Horticultural Fellowship Awards

Interim Report Form

Project title:	Maintaining and developing capability in vegetable crop pathology
Project number:	CP 113
Project leader:	Dr John Clarkson, Warwick Crop Centre, University of Warwick
Report:	Annual Report, October 2017 (Year 4)
Previous report:	Annual Report, October 2016 (Year 3)
Fellowship staff: (“Trainees”)	Dr John Clarkson & Dr Andrew Taylor
Location of project:	Warwick Crop Centre
Industry Representative:	N/A
Date project commenced:	1st November 2013
Date project completed (or expected completion date):	31/12/19

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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.

Dr Andrew Taylor
Research Fellow
Warwick Crop Centre, University of Warwick

Signature  Date: 19/10/17

Report authorised by:

Dr John Clarkson
Reader
Warwick Crop Centre, University of Warwick

Signature  Date: 19/10/17

CONTENTS

Progress Against Objectives

N.B. Grey shading refers to this reporting period

Objective	Original Completion Date	Actual Completion Date	Revised Completion Date
1.1 Determine pathogenicity of a range of *Fusarium oxysporum* isolates on onion and complete DNA sequencing of a range of housekeeping genes.	31/10/15	31/10/15	
1.2 Extract DNA, prepare libraries and carry out whole genome sequencing of *F. oxysporum* f.sp. *cepae* (FOC) isolates	31/10/17	31/10/17	
1.3 Bioinformatic analyses of FOC isolate genomes and identification of potential primers for FOC diagnostics.	31/10/17	31/10/17	
1.4 Test FOC diagnostic primers *in vitro*	31/10/18		
1.5 Test FOC diagnostic primers using soil and bulb samples.	31/10/18		
1.6 Test published PCR diagnostic for *Sclerotium cepivorum*	31/10/16	31/10/16	
1.7 Check existing *Pythium violae* specific primers using contemporary isolates / soil samples from carrot fields	31/10/14	31/10/14	
1.8 Develop qPCR for *P. violae* using WCC Roche Lightcycler	31/10/14	31/10/14	
1.9 Quantify *P. violae* in soil samples from AHDB Horticulture project FV 405 and other samples where available.	31/10/15	31/10/16	

Objective	Original Completion Date	Actual Completion Date	Revised Completion Date
1.10 Identify potential primers for *Itersonilia* diagnostics from existing gene sequences (or whole genome sequence).	31/10/16	31/10/16	
1.11 Test *Itersonilia* primers *in vitro*.	31/10/17	31/10/17	
1.12 Test the newly developed *Itersonilia* diagnostic test on infected parsnip seed lots and compare with the industry standard agar plate test.	31/10/17	31/10/17	
1.15 Test a range of *S. cepivorum* isolates for the presence of published pathogenicity genes	31/10/17	31/10/17	
1.16 Test the ability of sclerotia to germinate for a range of *S. cepivorum* isolates using an established assay based on diallyl disulphide (DADS)	31/10/17	31/10/17	
2.1 Collect new isolates of *Sclerotium cepivorum*, *Peronospora destructor* (onion downy mildew), *Botrytis squamosa* (botrytis leaf blight) and *Botrytis allii* (neck rot of onion)	31/10/15	31/10/15	
2.2 Confirm identity and characterise isolates from 2.1 by gene sequencing	31/10/16	31/10/16	
2.3 Develop appropriate plant infection tests and confirm pathogenicity of isolates from 2.1	31/10/17	31/10/17*	
3.1. Gain experience with lettuce pathogens such as *B. cinerea* and *B. lactucae* through a	31/10/18		

Objective	Original Completion Date	Actual Completion Date	Revised Completion Date
work programme to be developed with Katherine Denby and Eric Holub.			
3.2. Gain experience with brassica pathogens such as Turnip Mosaic Virus, *Albugo candida Hyaloperonospora brassicae* and *Xanthomonas campestris* through a work programme to be developed with Eric Holub and John Walsh.	31/10/18		
3.3. Gain experience of other pathogens such as *Pythium ultimum*, *Oidium. neolycopersici* through existing projects (John Clarkson)	31/10/17	31/10/17	
4.1. Synthesise Dez Barbara's unpublished work on carrot/parsnip viruses	31/10/15	31/10/15	
5.1. Attend relevant research project meetings.	Ongoing	Ongoing	
5.2. Present a poster at an industry meeting or event.	31/10/16	31/10/16	
5.3. Give a talk at an industry meeting or event.	31/10/17	31/10/17	
5.4. Work-shadowing of at least one industry collaborator.	31/10/17	31/10/17	
6.1. Contribute to writing at least one research proposal	31/10/17	31/10/17	
6.2. Initiate at least two research proposals and obtain funding for one.	31/10/18		
7 Test published (and unpublished) methods	31/10/18		

Objective	Original Completion Date	Actual Completion Date	Revised Completion Date
for extraction of DNA from larger quantities of soil.			
8 Isolate and confirm identity of the causal agent of onion pink root disease	31/10/17	31/10/17	
ADDITIONAL MILESTONE- Molecular characterisation of *F. oxysporum* f. sp. *narcissi* isolates. This will involve PCR amplification and sequencing of effector genes in a range of isolates.	31/10/16	31/10/16	

Summary of Progress

1.2/1.3: In collaboration with NIAB-EMR, the genomes of *F. oxysporum* isolates from onion were assembled and annotated, allowing the design of potential diagnostic qPCR primers based on the SIX5 gene.

1.11/1.12: Primers for *Itersonilia* were successfully designed and tested against a range of organisms found on parsnip seed and specificity confirmed. However, there was an incomplete correlation between the molecular test and the industry standard seed test.

1.15: *S. cepivorum* isolates were shown to contain the same compliment of pathogenicity genes with identical sequences.

1.16: Sclerotia from seven *S. cepivorum* isolates from diverse locations varied in germination response depending on conditioning treatment and exposure to diallyl disulphide (DADS). Conditioning sclerotia in soil stimulated germination of sclerotia overall while DADS resulted in earlier germination.

2.3: Plant infection tests were developed for onion downy mildew and pink root. An existing plant inoculation procedure will be tested for white rot following analysis of sclerotial germination data.

3.3: Throughout the fellowship, experience has been gained of a wide range of pathogens.

5.3: In addition to previous talks at industry events (see year 3 report) an invited talk was given at the Narcissus Growers workshop (Spalding, 25/05/17)

5.4: Work shadowing was completed with representatives from both carrot and onion industries. In January 2015 two days were spent working at Hobson Farming, gaining experience of carrot harvesting, processing and sampling for disease. In September 2017, a day was spent shadowing Andy Richardson (Allium and Brassica Centre) and involved visits to several onion growers and stores. In addition, Fusarium basal rot and pink root was observed in the field.
6.1: Contributions were made to a BBSRC LINK proposal with Hazera seeds titled: Development of resistance to Fusarium basal rot in onion and functional analysis of *Fusarium oxysporum* effectors.
8: Isolates collected from onion plants with pink root symptoms confirmed their identity as *Setophoma terrestris*. Additional isolates have recently been collected from Bedfordshire.

Milestones not being reached

Whilst all milestones have been reached, additional work is ongoing as part of 2.3.

Do remaining milestones look realistic?

Due to changes in the end date of the fellowship, additional milestones will be added and the remaining milestones reviewed. This will be discussed and agreed with AHDB.

Training undertaken

- Attended a seminar at Warwick by Professor Robin May, University of Birmingham: Understanding host immune manipulation by a lethal fungal pathogen (30th Nov 2016)
- Attended a seminar at Warwick by David Studholme, University of Exeter: Genome gazing: bacteria, oomycetes and maybe a crop plant (8th Dec 2016)
- Part of a team of researchers that visited Crops for the Future in Malaysia, taking soil samples from oil palm plantations and extracting DNA in the labs.
- Attended and presented poster titled 'Understanding pathogenicity and resistance in the *Fusarium oxysporum*-onion pathosystem' at BBSRC HAPI conference in Manchester (8-9th March 2017)
- Attended and gave talk titled '*Fusarium oxysporum*: a major pathogen of multiple crops causing basal rot of *Narcissus*' at Narcissus Growers workshop (Spalding, 25th May 2017)

- Attended Practical diagnosis of plant pathogens course at RHS Harlow Carr (3rd May 2017)
- Met with Steve Millington from Optagene and discussed LAMP. Learnt about how to set up assays (24th May 2017)
- Attended seminar at Warwick by John Pickett: Global food security: removing production constraints with GM but learning from nature
- Attended and gave talk titled 'Understanding the genetic control of pathogenicity and resistance for *Fusarium oxysporum* in onion' at the International Horticulture Conference at NIAB-EMR (17th – 19th July 2017)
- Attended and gave talk titled 'Understanding the genetic control of pathogenicity and resistance for *Fusarium oxysporum* in onion' at the BSPP Presidential meeting in Nottingham (11th – 13th Sept 2017)
- Hosted seminar by John Fraser (DOW Agrosciences) The Strategic Role of Wellesbourne in a Global Business (14th Sept 2017)
- Work shadowing with Andy Richardson (Allium and Brassica Centre) – visited onion stores and fields (20th Sept 2017)

Expertise gained by trainees

- Improved communication skills
- Soil sampling in plantations
- DNA extraction from diverse soils
- Improved understanding of the Narcissus industry
- Improved practical plant pathology skills including diagnosis of plant pathogens
- Primer and assay design for LAMP assays from diverse plant samples
- Improved knowledge of the onion industry and onion storage
- Greater understanding of global plant pathology and horticulture research
- An understanding of commercial production of stocks and a better understanding of Fusarium wilt in a commercial situation

Other achievements in the last year not originally in the objectives

- In February 2017, I was part of a team of researchers that visited Crops for the Future (CFF) in Malaysia. During this trip, soil samples were taken from different oil palm plantations and DNA extracted in the CFF labs. This DNA is currently being analysed using whole amplicon sequencing to examine the microbial community around oil palm roots.

- A paper titled: Resistance to *Sclerotinia sclerotiorum* in wild Brassica species and the importance of *Sclerotinia subarctica* as a Brassica pathogen was published in Plant Pathology.
- Contributed to a paper titled: Climate-linked shifts in host association patterns shaped host range diversity in a fungal pathogen clade, submitted to Molecular Ecology (accepted pending revisions). As part of this study, I amplified and sequenced a range of housekeeping genes from isolates of *Sclerotinia subarctica*, *Sclerotinia nivalis* and *Sclerotium cepivorum*.
- A paper titled First Report of *Fusarium oxysporum* causing a Vascular Wilt of Statice (*Limonium sinuatum*) in the U.K. was published in Plant Disease.
- A paper titled: Pan genomic analysis reveals lineage-specific regions and novel effector candidates in *Fusarium oxysporum* f. sp. *cepae* submitted to Scientific Reports.
- Visited a rocket grower with suspected *Fusarium* problems. It became apparent that clubroot was also an issue. *Fusarium* was isolated and identified (TEF sequencing) as *Fusarium redolens*. This confirms our previous findings from the same site and as far as we are aware is the first record of *Fusarium redolens* causing disease on rocket.
- Became an editor for Exchanges: the Warwick research journal (http://exchanges.warwick.ac.uk/index.php/exchanges).
- Maintained the quarantine field areas at Wellesbourne. Involved in a trial in the *Fusarium* area, being run by a chemical company.
- Designed and carried out an experiment to monitor SIX gene expression in planta following infection of Narcissus scales with FON. This produced excellent data which is being written into a publication.
- Following a meeting with Optigene Ltd, a Genie 2 machine was obtained on loan in order to carry out some Loop-mediated isothermal amplification (LAMP) assays. Primers were designed for FOC and Itersonilia and successful assays carried out.
- Started work as a postgraduate skills development mentor. This will involve mentoring MSc students on a range of research and transferrable skills.

Changes to Project

Are the current objectives still appropriate for the Fellowship?

Due to changes in the end date of the fellowship, additional milestones will be added and the remaining milestones reviewed. This will be discussed and agreed with the AHDB.

GROWER SUMMARY

Headline

The development of new and improved DNA based molecular tools will allow effective, quantitative monitoring of plant pathogens. Assays have been developed and tested for *Fusarium oxysporum* f. sp*. cepae* (FOC, causing onion basal rot) and *Itersonilia pastinacea* (causing parsnip canker). These will enable testing of seed (FOC, *Itersonilia*), soil, sets and bulbs in store (FOC). Whilst these currently remain as lab-based assays, preliminary testing has also shown that they have promise for the development of semi-quantitative Loop Mediated Isothermal Amplification (LAMP) assays using a portable instrument which paves the way for the development of in field tests.

Background

Onion diseases

Fusarium basal rot

Onion (*Allium cepa*) is an important horticultural crop which is cultivated by every agricultural nation. Soilborne diseases caused by *Fusarium oxysporum* formae speciales (isolates adapted to specific hosts, f. spp.) are major constraints to the production of many horticultural food crops worldwide including onion, leek, lettuce, tomato, brassicas, asparagus, cucurbits, peppers, coriander, spinach, basil, beans, peas, strawberry, watermelon and banana and also affect economically important non-food crops such as carnation and narcissus (Leslie and Summerell 2006) and stocks. *F. oxysporum* was recently identified as the 5th most important plant pathogenic fungus based on its economic and scientific impact (Dean et al, 2012). *F. oxysporum* f.sp. *cepae* (FOC) is one of the most important pathogens of onion crops and infects the roots and/or basal plate at any stage of plant development (Cramer, 2000; Taylor et al, 2013). This causes a damping-off symptom on seedlings and a basal rot on more mature plants resulting in severe pre and/ or post-harvest losses. In the UK, FOC is recognised mainly as being a problem at harvest and in store but in severe cases entire crops can be lost in the field. Economic losses due to FOC on onion are estimated at £10-11 million per year. FOC infection is favoured by warm temperatures and is predicted to get worse in Europe due to climate change (Cramer, 2000). It produces long-lived chlamydospores that survive in the soil for many years and hence control approaches have previously relied on the use of soil sterilisation, chemical fumigation, drenches with fungicides or seed treatments.

These approaches have in some cases been unsuccessful, have undesirable environmental effects and have been banned or are threatened by legislation governing restrictions in pesticide use. In the past it has been difficult to distinguish f. spp. of *F. oxysporum* and identification has relied on pathogenicity tests. However, work from *F. oxysporum* f. sp. *lycopersici*, the f. sp. infecting tomato (Lievens et al, 2009; Ma et al, 2010) has identified a set of pathogenicity related genes which are conserved in FOC (Taylor et al, 2016). Differences in the sequences of SIX genes between forma speciales of *F. oxysporum* can potentially be utilised to develop diagnostic assays which can be utilised to test soil and plant material for presence of the pathogen.

Allium white rot

Allium white rot, caused by the fungus *Sclerotium cepivorum*, is one of the most important diseases of Alliums, causing major losses for onion and garlic growers worldwide (Woodhall et al 2012). Initially, the root system becomes infected and as the infection progresses through the stem or bulb, leaf yellowing, stunting and plant death occurs (Crowe, 2008). The pathogen produces large numbers of sclerotia which can survive in the soil for up to 20 years, even without a plant host (Woodhall et al 2012). These sclerotia germinate in response to volatile compounds released by a new Allium crop, leading to new infection (Entwistle, 1990). In the past, pathogen detection was achieved by sieving soil to collect sclerotia (Crowe et al, 1980). A qPCR molecular based diagnostic has now been developed which involves extracting DNA from up to 1kg of soil (Woodhall et al, 2012). This method was tested in year 3 and shown to be effective at detecting *S. cepivorum*. It has been shown that sclerotia of *S. cepivorum* will germinate in response to diallyl disulphide (DADS), a volatile compound which is released into soil by Allium roots (Davis et al, 2007). In fact the addition of DADS has been used as a method to reduce the number of viable sclerotia in the soil – inducing them to germinate in the absence of an Allium host. Work from other species in the *Sclerotinacae* has shown that sclerotia will germinate more readily following a period of conditioning (Dillard et al, 1995). A similar biology is predicted for the sclerotia of *S. cepivorum* but this has not been widely studied.

Pink root

Onion pink root is a disease caused by *Setophoma terrestris*, a fungal pathogen formerly known as *Pyrenochaeta terrestris* or *Phoma terrestris* until a recent reclassification (de Gruyter et al 2010). The classic symptom is appearance of pink to red roots which will later become water soaked and eventually die (Nico and González Sánchez, 2012). The fungus will also infect other crops such as squash (Ikeda et al, 2012). Infection is favoured by warm temperatures and as such, pink root disease is generally a greater problem is warmer onion

growing regions such as the USA. However, it has been noted as a recent issue in the UK, particularly in onion seed production in glasshouse / polytunnel conditions where temperatures are higher than in a field.

Downy mildew

Onion downy mildew (*Peronospora destructor*) is a worldwide disease that causes severe damage to onion plants in cool moist conditions (Scholten et al, 2007). Infection leads to pale patches forming on the leaves which turn into purple lesions (Brewster, 2008). This is followed by sporulation, seen as a grey furry mass on the leaves, and leaf senescence. Once infection has occurred, damage can only be minimised by fungicides. It is listed as a high research priority by the British Onion Producers Research and Development Committee. Despite this, the only available varietal resistance comes from a single gene introgression from the wild species *Allium roylei* (Santero, Hazera seeds).

Parsnip canker

Parsnips are a speciality crop in the UK, covering an area of 3100ha with a value of £64M annually. The major constraint to production is crop losses associated with root canker diseases caused by fungal pathogens such as *Itersonilia*, *Cylindrocarpon* and *Mycocentrospora* spp. (Chappell, 2016). *Itersonilia pastinacae* is the primary cause of black canker in parsnip crops (Channon, 1963). It is a seed borne pathogen that produces ballistospores and chlamydospores that result in both foliar and root symptoms on parsnip. Root lesions have a brown/black colour and are visible on the crown or shoulder of the parsnip in autumn/winter (Channon, 1963). Foliar symptoms appear as brown lesions which eventually lead to a hole in the leaf.

Narcissus basal rot

Daffodil (Narcissus spp.) is one of the most widely cultivated bulb crops of temperate regions. The major production areas are the UK, Netherlands and USA although smaller areas are cultivated across the world (Hanks, 2002). In the UK, bulbs are particularly prone to infection by soil-borne pathogens due to the standard biennial growing system employed (Hanks, 2002). The most damaging pathogen is *Fusarium oxysporum*. f.sp. *narcissi* (FON), the cause of narcissus basal rot (Linfield, 1994). The symptoms include pale yellow leaf tips, soft bulbs, root rot and ultimately a bulb rot. Symptoms can occur in the field or on stored bulbs. If the genetic basis for pathogenicity could be identified in FON, then this information could be utilised to provide molecular tools for distinguishing it from other f. spp. and non-pathogenic

isolates, hence potentially allowing detection in soil and bulbs as part of developing a risk assessment strategy for Narcissus growers.

Summary

- A highly specific and reproducible molecular diagnostic assay (qPCR) was developed for FOC. This assay can be used to test seed, plant material and soil for FOC and is fully quantitative.
- A robust diagnostic qPCR assay was also developed for *Itersonilia*, with potential utility in rapid seed testing for disease risk. However, incomplete correlation with the industry standard seed test was observed so further development is required on this assay.
- Isolates of *S. cepivorum* were collected from across the UK and screened for the presence of range of pathogenicity-related genes. All isolates were identical, suggesting a clonal origin and showing a close relationship with other members of the *Sclerotinacae*. This could mean that a future control method would be effective against all isolates of *S. cepivorum*.
- *S. cepivorum* isolates differed in their sclerotial germination, a process which was strongly induced by conditioning sclerotia.
- Pathogenicity assays were successfully developed for onion downy mildew and pink root, allowing future resistance screening to be carried out.
- *Setophoma terrestris* was confirmed as the causal agent of onion pink root. This pathogen is favoured by warm temperatures and as such is one to be aware of in the UK considering current climate change models.
- FON SIX genes were shown to be expressed in daffodil plants following artificial inoculation, strongly indicating a role in pathogenicity. These genes may distinguish races of FON and the gene sequences could be used to develop a future molecular diagnostic test.
- Preliminary LAMP assays were developed for FOC and *Itersonilia*. This technique has the advantage that it is very rapid and can be carried out in the field. Further development is required, particularly for soil samples, but there is the possibility of future in field tests for both FOC and *Itersonilia*. These assays are classified as 'semi-quantitative'.

Financial Benefits

None to report

Action Points

None to report

SCIENCE SECTION

Introduction

Onion diseases

Fusarium basal rot

F. oxysporum f.sp. *cepae* (FOC) is one of the most important pathogens of onion crops and infects the roots and/or basal plate at any stage of plant development (Cramer, 2000; Taylor et al, 2013). This causes a damping-off symptom on seedlings and a basal rot on more mature plants resulting in severe pre and/ or post-harvest losses. In the UK, FOC is recognised mainly as being a problem at harvest and in store but in severe cases entire crops can be lost in the field. Economic losses due to FOC on onion are estimated at £10-11 million per year and FOC infection is favoured by warm temperatures and is predicted to get worse in Europe due to climate change (Cramer, 2000). FOC produces long-lived chlamydospores that survive in the soil for many years and hence control approaches have previously relied on the use of soil sterilisation, chemical fumigation, drenches with fungicides or seed treatments. These approaches have in some cases been unsuccessful, have undesirable environmental effects and have been banned or are threatened by legislation governing restrictions in pesticide use.

FOC is one of more than 120 *Fusarium oxysporum formae speciales* (isolates adapted to specific hosts) (Michielse & Rep, 2009) which are major constraints to the production of many horticultural food crops worldwide including onion, leek, lettuce, tomato, brassicas, asparagus, cucurbits, peppers, coriander, spinach, basil, beans, peas, strawberry, watermelon and banana and also affect economically important non-food crops such as carnation and narcissus (Leslie and Summerell, 2006). *F. oxysporum* was recently identified as the 5th most important plant pathogenic fungus based on its economic and scientific impact (Dean et al, 2012).

The genetically heterogeneous nature and lack of reliable morphological characters in this *F. oxysporum* complex means that distinguishing between different pathogenic f. spp. and also and between pathogenic and non-pathogenic isolates is difficult and can only be done using laborious and time consuming pathogenicity tests on different hosts. The factors which determine the host specificity and pathogenicity of different *F. oxysporum* f. spp. are poorly understood although recent studies have identified the role of secreted effector proteins (SIX genes) and mobile pathogenicity chromosomes in *F. oxysporum* f. sp. *lycopersici*, the f. sp. infecting tomato (Lievens et al, 2009; Ma et al, 2010). The genetic basis for pathogenicity

appears to be partially conserved in FOC with 7 of the 14 known SIX genes identified and predicted to have an important role in pathogenicity (Taylor et al, 2016). Differences in the compliment and sequences of SIX genes between *formae speciales* of *F. oxysporum* including FOC holds promise for the development of specific diagnostic assays for each f.sp. which could be utilised to test soil and plant material for presence of these important pathogens.

Allium white rot

Allium white rot, caused by the fungus *Sclerotium cepivorum*, is one of the most important diseases of Alliums, causing major losses for onion and garlic growers worldwide (Woodhall et al 2012). Initially, the root system becomes infected and as the infection progresses through the stem or bulb, leaf yellowing, stunting and plant death occurs (Crowe, 2008). The pathogen produces large numbers of sclerotia which can survive in the soil for up to 20 years, even without a plant host (Woodhall et al, 2012). These sclerotia germinate in response to volatile compounds released by roots from a new *Allium* crop (principally diallyl disulphide; DADs), leading to infection (Entwistle, 1990; Davis et al, 2007). DADS has also been used as a method to reduce the number of viable sclerotia in the soil through inducing them to germinate in the absence of an *Allium* host (Coley-Smith and Parfitt, 1986). Before *S. cepivorum* sclerotia can respond to DADs, they must also undergo a period of conditioning in soil to break dormancy but the precise factors involved in this process have yet to be defined (DEFRA, 2009). In the past, detection and quantification of *S. cepivorum* has relied on sieving soil to collect sclerotia (Crowe et al, 1980) but this is time-consuming, laborious and difficult to achieve for some soil types (e.g. peat soils). However, a qPCR molecular based diagnostic has now been developed (Woodhall et al, 2012) and this method was tested on infected soil in year 3 and shown to be effective.

Pink root

Onion pink root is a disease caused by *Setophoma terrestris*, a fungal pathogen formerly known as *Pyrenochaeta terrestris* or *Phoma terrestris* until a recent reclassification (de Gruyter et al 2010). The typical symptom is appearance of pink to red roots which will later become water soaked and eventually die (Noco and González Sánchez, 2012). The fungus can also infect other crops such as squash, melon, maize and up to 50 other species of the family Poaceae (Ikeda et al, 2012; Lević et al, 2012). It is often associated with other pathogens and known to occur in complexes, particularly on maize. Infection is favoured by warm temperatures and as such, pink root disease is generally a greater problem is warmer

onion growing regions such as the USA. However, it has been reported as a recent issue in the UK, particularly in onion seed production in glasshouse / polytunnel conditions where temperatures are higher than in the field.

Downy mildew

Onion downy mildew (*Peronospora destructor*) is a disease that causes severe damage to onion plants in cool moist conditions (Scholten et al, 2007). Infection leads to pale patches forming on the leaves which develop into purple lesions (Brewster, 2008). This is followed by sporulation, seen as a grey furry mass on the leaves, and leaf senescence. Once infection has occurred, damage can only be minimised by fungicides. It is listed as a high research priority by the British Onion Producers Research and Development Committee. Despite this, the only available varietal resistance comes from a single gene introgression from the wild species *Allium roylei* (Santero, Hazera seeds).

Parsnip canker

Parsnips are a speciality crop in the UK, covering an area of 3100ha with a value of £64M annually. The major constraint to production is crop losses associated with root canker diseases caused by fungal pathogens such as *Itersonilia*, *Cylindrocarpon* and *Mycocentrospora* spp. (Chappell, 2016). *Itersonilia pastinacae* is the primary cause of black canker in parsnip crops (Channon, 1963). It is a seed borne pathogen that produces ballistospores and chlamydospores that result in both foliar and root symptoms on parsnip. Root lesions have a brown/black colour and are visible on the crown or shoulder of the parsnip in autumn/winter (Channon, 1963). Foliar symptoms appear as brown lesions which eventually lead to a hole in the leaf.

Narcissus basal rot

Daffodil (*Narcissus* spp.) is one of the most widely cultivated bulb crops of temperate regions. The major production areas are the UK, Netherlands and USA although smaller areas are cultivated across the world (Hanks, 2002). In the UK, bulbs are particularly prone to infection by soil-borne pathogens due to the standard biennial growing system employed (Hanks, 2002). The most damaging pathogen is *Fusarium oxysporum*. f.sp. *narcissi* (FON), the cause of narcissus basal rot (Linfield, 1994). The symptoms include pale yellow leaf tips, soft bulbs, root rot and ultimately a bulb rot. If the genetic basis for pathogenicity could be similarly identified in FON as for FOC (see above) then this information could potentially be utilised to

provide diagnostic molecular tools for distinguishing it from other f. spp. and non-pathogenic isolates, hence potentially allowing detection in soil and bulbs.

Materials and methods

1.2 Extract DNA, prepare libraries and carry out whole genome sequencing of *F. oxysporum* f.sp. *cepae* (FOC) isolates / 1.3 Bioinformatic analyses of FOC isolate genomes and identification of potential primers for FOC diagnostics

This work was carried out in collaboration with NIAB-EMR. The highly pathogenic FOC isolate Fus2 (Taylor et al 2013; 2016) was selected for deep sequencing using PacBio technology. DNA extraction, library preparation, assembly and annotation was carried out as described by Armitage et al, (2017) and the assembled genome sequence imported into Geneious version 6.1 (Kearse et al 2012) for further analysis. A custom BLAST database was created and interrogated for the presence / absence of SIX genes. SIX gene sequences were extracted and aligned with publically available sequences extracted from NCBI BLAST and whole genome sequences from other *Fusarium* isolates (Kersey et al, 2016). Putative FOC-specific primers (based on SIX5) were designed manually following alignment with available sequences using MEGA version 7 (Kumar et al, 2015) and initial PCR and qPCR testing carried out as detailed in 1.11 with an annealing temperature of 60°C. For specificity testing, primers were tested against over 50 different *F. oxysporum* f.spp., *Fusarium* species, other common soil borne oomycetes / fungi as well as selected onion pathogens (Table 1) using both conventional and qPCR as described in 1.11. For conventional PCR, products were visualised by gel electrophoresis.

Table 1: Fungal isolates used for testing the specificity of a newly developed qPCR assay for FOC.

Species	f.sp.
Botrytis aclada	
Botrytis allii	
Botrytis cinerea	
Cylindrocarpon destructans	
Fusarium avenaceum	
Fusarium culmorum	
Fusarium equiseti	
Fusarium fujikuroi	
Fusarium graminerum	
Fusarium lactis	

Fusarium oxysporum	phaseoli	Bean	ATCC90245	USA
Fusarium oxysporum	pisi R1	Pea	FOP1	UK
Fusarium oxysporum	pisi R2	Pea	FOP2	UK
Fusarium oxysporum	pisi R5	Pea	FOP5	UK
Fusarium oxysporum	pisi	Pea	NRRL36311	Netherlands
Fusarium oxysporum	lini	Linseed		UK
Fusarium oxysporum	dianthi	Dianthus	BX13/113	UK
Fusarium oxysporum	narcissi	Narcissus	FOXN7	UK
Fusarium oxysporum	narcissi	Narcissus	FOXN139	UK
Fusarium oxysporum	freesia	Freesia	NRRL26990	Netherlands
Fusarium oxysporum	freesia	Freesia	NRRL26988	Netherlands
Fusarium oxysporum	cubense	Banana	E421A-3	UK
Fusarium oxysporum	cepae	Leek	L2-1	UK
Fusarium oxysporum	mathioli	Stocks	AJ182	UK
Fusarium oxysporum	gladioli	Gladiolus	NRRL 26993	Netherlands
Fusarium oxysporum	vasinfectum	Cotton	NRRL 25433	China
Fusarium oxysporum	melonis	Melon	NRRL 26406	Mexico
Fusarium oxysporum	conglutinans (Race 2)	Brassica/Arabidopsis	NRRL 54008	USA
Fusarium oxysporum	radicis-lycopesici	Tomato	NRRL 26381	USA
Fusarium oxysporum	lycopersici (race 1)	Tomato	AJ28	UK
Fusarium oxysporum	lycopersici (race 2)	Tomato	AJ30	UK
Fusarium oxysporum	lycopersici (race 3)	Tomato	NRRL 54003	USA
Fusarium oxysporum		Rocket	FR3	UK
Fusarium oxysporum		Statice	30A-9	UK
Fusarium poae		Pea	PG6	UK
Fusarium proliferatum			A8	UK
Fusarium proliferatum			A40	UK
Fusarium pseudocircinatum		Banana		UK
Fusarium redolens		Rocket	ML3	UK
Fusarium redolens		Onion	NL96	UK
Fusarium sacchari		Banana	18 RFB 2015	UK
Fusarium solani		Pea	PG14	UK
Fusarium tricinctum		Brassica	CO	UK
Itersonilia perplexans		Parsnip	IP 10	UK
Mycocentrospora acerina		Parsnip	Ma5	UK
Neonectria sp.		Snowdrop		UK
Phoma sp.		Unknown		UK
Plectosphaeralla cucumerina		Brassica	1O	UK
Pythium ultimum		Carrot	3b	UK
Pythium violae		Carrot	2C	UK
Rhizoctonia solani		Unknown	RS(A92-1)	UK
Sclerotinia sclerotiorum		lettuce	L6	UK
Sclerotium cepivorum		Onion	GS1	UK
Setophoma terrestris		Onion	PQF4	UK
Stemphylium sp.		Onion		UK
Trichoderma sp.		Onion	WR_11	UK
Verticillium sp.		Potato	PD693	UK

1.11 Test *Itersonilia* primers in vitro / 1.12 Test the newly developed *Itersonilia* diagnostic test on infected parsnip seed lots and compare with the industry standard agar plate test.

All *Itersonilia* primer pairs (developed from whole genome sequence as described in year 3 report) were tested as a potential diagnostic assay. Initial testing was carried out using conventional PCR using up to three *Itersonilia* isolates (IP9, IP13 and IP39) as well as a limited number of DNA samples from other fungi (including *F. oxysporum* and *M. acerina*). PCRs were carried out in 20µl reactions containing primers (0.5 µM), 5µl of RedTaq (Sigma) and 1µl of DNA with the following conditions: 1 cycle of 94°C for 2 mins followed by 35 cycles of 94°C for 45s, annealing for 30s and 72°C for 30s followed by 1 cycle of 72°C for 5 mins. PCR products (4µl) were run on a 1.2% agarose gel. To confirm that primers would amplify *Itersonilia* from different sources, PCR was carried out as described above using DNA from a collection of 30 diverse isolates (Table 2) provided by Lauren Chappell. To test primer specificity, PCR was then carried out using DNA from 14 other fungi isolated from parsnip seed and FOC isolate FUS2. Finally, qPCR was carried out using a dilution series of *Itersonilia* isolate IP35 ranging from 10 ng/µl-10 fg/µl and a limited number of non-target DNA samples from other fungi. qPCRs were carried out in 10µl reactions containing primers (0.4 µM unless stated), 5 µl SensiFAST™ SYBR® No-ROX Kit mastermix and 1 µl of DNA. Conditions were as follows: 1 cycle of 95°C for 3 mins followed by 45 cycles of 95°C for 5s and annealing for 10s and 72°C for 10s. All DNA dilutions were run in triplicate and a melt curve analysis carried out. Where a Taqman® probe was used, all reactions were run in triplicate with each reaction consisting of each primer (0.4 µM), probe (0.1 µM), 1 µl of DNA, and 5 µl of SensiFAST™ Probe No-ROX mix (Bioline). Cycling consisted of 1 cycle of 95°C for 3 mins followed by 45 cycles of 95°C for 5s and annealing for 30s.

Table 2: *Itersonilia* isolates and fungi from parsnip seed used for testing of specific PCR primers.

Isolate code	Fungal species	Isolated from	Source	Country
IP1	*I. pastinacae*	Parsnip Seed	Elsoms	England
IP2	*I. pastinacae*	Parsnip Seed	Elsoms	England
IP5	*I. pastinacae*	Parsnip Seed	NIAB/Elsoms	England
IP6	*I. pastinacae*	Parsnip Seed	NIAB/Elsoms	England
IP9	*I. pastinacae*	Parsnip Seed	Plant Health Services	England
IP10	*I. pastinacae*	Parsnip Seed	Elsoms	England
IP11	*Itersonilia sp*	Parsnip Root	Stockbridge House	England
IP12	*Itersonilia sp*	Parsnip Root	Stockbridge House	England

IP13	*Itersonilia sp*	Chrysanthemum	Stockbridge House	England
IP14	*Itersonilia sp*	Chrysanthemum	Stockbridge House	England
IP16	*Itersonilia sp*	Dill	Stockbridge House	England
IP23	*I. pastinacae*	Parsnip Seed	Elsoms	England
IP29	*I. pastinacae*	Parsnip Root	VCS	England
IP31	*I. pastinacae*	Parsnip Seed	Elsoms	N.Zealand
IP34	*I. pastinacae*	Parsnip Seed	Elsoms	N.Zealand
IP36	*I. pastinacae*	Fennel	Middlesex	England
IP37	*I. pastinacae*	Dill	Middlesex	England
IP38	*I. pastinacae*	Parsley	Middlesex	England
IP39	*Itersonilia sp.*	Parsnip Root	Cupar, Fife	Scotland
IP40	*Itersonilia sp.*	Parsnip Root	Elsoms	Sweden
IP41	*Itersonilia sp.*	Parsnip Root	Nottingham	England
IP50	*I. pastinacae*	Parsnip Seed	Elsoms	France
IP51	*I. pastinacae*	Parsnip Seed	Elsoms	France
NS1	*Alternaria alternata*	Parsnip Seed	Elsoms	England
NS2	*Cladosporium tenuissimum*	Parsnip Seed	Elsoms	England
NS3	*Penicillium chrysogenum*	Parsnip Seed	Elsoms	England
NS4	*Alternaria sp.*	Parsnip Seed	Elsoms	England
NS5	*Phoma fungicola*	Parsnip Seed	Elsoms	England
NS6	*Alternaria sp.*	Parsnip Seed	Elsoms	England
NS7	*Trichoderma hamatum*	Parsnip Seed	Elsoms	England
NS8	*Cladosporium cladosporoides*	Parsnip Seed	Elsoms	England
NS9	*Clladosporium cladosporoides*	Parsnip Seed	Elsoms	England
NS10	*Penicillium olsonii*	Parsnip Seed	Elsoms	England
NS11	*Alternaria infectoria*	Parsnip Seed	Elsoms	England
NS12	*Alternaria infectoria*	Parsnip Seed	Elsoms	England
NS13	*Fusarium chlamydosporum*	Parsnip Seed	Elsoms	England
NS14	*Sporobloromyces ruberrius*	Parsnip Seed	Elsoms	England

In order to compare molecular and conventional tests, seven infested seed lots (Elsoms Seeds, UK) were tested using a standard *Itersonilia* seed test on malt dextrose agar plates. Ten spots of petroleum jelly were spotted on to the lid of the petri dish (20 plates per seed batch) and seeds stuck to each spot, ensuring that the external surface faced the agar. The lids were replaced and plates incubated at 20°C +/- 2, 12:12hrs light: dark. After 5 days, the plates were examined for *Itersonilia* colonies underneath each seed. *Itersonilia* has a distinct appearance, the colony is off-white and ‘spikey’, the mycelium is very directional towards the light and will generally all be pointing the same direction. Where there were no *Itersonilia* colonies on the plates, seeds were also examined as *Itersonilia* can remain on the seed in the form of chains of chlamydospores, which have a distinctive ‘candy floss’ appearance.

For the molecular test, DNA was extracted from 200 seeds from each seed batch. These were initially placed in a sterile 50 ml tube, 25 ml of sterile water added and shaken vigorously for 1 min (by hand). The suspension was then sieved, collecting the liquid in a beaker and rinsing the tube (~10ml) and the seeds (~5 ml) with further sterile water. The filtrate was then poured into a sterile 50 ml tube, and the beaker rinsed with 5 ml of sterile water which was added to the 50 ml tube. Tubes were centrifuged at 4100 rpm for 15 min, the supernatant removed and the pellet resuspended in < 2 ml of liquid. This was then transferred to a 2 ml screw cap tube and centrifuged at 12,000 rpm for 10 min. The supernatant was transferred to a lysing matrix A tube (MPbio), 400 µl Buffer AP1 added and homogenisation carried out using a Tissuelyser (Qiagen) set at 30 Hz for 1 min (repeated). DNA was then extracted using a DNeasy plant mini kit (Qiagen), following the manufacturer's guidelines and eluting in 50 µl of elution buffer. PCR was then carried out using Iter COAT F1/R1 primers as previously described and products run on a 1.2% agarose gel. qPCR was then carried out using the same primer pair and the methods previously described. The results from the seed test and qPCR was compared using Pearson's correlation co-efficients in MS Excel.

1.15 Test a range of *S. cepivorum* isolates for the presence of published pathogenicity genes

Seven selected *S. cepivorum* isolates from diverse locations (Table 3) were screened for the presence / absence of putative effector genes using published primers for; acid protease 1, aspartyl protease, oxaloacetate acetylhydrolase, zinc finger transcription factor and polygalacturonase 1, 2, 5 and 6 (Andrew et al., 2012) with PCRs carried out as described in section 1.11. Amplicons were sequenced using Sanger sequencing (GATC Biotech).

Table 3. *S. cepivorum* isolates tested for presence / absence of pathogenicity-related genes.

Isolate	Location
GS1	Littleport, Cambs
RUG1-1	Rugby, Warks
RUG2-2	Rugby, Warks
JD1	Methwold Hythe, Norfolk
WR3	Wellesbourne, Warks
LG11	Loosegate, Lincs
WRAR13	Aswick Grange, Lincs
FRONTIER10	Holbeach, Lincs

1.16 Test the ability of sclerotia to germinate for a range of *S. cepivorum* isolates using an established assay based on diallyl disulphide

Sclerotia were produced on maize meal / sand (DEFRA, 2009) for seven *S. cepivorum* isolates (Table 3) with one isolate (GS 1) used initially to test the method. Fine sliver sand (60 g) was placed in a disposable 90 mm Petri dish and moistened with tap water to be just over saturated. Squares of nylon membrane were cut and placed onto the surface of the wet sand allowing the membrane to absorb the excess water present. Test sclerotia were placed in an 8 x 8 pattern onto the surface of the membrane and the Petri dish placed into a sealed plastic sandwich box (two Petri dishes per box, no lid) with a bijou bottle containing 0.5% diallyl disulphide (DADS, Sigma) in 1 ml of Triton X (5 g Triton X in 250 ml of sterile water). Control treatments (no DADs) were set up with a bottle containing 1 ml Triton X solution only. Boxes were incubated at 15°C in the dark with control boxes kept in a different location to the DADS boxes. Eruptive germination of sclerotia was recorded twice weekly for approximately eight weeks. During the recording period, the sand in the Petri dishes was maintained at a high moisture level at all times.

Due to some contamination of the sand with other fungi using this method, and also with a modified approach where the experiment was set using either autoclaved or non-autoclaved silver sand / horticultural grit, it was found that sterile vermiculite (4.8 g of 3 x autoclaved fine vermiculite per Petri dish) was the best medium to use for sclerotial germination assay. This method was then used to determine the effect of both a conditioning treatment and DADS on the sclerotial germination of seven *S. cepivorum* isolates. For the conditioning treatment, sclerotia produced as described previously were placed in nylon mesh bags and buried in 400 g of silty clay loam soil (air dried, sieved to 4mm, Dunnington Heath Series, Wellesbourne) in plastic boxes and water added to achieve 22% moisture. Boxes were incubated at 15°C in the dark and after 12 weeks, mesh bags were removed and sclerotia retrieved by washing over 500 and 212 µm sieves under running tap water. Sclerotia retained in the 212 µm sieve were gently rubbed with a rubber bung to eliminate those that were soft or degraded. Two Petri dishes containing a total of 64 sclerotia (8 x 8 grid) were tested for each *S. cepivorum* isolate / treatment combination and germination recorded twice a week. Percentage germination data after 10 d and seven weeks were then analysed using ANOVA in Genstat.

8. Isolate and confirm identity of the causal agent of onion pink root disease

Onions with typical pink root symptoms were observed at Wellesbourne (Fig. 1) and fungal isolations carried out by washing roots, surface sterilising in 70% ethanol for 1 min, rinsing

twice in sterile water then plating 1 cm sections on PDA containing 20 µg/ml chlorotetracycline. Molecular identification was carried out by PCR (as previously described, primer sequences in Table 4), initially using ITS1F and ITS4 primers (Gardes and Bruns, 1993) and subsequently using NS1 / NS4 primers (White et al., 1990) to amplify the 18S gene and LR7/LROR primers to amplify the 28S gene (Vilgalys and Hester, 1990). To fully resolve species identity, a concatenated alignment of 18S and 28S sequences was created and a maximum likelihood tree constructed using MEGA version 7.

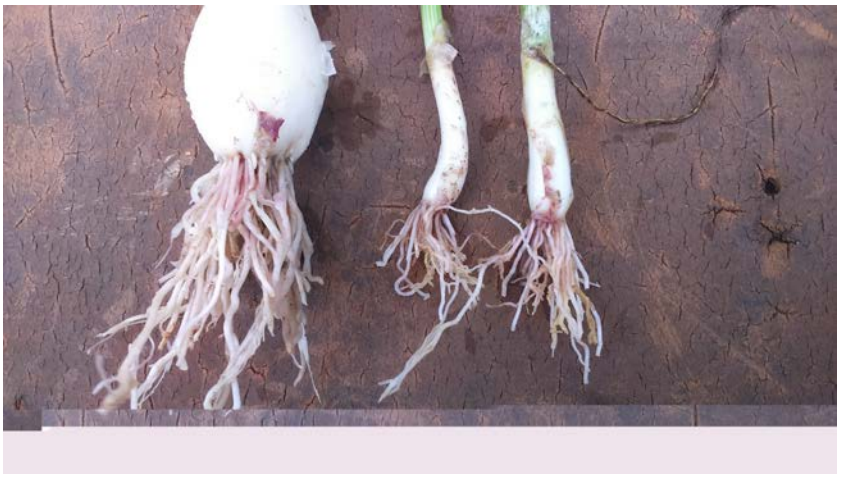


Figure 1: Pink root symptoms observed in onion plants used for isolation of *Setophoma terrestris*.

Table 4. Primer sequences and respective annealing temperatures.

Name	Sequences (5'-3')	Annealing temp. (°C)
NS1/NS4	GTAGTCATATGCTTGTCTC / CTTCCGTCAATTCCTTTAAG	48
LR7/LROR	TACTACCACCAAGATCT / ACCCGCTGAACTTAAGC	48
ITS1F/ITS4	CTTGGTCATTTAGAGGAAGTAA / TCCTCCGCTTATTGATATGC	55
FON qSIX13 F/R	ACAGCACGGGACAGCTTACA / CGTCAGAGGGGTAGCCACAT	60

2.3 Develop appropriate plant infection tests and confirm virulence of various pathogens of onion

Downy mildew

Onions with symptoms of downy mildew were obtained from four different field sites: Rugby (two different fields), Luddington and Wellesbourne. Plants were transplanted into pots and placed in an 'infection tent' within a cooled (20°C) glasshouse to maintain 100% humidity and induce sporulation. A humidifier was placed in the tent and set to run for 30 min at the start of the dark period and 30 min halfway through the dark period. Following one overnight period, spores were collected using two different methods. The first method used a vacuum pump to remove the spores from the leaves which were then stored at -20°C. The second involved excising leaf sections with sporulation, placing in tubes and storing at -20 or -80°C. Fresh spores collected by the vacuum method were also used to re-infect healthy plants. Here, a spore suspension was prepared in water and 0.01% Tween and adjusted to 3×10^4 spores/ml using a haemocytometer after which it was sprayed onto onion plants until run off. Leaf wetness was then maintained (humidifier on constant) for 24 hours to allow infection, after which plants were removed from the tent and observed for symptoms after 7-14 days. Plants with downy mildew symptoms were replaced back in the tent and sporulation induced as previously described.

Pink root

Inoculum was produced on a sterile compost and wheat bran mix for two *S. terrestris* isolates (P1 and P4, both from Wellesbourne) as described previously for FOC (Taylor et al, 2013) and the number of colony forming units per g calculated following dilution plating. Inoculum was then mixed with Levington's F2 compost to give final concentrations of 1×10^4 and 1×10^5 cfu/g. The infested compost mix was then used to fill 7 cm pots (six pots per concentration for P4 and 12 pots for P1). Control pots (12) were filled with clean F2 compost only. Five week old onion seedlings (cv. Hytech) were then transplanted into the pots which were placed in a randomised block design in a temperature controlled glasshouse (25°C day, 18°C night) for four weeks. Plants were then removed and scored for the presence / absence of pink roots.

White rot

To be completed in year 5.

Additional work: Determine the expression of FON SIX genes in planta

Narcissus bulbs (cv. Carlton) were obtained and any with disease symptoms discarded. All outer dried scales were removed and inner scales separated to obtain 1-2 mm thick tissue sections which were cut into 2 x 2 cm squares and surface sterilised by immersing in 70% ethanol for 2 mins before rinsing twice in sterile distilled water. Scale sections were placed in sealed clear plastic boxes (three per box) on moist chromatography paper (Whatman 3MM, Fisher Scientific, UK). FON isolate FON63 (containing SIX7, SIX9, SIX10, SIX12, SIX13 genes) was grown on PDA for 7 d and 5 mm mycelial plugs removed from the actively growing edge using a cork borer which were then placed mycelium side down in the centre of each of the detached scales. Mock inoculations were also set up using plugs of PDA. Four replicate samples were taken each day from 0-6 dpi by removing a 10 mm piece of scale tissue from around the inoculation point with a cork borer. Agar plugs were then removed and discarded and the tissue samples flash frozen in liquid N before storing at -80°C. RNA was extracted using the Spectrum plant total RNA extraction kit (Sigma) following the manufacturers guidelines for a starchy storage organ. DNA was removed using DNase I (Sigma) and first strand cDNA synthesized from 300 ng of RNA using Superscript II reverse transcriptase (Life Technologies) following the manufacturers' guidelines. The expression of the five SIX putative effector genes as well as two housekeeping genes (EF1α and β-TUB) was quantified using previously published primers and cycling conditions (Taylor et al, 2016) with the exception of SIX13 where new primers were designed (FON qSIX13 F/R, Table 4). Quantitative PCR was carried out using a Roche Lightcycler 480 and expression determined using a standard curve method and normalized to the geometric mean of EF1α and β-TUB.

Additional work: development of LAMP assays for FOC and *Itersonilia*

<u>FOC</u>

Two sets of specific primers were designed for FOC, both based on the SIX5 gene. One set (six primers) was designed using the package Lamp Designer (Premier Biosoft) and the other set (four primers) using the free online tool Primer Explorer (https://primerexplorer.jp/e/). Primer mixes were made up as follows; F3 / B3 primers at 2 µM, LoopF / LoopR primers at 4 µM; FIP / BIP primers at 16 µM and used in a 25 µl LAMP reaction mix containing 15 µl of isothermal mastermix (Optigene), 2.5 µl of primer mix and up to 5 µl of DNA. Assays were run in a Genie II LAMP instrument (Optigene) at 65°C for up to 90 minutes and included a melt curve step of 98°C for 1 min decreasing to 80°C by 0.05°C per second. Initial testing was carried out using a dilution series of FUS2 DNA (10ng/µl – 0.01ng/µl). DNA extractions were then carried out from clean and infected onion root, bulb and basal plate material using

a plant DNA extraction kit (Optigene), following the manufacturers guidelines. DNA extractions were also carried out from a soil sample from the FOC area of the quarantine field at Wellesbourne using either the plant kit or a KOH method as detailed in the manufacturer's instructions for the LNL isothermal mastermix (Optigene). For KOH extractions, the LNL mastermix was used as opposed to the isothermal mastermix. DNA extracted from quarantine field soil from a different area was used as a positive control.

Itersonilia

A set of primers was designed, based on Acetyl Co-A transferase (COAT), using the Lamp Designer package and reactions set up as described for FOC. DNA was extracted from a parsnip seed batch known to be infected with *Itersonilia* (~50% infection) using a Plant extraction kit. Initially, this was attempted using 10, 20 or 30 seeds while DNA extractions were subsequently carried out from seven seed batches using 30 or 40 seeds per extraction. Finally, a larger scale extraction was attempted using 100 seeds and also increasing the recommended volume of lysis buffer.

Results

1.2 Extract DNA, prepare libraries and carry out whole genome sequencing of *F. oxysporum* f.sp. *cepae* (FOC) isolates / 1.3 Bioinformatic analyses of FOC isolate genomes and identification of potential primers for FOC diagnostics

A high quality genome sequence of FUS2 was generated using PacBIo long read sequencing and a robust genome assembly allowed confirmation of SIX gene presence / absence and identification of potentially novel effectors. The presence of SIX3, 5, 7, 9, 10, 12 and 14 and absence of all other SIX genes in FOC was confirmed as reported by Taylor et al. (2016) but two copies of both SIX3 and SIX9 were observed in the genome. The two copies of SIX3 were identical while the two copies of SIX9 differed by only 3 base pairs. As there was only a single copy of SIX5 and this gene has only previously been identified in FOL, this provided a good target for designing FOC diagnostic primers. When tested, the primers were specific to FOC with no amplification of DNA from any of the other fungi / oomycetes tested with the exception of an *F. oxysporum* isolate from leek which has been subsequently shown to be pathogenic on onion and hence designated as FOC (Fig. 2). Results from the qPCR showed the same level of specificity (data not shown).

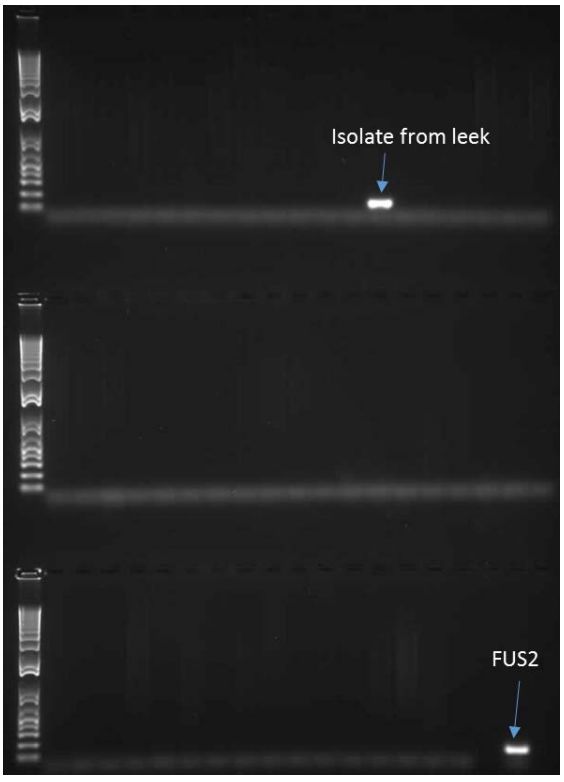


Figure 2. Gel electrophoresis image showing results of PCR using FOC SIX5 primers against a range of fungi. All lanes with no band were non-target species as listed in Table 1.

1.11 Test *Itersonilia* primers in vitro / 1.12 Test the newly developed *Itersonilia* diagnostic test on infected parsnip seed lots and compare with the industry standard agar plate test.

A range of different potential specific primers for *Itersonilia* were tested in different types of PCR assay (summarised in Table 5) with the best results observed for primer pair Iter COAT F1/R1 targeting the acetyl co-A transferase gene. These newly developed primers produced a strong, single product from all *Itersonilia* isolates tested (Fig. 3) and showed a high level of specificity. Some very weak non-specific amplification was noted for FOC isolate FUS2 but it should be noted that this was a crude extract DNA sample.

Table 5. Summary of PCR primers tested as a potential *Itersonilia* diagnostic assay.

Primer pair	Target gene	Annealing temp. (°C)	Assay type	Result
Iter FOR2 / REV2	tRNA methyl transferase	64	SYBR	Failed specificity test
Iter FOR2/REV2	tRNA methyl transferase	62	Taqman®	Poor amplification
Iter FOR3/REV3	tRNA methyl transferase	60	SYBR	Failed specificity test
Iter FOR3/REV3	tRNA methyl transferase	60	Taqman®	Poor amplification
Iter TEF	Translation elongation factor 1-α	62	SYBR	Do not amplify from all isolates
Iter COAS F2/R2	Acetyl Co-A synthase	65	SYBR	Mixed PCR product
Iter COAT F1/R1	Acetyl Co-A transferase	63	SYBR	Working assay

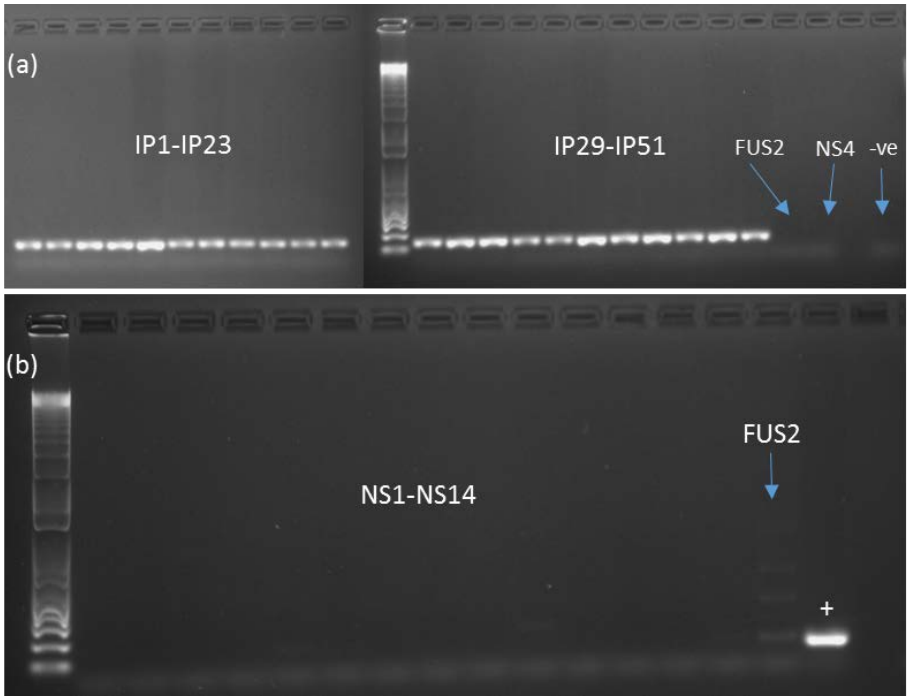


Figure 3. PCR testing of COAT F1/R1 primers against (a) 22 *Itersonilia* isolates and (b) a range of non-specific (NS) DNA samples from fungi commonly found on parsnip seed.

The newly developed primers also resulted in an efficient qPCR assay with quantification over a wide dilution series with a single peak in the melt curve (Fig. 4). The assay had low error (0.019 where <0.1 is desirable), a very high efficiency (1.96 where 2 is optimal) and a good slope (-3.42, where an assay with 100% efficiency has a slope of -3.32).

The DNA extracted from different parsnip seed batches was tested for *Itersonilia* using the newly developed assay. A range of DNA quantities were obtained, ranging from 0.7-111.8 pg/µl (Table 6). Using data generated from the standard seed test carried out both in house and at Elsoms Seeds, there was a partial correlation (r=0.50 / 0.53, Fig. 5) with the molecular test, but this was not statistically significant. The correlation was incomplete mainly due to unexpected seed test and / or qPCR test results associated with seed batches 2 (low DNA detection, 46% infection on Elsoms test) and 7 (low DNA detection but high infection on in-house test).

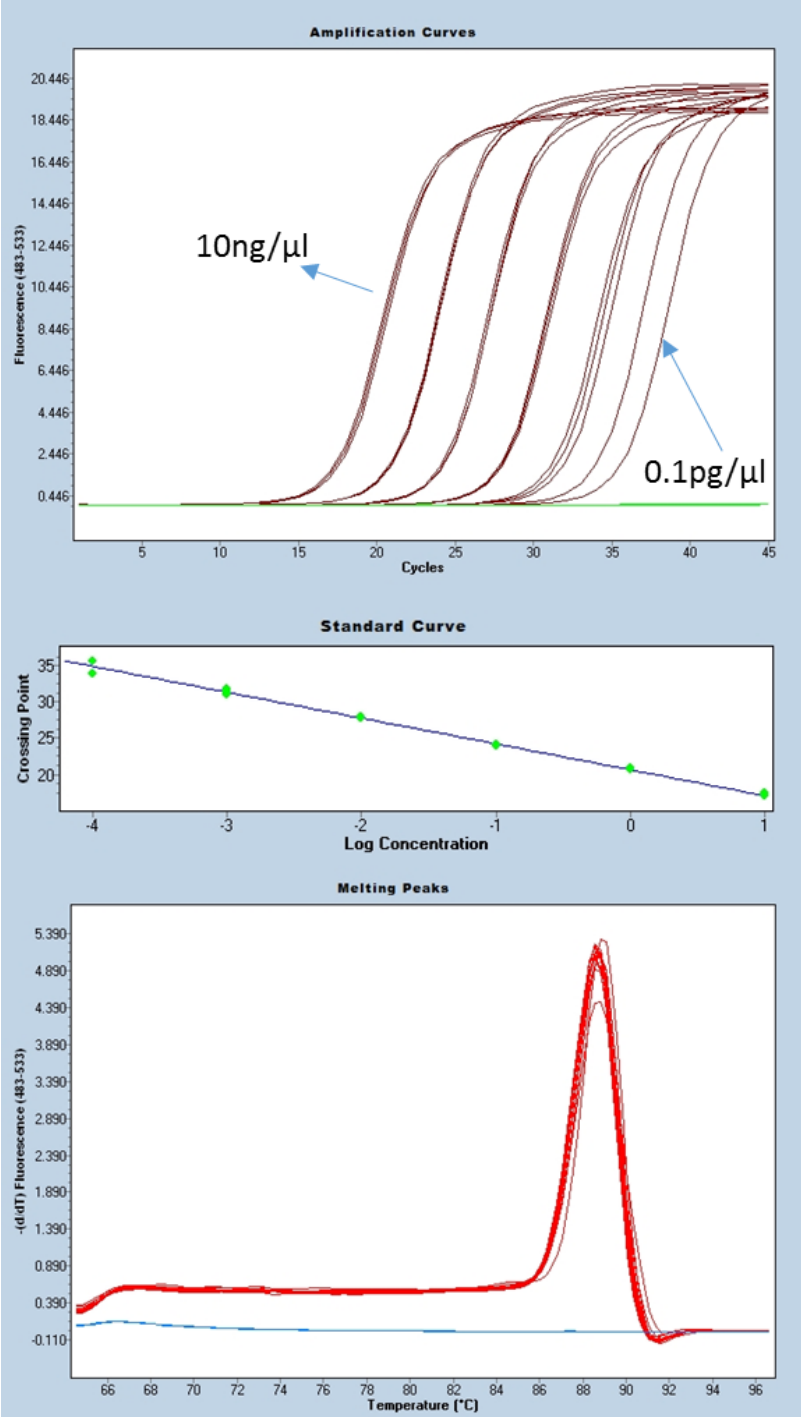


Figure 4. Quantitative PCR amplification, standard curve and melt peaks for COAT F1/R1 primers using a dilution series of *I. pastinacae* isolate IP34 DNA as a template.

Table 6. Quantification of *Itersonilia* DNA in seven parsnip seed batches using qPCR. Where a non-target organism was included, 1µl genomic DNA (10ng/µl) was used per reaction.

Sample	source	Inferred DNA quantity (pg)	% seed infection	% seed infection Elsoms
1	parsnip seed	79.6	87.5	77
2	parsnip seed	0.7	1.5	46
3	parsnip seed	39.2	10.5	0
4	parsnip seed	58.4	2.5	0
5	parsnip seed	38.7	0	14
6	parsnip seed	111.8	74.5	61
7	parsnip seed	0.1	61.5	2
NS3	*P. chrysogenum*	0.1		
NS13	*A. infectoria*	0.0		
GS1	*S. cepivorum*	0.0		
HL	*P. violae*	0.0		
MA6	*M. acerina*	0.0		
Blank	Sterile water	0.0		

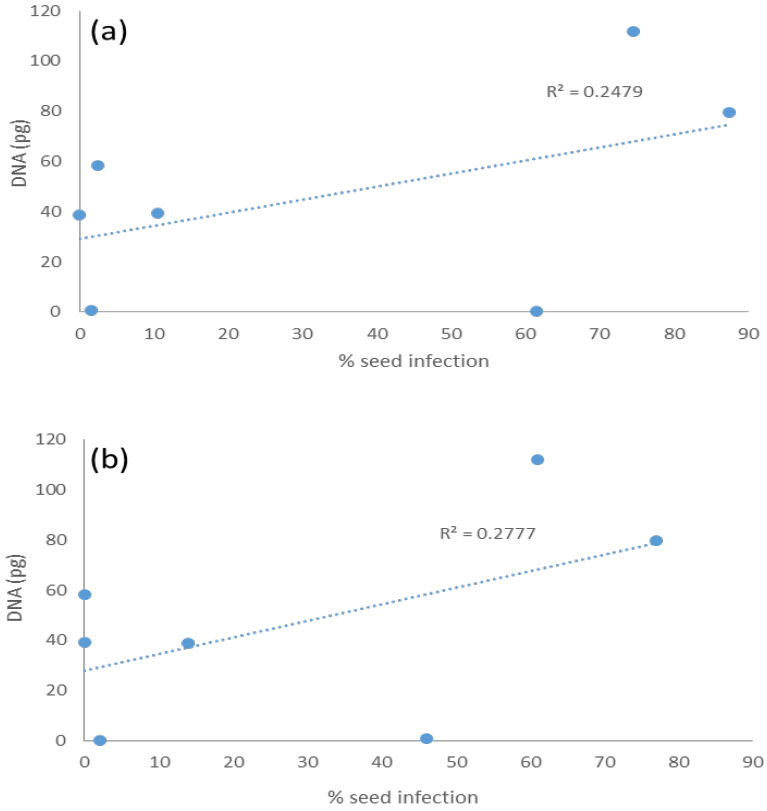


Figure 5. Correlation between *Itersonilia* DNA concentration detected by qPCR and percentage seed infection as measured by the standard seed test carried out either (a) in house or (b) by Elsoms Seeds.

1.15 Test a range of *S. cepivorum* isolates for the presence of published pathogenicity genes

All *S. cepivorum* isolates were found to contain at least six of the eight published pathogenicity genes (Table 7). Multiple bands were observed for the remaining two genes so it was possible that these are also present. The pattern of presence / absence of these genes for UK isolates was very similar to isolate LMK1 from the Netherlands but was different from isolate LMK71 from the USA which lacks PG3 and PG6.

Table 7. Presence / absence of a set of pathogenicity-related genes in isolates of *S. cepivorum*.

Isolate	Location	PG3	PAC1	PG1	ASPS	ACP	PG5	PG6	OAH
GS1	Littleport, Cambs	+	+	M	+	M	+	+	+
RUG1-1	Rugby, Warks	+	+	M	+	M	+	+	+
RUG2-2	Rugby, Warks	+	+	M	+	M	+	+	+
JD1	Methwold, Norfolk	+	+	M	+	M	+	+	+
WR3	Wellesbourne, Warks	+	+	M	+	M	+	+	+
LG11	Loosegate, Lincs	+	+	M	+	M	+	+	+
WRAR13	Aswick Grange, Lincs	+	+	M	+	M	+	+	+
FRONTIER10	Holbeach, Lincs	+	+	M	+	M	+	+	+
LMK71*	USA	-	+	+	+	-	+	-	+
LMK1*	Netherlands	+	+	+	+	-	+	+	+

*Andrew et al (2012); M=multiple bands observed on gel. ACP, acid protease 1; ASPS, aspartyl protease; OAH, oxaloacetate acetylhydrolase; PAC1, zinc finger transcription factor; PG, polygalacturonase.

Sequences were obtained for all *S. cepivorum* PCR amplicons and phylogenetic trees constructed including sequences from other *Sclerotinacae* fungi. Sequences of the pathogenicity genes were identical across all of the UK isolates while the phylogeny based on ASPS separated *S. cepivorum* from other *Sclerotinacae* species. UK *S. cepivorum* isolates also had identical sequences to isolates *S. cepivorum* LMK1 and LMK71 from the USA and Netherlands respectively (Fig. 6).

The phylogenies based on both PG6 (Fig. 7) and PG3 (Fig. 8) separated *S. cepivorum* isolates from other *Sclerotinacae* with the UK isolates clustering with LMK1 (Netherlands). A single base difference was observed for both genes.

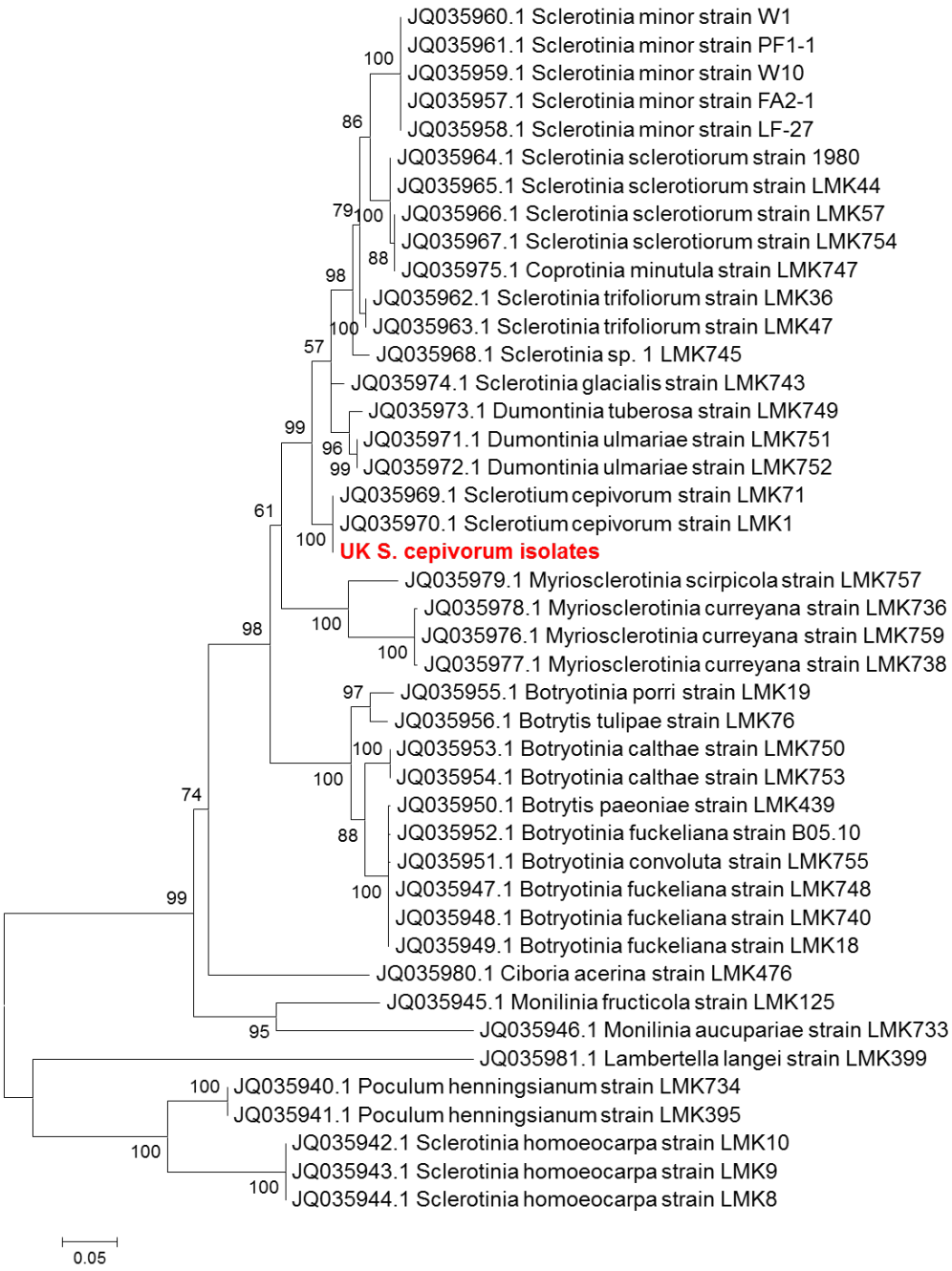


Figure 6. Maximum likelihood phylogenetic tree of *S. cepivorum* isolates and other *Sclerotinacae* species isolates based on aspartyl protease gene (ASPS). Numbers represent bootstrap values (1000 replicates). Scale bar indicates 0.05 substitutions per site.

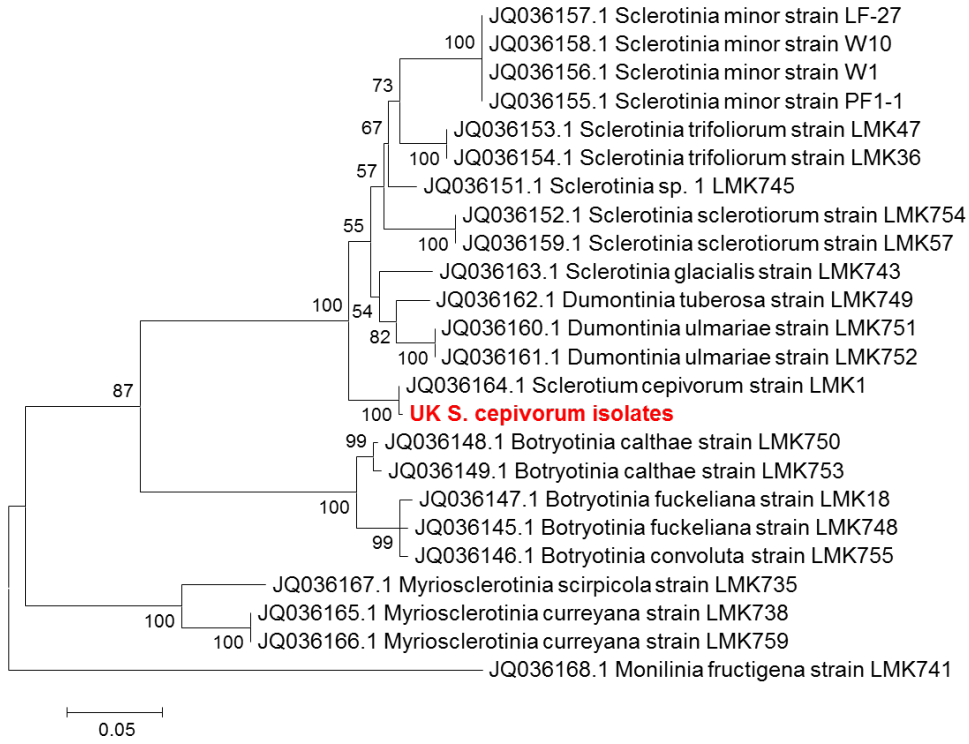


Figure 7. Maximum likelihood phylogenetic tree of *S. cepivorum* isolates and other *Sclerotinacae* species based on polygalacturonase PG6. Numbers represent bootstrap values (1000 replicates). Scale bar indicates 0.05 substitutions per site.

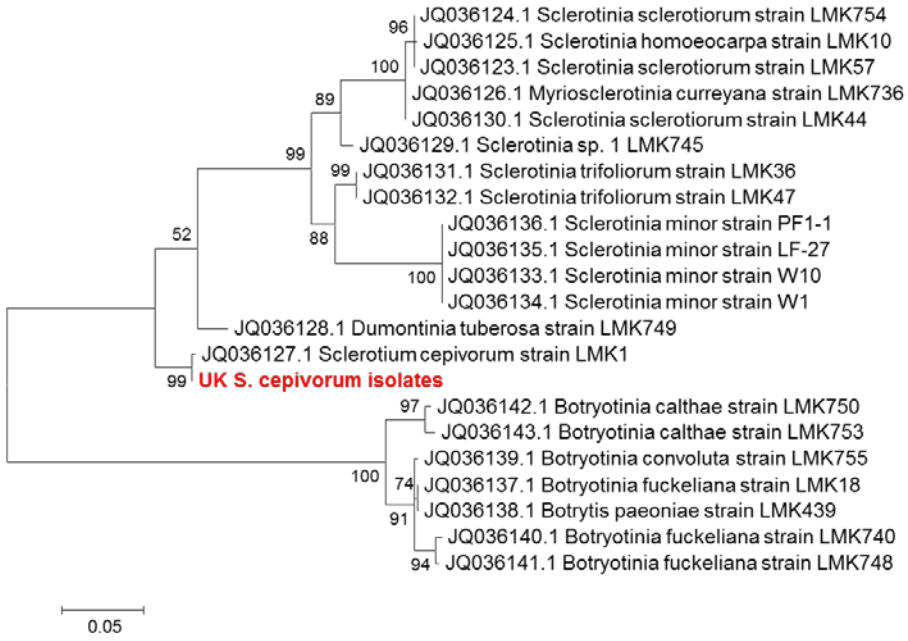


Figure 8. Maximum likelihood phylogenetic tree of *S. cepivorum* isolates and other *Sclerotinacae* species based on polygalacturonase PG3. Numbers represent bootstrap values (1000 replicates). Scale bar indicates 0.05 substitutions per site.

1.16 Test the ability of sclerotia to germinate for a range of *S. cepivorum* isolates using an established assay based on diallyl disulphide

Significant differences in germination of sclerotia was observed between *S. cepivorum* isolates at both 10 and 49 days (P <0.001, Fig. 9). All isolates exhibited a high level of germination with WR3 the highest for all treatments, reaching a mean final percentage germination of almost 100%. After 10 days, there was a significant effect of both conditioning and DADS on percentage germination observed across all isolates (P<0.001). However, after 49 days, whilst there was still a strong effect of conditioning (P<0.001), the effect of DADS was not significant. Indeed, sclerotia which had been conditioned but not exposed to DADS exhibited higher germination levels than those that had been exposed to DADS treatment (significant at 5% level but not at 1% level). *S. cepivorum* isolate WR3 exhibited a high level of germination, even without conditioning and was selected for subsequent pathogenicity assay development.

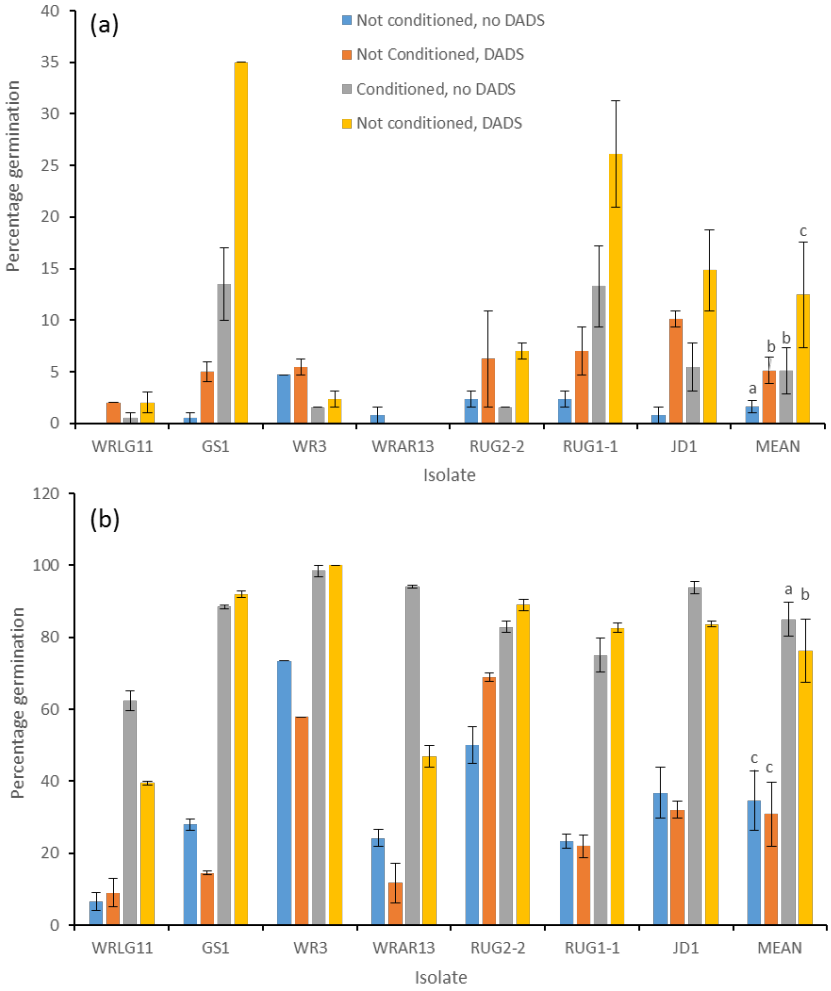


Figure 9. Germination of *S. cepivorum* sclerotia after (a) 10 days and (b) 7 weeks for different combinations of conditioning and DADS treatments. Error bars represent the SEM. Letters indicate a significant difference following ANOVA analysis.

8. Isolate and confirm identity of the causal agent of onion pink root disease

Visual examination of fungal colonies isolated on PDA from onion roots with pink root symptoms identified putative isolates of *Setophoma terrestris* and this was confirmed by molecular analysis. Identification based on ITS sequence was ambiguous but was clear using 18S / 28S (Fig. 10). The concatenated phylogenetic tree based on 18S and 28S sequences clearly identified the new isolates as *Setophoma terrestris* and these were identical to CBS strains from Senegal, North America and one of unknown origin.

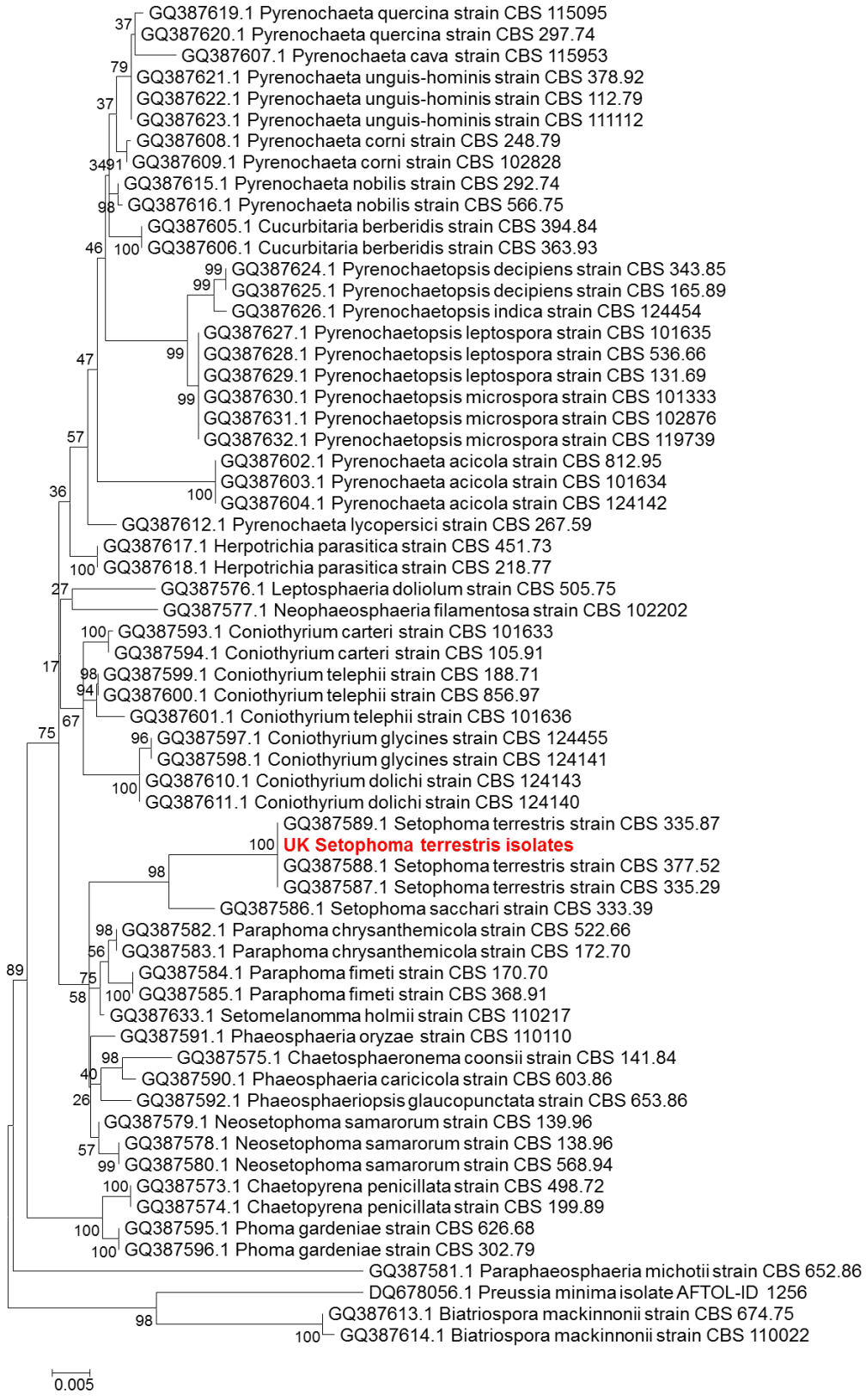


Figure 10. Maximum likelihood phylogenetic tree of *Paraphoma, Pyrenochaeta* and *Pyrenochaetopsis* species based on concatenated 18S/28S sequences. Numbers represent bootstrap values (1000 replicates). Scale bar indicates 0.005 substitutions per site.

2.3 Develop appropriate plant infection tests and confirm virulence of various pathogens of onion

Downy mildew

P. destructor isolates collected and frozen at -80°C were not viable after only one month in storage. However, when freshly collected *P. destructor* spores were used for inoculation (as described previously), typical downy mildew symptoms were observed and sporulation occurred after plants were exposed overnight to high humidity conditions. The approach for spore collection and infection can therefore be used for future pathogenicity / resistance testing.

Pink root

Pink root symptoms were successfully induced in all onion plants following inoculation of the compost with both *Setophoma terrestris* isolates at both concentrations (Fig. 11). All control plants had healthy root systems with no signs of infection.

Figure 11: Symptoms of pink root disease of onion following inoculation with *Setophoma terrestris*. (a) non-inoculated control (b) isolate PQF1 (c) isolate PQF4.

Additional work: Determine the expression of FON SIX genes *in planta*

All five SIX genes were expressed in a similar pattern *in planta* following inoculation of daffodil bulb tissue with FON (Fig. 12). As an example, significant upregulation of SIX7 was observed with a peak of expression at 3 dpi, a time-point where browning symptoms were just developing in the scale tissue. However, by the end of the timecourse (6 dpi) when tissue had become very necrotic, all SIX genes exhibited reduced expression levels.

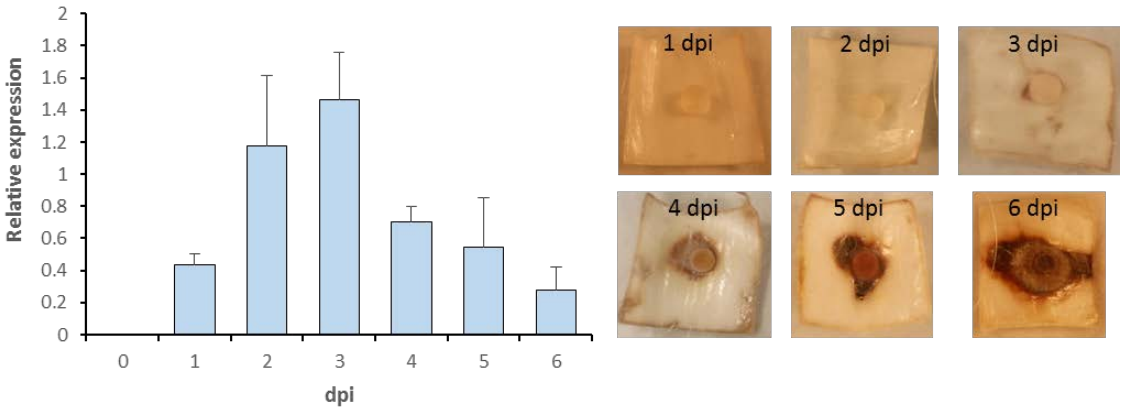
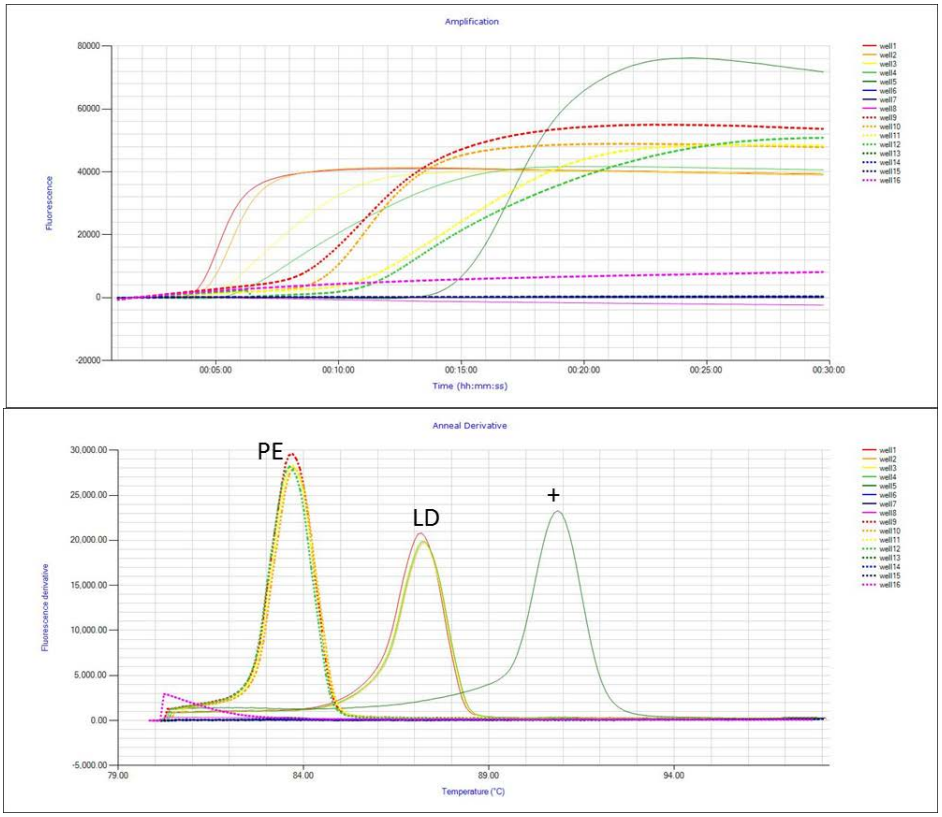


Figure 12. Relative expression of SIX7 for *F. oxysporum* f.sp. *narcissi* on detached *Narcissus* scales from 0-6 dpi. Error bars represent the SEM of four independent replicates.

Additional work: development of LAMP assays for FOC and *Itersonilia*

FOC LAMP assay

Primer sets designed using both Lamp Designer (LD) and Primer Explorer (PE) software resulted in successful LAMP assays for FOC (Fig. 13). LD primers produced an earlier peak in the reaction, after only 5 min for the most concentrated DNA sample compared to 10 min with the PE primers. This was consistent with the theory that a six primer set should be more efficient than four primers. However, a peak was also observed for FOL with the LD primers after 18 min with a 10 ng/µl DNA sample suggesting that these primers lack specificity. However, a reaction peak for FOL was not observed for the PE primers and hence these primers could be used for a FOC specific assay.



Well	Sample	time
1	10ng	05:00
2	1ng	05:30
3	0.1ng	06:00
4	0.01ng	07:30
5	+ve control	16:15
6	6	
7	7	
8	-ve control	
9	10ng	10:15
10	1ng	10:30
11	0.1ng	12:45
12	0.01ng	12:45
13	13	
14	14	
15	15	
16	-ve control	

Figure 13. LAMP assay development for FOC. Amplification traces (top) showing accumulated florescence (related to presence of FOC DNA) over time and melt peaks (bottom) are shown for PE and LD primers.

The PE primer assay was used to test DNA extracted from a range of plant and soil samples for the presence of FOC. DNA from FOC infected bulb and basal plate samples resulted in clear, early amplification of FOC DNA (Fig. 14) while two of the three soil DNA samples (from the Wellesbourne Fusarium quarantine area), known to be positive for FOC based on conventional PCR carried out previously, were also positive in this assay. A sample from infected roots was also positive for FOC DNA but with quite late amplification. All symptomless onion tissue and all crude soil extracts (KOH extraction) were negative for the presence of FOC.

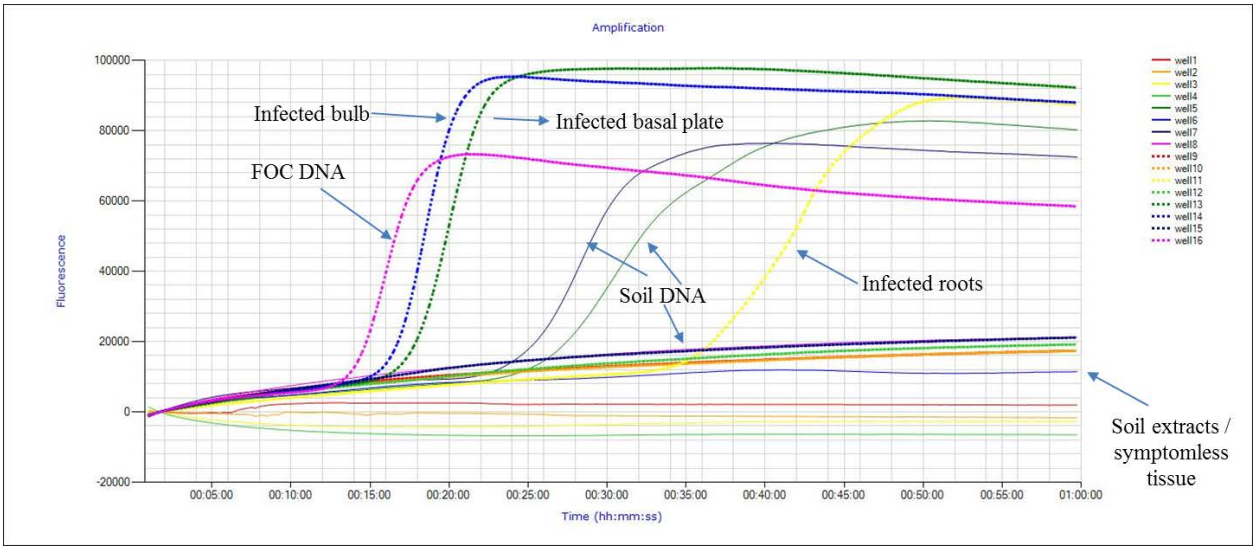


Figure 14. FOC LAMP assays using bulb, root, basal plate and soil samples. Amplification traces represent accumulated florescence related to presence of FOC DNA over time.

Itersonilia LAMP assay

The *Itersonilia* LAMP assay was initially tested using a dilution series of isolate IP34 DNA and was found to be effective (data not shown). When DNA from 30, 20 and 10 seeds was extracted from a parsnip seed lot with 62% *Itersonilia* infection was tested, earlier amplification of *Itersonilia* DNA was observed as expected for extracts from increasing numbers of seeds (Fig. 15).

However, when seven parsnip seed lots with different levels of *Itersonilia* infection were tested using DNA extracts from differing numbers of seeds, some conflicting results were observed (Table 8). The highly infected seed lot 1 (62% infection) was consistently positive in the LAMP assay irrespective of seed number with an amplification times of 28-32 mins. Seed lot 2 (20% infection) was also positive for all seed numbers but with a much later amplification for 100 seeds (52 min) than for 30 and 40 seeds (31-32 min). Seed lot 5 (25% infection) was positive for *Itersonilia* DNA for assays with 40 and 100 seeds (41 min) but not for 30 seeds. Seed lots 3 (67% infection) and 4 (51% infection) were badly affected by non-specific amplification. Seed lots with the lowest level of infection (7 and 8) generally tested negative. In summary, as was the case for the qPCR assay, an incomplete correlation was observed between percentage *Itersonilia* infection in the seed tests and the results of the LAMP assays.

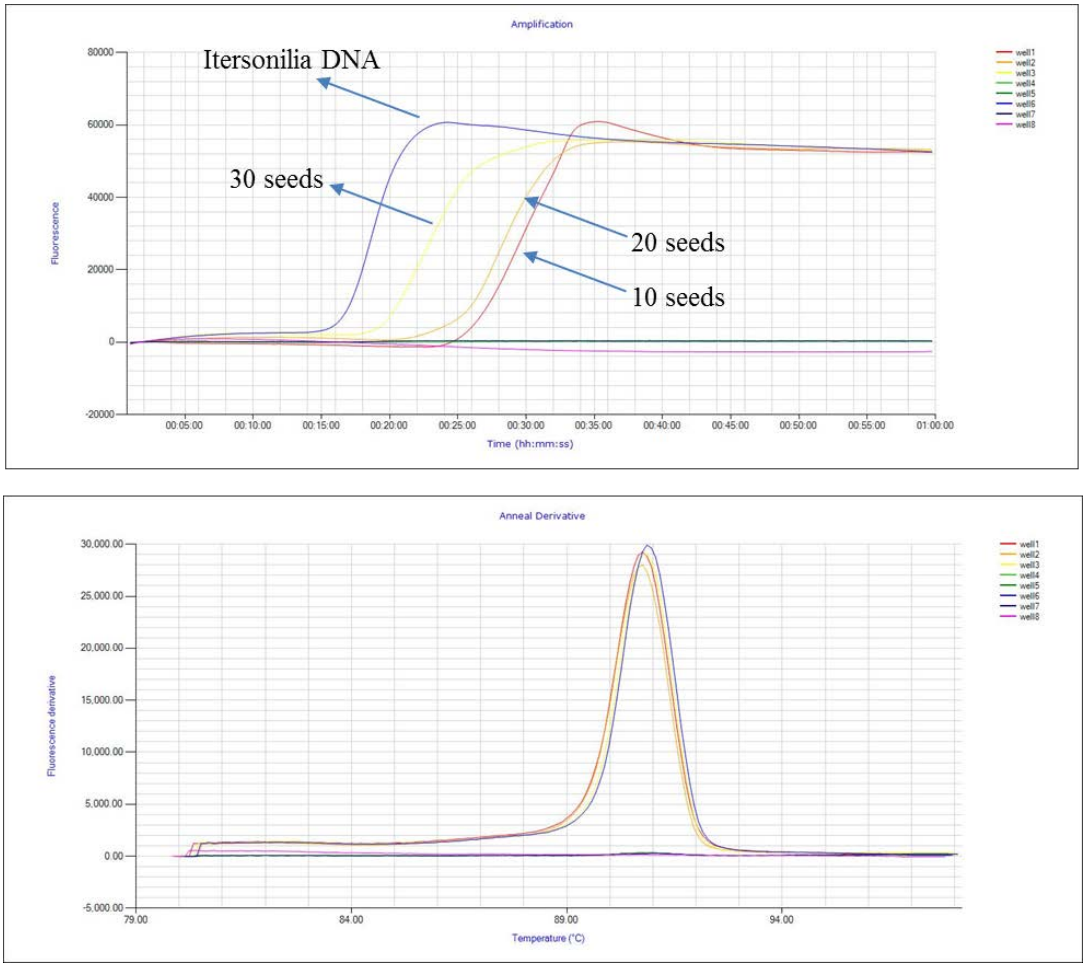


Figure 15. LAMP assays for *Itersonilia* infected parsnip seed. Amplification traces represent accumulated florescence related to presence of *Itersonilia* DNA (top) over time and melt peaks confirm identification as *Itersonilia* (bottom).

Table 8. Amplification times for different parsnip seed lots infected with *Itersonilia* for the LAMP assay compared to results from standard seed tests (data from Elsoms seeds).

Seed lot	30 seeds	40 seeds	100 seeds	Seed infection (%)
1	28:45	28:45	31:45	62
2	31:00	32:15	51:45	20
3	NS peak	NS peak	NS peak	67
4	NS peak	37:00	NS peak	51
5	-	41:00	41:00	25
6	-	-	NS peak	18
7	-	39:00	NS peak	17

NS = non-specific, non-target DNA

Discussion

1.2 Extract DNA, prepare libraries and carry out whole genome sequencing of *F. oxysporum* f.sp. cepae (FOC) isolates / 1.3 Bioinformatic analyses of FOC isolate genomes and identification of potential primers for FOC diagnostics

A high quality, long-read assembly of the FOC isolate FUS2 genome has been greatly beneficial for diagnostic development. PCR primers were designed based on the SIX5 single copy pathogenicity gene which is present in all FOC isolates and absent in all non-pathogenic *F. oxysporum* (Taylor et al. 2016). Furthermore, with the exception of FOL, no other *F. oxysporum* f.sp. or any other fungal species has been reported to contain homologues of SIX5. Specificity testing confirmed that the PCR assay only amplifies DNA from FOC and preliminary testing from soil and onion bulbs affected by basal rot has suggested that this diagnostic shows promise for detection of the pathogen in seed, sets, bulbs or soil and this will be tested further. A FOC-specific assay based on the SIX3 gene has been previously reported (Sazaki et al 2015). However, the FOC genome analysis revealed two copies of SIX3 which may mean that some isolates may only carry a single copy, complicating any quantitative analyses. In addition, when we tested this SIX3 based assay, amplification was inefficient, possibly suggesting some secondary structure in the product (data not shown).

1.11 Test *Itersonilia* primers in vitro / 1.12 Test the newly developed *Itersonilia* diagnostic test on infected parsnip seed lots and compare with the industry standard agar plate test.

The newly developed ITER COAT primers showed good potential for a specific diagnostic for *Itersonilia*. Primers appear to be specific and amplified from all isolates of *Itersonilia* tested, including isolates from different countries and plant hosts. Further specificity testing with a wider range of fungi is required to confirm this, but DNA from other organisms commonly associated with parsnip seed was not amplified. Despite good specificity, there was a lack of complete correlation between the qPCR assay and the conventional seed test. Some samples had a positive PCR and a negative seed test which might be explained by the presence of dead *Itersonilia* on seeds. However, other samples were negative in the PCR but positive for the seed test which is difficult to explain. It may be that the delay between the seed test being carried out and the DNA extraction allowed the *Itersonilia* levels to decline which has been observed previously. More testing will be carried out on further batches of parsnip seed in order to try and define a more complete correlation between seed test and

qPCR. If this were achieved, then such a rapid molecular test could potentially save seed companies both time and money as conventional seed testing can be very labour intensive.

1.15 Test a range of *S. cepivorum* isolates for the presence of published pathogenicity genes

All UK isolates of *S. cepivorum* contained the same compliment of pathogenicity genes and the sequences of these genes were all identical. This suggests a general lack of variation in the pathogen and a clonal origin for *S. cepivorum* in the UK. Whilst the genetic variability of *S. cepivorum* has not been extensively studied, genetically distinct clones have been identified and evidence for clonal spread of the pathogen has been previously documented (Couch and Kohn, 2000). As there is also no sexual stage of the pathogen the development of resistance to fungicides is likely to be slower. UK *S. cepivorum* isolates were very closely related to an isolate from the Netherlands (Andrew et al. 2012), suggesting a common origin. In contrast, a USA isolate lacked two of these pathogenicity genes, potentially suggesting a different divergent pathogen population.

All UK isolates of *S. cepivorum* also contained genes coding for aspartyl protease (ASPS) and an acid protease (ACP1). These have been shown to be expressed in *S. sclerotiorum*, during early infection and also accumulate during the expansion of lesions (Poussereau et al. 2001a and 2001b). A similar role for these genes in *S. cepivorum* is therefore hypothesised. The *S. cepivorum* isolates also contained polygalacturonase genes which again have been reported to be important in early infection of plants by *S. sclerotiorum* (Kasza et al 2004). OAH genes are involved in the production of oxalic acid in filamentous fungi (Andrew et al. 2012) which has been shown to be a major pathogenicity factor in *S. sclerotiorum* (Dickman and Mitra 1992). Both *S. cepivorum* isolates described by Andrew et al. (2012) were shown to produce high levels of oxalic acid and a role in pathogenicity is therefore highly likely. Finally, another gene (PAC1), shown to be present in all *S. cepivorum* isolates is a transcription factor controlling the expression of other genes involved in pathogenicity (Andrew et al 2012). In summary, isolates of *S. cepivorum* from the UK likely use a set of pathogenicity genes common with many other members of the *Sclerotinacae* to infect plants. This suggests that *S. cepivorum* has the 'tools' to infect a range of plant species but specificity is conferred through sclerotia only germinating in response to *Allium* plant root exudates.

1.16 Test the ability of sclerotia to germinate for a range of *S. cepivorum* isolates using an established assay based on diallyl disulphide

In laboratory tests, a very high level of sclerotial germination was observed for *S. cepivorum* with some significant differences between isolates. The ability of DADS to stimulate germination of *S. cepivorum* sclerotia is well documented (Davis et al 2007) and although this was evidenced by quicker germination at an early time-point, there was no effect on final germination levels compared to no DADS treatments. There was however a clear effect of conditioning in soil which stimulated early germination which has previously been reported to break constitutive dormancy of sclerotia (Gerbrandy 1992). The high germination levels of sclerotia not exposed to DADS is surprising although it has been suggested that drying can also trigger germination (Gerbrandy 1992). Further work will test this hypothesis by repeating the test with freshly harvested *S. cepivorum* sclerotia.

8. Isolate and confirm identity of the causal agent of onion pink root disease

Setophoma terrestris was confirmed as the cause of onion pink root and isolates were obtained from onions grown in Wellesbourne and Bedfordshire. This pathogen has been reclassified several times and our sequence data supports the classification as *Setophoma terrestris* (De Gruyter et al 2010). Isolates were found to have identical 18S and 28S sequences to strains from Senegal, North America and one of unknown origin, suggesting a common origin. This pathogen is more prevalent in warmer climates, but may become a greater problem for UK onion production in the future due to climate change.

2.3 Develop appropriate plant infection tests and confirm virulence of various pathogens of onion

Inoculation methods were successfully developed for onion downy mildew and pink root, confirming pathogenicity of isolates and providing the necessary knowledge and resources for future work to evaluate different control treatments or resistance screening of onion germplasm.

Additional work: Determine the expression of FON SIX genes in planta

All the five FON SIX genes identified were shown to be expressed in Narcissus tissue and significant upregulation was observed, strongly suggesting a role for these genes in pathogenicity. In FOL, it is known that these SIX genes are secreted into the xylem sap during infection and are predicted to play a role in pathogenicity (Schmidt et al 2013). Through

gene knock-out experiments, several of these genes have been shown to directly affect virulence. The exact mechanism is unknown but it is likely that these genes have a similar function in FON, facilitating infection. Gene knockout experiments would be required to confirm the role of FON SIX genes in pathogenicity and it is also likely that other effectors, are also involved in infection.

Additional work: development of LAMP assays for FOC and *Itersonilia*

Preliminary LAMP assays were developed for both FOC and *Itersonilia*. Whilst both assays require further development, there is some clear potential for rapid field lab or in-field testing in the future, particularly for FOC. LAMP machines (particularly the Genie III) are portable and are designed for rapid, on site analysis. A LAMP-based test for *Itersonilia* would be highly beneficial as the current seed test is very labour intensive but the preliminary assays developed so far require more rigorous specificity testing and some primer modification to remove the non-specific product observed from some seed lots. An area of concern for the FOC assay is the problems associated with soil samples. Whilst LAMP is reported to be less sensitive to inhibition than PCR, soil samples contain such a high level of inhibitors that a different extraction protocol is required. Discussions with Optigene are ongoing with a view to solving this problem.

Conclusions

- A high quality FOC genome sequence has provided crucial genomic information for this pathogen and allowed the design of a specific diagnostic assay based on a pathogenicity gene.
- A robust diagnostic assay for *Itersonilia* was developed but an incomplete correlation with the industry standard seed test was observed.
- A range of identical pathogenicity-related genes were found in UK isolates *of S. cepivorum* suggesting a clonal origin and demonstrating a close relationship with other members of the *Sclerotinacae*,
- Sclerotia from different *S. cepivorum* isolates differed in their germination response, and this process was strongly induced by conditioning sclerotia in soil.
- Pathogenicity assays using lab-produced inoculum were successfully developed for onion downy mildew and pink root, providing a resource for future testing of new control treatments and resistance screening.
- *Setophoma terrestris* was confirmed as the causal agent of onion pink root

- FON SIX genes were shown to be expressed and upregulated in planta following artificial inoculation
- Preliminary LAMP assays were developed for FOC and *Itersonilia*

Knowledge and Technology Transfer

- Meeting with Bayer seeds (USA) to discuss a *Fusarium* project. A project was initiated and a person from Bayer will visit Warwick in October 2017.
- Meeting with Tozer seeds to discuss *Fusarium* in celery (5th Dec 2016)
- Part of a team of researchers that visited Crops for the Future in Malaysia, taking soil samples from oil palm plantations and extracting DNA in the labs, exchanging knowledge on soil sampling and DNA extraction (Feb 2017)
- Attended and presented poster titled 'Understanding pathogenicity and resistance in the *Fusarium oxysporum*-onion pathosystem' at BBSRC HAPI conference in Manchester (8-9th March 2017)
- Attended and gave talk titled '*Fusarium oxysporum*: a major pathogen of multiple crops causing basal rot of *Narcissus*' at Narcissus Growers workshop (Spalding, 25th May 2017)
- Attended and gave talk titled 'Understanding the genetic control of pathogenicity and resistance for *Fusarium oxysporum* in onion' at the International Horticulture Conference at NIAB-EMR (17th – 19th July 2017)
- Visited Blackdown growers to discuss *Fusarium* on rocket (3rd Aug 2017)
- Visited UK Cut Flower Centre (Holbeach St Johns) to inoculate a polytunnel with *Fusarium oxysporum* f. sp. *mathioli* and monitor disease (various dates)
- Visited a nursery (Boston, Lincs) growing stocks, statice and Celosia. Plants were sampled for *Fusarium* and disease management discussed (9th Aug 2017)
- Attended and gave talk titled 'Understanding the genetic control of pathogenicity and resistance for *Fusarium oxysporum* in onion' at the BSPP Presidential meeting in Nottingham (11th – 13th Sept 2017)
- Work shadowing with Andy Richardson (Allium and Brassica Centre) – visited onion stores and fields, exchanging knowledge on Fusarium with onion growers (20th Sept 2017)
- Aided with commercial fungicide trial in Wellesbourne Fusarium area

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