Grower Summary

FV 432

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

Final 2019

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GROWER SUMMARY

Headline

- A method to capture oospores from soil and improve detection of *Pythium violae* has been developed.
- Artificial inoculation of pot-grown carrots in the glasshouse with *P. violae* consistently resulted in the formation of small, stubby and stunted carrots with some typical cavity spot lesions observed on roots.
- Artificial inoculation in the field resulted in high cavity spot incidence on roots.

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 & 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 led to the discovery that the oomycete *Pythium* was the causal agent (Groom & 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 & White, 2002). In the UK, *P. violae* is now thought to be the most significant cause of cavity spot (White, 1986; Groom & Perry, 1985), although *P. sulcatum* is also known to be associated with the disease (White, 1988; Lyons & 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 & 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 & 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 & 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 & 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 & 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 & 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 Three:

1. Develop effective tools for *P. violae* research:
    i) Continue collection and characterisation of multiple isolates of *Pythium;* produce phylogenetic trees to establish the genetic variation within *Pythium* species.
    ii) Develop, refine and test a new oospore capture method for quantification of *P. violae* in larger soil samples.
    iii) Develop *P. violae* inoculation systems for carrot seedlings and mature plants in glasshouse, and field experiments.

Summary

Objective 1 i) *Pythium* isolate collection and characterisation

Cavity spot infected carrots were collected from grower sites throughout the country between October 2014 and April 2015. 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 (Hales & Clarkson, 2016). Since then, further isolates were obtained in 2016/17 and results from a 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).

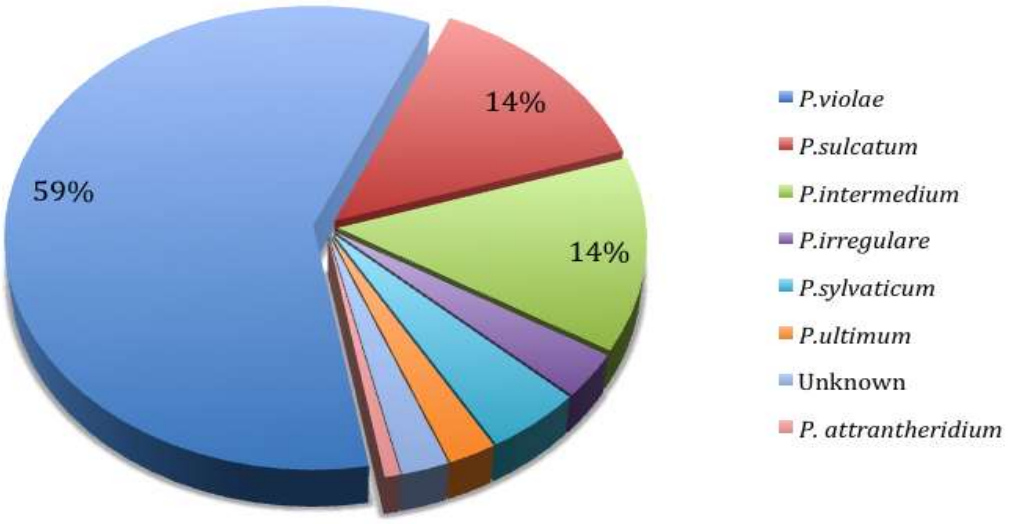

Figure 2: Relative proportions of different *Pythium* species identified from 125 isolates from carrot based on sequence of the ITS regions of the rDNA.

Objective 1 ii) Refining/testing new 'oospore capture' method

A new method for separating *P. violae* oospores from sand/soil by sucrose centrifugation and filtration was developed and refined. Soil/sand samples were suspended in water and sonicated to release oospores before adding to a saturated sucrose solution where oospores become suspended. These are then captured on a filter which can then be used for detection by DNA extraction and PCR. This new method enabled extraction of 50% more oospores than a previous 'standard' method. Combined with the *P. violae* specific qPCR, this method should allow more accurate quantification of oospores in soil and assessment of *Pythium violae* dynamics.

Objective 1 iii) Artificial inoculation

Seedling experiments

Sand and liquid-based *P. violae* inoculum were used to inoculate carrot seedlings in a controlled environment. *P. violae* oospore inoculum produced in V8 liquid culture did not result in any seedling mortality when applied either at sowing or to seedlings in autoclaved sand. However, when applied to seed or seedlings in non-autoclaved sand, up to 50% seedling mortality occurred, including in uninoculated control treatments. This suggested that seedling death was not due to *P. violae* and likely due to a contaminant. Similarly, a *P. violae* solid oospore inoculum showed high levels of seedling mortality including the uninoculated control treatments. Given the variability of this approach and recurrent problems with contamination, a new method is required to enable reliable infection of seedlings with *P. violae*.

Glasshouse experiments

Sand-based solid *P. violae* inoculum was used to initiate infection of carrots in pot-based glasshouse experiments, carried out jointly as part of AHDB project FV 391a. Inoculation of the growing media using this solid substrate at different rates resulted in some seedling death, reduced seedling size and a decrease in growth of foliage. However, at harvest, the principal effect of *P. violae* inoculation was the formation of small, stubby and stunted carrots with a much-reduced weight compared to the uninoculated control plants. These infected carrots were also characterised by a long hairy brown tap root with increased lateral root formation, many of which were collapsed. Typical cavity spot lesions were also observed in a large proportion of these stubby carrot roots (Fig. 3). *P. violae* could also be consistently isolated from the infected tap roots and cavity spot lesions, confirming that these symptoms were due to the inoculation. Generally, there was no clear effect of oospore concentration on the severity of any of these symptoms associated with *P. violae* inoculation. This method therefore shows promise for infection of carrots with *P. violae* but needs refining in order to reduce variability in disease levels and increase the number of cavity spot lesions.

Figure 3: Typical cavity spot lesions on carrot roots caused by *P. violae* following artificial inoculation.

Field experiment

A preliminary field experiment was carried out whereby macrocosms (concrete pipes sunk in the ground) were filled with a soil / sand mix, artificially inoculated with the same *P. violae* solid substrate inoculum as used in the glasshouse experiments and carrots sown. In this situation, there was no effect of pathogen inoculation on either seedling survival or subsequent carrot growth but at harvest, a large proportion of the carrots (up to 40%) were affected by typical cavity spot symptoms (Fig. 4). The sand based solid *P. violae* inoculum therefore shows promise for inducing cavity spot on a field scale.

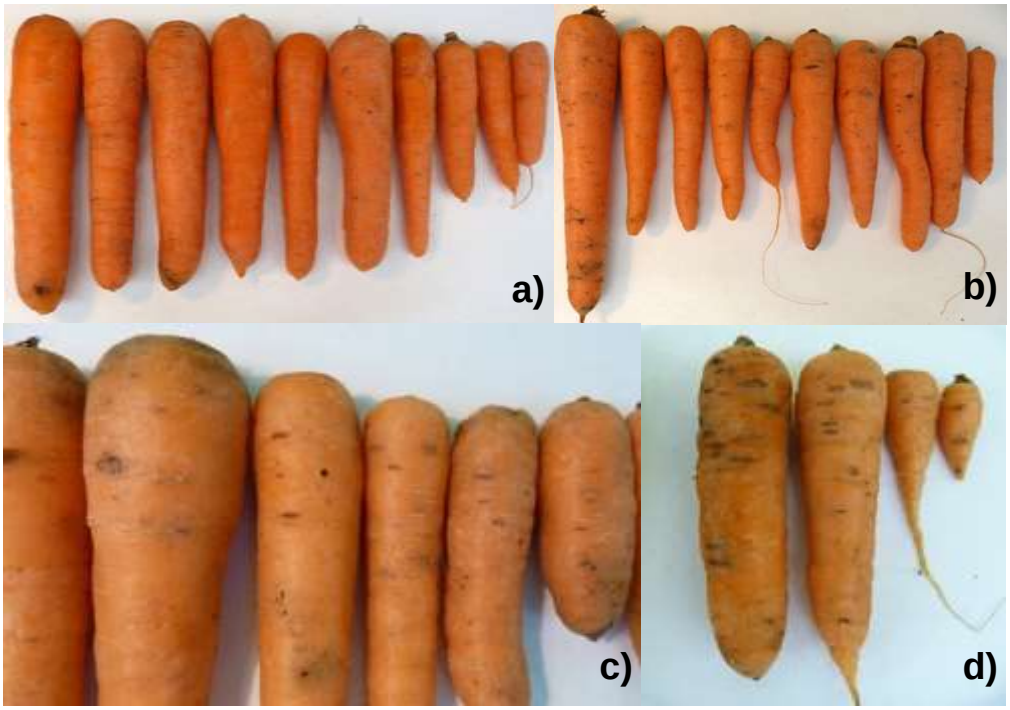

Figure 4: Carrots harvested from field macrocosms a) uninoculated control and b) inoculated with 50 *P. violae* oospores g^{-1}; close up view of carrots to show typical cavity spot lesions for carrots inoculated with c) 30 *P. violae* oospores g^{-1} and d) 50 *P. violae* oospores g^{-1}.

Financial Benefits

If artificial inoculation with *P. violae* can be refined to reduce variation, this approach may allow much more reliable testing of new control products and approaches, hence resulting in considerable financial benefits associated with a reduction in the number of failed field trials.

Action Points

None at this time.