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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 germinate 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.
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
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.
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.

**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 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.
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 germinate 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).
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
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
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.
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**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Full Field Rate (per soil box)</th>
<th>Half Field Rate (per soil box)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Brassica juncea</em> ‘Vitasso’</td>
<td>47g</td>
<td>23.5g</td>
</tr>
<tr>
<td>2. <em>Brassica juncea</em> ‘Pacific Gold’</td>
<td>47g</td>
<td>23.5g</td>
</tr>
<tr>
<td>3. <em>Sinapis alba</em> ‘Brisant’</td>
<td>47g</td>
<td>23.5g</td>
</tr>
<tr>
<td>4. <em>Brassica juncea</em> ‘Caliente 99’</td>
<td>47g</td>
<td>23.5g</td>
</tr>
<tr>
<td>5. <em>Raphanus sativus</em> ‘Terranova’</td>
<td>47g</td>
<td>23.5g</td>
</tr>
<tr>
<td>6. <em>Eruca sativa</em> ‘Nemat’</td>
<td>47g</td>
<td>23.5g</td>
</tr>
<tr>
<td>7. Perlka® (Calcium cyanamide)</td>
<td>0.43g</td>
<td>0.43g</td>
</tr>
<tr>
<td>8. Biofence (mustard meal pellets)</td>
<td>1.4g</td>
<td>1.4g</td>
</tr>
<tr>
<td>9. Contans WG (<em>Coniothyrium mimitans</em>)</td>
<td>0.4g</td>
<td>0.4g</td>
</tr>
<tr>
<td>10. Untreated</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11. <em>Brassica napus</em> ‘Temple’</td>
<td>47g</td>
<td>23.5g</td>
</tr>
</tbody>
</table>

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
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](image-url)
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](image)

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

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.

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
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.](image-url)
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](image_url) - 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).
Table 2 - Varieties being used in whole carrot plant inoculation trial, and their growth habits and sclerotia production on roots.

<table>
<thead>
<tr>
<th>Carrot Diversity Set No.</th>
<th>Group Name</th>
<th>Root position in soil</th>
<th>Leaf growth habit</th>
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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.
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...
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.
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.

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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.
**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.
- Presenting at AAB IPM conference, October 2012.
References


