Project title:	Carrots: Control of carrot cavity spot through the use of pre-crop green manures/biofumigation.
Project number:	FV 405
Project leader:	John Clarkson, Warwick Crop Centre, School of Life Sciences, University of Warwick, Wellesbourne Campus. Wellesbourne, Warwick, CV35 9EF.
Report:	Final Report
Previous report:	Annual Report 2014
Key staff:	Gill Prince (Warwick) Claire Handy (Warwick) James Howell (VCS) Steve Ellis (ADAS) Andrew Mead (Rothamsted)
Location of project:	Warwick Crop Centre
Industry Representative:	Ian Holmes
Date project commenced:	01/09/2012
Date project completed (or expected completion date):	30/06/2016

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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.

John Clarkson

Principal Research Fellow

Warwick Crop Centre, School of Life Sciences, University of Warwick.

Signature: 

Date: 30/06/2016

Report authorised by:

Rosemary Collier

Director

Warwick Crop Centre, School of Life Sciences, University of Warwick.

Signature: 

Date: 30/06/2016

CONTENTS

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.

SCIENCE SECTION

Introduction

Cavity spot and Pythium violae

Cavity spot is the major disease of carrots in the UK and is caused mainly by *Pythium violae* and less frequently by *P. sulcatum*. In other countries, *Pythium* species such as *P. vipa* and *P. intermedium* have also been associated with the disease. In addition, other fungi may cause similar root lesions or invade cavities e.g. *Cylindrocarpon destructans*, *Mycocentrospora acerina*.

Control of cavity spot is difficult and although application of appropriate fungicides can reduce disease by more than 50%, these must be applied early before disease is apparent and enhanced degradation of these products may also be a problem in some soils. 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. Long rotations between carrot crops may be beneficial although some reports have suggested that there was no build-up of cavity spot disease over four years of continuous carrot cropping (Anon, 2009). Many other plant species including other crop plants and weeds can also potentially be asymptomatic hosts of *P. violae* (Kretzschmar, 2009).

Although cavity spot has been studied in a number of different Defra and AHDB projects over the years, the dynamics of the pathogen have only begun to be addressed recently, following the development of a specific PCR test for *P. violae* in Norway (Klemsdal et al., 2008) and its validation and use in UK-focused research (e.g. Anon, 2009). However, the PCR test could not be used to reliably predict disease before carrot planting (Barbara, 2007), or in the autumn to assess disease risk in strawed down crops, as there was evidence that final levels of disease are to a large extent driven by environmental conditions such as soil moisture (Barbara, 2010; Martin, 2011). Hence, initial *P. violae* inoculum levels may be less important. The typical dynamics of the pathogen mirrors the development of the carrot crop, being undetectable in early spring, rising to a peak as the roots begin to mature in July / August, and rapidly declining in October / November (Anon, 2009). However, this pattern may vary from year to year, the year-round dynamics of *P. violae* has yet to be explored and the sensitivity of the PCR test has not been clearly established.

Biofumigation and green manure crops

Interest in the potential use of biofumigation and green manure crops to control soilborne diseases such as *P. violae* has increased in recent years. 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 (Kretzschmar, 2009), a clover/ryegrass mix or a potato crop grown before carrots was found to reduce cavity spot disease whereas other preceding crops such as forage rape and wheat maintained or enhanced *P. violae* levels. 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 crops with high glucosinolate levels 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. Glucosinolates occur at high levels in certain *Brassica* and related crucifer crops such as mustards, for instance brown mustard (*B. juncea*) produces the glucosinolate sinigrin (allyl glucosinolate) which is converted to allyl isothiocyanate while white mustard (*Sinapis alba*) produces the glucosinolate sinalbin (hydroxybenzyl glucosinolate) which is converted to hydroxybenzyl isothiocyanate. Biofumigation using a brown mustard (*Brassica juncea*) previously resulted in good control of cavity spot (Anon, 2009), but in this case there was seemingly little effect on pathogen levels as detected by PCR. This suggested a different mode of action such as the build-up of suppressive soil microbiota following incorporation of the biofumigant. The potential importance of microbiota generally for the suppression of cavity spot was also supported by the observation that soil pasteurisation using the fertiliser calcium cyanamide resulted in an increase in cavity spot disease.

Project aims and summary of phase 1 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*. 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 (cv. Nairobi) drilled approximately two weeks after incorporation. 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. 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 Cottage Field, little disease developed across both autumn and spring-sown biofumigant / green manure experiments during the season or in the larger root samples harvested pre- and post- strawing down of the carrot crops in November 2013 and March 2014 where 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 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^{-1} 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 had 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* 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*.

Materials and methods

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

Field experiments to test the effect of different biofumigant crops on cavity spot disease and the dynamics of *P. violae* were set up at three locations; i) Cottage Field, Wellesbourne (Warwickshire; sandy silt / clay pH 6.0); ii) Wretham (Norfolk; sandy loam pH 6.9) and iii) Gooderstone (Norfolk; sandy loam; pH 8.1) with the latter two sites managed by Vegetable Consultancy Services (VCS). Effects of these treatments on free-living nematodes and carrot fanging were also assessed, with Steve Ellis (ADAS High Mowthorpe) carrying out the nematode enumeration.

Biofumigant crops brown mustard (Caliente 99), white mustard (Brisant) and radish (Terranova) were established in each location in September 2014 in 8 rows within 1.83 m beds, at seed rates shown in Table 1. Crops were fleeced / netted until incorporation to prevent pigeon/rabbit damage and protect the plants from frost damage. At each field site there were four replicate plots per treatment (15 m x 1.83 m) each separated by 2 m arranged in a Latin square design arranged in 4 rows (across beds) x 4 columns (down beds) with each

treatment represented once in each row across the 4 beds. Each treatment bed was also separated by a complete untreated (fallow) bed.

In April 2015, biofumigants were crushed / chopped, when the crops were almost at full flower (corresponding to when maximum glucosinolate levels occur), using a Khun flail topper (Wellesbourne) or a tractor mounted flail mower (Wretham, Gooderstone) and incorporated using a bed former (Wellesbourne) or a single bed rotovator (Wretham, Gooderstone). The plots at the Norfolk sites were sealed with a tractor mounted water filled roll and irrigation then applied immediately to all treatments, ensuring that adequate moisture was present to allow the conversion of glucosinolates to ITCs. Irrigation was also applied at Wellesbourne. Approximately two weeks after incorporation of biofumigant crops, carrots (cv. Nairobi) were drilled in four rows at a rate of 80 seeds per linear m in all the beds over the experimental area at each of the three field experiment locations. All plots were irrigated regularly for seedling establishment and to encourage development of cavity spot. Soil and carrot root samples, as described in detail below, were taken throughout the season for PCR detection of *P. violae* and assessment of cavity spot disease levels during crop development. Soil samples for nematode enumeration were collected just prior to incorporation of biofumigants, at carrot drilling and at harvest. The main assessments of cavity spot disease levels were also carried out for two large harvests of carrot roots (600 roots per treatment) at Wretham and Gooderstone January-March 2015. Limited samples of roots were also taken at Wellesbourne in December 2015.

Table 1. Summary of biofumigant crop treatments, sowing and incorporation dates and subsequent carrot crop sowing dates

	Site 1 Cottage Field, Wellesbourne	**Site 2 VCS Site 1, Wretham**	**Site 3 VCS Site 2, Gooderstone**
Biofumigant crop			
Sowing date	09/09/2014	22/09/2014	23/09/2014
Seed rate brown mustard (Caliente 119)	1.5 g m^{-2}	1.5 g m^{-2}	1.5 g m^{-2}
Seed rate white mustard (Brisant)	2.5 g m^{-2}	2.5 g m^{-2}	2.5 g m^{-2}
Seed rate radish (Terranova)	2.5 g m^{-2}	2.5 g m^{-2}	2.5 g m^{-2}
Incorporation date	22/04/2015	29/04/2015	01/04/2015
Carrot crop			
Drilling date	08/05/2015	29/05/2015	17/04/2015
Harvest 1	01/12/2015	11/01/2016	12/01/2016
Harvest 2	-	03/03/2016	24/02/2016

PCR detection of P. violae and cavity spot disease monitoring

Soil samples for PCR detection of *P. violae* were taken from bare soil, from between plants of developing crops, or from around carrots roots lifted at regular intervals during the growing season depending on the time of year (Table 2). Samples were taken between September 2014 (drilling of biofumigants) and February 2016 (final main cavity spot disease assessment), and therefore included samples pre- and post- biofumigant incorporation and during development of the carrots crops. For soil samples taken in the absence of a crop or early on in crop development, a total of approximately 10 g of soil (from a depth of 8-12 cm) was taken from a five point 'W' sampling of each plot (2 g per sampling point), air dried for two days on the lab bench at room temperature and thoroughly mixed in a plastic bag before DNA extraction. For soil samples taken from around carrot roots, 20 roots were lifted from a five point 'W' sampling for each of the four replicate plots to give a total of 80 roots per treatment and any large lumps of soil attached removed and discarded before air drying for two days at room temperature. The soil from around each of the 20 carrots was then brushed off, combined and thoroughly mixed before DNA extraction. DNA extractions from all soil samples were carried out using 0.25 g soil and the PowerSoil DNA Isolation Kit (Camb Bio, UK). A ceramic bead (MP Biomedicals SAS, UK) was added to each soil sample before the samples were shaken in a FastPrep cell disruptor for 45 s at a speed of 5 m/s. DNA samples were then stored at -20°C and subsequently used for PCR detection of *P. violae* in 20 µl reactions using the specific primers published by Klemsdal *et al.* (2008). Amplified PCR products were visualised by gel electrophoresis with an expected product size for *P. violae* of 352 bp. Thermocycling parameters for the PCR reactions were 93°C for 2 min, followed by 40 cycles of 93°C 60s', 60°C 60s, 72°C 60s and finally 72°C for 10 min. Cavity spot lesions were also counted on each of the 20 carrot roots that were sampled from treatment plots for *P. violae* PCR detection as a way of monitoring disease levels over time.

Main cavity spot disease assessments

Two large harvests of 150 roots per plot (600 roots per treatment) were carried out in January-March 2016 at the Wretham and Gooderstone field sites to fully assess the effect of the biofumigant crop treatments on cavity spot disease (Table 1). In the first of these harvests, carrots were graded as exhibiting low, moderate or high cavity spot levels corresponding to 1-3, 3-7 and >7 lesions / carrot respectively and at the later carrot harvest, the number of lesions per carrot were assessed as well as yield and marketability. Data were analysed by standard ANOVA using Genstat with angular transformation of percentages. Limited samples of roots from some plots were also taken at Wellesbourne in December 2015 for assessment but were not included in the analysis.

Free-living nematode and carrot fanging assessments

Soil samples for free-living nematode enumeration from each biofumigant treatment plot were taken just before incorporation of biofumigants and approximately 14 days later, a few days before sowing of subsequent carrot crops (Table 2). Plots from Wretham and Gooderstone sites were also sampled again prior to the final harvest of carrot roots. Samples consisted of approximately 2 kg soil from a 5 point 'W' sampling from each plot (400 g / point) at 8-12 cm depth, and free-living nematodes counts for *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) determined. The number of fanged carrots was also carried out for the final carrot harvests at Wretham and Gooderstone and the limited root harvest at Wellesbourne.

Table 2. Summary of *P. violae* PCR detection, cavity spot disease, fanging and free-living nematode count assessments

Date	Wellesbourne				Wretham				Gooderstone			
	***P. violae* PCR**	**Cavity spot disease**	**Fanging**	**Nematode count**	***P. violae* PCR**	**Cavity spot disease**	**Fanging**	**Nematode count**	***P. violae* PCR**	**Cavity spot disease**	**Fanging**	**Nematode count**
09/09/14	root soil	-	-	-	-	-	-	-	-	-	-	-
09/09/14	**Biofumigants sown**				-	-	-	-	-	-	-	-
22/09/14	-	- - - -	-	-	root soil	-	-	-	-	-	-	-
22/09/14	-	-	-	-	**Biofumigants sown**				-	-	-	-
23/09/14	-	-	-	-	-	-	-	-	root soil	-	-	-
23/09/14	-	-	-	-	-	-	-	-	**Biofumigants sown**			
02/10/14	root soil	-	-	-	-	-	-	-	-	-	-	-
15/10/14	-	-	-	-	root soil	-	-	-	root soil	-	-	-
23/10/14	root soil	-	-	-	-	-	-	-	-	-	-	-
05/11/14	-	-	-	-	root soil	-	-	-	root soil	-	-	-
13/11/14	root soil	-	-	-	-	-	-	-	-	-	-	-
26/11/14	-	-	-	-	root soil	-	-	-	root soil	-	-	-
04/12/14	root soil	-	-	-	-	-	-	-	-	-	-	-
17/12/14	-	-	-	-	root soil	-	-	-	root soil	-	-	-
07/01/15	-	-	-	-	root soil	-	-	-	root soil	-	-	-
15/01/15	root soil	-	-	-	-	-	-	-	-	-	-	-
28/01/15	-	-	-	-	root soil	-	-	-	root soil	-	-	-
05/02/15	root soil	-	-	-	-	-	-	-	-	-	-	-
18/02/15	-	-	-	-	root soil	-	-	-	root soil	-	-	-
26/02/15	root soil	-	-	-	-	-	-	-	-	-	-	-
18/03/15	-	-	-	-	root soil	-	-	-	root soil	-	-	-
19/03/15	root soil	-	-	-	-	-	-	-	-	-	-	-
01/04/15	-	-	-	-	-	-	-	-	root soil	-	-	root soil
01/04/15									**Biofumigants incorporated**			
09/04/15	root soil	-	-	-	-	-	-	-	-	-	-	-
13/04/15	-	-	-	-	root soil	-	-	-	root soil	-	-	-
17/04/15									**Carrots drilled**			
21/04/15	root soil	-	-	root soil	-	-	-	-	-	-	-	root soil
22/04/15	**Biofumigants incorporated**				-	-	-	-	-	-	-	-
29/04/15	-	-	-	-	root soil	-	-	root soil	-	-	-	-

Date	Wellesbourne				Wretham				Gooderstone			
29/04/15	-	-	-	-	**Biofumigants incorporated**							
07/05/15	root soil	-	-	root soil	-	-	-	-	-	-	-	-
08/05/15	**Carrots drilled**				-	-	-	-	-	-	-	-
19/05/15	-	-	-	-	-	-	-	-	root soil	-	-	-
21/05/15	root soil	-	-	-	-	-	-	-	-	-	-	-
29/05/15	-	-	-	-	root soil	-	-	root soil	-	-	-	-
29/05/15	-	-	-	-	**Carrots drilled**				-	-	-	-
04/06/15	root soil	-	-	-	-	-	-	-	-	-	-	-
19/06/15	-	-	-	-	root soil	-	-	-	root soil	-	-	-
15/07/15	-	-	-	-	root soil	-	-	-	20 carrots	20 carrots	-	-
06/08/15	-	-	-	-	20 carrots	20 carrots	-	-	20 carrots	20 carrots	-	-
24/08/15	-	-	-	-	20 carrots	20 carrots	-	-	20 carrots	20 carrots	-	-
15/09/15	-	-	-	-	20 carrots	20 carrots	-	-	20 carrots	20 carrots	-	-
07/10/15	-	-	-	-	20 carrots	20 carrots	-	-	20 carrots	20 carrots	-	-
27/10/15	-	-	-	-	20 carrots	20 carrots	-	-	20 carrots	20 carrots	-	-
26/11/15	-	-	-	-	20 carrots	20 carrots	-	-	20 carrots	20 carrots	-	-
08/01/16	-	-	-	-	20 carrots	20 carrots	-	-	-	-	-	-
11/01/16	-	-	-	-	**Carrot harvest 1**				-	-	-	-
12/01/16	-	-	-	-	-	-	-	-	**Carrot harvest**			
03/02/16	-	-	-	-	20 carrots	20 carrots	20 carrots		20 carrots	20 carrots	20 carrots	-
23/02/16	-	-	-	-	-	-	-	-	root soil	-	-	root soil
24/02/16	-	-	-	-	-	-	-	-	**Carrot harvest**			
03/03/16	-	-	-	-	root soil	-	-	root soil	-	-	-	-
03/03/16	-	-	-	-	**Carrot harvest 2**				-	-	-	-

HPLC analysis of biofumigant crop glucosinolates

Leaf samples from each plot of the biofumigant crops at each field site were taken on the day of incorporation to quantify the levels of sinigrin, sinalbin and glucoraphenin glucosinolates at flowering. The leaf samples were placed in an oven at 80°C for 24 hours, then milled to a fine powder (Brook Compton Series 2000 mill, England) and stored in sealed plastics bags at -20°C. Quantification of the main GSL in each biofumigant crop species was carried out using a simplified extraction and HPLC method as described by Clarkson (2013).

PCR detection of Pythium violae in a commercial carrot crop and cavity spot disease assessment

Due to the failure of the carrot crop to establish at Wellesbourne, 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 (Fig. 1). Carrots (cv. Nairobi) were drilled on the 26th May 2015 at a seed rate of 2.146 million seeds/ha. There were twelve replicate plots (6 m x 1.83 m) arranged in 3 rows (across beds) x 4 columns (down beds). Twenty root samples (lateral roots only in larger plants) from four points within each plot to give a total 240 roots per sampling occasion, were collected throughout the season for PCR detection of *P. violae* and to assess cavity spot disease levels (Table 3).

Figure 1. Commercial carrot field used for PCR detection of *P. violae*

Roots from carrot seedlings or more mature plants were washed in tap water to remove adhering soil, and lateral roots excised from the tap root (for more mature plants) and freeze dried. The freeze-dried material was ground by placing in a FastPrep cell disruptor with a ceramic bead (MP Biomedical SAS, UK) with shaking for 40s at 6 m/s and DNA extractions carried out using 0.1 g of ground root sample and the DNeasy Plant mini kit (Qiagen, UK)

with the addition of Polyvinyl polypyrrolidone (PVPP; Sigma, UK) to the lysis buffer. DNA samples were then stored at -20°C and subsequently used for PCR detection of *P. violae* in 20 µl reactions using the method described previously. The number of cavity spot lesions were also recorded on each of the carrot roots sampled in more mature plants.

Table 3. Summary of *P. violae* PCR detection and cavity spot disease assessments for a commercial carrot crop

Date	**Commercial carrots, Mansfield, Nottinghamshire**		
	PCR	**Cavity Spot**	**Fanging**
26/05/2015	carrots sown		
24/06/2015	lateral roots	240 seedlings	240 seedlings
08/07/2015	lateral roots	240 seedlings	240 seedlings
23/07/2015	lateral roots	240 seedlings	240 seedlings
05/08/2015	lateral roots	240 carrots	240 carrots
19/08/2015	lateral roots	240 carrots	240 carrots
02/09/2015	lateral roots	240 carrots	240 carrots
23/09/2015	lateral roots	240 carrots	240 carrots
14/10/2015	lateral roots	240 carrots	240 carrots
26/10/2015	carrots strawed down		
04/11/2015	lateral roots	240 carrots	240 carrots
25/11/2015	lateral roots	240 carrots	240 carrots
16/12/2015	lateral roots	240 carrots	240 carrots

Results

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

Biofumigant crop growth and glucosinolate levels

The autumn-sown biofumigants initially established well over the warm autumn but the taller white mustard crop at Wellesbourne and Gooderstone suffered extreme frost damage, despite fleece protection. Incorporation times were 22/04/15, 29/04/15 and 01/04/15 for Wellesbourne, Wretham and Gooderstone field sites respectively. The earlier incorporation date at Gooderstone (Fig. 2A) was due to the grower requiring an earlier subsequent drilling of carrots and meant that biomass was lower compared to Wretham (Fig. 2B).

Figure 2. Incorporation of biofumigant crops at Gooderstone (A) and Wrethem (B)

Although crops at Wrethem were approaching flowering by the end of April generally biomass over all the sites was low with the exception of radish at Wretham. Mean biomass for brown mustard and radish crops were 0.67, 0.96, 0.27 kg m^{-2} and 1.46, 3.69, 0.63 kg m^{-2} for Wellesbourne, Wretham and Gooderstone respectively and for white mustard were 0.90 and 0.05 kg m^{-2} for Wretham and Gooderstone respectively (Fig. 3A). Mean glucosinolate levels were also generally low and for brown mustard (sinigrin) and radish (glucoraphenin) crops were 3.3, 3.4, 1.2 µmol g^{-1} and 2.81, 1.34 and 0.21 µmol g^{-1} for Wellesbourne, Wretham and Gooderstone respectively while for white mustard (sinalbin) were 1.22 and 1.25 µmol g^{-1} for Wretham and Gooderstone respectively (Fig. 3B).

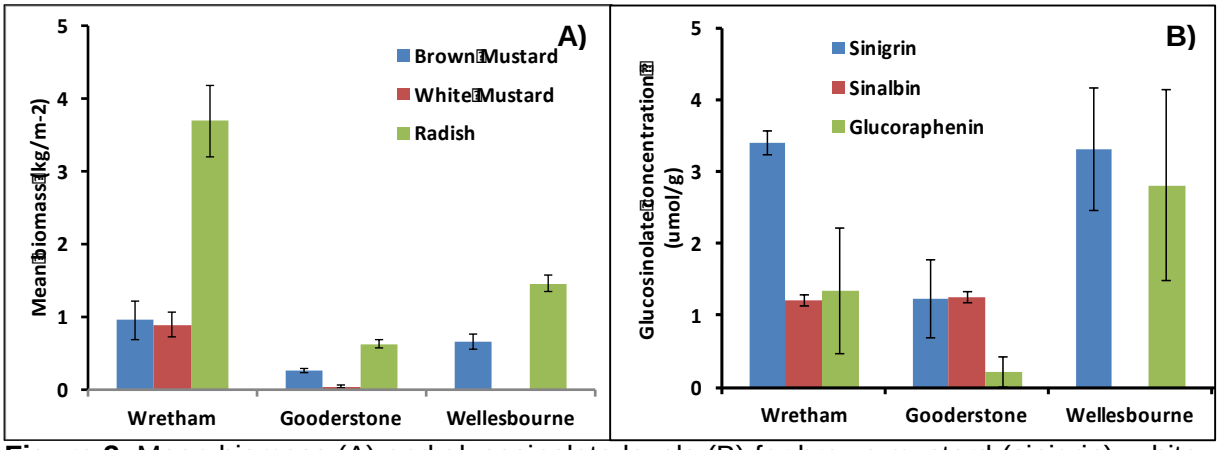

Figure 3. Mean biomass (A) and glucosinolate levels (B) for brown mustard (sinigrin), white mustard (sinalbin) and radish (glucoraphenin) crops from Wellesbourne, Wretham and Gooderstone at incorporation. Error bars represent the standard error of the mean

PCR detection and dynamics of *P. violae*

P. violae was detected by PCR in all biofumigant treatment and untreated control plots for at least one sampling time over the duration of the experiments at both Wretham and Gooderstone (Fig. 4). 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 in July and August at both field sites. 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 (Fig. 4). 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.

Development of cavity spot

Cavity spot levels in the same twenty roots used for PCR detection remained low throughout the season until the last sampling in February 2016, and the majority only had one lesion per root (Fig. 5). There was therefore no clear effect of any of the treatments on disease levels for these small root samples. The greatest incidence of cavity spot for both sites was at the

last sampling date on 03/02/16 ranging from 23-34% at Wretham and 12-23% at Gooderstone (Fig. 5).

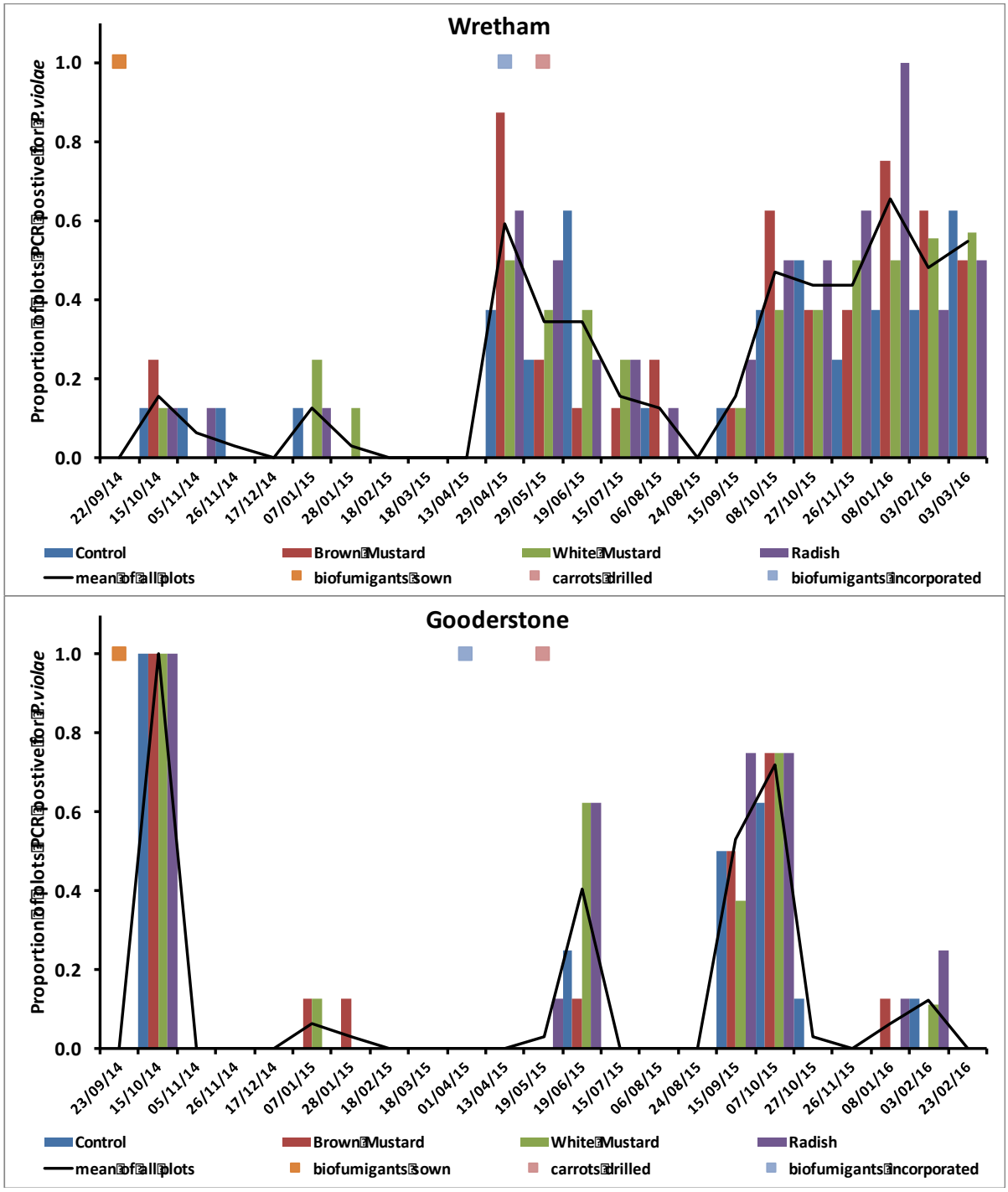

Figure 4. Proportion of plots (out of a maximum of four) positive for *P. violae* using PCR for Wretham and Gooderstone. Samples for PCR were from bare soil or soil from around plants (September 2014 to June 2015 at Wretham; September 2014 to July 2015 at Gooderstone) or from around carrots roots (August 2015 to March 2016 at Wretham; July 2015 to February 2016 at Gooderstone

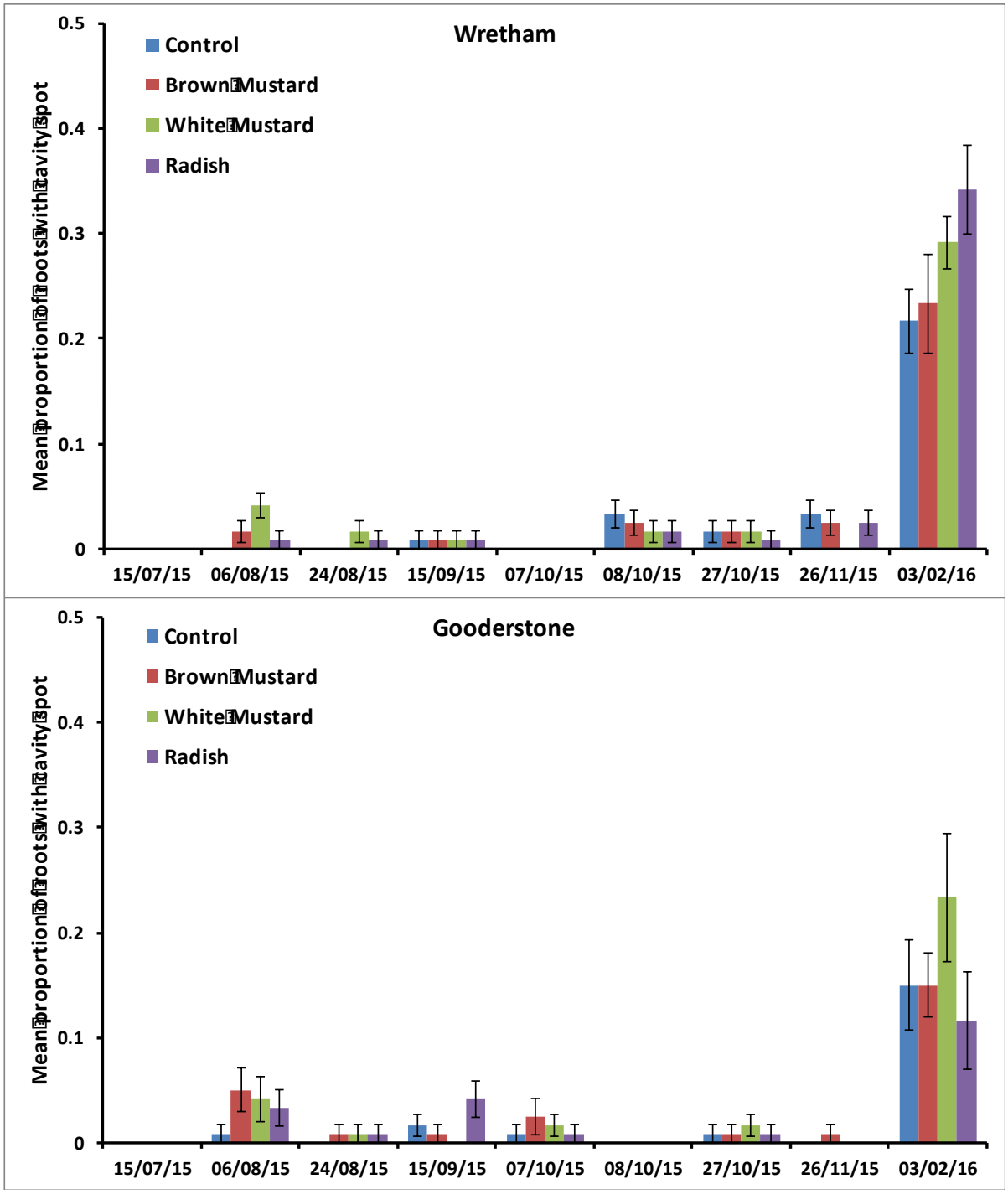

Figure 5. Cavity spot incidence (mean proportion of roots affected) over time for biofumigant crops at Wretham and Gooderstone. Samples were 20 carrot root samples per plot (80 roots per treatment). Error bars represent the standard error of the mean

Main cavity spot disease assessments

Results from the first harvest assessment in January 2016 showed that cavity spot disease had a higher incidence at Wretham (approx. 70% of carrots affected) compared to Gooderstone (approx. 25 % of carrots affected). Of the carrots affected, those at Wretham also had a greater number with moderate-severe cavity spot (21%) than at Gooderstone (4%) (Fig. 6 A&B). However, there was no significant effect of any of the biofumigant crops on disease levels at either field site following statistical analysis by ANOVA.

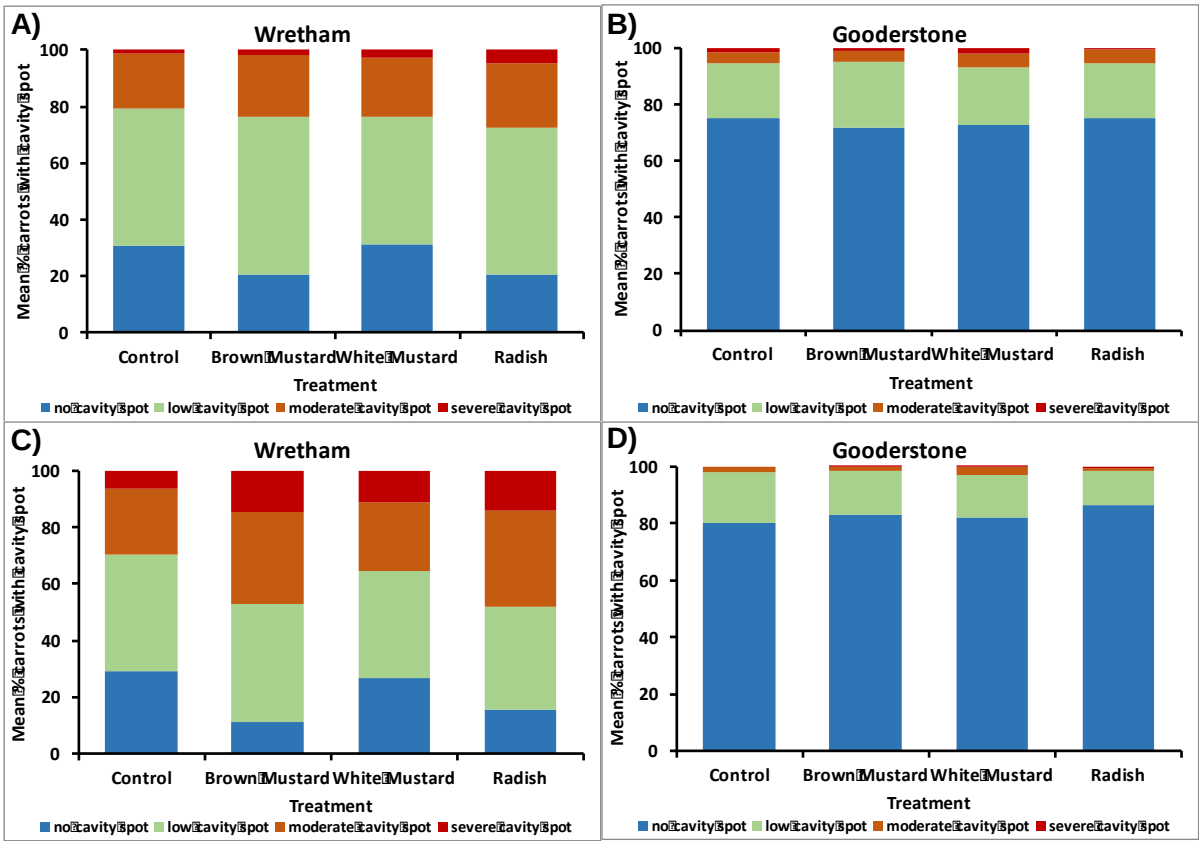

Figure 6. Effect of biofumigant crops on cavity spot disease for 150 roots per plot (600 roots per treatment) harvested on A)11/01/16 at Wretham, B) 12/01/16 at Gooderstone, C) 03/03/2016 at Wretham, and D) 24/02/2016 at Gooderstone. Low, moderate and high cavity spot levels correspond to 1-3, 3-7 and >7 lesions / carrot respectively

Cavity spot incidence remained at similar levels at the second later carrot harvest in late February / early March at both sites, with higher levels Wretham (approx. 79% of carrots affected) than at Gooderstone (approx. 20% of carrots affected). However, the severity of cavity spot increased at Wretham compared with the first harvest with approx. 40% of carrots with moderate-severe cavity spot, while the severity at Gooderstone remained at approx. 2% of carrots with moderate-severe cavity spot (Fig. 6 C&D). Again, there was no significant effect of any of the biofumigant crops on disease at either field site following ANOVA.

Similarly, there was no significance difference between any of the treatments for percentage total or marketable yield. No significant levels of cavity spot were observed on the limited root harvest at Wellesbourne.

Effect of biofumigant crops on free-living nematodes and carrot fanging

Free-living nematode counts were variable between sites. *Trichodorus, Tylenchorynchus / Helicotylenchus, Heterodera, Pratylenchus* and *Longidorus* were found at all sites at one or more of the sampling times while *Ditylenchus* was only detected at Gooderstone, *Meloidogyne* only at Wellesbourne and *Xiphinema* was not detected at any of the sites at any sampling time (Fig. 7).

At Wellesbourne, only *Tylenchorynchus / Helicotylenchus, Longidorus* and *Meloidogyne* were detected at higher levels. *Tylenchorynchus / Helicotylenchus* levels ranged from 2212-3950 nematodes L^{-1} soil pre- incorporation and 2700-3637 nematodes L^{-1} soil post incorporation. *Longidorus* levels ranged from 356-511 nematodes L^{-1} soil pre- incorporation and rose to 465-632 nematodes L^{-1} soil post incorporation. *Meloidogyne* was only detected pre-incorporation at mean levels of 43-75 nematodes L^{-1} soil. There was no apparent effect of biofumigant treatment (Fig. 7).

The major species detected at Wretham were *Trichodorus, Tylenchorynchus / Helicotylenchus and Pratylenchus.* There was no apparent effect of biofumigant treatment but numbers of *Trichodorus a*nd *Tylenchorynchus / Helicotylenchus* generally decreased post incorporation with mean levels ranging from 50-125 and 406-481 nematodes L^{-1} soil pre-incorporation and 0 and 37-56 nematodes L^{-1} soil post incorporation respectively (Fig. 7).

For Gooderstone, levels of *Trichodorus, Tylenchorynchus / Helicotylenchus and Pratylenchus* recorded in the assessment pre-incorporation of the biofumigants were reduced in all treatments including the untreated control in the post- incorporation assessment 14 days later. Over all the treatments, *Trichodorus, Tylenchorynchus / Helicotylenchus* and *Pratylenchus* were reduced from mean levels of 286, 800 and 1459 nematodes L^{-1} soil to 23, 298 and 734 nematodes L^{-1} soil respectively. There was no apparent effect of particular treatments. Levels of nematodes detected at carrot harvest were generally low for all species, except with *Heterodera*, where mean levels increased from 71 nematodes L^{-1} soil to 89 nematodes L^{-1} soil (Fig. 7).

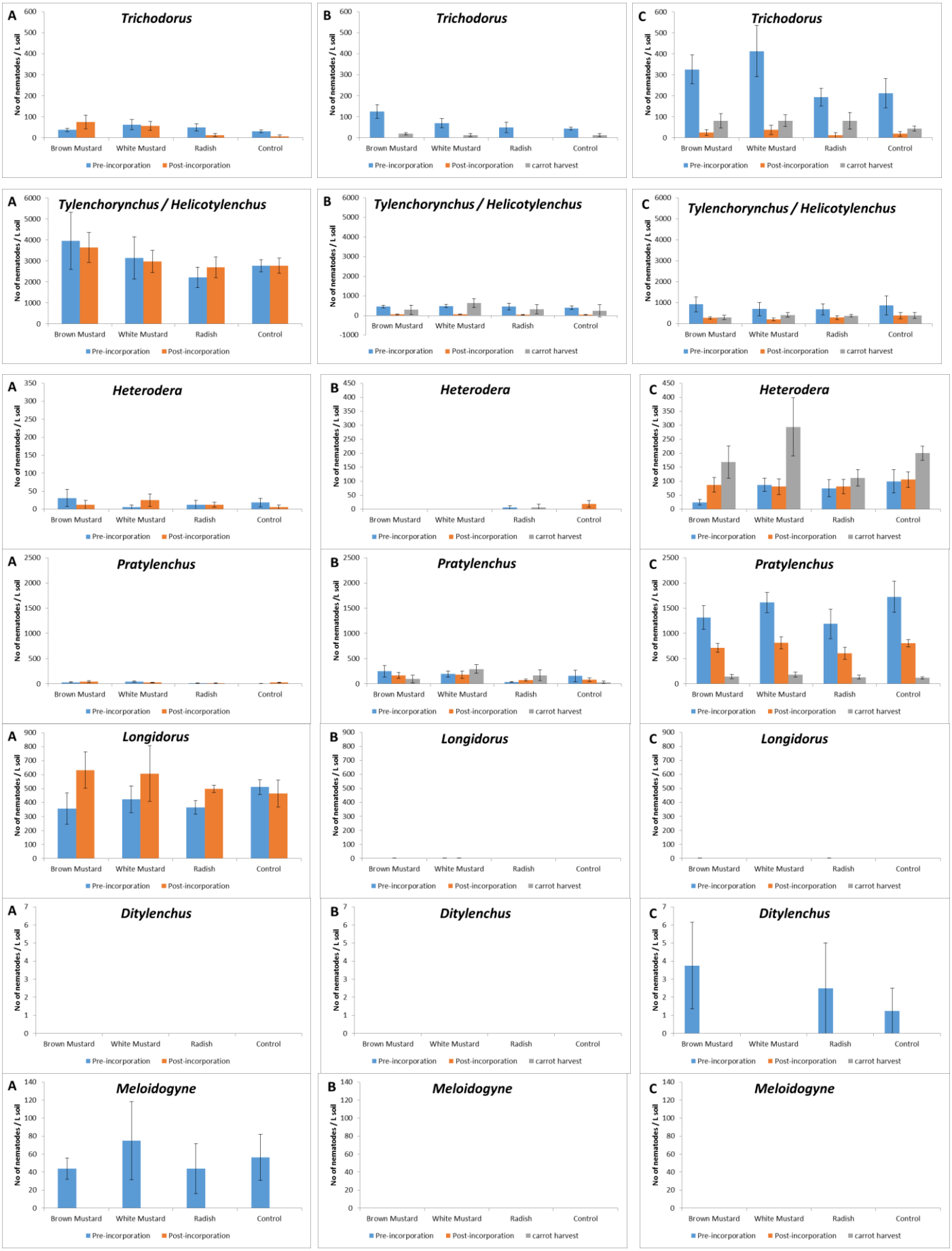

Figure 7. Free-living nematode counts pre and post-incorporation and at carrot harvest for biofumigant crops at Wellesbourne (A), Wretham (B) and Gooderstone (C). Error bars are standard error of the mean

The proportion of fanged carrot roots in the 20 carrot root samples over the season was inconsistent and was at very low levels (<1.2%) and there was no clear effect of any biofumigant treatment (data not shown). Similarly there were low levels of fanging observed (<2%) in the final large carrot root harvest on 03/03/16 (Wretham) and 24/02/2016 (Gooderstone). However, greater levels of fanging were observed in carrot root samples harvested on 01/12/15 at Wellesbourne (15-24%; Fig. 8).

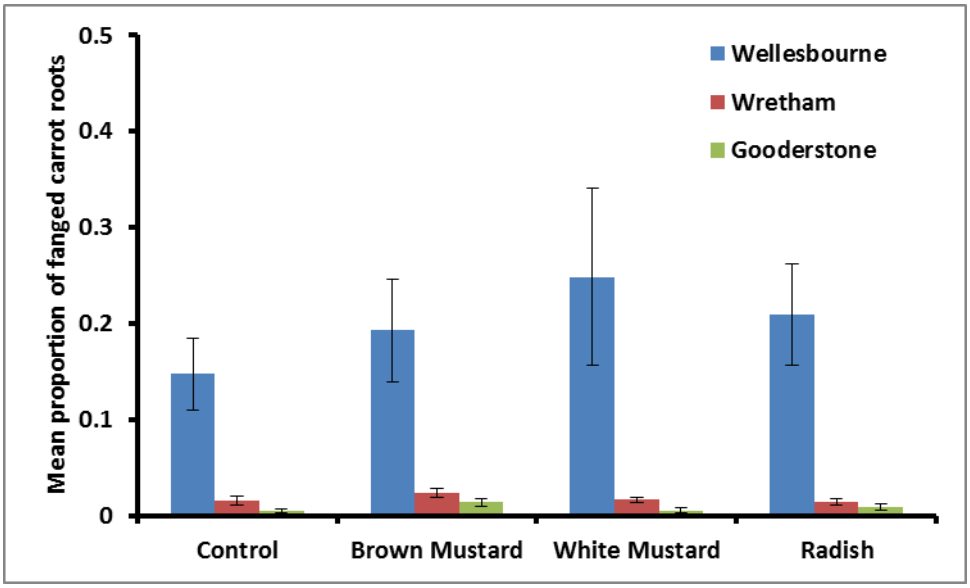

Figure 8. Mean proportion of fanged carrots for a maximum of 200 carrot root samples per plot (800 roots per treatment) at carrot harvest on 01/12/2015 at Wellesbourne, 03/03/2016 at Wretham and 23/02/2016 at Gooderstone. Errors bars are standard error of the mean

PCR detection of Pythium violae in a commercial carrot crop and cavity spot disease assessment

PCR detection and dynamics of *P. violae*

The levels of *P. violae* detected by PCR in the lateral roots of all plots were very low over the duration of the monitoring period, with *P. violae* being detected in only one sample of the 48 samples tested on one occasion (25/11/15), which was ca. 4 weeks after the carrots were strawed down (Fig. 9).

Development of cavity spot

Cavity spot levels in the same 240 roots used for PCR detection remained low throughout the season (Fig. 9). In general, only 1-4 carrots showing symptoms of cavity spot were detected over the monitoring period and there was one small peak of detection on roots collected on 19/08/15, in which a total of nine carrots from five plots showed symptoms of cavity spot. Of those affected roots, the majority only had only one lesion.

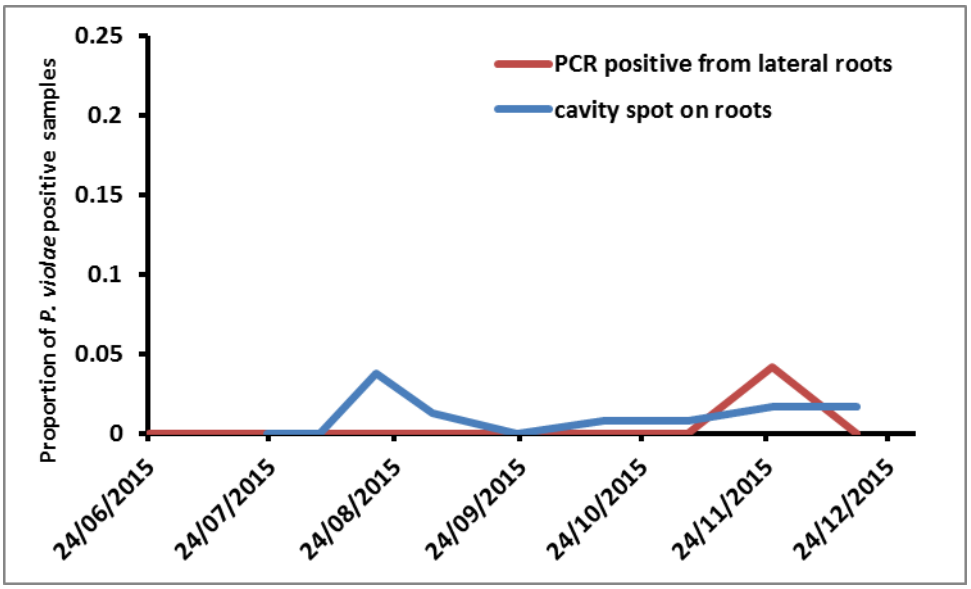

Figure 9. Proportion of seedling roots / lateral root samples positive for *P.violae* using PCR and roots with cavity spot symptoms

Discussion

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

Biofumigant crop growth and glucosinolate levels

Although the autumn-sown radish overwintered quite well, the brown and white mustards were more liable to frost with white mustard at Wellesbourne and Gooderstone suffering severe damage despite the use of fleece. Flowering of the biofumigants generally occurred in late April (although there was several weeks difference in flowering time between sites) and this was therefore the time when the crops were incorporated as it is well established that higher glucosinolate levels in mustards and other brassica crops occur at full flowering (Clossais-Besnard & Larher, 1991; Bellostas et al., 2007). However, both biomass and glucosinolate levels in the biofumigants were low. 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}; Clarkson et al., 2015). In a UK study investigating the suppression of potato cyst nematode (PCN), summer and autumn mustard and radish crops were established in late July and late September and incorporated early October and early April respectively (Ngala et al., 2014). The summer mustard and radish crops yielded 2.1 an 3.2 kg m^{-2} respectively while the winter crops yielded 1.03 and 1.62 kg m^{-2} respectively which is comparable to this study. Futhermore, the summer crops resulted in PCN suppression whereas the winter crops did not, hence further demonstrating that adequate biomass is essential for effective biofumigation. Even higher biomass levels of 7-18 kg m^{-2} (70-180 t ha^{-1}) have been achieved in the UK for mustard crops sown in early September and incorporated mid-November for suppression of PCN (Watts et al., 2014).

Glucosinolate levels for brown mustard, white mustard and radish were 2.6, 1.2 and 1.3 umol g^{-1} dw respectively. In the same PCN study of Ngala et al (2014) above, glucosinolate levels for brown mustard and radish in the summer crops were 49 and 50 umol g^{-1} dw respectively while for the winter crops were 10 and 14 umol g^{-1} dw. In other studies using field grown *B. juncea* and *B. napus*, glucosinolate levels were 20-35 µmol g^{-1} dw (Sarwar et al., 1998; Steindal et al., 2013). In a review of multiple studies, levels for sinigrin and sinalbin in field crops were reported in the range 0.1-18.7 µmol/g and 9.0-14.4 µmol/g respectively (Kirkegaard & Sarwar, 1998). It is clear therefore that both biomass and glucosinolate levels can vary substantially depending on growth conditions and it has been demonstrated that both lower temperature and light levels decrease the latter (Sarwar et al., 1998; Smolinska

and Horbowicz, 1999; Steindal et al., 2013). Overall therefore, it can be concluded that the short growing window and suboptimal environmental conditions associated with overwintering and incorporating 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.

PCR detection and dynamics of *P. violae*

There was no clear effect of any of the biofumigant treatments on *P. violae* as PCR detection showed that the dynamics of the pathogen was similar to that in the untreated control plots at both Wretham and Gooderstone. Although *P. violae* dynamics varied between the the two field sites, especially initially where there were high levels detected at Gooderstone which quickly decreased, there were some common trends in that *P. violae* showed two subsequent peaks of detection corresponding approximately to biofumigant incorporation and carrot drilling (May / June 2015) and the carrot crop maturing in September / October 2015. A high level of detection was then sustained at Wretham but not at Gooderstone and this corresponded with the higher disease levels observed at Wretham (see next section). Between these peak times of detection, which included the active growing period of carrot crops in July and August 2015, *P. violae* was at low or negligible levels. Results from the first phase of this project also showed a peak of *P. violae* detection in late September (2013) while previously, a general increase in pathogen levels had been observed as carrot crops matured, peaking a month earlier in August (Anon, 2009). This difference may be due to variation in carrot crop development as it has been reported that more mature carrots are more prone to infection by *P. violae* (Vivoda et al., 1991). Overall, results using the PCR test in this project and previous work by Dez Barbara (Anon, 2009) suggests that this approach can give some indication of the period when *P. violae* is multiplying on carrots as they mature, but detection at other times of year has been very variable and a complete absence of detection before planting of a crop that subsequently develops cavity spot or afterwards once the crop is harvested has also been observed. This calls into question the sensitivity of the PCR test and also the variability associated with processing such small soil samples. These problems are however being addressed in PhD project FV 432.

Development of cavity spot and effect of biofumigants

In the small samples of 20 carrots taken during the growing season and used for *P. violae* detection, few cavity spot lesions were observed at either field site until the last samples in early February 2016 where incidence was 23-34% at Wretham and 12-23% at Gooderstone. The two large harvest assessments a few weeks later indicated that final levels of cavity spot incidence were high at Wretham (70-79%) and moderate at Gooderstone (20-25%). Correspondingly, the severity of disease was also greater at Wretham where approx. 40% of carrots had moderate-severe cavity spot (>3 lesions per root). At both sites therefore, cavity spot development occurred late in the season with disease continuing to develop under straw. The higher disease pressure at Wretham and the development of cavity spot over the winter period was also supported by the PCR results where levels of *P. violae* detection were generally greater than at Gooderstone particularly in the period September 2016-March 2016. This suggests that 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.

Despite good cavity spot development at both field sites, there was no significant effect of any of the biofumigant crop treatments on disease incidence or severity with levels very similar across treatments including the untreated control. One explanation for this is that although *P. violae* mycelium is very sensitive to ITCs released from the biofumigant plants tested here (as reported in phase 1), they may not be effective in killing oospores which allow the pathogen to survive for long periods in soil. Alternatively, and perhaps more likely, is that the biomass and glucosinolate levels were just too low to be effective as suggested above. Further research would be needed to determine if biofumigants grown in optimum conditions can reduce cavity spot.

Effect of biofumigant crops on free-living nematodes and carrot fanging

Of the nematode species detected, *Trichodorus* and *Longidorus* spp. are potentially the most damaging to carrots. Anecdotal thresholds suggest that numbers of *Trichodorus* spp. in excess of 200 L^{-1} soil and of *Longidorus* spp. in excess of 50 L^{-1} soil would justify nematicide treatment (Dr Steve Ellis, personal communication). Numbers of *Trichodorus* exceeded this level at Gooderstone post-incorporation but fell below the damaging threshold in all treatments by carrot harvest. Anecdotal thresholds for *Tylenchorynchus* spp. and *Pratylenchus* spp. are estimated to be about 10,000 L^{-1} soil and 2,500 L^{-1} soil respectively so both these species were below levels thought to be damaging for all the sampling carried out at both Wretham and Gooderstone. However, at Wellesbourne where both *Longidorus* and *Tylenchorynchus* were above threshold, there was considerably more carrot fanging (15-

24%) than observed at the Norfolk field sites, suggesting that these free-living nematode species may have caused this damage. Overall however, increases, decreases or no change in nematode levels between pre- and post-incorporation sampling depended on the species and field site rather than the biofumigant treatment as the same changes were observed in untreated control plots. This suggests that the changes in population levels were due to the tilling operations involved in bedforming and drilling the carrot crops. A similar effect was observed in year 1 of the project although levels declined in all nematode species. None of the biofumigant treatments reduced or increased fanging.

PCR detection of Pythium violae in a commercial carrot crop and cavity spot disease assessment

In the commercial carrot crop, there was little PCR detection of *P. violae* on carrot seedling roots or lateral roots of more mature carrots and negligible cavity spot disease observed in the root samples or in any of the surrounding crop (Ian Holmes, personal communication). Due to the low pathogen levels it was therefore not possible to determine if seedlings or lateral roots are a significant route of pathogen infection.

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.

Knowledge and Technology Transfer

- 21/11/13: Presentation at the UK Carrot and Onion Conference: Challenges for control of cavity spot and Sclerotinia in carrot.
- Updates on the project were given regularly at BCGA / AHDB panel and technical meetings Oct 2013, Jan 2014, June 2014, March 2015.

References

Anon, (2009) Factors affecting the inoculum potential of soilborne plant pathogens. Defra final report for project HH3230SFV.

Barbara D.J. (2007) Assessing the effectiveness of a Norwegian developed PCR assay for the prediction of carrot cavity spot levels by measuring soil levels of five *Pythium* species. Horticultural Development Company final report for project FV 05g.

Barbara D.J. (2010). Carrot cavity spot (i) using quantitative PCR to predict disease in strawed crops; (ii) controlling moisture for optimum disease management. Horticultural Development Company final report for project FV 353.

Bellostas, N., Sørensen, J.C. & Sørensen, H. (2007). Profiling glucosinolates in vegetative and reproductive tissues of four Brassica species of the U-triangle for their biofumigation potential. Journal of the Science of Food and Agriculture 87: 1586-1594.

Clarkson, J.P. (2013). Pathogen diversity, epidemiology and control of Sclerotinia disease in vegetable crops (PhD). Horticultural Development Company annual report for project CP 080.

Clarkson, J.P., Michel, V. & Neilson, R. (2015). Biofumigation for the control of soil-borne diseases. [Online]. http://ec.europa.eu/eip/agriculture/sites/agri-eip/files/9_eip_sbd_mp_biofumigation_final_0.pdf. Soil borne disease focus group.

Clossais-Besnard, N. & Larher, F. (1991). Physiological role of glucosinolates in *Brassica napus*. Concentration and distribution pattern of glucosinolates among plant organs during a complete life cycle. Journal of the Science of Food and Agriculture 56: 25-38.

Kirkegaard, J.A. & Sarwar, M. (1998). Biofumigation potential of Brassicas. Plant and Soil, 201: 71-89.

Klemsdal S.S., Herrero M.L., Wanner L.A., Lund G., Hermansen A., (2008). PCR-based identification of *Pythium* spp. causing cavity spot in carrots and sensitive detection in soil samples. Plant Pathology 57: 877-86.

Kretzschmar, A. (2009). Carrot cavity spot: the effects of non-carrot crops on levels of relevant *Pythium* spp. in the soil (PhD). Horticultural Development Company final report for project CP 46.

Martin, D. (2011) Carrots: BCGA Incidence of cavity spot in commercial crops. Horticultural Development Company final report for project FV 373.

Ngala, B.M., Haydock, P.P.J., Woods, S. & Back, M.A. (2014). Biofumigation with *Brassica juncea, Raphanus sativus* and *Eruca sativa* for the Management of Field Populations of the Potato Cyst Nematode Globodera pallida. Pest Management Science 71: 759-769.

Sarwar, M., Kirkegaard, J.A., Wong, P.T.W., Desmarchelier, J.M., (1998). Biofumigation potential of brassicas. *Plant and Soil* **201**, 103-12.

Smolinska, U. & Horbowicz, M. (1999). Fungicidal activity of volatiles from selected cruciferous plants against resting propagules of soil-borne fungal pathogens. Journal of Phytopathology 147: 119-124.

Steindal, A.L.H., Mølmann, J., Bengtsson, G.B. & Johansen, T.J. (2013). Influence of day length and temperature on the content of health-related compounds in broccoli (*Brassica oleracea* L. var. *italica*). Journal of Agricultural and Food Chemistry 61: 10779-10786.

Vivoda, E., Davis, R. M., Nunez, J. J., & Guerard, J. P. (1991). Factors affecting the development of cavity spot of carrot. Plant disease 75: 519-522.

Watts, W.D.J., Grove, I.G., Tomalin G.R & Back M.A. (2014). Field screening of biofumigant species for the reduction of potato cyst nematodes (*Globodera* sp.). Aspects of Applied Biology 126: 1-7.

