Grower Summary

FV 448a

Development of an early warning test indicating the risk of Cavity Spot in Carrot. Phase 2: Validation

Final 2017

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Project title:	Development of an early warning test indicating the risk of Cavity Spot in Carrot. Phase 2: Validation
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GROWER SUMMARY

Headline

Prevent losses caused by *Pythium violae* by using a practical molecular test determining the risk of cavity spot in carrots.

Background

Cavity spot is a major disease in the UK and is mainly caused by *Pythium violae*. Cavity spot reduces harvest quality. Cavity spot carrots are not acceptable for packing, but are used at low levels in processing. Severe infections (either high incidence or deep lesions), would be rejected from the processing market.

An early indication for cavity spot would be of great value, as it can be used as a decision support system. The test has to assess risk on cavity spot at two cost adding moments: before distribution of straw and before fields are covered. Selecting low risk fields will reduce losses and leads to less costs for labour and straw.

Development of diagnostic methods enabling early detection of cavity spot would be of great importance in limiting the economic losses. Assays have been developed to detect *Pythium* species in the soil or in carrot tissue (White *et al.*, 1996; Klemsdal *et al.*, 2008; Barbara D.J. *et al.*, 2010). However, a positive result on *Pythium violae* in the soil or on carrots is no guarantee that cavity spot will occur as the vitality status of the carrot plays an important role too. To predict whether a certain field of carrots will develop cavity spot, it is necessary to look at the crop status.

The aim of this project was to identify cavity spot specific indicator genes from the most prominent carrot cultivar in the UK 'Nairobi'. These genes would be used to develop a practical test that quantifies the expression of those genes to determine the risk of cavity spot at an early stage.

Summary

The intended time to develop a molecular test to detect cavity spot at an early stage using carrot specific genes was expected to take two years.

Year 1

In collaboration with carrot growers Poskitt, Strawson and seed company Elsoms Seeds, NSure collected samples from the carrot variety 'Nairobi' in September and October 2015 from various fields in Nottinghamshire and Yorkshire. Based on the quality evaluation results, NSure selected two fields that showed a low occurrence of cavity spot and two fields with a high occurrence. Frozen samples collected from those fields were studied in detail by RNA

sequencing (RNA-Seq). By using this method, NSure was able to examine the activity of all genes. By comparing the low risk samples to the high risk samples, a longlist of potential indicator genes was created that could be suitable to predict the occurrence of cavity spot. A set of putative indicator genes were scrutinized by testing their predictive power on samples collected from other fields by quantitative RT PCR (qPCR). Several potential indicator genes, survived this testing phase, although it should be mentioned that correlation between the gene expression profiles of the genes that surpassed the testing phase to the occurrence of cavity spot was not perfect. The limited sample collection as well as the low number of quality assessments made it difficult to value the results.

Year 2

In the second year of the project, the putative indicator genes were validated in a new sample-set. Like previous year, samples were collected from various fields in Yorkshire and Nottinghamshire growing Nairobi. In parallel, samples were collected from two trials in which 6 carrot varieties intentionally were infected with *Pythium violae*, *Pythium sulcatum* or a control treatment. Based on the inoculation trials, it could be determined whether the potential indicator genes responded in a similar way in other varieties. Furthermore, it could be studied if *Pythium violae* and *Pythium sulcatum* alter the expression of the potential cavity spot indicator genes in a similar manner. Like previous year, it turned out that it was quite difficult to obtain a reliable sample collection and sufficient quality evaluation results. A complete sample-set was obtained from the commercial fields in Nottinghamshire, but in the fields also a lot of other pathogens were present that complicated the validation. Due to circumstances, a complete sample-set from the commercial fields in Yorkshire could not be obtained. In case of the inoculation trials several complications were encountered. Based on the control treatments, it could be deduced that there was already *Pythium* present in the fields. Furthermore, the occurrence of cavity spot was highly variable between the replicates as well as the presence of other pathogens disturbed the quality evaluation and also the validation.

Regarding the inoculation trial performed on the premises of Elsoms Seeds, no significant differences were observed in susceptibility between the tested varieties towards *Pythium sulcatum* and *Pythium violae*. In the other inoculation trial, a significant difference was observed between Nerac and Nairobi in the presence of *Pythium sulcatum* (Figure 1). Although not significant, this trend was also observed between Nerac and Nairobi in the control and *Pythium violae* treated plots. Nairobi seemed to be more susceptible than Nerac. Based on the severity scale, Nipomo was also more susceptible in the presence of *Pythium sulcatum* than Nerac. Within this trial, there appeared to be a trend that Nerac, Norfolk, Norway had a similar tolerance level against cavity spot whereas Nipomo, Newark and Nairobi seemed to

be the more susceptible varieties. The results were similar to those obtained in a cavity spot field trial performed by Bejo Seeds in the Netherlands in 2015 (personal communication).

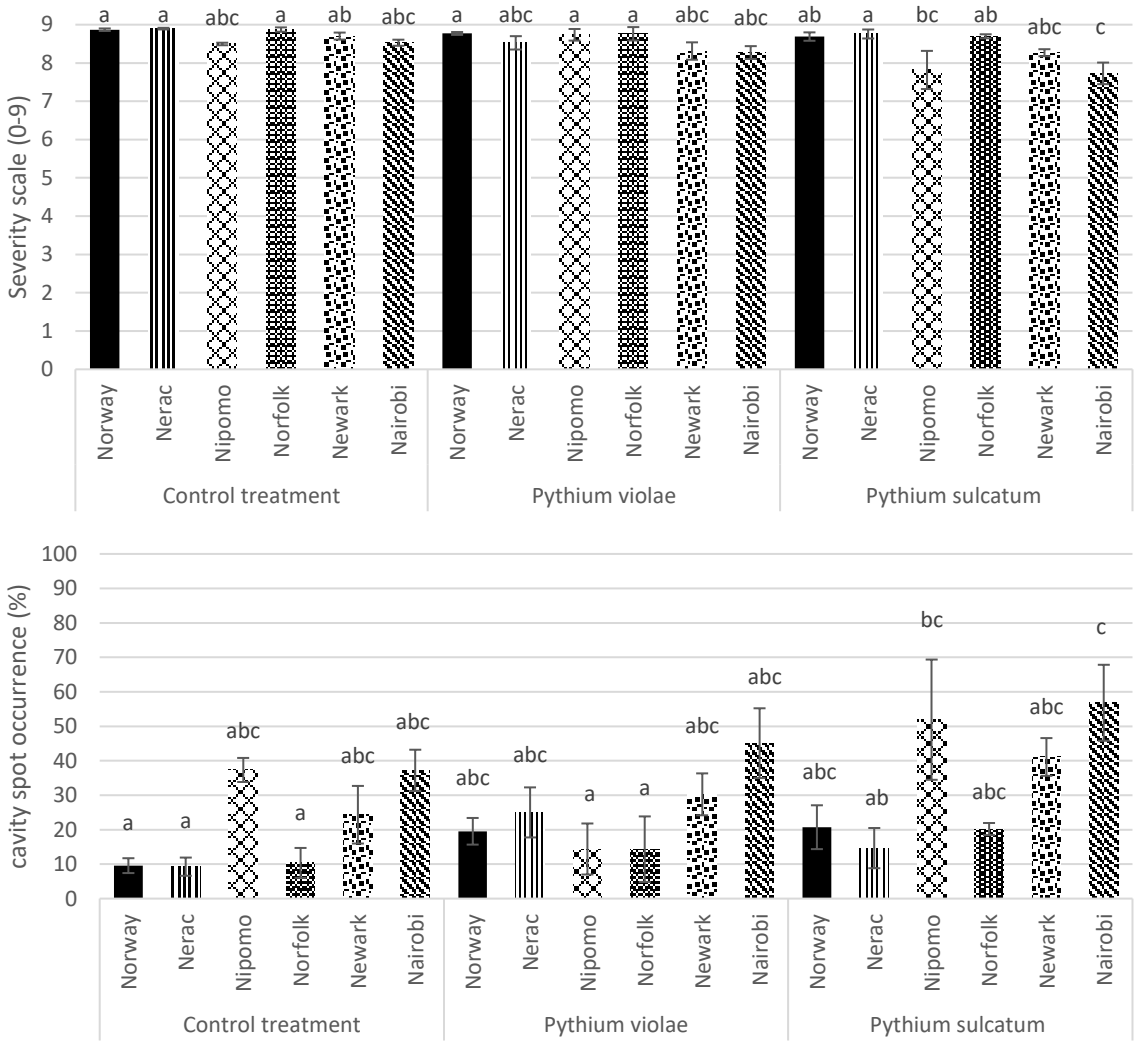

Figure 1. Cavity spot measurements conducted on the inoculation trial performed in Nottinghamshire. Top) Severity scale which ranges from 0 to 9; with 9 no symptoms observed and 0 very severely infected. Bottom) Cavity spot occurrence based on the total number of infected carrots (%). Measurements were conducted in November 2016. Each bar represents the mean based on four repetitions. The error bar represents the standard error. Bars with different letters are significantly different (Tukey's HSD, $p<0.05$).

The expression of the potential indicator genes, was first checked in the frozen samples collected from the commercial fields in Nottinghamshire 2016. Several genes were discarded due to a poor correlation to the occurrence of cavity spot and towards each other. Eleven genes surpassed this validation. Interestingly, 5 out of 11 genes were predicted to be associated with ethylene signalling and 3 other genes seem to respond to (a)biotic stresses. The plant hormone ethylene is involved in many developmental processes, including plant-

pathogen interactions. Root invasion by Pythium spp. is characterized by degradation of host cell walls and plants may respond actively to a Pythium invasion by thickening and lignification of the wall. Ethylene has shown to alter lignification, cell wall synthesis and cell wall composition (Geraats et al., 2002). Furthermore, ethylene insensitive tobacco and Arabidopsis mutants showed increased susceptibility to Pythium spp. (Geraats et al., 2002).

In Figure 2, the gene expression profiles are shown of 5 potential indicator genes and they show a quite similar gene expression pattern towards each other. For the majority of the commercial fields in Nottinghamshire, the gene expression profiles were quite comparable irrespective of the time (August/October) they were collected. For certain fields, variation was observed between the replicates or time points demonstrating that there may have been some heterogeneity within the field.

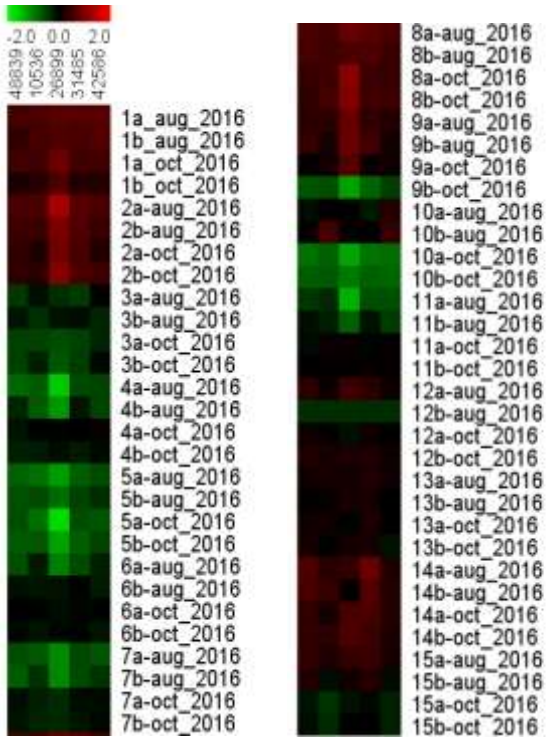

Figure 2. Heatmap representing colour-coded expression levels of 5 potential cavity spot indicator genes collected from commercial fields in Nottinghamshire. Low expression of the gene is indicated in red, high expression in green. The numbers indicate field numbers, a/b the replicates and aug/oct samples collected in August/October.

Regarding the commercial fields in Nottinghamshire, NSure was not able to identify all the high risk fields based on the gene expression patterns of the potential indicator genes. Although the correlation of the potential indicators to the occurrence of cavity spot was not all-decisive, the genes still looked promising especially regarding the predicted function. The set of genes were further validated in samples collected from commercial fields in Yorkshire and

from the inoculation trials. For most samples measured, similar gene activity profiles were observed between the genes as was observed for the Nottinghamshire samples. Nevertheless, correlation of the gene expression profiles of the potential indicators with the occurrence of cavity spot was low. This questions whether these genes are truly cavity spot indicators. The RNA-Seq study performed in the first year was re-evaluated again to find new indicator genes. Some new potential genes were identified, but in the end they did not pass the qPCR validation.

This project made clear that it is difficult or perhaps impossible to find specific genes for cavity spot. It could be that there are no specific genes that are solely altered upon a *Pythium* infection. On the other hand, it could be that the variability within our sample-set complicated the identification of specific genes. A different approach could possibly lead to specific indicator genes. An inoculation trial with *Pythium violae* (mainly found in the UK) should be set up under tight controlled conditions (greenhouse) to assure the absence of pathogens and subsequently RNA-Seq should be performed to identify genes that are altered upon a *Pythium violae* infection. After identification, the potential indicator genes should be monitored tightly in the field in combination with other measures over the years to find genuine patterns and cavity spot indicator genes.

Financial Benefits

Carrot is one of the major crops in the UK. The total cultivated area exceeds 9000 ha 60% of the acreage, approx. 5500 ha, is stored under straw. One hectare results on average in a gross income of £8000. This means that the total turnover of covered carrots is approx. £44million.

Losses due to cavity spot vary between years and geographical regions. Till recently, Scotland for example, had no occurrence of cavity spot. Other regions have more severe problems. In some fields the damage exceeds 40%. On average, cavity spot destroys 3 - 7% of the yearly yield, resulting in a loss between £1.25 and £3million. However, this percentage seems to increase over the years. In 2014 for example, the percentage was estimated to be between 5 and 10%, which almost doubled the losses.

The average cost of covering consist of straw (£3000 per ha) and logistics (transport and covering). In total the costs for covering are approx. £4000 per ha. It is clear that a high cavity spot occurrence means that a grower will not earn (instead: will lose) money on those batches. A predictive test that determines high risk fields, will support a grower to pick only low risk fields for covering.

A predictive test would make UK carrot industry much more profitable in picking the ‘safest’ crops to store over the shorter or longer term.

Action Points

There is no clear change of practice for the growers as no reliable indicator genes were identified within this project.

