Grower Summary

CP 080

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

Annual 2013

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Before using all pesticides check the approval status and conditions of use.

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Further information

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Headline

In trials *Raphanus sativus* 'Terranova' reduced carpogenic germination of *S. sclerotiorum* sclerotia to produce apothecium by 73% in comparison to the untreated control, which compares well to the positive control Perlka® which reduced germination by 82%.

Background

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). 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 *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). 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 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 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) 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). 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 also been much research on biological control, with the fungus *Coniothyrium minitans* (which parasitises 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 and 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 aims and objectives of this project are:

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

Objectives:

i. To determine the effect of organic soil amendments 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 diversity, epidemiology and control of *Sclerotinia subarctica.*

Summary

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

Results from trials showed that the majority of biofumigant crops tested can significantly reduce carpogenic germination of *S. sclerotiorum* (Figure 1), and the results from *in vitro* trials testing direct effects of biofumigants on mycelial growth and carpogenic germination suggests that this is caused by fungitoxic isothiocyanates being released from the plant material. However, although the low glucosinolate oilseed rape cultivar, *Brassica napus* 'Temple' reduced carpogenic germination, HPLC analysis confirmed that it does not contain significant levels of glucosinolates. This suggests that there are other volatile compounds being released from 'Temple' which affects the germination of sclerotia.

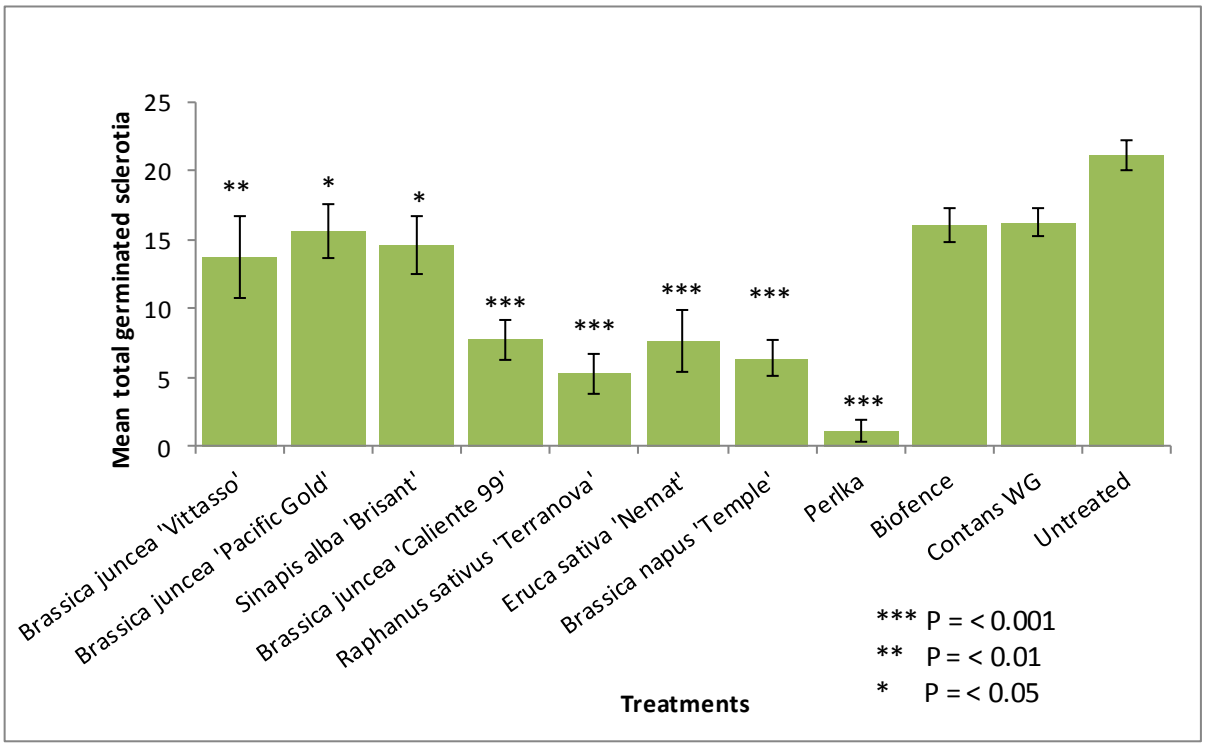

Figure 1 – The effect of biofumigant crops at full field rates, *Brassica napus* 'Temple', Perlka®, Biofence and Contans WG on final germination of *S. sclerotiorum* sclerotia after 150 days in a soil box experiment at 15°C.

The HPLC analysis showed a clear difference in the glucosinolate quantities in biofumigant crops grown at different times of year in the polytunnel. Further examination of this data will indicate whether this is due to changing temperatures across the cropping dates, or pest damage. A current polytunnel trial will help to assess the effectiveness of the biofumigant crops on carpogenic germination in a more realistic setting, as well as determining effects on subsequent disease incidence.

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

Carrot root inoculations repeated as in year one showed that there is consistent variation in the number and size of sclerotia produced by two different isolates of *S. sclerotiorum* (L6 and L44). 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. Some of the cultivars in the trial produced very few sclerotia for either *S. sclerotiorum* isolate and may therefore be suitable for future breeding work. Whole carrot plant inoculation trials 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.

Objective 4 - Epidemiology and control of Sclerotinia subarctica

The results from the microsatellite genotyping of *S. subarctica* isolates collected in 2012 shows that there is considerable diversity amongst isolates from Scotland, in comparison to isolates from England (Herefordshire). This could indicate that sexual reproduction is occurring in Scotland where the conditions may be more favourable for this species. Research investigating the chilling time required for rapid carpogenic germination will help determine the conditions required by this species, although mycelial growth trials showed no significance differences between *S. subarctica* isolates and a *S. sclerotiorum* isolate in their response to temperature.

Conclusions

- In soil box trials *Raphanus sativus* 'Terranova' reduced carpogenic germination of *S. sclerotiorum* sclerotia by 73% in comparison to the untreated control which compares well to the positive control Perlka® which reduced germination by 82%
- The best overall control in soil box trials and direct *in vitro* tests was achieved by *Brassica juncea* 'Caliente', which also had the highest levels of the glucosinolate sinigrin out of all the brown mustards.
- Roots from some carrot lines produce very few sclerotia while others from whole plant tests show slow disease progression after inoculation and could therefore be used in future breeding programs.
- Results from genotyping 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 the project.

Action Points

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