Grower Summary

FV 432

Understanding the ecology and epidemiology of Pythium violae to enable disease management in carrot crops.

Annual 2015

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Project title:	Understanding the ecology and epidemiology of *Pythium violae* to enable disease management in carrot crops.
Project number:	FV 432
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Report:	Annual report, September 2015
Previous report:	N/A
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Date project commenced:	1 October 2014
Expected completion date:	30 September 2018

GROWER SUMMARY

Headline

- *Pythium violae* and *Pythium sulcatum* are the most common species associated with cavity spot of carrot.
- An improved soil sampling and detection method has been developed allowing 10 g of soil to be used for *P. violae* detection.
- Artificial inoculation of carrot seedlings with *P. violae* oospores resulted in damping off and indicated the importance of inoculum concentration on disease development.

Background

Cavity spot disease of carrot

Cavity spot is the most important disease problem for carrot growers and regularly results in losses of £3-5 million per season (Martin, 2013). The disease was first recognised in the UK from 1960 and has been reported widely across the globe (Hiltunen and White (2002). Typical symptoms on carrot are dark, sunken elliptical lesions which result in an unmarketable crop (Fig. 1).

Figure 1. Symptoms of cavity spot.

In the 1980's the fungicide metalaxyl was found to reduce the severity of cavity spot (Lyshol et al., 1984) and the discovery that the oomycete *Pythium* was the causal agent (Groom and Perry, 1985). A range of *Pythium* species have since been associated with the disease in different parts of the world including *P. violae*, *P. sulcatum*, *P. ultimum* and *P. irregulare* (Hiltunen and White, 2002). In the UK, *P. violae* is now thought to be the most significant cause of cavity spot (White, 1986, Groom and Perry, 1985), although *P. sulcatum* is also known to be associated with the disease (White, 1988, Lyons and White, 1992). Although *P. violae* is reported to be the major *Pythium* species causing cavity spot in the UK, it is still unclear whether the proportion of different *Pythium* species causing disease varies between different fields or carrot growing areas. The symptoms of cavity spot can also vary significantly, from small clean and dry looking shallow lesions to large dark lesions (Fig. 1). It is unclear however whether this variation is caused by environmental factors or is related to the species or isolate of *Pythium* causing the infection.

Control of cavity spot

In the absence of resistant carrot cultivars, the fungicide metalaxyl has been the primary means of managing cavity spot. Since the first report of this fungicide's utility in combating disease (Lyshol et al., 1984), control has largely improved (Hiltunen and White, 2002), but recently, results have been variable and defining the most appropriate time of application is proving challenging (Gladders, 2014). Some of this variability in control may be due to the enhanced degradation of the active molecule by microbes in the soil (Davison and McKay, 1999). New fungicide treatments have been tested recently (Gladders, 2014) but results were disappointing and demonstrating efficacy was hampered by lack of high enough disease levels in many of the trials. The dependency on metalaxyl as the single fungicide for control of cavity spot is of concern as its long-term sustainability is questionable.

Pythium violae

As indicated above*, P*. *violae* is the principal plant pathogen associated with cavity spot in the UK and is in the class Oomycota, making it distinct from 'true fungi'. The genus *Pythium* contains a large number of species, most of which are plant pathogens (Hendrix and Campbell, 1973). *P. violae* can infect many plant species including wheat, alfalfa and cucumber, although it does not cause disease in all of these hosts (Schrandt et al., 1994). It may also utilise a variety of weed hosts (Barbara, 2010, Kretzschmar, 2010). The ability of *P. violae* to exploit a wide range of hosts may explain why long rotations between carrot crops may sometimes be ineffective as a management strategy.

P. violae epidemiology

Detection and isolation of *P. violae* both from the soil and from carrots can be difficult as it has a very heterogeneous distribution in soil, and secondary infections can also occur on carrots (Hiltunen and White, 2002). Representative sampling is challenging as 0.25 g of soil is routinely used for DNA extraction and detection limits are unclear. Previous work studying *P. violae* dynamics by Barbara and Martin (2007) used a PCR assay developed by Klemsdal (2008) to monitor five *Pythium* species in field sites but no predicative information was obtained that would be useful to growers. A DEFRA funded project (Anon., 2009) which followed the dynamics of *P. violae* using a semi-quantitative PCR suggested that *P. violae* was usually undetectable in soil pre-planting, but increased from low levels in April in newly sown carrot crops to reach a peak in late August/September as the plants matured, before disappearing from the soil at an unpredictable and variable rate. It is unlikely though that *P. violae* does not survive in the soil as it produces oospores, and hence the failure to detect the pathogen pre-planting and post harvest may be due to issues with sampling or the sensitivity

of the PCR test. The production of oospores by the pathogen allows survival in soil for many years and also provides the primary inoculum for infection (Stanghellini and Burr, 1973, Hall et al., 1980). However, further investigation of the early infection events of carrots is needed, as information regarding oospore germination, infection routes and the effect of inoculum concentration on disease development, is sparse. The effect of environmental factors on disease development in the field has also been studied, with rainfall (soil moisture) and temperature (Barbara, 2010, Martin, 2013) being identified as particularly important. However quantifying these effects has been challenging, mainly due to the variability in results between different years and locations.

Artificial inoculation

Cavity spot research continues to be hampered by a lack of effective and reproducible methods to induce cavity spot symptoms in pot-grown carrots or in the field. The lack of knowledge concerning inoculum levels needed to induce disease and the ability to accurately quantify the pathogen in soil has also hindered progress. A number of methods have been investigated in an attempt to artificially inoculate carrots but with only limited success (Suffert and Montfort, 2007, Kretzschmar, 2010).

PhD Aims and objectives

The overall aim of this PhD project is to develop understanding of cavity spot disease by studying the biology, ecology and epidemiology of the main causal agent *Pythium violae*.

Specific objectives in Year One:

1. Develop effective tools for *P. violae* research:

    a) Collect and characterise multiple isolates of *Pythium.*

    b) Develop a more robust and accurate PCR test for *P. violae*.

    c) Develop an improved DNA extraction method and / or sampling from soil.

    d) Develop a reproducible *P. violae* inoculation system for carrot.

2. Investigate *P. violae* dynamics, ecology and interactions with soil microbiota:

    a) Assess the dynamics of *P. violae* on carrot crops throughout the year.

Summary

Objective 1a) Pythium isolate collection and characterisation

From October 2014 through to April 2015, cavity spot infected carrots were collected from grower's sites throughout the country. Over 140 *Pythium* isolates were obtained from these samples and the species identified through PCR and DNA sequencing. Initial results from 78 isolates found that *P. violae* was predominant, comprising 64% of isolates followed by *P. sulcatum* (17%), *P. intermedium* (5%) and *P. sylvaticum* (3%) (Fig. 2).

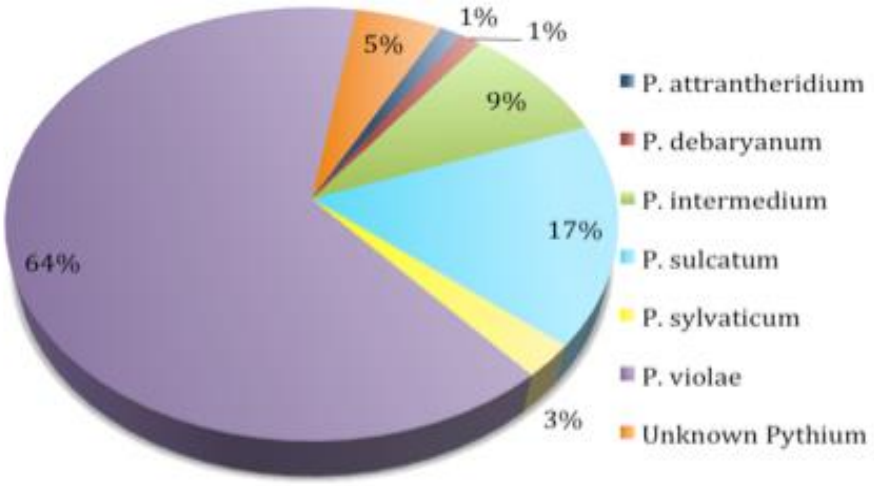
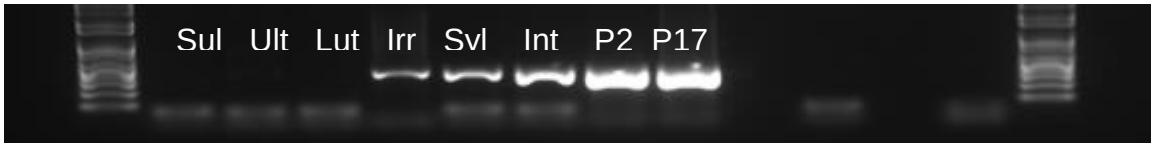


Figure. 2. Relative proportions of *Pythium* species identified from 78 isolates based on sequence of the ITS regions of the rRNA gene.

Objective 1b) Developing specific primers for PCR detection and quantification of P. violae

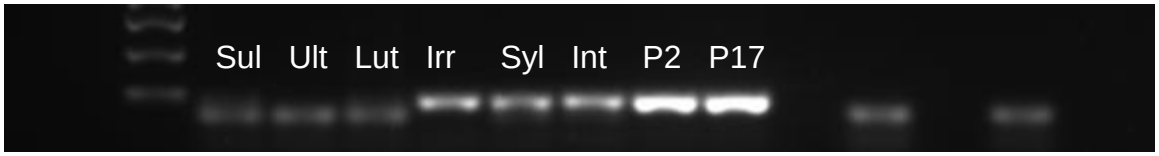
A number of different PCR primer pairs have been tested under a range of conditions. It was found that some primers cross-reacted with other *Pythium* species (Fig. 3 A, B). Development of new primer pairs has provided more promising results and are more specific to *P. violae* (Fig. 3 C).



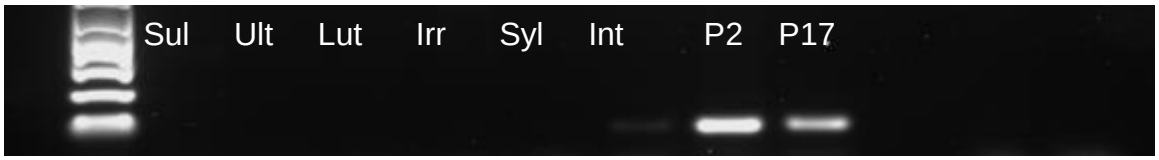
A

B

Figure 3. Gel



C



electrophoresis showing amplification of *Pythium* species using PviolF/R primers (A), PviolNEWF/R primers (B) and OCM1 1128F/R primers (C). Sul=*P. sulcatum*, Ult= *P. ultimum,* Lut=*P. lutarium,* Irr=*P. irregulare,* Syl=*P. sylvaticum, Int=P. intermedium*, P2, P.7= *P. violae*.

Objective 1c) Improvement of soil sampling technique and refinement of DNA extraction protocol

A new method based on 'oospore capture' from soil by sucrose centrifugation and filtration has been developed to allow 10 g of soil to be tested for *P. violae* by PCR. Soil samples from commercial sites have been tested with this method and oospores have been visualised under light microscopy. Field soil from two field sites was shown to contain between 18 and 280 oospores/g (Table 1). Combined with PCR, the methodology can detect as little as one oospore in 10 g soil with more reliable detection of 25 oospores in 10 g.

Table 1. Average numbers of oospores/gram in 10 g of soil following oospore capture.

Sample	**Site**	**Average spores/gram**
A	Commercial field site containing cavity spot-infected strawed carrots	280
B	Commercial field site 3 months after cavity spot infected carrots harvested	57

Objective 1d) Develop a reproducible *P. violae* inoculation system for carrot

Production of a sand-based *P. violae* inoculum has been developed to test artificial inoculation of carrot seedlings and more mature plants. Initial results where inoculum was further mixed with course sand into which carrot seeds were planted indicated that 200 oospores/g leads to

damping-off and high mortality in carrot seedlings after 5 weeks at 14°C. Further experiments will establish the relationship between oospore levels and carrot seedling mortality as well as levels required to allow seedling survival, but induction of cavity spot symptoms in mature plants.

Objective 2a) Assess the dynamics of *P. violae* on carrot crops throughout the year

A *P. violae* monitoring trial has been set up in a commercial field site for soil sampling from January 2015 to January 2016, with the aim of using PCR to detect/quantify *P. violae* over time. Soil samples from around growing carrot plants (January – June) or from around harvested carrot roots (June onwards) have / are being collected for storage and later analysis using oospore capture and PCR detection.

Conclusions

- Over 140 isolates of *Pythium* have been collected from cavity spot infected carrots obtained from different locations. *P. violae* was identified as the predominant species present (64% of isolates).
- Development of new primers for use in conventional and quantitative PCR is on-going and show increased specificity to *P. violae*.
- An improved soil sampling and detection method has been developed allowing 10 g of soil to be used for *P. violae* detection. Sensitivity of detection is less than 25 oospores in 10 g soil.
- Initial results suggest that 200 *P. violae* oospores/g causes complete death of carrot seedlings 5 weeks after sowing.

Financial Benefits

Financial benefits have yet to be established – further details on this expected at the end of year 2 of the project.

Action points

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