Grower Summary

FV 405

Carrots: Control of carrot cavity spot through the use of pre-crop green manures/biofumigation

Final 2017

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The results and conclusions in this report may be based on an investigation conducted over one year. Therefore, care must be taken with the interpretation of the results.

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GROWER SUMMARY

Headline

Autumn-sown overwintered biofumigant crops of brown mustard, white mustard and radish had no effect on cavity spot disease of carrots sown after incorporation the following spring. This may have been due to lack of sufficient biofumigant biomass and glucosinolate content, indicating that these type of crops are better suited to a different time in the rotation. There was no apparent effect of biofumigants on detection of *P. violae* and highest levels of the pathogen were detected in May / June and September / October.

Background

Cavity spot is the major disease of carrots in the UK and is caused mainly by *Pythium violae* and less frequently by *P. sulcatum*. Control of cavity spot is difficult and currently relies on application of metalaxyl. In the absence of any other approved products and the cost of bringing new actives to market that meet the increasingly stringent registration requirements, there is an urgent need to identify new approaches to disease control. The interest in the potential use of biofumigation and green manure crops to control soilborne diseases such as *P. violae* has increased in recent years. Biofumigation involves crushing and incorporating specific crops with high glucosinolate levels into the soil. This process, when carried out under high soil moisture conditions, allows the conversion of the glucosinolate compounds to isothiocyanates (ITCs) which are toxic to a range of soil microorganisms. Glucosinolates occur at high levels in certain *Brassica* and related crucifer crops such as mustards and radish. Biofumigation using a brown mustard (*Brassica juncea*) previously resulted in good control of cavity spot (Anon, 2009).

Project aims and summary of first phase results

The main aim of this project was therefore to test the effect of biofumigant mustards and green manure crops on the dynamics of *P. violae* and cavity spot disease. A secondary aim was to determine effects on free-living nematodes and assess fanging.

In the first phase of the project, two field experiments were carried out in Cottage Field at Wellesbourne (where cavity spot was previously known to develop) to test the effect of different biofumigant / green manure crops on cavity spot disease and the dynamics of *P. violae*. At the same time, the effects of these treatments on free-living nematodes and carrot fanging were also assessed. The first experiment established biofumigant / green manure crop treatments (two mustards, wheat, clover-rye mix, forage rape) in September 2012 which were incorporated in late May 2013 while a second experiment established biofumigant treatments (two mustards) in March 2013 which were incorporated in early June 2013. For both experiments, biofumigants / green manures were sown in beds and carrots drilled approximately two weeks after incorporation. Soil and / or small carrot root samples were taken during the season for PCR detection of *P. violae*, counts of free-living nematodes and to assess cavity spot disease levels and fanging. The main assessments of cavity spot and fanging were carried out for two large harvests of carrot roots before and after strawing down of the carrot crops. In addition, sclerotia of *Sclerotinia sclerotiorum* were also buried in the treatment plots and monitored to determine if biofumigant crops could suppress germination.

Results from this first phase of work indicated that *P. violae* could be detected by PCR in all treatment plots for at least one sampling time over the duration of the autumn and spring-sown experiments. However there was no effect of biofumigation / green manure treatments on the pathogen and generally, *P. violae* dynamics followed a similar pattern irrespective of treatment. Three peaks of pathogen detection were observed in February 2013 (biofumigants/green manures semi-mature), September 2013 (subsequent carrot crops mature) and March 2014 (post carrot crop strawing down). The general increase in *P. violae* levels in late autumn as the carrot crops matured had been observed previously (Anon, 2009). Although cavity spot has been observed at moderate-high levels in the past in the field at Wellesbourne, little disease developed across both autumn and spring-sown biofumigant / green manure experiments during the season and in the larger root samples harvested pre- and post- strawing down of the carrot crops in November 2013 and March 2014. Due to these low disease levels, it was therefore not possible to determine any effect of the biofumigant/green manure treatments on cavity spot disease.

For the free-living nematodes, initial counts showed that *Trichodorus* spp. (stubby root), *Tylenchorynchus* / *Helicotylenchus* spp. (stunt / spiral), *Pratylenchus* spp. (root lesion) and *Longidorus* spp. (needle) nematodes were all present in Cottage Field while other free-living nematode types were absent or below detectable levels. Only *Longidorus* spp. were present in sufficient numbers (>200/L soil) to cause damage throughout the field experiments. For both autumn and spring experiments, numbers of these and all the other nematode spp. declined considerably following incorporation of the biofumigants / green manures but this effect was observed for all the treatments including the untreated (fallow) control. This led to the conclusion that the treatments themselves had no effect on free-living nematodes and hence the decline may have been due to other factors, most likely the tilling operations involved in incorporating the treatments and drilling subsequent carrot crops.

Finally, biofumigants / green manures has little or no effect on germination of *S. sclerotiorum* sclerotia which was in contrast to findings by Clarkson (2013) under controlled conditions (in enclosed boxes) where a brown mustard significantly reduced carpogenic germination by 63% compared to the untreated control.

Aims in phase 2

Due to the low levels of cavity spot at the single field site in phase 1, it was decided after consultation with AHDB, the grower representative and the BCGA to test the biofumigants over multiple field sites in order to mitigate the risk of low cavity spot disease levels. Hence in this second phase of the work, field experiments were carried out at three sites to test the effect of brown mustard, white mustard and radish on cavity spot and free living nematodes. Crops were established in Autumn 2014 with carrots sown in Spring 2015, and as before, soil and root samples were tested for the presence of *P. violae* by PCR over the course of the experiments. Cavity spot disease was assessed in two large harvests January-March 2016 and effects of the treatments on free-living nematodes, and carrot fanging were also assessed. Due to poor carrot seedling emergence at Wellesbourne, this site was abandoned and hence an additional commercial carrot crop near Clipstone (Nottinghamshire) was monitored using PCR to specifically examine colonisation of seedlings and lateral carrot roots by *P.violae*.

Summary

Field experiments to determine the effect of biofumigants on cavity spot, Pythium violae levels and free living nematodes

Field experiments were set up at three different field sites at Wellesbourne (Warwickshire), Wretham (Norfolk) and Gooderstone (Norfolk) to test the effect of different biofumigant crops on cavity spot disease and the dynamics of *P. violae*. At the same time, the effects of these treatments on free-living nematodes and carrot fanging were also assessed. At each site, biofumigant crops of brown mustard (Caliente 99), white mustard (Brisant) and radish (Terranova) were established in September 2014, protected with fleece, and chopped/incorporated in April 2015 after which carrots were sown approx. two weeks later. Leaf samples from brown / white mustards and radish were taken to quantify the levels of glucosinolates using HPLC just prior to incorporation. Carrot crops established well at the Norfolk sites, but seedling emergence at the Wellesbourne site was poor due to adverse seedbed conditions and hence, after consultation with AHDB and the grower representative, this experiment was abandoned. For the remaining two sites, a total of 8 soil and / or small carrot root samples (160 roots per treatment) were collected for each treatment at regular intervals from September 2014 to March 2016 for PCR detection of *P. violae* and to assess cavity spot disease levels. Two main assessments for cavity spot and fanging in each treatment were then carried out for large harvests of carrot roots (600 roots per treatment)

January-March 2016 following strawing down. Levels of free living nematodes were determined pre- and post-incorporation of biofumigants and prior to final harvest.

Monitoring of Pythium violae in a commercial crop

As the Wellesbourne experiment was abandoned, a commercial carrot crop near Mansfield, Nottinghamshire was monitored for *P. violae* using PCR to test the hypothesis that *P. violae* infects seedlings or through lateral roots in larger plants. Here, roots from a total of 240 carrot seedlings or lateral roots from more mature plants were collected on each of 11 occasions from emergence to harvest in (May to December 2015) for analysis.

Results and conclusions

Biofumigant growth and glucosinolate levels

Biofumigant crops initially established well in the warm Autumn of 2014 but despite fleece protection, the white mustard crop at Wellesbourne in particular suffered severe frost damage. Mean biomass levels of brown mustard, white mustard and radish over all crops was 0.67, 0.48 and 1.5 kg m^{-2} with a maximum at the Wretham site of 0.96, 0.90 and 3.69 respectively. In comparison, the minimum (anecdotal) recommended biomass level for effective biofumigation is approx. 5 kg m^{-2} (50 tonnes ha^{-1}). Other UK studies have shown that summer biofumigant crops can yield 2.1 – 3.2 kg m^{-2} compared to 1.0 - 1.6 kg m^{-2} for winter crops and yields as high as 7-18 kg m^{-2} have been recorded under optimum conditions. Glucosinolate levels were also low with means of 2.6, 1.2 and 1.3 µmol g-1 for brown mustard, white mustard and radish respectively. This compares to published levels of 20-50 µmol g^{-1} for biofumigant crops grown in more optimum conditions. The most likely reason for this was the combination of low temperature and short day-length in winter / early spring, both of which have been demonstrated to reduce biomass and glucosinolate production**.**

It can be concluded therefore that the short growing window and suboptimal environmental conditions associated with overwintering biofumigants before carrot drilling results in low biomass and glucosinolate content reducing potential disease control efficacy. In the absence of truly winter-hardy biofumigant crops which can produce adequate glucosinolate levels during this period, a better strategy would be to grow them in late summer and incorporate them much earlier before carrot drilling the following Spring. This may be problematic however for growers on rented land which may not be available at this time.

Development of cavity spot and P. violae levels

Cavity spot levels in the small carrot samples collected during the season remained low until February 2016 when the incidence of cavity spot was 23-34% at Wretham and from 12-23% at Gooderstone over all the treatments. Generally, *P. violae* dynamics followed a similar pattern over all the treatment plots including the untreated control and hence there there was no clear effect of the biofumigation crops on the pathogen. Although the dynamics of *P. violae* varied between the two field sites, there were some general trends. Following biofumigant sowing in late September 2015, detection of *P. violae* decreased over the winter and early spring period at Gooderstone where all the plots initially tested positive for the pathogen and was also at a low level at Wretham during this period. Subsequently there were two peaks of detection in May / June 2015 at the time of biofumigant incorporation and carrot drilling (Wretham) or just after (Gooderstone) followed by a decline in *P. violae* levels at both field sites in July and August. Detection then increased to another peak in September / October 2015 as the carrot crop matured further at both field sites. For Wretham, *P. violae* was detected continuously thereafter in approximately half of the plots until the carrot harvests in February / March 2016 while at Gooderstone, there were low levels of the pathogen in November before some further detection towards harvest. Between these peaks of detection, *P. violae* was not present or only observed for a small number of plots. Overall, the pathogen was detected more frequently at the Wretham site especially leading up to harvest, and this corresponded with a high incidence of cavity spot symptoms (see next section).

Effect of biofumigants on cavity spot

Cavity spot developed on both carrot crops at the two Norfolk field sites with a mean incidence over all treatments of 79% Wretham and 20% at Gooderstone by the second large root harvest. Severity of cavity spot was also high for Wretham with a mean of 40% of carrots having moderate-severe symptoms (>3 lesions per carrot) by the second harvest. However, there was no effect of the biofumigant crops on cavity spot as levels were very similar across all treatments including the untreated control. This could be due to low biomass and glucosinolate levels.

Effect of biofumigants on free-living nematodes and fanging

Levels of different free-living nematodes *Trichodorus* spp. (Stubby Root), *Tylenchorynchus / Helicotylenchus* spp. (Stunt / Spiral), *Heterodera* spp. (Cyst), *Pratylenchus* spp. (Root Lesion), *Longidorus* spp. (Needle), *Xiphinema* spp. (Dagger), *Ditylenchus* spp. (Stem) and *Meloidogyne* spp. (Root knot) varied between field sites but overall there was no consistent effect of biofumigant crops on these organisms. There was also no clear effect of biofumigant crops on fanging.

PCR detection of Pythium violae in a commercial carrot crop

In the commercial crop, levels of *P. violae* detected by PCR in seedling or lateral roots of more mature plants were very low over the duration of the monitoring period, with *P. violae* being detected in only one sample on one occasion (25/11/15), approx. 4 weeks after the carrots were strawed down. Correspondingly there were negligible levels of cavity spot symptoms on roots or in the surrounding commercial crop.

Conclusions

- Biofumigant crop plants of brown mustard, white mustard and radish had low biomass and glucosinolate levels when incorporated in late April after sowing the previous September due to sub-optimal growing conditions over winter. White mustard was liable to severe frost damage despite fleece protection. Biofumigants should therefore be grown at a different time in the rotation, preferably in mid-late summer following a winter barley crop for instance.
- *P. violae* detection by PCR in field experiments was high in May / June but declined in July and August. There was then an increase in pathogen detection in September as the carrot crop matured further. A high level of detection at the field site at Wretham corresponded to greater cavity spot disease suggesting the PCR test can identify when the pathogen is multiplying and disease pressure is high. The PCR approach is being developed further in AHDB PhD project FV 432 to improve sensitivity and quantification.
- Biofumigant crop plants had no effect on cavity spot disease either due to low biomass and glucosinolate levels, or because the ITCs released had no effect on pathogen oospores. Further research would be needed to determine if biofumigants grown in optimum conditions can reduce cavity spot.
- Biofumigant crop plants had no effect on free-living nematodes or on the level of carrot root fanging.
- *P. violae* was detected at very low levels in a commercial crop which did not develop significant levels of cavity spot. This again suggests that the PCR test is a valid approach to assessing disease pressure.

Financial Benefits

Currently, no conclusions can be drawn concerning the potential economic benefits of growing biofumigants crops for control of cavity spot disease.

Action Points

Biofumigant crops tested here are unsuitable for growing over winter for control of cavity spot in a following spring carrot crop due to low biomass and glucosinolate content. Growers should consider a mid-late summer planting of a biofumigant crop to take advantage of better growing conditions in order to maximise biomass and glucosinolate production if using within a rotation, e.g. following a winter barley crop.