Grower Summary

FV 405

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

Annual 2014

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.

Use of pesticides

Only officially approved pesticides may be used in the UK. Approvals are normally granted only in relation to individual products and for specified uses. It is an offence to use non-approved products or to use approved products in a manner that does not comply with the statutory conditions of use, except where the crop or situation is the subject of an off-label extension of use.

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

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Project Title:	Carrots: Control of carrot cavity spot through the use of pre-crop green manures/biofumigation.
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GROWER SUMMARY

Headline

Pythium violae was detected in soil from around mature carrots in crops drilled after the incorporation of different biofumigant and green manure crops. However, cavity spot disease failed to develop significantly in any of these treatments or the untreated control. The potential of these crops against cavity spot could not therefore be determined due to the low disease levels.

Background

Cavity spot is the major disease of carrots in the UK and is caused mainly by *Pythium violae*. Control of the disease largely relies on early application of fungicides but enhanced degradation of these products may also be a problem in some soils. Long rotations between carrot crops are also recommended. Although cavity spot has been studied in a number of different projects over the years, the dynamics of the pathogen have only begun to be addressed recently, following the development of a specific DNA-based PCR test for *P. violae* and its use in UK-focused research (Anon, 2009). However, this test cannot be used to predict disease before carrot planting (Barbara, 2007), or in the autumn to assess disease risk in strawed down crops, as there is evidence that final levels of disease are to a large extent driven by environmental conditions such as soil moisture (Barbara, 2010; Martin, 2011).

Recently, there has been increased interest in the potential use of biofumigation and green manure crops to control soilborne diseases such as *P. violae*. Green manure crops aim to enhance soil health and fertility with the potential added benefit of encouraging soil microbial activity which may suppress certain plant pathogens. In previous work, a clover/ryegrass mix or a potato crop grown before carrots reduced cavity spot disease whereas other preceding crops such as forage rape and wheat maintained or enhanced *P. violae* levels (Kretzschmar, 2009). It was proposed that this effect was due to the relative ability of different plants or their associated microbiota to sustain or suppress the pathogen.

Biofumigation involves crushing and incorporating specific biofumigant crops into the soil. This process, when carried out under high soil moisture conditions, allows the conversion of glucosinolate compounds to isothiocyanates (ITCs) which are toxic to a range of soil microorganisms. Biofumigation using a brown mustard resulted in good control of cavity spot in one experiment previously (Anon, 2009), but there was seemingly little effect on *P. violae* levels as detected by PCR. This suggested that disease suppression may actually be due to the build-up of competing soil microbiota following incorporation of the biofumigant, a hypothesis also supported by the observation that soil pasteurisation using the chemical fertiliser calcium cyanamide resulted in an increase in cavity spot (Anon, 2009).

In addition to cavity spot caused by *P. violae*, carrot crops are also increasingly affected by Sclerotinia disease caused by *Sclerotinia sclerotiorum*. *S. sclerotiorum* survives in the soil as sclerotia which when brought close to the soil surface germinate carpogenically to produce mushroom-like apothecia. These then release air-borne ascospores which infect plants. Additional problems in carrot crops such as fanging and other root disorders may also occur due to free-living nematodes (particularly stubby-root and needle nematodes), although there is still scant evidence for the extent and type of symptoms they cause (Ellis, 2000). Biofumigation / green manures may therefore also have some benefit against these other carrot problems.

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. Secondary aims of the project were to 1) determine the dynamics of free-living nematodes during the carrot growing season and assess fanging and 2) test the effect of biofumigant mustards and green manure crops on germination of *S. sclerotiorum* sclerotia

Summary

Field experiments and assessments

Two field experiments were carried out in Cottage Field at Wellesbourne (where cavity spot was previously known to develop), primarily 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, carrot fanging and germination of *S. sclerotiorum* sclerotia 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. The second 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. Mustard cultivars used were the brown mustard Caliente 119 and the white mustard Brisant, both of which have been bred for high glucosinolate content. The forage rape cv. Hobson was included as a low glucosinolate Brassica ‘control’, while the wheat cv. Consort and the clover rye mix (cvs. Hobson / Merwi)

were included as they had previously been shown to maintain / suppress cavity spot respectively. Biofumigant / green manure crops were netted or fleeced to prevent pigeon/rabbit damage, subsequently crushed / chopped using a flail topper and incorporated using a bed former when the mustard crops were at full flower (when maximum glucosinolate levels occur). Irrigation was applied immediately to all treatments, ensuring adequate moisture to allow the conversion of glucosinolates to ITCs in the two mustard crops. For the autumn experiment only, sclerotia of *S. sclerotiorum* were buried in each treatment plot immediately after incorporation (but before irrigation) to assess germination and production of apothecia. Following incorporation of the autumn / spring biofumigant / green manure crops, carrots (cv. Nairobi) were drilled and all plots irrigated regularly to encourage development of cavity spot. Leaf samples from brown / white mustards and forage rape were taken to quantify the levels of glucosinolates using HPLC. Soil and / or small carrot root samples (40 roots per treatment) 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 (160 roots per treatment) before and after strawing down of the carrot crops.

Direct effect of biofumigant plants on *P. violae*

Additional work was carried out as part of a University of Warwick Summer Studentship to determine if ITC volatiles released from selected biofumigants including brown and white mustards had a direct effect on *P. violae in vitro*. For each biofumigant, the growth of *P. violae* mycelium was assessed on agar in an inverted Petri dish where water was added to dried, milled plant material in the lid to liberate ITCs.

Results and conclusions

Biofumigant / green manure crop growth and glucosinolate levels

The autumn-sown biofumigants / green manures over-wintered well but a cold spring resulted in a relatively late flowering time and initially a slow development of the spring-sown mustard crops, even though these were fleeced. Autumn and spring-sown mustard crops were in full flower in late May / early June respectively. This is the optimum time for incorporation (highest glucosinolate levels) and hence for carrot growers who drill mainly in April and May, or even earlier, the period for growing mustard biofumigants to this stage is short. Glucosinolate levels in the brown and white mustard crops were also lower compared with those obtained using the same mustard types when gown in a polytunnel in June

(Clarkson, 2013). 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 glucosinolate production.

Dynamics of *P. violae*

P. violae was detected by PCR in all treatment plots (from soil or soil from around carrots) for at least one sampling time over the duration of the autumn and spring-sown experiments (Fig. 1). However, based on the number of plots per treatment testing positive for the pathogen, there was no clear effect of biofumigation / green manure treatments. Generally, *P. violae* dynamics followed a similar pattern for all the treatments plots and there were three sampling times for the autumn-sown experiment where the pathogen was detected in most plots; 19/02/13 (biofumigants/green manures semi-mature), 20/09/13 (subsequent carrot crops mature) and 06/03/14 (post carrot crop strawing down). The general increase in *P. violae* levels in late autumn as the carrot crops matured has been observed previously (Anon, 2009). However, an apparent decrease in the pathogen in October / November 2013 in the presence of overwintered carrots is unexpected but examination of recorded weather data suggests that there was a drier period during this time (data not shown). Detection of *P. violae* in February 2013 in the absence of carrots was also unexpected and requires further investigation.

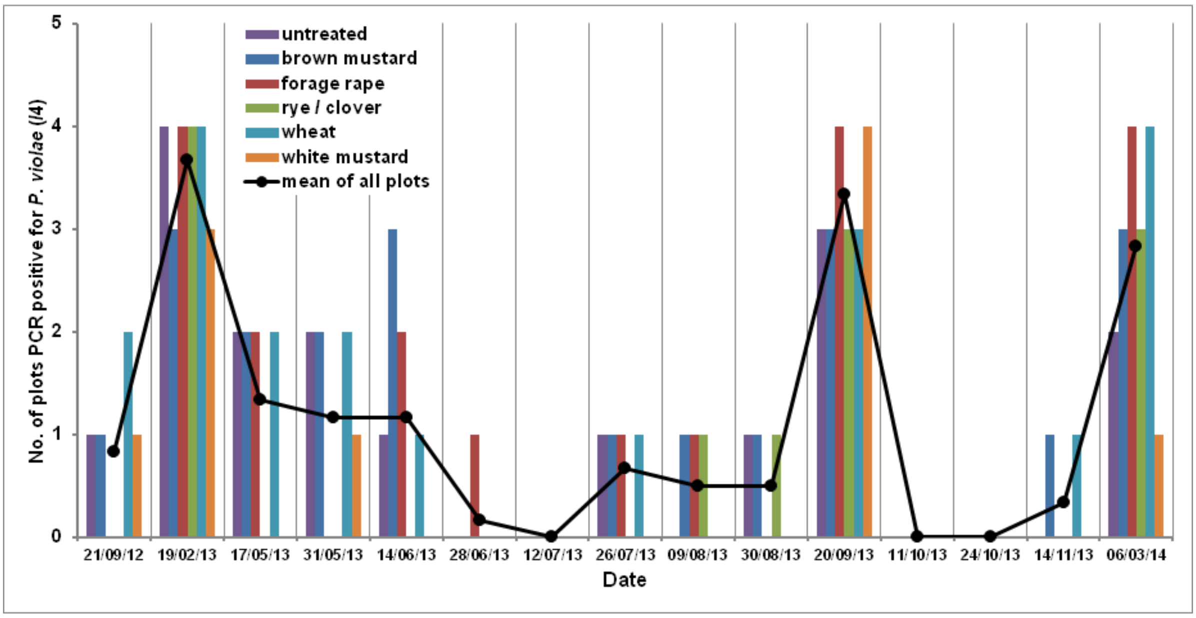

Figure 1. Number of plots (out of a maximum of four) positive for *P. violae* using PCR for autumn-sown biofumigant / green manure crops. Samples for PCR were from bare soil or soil from around plants or from around carrots roots.

Development of cavity spot and effect of biofumigants / green manures

Cavity spot levels as recorded for the same ten roots used for PCR detection remained low throughout the season for both autumn and spring-sown biofumigant / green manure experiments with an average of less than two lesions per root. Cavity spot incidence (presence of one lesion or more) was variable and ranged between 40 and 70%. Similarly, cavity spot levels in the larger root samples (160 roots per treatment) harvested pre- and post- strawing down of the carrot crops on 14/11/13 and 06/03/14 were also low for both experiments. Here, the average number of lesions per root was less than one and cavity spot incidence (presence of one lesion or more) varied between 23 and 42%. Due to these low disease levels, it was not possible to determine any effect of the treatments.

Effect of biofumigants / green manures on free-living nematodes and carrot fanging

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. However, 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.

Effect of biofumigants / green manures on germination of *S. sclerotiorum* sclerotia

In the autumn-sown experiment, final mean percentage germination for buried *S. sclerotiorum* sclerotia were 65, 61, 73, 54, 57 and 71% for brown mustard, white mustard, forage rape, rye / clover mix, wheat and untreated control treatments respectively. Statistical analysis showed that there was a small significant effect on germination for the rye/grass clover treatment only. This 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.

Direct effect of biofumigant plants on *P. violae in vitro*

All biofumigant treatments significantly inhibited the growth of *P. violae* by more than 50% on agar. The brown and white mustards tested completely inhibited growth of *P. violae* (Fig. 2). This is the first evidence that such biofumigants may have a direct effect on the pathogen rather than potentially reducing disease through increased microbial activity as previously suggested.

Overall conclusions

Although ITCs from mustard biofumigants can inhibit both *P. violae* and *S. sclerotiorum*, it is clear that demonstrating this effect in the field is challenging. The primary problem was the low levels of cavity spot developing in experiments despite the extensive use of irrigation in an attempt to promote disease. However, it is important to note that the efficacy of biofumigation can be affected by any agronomic and environmental factors which result in reduced growth and low glucosinolate levels in the biofumigants, poor conversion of glucosinolates to ITCs due to inefficient crushing / incorporation of plant material and inadequate soil moisture levels as well as potential loss of ITCs through escape of volatiles in the air or dissolved in percolating water. The latter could be prevented by the use of polythene covers post-incorporation as used in the original work which demonstrated that brown mustards could reduce cavity spot (Anon, 2009) but this was deemed impractical and potentially costly by the industry.

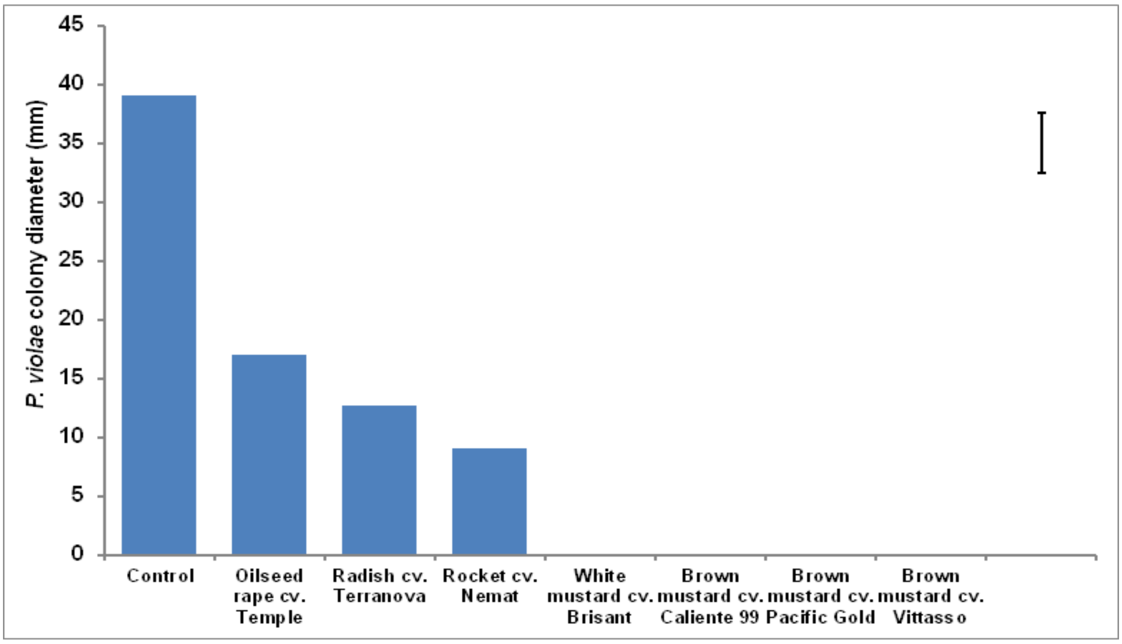

Figure 2. Effect of biofumigants on growth of *P. violae* on agar after 7 days at 20°C. Bar indicates least Significant Difference (LSD, $P \leq 0.05$) between treatments.

Recommendations for work in Year 2 for consideration by BCGA

1) Include one or two grower sites to test selected biofumigants / green manures to reduce the risk of low cavity spot levels.

2) Reduce the number of biofumigant / green manure treatments to allow increased monitoring of *P. violae* dynamics by PCR detection and more accurate assessment of cavity spot disease levels through increased sampling of larger plots.

3) Reconsider using polythene covers to maximise biofumigation potential of mustards.

4) Cease monitoring of free-living nematodes and fanging to increase project focus on cavity spot and free up resources.

Financial Benefits

None at this time.

Action Points

None at this time.