Grower Summary

CP 196

Defining relationships between F. *oxysporum* inoculum level, quantitative molecular diagnostics, microbial community composition and basal rot development in different soils to enable disease prediction in bulb onions

Final Report

Project title:	Defining relationships between *F. oxysporum* inoculum level, quantitative molecular diagnostics, microbial community composition and basal rot development in different soils to enable disease prediction in bulb onions
Project number:	CP 196
Project leader:	John Clarkson, Warwick Crop Centre, University of Warwick
Report:	Final report
Previous report:	N/A
Key staff:	Alison Jackson (Warwick) Helen Bates (NIAB-EMR) Julie Bennett (NIAB-EMR) Greg Deakin (NIAB-EMR) Andrew Armitage (NIAB-EMR)
Location of project:	University of Warwick and NIAB-EMR
Industry Representative:	N/A
Date project commenced:	14/10/2019

DISCLAIMER

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

John Clarkson

Reader

University of Warwick

Signature: John Clarkson Date: 27/02/2020

GROWER SUMMARY

Headline

Detection of *Fusarium oxysporum* f.sp. *cepae* (FOC) causing onion basal rot using qPCR was related to levels of pathogen inoculum and disease development in artificially inoculated soils. A similar threshold of detection for FOC was evident when amplicon sequencing was employed as an alternative approach to quantify the pathogen at the same time as characterising the wider microbial community.

Background

This project builds on previous AHDB and BBSRC supported work where whole genome sequencing, assembly and bioinformatic analyses enabled the development of specific DNA-based quantitative PCR (qPCR) diagnostic tests for some of the most significant and economically damaging *Fusarium* pathogens in UK horticulture: *F. oxysporum* f.sp. *cepae* (FOC, onion), *F. oxysporum* f.sp. *matthiolae* (FOM, stocks) and *F. oxysporum* f.sp. *narcissi* (FON, *Narcissus*). Furthermore, using similar bioinformatic analyses, an innovative amplicon sequencing approach was developed which allowed the abundance and identity of different components of the microbial community to be identified simultaneously from the same DNA sample. This approach specifically identifies pathogenic and non-pathogenic *Fusarium* species, as well as individual pathogenic *F. oxysporum* f.spp. including FOC, alongside members of the wider fungal and bacterial microbiota. Although amplicon sequencing is now used extensively in research, its practical application to assess soil health or determine the presence of pathogens in soil is largely unproven.

The approach in this project was to focus specifically on FOC which continues to cause ever increasing concern to growers as basal rot disease levels in bulb onions continue to escalate year on year. Having developed a specific DNA-based qPCR assay for this pathogen previously, one of the main objectives of the project was to determine if this test could accurately quantify different levels of FOC inoculum in soil and hence predict the risk of disease development as a practical tool for growers. This would indicate whether pathogen-free or low pathogen field sites could be identified with confidence and hence whether a pre-planting soil test to assess basal rot disease risk was feasible. Another objective was to assess whether FOC root colonisation could be detected and quantified early in crop development as another way of potentially assessing risk of disease. In

practice, detecting FOC in seedlings or young plants before the expression of symptoms in the field would allow onion growers to manage their crop appropriately and make decisions about harvest, post-harvest curing / drying and storage.

A final objective was to use the amplicon sequencing method developed previously to determine the abundance and identity of *Fusarium* spp., fungi and bacteria that were present in the different soils and also examine whether this approach was sensitive enough to effectively distinguish between the different FOC inoculum levels. This would determine whether in the future, amplicon sequencing could be used to accurately determine pathogen levels at the same time as assessing soil health based on microbial community analysis.

In summary, the main aim of the project was to define relationships between FOC inoculum level, the amount of FOC DNA as measured by a quantitative qPCR test and onion basal rot disease development for different soils. A secondary aim was to examine onion root colonisation by FOC and determine if different soils reduced the rate of FOC disease development and if so, identify the components of the microbial community that may be responsible.

The main project objectives were to:

1) Determine the effect of different FOC inoculum levels on onion basal rot disease development for different soils
2) Quantify the amount of FOC DNA for different inoculum levels using qPCR in different soils and define relationships with onion basal rot disease development
3) Determine the rate of onion root colonisation by FOC for different soils
4) Establish the potential of amplicon sequencing to quantify levels of FOC inoculum for different soils and identify components of the microbial community that may reduce the rate of onion basal rot disease development

Summary

Four different onion growing soils (NOTTS 1, NOTTS 2, CAMBS and LINCS) including two of the same type with and without previous (grower) application of poultry manure (NOTTS1 and NOTTS2 respectively) were shown to result in significantly lower levels of basal rot disease development in onions compared to a peat-based growing compost when inoculated with different concentrations of FOC inoculum in a glasshouse experiment. However, disease development in compost was lower than expected most likely due to suboptimal environmental conditions in the glasshouse at the time of year it was necessary to carry out the experiment (November-January). Nonetheless, the results suggested that the physical and / or biological properties of the soils were less conducive to disease than

compost. Basal rot development in NOTTS 1 and NOTTS 2 soils (sandy loam) was similar (although slightly higher for NOTTS 1) and only extensively developed for the highest FOC inoculum levels (2 x 10^5 and 2 x 10^6 cfu g^{-1} soil). This indicated that the previous (grower) addition of poultry manure for NOTTS1 which resulted in relatively greater levels of NPK, organic matter and microbial biomass than NOTTS 2 did not suppress basal rot. Much higher levels of organic matter and microbial biomass were found in the CAMBS (clay) and LINCS (sandy, silt loam) soils with the former having much higher levels of N and K compared to the others. The CAMBS soil was also the most suppressive to basal rot disease development but it was not clear if this was related to these factors.

As well as differing in their physical properties, the four soils could also be distinguished based on the structure, diversity and identity of components of the bacterial, fungal and *Fusarium* spp., communities as measured by amplicon sequencing. Overall bacterial diversity was greater in NOTTS1 than for the other soils but fungal diversity was similar. The main components of the bacterial and fungal communities comprised of genera commonly found in soil. Analysis of *Fusarium* spp. and related species present identified pathogenic species including *Ilyonectria. radicicola* (*Cylindrocarpon destructans*), *F. redolens*, *F. solani*, *F. equiseti* and *F. proliferatum*. *F. redolens* and *F. proliferatum* have previously been associated with onion basal rot in addition to FOC. Given that basal rot disease development was generally similar between soils, then no specific components of microbial communities could be identified as being associated with basal rot disease suppression. Ideally a much wider range of soils and onion production systems would need to be examined with more detailed sampling to determine physical or microbial factors associated with differing levels of basal rot.

Using the qPCR, it was clear that detection of FOC in soil was only consistent for inoculum levels ≥1x10^4 cfu g^{-1} soil across all the four soils. However, basal rot disease development in soil only occurred at inoculum levels ≥1x10^5 cfu g^{-1} so for this experiment at least, this suggests that the qPCR would be able to predict disease in a pre-planting test. As conditions for disease development in this experiment were sub-optimal due to the time of year the experiment was carried out, a different disease outcome might have occurred if the experiment was repeated in better light / higher temperatures in the summer. In addition, disease development in an inoculated pot system may differ from that under commercial field conditions.

Despite lower disease levels than expected in the experiment, with first disease symptoms observed after 20-30 days, colonisation of onion roots by FOC was detected by qPCR as early as 3 days and more consistently after 13 days at inoculum levels ≥1x10^5 cfu g^{-1} soil. FOC was also less consistently detected on onion roots at 1x10^4 cfu g^{-1} and overall

therefore the threshold for detection was similar as for soil. However, the ability to detect FOC at an early stage in plant development before symptoms occur suggests that using the qPCR to identify the pathogen on onion seedling roots in the field may be another approach to predict disease risk in growing crops. Moreover, if onion seedlings could be used to 'bait' FOC from soil in pre-planting tests, this might with some further development increase the sensitivity of the qPCR detection.

As expected, the amplicon sequencing approach was appropriate for characterising and defining the relative abundance of bacteria and fungi in the different soils. Targeting the TEF gene was particularly effective for identification of *Fusarium* and related spp. and background levels of a number of potentially different pathogenic spp. (including *F. oxysporum*) were identified across the different soil types. When FOC was introduced into these soils, the pathogen was successfully detected using different gene targets including ITS (to genus level), TEF (to species level) and OG4952 (to *F. oxysporum* f.sp. level) with a similar detection threshold as for the qPCR ($\geq 1x10^4$ cfu g^{-1} soil) across all the four soils.

In summary, this small project was successful in establishing the level of FOC detection achievable by qPCR and relating this to inoculum concentration and basal rot disease development. Furthermore, it showed that the amplicon sequencing approach has potential to detect FOC at a similar detection threshold and provide further information of other *Fusarium* pathogens and the wider microbial community.

Financial Benefits

The implementation of an effective pre-planting qPCR test for onion basal rot would allow growers to select disease-free or low-disease fields resulting in reduction in losses and concomitant economic benefits. However, further work is required to establish the utility of the qPCR in a range of commercial situations.

Action Points

None at this time.