Grower Summary

FV 432

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

Annual 2016

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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 2016
Previous report:	Annual report, September 2015
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Date project commenced:	1 October 2014
Date project completed (or expected completion date):	30 September 2018

GROWER SUMMARY

Headline

- *P. violae* is the most common species associated with cavity spot of carrot.
- Methods have been developed to improve sampling and detection of *P. violae* from soil.
- Inoculation of carrot seedlings and mature plants has resulted in disease development but with some variability.

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 in 1960 and has been reported widely across the globe Hiltunen and White (2002). Typical symptoms on carrot are dark, sunken elliptical lesions that 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 concerning as its long-term sustainability is questionable.

Pythium violae

As indicated above, *P*. *violae* is thought to be 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 only 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 the inoculum levels required 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).

Aims of the PhD project

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

Objectives in Year Two:

1. Develop effective tools for *P. violae* research:
    i) Continue collection and characterisation of multiple isolates of *Pythium;* conduct pathogenicity tests and whole genome sequencing to help understand the genetic basis of pathogenicity.
    ii) Develop a more robust and accurate PCR test for *P. violae* suitable for use with quantitative PCR
    iii) Develop a *P. violae* inoculation system for seedling and mature plant trials

2. Investigate *P. violae* dynamics, ecology and interactions with soil microbiota:
    i) Assess the dynamics of *P. violae* on carrot crops throughout the year.

Summary

Objective 1i) *Pythium* isolate collection and characterisation

From October 2014 through to April 2015, cavity spot infected carrots were collected from grower sites throughout the country. Approx. 80 *Pythium* isolates were obtained from these samples and the species identified through PCR and DNA sequencing of the internal transcribed spacer regions (ITS) of the rDNA (see Annual Report 2015 for details). Since then further isolates have been obtained and results from a current total of 125 isolates indicated that *P. violae* was the predominant species associated with cavity spot lesions, comprising 59% of isolates followed by *P. sulcatum* (14%) and *P. intermedium* (14%) (Fig. 2).

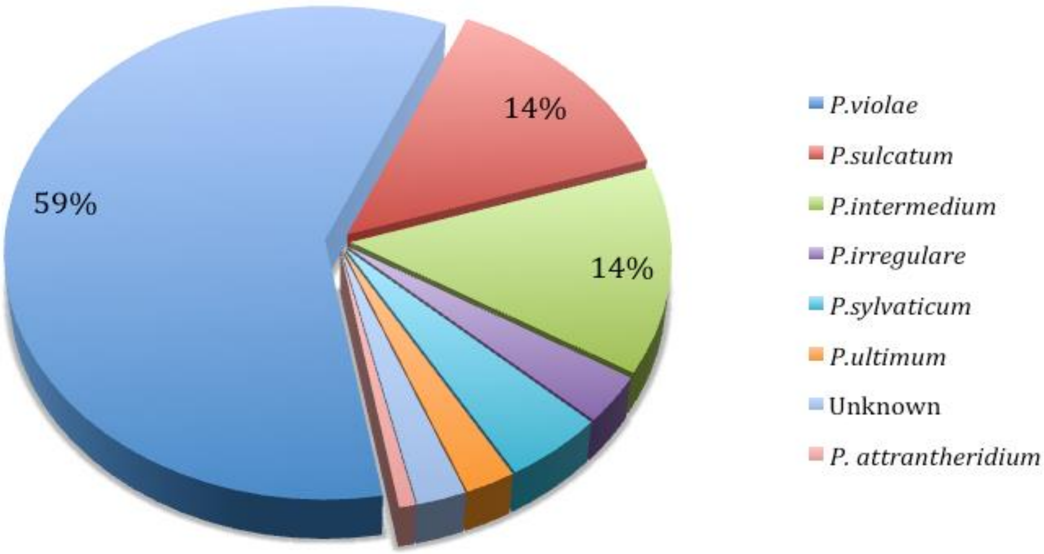

Figure 2. Relative proportions of different *Pythium* species identified from 125 isolates based on sequence of the ITS regions of the rDNA.

Objective 1ii) Improvement in soil sampling and detection

A new method based on 'oospore capture' from soil by sucrose centrifugation and filtration was developed to allow 10 g of soil to be tested for *P. violae* by PCR (see Annual Report 2015 for details). Following this, a number of different PCR primer pairs have been developed and tested under a range of conditions, and found to be specific to *P. violae.* The latest primer pair, AT_ITS FOR/REV1 was specific and suitable for qPCR.

Objective 1 iii) Artificial inoculation

Production of a sand-based *P. violae* inoculum was developed and tested in carrot seedling experiments previously (see Annual Report 2015 for details) and this year further repeat experiments were carried out as well as pot-based mature plant trials as part of project FV

391a. Although results have been variable, damping off has been induced in seedlings (Fig. 3), while reduced yield and cavity spot symptoms were observed in the mature plant trials (Fig. 4).

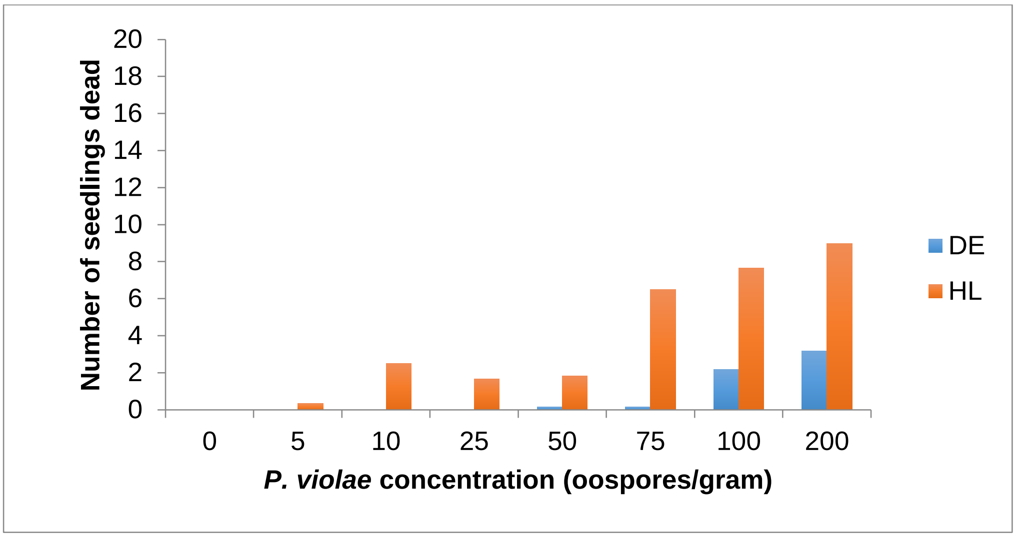

Figure 3. Carrot seedling death from damping off at 10 weeks post sowing for two *P. violae* isolates (DE, HL) at concentrations of 0-200 oospores/gram growing media.

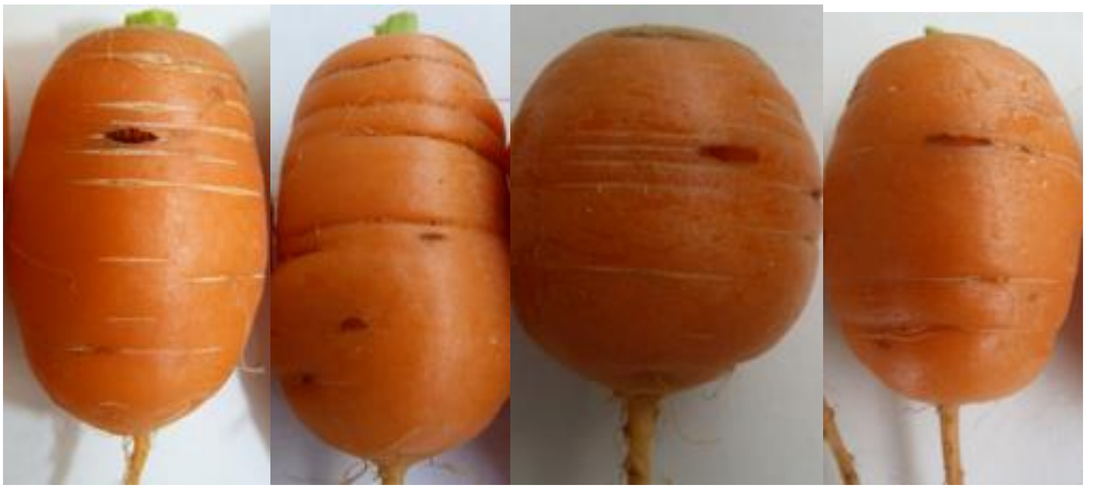

Figure 4. Cavities produced on roots from pot-based mature plant trial after artificial inoculation with a range of *Pythium* concentrations as part of FV 391a.

Financial Benefits

Financial benefits have yet to be established – further details on this are expected at the end of year 3 of the project.

Action Points

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