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Project leader:	John Clarkson, University of Warwick
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Location of project:	Warwick Crop Centre, School of Life Sciences, University of Warwick Wellesbourne Campus
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Date project commenced:	01/04/2020

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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.

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TABLE OF CONTENTS

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.

SCIENCE SECTION

Introduction

Fusarium oxysporum is the most widely dispersed and economically important plant pathogenic species in the *Fusarium* genus as it infects numerous hosts and causes extensive crop losses (Leslie and Summerell, 2006). In 2012 it was identified as 5th in a list of the top 10 fungal plant pathogens in terms of scientific and economic importance (Dean et al., 2012). *F. oxysporum* is responsible for a wide range of plant diseases, usually causing a vascular wilt but also root and bulb rots (Edel-Hermann and Lecomte, 2019). *F. oxysporum* is a species complex with over 150 'special forms' (*formae speciales*; f. spp.), adapted to infect specific hosts plants which affects many agriculturally important crops such as lettuce, onion, tomato, banana, leek, peas, melon and narcissus (Edel-Hermann and Lecomte, 2019). The complex also includes non-pathogenic *F. oxysporum* isolates. As *F. oxysporum f.spp.* are polyphyletic (Fourie et al., 2011; van Dam et al., 2018), isolates from one f. spp. may be more closely related to isolates infecting other hosts than to each other (O'Donnell et al., 1998) resulting in them being difficult to distinguish. The need for pathogenicity tests to determine host range and pathogenicity is very costly and time consuming. *TEF* is reliably used to identify *F. oxysporum* (Geiser et al., 2004) but cannot distinguish between f. spp.; therefore molecular approaches using effector gene targets have been investigated. Genes associated with pathogenicity in *F. oxysporum* f. spp., such as *Secreted In Xylem* (*SIX*) genes and other putative effectors have been the focus for developing molecular diagnostics due to their presence / absence or difference in sequence between different f. spp. (Lievens et al., 2009; van Dam et al., 2016).

Fusarium disease of lettuce

Fusarium wilt of lettuce is caused by *F. oxysporum* f. sp. *lactucae* (FOL) and affects lettuce production across many countries (Gordon and Koike, 2015). Symptoms of Fusarium wilt in lettuce are yellowing and necrosis of leaves, stunting and wilting of plants and reddish-brown/black necrosis of vascular tissue (Matheron, 2015). There are four cultivar specific races, with races 1 and 4 being the most widespread and the only two identified in Europe (Gilardi et al., 2017a). FOL race 4 (FOL4) was first characterised in the Netherlands (Gilardi et al., 2017a) and was subsequently reported in Belgium (Claerbout et al., 2018), the UK and Ireland (Taylor et al., 2019a) and very recently in Italy (Gilardi et al., 2019). So far FOL4 has been restricted to protected lettuce (grown under cover: polytunnels or glasshouses) and therefore particularly affects those types grown in this enviroment such as butterhead and Little Gem which are high susceptible. However, some FOL4 resistant cultivars are beginning

to be introduced but have yet to be widely used and evaluated for the degree of resistance and quality. FOL, like many other *F. oxysporum* f. spp. produces long lived chlamydospores (Gordon and Koike, 2015), enabling inoculum levels to increase over time, especially in the case of FOL4 as protected lettuce production can be intensive with up to six crops a year in the same soil (Taylor et al., 2019a). Generally, FOL is managed with good crop hygiene procedures to prevent spread between fields, glasshouses and farms, crop rotations and soil treatments (chemical and heat/steam treatments) to try to reduce the pathogen pressure in the soil (Matheron, 2015).

Fusarium disease of onion

Fusarium oxysporum f. sp. *cepae* (FOC) is the causal agent of Fusarium disease in onion and symptoms can include seedling damping off, root/stem rot in immature plants and most importantly basal rot of bulbs resulting in significant losses (Entwistle, 1990; Taylor et al., 2013). In the UK, 2-6% of the onion bulb crop (8779 ha valued at approx. £132M in 2018; Defra Horticulture Statistics, 2019) is lost each year in the field with a corresponding economic value of £7.9M. In addition, basal rot also occurs in storage as onions which appear healthy when harvested in the field go onto develop disease (Cramer, 2000). As with FOL, Fusarium disease in onion is very difficult to control, and previous work has shown that even if fungicide seed treatments are available, they do not provide long lasting efficacy resulting in bulbs developing disease later in the season (Cramer, 2000),. Onion diversity sets have been developed to improve future onion breeding by identifying accessions with high levels of basal rot resistance (Taylor et al., 2019b), however, currently, there are no commercial onion varieties resistant to basal rot. Previous work at Warwick Crop Centre has developed a reliable diagnostic tool for identifying FOC in soil (Clarkson, 2018).

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 *F. oxysporum*

As mentioned previously, control of *F. oxysporum* is challenging, requiring long crop rotations and good hygiene practices to reduce inoculum concentration and prevent spread. However, even crop rotations are becoming difficult to manage as there is increasing evidence that *F. oxysporum* f. spp. can proliferate on non-host reservoir crops, enabling inoculum levels to remain high (Leoni et al., 2013; Scott et al., 2014). Black bean and corn (maize) were among the highest of 13 crops to enable FOC proliferation (Leoni et al., 2013), whereas broccoli, cauliflower and spinach were all found to support colonisation of FOL, in addition to resistant lettuce cultivars (Scott et al., 2014). Fungicide treatment can help control some level of disease, especially seed borne infection or seedling blight, however, their use for control in soil is very limited (Cramer, 2000; Gilardi et al., 2005). For FOC, seed treatments such as fludioxonil ± metalaxyl are about to be withdrawn, but they did not provide long term control of FOC and offer no protection for bulbs from basal rot (Clarkson, 2018). In lettuce, azoxystrobin and fosetyl-aluminium have been shown to offer some reduction in disease severity (Gilardi et al., 2016), however, once FOL is present in the soil, the likelihood of complete control with fungicides is very low and they are only really useful as a preventative measure.

Managing soil health and a diverse microbial community has been shown to be important in combating *F. oxysporum*. Soils with high organic matter content were found to be more suppressive to flax wilt (caused by *F. oxysporum* f. sp. *lini*) due to the higher microbial biomass' resilience against disturbances, enabling it to maintain a more stable environment and suppress pathogens from proliferating (van Bruggen et al., 2015). Therefore, it has been suggested that organic amendments could be applied to soils to stimulate a diverse community of bacteria and fungi which aid in disease suppression (Baum et al., 2015).

Soil disinfection such as steaming/heat treatments and chemical fumigation can use used in protective cropping systems like glasshouses/polytunnels to help lower the inoculum levels of pathogens (Gullino et al., 2003). Steam sterilisation can be expensive as it's an energy intensive process, however, it has proven an effective method of controlling Fusarium wilt of tomato (Luvisi et al., 2008). However, an advantage of steam sterilising is that cropping can occur soon after treatment, unlike with chemical fumigation. Although these treatments are equally expensive, products such as dazomet (Basamid, Certis, UK) have been shown to control fungi, pests, nematodes and weeds. It has been shown to be effective against FOL,

reducing incidence of Fusarium wilt of lettuce by up to 91% (Gilardi et al., 2017b). Soil sterilants need to be applied regularly, every three or so cropping cycles (or less) as pathogen levels build up over time. As before, diverse microbial communities in soil play a significant role in disease suppression, and therefore these approaches have been reported to enable pathogens to establish quickly in the absence of a biologically rich environment (O'Neill et al., 2005). A combination of soil sterilisation followed by the application of organic amendments could provide a solution to lower disease pressure and prevent *F. oxysporum* re-establishing rapidly.

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 the risk of 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 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

Materials and methods

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

A glasshouse experiment was set up to determine the relationship between the concentration of *Fusarium oxysporum* f.sp. *lactucae* race 4 (FOL4) inoculum, disease development and the amount of FOL4 DNA measured at the start of the experiment (qPCR assay) in different soils. The methods used were based on a similar study of *Fusarium oxysporum* f.sp. *cepae* disease development in onion (AHDB project CP 196).

Soil selection and analysis

Two *Fusarium*-free soils (tested using FOL4 specific qPCR assay, as below) were collected from protected lettuce growers with a third soil (Soakwaters) obtained from Warwick Crop Centre (Wellesbourne; Table 1). Soils were air dried for 3-4 days and sieved (4 mm mesh) to remove large particles.

Table 1. Locations of soils from UK lettuce growers (1 and 2) and from Warwick Crop Centre

Soil No.	Location
1	Lancashire
2	North Yorkshire
3	Warwick Crop Centre, Warwickshire

Soil physical and chemical analysis

Sieved soil was dispensed into 11 cm pots, watered and left at 25˚C for 6 days (16 hr light). Samples of each soil were analysed by NRM laboratories (Bracknell, UK) to determine the pH; N, P, K, Mg, organic matter content, and CO_2 respiration (Solvita test).

Lettuce wilt disease development glasshouse assay

The three soil types from above (Table 1) and also M2 compost were mixed with medium grade vermiculite in a ratio of 4:1 (soil:vermiculite) and moistened to aid inoculum mixing. A bran/compost inoculum of FOL4 isolate AJ516 was prepared as described by Taylor et al. (2013) and mixed into each of the three soil types as well as M2 compost to achieve five ten-

fold increases in concentrations from 2 x 10^2 - 2 x 10^6 cfu g^{-1} soil/compost. The infested soil/compost was dispensed into 9 cm pots and 2-3 week old lettuce seedlings (cv. Temira) transplanted into each pot. An untreated control (non-inoculated soil/compost) was included for each soil type and compost. Pots were arranged in a randomised block design in a glasshouse compartment set at 25°C day, 18°C night, 16 h day-length. Plants were watered from below as needed and supplemented with fertiliser (Vitax 2:1:4, diluted 100-fold) once/twice a week (depending on how often they needed watering). Plants were scored twice weekly for wilt symptoms (Table 2) and after 6-7 weeks (or when control plants started to senesce), lettuce were harvested, the roots dissected longitudinally and scored for degree of internal vascular browning (Table 2). Lettuce heads were weighed (fresh weight) and dried for 3-4 days at 80˚C before re-weighing to determine dry weight. Some plants which died from FOL4 infection early on in the experiment were removed to prevent the spread of opportunistic secondary pathogens, and therefore some fresh and dry weights were not captured.

Table 2. Scoring scales for lettuce Fusarium wilt and internal vascular browning.

Score	Disease/ wilt scale
0	Healthy
1	1 – 2 leaves wilted
2	10% wilt (3 leaves or more)
3	10 – 25% wilt
4	25 – 50% wilt
5	50 – 75% wilt
6	75 – 99% wilt
7	100% wilt
8	Dead

Score	Vascular browning scale
0	No browning
0.5	Very mild vascular browning
1	Mild vascular browning
2	Vascular browning
3	Severe vascular browning (SVB)
3.5	Completely hollowed SVB
4	Dead

DNA extraction and qPCR analysis

DNA was extracted from the three soils spiked with different concentrations of FOL4 using the GeneAll Exgene Soil SV kit (Cambio, Cambridge, UK) following the manufacturers protocol, with the following modifications: 1) 550 µL of SL buffer and 200µl of SDW was added to 500 mg of soil, which was homogenised in a Powerbead tube using a FastPrep-24 (MP Biomedicals, Cambridge, UK) machine set at 5.5 m s^{-1} for three cycles of 25 seconds; 2) spin columns were incubated for 5 min after the addition of buffer EB before elution. Following extraction, DNA diluted 1:6 with TE Buffer and used for qPCR analysis with FOL4 specific primers g23490 F3/R (AHDB project FV/PE 458). qPCR was carried out using a QuantStudio 5 (384-well) machine (Applied Biosystems) using 20 µL reactions containing both primers (final concentration 0.4 µM), 10 µl Power SYBR™ Green PCR Master Mix (Applied Biosystems) and 1 µl of DNA. Conditions were as follows: 1 cycle of 95°C for 120s followed

by 45 cycles of 95°C for 3 s, and 60°C for 30 s. All samples were run in triplicate and a melt curve analysis carried out. The concentration of FOL4 DNA was in each sample was calculated as pg mg^{-1} of dry soil.

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

In order to determine the utility of a molecular qPCR test for FOC in assessing disease risk and also to examine pathogen dynamics, soil samples were collected from different commercial onion fields pre-planting and from two sites both pre-planting and at seven different time points during the season. Fusarium disease was assessed during the season and in harvested bulbs with the aim of comparing these data with quantification of FOC in the soil by qPCR.

Soil sampling

Soil samples were collected pre-planting from 12 commercial onion fields with different predicted Fusarium disease risks by Vegetable Consultancy Services (VCS) and the Allium and Brassica Centre (ABC). In addition, a further two sites with high Fusarium disease risk were sampled pre-planting and also at seven time points during the season (Table 3). Samples were initially stored at -20˚C, before being transferred to 4˚C prior to processing. For each field site, soil sampling was conducted by collecting and pooling four samples across four rows per bed (labelled A-D, Figure 1), from a total of six beds per field (total of 24 samples per field at each time point).

Fusarium disease assessments

At each field site, Fusarium disease assessments (proportion of plants with yellowing / wilting or plant death) were carried out during the season by ABC and VCS at seven time points in 1 m areas of bed in each of the 24 areas where soil was sampled. For each site, the presence / absence of basal rot was also assessed in harvested bulbs in storage from each of the 24 areas.

DNA extraction and qPCR

Soil samples from the onion fields were dried before being sieved (4 mm and 2 mm mesh). Based on the field disease data provided by ABC (Figure 8) and VCS (Figure 9), the sites RX2 and FP1 (ABC) and RIS and WRE (VCS) were initially used for DNA extraction and qPCR as they developed Fusarium disease (Table 3). The four samples A – D (Figure 1) from each of the six locations per field were pooled and mixed vigorously before taking a 500 mg sample for DNA extraction using the GeneAll Exgene Soil SV kit (Cambio, Cambridge, UK) as in Objective 1. DNA was diluted 1 in 2 before using 1 µL in qPCR reactions (20 µL reactions) set up as above but with q*SIX*5 primers (Taylor et al., 2016) at a final concentration of 0.6 µM. All samples were run in triplicate and a melt curve analysis carried out. The concentration of FOC DNA in each sample was calculated as pg mg^{-1} of dry soil.

Table 3. Soil samples collected from various sites across UK onion growing fields by Allium and Brassica Centre (ABC) and Vegetable Consultancy Services (VCS). Highlighted cells indicate samples used for FOC qPCR

Company	Site code	Sample date	
ABC	RX1	02/04/2020	**Pre-planting soil samples (12 field sites)**
ABC	RX2	02/04/2020	
ABC	RX3	02/04/2020	
ABC	CAMB	07/04/2020	
ABC	FP2	10/04/2020	
ABC	NF1	13/04/2020	
VCS	GED	03/04/2020	
VCS	STU	06/04/2020	
VCS	SCO	15/04/2020	
VCS	RIS	16/04/2020	
VCS	LAR	20/04/2020	
VCS	ELV	27/04/2020	
ABC	FP1	10/04/2020	**Pre-planting and time point soil samples (2 field sites)**
ABC	FP1	21/05/2020	
ABC	FP1	02/06/2020	
ABC	FP1	15/06/2020	
ABC	FP1	01/07/2020	
ABC	FP1	15/07/2020	
ABC	FP1	27/07/2020	
ABC	FP1	11/08/2020	
VCS	WRE	17/04/2020	
VCS	WRE	23/05/2020	
VCS	WRE	04/06/2020	
VCS	WRE	18/06/2020	
VCS	WRE	02/07/2020	
VCS	WRE	17/07/2020	
VCS	WRE	31/07/2020	
VCS	WRE	16/08/2020	

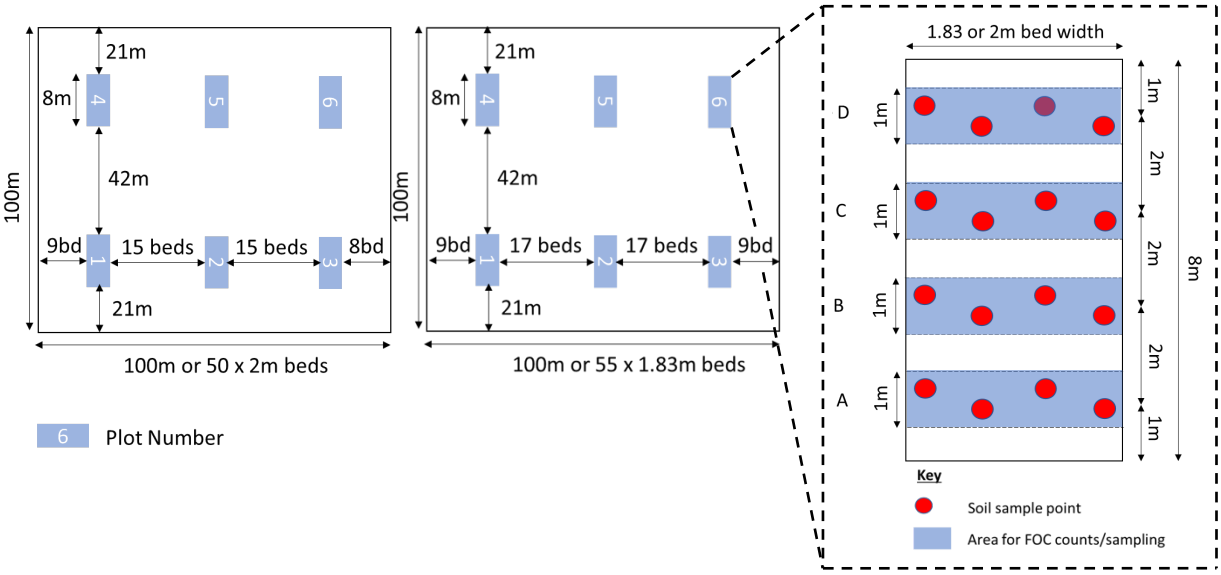

Figure 1. Layout of the 6 plots in each field site (1 ha area) for soil sampling and the positions of the 4 sampling areas within each of the 6 plots. Red circles represent the locations in which soil samples were collected. Samples were pooled across each area A-D.

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

Experiments were conducted to determine if FOC could be detected in onion bulbs by qPCR where there were clear symptoms of basal rot but also when disease was not apparent. Onion bulbs from commercial fields or stores were assessed by VCS and ABC for Fusarium basal rot disease status (healthy, 'corky' basal plate, soft basal plate) and subsequently cut open. Additionally, the presence of FOC was determined by agar plating and qPCR, using samples from the same bulbs.

Onion bulbs which had clear symptoms of Fusarium basal rot (soft basal plate), a 'corky' basal plate or appeared healthy (approx. 300 bulbs per symptom type) were collected by VCS and ABC from five different sites. In the laboratory, onions were sliced in half, photographed and samples taken from the basal plate and scales taken from both halves of the bulb. Samples from one half were surfaced sterilised for 45 s in 70% ethanol, washed twice with SDW, then plated on Potato Dextrose Agar (PDA) plates containing chlortetracyline (2 mL L^{-1}). Plates were then incubated at room temperature for 3-4 days and any Fusarium-like colonies sub-

cultured onto PDA + chlortetracycline. The remaining samples were transferred to a 2 mL tube and flash frozen in liquid nitrogen, before being stored at -80˚C.

Following re-assessment of basal rot disease status for the dissected onion bulbs, a selection of the basal plate samples from these bulbs stored at -80˚C (ranging from healthy to severely diseased from each site) were used for FOC qPCR. Samples were ground in a pestle and mortar in liquid nitrogen and 20 mg weighed out into a Lysing matrix A tube (MP Biomedicals). DNA extraction was performed using a DNeasy plant mini kit (Qiagen, UK) in accordance with manufacturer's protocol with a minor modification whereby the sample was first homogenised in a in a FastPrep-24^{TM} machine set at 6 ms^{-1} for 40 s. DNA from the infected samples (mild, intermediate and severe disease) were diluted 1 in 2 as a preliminary test showed they were too concentrated. qPCR reactions were set up as in Objective 2 using the same q*SIX*5 primers, except with 2 µL of DNA in 20 µL reactions. All samples were run in triplicate and a melt curve analysis carried out.

Finally, to determine if onions from the corky or healthy disease categories could develop basal rot, bulbs from the same batches from each site were placed in sealed plastic bags (10 per bag) and incubated at 20˚C for eight weeks in the dark. They were then cut in half, photographed and assessed for disease symptoms on a scale of 0-5 (Table 4).

Table 4. Fusarium basal rot disease score scale

Score	Description
0	Healthy
1	Basal plate browning
2	up to 10% bulb affected
3	10-25% bulb affected
4	25-50% bulb affected
5	>50% bulb affected

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

This preliminary work aimed to develop a system to examine the build-up of FOL4 inoculum in sterile and non-sterile soil in order to allow testing of treatments to mitigate this in the future.

Soakwaters soil (as used in Objective 1) was sieved (4 mm mesh) and air dried for 2 days. The moisture content of the soil was determined, and water was added so the soil held together when compressed by hand (around 12% moisture content). Half of the soil was sterilised in a Camplex Plantcare Soil Steriliser (Cat no, HD5116, Thermoforce Ltd, Essex, UK) following the manufacturers operational instructions. The soil is heated to 71˚C (in approx. 1.25 hours), then is switched off and remains sealed until the temperature reaches 82˚C (approx. 1.5 hours). The other soil sample was left unsterilised. Soil was then mixed 4:1 with medium grade vermiculite for both the sterilised and non-sterilised soil. FOL4 inoculum was prepared as before (Objective 1) and used to prepare a serial dilution in soil in order to inoculate sterilised/non-sterilised soil at the final concentrations of 2 x 10^2 and 2 x 10^3 cfu g^{-1}. These levels of pathogen were shown previously (Objective 1) to cause little or no disease. Non-inoculated soil was also included for both sterilised and non-sterilised treatments. Soil was used to fill 9 cm pots and 2-3 week old lettuce plants (cv. Temira) transplanted into each pot (12 pots per treatment). Pots were arranged in a randomised block design in a controlled environment cabinet set at 25˚C with 16 hr light. After around 10 weeks, the lettuce were removed, dissected and assessed for vascular browning caused by FOL4 (Table 2). The heads of the lettuces were then discarded and the roots and soil from the 12 plots per treatment combined and mixed. Additional soil was sterilised (as above), mixed 4:1 with vermiculite and used to dilute (1:1) the remaining soil for each treatment from the first experiment. Non-sterilised / sterilised soil was added to the non-sterilised / sterile soil from the previous FOL4 inoculated or non-inoculated treatments in a 1:1 (v/v) ratio, and used to fill 11 cm pots, to take into account the extra root material in the soil. Again, 2-3 week old lettuce (cv. Temira) plants were transplanted into each pot (12 pots per treatment, apart from the sterilised control which had 11). After around 8 weeks, the lettuce were harvested and assessed for disease as before, and the soil used for a third round of lettuce inoculations.

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

Overview

We have previously demonstrated the ability to identify the presence of different *Fusarium oxysporum* f. sp. *cepae*, *narcissi* and *matthioli* (FOC, FON, FOM) in soil samples from diseased fields using targeted amplicon sequencing as part of AHDB project FV POBOF 452 (Clarkson, 2019). Subsequently, as part of AHDB project CP 196, we demonstrated that FOC could be identified using amplicon sequencing in soil samples inoculated with FOC spores at an inoculum level > $2x10^3$ spores g^{-1} soil which was a level at, or just below which disease symptoms developed. The current project builds on this work by extending the repertoire of f. sp. identification to include *Fusarium oxysporum* f sp. *lactucae* (FOL) and attempting to identify and quantify the amount of pathogen and other microbes in the soil.

Design of 16S, ITS and TEF spike-in plasmids

Synthetic DNA spike-ins (Tkacz, 2018) can be used to much better quantify organisms detected using amplicon sequencing. Hence, spikes were designed based on the targets used in this project for identification of bacteria (16S), fungi (ITS) and *Fusarium* species (TEF). Each artificial sequence target contained random sequences of the same average length and GC content as the *in vivo* targets flanked by the primer sequences used for these targets in this and previous studies. The random DNA generator https://www.faculty.ucr.edu was used to generate the spike sequences. Spikes were synthesised using the Invitrogen Geneart service and supplied cloned into plasmid pMA-T and as glycerol stocks in *E. coli*.

Design and testing of gene targets for amplicon sequencing for FOL

Following genome sequencing and assembly of FOL race 1 (FOL1) and race 4 (FOL4) isolates, a range of potential pathogenicity-related genes were identified. Initially this was to develop FOL and FOL4 specific diagnostics in AHDB project FV PE 458. For amplicon sequencing, it is necessary to identify a pathogenicity gene present in multiple *F. oxysporum* f.spp. but which differs in sequence between them. Then, this gene can be amplified by PCR and sequenced to identify several *F. oxysporum* f.spp. at the same time in a single sample. However, such target genes identified previously for simultaneous identification of FOC, FOM and FON by amplicon sequencing in AHDB project FV PO BOF 452 (OG4952 and OG13890) were unfortunately found to be absent in FOL and hence new target genes were required.

The presence of FOL pathogenicity genes was therefore assessed for 141 *Fusarium* genomes and three were selected as potential targets for amplicon sequencing (g19096, g19133 and g7788). Primers were designed to amplify regions in these genes of approximately 300bp, each of which, in combination with other gene targets would allow identification of FOL. The primers were tested for specificity against purified FOL DNA and also soil samples spiked with FOL4 from Objective 1.

Soil DNA extraction

Soil DNA extractions were carried out as previously described using the Soil SV kit (GeneAll, UK) for the six different FOL4 inoculum concentrations in the three different soil types from Objective 1. (D1=0, D2=2x10^2, D3=2x10^3, D4=2x10^4, D5=2x10^5, D6=2x10^6 cfu g^{-1} soil). As DNA extraction efficiencies can vary between repeat extractions even within the same soil type, this effect was minimised by carrying out three extractions for each soil sample (500mg soil / extraction) and pooling. To remove potential PCR inhibitors and produce better quality DNA samples for sequencing, a clean-up step using Mag-Bind Total Pure NGS (Omega Bio-Tek) was carried out for the DNA samples before diluting to 2 ng μl^{-1} for use in amplicon sequencing PCR reactions. Samples were made up to 120µl by the addition of nuclease free water, an equal volume of magnetic beads was added to each sample, rotated at room temperature for 5 min to bind DNA and then placed on a magnet rack for 5 min to separate the DNA bound to the beads from the supernatant. The supernatant was discarded, and the beads washed twice with 200µl 80% ethanol. The beads were dried for 30 s in a fume hood followed by re-suspension in 50µl Tris-EDTA (10mM Tris HCl, 1mM EDTA, pH8) preheated to 50°C, the tube was flicked to mix and incubated at 50°C for 10 min with occasional flicking. The samples were placed on a magnetic rack for 5 min and the supernatant containing the DNA was removed to a new tube.

DNA library preparation, PCR and amplicon sequencing

DNA library preparation, PCR and amplicon sequencing was as described in AHDB project report CP 196 (Clarkson, 2020; Figure 2). The only difference was that gene target OG13890 was omitted and replaced by target g19096 to identify FOL. All other gene targets were the same (OG4952, TEF1a, ITS, 16S). Briefly, first round target PCR reactions were carried out using 5µl containing 10 ng of soil template DNA in a 25µl reaction using KAPA HiFi HS 2x master mix (Roche, UK). Libraries were pooled for barcoding (ITS with 16S, TEF with OG4952 and g19096) as described previously. Barcoded libraries were bead-cleaned and normalised to at 4nM before being pooled (4µl each TEF/16S barcode and 2µl of each

TEF/OG4952/g19096) for sequencing and loaded at 8pM on to the Illumina MiSeq V3 600 cycle flow cell with 20% PhiX spiked in.

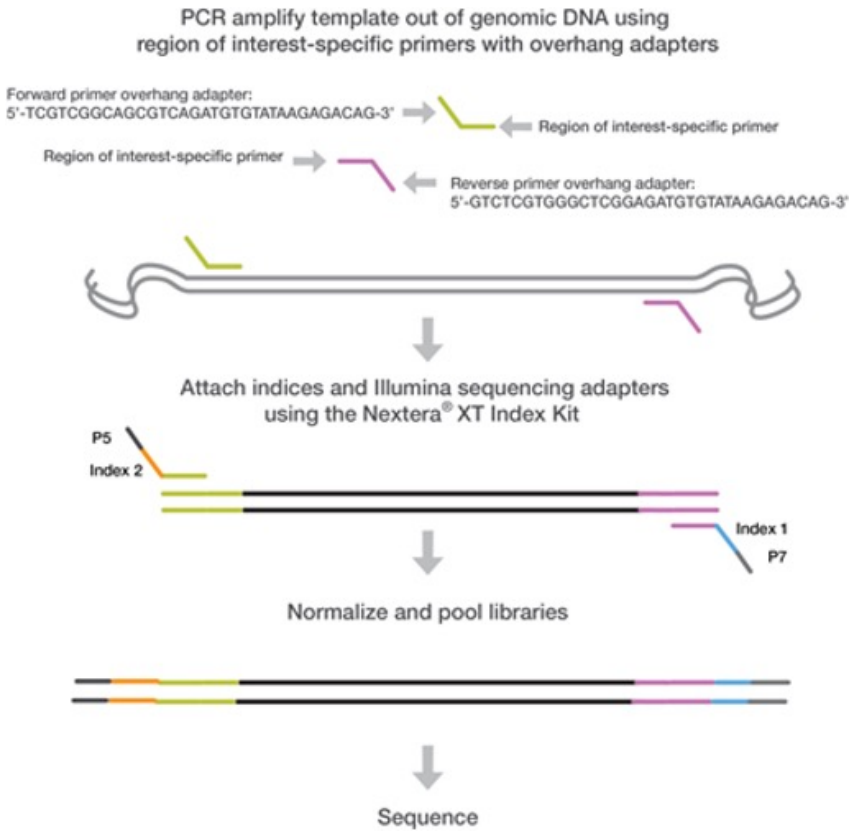

Figure 2. Illumina dual-index, 2-step PCR approach used to carry out amplicon sequencing. Dual-indexing reduces mis-tagging events and enables up to 96 libraries to be pooled for sequencing on a single flow cell.

Amplicon sequence data analysis pipeline

The sequence data generated was analysed as described previously in AHDB project FV PO BOF 452 (Clarkson, 2019; Figure 3). Illumina sequence reads were demultiplexed according to their barcodes and each read from the sample assigned to a target PCR amplicon based on 100% identity to one of the primer sequences for that target. Forward and reverse reads of a sequence were merged into a single sequence and the primer sequences removed. Reads were quality filtered as previously described and remaining reads were retained for Operational Taxonomic Unit (OTU, clustered sequences based on a shared similarity threshold) identification and quantification. Amplicon sequence variants (ASVs, single rather than DNA sequences) using were produced from all reads attributed to a locus using the UNOISE3 option in USEARCH. Taxonomy was assigned to ASVs by searching against

reference sequence databases (RDP 16S v. 16 and UNITE ITS v.7.2) using the SINTAX algorithm in USEARCH software (Edgar 2016). Reads were quantified against the identified OTUs and summarised by genus, species or *F. oxysporum* f. spp. Reads were mapped using USEARCH at 97% sequence identity for 16S and ITS and 100% identity for mapping to TEF1a, OG4952 and g19096. To account for differences in sequencing depth (number of total reads) between samples, sequence counts for 16S and ITS were 'normalised' using a statistical sub-sampling approach (rarefaction) . For TEF and *F. oxysporum* f. sp. amplicons, reads were normalised to 1000 reads per sample. Counts from three technical replicates were combined to produce a mean and standard error of the mean was also calculated.

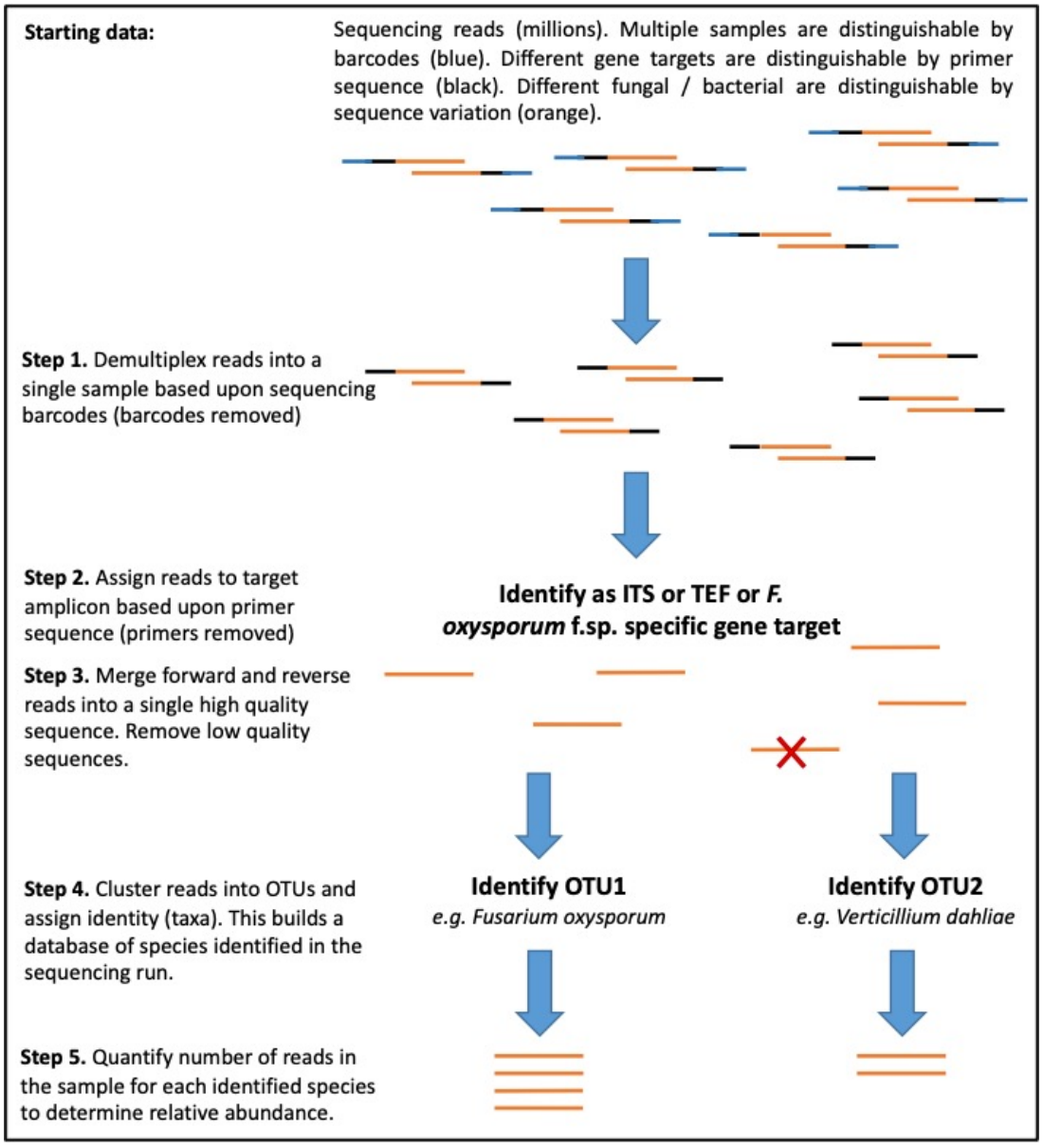

Figure 3. Summary of steps used in the analysis of amplicon sequence data.

Results

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

Soil analyses

There were some notable differences in the physical properties between the three soil types used in the FOL4 inoculum concentration experiment. Soil 1 was found to be peaty and contained the highest percentage of organic matter at 22.4%, compared to only 6.4% (Soil 2) and 2.5% (Soil 3). It also contained much greater concentrations of P, Nitrate N and available N compared to the other two (Table 5). Generally, Soil 3 contained the lowest concentrations of most minerals, including P, Mg, Nitrate, Ammonia and available N; however, it had the highest concentration of K out of the three soils (Table 5).

Table 5. Physical properties of UK lettuce growing soils.

	Soil 1	**Soil 2**	**Soil 3**
Major soil classification	Peaty	Medium	Medium
Textural Classification	Silty clay	Sandy loam	Sandy clay loam
Soil pH	6.2	6.4	6.1
P index	7	5	4
K index	3	2-	3
Mg index	4	4	1
P (mg/L)	165	89.4	66.4
K (mg/L)	247	128	341
Mg (mg/L)	248	189	83.9
Nitrate N (mg/kg)	362.2	87.65	26.81
Ammonium N (mg/kg)	1.25	1.12	1.07
Available N (kg N/ha)	1362.9	332.9	104.5
CO2 respiration (mg/kg)	123	37	55
OM (%)	22.4	6.4	2.5

Lettuce wilt disease development assay

Fusarium wilt symptoms developed more slowly across the different soils compared to M2 compost, although all growing media were able to facilitate FOL4 infection (Figure 4). There was a clear disease response in relation to the concentration of FOL4 inoculum, with the highest wilt scores being recorded in lettuce which received the highest level (2×10^6 cfg g^{-1}) across all soil types and compost (Figure 4). It was clear that the rate of disease development was greater in compost, with most lettuce recorded as dead for the highest FOL concentration after 35 days post inoculation. In the soils with the highest FOL concentration, lettuce survived

until the end of the experiment (45 days post inoculation), although they were severely wilted. However, lettuce in the Soil 2 were more severely wilted compared to those in Soil 1 and Soil 3, and the rate of disease development was also greater in Soil 2 (Figure 4). Some plant senescence was observed in the control plants in all soils/compost, resulting in similar disease scores to some of the lower concentrations of FOL4, especially in Soil 3 (Figure 4).

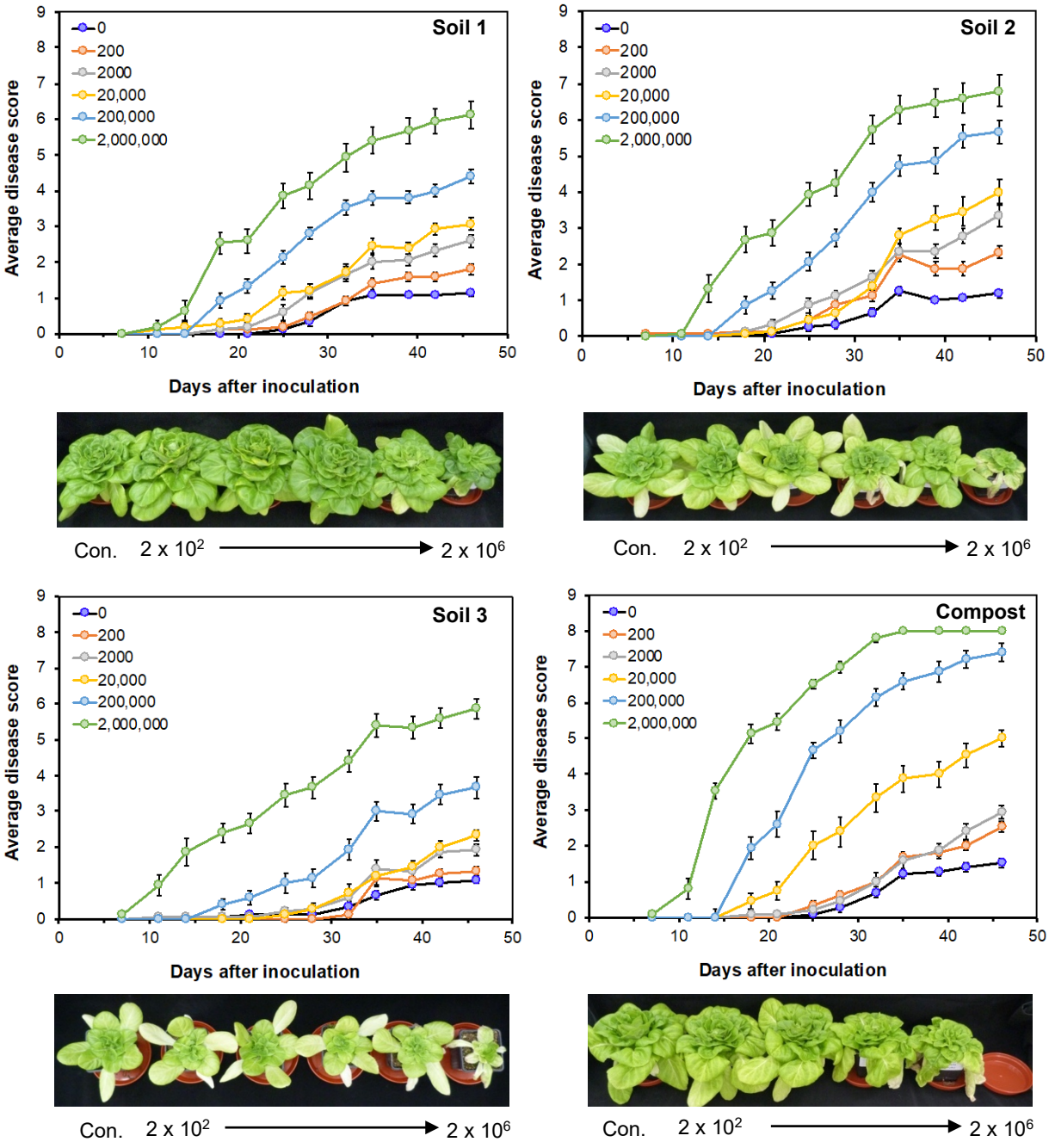

Figure 4. Effect of *Fusarium oxysporum* f. sp. *lactucae* race 4 (FOL4) inoculum levels on wilt development in lettuce for three soil types and compost. 0 cfu g^{-1} (purple line) refers to the non-inoculated control.

The differences between FOL4 concentrations were also clearly observed when comparing the vascular browning scores at lettuce harvest. Although some very mild vascular browning was observed in some of the lettuce from non-inoculated control treatments (Figure 5), severe disease symptoms were observed at the two higher concentrations of FOL4 for all three soils (Figure 2). However, for all FOL4 levels (2 x 10^2 to 2 x 10^6 cfu g^{-1} soil), Soil 3 resulted in the lowest vascular browning scores, suggesting that this soil was the least favourable for disease development. There were no differences between Soil 1 and Soil 2 soils in the severity of vascular browning for each FOL4 concentration, but in all soils the severity of vascular browning clearly increased with the concentration of FOL4 (Figure 5).

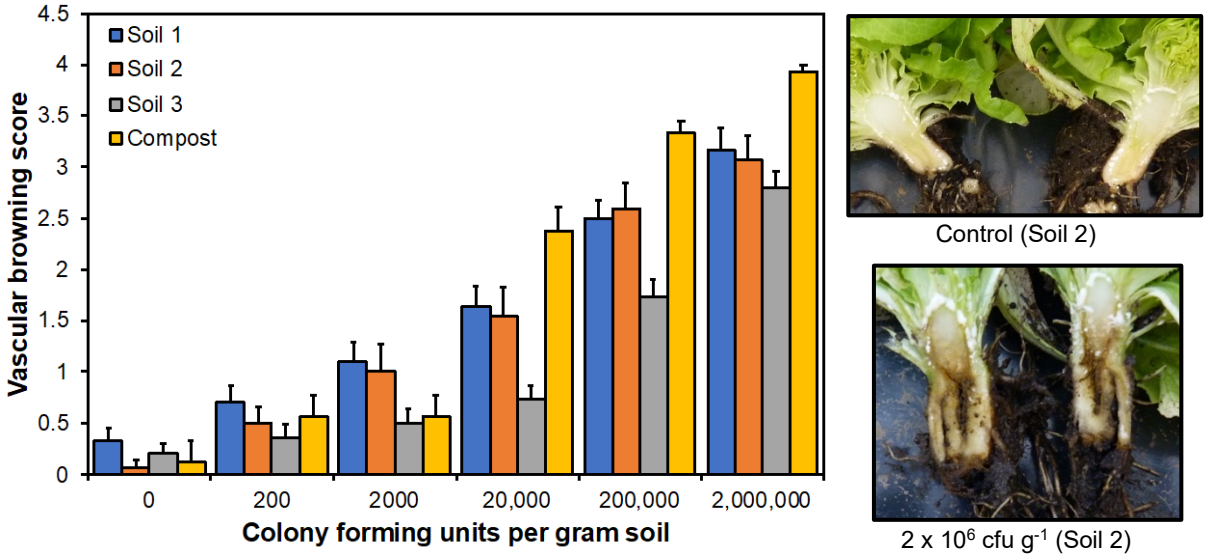

Figure 5. Effect of *Fusarium oxysporum* f. sp. *lactucae* race 4 (FOL4) inoculum levels on vascular browning disease score for three soil types (Soil 1, 2 and 3) and compost. Images show vascular browning symptoms for the control and 2 x10^6 cfu g^{-1} treatments in Soil 2.

Even though the same lettuce cultivar was grown in all the soils, their sizes varied widely. The lettuce grown in the sandy clay loam of Soil 3 were all considerably smaller than in all the other soils at every concentration of FOL4 inoculum (Figure 4, Figure 6). Although this soil seemed to suppress disease more than the other soils (Figure 5), the lettuce were smaller and would be unmarketable at this size. Of the three soils, the peaty silty clay of Soil 1 produced the largest lettuce, which was most likely due to the higher organic matter content and the much higher levels of N and P compared to the other soils (Figure 6, Table 4). In all soils and compost, the size of the lettuce decreased considerably at higher FOL4 doses, consistent with the wilt and vascular browning scores above (Figure 4, Figure 5).

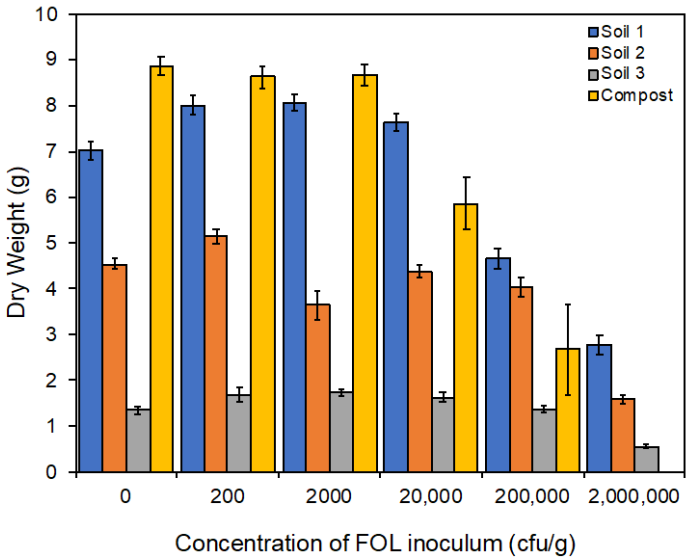

Figure 6. Mean dry weight of lettuce heads after being grown in three different soils and compost, inoculated with FOL race 4 at a range of concentrations from 2 x 10^2 to 2 x 10^6 cfu g^{-1} soil.

FOL4 qPCR analysis

The quantification of FOL4 DNA by qPCR analysis in the three soil types (excluding compost) revealed an increase in detectable FOL4 DNA in the soils with increasing FOL4 inoculum concentrations (Figure 7 A). FOL4 DNA was reliably detected in all soils inoculated with concentrations of 2 x 10^4 cfu g^{-1} and above while in Soil 2 and Soil 3, FOL4 DNA was also detected at low levels in the 2 x 10^3 cfu g^{-1} inoculum concentration (Figure 7 A). Even though the disease scores for wilt (Figure 4) and vascular browning (Figure 5) were relatively low for FOL4 inoculum levels of 2 x 10^3 cfu g^{-1} and 2 x 10^4 cfu g^{-1}, the pathogen was clearly detectable in the soil. There was clear correlation between the concentration of FOL inoculum, the concentration of FOL4 DNA detected in the soil and the disease severity scores (wilt and vascular browning (Figure 7 B).

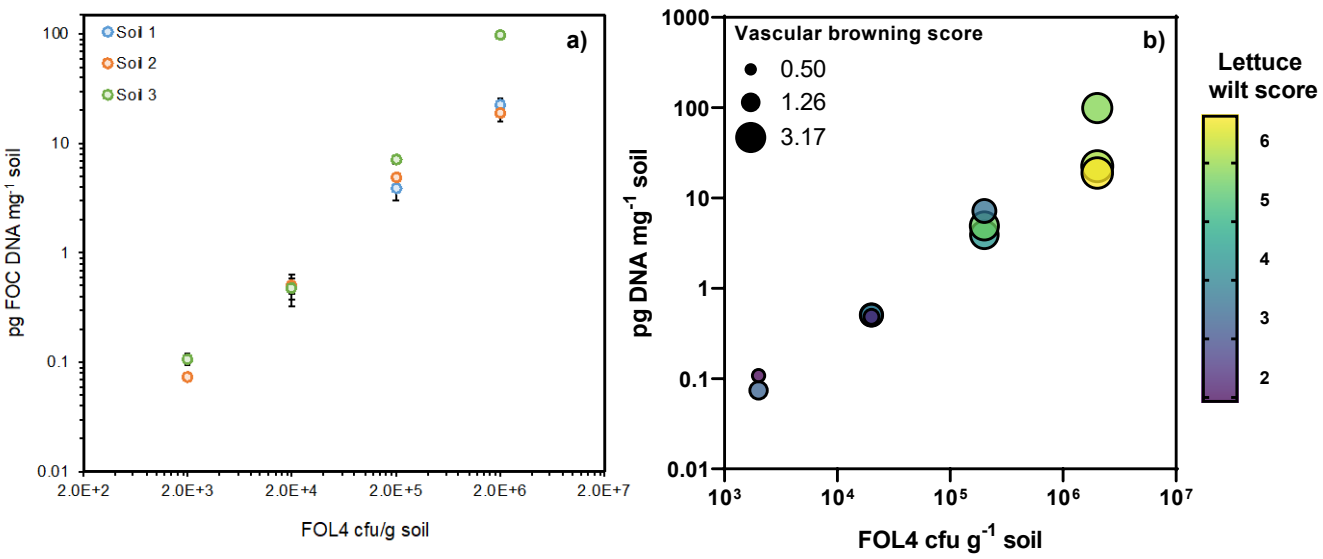

Figure 7. a) Amount of FOL4 DNA detected (pg mg-1 dry soil) in three inoculated soils at concentrations ranging from 2 x 10^2 to 2 x 10^6 cfu g-1 soil. No FOL4 DNA was detected below 2 x 10^3 cfu g^{-1}. Error bars represent SEM. b) Correlation between FOL4 inoculum concentration, FOL4 DNA detected in soil and the disease severity scores (wilt and vascular browning score).

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

Fusarium disease assessments

Only two of the field sites monitored by ABC developed Fusarium disease during the assessment time, RX2 and the intensively sampled site FP1 (Figure 8). RX2 developed the highest level of disease with 27% of onions showing disease symptoms at harvest in plot 4 (Figure 8), with disease developing rapidly between June and August 2020. Other plots at this site had lower levels of disease (all less than 5%) at harvest (Figure 8). However, onions from plot 3 and 6 both developed much higher levels of disease in store with 66% and 68% of onions becoming infected. Only 30% of onion bulbs from plot 4 went onto to develop disease in storage (Figure 8). There were much lower disease levels at site FP1 with <7% disease development in all plots by harvest. However, after being in storage up to 40% of onion bulbs developed Fusarium disease (Figure 8).

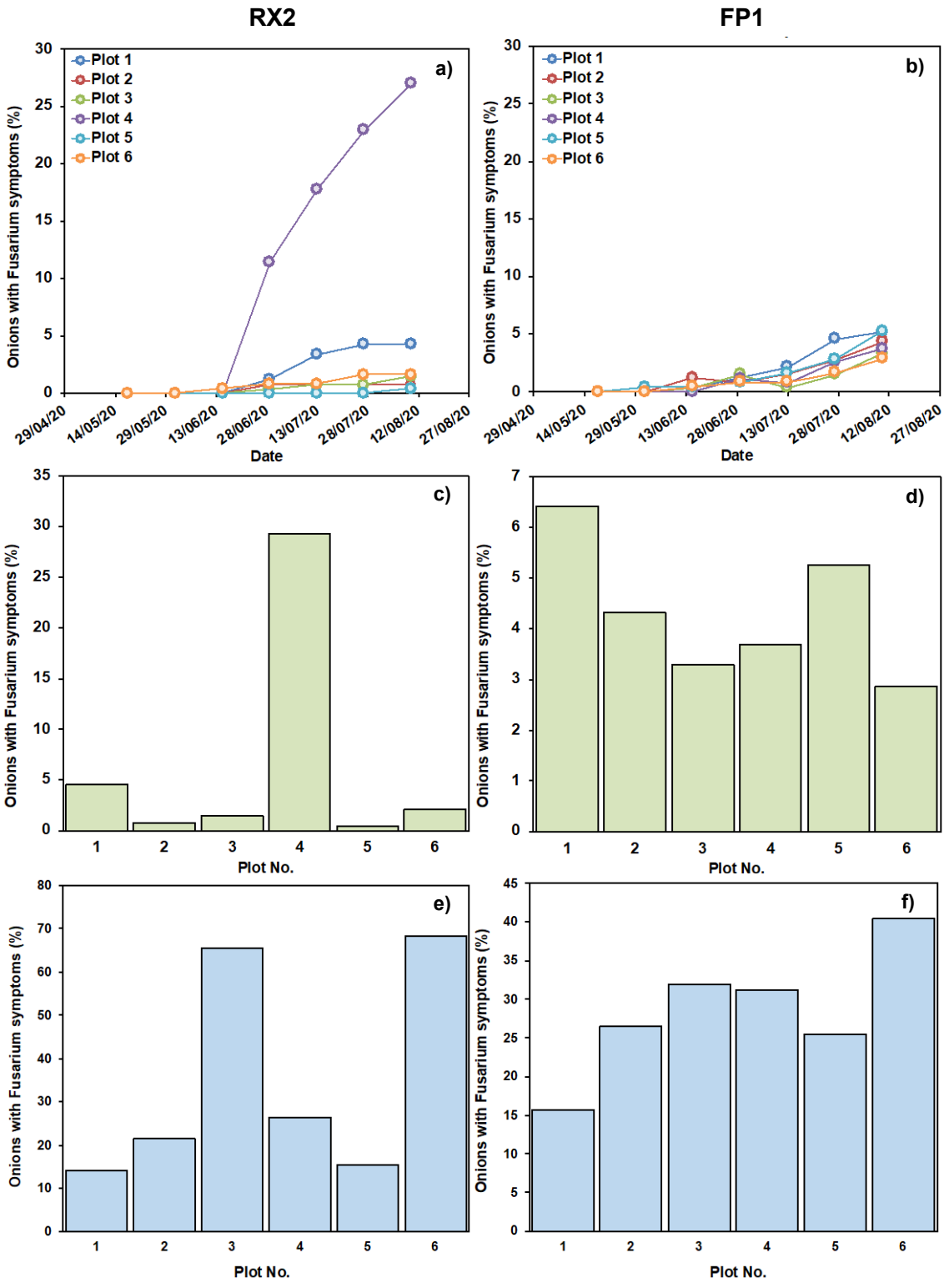

Figure 8. ab) Fusarium disease development over time, cd) the percentage of onion bulbs with symptoms at harvest and ef) the percentage of onion bulbs that developed symptoms of basal rot in storage, for two field sites (RX2 and FP1) sampled by the Allium and Brassica Centre (ABC).

Levels of Fusarium disease were much lower at the VCS sites compared to ABC. The two sites with the highest levels of Fusarium were RIS and WRE with 2.9% and 1.9% respectively of onions with symptoms during the assessment period May - August 2020 (Figure 9). However, at harvest (September 2020), 20% and 31% of onions had developed disease at each site respectively (Figure 9). This increase could be due to better assessments on bulbs after they have been removed from the soil, rather than scoring the leaves for disease symptoms during the growing season. After curing and storage (November 2020), 62% of onion bulbs from plot 1 (WRE) were classed as developing basal rot disease (Figure 9).

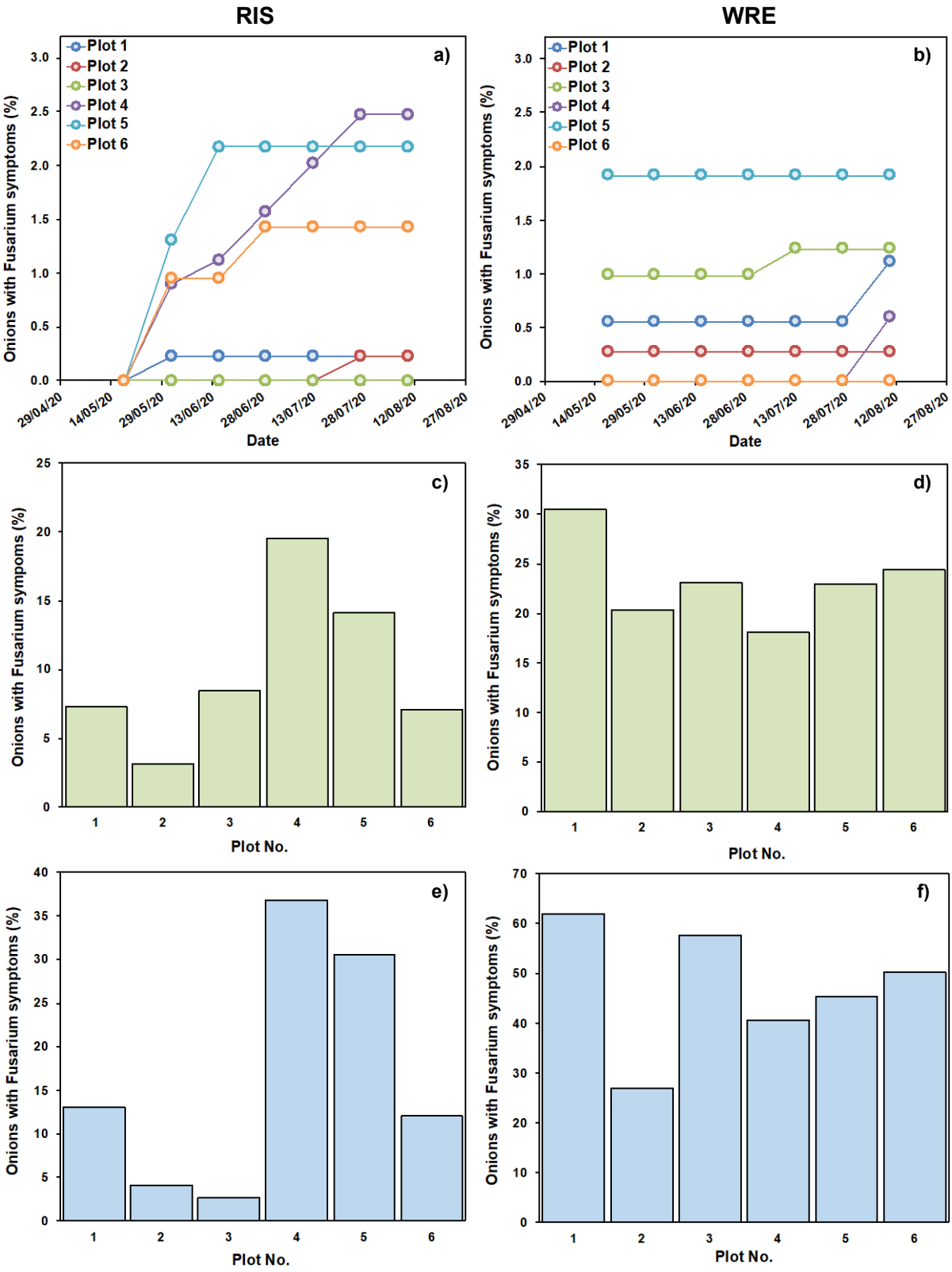

Figure 9. ab) Fusarium disease development over time, cd) the percentage of onion bulbs with symptoms at harvest and ef) the percentage of onion bulbs that developed symptoms of basal rot in storage, from two field sites (RIS and WRE) sampled by the Vegetable Consultancy Services (VCS).

qPCR analysis on soil samples

The two ABC sites (RX2 and FP1) selected for soil analysis using qPCR had relatively low levels of disease through the growing season and at harvest (Figure 8). When the four samples per plot (1-6) were pooled and used for qPCR analysis, there was no amplification of FOC DNA in any of the plots for RX2 where soil samples were only taken just before sowing. For FP1 where samples were taken throughout the cropping period, FOC DNA was only detected at low levels in one plot (plot 4) at sowing (10.4.20; Figure 10). The amount of FOC DNA being detected then varied across the onion crop growing period, but was detected in all plots in at least four of the eight sampling times (Figure 10). Plot 6 sampled on 2.6.20 had the highest concentration of FOC DNA, but then decreased in the following sampling time points (Figure 10). Although disease levels in field were low, onions bulbs harvested from plot 6 developed the highest levels of basal rot in store (40%; Figure 8). Overall, the amount of FOC DNA detected in the field was low and on the limit of detection for the qPCR assay.

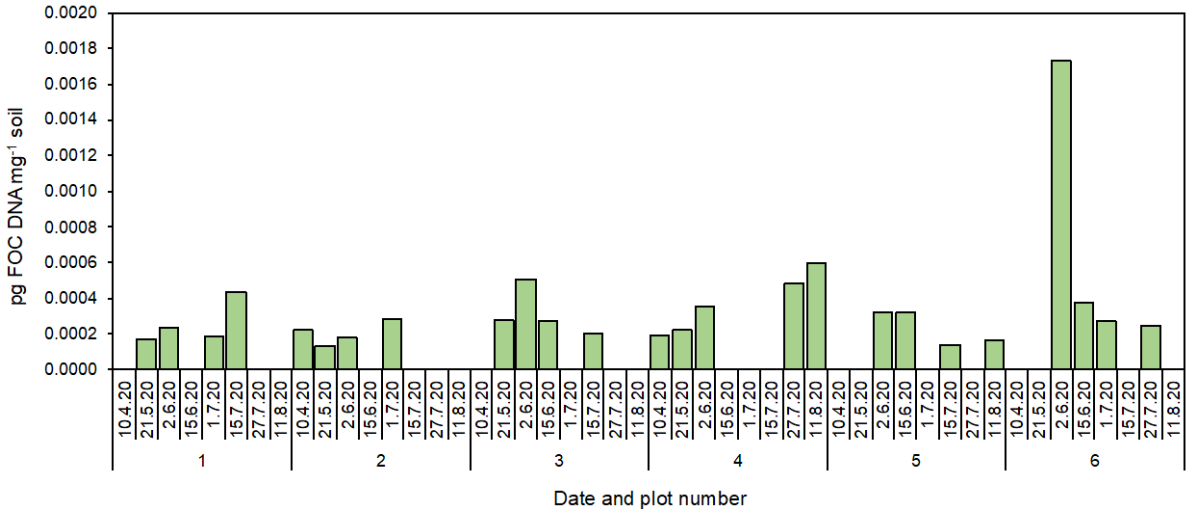

Figure 10. Amount of FOC DNA (pg mg^{-1} dry soil) in soil samples from ABC field site FP1. Soil samples from six plots were collected over eight time points during the growing season).

As for ABC, the two field sites from VCS (RIS and WRE) developed low levels of Fusarium disease during the growing season. There was no amplification of FOC DNA from RIS soil samples taken just before sowing and only a maximum of three time point samples from WRE had amplification of FOC DNA (Figure 11). There was no amplification of FOC DNA for plot 2 (WRE) at any time point, while FOC was only detected at one time point for plots 1, 4 and 6 (Figure 11). Samples from plot 3 had the highest levels of FOC DNA detected (02/07/20) but this decreased over subsequent time points. Overall, as for the ABC sites, low levels of FOC DNA were detected and disease levels over the growing period were low.

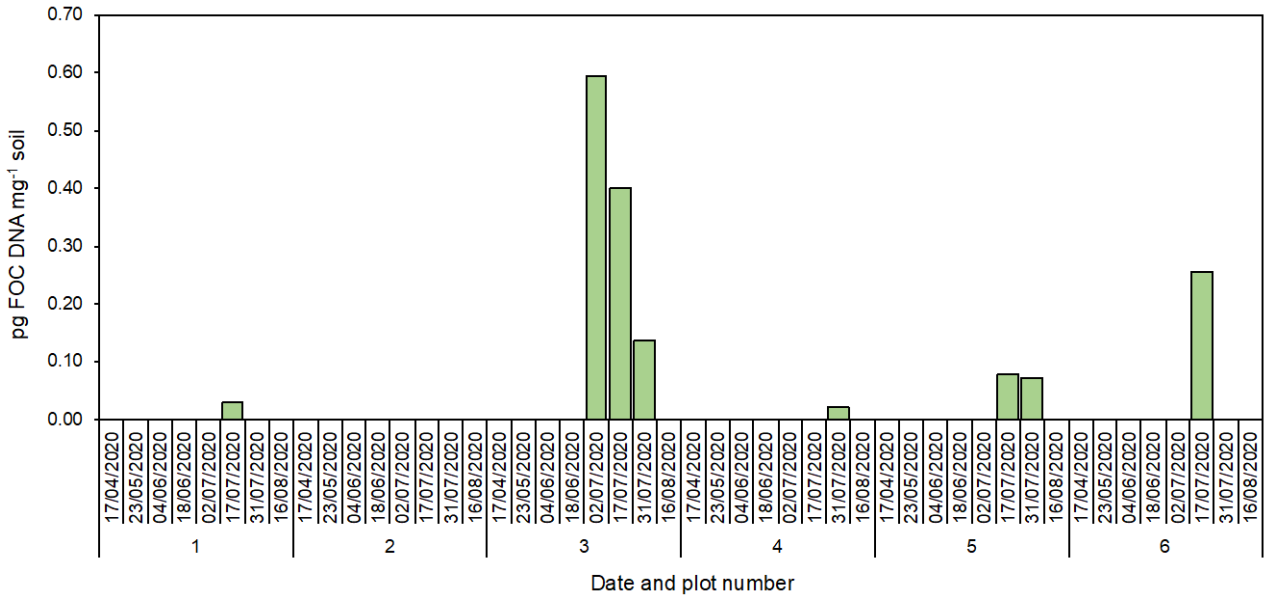

Figure 11. Amount of FOC DNA (pg mg^{-1} dry soil) in soil samples from VCS field site WRE. Soil samples from six plots were collected over the growing season (8 time points), but only three time point are displayed.

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

Once onion bulbs initially classified by ABC and VCS for the different symptom types (healthy, corky, infected) were dissected to more clearly assess basal rot, each was reclassified into seven disease categories based on their internal appearance (Table 6, Figure 12).

Table 6. Reclassification categories assigned to onion bulbs from ABC and VCS.

Symptom	Abbreviation	Description
Healthy	H	No symptoms (includes darkening in basal plate)
Missing basal plate/healthy	MBP	Bulbs missing lower basal plate but healthy
Corky	C	“Cork” like texture to internal basal plate
Mild Fusarium	M	Brown basal plate and infection just moving up into scales
Intermediate Fusarium	I	Infected bulb <50% of scales affected
Severe Fusarium	S	Infected bulb >50% of scales affected
Split base	SB	Any split base- including corky and non-corky basal plates

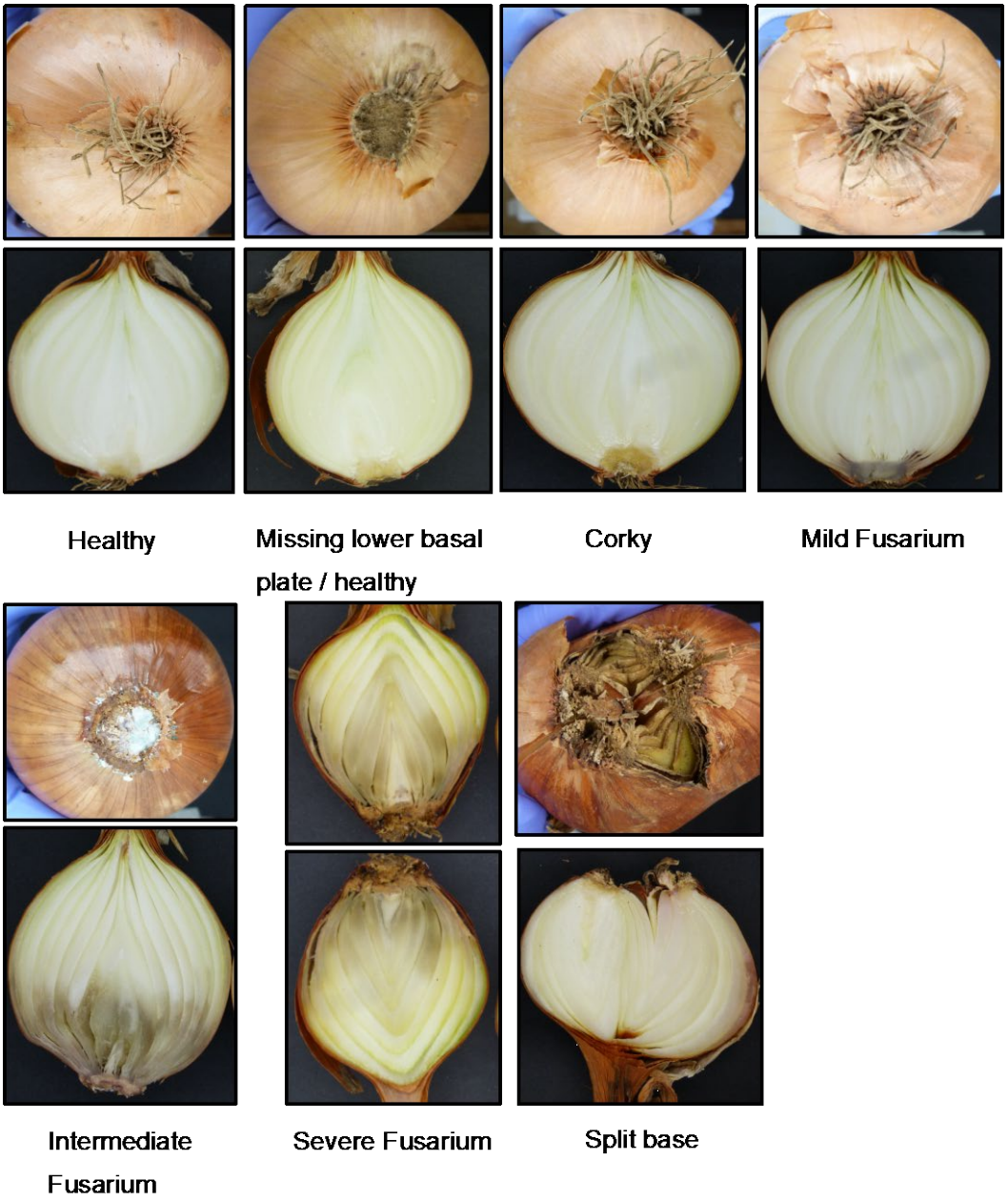

Figure 12. Disease classification of onion bulbs based on internal symptoms of Fusarium basal rot.

The number of onion bulbs in each disease category from ABC and VCS varied between sites. Over 60% of the bulbs from ABC were healthy, with the rest all showing either mild, intermediate or severe basal rot symptoms (Figure 13). Bulbs described as having a corky basal plate were found in all sites from VCS, compared to none displaying this symptom from ABC (Figure 13). VCS site 1 had no obviously diseased bulbs, but many had a split basal plate. Interestingly, there were some bulbs from VCS sites 3 and 4 which appeared to be missing their lower basal plate; however, these bulbs also had no visible internal Fusarium basal rot symptoms (Figure 13).

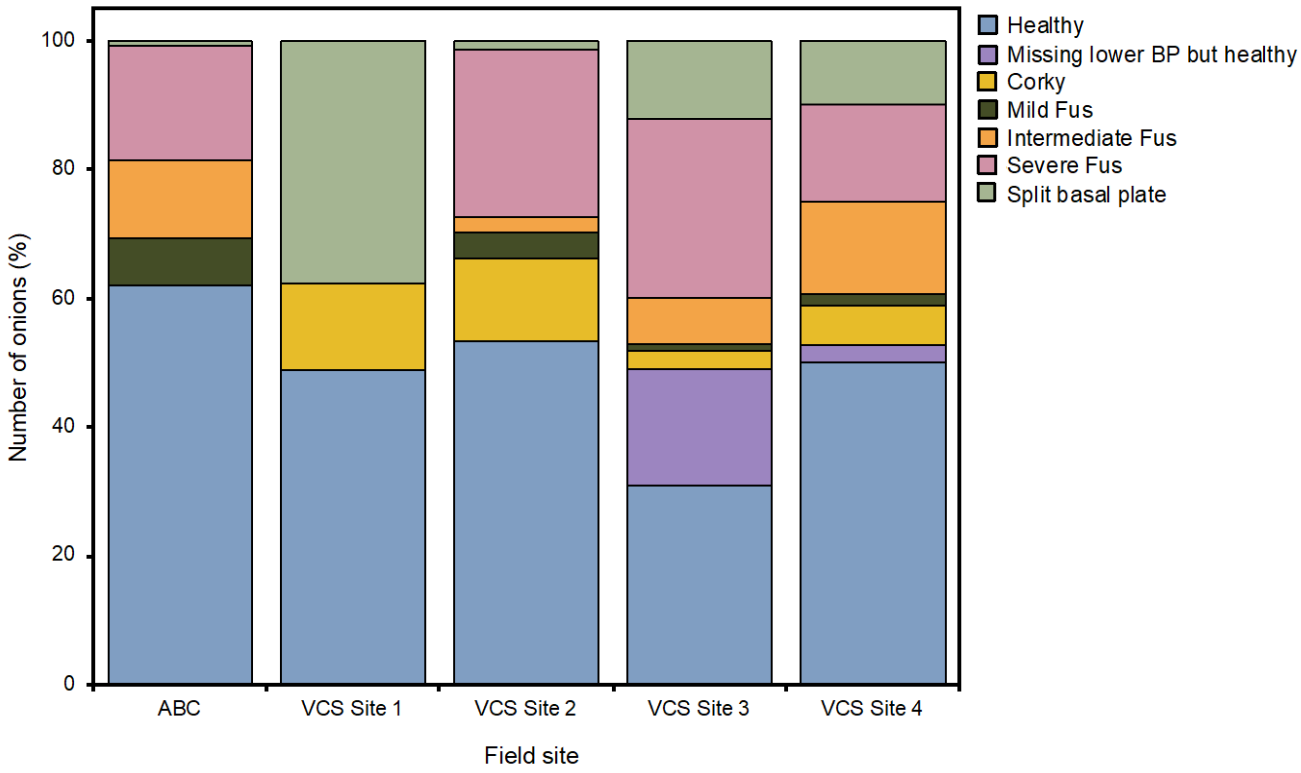

Figure 13. Percentage of onion bulbs from five sites (ABC and VCS) with different severities of Fusarium basal rot. Bulbs ranged from healthy to severe disease, included those with missing or split basal plates.

Overall, *Fusarium* was could be isolated from onion bulbs in every disease category including healthy bulbs. (Figure 14). *Fusarium* was isolated from 100% of the basal plates from bulbs classified as having mild, intermediate and severe basal rot disease, with only a few exceptions where bulbs were severely degraded (severe disease) where agar plates became overwhelmed with contaminating bacteria and mites (Figure 14). This was also generally the case for isolations from the scales of diseased bulbs. In corky bulbs from all sites (other than VCS site 1) *Fusarium* was only isolated from basal plates (scales showed no symptoms), with VCS site 3 resulting in the pathogen being isolated from all samples although there were only three bulbs classed as corky from this site (Figure 14). Interestingly, the onion bulbs classed as missing the basal plate (but healthy) and split bases were positive for *Fusarium*, even though they usually had no clear basal rot symptoms. However, these could have been other opportunistic *Fusarium* species, which do not cause serious disease in onion.

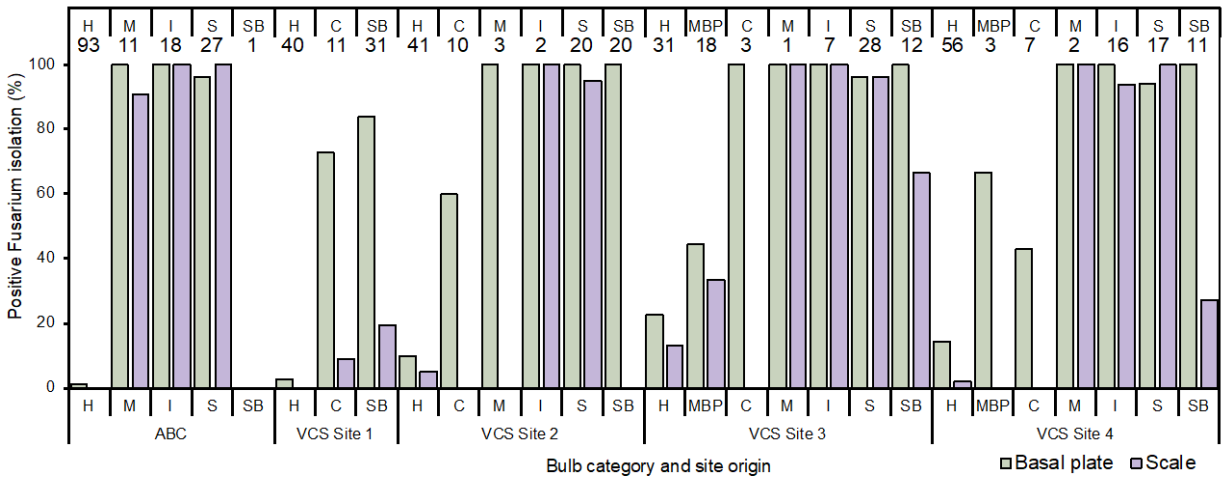

Figure 14. Onion basal plates and scales with a positive Fusarium isolation from bulbs with different visual symptoms: H = Healthy, MBP = Missing basal plate but healthy, C = Corky, M = Mild disease, I = Intermediate disease, S = Severe disease, SB = split basal plate. Bulbs were obtained from ABC and four sites from VCS.

A selection of the basal plate samples from onion bulbs with different disease symptoms from each site were then used for detection of FOC by qPCR. The presence of FOC DNA generally matched the results from the agar isolations for these bulbs (Table 7). In the case of healthy bulbs from ABC, the qPCR was able to detect FOC in three bulbs, whereas with the isolations only 1 Fusarium colony was detected. In cases where the qPCR numbers are lower than the number of isolations this could be due to the assay not being sensitive enough or that another Fusarium species, such as *F. solani* or *F. redolens* (as was found, data not shown), or another *F. oxysporum* (not FOC).

Table 7. Detection of FOC in basal plate tissue by agar plating and qPCR for onion bulbs with different basal rot symptoms.

Supplier	Site	Disease classification	No. bulbs used for qPCR	No. bulbs with *Fusarium* isolated	No. bulbs FOC qPCR positive
ABC	N/A	Healthy	8	1	3
ABC	N/A	Mild	6	6	6
ABC	N/A	Intermediate	6	6	6
ABC	N/A	Severe	10	10	10
VCS	SITE 1	Healthy	4	1	0
VCS	SITE 1	Corky	11	9	7
VCS	SITE 2	Healthy	12	4	4
VCS	SITE 2	Corky	10	6	0
VCS	SITE 2	Mild	3	3	2
VCS	SITE 2	Intermediate	2	2	2
VCS	SITE 2	Severe	2	2	2
VCS	SITE 3	Healthy	13	7	0
VCS	SITE 3	Missing BP	8	4	0
VCS	SITE 3	Corky	3	3	0
VCS	SITE 3	Mild	1	1	1
VCS	SITE 3	Intermediate	4	4	4
VCS	SITE 3	Severe	3	3	3
VCS	SITE 4	Healthy	11	5	2
VCS	SITE 4	Corky	7	3	3
VCS	SITE 4	Mild	2	2	2
VCS	SITE 4	Intermediate	4	4	4
VCS	SITE 4	Severe	2	2	2

Onion bulbs which were either healthy or corky (as designated by ABC / VCS) were incubated for eight weeks to determine if any basal rot would develop which was assessed following dissection. Both healthy and corky onion bulbs developed basal rot symptoms, but disease levels varied (Table 8). Those bulbs originally designated healthy generally remained healthy or had lower levels of basal rot (mostly scores of 0-3), whereas most of the corky bulbs developed basal rot (Table 8) and generally had higher disease scores (mostly scores of 2-4). VCS site 3 had the a large proportion of healthy bulbs (27 out of 33) develop basal rot in storage, and had bulbs with the highest average disease score (Table 8), apart from VCS site 4 corky bulbs.

Table 8. Fusarium basal rot scores of onions from different field/store sites after being incubated for 8 weeks at 20˚C. Scores range from 0 (healthy) to 5 (>50% disease in scales). Error bars represent standard deviation.

Site	Bulb type	Total bulbs	Number healthy	Number infected	Average basal rot score	SD
ABC	Healthy	50	18	32	1.4	1.2
	Corky	50	18	31	1.6	1.5
VCS Site 1	Healthy	40	32	8	0.4	0.9
	Corky	24	1	23	1.6	1.3
VCS Site 2	Healthy	48	23	25	0.9	0.9
VCS Site 3	Healthy	33	6	27	2.2	1.4
	MBP	10	1	9	2.3	1.3
VCS Site 4	Healthy	60	24	36	1.5	1.5
	Corky	20	4	16	2.5	1.8

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

As expected, in the first lettuce planting where sterilised and unsterilised soil was inoculated with low levels of FOL4, there were no visible Fusarium wilt symptoms but there were early signs of vascular browning (score >1) at harvest, especially in the sterilised soil inoculated at 2 x 10^3 cfu g^{-1} (Figure 15).

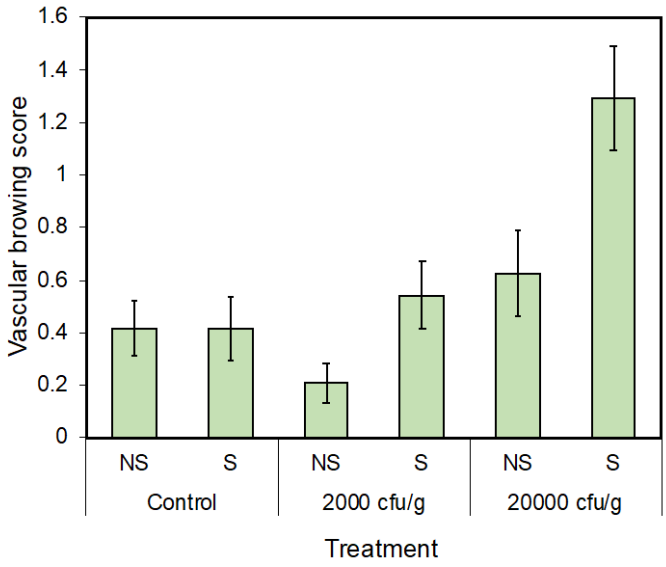

Figure 15. Vascular browning score at harvest for a first crop of lettuce grown in FOL4 inoculated soil (2 x10^2 and 2 x 10^3 cfu g^{-1}) which was either non-sterilised (NS) or steam sterilised (S).

In the second lettuce crop, Fusarium wilt symptoms were observed early on, and resulted in high levels of disease after eight weeks (Figure 16). This demonstrated that the level of inoculum in the soil had increased for both FOL4 inoculum concentrations with successive crops (Figure 16). This was particularly evident for the sterilised soil which resulted in considerably more wilt than in the non-sterilised soil at the same concentration, especially for the 2 x 10^2 cfu g^{-1} concentration (Figure 16). There was only a small difference between the sterilised and non-sterilised soils in the percentage wilt in lettuce grown in the 2 x 10^3 cfu g^{-1} concentration. It was also noted that the lettuce grown in sterilised soil without FOL inoculum in the second lettuce planting were consistently larger than those in the unsterilised soil.

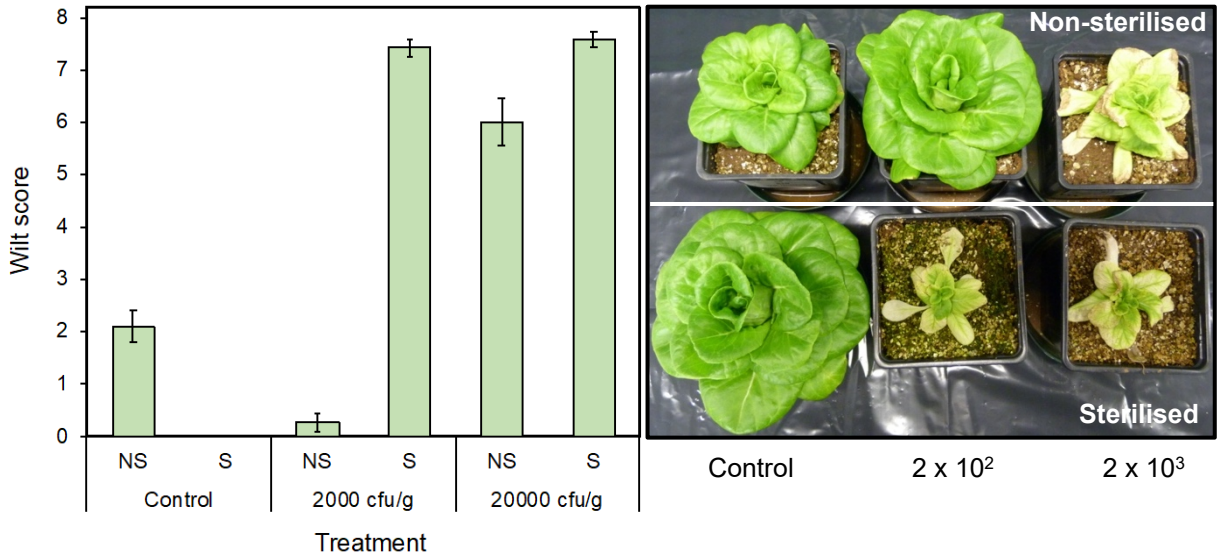

Figure 16. Mean Fusarium wilt score for a second crop of lettuce grown in FOL4 inoculated soil at initial concentrations of 2 x10^2 and 2 x 10^3 cfu g^{-1}, which was either non-sterilised (NS) or steam sterilised (S). Images show examples of lettuce size from all the treatments, and the wilt symptoms seen in some of the concentrations.

When the second lettuce crop was harvested, there was significantly more vascular browning due to FOL4 in lettuce grown in the 2 x10^2 cfu g^{-1} sterilised soil than for the non-sterilised soil (Figure 17 A), suggesting pathogen inoculum levels had built up more quickly in the sterilised soil. This was also apparent when the lettuce dry weights were examined, in that lettuce plants were bigger in the non-sterilised soil treatments than the sterilised (Figure 17 B). At the higher FOL4 inoculum concentration (2 x 10^3 cfu g^{-1}) there was no difference in the vascular browning score between the two soil treatments (Figure 17 A); however, lettuce dry weight in the non-sterilised soil was still greater than for the lower FOL4 inoculum concentration (Figure 17 B). This suggests that lettuce succumbed to disease later in the non-sterilised soil treatment compared to the sterilised soil and therefore that inoculum levels are likely higher in the latter.

Unexpectedly in this second lettuce crop, there were high levels of Fusarium wilt and some vascular browning for plants grown in the non-sterilised uninoculated control treatment. This is most likely due to FOL4 contamination when setting up or harvesting the experiments so better hygiene needs to be employed for future experiments.

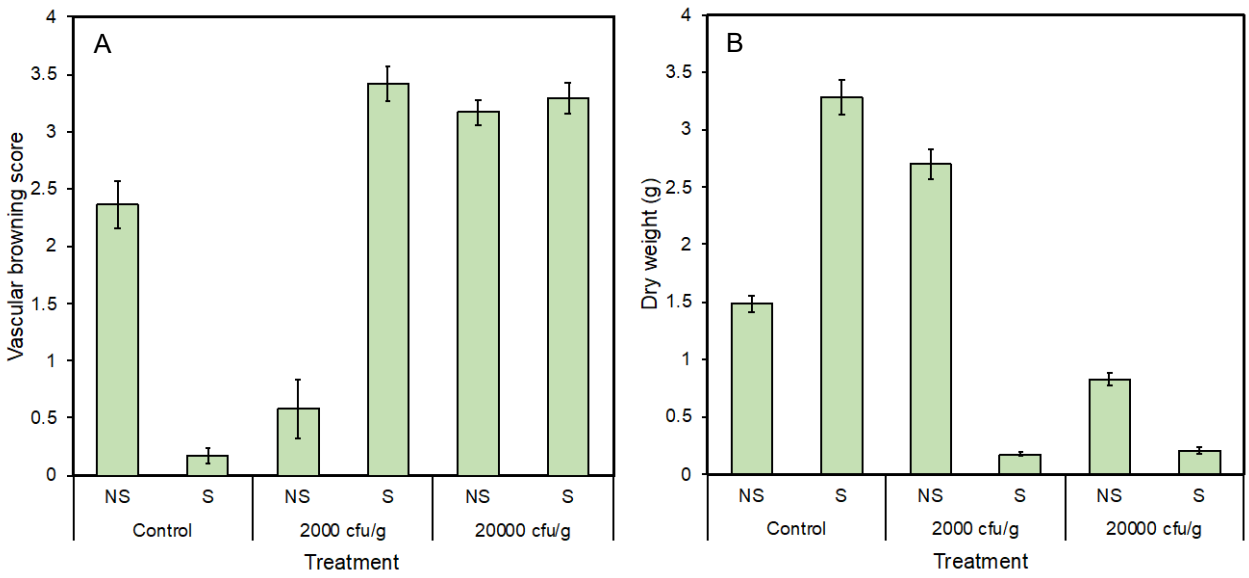

Figure 17. A) Vascular browning score and B) dry lettuce head weight for a second crop of lettuce grown in FOL4 inoculated soil at initial concentrations of 2 x10^2 and 2 x 10^3 cfu g^{-1} which was either non-sterilised (NS) or steam sterilised (S) before inoculation.

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

Design of 16S, ITS and TEF spike-in plasmids

While carrying out the detailed experiment design for this step, it was determined that this approach would not be appropriate due to the additional time and resources required. The plasmid spike-ins initially have to be titrated in for each soil type to determine the final level of spike-in, requiring at least one additional sequencing flow cell for this step. In addition, the spike-in levels need to be added at read-depths of 200-800 reads / 1000, which would again double the number of flow cells used for the experiment. It was therefore decided to abandon this method of quantification Instead, it may be possible to use these plasmids in a qPCR assay to quantify the target amplicons in the soil samples.

Design and testing of gene targets for amplicon sequencing for FOL

Primers were designed to the three candidate target genes and tested for specificity against FOL DNA and DNA extracted from soil samples from Objective 1 spiked with FOL4. It was found that the only suitable gene target was g19096. This was found to produce a single PCR band using DNA from the different soil samples. Although this gene target sequence for FOL is also identical to that found in *F. oxysporum* f.sp. *tulipae* (FOT) and FOM (Figure 18), FOL can be reliably identified in amplicon sequencing by using this amplicon in combination with the other OG4952 gene target (which amplifies FOT and FOM but not FOL).

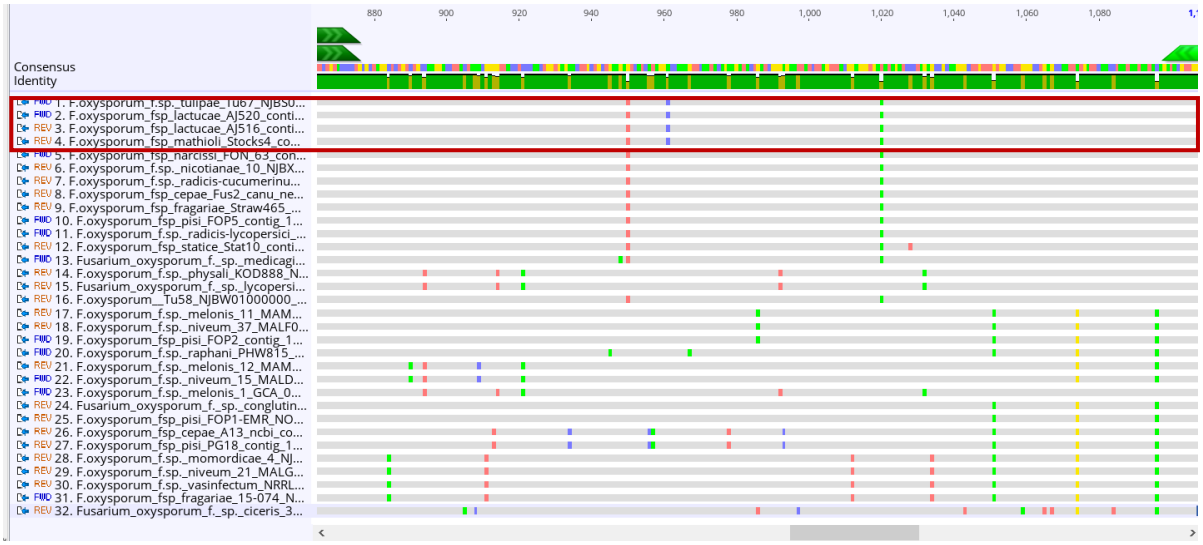

Figure 18. Pattern of sequence variation associated with target amplicon g19096 for different *F. oxysporum* f.spp. Note same pattern of single nucleotide polymorphisms for FOL, FOM and FOT (red box).

Amplicon sequencing of DNA from soils inoculated with FOL

Amplicon sequencing was successful for all of the three soils from Objective 1 (S1 = Soil 1, S2 = Soil 2, S3 = Soil 3) inoculated with different concentrations of FOL (D1=0, D2=$2x10^2$, D3=$2x10^3$, D4=$2x10^4$, D5=$2x10^5$, D6=$2x10^6$ cfu g^{-1} soil). Results from Soil 1 and Soil 2 are presented here for bacterial (16S) and fungal (ITS) soil composition while results for Soil 1 only are presented for *Fusarium* spp. (TEF) and *F. oxysporum* f. spp. composition (g19096, OG4952). The remaining results are currently being analysed and will be presented in the next report.

Identity of bacteria using 16S amplicon sequencing in Soil 1 and Soil 2

As expected, many bacterial genera were present in the soil samples with differences between soil type (Figure 19) and different FOL inoculum levels (data not shown). *Gemmatimonas* was the most common bacterial genus in both Soil 1 and Soil 2. There were distinct patterns for the remaining bacterial genera. Of the most abundant 25 genera for Soil 1 and Soil 2 only 16 are shared and these showed different patterns of abundance with 9 out of the 25 distinct for each soil type (Figure 19). Notably, in four out of the six samples for Soil 2, *Pseudomonas* was the second most abundant whereas this was much less abundant in Soil 1 samples.

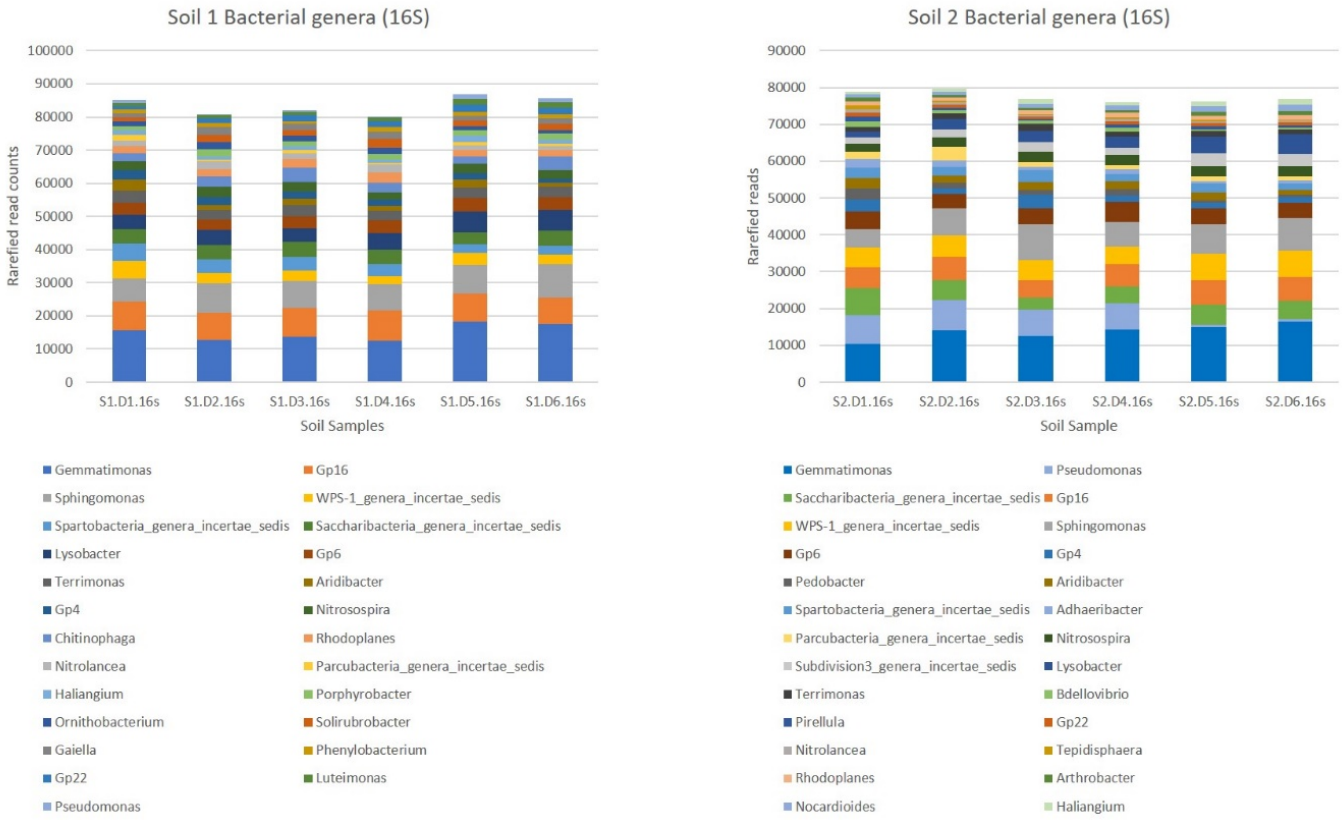

Figure 19. Relative abundance of the top 25 most abundant bacteria in Soil 1 and Soil 2 using 16S sequencing for the uninoculated control soil samples. The key shows the genera listed by abundance from top left to right going down.

Identity of fungal genera using ITS amplicon sequencing in Soil 1 and Soil 2

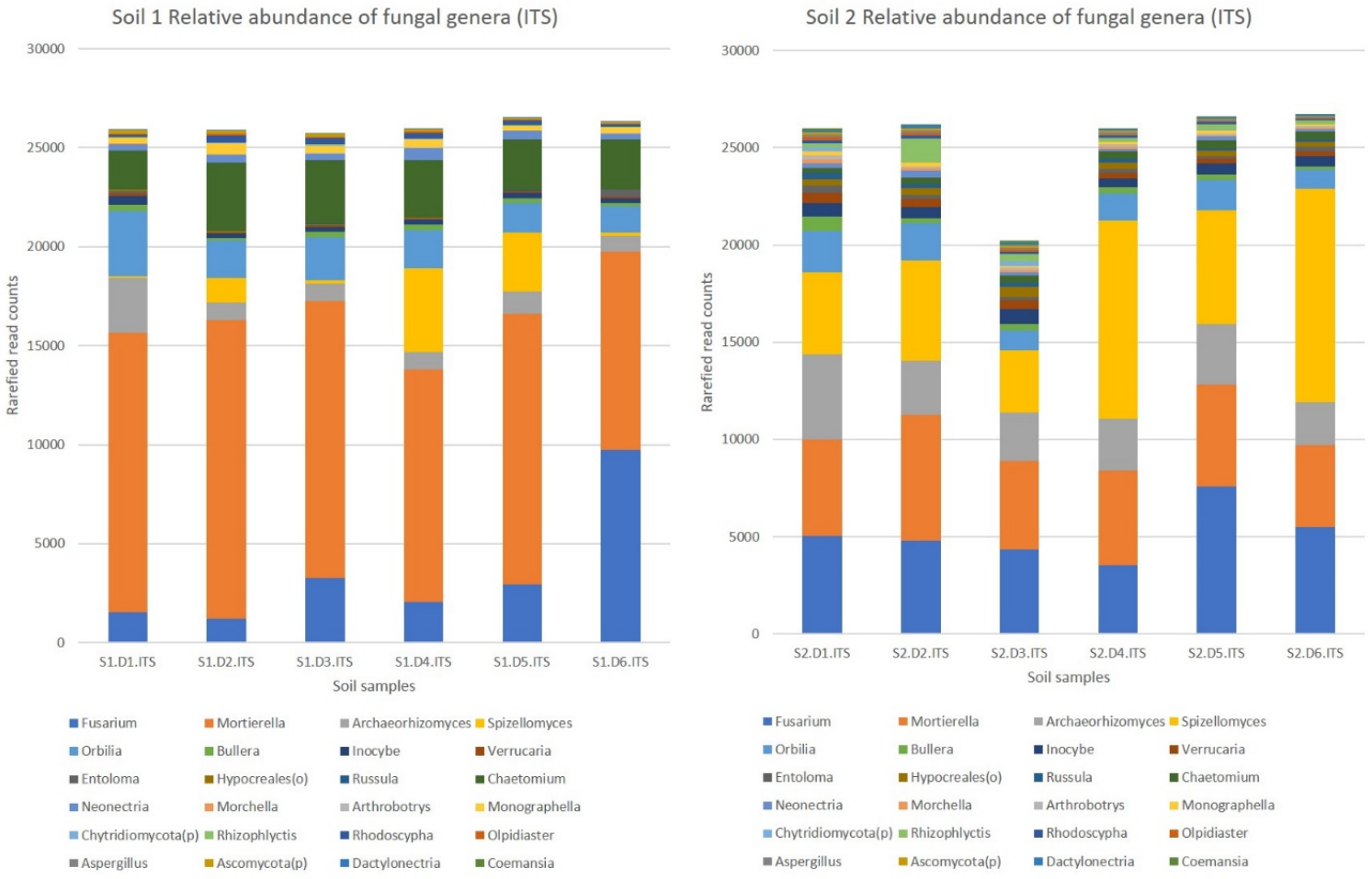

Figure 20. Relative abundance of the top 24 most abundant fungal genera in Soil 1 and Soil 2 using ITS sequencing for different inoculum concentrations of FOL (D1=0, D2=2x10^2, D3=2x10^3, D4=2x10^4, D5=2x10^5, D6=2x10^6 cfu g^{-1} soil).

Abundance of *Fusarium* species found in Soil 1 samples using TEF amplicon sequencing

Although the TEF amplicon sequencing identified the presence of several *Fusarium* species, *F. oxysporum* was the dominant species at all inoculum levels (Figure 21). The large error bars for abundance of *F. oxysporum* in the uninoculated control (D0) and 2x10^2 cfu g^{-1} FOL inoculum concentration (D1) and the other species in all samples, indicated that these were present at low background levels and the stochastic nature of PCR on rare templates is causing this variation. For FOL inoculum levels > 2x10^3 cfu g^{-1} (D3) *F. oxysporum* was detected at high levels with little variation as might be expected indicating that the pathogen is outcompeting all the other *Fusarium* spp. present at background levels in the soil.

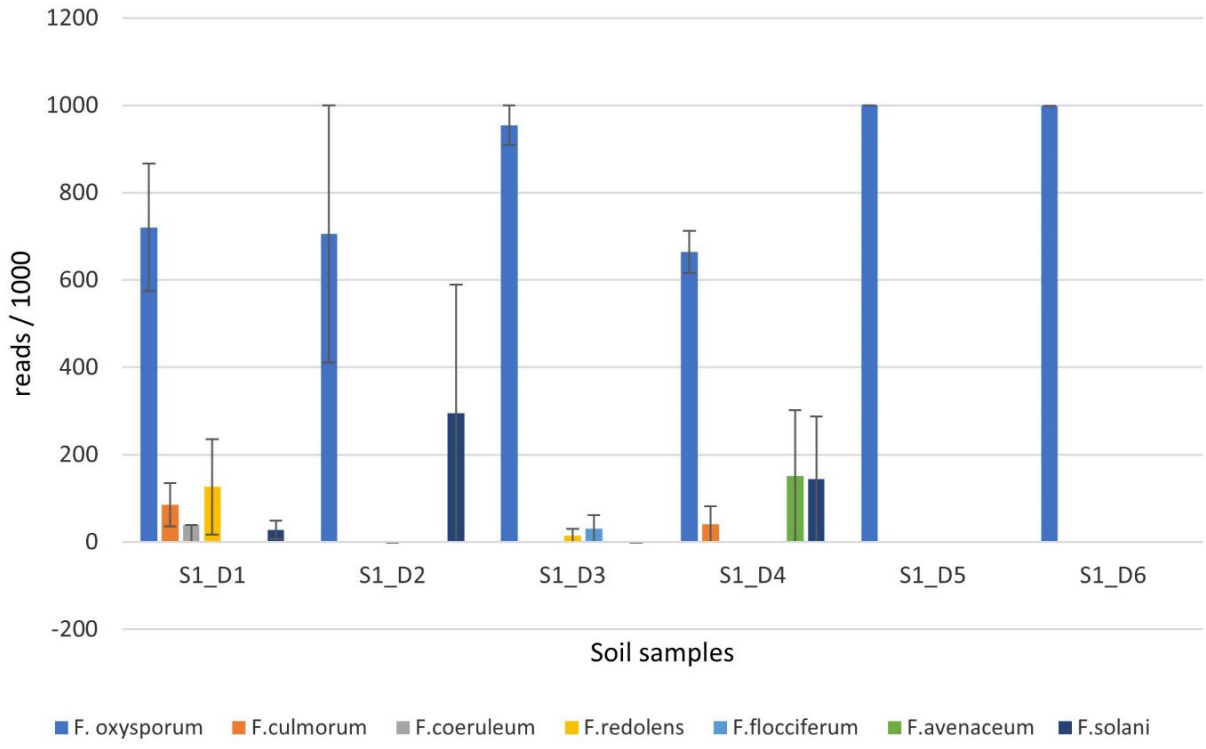

Figure 21. Relative abundance of *Fusarium* spp. using TEF sequencing in Soil 1 for different inoculum concentrations of FOL (D1=0, D2=2x10^2, D3=2x10^3, D4=2x10^4, D5=2x10^5, D6=2x10^6 cfu g^{-1} soil).

Abundance of *F. oxysporum* f.spp. in Soil 1 samples using g19096 and OG4952 amplicon sequencing

Amplicon sequencing of g19096 identified the presence of *F. oxysporum* f.spp. in all Soil 1 samples with a high abundance of FOL at high levels of inoculum (Figure 22a). In the uninoculated control (D0) and at low levels of FOL4 inoculum (2x10^2 cfu g^{-1}, D1; 2x10^3 cfu g^{-1}; D2) the most common amplicon sequence was that associated with several different *F. oxysporum* f.spp. including FOC, FON, f.sp. *nicotiana*, f.sp. *radicis-cucumerenin* and others but not FOL. At the inoculum level of 2 x 10^3 cfu g^{-1} soil (D3), the amplicon sequence associated with FOL4, FOM and FOT is first identified at approximately equal abundance with the other background sequence. For FOL4 inoculum levels > 2 x 10^4 cfu g^{-1} soil) the amplicon sequence associated with FOL sequence was most abundant. This indicates that using the g19096 gene target for amplicon sequencing effectively detects FOL4 at inoculum levels >2 x 10^3 cfu g^{-1} soil.

Although target OG4952 is not present in FOL, it can be used in combination with g19096 to distinguish FOL from FOM, FOT and other *F. oxysporum* f.spp which have the same g19096 sequence as FOL. Amplicon sequencing of OG4952 revealed the presence of the sequence

associated with FOC in all Soil 1 samples (Figure 22b). Although sequences associated with FOM and *F. oxysporum* f.sp. *pisi* race 1 (FOP1) were also present, these were sporadic and highly variable suggesting they were present at very low levels. The sequence associated with FOM or FOT was not detected and indicates that the identification of FOL by the g19096 amplicon was not due to the presence of FOM or FOT in the sample. Together the results from OG4952 and g19096 suggest that FOC may be present at low levels in Soil 1. To investigate this further, PCR was carried out on all S1 samples using FOC specific primers developed in AHDB project FV POBOF 452. Preliminary results indicated the presence of FOC in some samples, but this was not consistent. However, this was most likely due to levels close to the limit of qPCR detection.

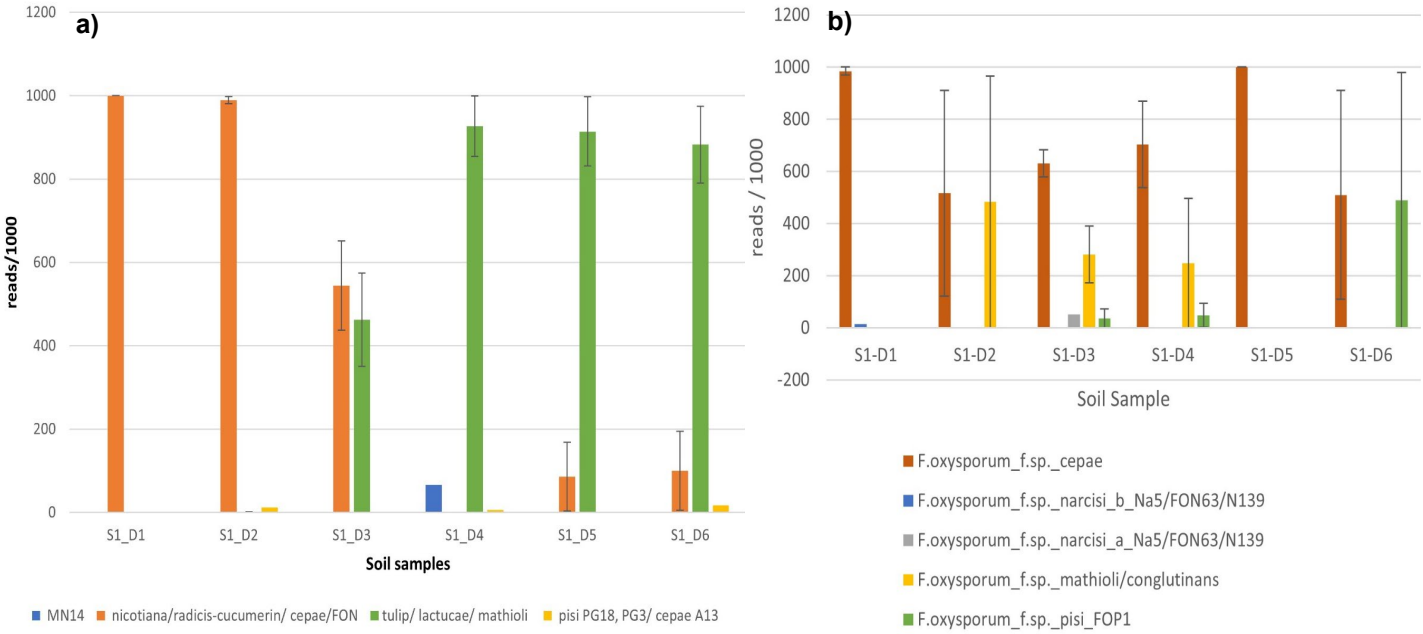

Figure 22. Relative abundance of *F. oxysporum* f.spp. in Soil 1 for different inoculum concentrations of FOL (D1=0, D2=2x10^2, D3=2x10^3, D4=2x10^4, D5=2x10^5, D6=2x10^6 cfu g^{-1} soil) for a) gene target g19096 and b) gene target OG4952.

Discussion

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

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 two soils obtained from growers varied in their physical composition, with Soil 1 having a much higher organic matter content compared with Soil 2 which in turn was greater than Soil 3 from Warwick Crop Centre. Even though it has been reported previously that soil with high OM content was more suppressive to Fusarium diseases (van Bruggen et al., 2015), this was not the case in this experiment with disease levels in lettuce grown in Soil 1 slightly lower than in those in Soil 2. A clear relationship between FOL4 inoculum concentration and disease development in lettuce in different soils was established and this could also be related to the results of the qPCR measuring the amount of FOL4 DNA. Detection of FOL4 by qPCR was possible down to a level of 2 x 10^3 cfu g^{-1} which resulted in relatively low levels of disease. This suggests that molecular diagnostics can be used to identify soils with a high risk of Fusarium wilt disease development in lettuce. 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

Relatively low levels of Fusarium basal rot disease developed at the onion field sites sampled. 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 detectable in soil at low levels and intermittently during the season using qPCR for the two intensively sampled sites where disease developed. However, FOC could not be detected in the soil samples taken at drilling for any site where disease developed. This suggests that initial inoculum levels were too low to detect by qPCR at planting and that a better approach would be detect the pathogen during the season to improve prediction of the risk of basal rot at harvest or in store. Further tests on additional 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

Detection of FOC by isolation onto agar or qPCR was carried out for onion bulbs which were classified by VCS an ABC as healthy, corky or infected. Results indicated that FOC could be detected by both methods for clearly infected, corky and some asymptomatic bulbs. Although most isolates grown on agar from infected bulbs were morphologically similar to FOC, a few were identified as *F. redolens*, *F. solani* and *F. avenaceum* (data not shown). These are known to be generalist pathogens and could have been present on the outside of bulbs or have been colonising lower basal plate sections, although some reports have linked these species to disease in onion (Bayraktar and Dolar, 2011; Haapalainen et al., 2016). Further work will now potentially focus on analysing onion bulb samples which are apparently healthy but go on to develop disease in store to further validate the qPCR approach as a tool for assessing risk of basal rot development in harvested bulbs.

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

In preliminary experiments to develop a system to examine FOL4 inoculum build up in sterilised and non-sterilised soils there were clear differences in the amount of disease observed. Even though low concentrations of FOL4 were used in the first round of lettuce production (2 $x10^2$ and 2 x 10^3 cfu g^{-1}) which caused no visible wilt, there was mild vascular browning in the tap root. After the second crop there was visible wilt in plants with a dramatic difference in the sterilised and non-sterilised soil initially inoculated at 2 $x10^2$ cfu g^{-1}. This suggests that FOL builds up in sterilised soil far more quickly than in non-sterilised soil. This system is therefore an effective way of examining treatments to prevent the build-up of FOL4. This approach will be further refined using different soils in Year 2 and begin to test different treatments in Year 3.

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 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 FOM or FOT. However, by also amplifying another gene target, OG4952 which is not present in FOL, the presence and abundance of FOL can be successfully be determined. 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.

Conclusions

- Objective 1: A clear relationship between FOL4 inoculum concentration, pathogen DNA levels and Fusarium wilt disease development in lettuce was established. This was help determine the risk of disease development from qPCR analysis of soil samples.
- Objective 2: FOC could be detected in soil samples from commercial onion fields using qPCR during the season but results were variable due to relatively low levels of basal rot developing. No FOC was detected at planting suggesting a different approach to utilising qPCR is required to better assess disease risk.
- Objective 3: Molecular qPCR diagnostics was effective at detecting FOC in onion bulbs even in the absence of visible symptoms. This approach is therefore promising for assessing if bulbs might develop basal rot before they go into store.
- Objective 5: A method was successfully developed to examine FOL4 inoculum build up in sterilised and non-sterilised soil after successive lettuce crops. This will be used to test different treatments to suppress proliferation of the pathogen.
- Objective 6: Amplicon sequencing was successfully employed to quantify FOL4 alongside other components of the soil microbial community

Knowledge and Technology Transfer

- Presentation at AHDB soil disinfestation webinar (04/11/20) – John Clarkson
- Presentation at AHDB Narcissus webinar (11/11/20) – John Clarkson
- Presentation at Plant and Crop Theme Seminar (PACTS), School of Life Sciences, University of Warwick (31/03/21) - Sascha Jenkins

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