Grower Summary

CP 080

Pathogen diversity, epidemiology and control of Sclerotinia disease in vegetable crops

Final 2014

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GROWER SUMMARY

Headline

In trials biofumigant crops reduced germination of *S. sclerotiorum* sclerotia by up to 70%, but were less effective against larger sclerotia. *S. subarctica*, a related species, has been identified on numerous crop hosts in Scotland.

Background

In the UK, carrot growers suffer estimated annual crop losses in excess of six million pounds due to Sclerotinia disease, with marketable yield predicted to be reduced by one tonne per hectare for each 1% increase in diseased roots (McQuilken, 2011; AHDB Horticulture Factsheet 19/11). Additionally, lettuce growers in the UK face potential crop losses of 15%, worth an estimated £12 million, due to Sclerotinia disease (DEFRA, 2013).
S. sclerotiorum has a host range of over 400 plant species including important horticultural crops such as oilseed rape, lettuce, potato, peas, beans, sunflower, celery and some vegetable brassicas such as cabbage and swede. Many wild hosts and broad leaved weed species can also be infected including dandelion, fat hen, thistle and buttercup species.

The Pathogen - *Sclerotinia sclerotiorum*

Sclerotinia sclerotiorum (Lib.) de Bary is a plant pathogenic fungus that affects many economically important crops (Hegedus & Rimmer, 2005), with a world-wide distribution (Purdy, 1979) and a wide host range of over 400 plant species (Boland & Hall, 1994). Crops susceptible to sclerotinia disease include lettuce, oilseed rape, beans, peas, potatoes and carrots (Saharan & Mehta, 2008).
The long term survival structures for *S. sclerotiorum* are small black resting bodies called sclerotia (Willetts & 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 & 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 & 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 Alaska (Winton *et al.*, 2006). The symptoms caused by *S. subarctica* are very similar to *S. sclerotiorum* and therefore the former may be undetected in crops in the UK. One aim of this work was 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) and other crops. 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

The most common approach to control of *S sclerotiorum* is to apply fungicides with the aim of killing ascospores before they infect plants, with the best protection obtained by spraying before canopy closure (McQuilken, 2011). Timing of spraying is critical to the effectiveness of protection provided by fungicides, and hence the extent of control by fungicides can be variable. Also, some of the effective active ingredients in fungicides currently used routinely against Sclerotinia disease such as boscalid, carbendazim, cyprodinil, fludioxonil (Matheron & Porchas, 2008), azoxystrobin and difenoconazole are classed as medium to high risk for resistance (McQuilken, 2011). Clipping of carrot foliage to prevent lodging and hence plant to plant spread of infection between beds has also been 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). New control methods to reduce the viability of *S sclerotiorum* sclerotia in the soil would therefore be useful as a more long-term and sustainable control strategy.

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. There has also been much research on biological control, with the fungus *Coniothyrium minitans* which

parasitizes the sclerotia of *S. sclerotiorum,* now 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 potentially 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* (brown mustard) was found to be the only cruciferous plant to delay germination of *S. sclerotiorum* sclerotia in one study, (Smolinska & Horbowicz, 1999) yet *Brassica oleracea* var. *caulorapa* (kohlrabi) reduced mycelial growth in another (Fan *et al.*, 2008). Another study found that a blend of *Brassica napus* (oilseed rape) and *Brassica rapa* (field mustard) reduced the viability of sclerotia in the soil (Geier, 2009).

The main aim and objectives of the project were:

Aim: To identify potential new soil treatments for control of sclerotinia disease and to assess pathogen diversity.

Objectives:

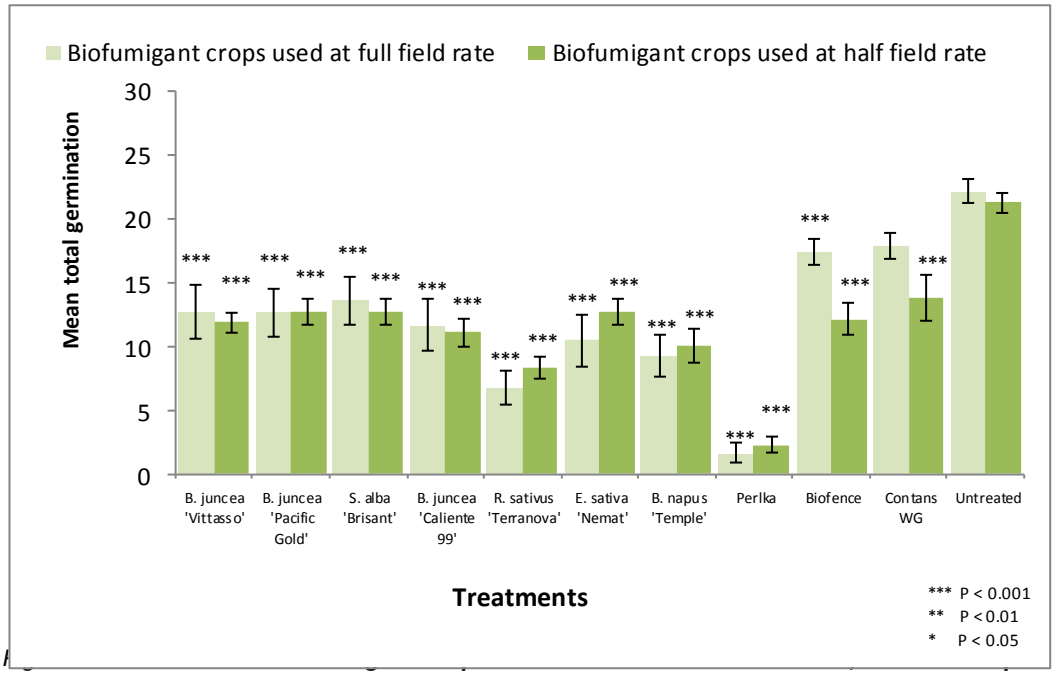
i. To determine the effect of different biofumigation crops on the germination and survival of sclerotia of *Sclerotinia sclerotiorum*.
ii. To evaluate carrot varieties for susceptibility to *Sclerotinia sclerotiorum* and quantify production of sclerotia by different *S. sclerotiorum* genotypes.
iii. To investigate the incidence, diversity and epidemiology of *Sclerotinia subarctica.*

Summary

Objective 1: To determine the effect of biofumigation on the germination and survival of sclerotia of *Sclerotinia sclerotiorum*

Results from laboratory experiments showed that some biofumigant crops can significantly reduce carpogenic germination of *S. sclerotiorum* (Figure 1), with the most effective in soil box tests being *Raphanus sativus, which* reduced germination by 70%. The results from *in vitro* experiments testing direct effects of biofumigants on mycelial growth and carpogenic germination suggested that this reduction was caused by fungitoxic isothiocyanates being released from the plant material. Results from polytunnel experiments showed that three *Brassica juncea* biofumigation crops reduced germination of *S. sclerotiorum* sclerotia, but this effect was not statistically significant, most likely due to insufficient germination in the

control plots. Further work on methodology is required to enable assessment of biofumigant crops in a non-laboratory setting. An HPLC analysis showed a clear difference in the glucosinolate quantities in biofumigant crops grown at different times of year, with later sown crops (harvested June to November) having generally lower levels.



'Temple', Perlka®, Biofence and Contans WG on final germination of* S. sclerotiorum *sclerotia after 150 days in soil box trials.

Objective 2: To evaluate carrot varieties for susceptibility to *Sclerotinia sclerotiorum* and quantify production of sclerotia by different *S. sclerotiorum* genotypes

Carrot root inoculation experiments showed that the number of sclerotia produced on carrot roots is significantly affected by *S. sclerotiorum* isolate, possibly related to the survival strategy of the individual isolate. The weight of individual sclerotia produced by different isolates is influenced by carrot accession, but not by *S. sclerotiorum* isolate. This may be due to the nutritional content of plant tissue, or due to variations in the permeability and integrity of root cell membranes. Some of the cultivars in the experiment produced very few sclerotia for either *S. sclerotiorum* isolate and may therefore be suitable for future breeding work. Whole carrot plant inoculation tests indicated that 'Little Finger' and 'Brasilia' may also be suitable cultivars for such a program, as they showed the slowest disease progression down the petiole compared to other carrot varieties such as Nairobi, Chantenay, Eskimo and Narbonne. However, detached leaf inoculations produced results which were not

correlated with the whole plant inoculations, with 'Brasilia' having the fastest rate of disease progression. This latter assay may not therefore be suitable for screening carrot varieties for resistance to *S. sclerotiorum.*

Objective 3: To investigate the diversity and epidemiology of *Sclerotinia subarctica*

S. subarctica was identified in East Scotland on numerous crop hosts in several locations whereas previously it had only been found in one location in England, on meadow buttercup. Population structure analysis showed that *S. subarctica* is genetically diverse in Scotland, and shared genotypes with isolates from Norway. The limited geographical distribution of *S. subarctica* may be due to its lower tolerance to high temperatures for mycelial growth compared with *S. sclerotiorum*, as well as its requirement for a longer period of cold conditioning before carpogenic germination can occur.

Although the full host range and epidemiology of *S. subarctica* are unknown, production of apothecia from germinating sclerotia has been observed in the lab and we assume therefore that ascospores are the main inoculum sources as for *S. sclerotiorum*. It is therefore very likely that fungicides applied for control of *S. sclerotiorum* will also control *S. subarctica.*

Conclusions

- In soil box experiments *Raphanus sativus* 'Terranova' reduced carpogenic germination of *S. sclerotiorum* sclerotia by 70% in comparison to the untreated control which compares well to the positive control Perlka® which reduced germination by 93%.
- Following inoculation with *S. sclerotiorum*, roots from some carrot lines produced very few sclerotia while others from whole plant tests showed slow disease progression and could therefore be used in future breeding programs.
- *S subarctica* is present on numerous crop and wild hosts in East Scotland, Norway and Sweden, often occurring in sympatry with *S. sclerotiorum*. *S. subarctica* genotypes were shared between Scotland and Norway, and between crop plants and meadow buttercup.
- Mycelial growth experiments indicated that *S. subarctica* has a lower tolerance to high temperatures for mycelial growth compared with *S. sclerotiorum*, as well as

requiring a longer period of cold conditioning at 5°C for rapid carpogenic germination, which may indicate adaptation colder climates.

Financial Benefits

Given the losses experienced in carrot and other susceptible crops, the potential financial benefits of reducing *S. sclerotiorum* inoculum through biofumigation or use of less susceptible cultivars could be substantial in the future. The next step is to confirm the efficacy of biofumigation in the field and to develop an integrated approach to Sclerotinia control which could include other techniques such as disease forecasting, canopy clipping (for carrot) and biological control using Contans.

Action Points

Biofumigation may be a useful addition to an integrated disease management system for *Sclerotinia sclerotiorum* - further polytunnel and field experiments are required to establish the best time of year to grow the biofumigation crop to maximize glucosinolate levels and biomass, as well as to determine the most effective method of incorporation.