Grower Summary

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

CP 204

Annual report 2022

Project title:	Diagnostic tests to assess Fusarium disease risk, select rotation crops and monitor microbial communities
Project number:	CP204
Project leader:	John Clarkson, University of Warwick
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Key staff:	Sascha Jenkins Sarah van Amsterdam Helen Bates
Location of project:	Warwick Crop Centre, School of Life Sciences, University of Warwick Wellesbourne Campus
Industry Representative:	Sam Rix
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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: 23/03/22

Helen Bates

Research Scientist

University of Warwick

Signature: *Helen Bates* Date: 23/03/22

Report authorised by:

John Clarkson

Professor

University of Warwick

Signature: *John Clarkson* Date: 23/03/22

GROWER SUMMARY

Headline

Fusarium oxysporum DNA concentration correlates highly with disease development and inoculum concentration in *formae speciales* affecting lettuce and Narcissus. DNA of *F. oxysporum* affecting onion was detected in field soil, onion roots and basal plates using molecular diagnostics.

Background

Fusarium oxysporum is a soil borne 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 are investigating 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 daffodil

Fusarium basal rot of *Narcissus* is caused by *F. oxysporum* f. sp. *narcissi* (FON). It infects the roots or damage**s** basal plates resulting in soft and rotting bulbs, which leads to bulbs not sprouting or producing short lived or early senescing foliage with few or no flowers (Taylor et al., 2019a). It is a problem for UK growers as bulbs are lifted after 2 years and used as replanting stock, therefore maintaining inoculum levels or allowing it to spread (Hanks, 2013; Taylor et al., 2019a).

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 and prevent disease occurring. These 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, such as Trianum and T34, or Calcium cyanamide (Perlka) 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 are utilising molecular diagnostics developed at Warwick Crop Centre in a previous AHBD 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 inoculations 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 (Year 1 report, current report; objective complete).
2. Evaluate the use of molecular diagnostics to detect and assess the risk of Fusarium disease in onion, lettuce and Narcissus (Year 1 report, current report).
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 (Year 1 report, current report).
4. Determine the colonisation of non-host plants by *F. oxysporum* pathogens to identify suitable rotation crops (To be carried out in 2022 - Year 3).
5. Investigate the feasibility of establishing Fusarium-suppressive microbial communities and biological control agents in protected cropping systems (Year 1 report, current report).

6. Employ amplicon sequencing to quantify *F. oxysporum* pathogens alongside suppressive components of the soil microbial community (Year 1 report, current report).
7. Evaluate crop protection products for control of FOC in field experiments (to report in 2023; one field trial carried out in Year 2, remaining 2 field trials to be carried out in 2022).

Summary

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

In the last report, we demonstrated a strong positive correlation between FOL4 inoculum concentration, disease symptoms in lettuce and the amount of DNA detected in the soil. This has been further confirmed by extracting DNA from the roots of lettuces grown in the inoculated soils, which after 13 days, contained approximately 100 pg pathogen DNA mg^{-1} root dry weight for the highest dose.

A dose response experiment was also conducted to determine the relationship between FON inoculum, disease development in Narcissus and DNA concentration. Bulbs were planted into two FON inoculated soils and a compost mix suitable for growing daffodils at different concentrations between 1 x 10^2 - 1 x 10^6 cfu g^{-1} soil. Currently, daffodils have just finished flowering and therefore disease development in the bulbs has yet to be assessed. However, there was a strong positive correlation between the concentration of FON inoculum and the amount of pathogen DNA present in the soil and in Narcissus roots.

This work enables us to determine the critical level of FOL4 / FON inoculum needed to cause disease in different soils, and to be able to reliably quantify these concentrations using molecular diagnostics.

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

Soil / onion root sampling 2020 and detection of FOC

Onion root samples were collected from two intensively sampled commercial fields at different timepoints during the 2020 growing season with moderate to high levels of Fusarium. DNA was extracted from each sample and used for qPCR analysis to determine the level of FOC in the roots. This was compared with the quantity of FOC DNA in soil samples taken at the same timepoints (see 2021 report) and to Fusarium disease assessments conducted throughout the season, at harvest and after storage. FOC DNA was detected in all root samples from different time points using molecular diagnostics and it was concluded that detection of the pathogen in onion roots was more informative and reliable than detection in soil. This will be further tested using 2021 samples.

Soil and onion root sampling 2021 and detection of FOC

Bulb onions in fields selected for sampling in 2021 developed very low levels of Fusarium basal rot and only a small percentage of bulbs went onto develop symptoms post store. The onion roots and soils sampled from this season are still being processed, but it is predicted that low levels of FOC DNA may be detected due to the low levels of disease observed.

Onion root baiting – 2021 soils

Growing onions in soil sampled pre-drilling was another new approach used to try and improve detection of FOC in soil. The rationale was that detection of FOC could be improved by 'baiting' the pathogen from soil as it quickly colonises and multiplies on onion roots. Hence, onion seeds were sown in soil collected from each of the same fields used for sampling above (mixed with vermiculite), and after 5-6 weeks were plants were harvested, roots washed and then frozen before extracting DNA and performing qPCR with the FOC specific diagnostic assay. Although, onion roots from only one field had detectable levels of FOC DNA, this could be due to there being very little disease pressure for any of the field sites and the method will be improved and refined in 2022.

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

In Year 1, apparently healthy onion bulbs and others with different levels of basal rot were used for FOC detection to establish the utility of the FOC specific PCR assay. This year, the focus was only on apparently healthy bulbs to investigate if PCR could detect FOC in symptomless bulbs to determine the risk of Fusarium development in store. Onion bulbs with no symptoms of Fusarium disease were obtained from 4 field sites (2 high and 2 low risk of developing basal rot) and the basal plate excised and frozen. These were used for DNA extraction and qPCR with the FOC specific diagnostic assay. Additional bulbs were incubated at 20˚C for 5-6 weeks (conditions favourable for Fusarium development) to determine if any disease would develop in apparently healthy bulbs. None of the bulbs tested positive for FOC infection with qPCR, and only a maximum of 14% of bulbs from one field site displayed symptoms after incubation (other sites ranged from 0-6%). This approach therefore requires additional work as we were unable to correlate qPCR detection with symptoms in storage due to the lack of bulbs developing symptoms.

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

An experiment was set up to determine how FOL inoculum builds up in successive lettuce crops grown in sterilised and non-sterilised FOL inoculated soil and if the addition of soil treatments (Perlka, Trianum G and T34 Biocontrol) can slow or halt this process. Steam sterilised and non-sterilised soil was inoculated with FOL at 1 x 10^2 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 soil was then used to grow a second crop of lettuce (currently underway). Soil samples were collected at product application, lettuce transplanting (2 weeks later to allow activity of treatments) and at lettuce harvest. Some Fusarium wilt disease developed in lettuces grown in all the FOL-inoculated sterilised soil treatments in the first crop, with those treated with Perlka having the most severe symptoms. There was therefore no indication that Trianum or T34 reduced FOL inoculum level. FOL DNA was also consistently detected in inoculated

sterilised soils at lettuce harvest. In contrast, no FOL disease symptoms were observed in non-sterilised soils, suggesting that FOL is able to establish far more quickly in sterilised soil than non-sterilised soil, even in the presence of soil treatments such as Perlka, Trianum G and T34.

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

Amplicon sequencing is a technique used to identify all components of the microbial community by mass sequencing a common gene target. This approach was used successfully used to quantify the relative abundance of bacterial and fungal communities using 16S and ITS gene targets in two different soils (from lettuce growers) artificially inoculated with FOL4. TEF1a amplicon sequencing also enabled identification of different *Fusarium* spp. present in soils and showed that there was a high level of *F. oxysporum* naturally present in Soil 2 (not FOL4) while Soil 3 contained a variety of different *Fusarium* spp. Several gene targets were also identified to potentially enable multiple *F. oxysporum* f.spp. to be identified concurrently with a focus on FOL, FOC and FON. The gene target, g19096, successfully enabled identification of FOL4 in inoculated soils and a clear increase in the abundance of FOL4 in Soil 3 with increasing inoculum levels. However, the high naturally occurring level of *F. oxysporum* in Soil 2 masked the ability to clearly identify FOL4 using g19096 and also affected the usefulness of another gene target OG4952 for cross-identification. Further work will investigate the effectiveness of an additional gene target for FOL identification and will use cross-referencing of existing targets to more accurately identify different *F. oxysporum* f. spp. present in soil samples.

Conclusions

- **Objective 1:** A clear relationship between FOL4 inoculum concentration, FOL DNA levels in soil and lettuce roots and Fusarium wilt disease development in lettuce was established. In addition, a strong relationship was also found for *Narcissus*, between FON inoculum concentration and the quantity of DNA recovered in inoculated soils and colonised roots. These molecular diagnostics could be used to predict the level of inoculum in fields and determine the risk of severe disease.

- **Objective 2:** FOC is more reliably detected in onion roots than directly from soil for commercial field sites. Therefore, testing onion roots using a plant baiting approach or from field sampling is likely to be a better approach for assessing disease risk.
- **Objective 3:** Molecular qPCR diagnostics was effective at detecting FOC in onion bulbs even in the absence of visible symptoms from bulbs in 2020, however, in healthy bulbs collected in 2021, FOC was undetectable in basal plate tissue. This approach requires further investigation and bulbs from infected sites need to be used to ensure development of disease in store.
- **Objective 5:** The methodology developed previously to examine FOL4 inoculum build up in sterilised and non-sterilised soil after successive lettuce crops was successfully used to determine the effect of products (Perlka, Trianum G (T22) and T34) on disease development. Further lettuce crops will be grown successively to determine their effect in an intensive cropping situation.
- **Objective 6:** Amplicon sequencing successfully identified and quantified the relative abundance of bacteria, fungi and *Fusarium* spp. in soils inoculated with FOL4. Gene targets to identify multiple *F. oxysporum* f.spp. have also been identified but some technical challenges need to be solved for these to be used reliably.

Financial Benefits

None at this time.

Action Points

None at this time.