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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

In initial laboratory trials brown mustard *Brassica juncea* 'Vittasso' significantly reduced germination of sclerotia of *Sclerotinia sclerotiorum* by 61% in comparison with an untreated control. Other biofumigant crops also significantly reduced germination, indicating they may be useful as part of an integrated disease management program.

Background

The pathogen – *Sclerotinia sclerotiorum*

Sclerotinia sclerotiorum (Lib.) de Bary is a plant pathogenic fungus that affects many economically important crops world-wide (Hegedus and Rimmer, 2005), (Purdy, 1979) and a wide host range of over 400 plant species (Boland and Hall, 1994). Crops susceptible to Sclerotinia disease include lettuce, vegetable Brassicas, oilseed rape, beans, peas, potatoes and carrots (Saharan and Mehta, 2008).

The long term survival structures for Sclerotinia are small black resting bodies called sclerotia (Willetts and Wong, 1980) which when brought close to the soil surface germinate to produce mushroom-like apothecia. These then release air-borne ascospores which infect plants, upon which further sclerotia are formed and are returned to the soil (Bolton *et al.*, 2006). Sclerotia can also geminate to produce hyphae which can attack plant tissues directly (Bardin and Huang, 2001). The number of sclerotia produced by *S. sclerotiorum* on different plant tissues is variable and is an important factor in determining the inoculum levels in soil following an infected crop (Leiner and Winton, 2006).

A related species, *S. subarctica,* has been found in the UK (Clarkson *et al.*, 2010) on meadow buttercup and also more recently in a carrot crop in Scotland. Previously this pathogen has only been found in Norway (Holst-Jensen *et al.*, 1998) and in Alaska (Winton *et al.*, 2006). The symptoms caused by *S. subarctica* are very similar to *S. sclerotiorum* and therefore may be undetected in crops in the UK. One aim of this work is therefore to establish the distribution and ecology of this species in the UK, on both crops and wild hosts.

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Sclerotinia on carrot

This project will focus on Sclerotinia disease on carrots, as it is one of the most economically important diseases affecting carrot production worldwide (Kora *et al.*, 2005) and has been reported in over twenty carrot producing countries (Kora *et al.*, 2003). Previous research has shown differences in aggressiveness between isolates of *S. sclerotiorum* on carrots (Jensen *et al.*, 2008). Possible pre-harvest resistance has been shown in glasshouse trials with carrots, (Foster *et al.*, 2008) although it is thought that control of Sclerotinia disease in carrots is best obtained by preventing leaf infection and reducing the quantity of sclerotia in the soil (McQuilken, 2011).

Control of Sclerotinia disease

Fungicides are applied to kill ascospores before they infect plants, with the best protection obtained by spraying before canopy closure (McQuilken, 2011). The timing of spraying is critical to the effectiveness of protection provided by fungicides, so new control methods to reduce the viability of sclerotia in the soil would help to eliminate this issue. Also, some of the effective active ingredients in fungicides currently used routinely against Sclerotinia disease such as boscalid, carbendazim, cyprodinil, fludioxonil (Matheron and Porchas, 2008), azoxystrobin and difenoconazole are classed as medium to high risk for resistance (McQuilken, 2011).

Various non-organic soil amendments have been shown to inhibit sclerotial germination, such as potassium bicarbonate (Ordonez-Valencia *et al.*, 2009) and calcium cyanamide (Perlka®) (Huang *et al.*, 2006), but these are considered expensive by growers. Clipping of carrot foliage to prevent lodging and hence plant to plant spread of infection between beds was found to protect against Sclerotinia disease in carrots (Kora *et al.*, 2005), as does applying optimum amounts of nitrogen to limit canopy growth and lodging (McQuilken, 2011). There has been much research into biological controls, with *Coniothyrium minitans* being commercialised and marketed as 'Contans WG', although it does not always provide consistent results under field conditions (Fernando *et al.*, 2004).

It is thought that using *Brassica* green manure crops for biofumigation can provide control against Sclerotinia disease (Porter *et al.*, 2002), but further work is needed to establish which crops work against which pathogens, as *Brassica juncea* was found to be the only cruciferous plant to delay germination of *S. sclerotiorum* sclerotia in one study, (Smolinska and Horbowicz, 1999) yet *Brassica oleracea* var. *caulorapa* reduced mycelial growth in

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another (Fan *et al.*, 2008). A different study found that a blend of *Brassica napus* and *Brassica campestris* reduced the viability of sclerotia in the soil (Geier, 2009).

The aims and objectives of this project are:

Aims: To identify potential new soil treatments for control of Sclerotinia disease and to assess the impact of pathogen diversity on both aggressiveness and fungicide sensitivity.

Objectives:

i. To determine the effect of organic soil amendments on the survival of sclerotia of *Sclerotinia sclerotiorum*.
ii. To determine the aggressiveness of different *Sclerotinia* genotypes and species on commercial carrot varieties and quantify production of sclerotia.
iii. To evaluate the sensitivity of different *Sclerotinia* genotypes and species to fungicides.
iv. To investigate the epidemiology and control of *Sclerotinia subarctica.*
v. To carry out a population study of *S. sclerotiorum* on *Daucus carota* in the UK.

Summary of the results and main conclusions

Objective 1 - To determine the effect of organic soil amendments on the survival of sclerotia of *Sclerotinia sclerotiorum*.

Initial results show that some biofumigant crops can suppress carpogenic germination of *S. sclerotiorum*, hence reducing the number of apothecia produced. *Brassica juncea* 'Vittasso' provided the best control, reducing germination by 61% compared with the untreated control (Figure 1). Only a small reduction in germination was observed for mustard meal pellets (Biofence) and *Coniothyrium minitans* (Contans WG). Perlka® also performed well in the germination tests, as would be expected from previous research.

The results from a preliminary *in vitro* trial showed that *Brassica juncea* 'Pacific Gold' delayed or reduced mycelial growth of *S. sclerotiorum* on agar. Further such *in vitro* work is needed to establish whether the effect on sclerotia in the soil box experiments is due to the direct action of volatile gases being released from the plant material, or due to other effects such as increased microbial activity.

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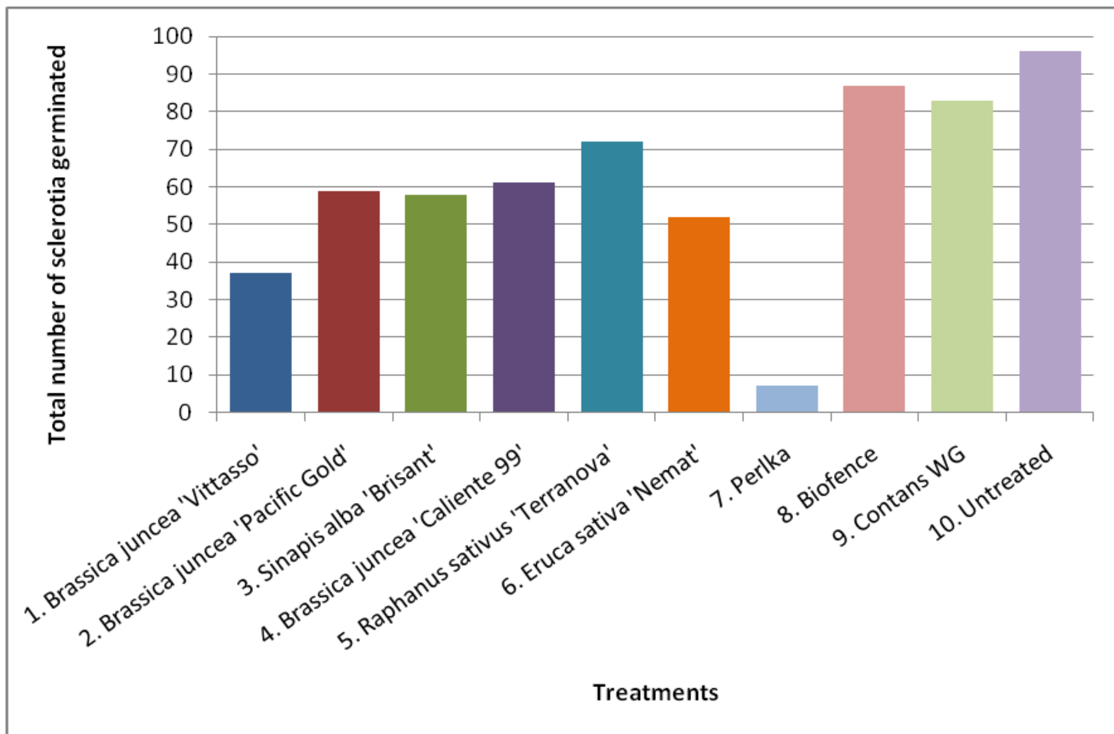

Figure 1 – The effect of biofumigant crops (treatments 1 to 6), Perlka®, Biofence and Contans WG on final germination of sclerotia after 150 days in a soil box biofumigation experiment.

Objective 2 - To determine the aggressiveness of different Sclerotinia genotypes and species on commercial carrot varieties and quantify production of sclerotia.

Roots from a carrot diversity set grown by the Genetic Resources Unit at Wellesbourne were inoculated with different isolates of *S. sclerotiorum.* Generally, isolate L6 produced smaller sclerotia in large numbers, and isolate L44 produced large sclerotia in small numbers. Some of the carrot accessions produced only a small quantity of sclerotia for both isolates, and these may be useful for any future breeding of new carrot varieties. The amount of sclerotia returned to the soil by an infected crop will therefore vary depending on the isolate causing the infection.

Whole carrot plant inoculation trials are currently underway to establish if there are any differences in susceptibility.

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Objective 4 - Epidemiology and control of *Sclerotinia subarctica.*

Preliminary results from studies using DNA based microsatellite markers show that there is considerable diversity in isolates of *S. subarctica* that have been obtained from Scotland, in comparison with those obtained from buttercups in Hereford. *S. subarctica* has been found in all sampling carried out in Scotland so far and it is hoped that further sampling in Scottish crops will indicate how prevalent this species is, particularly as symptoms of infection in the field appear to be the same as *S. sclerotiorum*.

Conclusions

- Initial results show that all but one of the biofumigant crops tested against *S. sclerotiorum* sclerotia significantly reduced carpogenic germination and production of apothecia.
- *Brassica juncea* 'Vittasso' reduced carpogenic germination of sclerotia by 61% in comparison to the untreated control.
- *Brassica juncea* 'Pacific Gold' completely inhibited mycelial growth of *S. sclerotiorum in vitro* and delayed growth at lower rates.
- Some carrot roots produce very few sclerotia and could be used in future breeding programs.
- Initial results suggest that *S. subarctica* isolates are more diverse in Scotland compared to those found in Herefordshire.

Financial Benefits

Financial benefits have yet to be established – further details on this expected at the end of year 2 of the project.

Action Points

Experiments are still underway to establish proof of concept, so no action points at present.

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SCIENCE SECTION

Introduction

The Pathogen – *Sclerotinia sclerotiorum*

Sclerotinia sclerotiorum (Lib.) de Bary is a plant pathogenic fungus that affects many economically important crops (Hegedus and Rimmer, 2005), with a world-wide distribution (Purdy, 1979) and a wide host range of over 400 plant species (Boland and Hall, 1994). Due to the large host range the symptoms caused by *S. sclerotiorum* vary, but the white fluffy mycelial growth is an early symptom. Pale or dark brown lesions may be seen on the base of stems of herbaceous plants, often quickly covered by white mycelium, or infection may begin on a leaf and move into the stem (Saharan and Mehta, 2008). Multiple genotypes of *S. sclerotiorum* have been identified in the UK, with one genotype being found more frequently than the rest, at different locations and on different crops and it is thought that the genotypes vary in their aggressiveness (Clarkson *et al.*, 2008).

The long term survival structures for *S. sclerotiorum* are small black resting bodies called sclerotia (Willetts and Wong, 1980) which when brought close to the soil surface germinate carpogenically to produce mushroom-like apothecia. These then release air-borne ascospores which infect plants, upon which further sclerotia are formed and are returned to the soil (Bolton *et al.*, 2006). Sclerotia can also geminate myceliogenically to produce hyphae which can attack plant tissues directly (Bardin and Huang, 2001). *S. sclerotiorum* therefore functions as both an airborne and soil borne pathogen. The longevity of sclerotia is variable, being influenced by many factors including the time and depth of burial (Duncan *et al.*, 2006), and soil type (Merriman, 1976). The number of sclerotia produced by *S. sclerotiorum* on different plant tissues is also variable and is an important factor in determining the inoculum levels in soil following an infected crop. An infected cabbage head was found to produce 250 to 500 sclerotia, (Leiner and Winton, 2006) while an infected carrot root produced up to 30 (Jensen *et al.*, 2008).

A related species *Sclerotinia subarctica* has been recently identified in the UK (Clarkson *et al.*, 2010) after previously only being found in Norway on wild hosts (Holst-Jensen *et al.*, 1998) and on vegetable crops in Alaska (Winton *et al.*, 2006). The symptoms caused by *S. subarctica* are very similar to *S. sclerotiorum* and therefore may be undetected in crops in the UK. Further work is required to establish the distribution and ecology of this species in the UK, on both crops and wild hosts (Clarkson *et al.*, 2010).

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Sclerotinia on Carrots

This project will focus on Sclerotinia disease on carrots, as it is one of the most economically important diseases affecting carrot production worldwide (Kora *et al.*, 2005) and has been reported in over twenty carrot producing countries (Kora *et al.*, 2003). It is a particular problem in temperate regions where carrots are stored for long periods (Kora *et al.*, 2005a). Previous research has shown differences in aggressiveness between isolates of *S. sclerotiorum* on carrots (Jensen *et al.*, 2008). Infection is normally via ascospores landing on damaged or senescing leaves, which then germinate and infect tissue. Spore release from apothecia can occur throughout the growing season from June to September, with optimal conditions for foliage infection being four days continuous leaf wetness with an air temperature of 10 to 18°C (McQuilken, 2011). It is suggested that under field conditions the pathogen enters the root via the crown of the plant (Jensen *et al.*, 2008), and trials show that it is unlikely that carrot roots are directly infected by mycelium germinating from sclerotia in the soil surrounding the carrot roots (Finlayson *et al.*, 1989).

Possible pre-harvest resistance has been shown in glasshouse trials with carrots, one defence mechanism being leaf abscission after infection of the petiole (Foster *et al.*, 2008) and a second being a structural barrier of lignin, diphenols, suberin flavanols, peroxidases and phenolases (Craft and Audia, 1962) which slow or stop progression of the pathogen from an infected petiole into the crown (Foster *et al.*, 2008). It is thought that control of Sclerotinia disease in carrots is best obtained by preventing leaf infection and reducing the quantity of sclerotia in the soil (McQuilken, 2011).

Control of Sclerotinia Disease

In the absence of resistant crop cultivars control methods for Sclerotinia disease include fungicides, soil solarisation, biofumigation and cultural practices (Bardin and Huang, 2001). Fungicides are applied to kill ascospores before they infect plants, with the best protection obtained by spraying before canopy closure (McQuilken, 2011). Some of the effective active ingredients in fungicides currently used routinely against Sclerotinia disease such as boscalid, carbendazim, cyprodinil, fludioxonil (Matheron and Porchas, 2008), azoxystrobin and difenoconazole are classed as medium to high risk for resistance (McQuilken, 2011). Even so, no resistance has been found to boscalid when tested against isolates of *Sclerotinia sclerotiorum* from China, but boscalid was not being used in China at the time of the studies (Wang *et al.*, 2009) (Liu *et al.*, 2009). Also, no resistance was found in Australian isolates either, where boscalid was the only fungicide registered for control in

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bean fields, where the isolates originated from (Jones *et al.*, 2011). Similarly, it was found that there has been no change in *S. sclerotiorum* sensitivity to boscalid since its introduction in Europe. However, there have been very few resistance studies carried out (Stammler *et al.*, 2007). Conversely, *S. sclerotiorum* isolates with resistance to carbendazim have been found in both China (Yin *et al.*, 2010) and in several regions of France (Kaczmar *et al.*, 2000), but none have yet been reported in the UK. No cross resistance was found between fludioxonil and carbendazim, suggesting that this active can be used in areas of carbendazim resistance (Kuang *et al.*, 2011).

Various non-organic soil amendments have been shown to inhibit sclerotial germination, such as potassium bicarbonate (Ordonez-Valencia *et al.*, 2009) and calcium cyanamide (Perlka®) (Huang *et al.*, 2006). Simply burying sclerotia to prevent carpogenic germination is effective at reducing disease (Williams and Stelfox, 1980), but a subsequent cultivation could bring viable sclerotia back to the soil surface (Mitchell and Wheeler, 1990). Clipping of carrot foliage to prevent lodging and hence plant to plant spread of infection between beds was found to protect against Sclerotinia disease in carrots (Kora *et al.*, 2005), as does applying optimum amounts of nitrogen to limit canopy growth and lodging (McQuilken, 2011). Soil solarisation reduces the numbers of sclerotia in the soil, and also reduces the ability of surviving sclerotia to germinate carpogenically (Phillips, 1990).

Inhibition of carpogenic germination of *S. sclerotiorum* sclerotia has been achieved using various organic soil amendments, including fish meal, bone meal, raw cattle manure (Huang *et al.*, 2002), fowl manure and lucerne hay (Asirifi *et al.*, 1994) and some amendments can be even more effective when combined with mycoparasites such as *Trichoderma* spp. or *Coniothyrium minitans* (Huang *et al.*, 2005). There has been much research into these biological controls, with *C. minitans* being commercialised and marketed as Contans WG, although it has not provided consistent results under field conditions (Fernando *et al.*, 2004). However, it has been found to significantly reduce carpogenic germination when used in conjunction with a commercial NPK fertiliser (Yang *et al.*, 2011).

Biocidal activity of plant extracts such as glucosinolates have been reported in literature since the 19th century. Many *Brassica* spp. produce significant levels of glucosinolates, which themselves are not fungitoxic (Manici *et al.*, 1997), but are hydrolysed in the presence of water and endogenous myrosinase enzyme to release isothiocyanates (ITCs) which have a wide range of biocidal characteristics (Kurt *et al.*, 2011) and are acutely toxic to several pathogenic fungi (Chew, 1987). It has been found that even when ITCs are present in concentrations too low to suppress mycelial growth they can delay fungal

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sporulation (Drobnica *et al.*, 1967) and some of these natural ITCs are superior to the synthetic fumigant metham sodium (methyl isothiocyanate) in their abilities to suppress fungi (Sarwar *et al.*, 1998). The definitive mode of action of ITCs inhibiting fungal growth and other microorganisms is not known, but some hypotheses are:

i. Inactivation of intracellular enzymes by oxidative breakdown of –S-S bridges (Zsolnai, 1966)
ii. Uncoupler action of oxidative phosphorylation suggested from the inhibition of oxygen uptake of yeasts by ITCs (Kojima and Oawa, 1971)
iii. Inhibition of metabolic enzymes by thiocyanate radical, indicated as a degradation product of ITCs (Banks *et al.*, 1986)

Using *Brassica* green manure crops for biofumigation can provide control against Sclerotinia disease (Porter *et al.*, 2002), but has not yet been shown to have a consistent significant effect on viability of sclerotia (Matthiessen and Kirkegaard, 2002). A study on a blend of *Brassica napus* & *Brassica campestris* showed a reduction in the level of viable sclerotia in the soil (Carr, 2003), so it seems issues surrounding methods and rates of incorporation need to be resolved in order to gain consistent results (Geier, 2009), particularly as synthetic pure ITCs significantly reduce sclerotial viability *in vitro* (Kurt *et al.*, 2011). Also, further work is needed to establish which crops work against which pathogens, as *Brassica juncea* was found to be the only cruciferous plant to affect sclerotial viability of *S. sclerotiorum* in one study, delaying myceliogenic germination by seven days (Smolinska and Horbowicz, 1999) yet *Brassica oleracea* var. *caulorapa* inhibited mycelial growth by 89.5% in another (Fan *et al.*, 2008).

The aims and objectives of this project are:

Aims: To identify potential new soil treatments for control of Sclerotinia disease and to assess the impact of pathogen diversity on both aggressiveness and fungicide sensitivity.

Objectives:

i. To determine the effect of organic soil amendments on the survival of sclerotia of *S. sclerotiorum*.
ii. To determine the aggressiveness of different *Sclerotinia* genotypes and species on commercial carrot varieties and quantify production of sclerotia.

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iii. To evaluate the sensitivity of different *Sclerotinia* genotypes and species to fungicides.
iv. To investigate the epidemiology and control of *Sclerotinia subarctica.*
v. To carry out a population study of *S. sclerotiorum* on *Daucus carota* in the UK.

Objective 1 – Organic soil amendments

Biofumigation Soil Box Trials

Materials and Methods

Soil box trials (four in total) were set up to test the effect of 10 treatments on the carpogenic germination of *S. sclerotiorum* sclerotia (Table 1). All biofumigant crops were used at either half or full field rate equivalents. Positive controls (Perlka® and Contans WG) and biofumigant treatment Biofence (mustard meal pellets) were used at full field rate to provide comparisons with biofumigation crops. Oilseed rape 'Temple' was used as a low glucosinolate *Brassica* control in trials two, three and four.

Table 1- Summary of treatments and rates used in soil box biofumigation trials

Treatments	Full Field Rate (per soil box)	Half Field Rate (per soil box)
1. *Brassica juncea* 'Vitasso'	47g	23.5g
2. *Brassica juncea* 'Pacific Gold'	47g	23.5g
3. *Sinapis alba* 'Brisant'	47g	23.5g
4. *Brassica juncea* 'Caliente 99'	47g	23.5g
5. *Raphanus sativus* 'Terranova'	47g	23.5g
6. *Eruca sativa* 'Nemat'	47g	23.5g
7. Perlka® (Calcium cyanamide)	0.43g	0.43g
8. Biofence (mustard meal pellets)	1.4g	1.4g
9. Contans WG (*Coniothyrium minitans*)	0.4g	0.4g
10. Untreated	-	-
11. *Brassica napus* 'Temple'	47g	23.5g

All crops were grown in a glasshouse at 22-26°C under lights (16 h days) and harvested within two weeks of first flowering. Compost (John Innes No 1) for use in experiments was passed through a 4mm sieve and pasteurised by autoclaving at 110°C for 30 minutes. Sclerotia of *S. sclerotiorum* isolate L6 were produced by inoculating wheat grain in flasks

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with mycelial agar plugs and incubating them at 18°C for six weeks. The sclerotia were harvested by floating off the wheat grain, and dried overnight in a laminar flow cabinet. These sclerotia were conditioned in pasteurised compost with 30% moisture at 5°C for 40 days.

Each biofumigant/soil treatment was mixed with pasteurised compost (plant material was macerated in a food processor first) and 350g of the compost/treatment mixture placed into a 600ml clear plastic box. Preconditioned sclerotia (30) were laid out in a grid pattern before adding another 50g of the mixture to cover the sclerotia. Water was added to give 30% moisture content, lids were then immediately placed onto the boxes and they were weighed before being incubated in a controlled environment room at 15°C with lights (14h day).

Four replicates of each treatment were set up in each trial, arranged in a randomised block design with four rows and 11 columns on a single shelf in the controlled environment room (Figure 2). Every 2 weeks the boxes were watered to bring them back to their original weight. The emergence of stipes or apothecia was recorded twice a week using a scale of 1 (stipe) to 4 (mature apothecium with wavy cap).

Figure 2 - Soil box trials laid out on shelving in a controlled environment room

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Results

Each soil box trial was run for at least 150 days to fully assess the effects of the biofumigation treatments. Therefore, at the time of writing only Trial 1 has finished, and these results have been (statistically) analysed using a Generalised Linear Model. The biofumigant crops substantially delayed carpogenic germination of the sclerotia (Figure 3), and all except *Raphanus sativus* 'Terranova' significantly reduced germination in comparison with the untreated control after 150 days (Figure 4). *Brassia juncea* 'Vittasso' provided the greatest reduction in germination (61%) compared to the untreated control. *Coniothyrium minitans* (Contans WG) and Biofence only slightly reduced overall germination in comparison with the untreated control, whereas Perlka reduced germination by 92%.

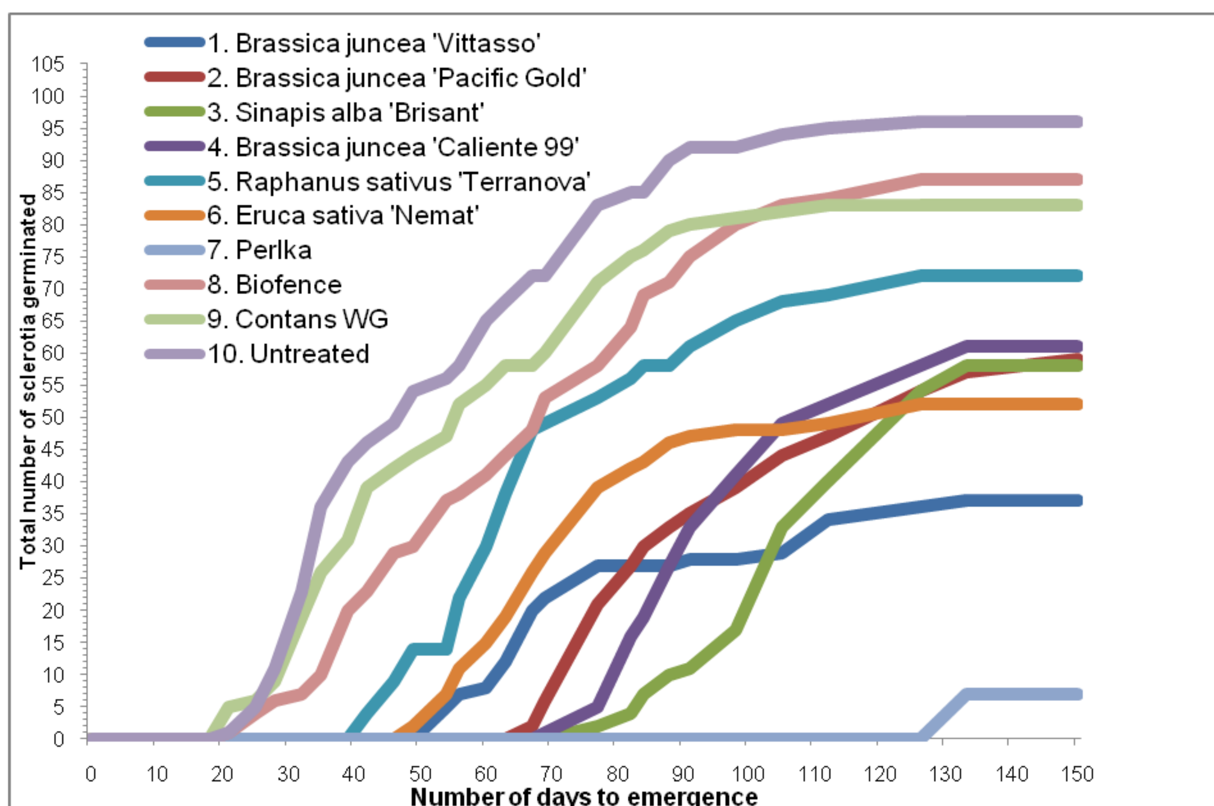

Figure 3 - Effect of biofumigation treatments on germination of sclerotia of *S. sclerotiorum* over 150 days in soil box Trial 1.

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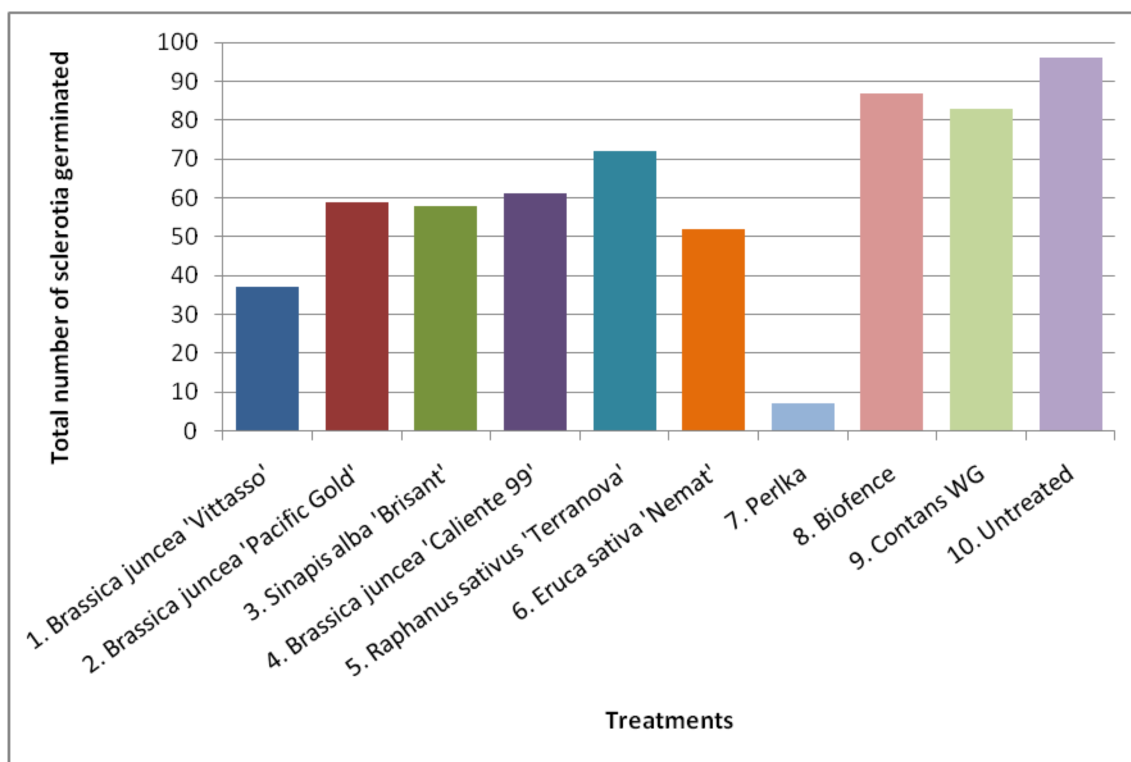

Figure 4 - The effect of biofumigant crops (treatments 1 to 6), Perlka®, Biofence and Contans WG on final germination of sclerotia after 150 days in a soil box biofumigation trial.

In vitro Biofumigation Trials

Materials and Methods

Initial trials were carried out to establish suitable methods for testing the biofumigant crops *in vitro*, to determine whether they reduced or suppressed growth of *S. sclerotiorum* on agar. *Brassica juncea* "Pacific Gold" (grown and harvested as for the soil box trials) was oven dried at 80°C for 24h, before being ground in a mill to a fine powder prior to use. One 5mm mycelial plug of actively growing mycelium from *S. sclerotiorum* isolate L6 was placed in the centre of a PDA plate. The plate was inverted, and different amounts of the dried plant material (0.1g, 0.25g, 0.5g and 1g) were placed in the lid of the Petri dish and water added. An untreated control was also set up. All Petri dishes were immediately sealed with parafilm (Figure 5) and placed into a growth room at 15°C in the dark. There were five replicates of each treatment and mycelial growth was assessed twice a day for 5 days using calipers to measure radial growth.

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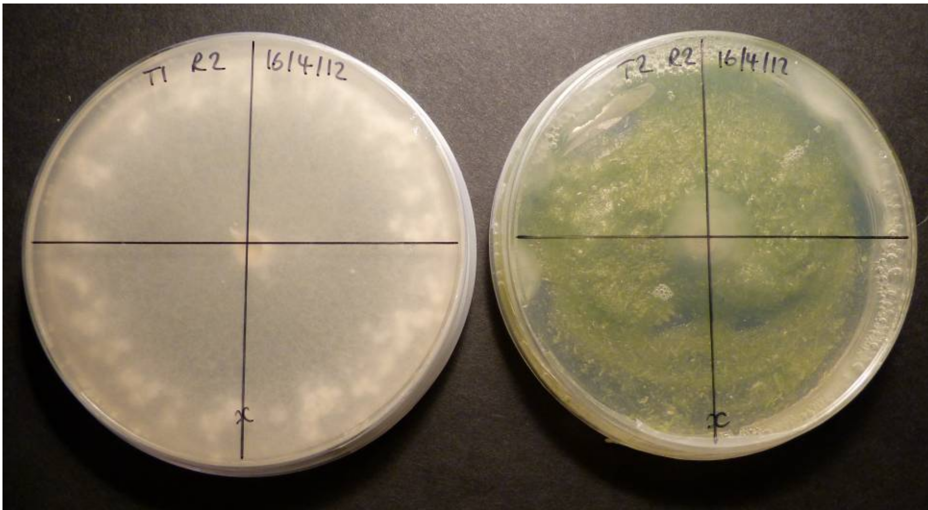

Figure 5 – Growth of *S. sclerotiorum in vitro* after 5 days. Untreated plate on the left and plate treated with 1g *Brassica juncea* 'Pacific Gold' dried plant material and RO water on the right.

Results

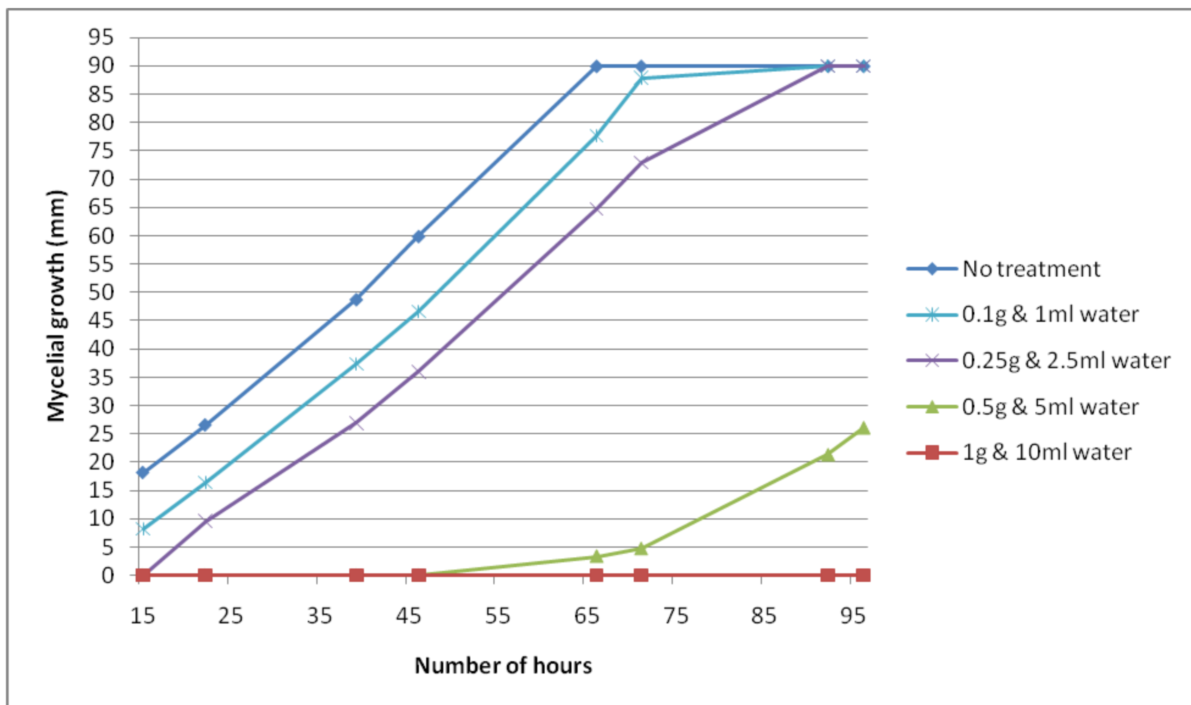

Figure 6 - Effect of oven dried *Brassica juncea* 'Pacific Gold' on mycelial growth of *Sclerotinia sclerotiorum* over 97 hours.

Mycelial growth of *S. sclerotiorum* isolate L6 was either delayed or completely inhibited by the biofumigant *B. juncea* ‘Pacific Gold’ (Figure 6). This method will be used in the future to

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assess the effects of all the biofumigant crops used in the soil box trials and a low glucosinolate oil seed rape will be used as a control.

Objective 2 – Aggressiveness of *Sclerotinia sclerotiorum* isolates and production of sclerotia

Carrot Root Inoculation

Materials and Methods

A trial was carried out to assess the production of sclerotia by two *S. sclerotiorum* isolates (L6 and L44) on roots from a carrot diversity set grown at the Wellesbourne site by the Genetic Resources Unit. Previously, isolate L6 has been found to produce large numbers of small sclerotia while isolate L44 produces small numbers of larger sclerotia. A 5mm plug of mycelium was placed into the centre of each carrot root which were incubated on damp tissue in bagged trays at 13°C (Figure 7). Four replicate roots for each of 88 accessions for each *S. sclerotiorum* isolate. Sclerotia were counted and weighed once they were mature.

Figure 7 - Carrot roots incubated on damp tissue, three weeks after being inoculated with *S. sclerotiorum* isolate L44.

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Results

Statistical analysis has been carried out using a restricted (or residual) maximum likelihood. Isolate L6 produced an average of 0.625 sclerotia per one gram of carrot root tissue, while L44 produced just over half that amount, at 0.341 sclerotia. Some of the accessions produced very few sclerotia for either *S. sclerotiorum* isolate, but generally more sclerotia were produced by isolate L6 than isolate L44 (Figure 8).

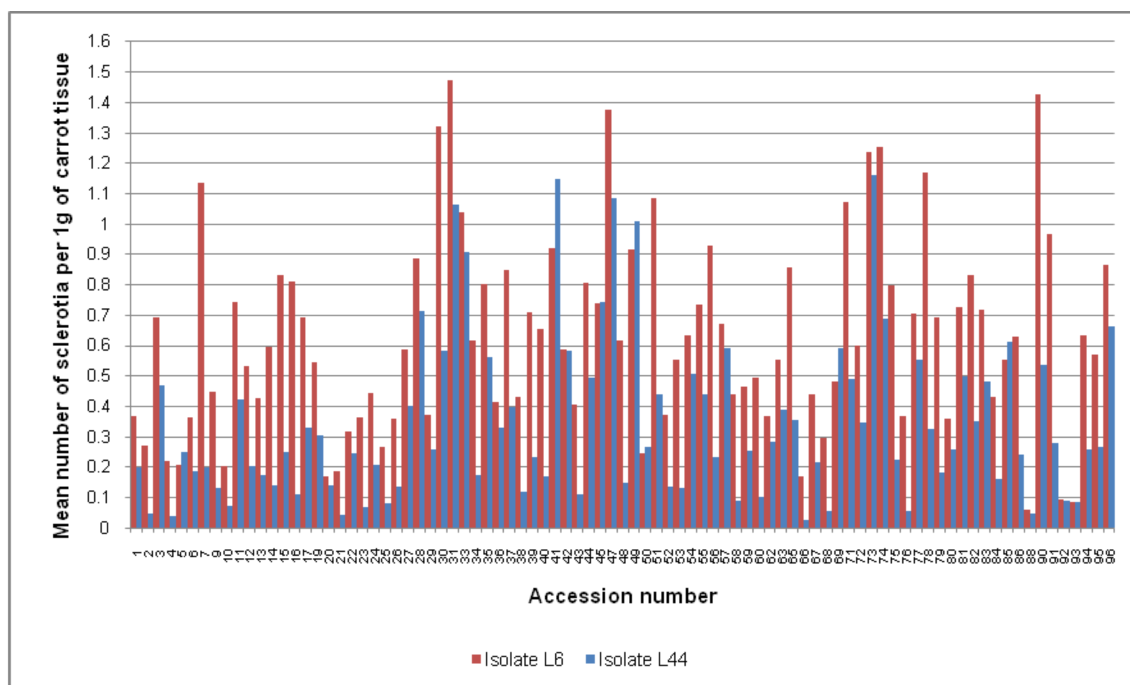

Figure 8 - Mean numbers of sclerotia produced per 1g of carrot tissue, for *S. sclerotiorum* isolates L6 and L44, organised in order of carrot accession number.

Whole Carrot Plant Inoculation

Materials and Methods

Whole carrot plant inoculations to assess the susceptibility of different cultivars and accessions to *S. sclerotiorum* are underway. The results from the carrot root inoculation trial, together with root position and leaf growth habit was taken into account to obtain a diverse range of varieties to trial (Table 2).

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Table 2 - Varieties being used in whole carrot plant inoculation trial, and their growth habits and sclerotia production on roots.

Carrot Diversity Set No.	Group	Name	Root position in soil	Leaf growth habit	L6 sclerotia production	L44 sclerotia production
7	Elite	Nairobi	shallow	semi-upright	high	low
	Elite	Chantenay	shallow - medium	upright	low - med	low
	Elite	Eskimo	deep	upright		
	Elite	Narbonne				
90	Mapping parent - wild	QAL	deep	upright	high	med
86	Mapping parent - elite	Brasilia	shallow	upright	med	low
93	Mapping parent	USDA 9304B	shallow	upright	low	low
92	Mapping parent	USDA 7262B	deep	upright	low	low
30	Wild	7159	deep	prostrate	high	med
51	Cultivated	Little finger	shallow	prostrate	high	med

Carrot plants were grown in 3L deep pots in a poly tunnel. At 18 weeks old, six plants of each cultivar were moved to a glasshouse and three leaves on each plant were inoculated by cutting off the leaf and placing a pipette tip with a mycelial plug inside onto the cut end (Figure 9). The plants were then covered with a plastic bag to maintain humidity for three days, and sprayed with water three times a day.

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Figure 9 - Carrot plants in a glasshouse with pipette tip inoculations of *S. sclerotiorum* on three petioles per plant.

The distance from where the petiole meets the crown of the plant to the edge of any lesion on the petiole was measured, and progression of infection into the crown of the plant was scored from zero (no infection in crown) to four (crown diseased and rotten). The plants were assessed twice a week, for a total of four weeks

Results

Data are still being collected in the first of 3 proposed trials.

Objective 4 – Epidemiology and control of *Sclerotinia subarctica*

Microsatellite Markers

Methods and Materials

Genomic DNA was extracted from freeze-dried mycelium for 23 *S. subarctica* isolates (collected in 2011) using a DNeasy plant mini kit. The isolates were then characterised

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using eight microsatellite markers in two separate multiplexed PCR reactions (4 loci per reaction) (Winton *et al.*, 2007). Primer mix 1 contained MS01, MS03, MS06 and MS08 and primer mix 2 contained MS02, MS04, MS05, and MS07. PCR amplification was carried out with thermocycling parameters of 95°C for 15 min; 35 cycles of 94°C for 30s, 55°C for 90s, 69°C for 75s; 60°C for 30min and then a hold at less than 12°C. All products were sized using an ABI Prism 3100 Genetic Analyser.

Results

The microsatellite data from the 2011 isolates has been compared with data from previous work carried out at Wellesbourne on isolates obtained in 2009 and 2010. All nine Scottish *S. subarctica* isolates from 3 different locations are different genotypes and are also different from any of the isolates collected in Herefordshire (Table 3). Only four genotypes were found within 33 isolates from Herefordshire.

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Table 3 - Size of PCR products from microsatellite marker analysis of *S. subarctica*, organised into genotypes. Isolates highlighted with the same colour are the same genotype.

Isolate	Year isolate obtained	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7	Locus 8	Genotype Number	
SC23	2011	333	189	178	364	146	371	375	193	1	Scottish isolates
SC2	2011	320	181	178	364	130	372	375	194	2	
SC27	2011	320	181	178	364	146	371	375	185	3	
SC17	2011	333	197	178	364	130	371	371	193	4	
SC25	2011	320	197	178	364	130	371	375	185	5	
Bo'mains 10 -1	2011	320	200	179	364	130	371	409	185	6	
SC30	2011	320	189	182	364	146	371	389	185	7	
Liel8-1	2011	320	200	184	364	146	372	375	185	8	
SC11	2011	320	200	193	364	146	372	409	193	9	
CC19-1	2011	333	189	174	376	142	383	389	193	10	Herefordshire isolates
CC2-4	2011	333	189	174	376	142	382	389	193	10	
HE8	2009	333	189	174	376	142	381	389	194	10	
HE14	2009	333	189	174		142	382	389	194	10	
CC18-1	2011	345	189	174	388	130	393	378	193	11	
CC23-1	2011	345	189	174	388	130	394	378	193	11	
CC7	2010	346	189	174	388	130	394	379	194	11	
CC1	2010	346	189	174	388	130	394	379	194	11	
HE19	2009	346	189	174	389	130	394	378	194	11	
HE3	2009	346	189	174	389	130	394	378	194	11	
HE2	2009	346	189	174	389	130	394	379	194	11	
CC46-2	2011	345	189	174	372	130	379	405	194	12	
CC15-1	2011	345	189	174	372	130	378	404	193	12	
CC13-2	2011	346	189	174	372	130	378	404	193	12	
CC47-2	2011	346	189	174	372	130	379	405	194	12	
CC16-3	2011	346	189	174	372	130	378	404	193	12	
CC22-1	2011	346	189	174	372	130	379	405	194	12	
CC48-1	2011	346	189	174	372	130	379	405	194	12	
CC5	2010	346	189	174	372	130	379	405	194	12	
CC4	2010	346	189	174	372	130	379	405	194	12	
HE13	2009	346	189	174	372	129	379	405	194	12	
HE16	2009	346	189	174	372	130	379	405	194	12	
HE10	2009	346	189	174	373	130	379	405	194	12	
HE21	2009	346	189	174	373	130	379	405	194	12	
HE5	2009	346	189	174	373	130	379	405	194	12	
HE6	2009	346	189	174	373	130	379	405	194	12	
HE27	2009	346	189	174	373	130	379	405	194	12	
HE1	2009	346	189	174	373	130	379	405	194	12	
HE25	2009	346	189	174	373	130	379	405	194	12	
CC6-2	2011	346	189	174	372	130	378	405		12	
CC21-1	2011	320	189	174	388	146	394	424	203	13	
CC11-1	2011	320	189	174	388	146	393	424	202	13	
HE4	2009	320	189	174	389	146	394	424	203	13	

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Discussion

Objective 1 - To determine the effect of organic soil amendments on the survival of sclerotia of *Sclerotinia sclerotiorum*.

Initial results from the soil box trials show that some biofumigant crops can significantly reduce carpogenic germination of *S. sclerotiorum*, and can also delay germination. If sclerotia are being weakened by biofumigation (hence the delay in germination) it may be possible to combine biofumigation treatments with biological control agents such as Contans WG to achieve better germination suppression/reduction. Further work on different cultivars of the best performing biofumigants (brown mustards) may help to highlight those most suitable for use against *S. sclerotiorum*. These can also be trialled against different isolates of *S. sclerotiorum,* as well as *S. subarctica* to see if this is a factor that affects the efficacy of the biofumigation.

Further *in vitro* work is needed to establish whether the effect on sclerotia in the soil box trials is due to ITCs being released from the plant material, or due to other effects such as increased microbial activity. This aspect has also been addressed by including a low glucosinolate content oilseed rape cultivar in soil box trials 2, 3 and 4. Additionally ground oven dried plant material will be used in future soil box trials to eliminate any variation in water content of the boxes, which was observed when using fresh plant material. This is caused by water being released by the plant material as it breaks down over time and may have an effect on viability/germination ability of the sclerotia.

Objective 2 - To determine the aggressiveness of different Sclerotinia genotypes and species on commercial carrot varieties and quantify production of sclerotia.

The carrot root inoculations showed that there is consistent variation in the number and sizes of sclerotia produced by the two different isolates of *S. sclerotiorum*. The size of sclerotia may affect their survival in soil, and the number of apothecia produced, therefore having a direct impact on the relative frequencies of each isolate. Further investigation is required to determine if any of the cultivars in the trial which produced very few sclerotia for either *S. sclerotiorum* isolate would do so consistently and therefore be suitable for future breeding work. The whole carrot plant inoculation trials may also indicate suitable cultivars for future breeding programs.

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Objective 4 - Epidemiology and control of *Sclerotinia subarctica.*

The results from the microsatellite marker data show that there is considerable diversity in isolates of *S. subarctica* in Scotland, in comparison to isolates from England (Herefordshire). This may indicate that sexual reproduction is occurring in Scotland where the conditions may be more favourable for this species. Further sampling in Scottish crops will indicate the prevalence of *S. subarctica* which is particularly important as symptoms of infection in the field appear to be the same as *S. sclerotiorum*. It is likely that *S. subarctica* remains undetected in many crops. Future work on growth and germination requirements will help to provide further ecological information on this newly identified species.

Conclusions

- Initial results show that all but one of the biofumigant crops tested against *S. sclerotiorum* sclerotia significantly reduced carpogenic germination and production of apothecia.
- *Brassica juncea* 'Vittasso' reduced carpogenic germination of sclerotia by 61% in comparison to the untreated control.
- *Brassica juncea* 'Pacific Gold' completely inhibited mycelial growth of *S. sclerotiorum in vitro* and delayed growth at lower rates.
- Some carrot roots produce very few sclerotia and could be used in future breeding programs.
- Initial results suggest that *S. subarctica* isolates are more diverse in Scotland compared to those found in Herefordshire.

Knowledge and Technology Transfer

- Abstract for University of Warwick School of Life Sciences Postgraduate Symposium, March 2012.
- Poster presented at 2012 HDC studentship conference, July 2012.
- Presenting at AAB IPM conference, October 2012.

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