordshire Growers Limited trial. Values are the mean of five replicate rows ± 1 standard error

(ii) Allium *white rot assessment*

No AWR was recorded in any of the plots.

Trial 3 – Moulton Bulb Company Limited (2008)

(i) Recovery of Trichoderma *spp. from field plots*

The background level of *Trichoderma* spp. in the field soil prior to compost application was 1.4 x 10^3 cfu g^{-1}. Table 24 details the level of *Trichoderma* spp. recovered from the various treatment plots at Moulton Bulb Company Limited throughout the growing season. Similar to the trials at Warwick HRI, a consistently high level of *Trichoderma* spp. was recovered from the green waste + *T. viride* S17A treatment plots throughout the growing season (Table 24). In contrast, the level of *Trichoderma* spp. in the other treatments remained consistently low at a background level.

Table 24: *Trichoderma* spp. recovered from the various treatment plots throughout the growing season (2008) at Moulton Bulb Company Limited. Values are the mean of three replicate plots ± 1 standard error

Treatment	***Trichoderma* spp. (cfu g^{-1})**	
	June	**August**
Control	1.0×10^3 (± 0)	1.5×10^3 (± 4.08×10^2)
Folicur	1.0×10^3 (± 0)	1.0×10^3 (± 0)
Green waste	1.0×10^3 (± 0)	1.0×10^3 (± 0)
Green waste + *T. viride* S17A	1.9×10^6 (± 1.50×10^5)	1.5×10^6 (± 1.70×10^5)

(ii) Emergence of sets

The emergence of sets, planted in April 2008, in 2 x 1 m lengths within the treatment plots at Moulton Bulb Company Limited is shown in Figure 52. Emergence in the various treatments was similar to the untreated control.

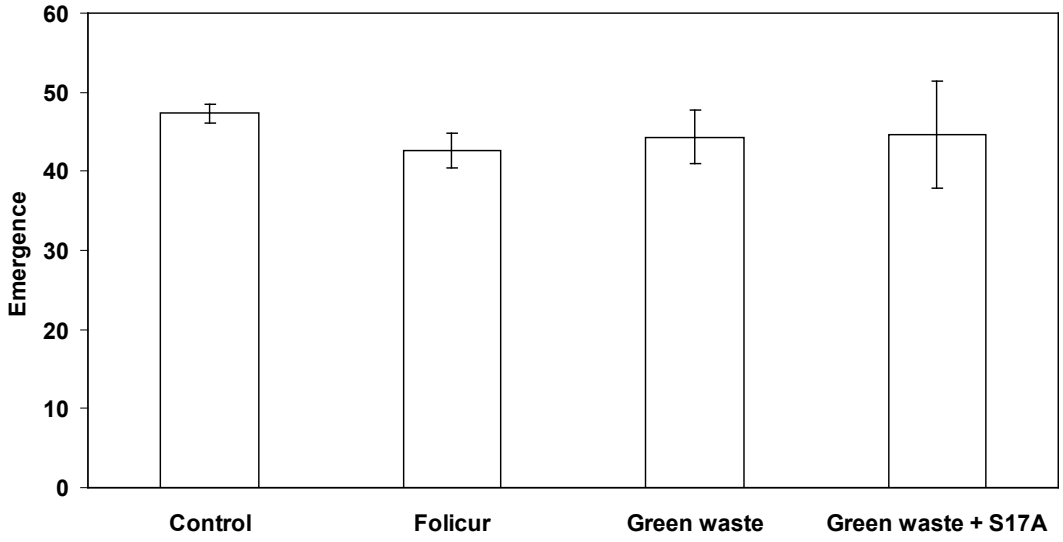

Figure 52: Emergence of sets (cv. Hercules), planted in April 2008, in 2 x 1 m lengths within the treatment plots at Moulton Bulb Company Limited. Values are the mean of three replicate plots ± 1 standard error

(iii) Allium *white rot assessment*

The AWR recorded in the treatment plots at Moulton Bulb Company Limited at harvest is shown in Figure 53. A low level of disease was recorded in all treatments. The green waste + *T. viride* S17A and green waste alone treatments reduced disease compared with the control and with the Folicur-treated sets.

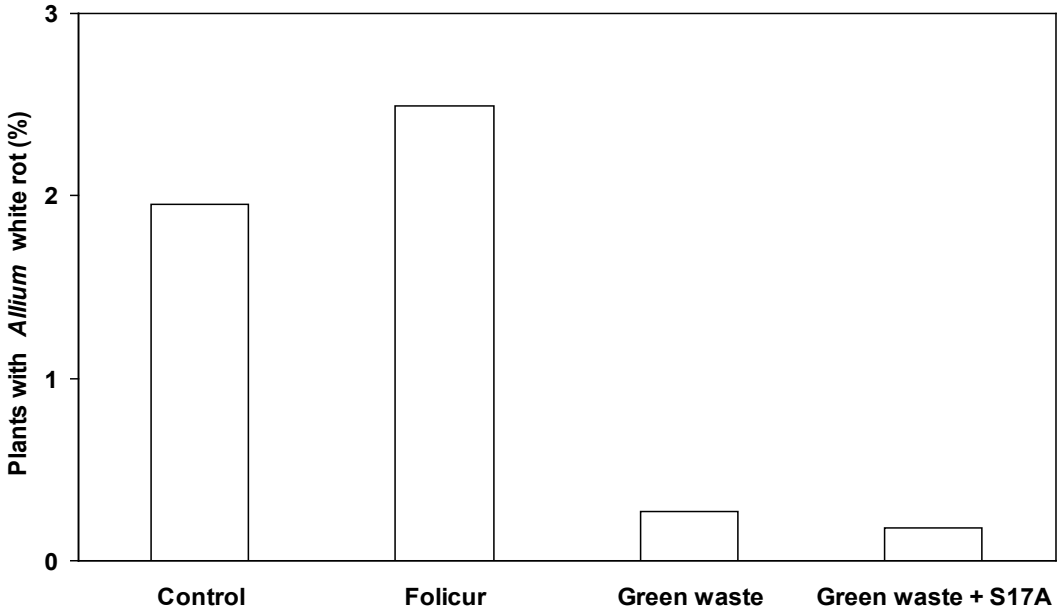

Figure 53: Onion plants (%), in the three centre rows within the treatment plots (*c.* 340 plants), infected with *Allium* white rot at harvest at Moulton Bulb Company Limited in 2008. Values are the mean of three replicate plots

(iv) Onion yield

The total yield of onions harvested from each of the treatments at Moulton Bulb Company Limited is shown in Figure 54. The yield from each of the treatments was very similar to the control.

Figure 55 shows the yield of bulbs in the three different size categories from the Moulton Bulb Company Limited trial. The compost treatments had no or very little effect on the yield from the three different size categories.

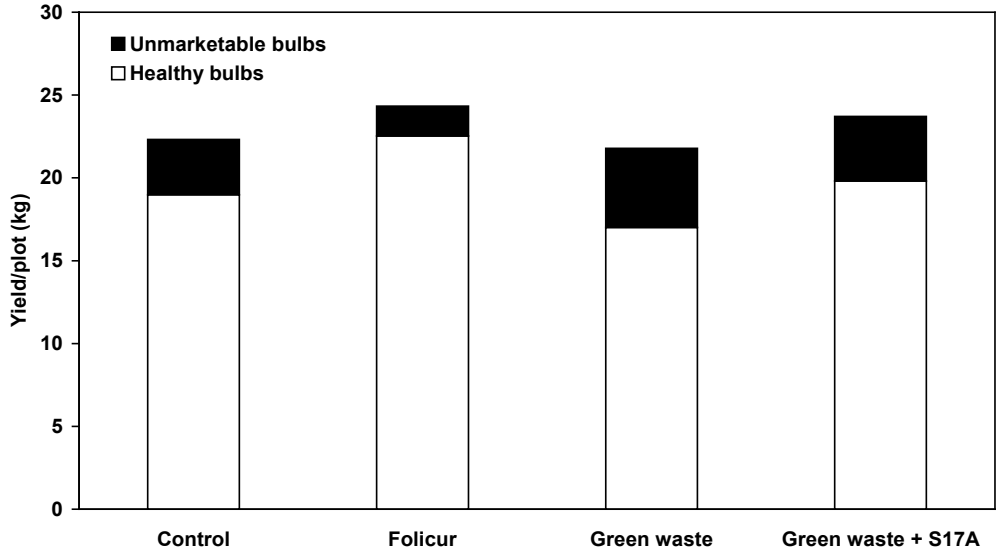

Figure 54: Onion yield (kg) from the various treatments at Moulton Bulb Company Limited in 2008. Values are the mean of three replicate plots. Unmarketable bulbs = diseased, damaged, poor growth or shrivelled

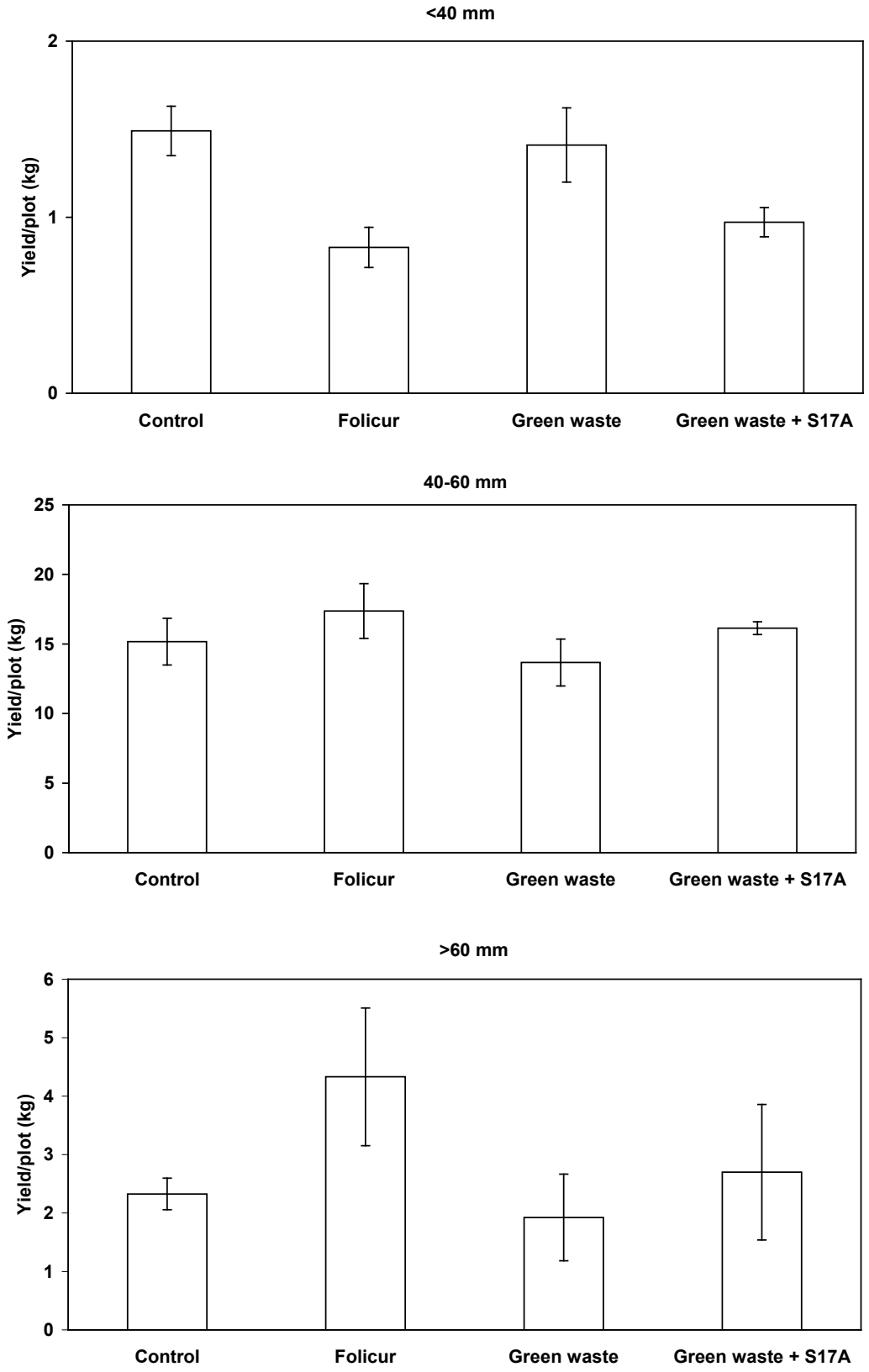

Figure 55: Healthy yield of onions from the various treatments in the three size categories, <40 mm, 40-60 mm and >60 mm diameter, at Moulton Bulb Company Limited in 2008. Values are the mean of three replicate plots ± 1 standard error

DISCUSSION

Colonisation of green waste compost by *T. viride* S17A (Milestone 1.2)

The preliminary *in vitro* growth studies on the effect of pH and conductivity on the growth of *T. viride* S17A (Milestone S1.3) indicated that the growth of *T. viride* S17A is sensitive to pH and conductivity. Growth was found to be fastest at low pH (pH 4.78) and conductivity (6.1 mS cm^{-1}), with growth inhibited at pH $\geq$ 6.68. Green waste compost of different ages, produced from various feedstocks (fruit, vegetables, parks and gardens waste), was sourced to provide a range of growing conditions for *T. viride* S17A. Despite the diversity of composts, the pH of all the green waste composts analysed was higher than the pH shown to inhibit the growth of *T. viride* S17A *in vitro*. However, in contrast to the *in vitro* results, *T. viride* S17A was found to grow well in all the composts tested and maintained a high level of colonisation over a 70 day period. The 1 year old Top soil conditioner from Organic Recycling Limited was selected to be used further in the project in glasshouse and field trials. However, the results from the green waste colonisation experiment indicated that *T. viride* S17A is not very specific in terms of the green waste compost on which it grows. Green waste compost can vary from region to region and even on one site throughout the year due to seasonal changes in feedstocks. The lack of green waste compost specificity of *T. viride* S17A is therefore a favourable characteristic that has potential to facilitate the widespread use of *T. viride* S17A-colonised composts.

Application of *T. viride* S17A to onion sets (Milestone 1.2)

T. viride S17A was successfully applied to onion sets. The treated sets delayed the onset of disease in the green waste-*T. viride* S17A Bioassay 1 (Milestone 2.1) and reduced AWR compared with the control in Field Trial 1 (Milestone 5.1). Set treatment with *T. viride* S17A therefore has potential as a control method for AWR. However, it is likely that *T. viride* S17A set treatment may need to be combined with another control method to reduce the amount of disease to an acceptable level. One possibility may be to combine *T. viride* S17A-treated sets with green waste compost application. In the glasshouse (Milestones 2.1, 2.2 and 4.1) and field trials (Milestones 5.1 and 5.2), *T. viride* S17A was recovered from all the *T. viride* S17A-colonised green waste compost treatments at the end of the onion growing period. In contrast, the *T. viride* S17A applied to onion sets did not proliferate in the soil and was not recovered at onion harvest. Application of green waste compost with *T. viride* S17A-treated sets may provide a food source for the organism on which it can grow and survive throughout the growing season. The combination of *T. viride*

S17A-treated sets and green waste compost was examined in the green waste-*T. viride* S17A bioassays (Milestones 2.1, 2.2 and 4.1) but the level of *T. viride* S17A recovered from these treatments was similar to the background level in the soil. Although the level of *T. viride* S17A on the sets at planting is high, it is not as high as the level in the *T. viride* S17A-colonised composts. In addition, the organism is concentrated on the sets and not in the surrounding soil into which roots grow. Application of a higher level of *T. viride* S17A to sets may increase the possibility of its proliferation in the surrounding soil/compost and survival throughout the growing season, and improve AWR control.

Effect of *T. viride* S17A-colonised green waste compost and onion sets on the control of *Allium* white rot (Milestones 2.1, 2.2 and 4.1)

T. viride S17A survived well in the green waste compost throughout the course of Bioassays 1 and 2. In Bioassay 1, no difference between the level of AWR in the control and any of the treatments was observed at the end of the bioassay. However, the *T. viride* S17A set treatments did delay the onset of disease in Bioassay 1 and control of AWR was observed with this treatment in the field (Milestone 5.1 – Warwick HRI Trial 1). In contrast, the Bioassay 1 results did not predict the control of AWR observed in the field with the *T. viride* S17A-colonised green waste compost (Milestones 5.1 and 5.2 – Warwick HRI Trials 1, 2 and 4). This may have been due to the difference between the controlled environmental conditions in the glasshouse and the variable conditions in the field. A higher rate (40%, v/v) of *T. viride* S17A-colonised green waste compost was included in Bioassay 2 to attempt to control AWR in the glasshouse. In addition, there was interest among the consortium in applying *T. viride* S17A-colonised green waste compost directly into the planting drill with sets. The green waste compost would therefore be concentrated in the planting zone rather than broadcast over the entire growing area, potentially exposing the sets to a high level of compost. The high rate of green waste compost used in Bioassay 2 would therefore give an indication as to whether this would impact on onion growth.

Preliminary *in vitro* experiments during year 1 indicated that growth of *T. viride* S17A was greatly reduced in the presence of Folicur (Milestone S1.3). The combination treatment in Bioassay 2 (40% *T. viride* S17A-colonised green waste compost with Folicur-treated sets) allowed the feasibility of combining Folicur with *T. viride* S17A in a real system to be examined. It has been shown that *T. viride* S17A colonises and survives well in green waste compost (Milestones 1.2 and 2.1). In allowing *T. viride* S17A to establish in the system prior to planting Folicur-treated sets

this may reduce any detrimental effects of the fungicide on its growth. In Bioassay 2, in contrast to Bioassay 1, all the green waste compost and *T. viride* S17A-colonised green waste compost treatments reduced AWR compared with the control, with the 40% green waste + *T. viride* S17A treatment being the most effective in controlling disease. The high rate of compost (40%) had no deleterious effect on onion growth. In both bioassays, the plants grown in the green waste compost alone and *T. viride* S17A-colonised green waste compost treatments were significantly heavier than those grown in soil alone. The survival of *T. viride* S17A was not affected by the presence of fungicide on the Folicur-treated sets in that a similar level of *T. viride* S17A was recovered from the *T. viride* S17A-colonised green waste compost and combination treatments.

Effect of sulphur-containing composted wastes on the viability of sclerotia of *S. cepivorum* and the control of *Allium* white rot (Milestones 3.1 and 3.2)

A number of sulphur-containing compounds were detected in the various compost treatments in Bioassays 1 and 2, including the known sclerotia germination stimulant, dipropyl disulphide. In both bioassays, volatiles from the OWCs stimulated germination of sclerotia over time, whereas no stimulation of germination was observed with the other treatments. The volatiles released from the high N poultry manure compost also had an effect on sclerotia in that viability was greatly reduced, suggesting that volatiles released from this treatment were toxic to sclerotia. The OWCs and high N poultry manure compost treatments also reduced the viability of sclerotia buried in these soil-compost mixtures. The high N poultry manure treatment effectively reduced AWR compared with the control in both bioassays. In contrast, despite the reduction in sclerotia viability recorded with the OWCs in both bioassays, there was variation in the effect of the OWCs on the control of AWR between Bioassay 1 and 2. In Bioassay 1, the OWCs had no effect on AWR whereas in Bioassay 2, the OWCs effectively controlled the disease. OWC has previously been shown to control AWR in the glasshouse and in the field. Previous work has used a 50% incorporation rate, twice as high as used in these bioassays, and the variation in the glasshouse results between bioassays may have been due to the 25% rate used being near to the limit to achieve effective disease control.

Similar variation between bioassays was observed with the Brassica composts although this may have been partly due to the more variable constituents of these composts between bioassays. In Bioassay 1, the Brassica compost appeared to stimulate the pathogen in that the level of disease in this treatment was much higher than in the control. This phenomenon has been observed before in the

field in that onions grown after a Brassica crop have been found to have a higher level of AWR (A. Stewart, pers. comm.) and this has implications in planning a crop rotation. In Bioassay 2 (Milestone 3.2), the low N poultry manure compost was included to prevent any potential phytotoxicity as a result of ammonia released from the high N poultry manure compost in Bioassay 1. Dräger tube measurements confirmed a lower level of ammonia was released from the low N compared with the high N poultry manure compost. However, unlike the high N poultry manure compost, the volatiles released from the low N poultry manure compost showed no effect on sclerotia viability or the control of AWR.

Field trials (Milestones 5.1 and 5.2)

Green waste and *T. viride* S17A trials

Similar to the glasshouse bioassay results (Milestones 2.1, 2.2 and 4.1), *T. viride* S17A survived in the presence of green waste compost at a high level under field conditions throughout the onion growing season. *T. viride* S17A-colonised green waste compost applied 2-4 weeks before set planting effectively controlled AWR in four field trials with very high disease pressure. However, similar to the results from the sclerotia viability bioassay (Milestone 2.1), *T. viride* S17A had no effect on sclerotia viability, suggesting that AWR control may have been due to an effect on the mycelium of *S. cepivorum*. The *T. viride* S17A-colonised green waste compost gave a comparable or higher onion yield to Folicur-treated sets in four field trials. In addition, all the green waste treatments in Trial 1 increased the yield of larger bulbs. A similar result was obtained in the *T. viride* S17A-green waste bioassays (Milestones 2.1, 2.2 and 4.1) in that the plants grown in the green waste compost treatments were significantly heavier than those grown in soil alone. These results indicate that colonised green waste compost not only offers an alternative to Folicur to control AWR, but may contribute to the nutrition of the crop. As well as the *T. viride* S17A-colonised green waste compost treatment, the *T. viride* S17A set treatment was shown to reduce AWR. The control achieved with the *T. viride* S17A set treatment was far less efficient than with the colonised compost.

Whilst control of AWR is the primary concern of this project, the cost of effective treatments is an important consideration. The colonised compost treatment was as effective as Folicur, but broadcast over the entire planting area, this control method is considerably more expensive than the use of Folicur. In addition, the level of compost applied (25% v/v, *c.* 72 t ha^{-1}) is above that permitted under the Nitrates Directive (30 t ha^{-1}). In an attempt to reduce the quantity of compost used and target

the treatment, preliminary trials are underway at Moulton Bulb Company Limited and G's Marketing Limited to determine the feasibility of applying compost within the planting drills at set planting. The results from the replant trials (Trial 4) offer the potential for a further saving in compost application in that *T. viride* S17A was found to survive from one growing season to the next and the *T. viride* S17A-colonised green waste compost continued to control AWR in the second growing season. This treatment may not therefore need to be applied yearly, and further work is required to determine for how many growing seasons its efficacy extends.

Sulphur-containing composted wastes trials

OWC applied at a 50% (v/v) incorporation rate has previously been shown to control AWR. Unfortunately there is insufficient onion waste to treat all AWR-infested land with this incorporation rate. One approach in this project to address this problem was to reduce the compost incorporation rate to 25%; the other was to examine the potential of other sulphur-containing composted wastes for controlling the disease. On the basis of the glasshouse bioassay results (Milestones 3.1 and 3.2), windrow OWC and poultry manure compost were selected to be used in the field trials. The glasshouse bioassays (Milestones 3.1 and 3.2) did not however consistently predict the effect of these composts in the field. Windrow OWC, applied at a 25% incorporation rate 18 months prior to onion planting, effectively controlled AWR in two field trials with very high disease pressure throughout the growing season. In addition, windrow OWC applied six months prior to planting effectively controlled AWR in one field trial. The windrow OWC treatments gave at least three times higher an onion yield than the yield from Folicur-treated sets and increased the yield of larger bulbs. Similar to the results from the glasshouse bioassays, both the windrow OWC and high nitrogen poultry manure compost reduced sclerotia viability in the field. However, in contrast to the bioassay results, no control of AWR was observed with the poultry manure compost in the field. Similar to the green waste and *T. viride* S17A trials, this result was presumably due to the difference in environmental conditions in the glasshouse and field.

CONCLUSIONS

Laboratory and Glasshouse Bioassays – *Trichoderma viride* S17A

- Growth of *T. viride* S17A *in vitro* is sensitive to pH and conductivity. Growth was fastest at the lowest pH (4.78) and conductivity (6.1 mS cm^{-1}) tested and decreased as these increased, with no growth at pH ≥ 6.68.
- Growth of *T. viride* S17A *in vitro* is inhibited by low concentrations (0.01%) of Folicur.
- The pH of all the green waste composts analysed was higher than the pH shown to inhibit growth of *T. viride* S17A *in vitro*. The conductivity of these composts (0.91-2.96 mS cm^{-1}) was well below the conductivity shown to reduce growth of *T. viride* S17A *in vitro*.
- Growth of *T. viride* S17A was not inhibited by the above neutral (>7) pH of the green waste composts. *T. viride* S17A colonised all the green waste composts selected, alone and with the addition of peat.
- Following application of *T. viride* S17A to soil and soil-green waste compost mixtures, its level remained high for 70 days after inoculation. The level was generally unaffected by amendment of soil with green waste composts or peat.
- *T. viride* S17A survived on onion sets at least up to three weeks post-inoculation.
- *T. viride* S17A survived in soil-green waste compost mixtures at a high level for 23 weeks.
- Incorporation of *T. viride* S17A-colonised green waste compost (25% or 40% v/v) into soil effectively reduced AWR.
- The addition of green waste compost to soil increased plant growth in the absence of the pathogen, *S. cepivorum*.
- Even in the absence of any obvious symptoms of AWR (mycelium, sclerotia), the pathogen *S. cepivorum* had a negative effect on plant growth. Plants grown in inoculated (sclerotia added) treatments had a lower plant weight than those grown in uninoculated treatments.

Laboratory and Glasshouse Bioassays – Sulphur-containing composts

- The sulphur content of the sulphur-containing wastes varied from 1280 mg kg^{-1} in the windrow OWC to 6850 mg kg^{-1} in the poultry manure compost. A number of sulphur-containing compounds were detected in these wastes.

- The known sclerotia germination stimulant, dipropyl disulphide, was detected in both the flask and windrow OWC; tert-butyl mercaptan was only detected in the poultry manure compost.

- Volatiles from onion waste compost stimulate the germination of sclerotia of *S. cepivorum.* High N poultry manure/prunings compost produced the most volatile sulphur compounds and ammonia and was most effective in reducing the viability of sclerotia.
- Onion waste compost and high N poultry manure/prunings compost effectively reduced sclerotia viability.
- Incorporation of OWC or high N poultry manure compost effectively reduced AWR.
- Onion waste and poultry manure composts showed no phytotoxicity to plant growth, 10 months after soil incorporation.

Warwick HRI Field Trials

Results from the glasshouse bioassays did not always predict the control results obtained in the field; this may be due to the difference in environmental conditions in the glasshouse and field.

Green waste and *Trichoderma viride* S17A trials

- *T. viride* S17A-colonised green waste compost applied 2-4 weeks before set planting effectively controlled AWR in four field trials in two years with very high disease pressure throughout the growing seasons.
- *T. viride* S17A set treatment reduced AWR except when the disease pressure was very high.
- *T. viride* S17A-colonised green waste compost gave a comparable or higher onion yield to Folicur-treated sets in the field trials.
- *T. viride* S17A survived at a high level under field conditions throughout an onion growing season in the presence of green waste compost.
- *T. viride* S17A had no effect on sclerotia viability after two weeks in contact with sclerotia in soil; control of AWR may have been due to an effect on *S. cepivorum* mycelium.
- *T. viride* S17A survived at high levels in the presence of green waste compost from one season to the next.

- *T. viride* S17A-colonised green waste compost applied in 2007 effectively controlled AWR, where *T. viride* S17A survived, in 2008 and gave a yield twice as high as any of the other treatments.

Sulphur-containing composted wastes trials

- Windrow OWC applied 18 months prior to planting effectively controlled AWR in two field trials with very high disease pressure throughout the growing season. Windrow OWC applied six months prior to planting also effectively controlled AWR in one field trial but not in another.
- The windrow OWC treatments gave at least three times higher an onion yield than the yield from Folicur-treated sets.
- The windrow OWC treatments increased the yield of larger bulbs.
- Windrow OWC and high nitrogen poultry manure compost reduced sclerotia viability after 10 months contact with sclerotia in soil.

Growers Field Trials

- *T. viride* S17A survived at high levels in the presence of green waste compost throughout the growing season at Moulton Bulb Company Limited and effectively controlled AWR.
- *T. viride* S17A survived at high levels in the presence of green waste compost throughout the growing season at Bedfordshire Growers Limited but had no effect on sclerotia viability after six months in contract with sclerotia in soil.

COST/BENEFIT ANALYSIS

The costs and benefits of *Trichoderma*-colonised compost treatments are compared with a conventional Folicur spray programme in five Scenarios (A-E) in Tables 25 to 29. The scenarios make the following assumptions:

- The level of AWR control, onion yield and size grades are comparable in the compost and Folicur treatments (the results here indicate that these factors may be better from the compost treatment).
- The compost treatment would be suitable for organic production; however, no additional value of the crop has been considered.
- In Scenarios A, B and C, AWR control using compost is for a single season; in Scenario D it is for two seasons, and in Scenario E, it is for four seasons.
- The costs for applying compost within the planting row are the same as for broadcasting (about £90/hectare).

- A slightly higher rate of *Trichoderma* spawn (0.2%) is needed at the lowest rate of compost application, otherwise a 0.15% rate is used.
- Where broadcast, compost is applied at the maximum rate allowed within a Nitrate Vulnerable Zone (NVZ) of 30 tonnes/hectare.
- The nutrient value of the compost applied within the planting row at 5 and 7 tonnes/hectare is 50 and 60% of the nutrient value of compost applied across the entire land at 30 t/ha.

The analysis in Scenario A indicates that applying *Trichoderma*-colonised compost at 30 t/ha would be £66/ha more expensive than a Folicur spray programme (Table 25). Applying colonised compost only within the planting row at 7 t/ha (Scenario B) would also be £28/ha more expensive than a Folicur spray programme (Table 26). However, applying colonised compost at 5 t/ha (Scenario C) would almost break-even with a Folicur spray programme (Table 27).

If *Trichoderma*-colonised compost, broadcast at 30 t/ha were effective in AWR control for 2 seasons (Scenario D), the treatment would be £34/ha cheaper than a Folicur spray programme (Table 28). This saving would increase to £154/ha over four seasons (Scenario E) if uncolonised (plain) compost was applied to land at 30 t/ha in Year 3 to "top-up" the food source for the *Trichoderma* applied in Year 1 (Table 29).

Table 25: Scenario A, 30 t compost / hectare, broadcast

	£ per hectare		
	COST	**SAVING**	**NET**
Unscreened compost, £4/tonne	120		
Delivery, 20 miles, tractor and trailer, £4/tonne	120		
Application cost, £3/tonne	90		
Trichoderma spawn, 0.15% @ £1.9/kg	86		
Inorganic fertiliser		250	
Folicur, 2 sprays @ £50 each		100	
TOTAL	**416**	**350**	**-66**

Table 26: Scenario B, 7 t compost / hectare, in planting rows

	£ per hectare		
	COST	**SAVING**	**NET**
Screened compost, £20/tonne	140		
Delivery, 20 miles, tractor and trailer, £4/tonne	28		
Application cost (same as broadcast)	90		
Trichoderma spawn, 0.15% @ £1.9/kg	20		
Inorganic fertiliser		150	
Folicur, 2 sprays @ £50 each		100	
TOTAL	**278**	**250**	**-28**

Table 27: Scenario C, 5 t compost / hectare, in planting rows

	£ per hectare		
	COST	**SAVING**	**NET**
Screened compost, £20/tonne	100		
Delivery, 20 miles, tractor and trailer, £4/tonne	20		
Application cost (same as broadcast)	90		
Trichoderma spawn, 0.2% @ £1.9/kg	19		
Inorganic fertiliser		125	
Folicur, 2 sprays @ £50 each		100	
TOTAL	**229**	**225**	**-4**

Table 28: Scenario D, 30 t compost / hectare, broadcast

AWR control efficacy for 2 seasons

	£ per hectare		
	COST	**SAVING**	**NET**
Unscreened compost, £4/tonne	120		
Delivery, 20 miles, tractor and trailer, £4/tonne	120		
Application cost, £3/tonne	90		
Trichoderma spawn, 0.15% @ £1.9/kg	86		
Inorganic fertiliser		250	
Folicur, 4 sprays @ £50 each		200	
TOTAL	**416**	**450**	**+34**

Table 29: Scenario E, 30 t *Trichoderma*-colonised compost / hectare, broadcast, in Year 1; 30 t Uncolonised compost broadcast in Year 3

AWR control efficacy for 4 seasons

	£ per hectare		
	COST	**SAVING**	**NET**
Unscreened compost, £4/tonne in Years 1 and 3	240		
Delivery, 20 miles, tractor and trailer, £4/tonne in Years 1 and 3	240		
Application cost, £3/tonne in Years 1 and 3	180		
Trichoderma spawn, 0.15% @ £1.9/kg (Year 1)	86		
Inorganic fertiliser in Years 1 and 3		500	
Folicur, 8 sprays @ £50 each		400	
TOTAL	**746**	**900**	**+154**

TECHNOLOGY TRANSFER

Publications

- LINK leaflet: Integrated *Allium* white rot control using composted onion waste and fungi HL0176
- R. Noble: Control of *Allium* white rot using composts. Proceedings of 2007 Carrot and Onion Conference, Peterborough
- Biocontrol agent targets AWR. The Vegetable Farmer, 1st January 2008, p24
- Composts rival fungicides for white rot control, HDC News, February 2008, p9
- Conference will highlight new control for onion white rot, Horticulture Week, 9th January 2009
- Science into practice. Onion white rot control with composted waste. Horticulture Week, 13th February 2009, p28, 34
- Fungus wins against white rot in the onion field. HDC News 151:9.

Presentations

- R. Noble: Carrot and Onion Conference, Peterborough, November 2007
- E. Coventry: Elsoms Seeds Bulb Onion Conference, November 2008
- R. Noble: UK Vegetable Conference, Peterborough, January 2009

Poster

- Integrated *Allium* white rot control using composts and *Trichoderma viride*. HortLINK event, London, November 2007

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APPENDICES

APPENDIX 1: BIOASSAY 1

Mean concentration (ppm) of volatiles detected by GCMS in the Kilner jar microcosms 3 days after set up

Volatile Detected	Mean concentration (n = 3)					
	Kirton Soil	Flask OWC	Windrow OWC	*Brassica*	Poultry	Sewage
Sulphur-containing volatiles						
Allyl methyl sulfide	0.0000	0.0951	0.7971	0.0257	0.0002	0.0000
Benzothiazole	0.9972	1.0239	1.4437	1.0434	1.0087	0.9161
Dimethyl disulfide	0.0210	0.2120	1.0874	0.6509	2.8717	0.0500
Dimethyl sulfide	0.0046	0.1624	21.4379	0.2384	0.4588	0.0000
Dimethyl Sulfoxide	0.0018	0.0649	1.0269	0.1156	0.0063	0.0310
Dipropyl, disulfide	0.0244	0.5060	8.1624	0.0206	0.0103	0.0072
Methyl propyl disulfide	0.0000	1.0258	2.0119	0.0198	0.0183	0.0000
Methyl-trans-propenyl-disulfide	0.0000	0.4758	0.0416	0.0214	0.0288	0.0031
Propane, 1-(methylthio)-	0.0065	1.1530	15.1932	0.0351	0.0792	0.0907
1-Propanethiol	0.0000	0.0049	1.7580	0.0000	0.0000	0.0000
1-Propene, 1-(methylthio)-(E)	0.0000	0.0945	1.0230	0.0264	0.0002	0.0089
1-Propene, 1-(methylthio)-, (Z)	0.0000	0.2534	3.1565	0.0229	0.0006	0.0000
1-Propene, 3-(methylthio)-	0.0000	0.0810	0.7886	0.0258	0.0006	0.0000
Sulfur dioxide	0.0000	0.3991	0.0000	0.3144	0.0000	0.0305
Thiophene, 2,5-dimethyl	0.0000	0.1341	0.0515	0.0340	0.0041	0.0112
Other compounds						
Acetic acid	0.4596	2.9577	0.7156	3.0146	0.5057	0.6351
Acetone	0.3854	1.9021	1.6099	0.6118	0.1498	0.0495
Acetophenone	0.0138	6.8599	6.4655	0.6066	0.0217	3.7935
Benzaldehyde	0.0925	3.9912	2.8084	0.8545	0.0925	2.8236
Benzene	0.4683	5.6252	2.5857	0.8636	0.5403	4.8057
Benzene, ethynyl-	0.0000	1.9256	0.9621	0.0010	0.0032	0.4537
Benzeneacetaldehyde	0.0533	7.5030	9.4090	0.3099	0.0189	2.3038
Benzoic acid	0.0000	3.2180	0.1634	1.8998	0.2476	1.2139
Benzoic acid, phenyl ester	0.0000	0.7549	0.0165	0.2341	0.0132	0.1664
Benzophenone	0.0000	3.8189	0.7323	1.5838	0.1774	2.0777
Butanoic acid	0.0225	0.0490	0.3695	0.0377	0.5662	0.0162
Butanoic acid	0.0092	0.2195	2.3667	0.5649	0.1009	0.1759
2-Butanol	0.0000	0.0000	20.1775	0.0000	0.0000	0.0000
2-Butanone	0.0000	0.7097	28.0950	0.3892	0.1862	0.0000
Cyclopentane, methyl-	0.0000	0.0000	0.0014	0.0000	0.0098	0.0188
Decanal	0.0982	0.0112	0.0614	0.1693	0.0677	0.0557
Decane, 2,2,7-trimethyl-	0.0000	0.0732	0.0799	0.0764	0.0568	0.0205
1,2-Dimethoxy-ethene	0.0000	0.0952	1.0175	0.0269	0.0014	0.0000
Ethanedione, diphenyl-	0.0007	0.0982	8.1562	1.8687	0.1263	0.0000
Ethanol	0.0000	0.0952	61.2088	0.5550	0.0000	0.0000
Ethanol, 2-phenoxy-	0.0048	0.0120	0.0140	0.0132	0.0040	0.0000
Ethanone, 1,2-diphenyl-	0.0220	0.0000	9.2863	0.2268	0.0541	0.0145
Ethylene, 1,1-diphenyl-	0.0000	0.4909	0.1002	0.0000	0.0008	0.0000
Hexane	0.0925	0.0247	0.9394	0.3694	0.0375	0.0326
1-Hexanol, 2-ethyl	0.0799	0.0817	0.0324	0.6959	0.0785	0.0408
Methyl ethyl ketone	0.0000	0.7090	28.8532	0.3886	0.2074	0.0000
Nonanal	0.0097	0.0000	0.0208	0.1152	0.0181	0.0139
Oxime-, methoxy-phenyl-	0.1546	0.0000	0.0186	6.6316	0.2271	0.2105
Pentane	0.4148	0.0742	1.6566	0.0813	0.1251	0.0403
Phenanthrene	0.0040	0.5846	0.1208	0.1840	0.0507	0.2730
Phenylethyne	0.0000	1.9239	0.9603	0.0000	0.0016	0.4543
1,2-Propanedione, 1-phenyl	0.0000	3.4162	0.7241	0.5534	0.3078	0.4876
Propanoic acid	0.0000	0.0159	0.0908	0.0000	0.0698	0.0080
Propanoic acid	0.0040	0.0203	0.1329	0.0922	0.0534	0.5184
Propanoic acid	0.0130	0.2724	1.9260	0.0990	0.0156	0.0187
1-Propanol	0.0000	0.0000	3.5869	0.0124	0.0000	0.0000
Styrene	0.0015	0.7132	0.5928	0.0354	0.0169	0.3655
[1,1':3',1"-Terphenyl]-2'-ol	0.0000	0.0000	0.0000	7.0093	0.0000	0.0000
Toluene	0.0047	0.2175	0.1469	0.0423	0.0837	0.1335
Tricyclo[3.1.0.0[2,4]]hex-3-ene-3-carbo	0.0241	1.9656	0.7901	0.1690	0.2270	1.4123
2,4,5-Trimethyl-1,3-dioxolane	0.0000	0.0565	0.0182	0.0000	0.0187	0.0000

APPENDIX 2: BIOASSAY 2

Mean concentration (ppm) of volatiles detected by GCMS in the Kilner jar microcosms 3 days after set up

Volatile Detected	Mean concentration (n = 3) Kirton Soil	Flask OWC	Windrow OWC	Brassica	Low N Poultry	High N Poultry
Sulphur-containing volatiles						
Allyl methyl sulfide	0.0000	0.0566	0.0000	0.0000	0.0000	0.0000
Benzothiazole	0.6905	2.7394	2.7406	0.8462	0.4588	1.8696
Dimethyl disulfide	0.0000	0.0000	0.0000	1.3956	0.0000	9.1692
Dimethyl sulfide	0.0000	18.9467	0.4800	10.6958	0.9050	129.1380
Dimethyl Sulfoxide	1.1936	0.0135	1.5271	0.0138	0.8725	0.0069
Dipropyl, disulfide	0.0000	0.0433	0.0000	0.0000	0.0000	0.0000
Methyl propyl disulfide	0.0000	1.6134	0.1258	0.0000	0.0000	0.0350
Methyl-trans-propenyl-disulfide	0.0000	0.0000	0.0210	0.0180	0.0000	0.0286
Propane, 1-(methylthio)-	0.0000	8.4062	0.1993	0.0000	0.0000	0.0000
1-Propanethiol	0.0000	0.3001	0.2020	0.0000	0.0000	0.1662
1-Propene, 1-(methylthio)-(E)	0.0000	0.0863	0.0000	0.0000	0.0000	0.0000
1-Propene, 1-(methylthio)-, (Z)	0.0000	0.0103	0.0000	0.0000	0.0000	0.0000
1-Propene, 3-(methylthio)-	0.0000	0.0752	0.0000	0.0000	0.0000	0.0000
Sulfur dioxide	11.8275	3.3367	9.0439	15.2585	0.0000	0.0000
Thiophene, 2,5-dimethyl	0.0000	0.2975	0.1903	0.0000	0.0000	0.0000
Other compounds						
Acetic acid	34.9696	29.5073	63.3894	26.3910	32.1034	25.6363
Acetone	0.0000	4.9417	8.0507	0.0000	0.0000	4.5258
Acetophenone	159.1437	18.7714	243.2809	3.3840	32.4916	2.4567
Benzaldehyde	61.1836	7.4239	102.8432	1.3218	19.2428	1.0433
Benzene	104.1597	16.4484	175.1487	68.8511	140.7815	6.2270
Benzene, ethynyl-	55.1148	1.1373	61.9181	0.4443	4.0867	0.2648
Benzeneacetaldehyde	217.2516	22.1036	374.8170	1.1483	30.4550	1.3431
Benzoic acid	12.4532	0.6119	0.0000	0.0000	0.0000	0.0000
Benzoic acid, phenyl ester	0.0874	19.8678	0.0304	0.0000	0.0000	0.0000
Benzophenone	13.3175	12.5333	0.0000	0.0000	1.0800	0.0000
Butanoic acid	0.0000	0.1876	0.0000	0.0093	0.3205	0.0000
Butanoic acid	35.0626	24.0605	15.1499	27.0339	14.2508	27.6141
2-Butanol	0.0000	0.3593	1.5711	0.0000	0.0000	0.0000
2-Butanone	1.9021	29.0494	6.3542	2.1547	0.0000	5.1467
Cyclopentane, methyl-	0.0000	0.3069	0.0000	0.0000	0.0000	0.0000
Decanal	0.0925	1.0790	1.7735	1.2297	1.1211	0.9133
Decane, 2,2,7-trimethyl-	0.0000	2.4661	0.0922	0.0000	0.0000	0.0000
1,2-Dimethoxy-ethene	0.0000	0.0923	0.0000	0.0000	0.0000	0.0000
Ethanedione, diphenyl-	0.7346	5.8191	3.8058	3.5965	0.7881	2.5081
Ethanol, 2-phenoxy-	0.0133	0.0000	0.0269	0.0301	0.0179	0.0000
Hexane	0.7594	26.4377	1.5026	122.8565	0.0000	5.9049
1-Hexanol, 2-ethyl	1.9875	5.4418	2.7354	7.6795	0.0000	2.4985
Methyl ethyl ketone	2.1471	30.0972	5.8667	1.3982	0.0000	4.9067
Nonanal	0.0000	0.2506	0.0000	0.8475	0.4584	3.8328
Pentane	0.0000	9.0456	4.2220	3.0244	0.0000	3.8910
Phenanthrene	0.0000	1.6549	0.0000	0.0000	0.0000	0.0000
Phenylethyne	55.1804	1.1655	61.9423	0.5295	4.0338	0.2872
1,2-Propanedione, 1-phenyl	12.1411	5.1504	21.1263	0.0000	0.0645	0.0000
Propanoic acid	0.6221	0.0511	0.0000	0.0000	0.3154	0.0000
1-Propanol	0.0000	0.0000	0.0000	0.0000	0.0176	0.0000
Styrene	14.0107	0.9781	17.7910	0.0310	10.5004	0.3824
[1,1':3',1"-Terphenyl]-2'-ol	0.0000	0.0477	0.0000	0.0886	0.0000	0.0000
Toluene	0.1936	0.0000	0.7639	0.0000	0.0108	0.2487
Tricyclo[3.1.0.0[2,4]]hex-3-ene-3-carbo	8.7985	0.5488	13.6879	0.2933	7.8519	0.8929
Undecane	2.1940	1.1499	0.1737	1.8347	0.8276	4.2072