Grower Summary

FV 391b

Carrots: Further development of artificial inoculation techniques for cavity spot caused by *Pythium violae*

Final 2018

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GROWER SUMMARY

Headline

Inoculation of pot-grown carrot plants in the glasshouse with a sand/oat *Pythium violae* oospore inoculum resulted in stunted carrots and cavity spot lesions but levels were variable. A millet seed mycelial inoculum resulted in much higher disease incidence. In field macrocosms, inoculation of carrots with the oospore inoculum resulted in high incidence of cavity spot.

Background

Cavity spot of carrots in the UK is principally caused by the soilborne oomycete pathogen *Pythium violae* and continues to be the most economically important disease for UK carrot growers with losses of at least £3-5 million per season. Management of the disease relies heavily on metalaxyl-M fungicide but its efficacy is variable, possibly due to enhanced degradation in some fields. The reliance on this single fungicide, its efficacy in controlling the disease and the potential withdrawal of approval in the future are major concerns for the industry and hence there is an urgent need to identify new actives or approaches for control. To address this, AHDB Horticulture projects have been commissioned to improve the management and control of cavity spot including testing of potential new fungicides, biological control treatments, pre-planting calcium applications and biofumigation (FV 391, FV 405). These projects were field based but despite best efforts results have not been forthcoming due to no, or low levels of cavity spot development in many of the trials. This has resulted in a failure so far to reliably identify any new actives or approaches to control of *P. violae*.

One solution to the problem of low cavity spot levels is to artificially inoculate carrots with *P. violae* in pots or the field to ensure high enough levels of disease development such that activity of control treatments can be assessed reliably. In a previous project (FV 391a) we developed methods to produce large numbers of *P. violae* oospores in both a liquid medium and a sand/oat-based solid substrate culture. However, initial investigations indicated that oospore germination on agar was negligible as has been observed previously by other researchers. Nonetheless, in two glasshouse experiments, artificial inoculation of the growing media using the *P. violae* solid substrate at different concentrations resulted in some seedling death, reduced seedling size and a decrease in growth of carrot 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. Typical cavity spot lesions were also observed in a large proportion of these stubby carrot roots.

These initial artificial inoculation experiments with *P. violae* in FV 391a were quite successful compared to previous attempts; however, results between experiments were variable. In this project we build on this initial work to address this variation and try and increase the number of cavity spot lesions observed on carrots artificially inoculated with *P. violae* both in the glasshouse and field. We hypothesised that differences in cavity spot disease levels or impact on carrot growth could be due to variation in oospore viability and their ability to germinate (in the presence of carrots) between different batches of *P. violae* inoculum or between different isolates. Hence lab-based experiments were designed to test this for *P. violae* and also *P. sulcatum*. A liquid *P. violae* inoculum was also tested in an initial pot-based glasshouse experiment but due to lack of carrot infection using this approach, further work investigated the ability of different *P. violae* and *P. sulcatum* isolates to induce cavity spot using the sand/oat solid inoculum employed successfully in FV 391a and also tested a millet-based inoculum as previously employed for *P. sulcatum*. Finally, the feasibility of producing solid inoculum of *P. violae* on a large scale in solid state fermentation for potential use in field experiments was assessed.

The overall aim of this project was therefore to further develop methods for producing *P. violae* inoculum and determine the potential to cause cavity spot disease. The specific objectives were:

1) Test vital stains and different chemical treatments to assess *P. violae* oospore viability and increase germination.
2) Test the efficacy of different rates of *P. violae* liquid and solid inoculum in producing cavity spot symptoms in pot grown carrots in the glasshouse.
3) Test the efficacy of different rates of solid *P. violae* inoculum in producing cavity spot symptoms in field grown carrots.
4) Examine the feasibility of large-scale *P. violae* inoculum production in solid state fermentation.

Summary

Methods to assess *Pythium* oospore viability and induce germination

Two methods were investigated to assess *P. violae* oospore viability as a way of checking the quality of inoculum, as this was hypothesised to be a potential source of the variation in cavity spot disease levels and effects on carrot growth observed in experiments in FV 391a. A vital stain method tested showed that live (viable) oospores from both *P. violae* and *P. sulcatum* stained purple in contrast to dead (non-viable) oospores which were stained black or colourless. However, there was some difficulty distinguishing between very dark purple (viable) and black (non-viable) oospores. A plasmolysis method where oospores were

incubated in a saline solution showed that the cytoplasm of live (viable) oospores contracted into a ball-like structure due to the resulting loss of water; in contrast, dead (non-viable) spores did not display this plasmolysis due to the lack of functional cell membranes. It was concluded therefore that the plasmolysis method was the most reliable for assessing *P. violae* oospore viability.

To test approaches for inducing *P. violae* oospore germination we first had to develop an effective method for producing *Pythium* oospore preparations free of mycelial fragments as live, residual mycelium hampers microscopic assessment of germination as it quickly overgrows oospores. This involved treatment with Glucanex, a mixture of lysing enzymes from *Trichoderma harzianum*, which effectively digested and deactivated mycelial fragments in *P. violae* and *P. sulcatum* oospore preparations whilst the walls of oospores were left intact. The survival of oospores during this process was confirmed as there was little or no loss of viability as assessed by the plasmolysis assay. It has also been suggested previously that lysing enzymes may also break oospore dormancy and enable germination but neither Glucanex treatment alone nor the addition of potassium permanganate (also reported to promote oospore germination in some *Phytophthora* and *Pythium* spp.) induced germination in *P. violae* oospores. In contrast, a high proportion of Glucanex-treated oospores of one *P. sulcatum* isolate germinated within 2 to 3 days. Only one concentration of $KMnO_4$ and one incubation period was tested in this study, and other factors such as desiccation, heat shock, exposure to soil and plant exudates may be important for *Pythium* oospore germination. The outcomes of this series of experiments highlighted that the requirements for oospore germination potentially vary considerably between *P. violae* and *P. sulcatum* isolates. Hence, more extensive research is required to investigate potential triggers of oospore germination in multiple isolates of *P. violae* and *P. sulcatum*.

Development of inoculation methods to produce cavity spot symptoms in pot grown carrots in the glasshouse

Experiments were carried out to continue the initial work in FV 391a to develop an artificial inoculation system for *P. violae* for pot-grown carrots in the glasshouse. An initial experiment using a liquid oospore inoculum resulted in no cavity spot symptoms and was also difficult to apply consistently. Further experiments therefore focussed on developing the use of a solid *P. violae* inoculum. Initially, an experiment was set up using a solid sand/oat inoculum to test the effect of metalaxyl seed treatment, firstly to investigate whether it would control cavity spot and secondly to determine if it could delay early infection of *P. violae* yet allow symptoms to develop later on in mature carrot roots. A follow up experiment using the same type of inoculum then evaluated the ability of different isolates of both *P. violae* and *P. sulcatum* to

induce cavity spot symptoms to examine if there were more virulent isolates that could induce higher levels of cavity spot than observed in previous tests.

Overall, the major finding from these first two glasshouse experiments was that artificial inoculation of carrots with the sand/oat *P. violae* oospore inoculum resulted in a reduction in seed germination and foliage development as well as the formation of short stubby carrots with a reduced root weight. Typical cavity spot lesions were also formed while tap roots were misshapen and brown. The pathogen was consistently isolated both from lesions and taproots, confirming that infection was successful. These observations were consistent with the results from FV 391a where the same range of symptoms was also evident. In addition, results from this project and FV 391a also indicated that increasing the concentration of *P. violae* oospores did not result in a corresponding increase in subsequent disease metrics across the range of 10-100 oospores g^{-1} soil. Significantly, the extent of disease indicators between experiments both in this project and FV 391a was variable despite careful quantification of *P. violae* inoculum. For instance, reduction in carrot weight for *P. violae* inoculated treatments was significant in both the experiments in FV 391a but not in the first two experiments in this project. Cavity spot incidence for *P. violae* was 0.75-23% and 5-20% in this project and FV 391a respectively, with corresponding disease severity of 1-3 and 1-2 lesions per carrot for those roots affected. Overall therefore, while inoculation of carrots with the *P. violae* sand/oat inoculum always results in some cavity spot disease, the level at which this occurs is unpredictable. In this project, similar growth effects on carrot and cavity spot symptoms were observed following inoculation with one isolate of *P. sulcatum* while another two failed to cause any infection.

The reasons for this variability in cavity spot disease are unclear and may be related to oospore viability, the ability of oospores to germinate or environmental factors (e.g. water availability) in the glasshouse. Viability and germinability of oospores used in these experiments was not directly assessed as the methods described above to achieve this were only developed in the latter stages of the project. However, viability of *P. violae* and *P. sulcatum* oospores is unlikely to have been a significant factor given subsequent results of the plasmolysis assay. Previous studies have also reported variation within-species variation in virulence for *P. violae* and *P. sulcatum*.

Finally, results from the experiment testing the effect of metalaxyl treated seed suggested that this had little overall benefit in reducing cavity spot which is in contrast to previously published work. However, there was some protective effect of metalaxyl at a *P. violae* inoculum level 1 oospore g^{-1} only, which may suggest some benefit at low disease pressure. There was also some evidence that in the absence of the pathogen, metalaxyl reduced carrot seed germination which has also been noted previously.

Given the variability in cavity spot disease development and plant growth effects in experiments using the sand/oat oospore inoculum, a new approach was tested in a third glasshouse experiment where a millet seed mycelium-based inoculum of *P. violae* and *P. sulcatum* was tested at two different concentrations. Although there was a significant carrot growth promoting effect of the millet itself at the high concentration of 50 mg g^{-1} soil, which was not evident at the low concentration of 5 mg g^{-1} soil, both levels of inoculum resulted in very high incidence of cavity spot for *P. violae* isolate HL with 49% and 82% carrots affected for low and high inoculum levels respectively. Cavity spot incidence was lower for *P. sulcatum* isolate P67 at the lower inoculum concentration (17%) while no carrots survived to maturity at the higher inoculum level due to extensive damping off. Overall therefore, the millet inoculum resulted in much higher cavity spot disease incidence and severity than observed in any of the experiments using the sand/oat inoculum either in this study or the previous project FV 391a. This suggests that the millet-based *Pythium* inoculum may be a better approach for inoculation of carrots in the glasshouse although further testing is required to confirm this.

Development of an inoculation method to produce cavity spot symptoms in field grown carrots

Experiments were set up over two years to test the effect of different concentrations of *P. violae* isolate HL sand/oat oospore inoculum on development of cavity spot disease in field macrocosms. Overall, this approach was extremely successful and in contrast to the pot-based experiments resulted in high cavity spot incidence ranging from 30.4-35.7% roots affected in the second year (2018) with similar levels in year 1. Again however, mean disease severity was low with a mean of 2-3 lesions per carrot. Nonetheless this is the first report of artificial inoculation with *P. violae* resulting in significant cavity spot disease under field conditions. These results may suggest that *P. violae* oospores germinate more readily in field soil than in the growing medium employed in the pot tests, perhaps due to exposure to certain soil factors or environmental conditions that are more conducive to activation of oospores and infection of carrot roots.

Conclusions

- High numbers of *P. violae* and *P. sulcatum* oospores can be produced in a liquid medium and solid substrate.
- A plasmolysis assay was developed to determine viability of *P. violae* and *P. sulcatum* oospores.
- Treatment with a Glucanex enzyme mix or potassium permanganate did not promote germination of *P. violae* oospores.

- Oospores of a *P. sulcatum* isolate germinated readily on agar in contrast to an isolate of *P. violae*
- Artificial inoculation of a compost / sand growing medium with a *P. violae* solid substrate sand/oat oospore inoculum resulted in successful infection of pot grown carrots. Symptoms included some seedling death, reduced seedling size, a decrease in growth of foliage and the formation of small, stubby and stunted carrots with brown tap roots and typical cavity spot lesions. *P. violae* was consistently isolated from tap roots and cavities.
- Metalaxyl seed treatment had little effect or no effect on cavity spot levels or on reducing effects on carrot plant growth in pot tests following artificial inoculation with a *P. violae* sand/oat oospore inoculum.
- Different isolates of *P. violae* and *P. sulcatum* varied in their ability to cause cavity spot symptoms or reduce carrot plant growth in pot tests following inoculation with sand/oat oospore inoculum.
- A solid substrate millet seed mycelial inoculum of *P. violae* resulted in high incidence of cavity spot level in pot tests.
- Artificial inoculation of soil contained in field macrocosms with the *P. violae* solid substrate sand/oat oospore inoculum resulted in a very high incidence of cavity spot.

Financial Benefits

Artificial inoculation in filed or glasshouse may now allow more reliable testing of new control products, hence resulting in considerable financial benefits associated with a reduction in the number of failed trials.

Action Points

None at this time.