Grower Summary

CP 204

Diagnostic tests to assess Fusarium disease risk, select rotation crops and monitor microbial communities

Annual report March 2021

Project title:	Diagnostic tests to assess Fusarium disease risk, select rotation crops and monitor microbial communities
Project number:	CP 204
Project leader:	John Clarkson, University of Warwick
Report:	Annual report, March 2021
Previous report:	N/A
Key staff:	Sascha Jenkins Alison Jackson Helen Bates
Location of project:	Warwick Crop Centre, School of Life Sciences, University of Warwick Wellesbourne Campus
Industry Representative:	Sam Rix, P G Rix Ltd.
Date project commenced:	01/04/2020

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.

Sascha Jenkins

Research Fellow

University of Warwick

Signature: *Sascha Jenkins* Date: 01/04/21

John Clarkson

Reader

University of Warwick

Signature: *John Clarkson* Date: 01/04/21

Report authorised by:

John Clarkson

Reader

University of Warwick

Signature: *John Clarkson* Date: 01/04/21

GROWER SUMMARY

Headline

DNA based qPCR diagnostics and other molecular approaches show promise for detection of *Fusarium oxysporum* pathogens of onion and lettuce in order to assess disease risk for growers. The use of these tests in commercial situations requires further optimisation.

Background

Fusarium oxysporum is a soilborne plant pathogen with a worldwide distribution and causes vascular wilt, root rot and bulb rot diseases in many economically important crops. *F. oxysporum* is a species complex comprised of over 150 *formae speciales* (f. spp.) which are adapted to infect specific crops such as onion, lettuce, leek, banana, tomato peas, brassicas and also narcissus and column stocks.

Fusarium wilt disease of lettuce

Fusarium wilt of lettuce, which is caused by *F. oxysporum* f. sp. *lactucae* (FOL), has spread to most production areas globally. There are four cultivar specific races, with race 1 and race 4 being the most widespread which cause severe economic losses in both field and protected crops respectively. In the UK, FOL race 4 (FOL4) was first identified in 2017 in Lancashire and Ireland, but has since spread to Cambridgeshire and Yorkshire, as well as locally within each area; so far FOL4 occurrence has been restricted to lettuce grown under protection. Disease symptoms include yellowing and necrosis of leaves, stunting and wilting of plants and reddish-brown/black necrosis of vascular tissue. There are currently no commercially available resistant cultivars and therefore rapid spread between growers is being prevented through hygiene measures such as rigorous cleaning of equipment and glasshouses, and by using foot dips/containment procedures for people moving from infected to clean areas. Growers have been mitigating disease impact through occasional use of the soil fumigant dazomet (Basamid), removal of contaminated soil or by abandoning affected growing areas.

Fusarium disease of onion

Fusarium basal rot of onion, caused by *F. oxysporum* f.sp. *cepae* (FOC), represents a major threat to the industry, with incidence levels increasing over the last few years. Recently basal rot losses have increased from 2-6% to over 10%, resulting in economic losses of approx. £13M. Many of these losses have been the result of disease developing in storage, where apparently healthy bulbs develop disease, which can result, in worse cases, to the whole consignment being abandoned, as it becomes too costly to extract affected bulbs. Fungicides may have limited effect as FOC, like other f. spp. is soilborne, therefore it can be difficult to

control once an area becomes infected. Early detection is key to prevent crops being grown in affected soils, to reduce disease incidence as much as possible, and prevent bulbs being contaminated before going into storage. Hence in this project we will investigate the use of molecular diagnostics as a tool to assess disease risk for bulb onions pre-planting and also during the cropping period.

Fusarium disease of Narcissus

Fusarium basal rot of Narcissus is caused by *F. oxysporum* f.sp. *narcissi* (FON) and is a major problem for UK daffodil growers. The industry is estimated to be worth £45M and 10% losses are not unusual with a corresponding value of £4.5M (Hanks, 2010). Control has been dependent on just two active substances, thiabendazole (Storite) and chlorothalonil (Bravo) applied to bulbs in hot water treatments used to control stem nematode. However, both these actives have recently been withdrawn for use although an EAMU is being developed for prochloraz which has proven effective against FON in previous research (Clarkson, 2014). Further potential new fungicides are being tested within AHDB's Sceptre+ project. Despite the regular application of fungicides by Narcissus growers, extensive losses are still common in certain parts of the production area and the long periods of time the crop is in the ground makes it vulnerable to basal rot irrespective of initial fungicide applications. Although historically some Narcissus cultivars were thought to be resistant to FON (e.g. St Keverne), this seems to have broken down and widely grown cultivars such as Carlton and Golden Harvest are susceptible.

Control of Fusarium diseases

As *F. oxysporum* is a soil borne plant pathogen, control is fundamentally difficult as it invades plants via the roots and produces long lived chlamydospores which can survive in the soil for many years. Crop rotations are one of the most successful ways to avoid build-up of inoculum in the soil to levels capable of producing disease. However, there is increasing evidence to suggest that *F. oxysporum* can proliferate on non-host crops, therefore maintaining levels of inoculum which continue to increase when the host is again grown in the rotation. Fungicides usually have little effect; however, soil sterilisation or chemical fumigation is often used in protected crops to try to prevent disease occurring. These techniques have been shown to reduce the levels of inoculum in the soil to below the required level for disease to occur, therefore reducing incidence and preventing losses. Unfortunately, they also negatively impact the microbial communities in soil which often act to suppress diseases and can therefore lead to *F. oxysporum* inoculum building up after fewer cropping cycles. This is a particular problem with crops grown under protection, such as lettuce, and multiple crops are often sown in the same location every year without rotation, therefore facilitating *F.*

oxysporum proliferation. This project aims to investigate the potential of soil sterilisation techniques to suppress disease, but also to determine their effect on microbial communities and how this interaction relates to levels of disease. One approach to mitigate the negative effects of soil sterilisation is to introduce biological control agents or soil amendments to encourage the recovery of microbial communities and suppress inoculum build-up; therefore the use of these treatments will be investigated in the project.

Project aims and objectives

In this project we will utilise molecular diagnostics developed at Warwick Crop Centre in a previous AHDB-funded project FV POBOF 452 to determine the risk of Fusarium disease with a focus on FOC causing basal rot in onion but also with some testing of samples from Narcissus and lettuce. Initially, the relationship between Fusarium inoculum concentration, disease development and the amount of DNA detectable in the soil will be established through glasshouse experiments involving lettuce and narcissus grown in inoculated soils (this has been done previously for onion). We will then use these diagnostic techniques to detect the different *F. oxysporum* pathogens in soil samples to establish whether molecular diagnostics can accurately detect and predict Fusarium diseases in the field. In addition, large scale artificial inoculation will be used to screen multiple non-host plants to determine the extent of colonisation by *F. oxysporum*, as crops which enable proliferation of the pathogen should be avoided in rotations. Finally, microbial communities have been shown to be important for suppression of disease; therefore, we will investigate how to establish healthy suppressive microbial communities through soil amendments and biological control agents. Amplicon sequencing will enable quantification of microbial communities in comparison with *F. oxysporum* populations.

The overall aim of the project is:

To use molecular methods to determine the risk of Fusarium disease, select effective rotation crops and monitor *F. oxysporum* pathogens and associated microbial communities.

This will be achieved through the following objectives:

1. Define a relationship between the amount of Fusarium DNA, Fusarium inoculum and disease development in soil
2. Evaluate the use of molecular diagnostics to detect and assess the risk of Fusarium disease in onion, lettuce and Narcissus

3. Evaluate the use of molecular diagnostics to determine the presence of FOC in harvested onion bulbs to assess the risk of disease development in store or pre-planting.
4. Determine the colonisation of non-host plants by *F. oxysporum* pathogens to identify suitable rotation crops
5. Investigate the feasibility of establishing Fusarium-suppressive microbial communities and biological control agents in protected cropping systems
6. Employ amplicon sequencing to quantify *F. oxysporum* pathogens alongside suppressive components of the soil microbial community
7. Evaluate crop protection products for control of FOC in field experiments

Summary

Objective 1: Define a relationship between the amount of Fusarium DNA, Fusarium inoculum and disease development in field soil (dose response)

Two Fusarium-free soils from lettuce growers and one soil from Wellesbourne were collected and inoculated with different concentrations of FOL4 between 2 x 10^2 - 2 x 10^6 cfu g^{-1} soil, and a non-inoculated control was also set up. A peat-based compost was also inoculated in the same way as a comparison. Lettuce seedlings were transplanted into pots containing the soil/FOL4 inoculum mixes and then monitored for disease symptoms. Samples of each of the inoculated soils were also taken at transplanting to quantify the FOL4 DNA levels by qPCR. High disease levels developed in all soils at FOL4 concentrations of > 2 x 10^5 cfu g^{-1} with more moderate disease at 2 x 10^4 cfu g^{-1} in some soils. The amount of FOL4 DNA detected in the soils could also be related to the level of disease observed, and detection was possible at inoculum levesl down to 2 x 10^3 cfu g^{-1} soil. Therefore, the critical level of FOL4 inoculum needed to cause disease in different soils was defined and also related to pathogen DNA levels which will enable the risk of Fusarium wilt to be assessed through soil testing. A comparable experiment to that carried out for FOL4 / lettuce will be conducted in Year 2 for FON / Narcissus to similarly determine the relationships between inoculum level, pathogen DNA concentration (as detected by qPCR) and disease development. FOL4 and FON qPCR tests will then be used to attempt to detect these pathogens in growers' soil samples.

Objective 2: Evaluate the use of molecular diagnostics to detect and assess the risk of Fusarium disease in onion, lettuce and Narcissus

Soil samples were collected from 12 different onion fields at sowing, including two fields which were also intensively sampled during the growing season. DNA was extracted from each sample and used for qPCR analysis to determine the level of FOC in the soil. Assessments were also conducted to measure disease development throughout the season, as well as the prevalence of basal rot in bulbs at harvest and in storage. Disease levels were generally low in the growing crops, but onions from some fields developed high levels of basal rot at harvest and in store. FOC DNA was detected at low levels in soil and intermittently using qPCR during the season for the two intensively sampled sites where disease developed. However, the pathogen was undetectable in the field soil samples taken at drilling. This suggests that FOC inoculum levels at drilling may be too low to detect by molecular diagnostics and that a better approach may be to detect the pathogen during the season to improve prediction of the risk of basal rot at harvest or in store. Further tests in multiple field sites and some optimisation of the assay will be carried out in Year 2.

Objective 3: Evaluate the use of molecular diagnostics to determine the presence of FOC in harvested onion bulbs to assess the risk of disease development in store

Onion bulbs classified by industry collaborators as healthy, with clear symptoms of basal rot or with basal plate deformities (e.g. corky / cracked) were collected from multiple fields and stores. Bulbs were cut in half, reclassified into categories based on internal appearance of disease symptoms and sections of the basal plate and scales plated onto agar to determine presence of FOC. Additional samples were flash frozen for DNA extraction and FOC qPCR analysis. *Fusarium* spp. with morphology typical of *F. oxysporum* was isolated from all clearly infected bulbs and also from some exhibiting the corky basal plate symptom and a few that appeared healthy. Similarly, qPCR also consistently detected FOC in the basal plates of infected bulbs, and also in some corky and healthy bulbs. Apparently healthy and corky onion bulbs from each site were also incubated for 8 weeks after which some developed basal rot but this varied in incidence and severity. Overall, these results demonstrated that qPCR can be used for detection of FOC in symptomless onion bulbs and hence shows potential for determining the risk of basal rot disease development in store.

Objective 4: Determine the colonisation of non-host plants by F. oxysporum pathogens to identify suitable rotation crops

This objective will be started in Year 2.

Objective 5: Investigate the feasibility of establishing Fusarium-suppressive microbial communities and biological control agents in protected cropping systems

Preliminary experiments were conducted to determine how FOL4 inoculum builds up in sterilised and non-sterilised soil when lettuce is grown repeatedly. Steam sterilised and non-sterilised soil was inoculated with two concentrations of FOL4 below the level required to cause significant disease (2 x 10^2 cfu g^{-1} and 2 x 10^3 cfu g^{-1} soil), dispensed into pots and lettuce seedlings transplanted. Mature lettuce were harvested, assessed for disease (internal vascular browning) and the soil diluted 1 in 2 with fresh sterilised/non-sterilised soil. This process was repeated twice and currently the third lettuce crop is being grown in the soil. FOL inoculum built up more quickly at the higher initial inoculum concentration and also in the sterilised soil. After just one lettuce crop, there was considerably more vascular browning in the sterilised soil (2 x 10^3 cfu g^{-1} soil) compared to the non-sterilised. After two crops this was evident at both original inoculum concentrations. Therefore, a system has been developed to understand the build-up of FOL4 inoculum in soil and will be used to examine how different soils and treatments may suppress the proliferation of FOL4 in soil.

Objective 6: Employ amplicon sequencing to quantify *F. oxysporum* pathogens alongside suppressive components of the soil microbial community

Amplicon sequencing was successful in quantifying the relative abundance of the bacterial, and fungal communities using standard 16S and ITS gene targets in the FOL4 infested soils from Objective 1. *Fusarium* spp. were also identified using the *Translation elongation factor 1* α (*TEF*) gene target and as expected a high abundance of *F. oxysporum* was detected in the FOL4 infested soils. A single gene target, g19096 was identified to detect the abundance of FOL, but the sequence is identical to that in some other *F. oxysporum* f.spp. However, by also amplifying another gene target, OG4952 which is not present in FOL, the presence and

abundance of FOL could be confirmed. Further work will now use this amplicon sequencing approach to investigate how different soils or soil treatments may suppress FOL4 in the system developed in Objective 5.

Financial Benefits

None at this time.

Action Points

None at this time.