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 2016 (Year 3)
Previous report:	Annual Report, October 2015 (Year 2)
Fellowship staff: ("Trainees")	Dr John Clarkson & Dr Andrew Taylor
Location of project:	Warwick Crop Centre
Industry Representative:	
Date project commenced:	1st November 2013
Date project completed (or expected completion date):	30th April 2019

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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.

Andrew Taylor

Research Fellow

Warwick Crop Centre, School of Life Sciences, University of Warwick

Signature:  Date: 28/10/16

John Clarkson

Principal Research Fellow

Warwick Crop Centre, School of Life Sciences, University of Warwick

Signature:  Date: 29810/16

Report authorised by:

Rosemary Collier

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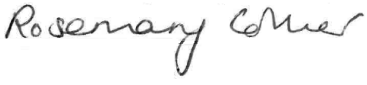
Warwick Crop Centre, School of Life Sciences, University of Warwick

Signature:  Date: 28/10/16

CONTENTS

PROGRESS AGAINST OBJECTIVES

Objectives

1. To train the Fellow as a plant pathologist with a focus on molecular techniques and
2. diagnostics through direct participation in research relevant to the industry.
3. To develop pathogen collections of molecularly characterised isolates with known virulence to enhance future capability for plant pathology research.
4. To train the Fellow as a plant pathologist with a broad knowledge of different pathosystems and associated plant pathology techniques.
5. To synthesise existing unpublished work/data generated by Dr Dez Barbara.
6. To give the Fellow experience of grower practice and disease problems through industry meetings, visits and contacts.

To mentor the Fellow in becoming an independent research leader and establish a role for him as a leading molecular plant pathologist in UK horticulture.

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		
1.3 Bioinformatic analyses of FOC isolate genomes and identification of potential primers for FOC diagnostics (end Yr 4).	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 *S. cepivorum*	31/10/16	31/10/16	
1.7 Check existing *P. 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	

Objective	Original Completion Date	Actual Completion Date	Revised Completion Date
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	31/10/16
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		
1.12 Test *Itersonilia* primers using soil and diseased parsnip roots.	31/10/17		
1.13 Extract DNA, prepare libraries and carry out whole genome sequencing of *Sclerotinia* spp. isolates including *S. subarctica*.	31/10/17		
1.14 Bioinformatic analyses of *Sclerotinia* spp. genomes.	31/10/18		
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		
2.4 Confirm identity and characterise parsnip canker pathogen isolates as obtained through AHDB Horticulture project FV 366 or BBSRC PhD project using gene sequencing	31/10/17		
3.1. Gain experience with lettuce pathogens such as *B. cinerea* and *B. lactucae* through a work programme to be developed with Katherine Denby and Eric Holub.	31/10/18		
3.2. Gain experience with brassica pathogens such as Turnip Mosaic Virus, *A. candida H. brassicae* and *X. 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 *P. ultimum*, *O. neolycopersici* through existing projects (John Clarkson)	31/10/17		

Objective	Original Completion Date	Actual Completion Date	Revised Completion Date
4.1. Synthesise Dez Barbara's unpublished work on carrot/parsnip viruses	31/10/15	31/10/15	
4.2. Analyse and publish virus sequences collected by Dez Barbara from carrots and wild umbellifers from a previous Defra project.	31/10/17		
5.1. Attend relevant research project meetings.	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	31/10/16	
5.4. Work-shadowing of at least one industry collaborator.	31/10/17		
6.1. Contribute to writing at least one research proposal	31/10/17		
6.2. Initiate at least two research proposals and obtain funding for one.	31/10/18		
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

1.6: A published diagnostic for *S. cepivorum* was tested and shown to be effective and specific.

1.8/1.9: An improved PCR-based detection assay for *P. violae* was developed and tested against a range of *Pythium* species as well as samples from project FV 405. The test was shown to be highly sensitive and specific and was capable of detecting low levels of *P. violae* in soil samples.

1.10: Potential primers for an *Itersonilia* diagnostic were developed and tested. These primers gave good amplification from a range of isolates and were suitable for a quantitative PCR assay. Initial work suggests that they are specific to *Itersonilia*.

2.1 / 2.2: Additional isolates of *P. destructor, S. cepivorum* and *Botrytis* spp. were collected. Isolate identities were confirmed using PCR and sequencing of the internal transcribed spacer

(ITS) regions of the rDNA. Based on cytochrome oxidase 1 and 2 (COX1 / COX2) sequences, all *P. destructor* isolates were identical to an isolate from Germany, suggesting a clonal origin. Based on the sequences of ITS, Glyceraldehyde 3-phosphate dehydrogenase (G3PDH), heat shock protein (HSP60), β-Tubulin, 28S and 18S, all *S. cepivorum* isolates had identical sequence to isolates from other countries, again suggesting a clonal origin. A total of six different *Botrytis* species were isolated from onions with foliar lesions, suggesting that many species may potentially be pathogenic. Using a combination of two loci (rDNA intergenic spacer region (IGS) and G3PDH), the different species can be effectively identified. Isolates from an historic collection were re-classified based on these loci.

5.2: Posters on the work carried out in the fellowship and on other *Fusarium* basal rot research were presented at the Onion and Carrot Conference in Peterborough. A presentation titled '*Fusarium* basal rot of onion' was given at the Elsoms onion conference at the Elveden estate.

Additional objective: A set of 30 *F. oxysporum* f. sp. *narcissi* isolates were characterised through sequencing three housekeeping genes and screening for the presence of 14 pathogenicity genes. Whilst isolates were almost identical based on housekeeping gene sequence, they differed in effector gene complement, suggesting a possible race structure.

Milestones not being reached

None

Do remaining milestones look realistic?

Yes

Training undertaken

- Attended and presented a poster on *Fusarium* basal rot research at the Elsoms Open Day (14th Oct 2015)
- Attended and presented posters on this fellowship and on other *Fusarium* basal rot research at the UK Carrot and Onion conference (4th-5th Nov 2015)
- Attended an SPSS training session (statistical analysis software) at the University of Warwick (14th Dec 2015)
- Two days training at East Malling Research in order to learn techniques for the transformation of *Fusarium oxysporum* (11-12th Jan 2016)
- Attended, gave an oral presentation titled 'Understanding pathogenicity and resistance in the *Fusarium oxysporum*-onion pathosystem' and presented a poster on the same subject at the HAPI conference in Cambridge (1-2nd March 2016).
- Attended and gave an oral presentation titled 'Understanding pathogenicity and resistance in *Fusarium oxysporum* f.sp. *cepae*' at the *Fusarium* satellite meeting (part of the European Fungal Genetics meeting) in Paris (3rd April 2016)
- Hosted a seminar by Joe Martin from AHDB (7th April 2016)
- Attended the Warwick Life Sciences postgraduate student symposium (18-19th April 2016)
- Attended a seminar on plant-pathogen interactions by Nick Talbot (University of Exeter) at Warwick (4th May 2016)
- Presented a seminar titled 'Understanding the genetic control of pathogenicity and resistance for *Fusarium oxysporum* in onion' in the Warwick Crop Centre seminar series (19th May 2016)
- Hosted a guest seminar by Tom Pope (entomology) from Harper Adams University (26th May 2016)
- Attended and gave a presentation titled '*Fusarium* basal rot of onion' at the Elsoms onion conference (Elveden estate, 6th June 2016)
- Visited Whiteheads nursery in Boston to observe and sample *Fusarium oxysporum* from stocks and statice (22nd June 2016)
- Attended and presented a poster on *Fusarium* basal rot at to the New Frontiers in Crop Research event in London (20th Oct 2016).

- Expertise gained by trainee
- Improved presentation and communication skills
- Increased knowledge of industry problems and how to work towards solutions
- Increased understanding of the onion industry
- Increased understanding of the carrot industry
- Increased knowledge of statistical analysis of data
- Ability to genetically transform *Fusarium oxysporum* (and potentially other organisms)
- Increased knowledge of plant-pathogen interactions
- Greater knowledge of worldwide *Fusarium* research
- Ability to identify and isolate *Fusarium* from other hosts including stocks and statice
- Ability to inoculate on a field scale
- Improved networking skills
- Improved knowledge of probe-based qPCR assays
- Culturing and storing different pathogens including *Botrytis* spp., *Itersonilia* spp. and *Mycocentrospora acerina*.

Other achievements in the last year not originally in the objectives

- A paper entitled: 'Identification of pathogenicity-related genes in *Fusarium oxysporum* f. sp. *cepae*' was published in Molecular Plant Pathology
- *Fusarium oxysporum* was isolated from stocks and statice and shown to be pathogenic on the respective hosts. This led to the production of a new disease report entitled 'First report of *Fusarium oxysporum* causing a vascular wilt of statice (*Limonium sinuatum*) in the UK,' which was accepted for publication in Plant Disease (pending revisions).
- Further developed disease areas in the Wellesbourne Quarantine Field for *Fusarium* and white rot on onion and *Sclerotinia* on lettuce. These will be valuable resources for future projects and/or for commercial trials. See Science section results for further details.
- Molecular characterization of a range of *Fusarium oxysporum* f. sp. *narcissi* isolates using primers for putative effector genes. See science section for details.
- The molecular characterisation of pathogens from onion (objective 2.2) was extended to several genetic loci and phylogenies were produced to assess variation between isolates as well as to compare the isolates with publically available sequence data.

Changes to Project

Are the current objectives still appropriate for the Fellowship?

No, some changes are proposed based on work that is now being carried out in other projects. Objectives 1.13 and 1.14 relating to *Sclerotinia* genome sequencing and analysis is now being carried out as part of a PhD project at the University of York. Objective 2.4 (molecular identity of parsnip canker pathogens) was fully completed in a PhD project (Lauren Chappell). Objective 4.2 (publishing carrot virus sequences) will now being carried out by Adrian Fox at FERA using data summarised and analysed in the first part of this fellowship as well as additional data on another virus that he has collected. Some new objectives are therefore proposed:

- Test published (and unpublished) methods for extraction of DNA from larger quantities of soil. Current methods using commercial kits rely on 0.25-0.5g of soil which is not representative of a pathogen in a field which is often unevenly distributed. This can frequently lead to inconsistent results in soil diagnostic tests and as such am improved method would be highly beneficial (new objective 7, 31/10/18).
- Test the newly developed *Itersonilia* diagnostic test on infected parsnip seed lots and compare with the industry standard agar plate test. This will be done as opposed to testing soil samples as this is more relevant to industry needs following discussions with Elsoms Seeds (amended objective 1.12, 31/10/17).
- Isolate and confirm identity of the causal agent of onion pink root disease. Onion plants with pink roots were observed at Wellesbourne and there are also anecdotal reports of observations in commercial crops. This symptom is presumed to be caused by *Phoma terrestris* as reported in the literature and as this pathogen is favoured by warm temperatures it may be a future problem for UK onion production due to climate change (new objective 8, 31/10/17).
- Test a range of *S. cepivorum* isolates for the presence of published pathogenicity genes which have been reported previously (Andrew *et al* 2012) (new objective 1.15, 31/10/17).
- Test the ability of sclerotia to germinate for a range of the new *S. cepivorum* isolates using an established assay based on diallyl disulphide (new objective 1.16, 31/10/17).

GROWER SUMMARY

Headline

The development of new and improved molecular tools for pathogen detection will allow for effective, quantitative monitoring of pathogens in seed, fields and in stores. Assays have been developed and tested for *Pythium violae* (cavity spot of carrot), *Itersonilia pastinacea* (parsnip canker) and *Sclerotium cepivorum* (*Allium* white rot).

Background

Onion diseases

Allium white rot

Allium white rot, caused by the fungus *Sclerotium cepivorum,* is one of the most important diseases of *Allium*s, 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 molecular based diagnostic has now been developed which involves extracting DNA from up to 1kg of soil (Woodhall *et al* 2012). However, this method has not been widely tested. Once tested, this diagnostic assay could be used to test soil for levels of *S. cepivorum* DNA in order to assess disease risk.

Botrytis leaf blight / Botrytis neck rot

Botrytis species cause a range of diseases on onions including leaf blight and neck rot. Leaf blight is caused predominantly by *B. squamosa* leading to lesions which are whitish in colour, 1 to 5 mm in length and surrounded by a white halo (Tremblay *et al* 2003). Neck rot can be caused by a number of species, the main characterised species being *B. allii*, *B. aclada* and *B. byssoidea* (Chilvers and du Toit 2006). Symptoms of neck rot include soft, watery decay, mycelial growth in the bulb and sclerotia on the outer scales (Khan *et al* 2013). Infection may be latent in the field and occur after several months of storage. The pathogen can enter the bulb through several routes including through flowers which can lead to seed transmission (Maude and Presly 1977). Many closely related *Botrytis* species are difficult to separate by culturing so the development of molecular techniques would be beneficial in order to rapidly identify species and deploy appropriate control measures.

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 Association Research and Development Committee. Despite this, there is no information on the diversity of *P. destructor* isolates in the UK and very limited information worldwide.

Cavity spot of carrots

Cavity spot of carrots (caused predominantly by *Pythium violae*) is the most damaging disease for UK carrot growers. Infection leads to small, sunken, elliptical lesions and eventually the skin ruptures to form an open cavity (Hiltunen and White 2002). Currently, the only control option is the use of metalaxyl as a drench applied 6 weeks after drilling, which is not always effective. The biology of *P. violae* is poorly understood and it is difficult to isolate conventionally from soil. Moreover, whilst the pathogen is present in newly formed (and still closed lesions), once these are open and exposed to the soil secondary infections occur and the pathogen is difficult to isolate from mature lesions. A rapid and specific diagnostic assay for *P. violae* and the other species potentially involved in cavity spot would be a valuable tool for further study of the disease.

Parsnip canker

Parsnips are a speciality crop in the UK, covering an area of 3,100ha 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.

Mycocentrospora acerina infects a range of plant species including lettuce, carrot, celery and parsnip (Chappell 2016). It is widely known as a storage disease, causing liquorice rot of carrots and celery. It is thought to survive in the soil as chlamydospores and may be transmitted through earthing-up, splash dispersal or infected seed. The symptoms on parsnip

are similar to those caused by *Itersonilia*, but lesions tend to be purplish-black in appearance (Channon 1965). Currently, there are no molecular diagnostic tests from *Itersonilia* or *M. acerina*. The development of such tests would be highly beneficial for seed, soil and root testing.

Narcissus basal rot

In the UK, daffodil bulbs are particularly prone to infection by soil-borne pathogens due to the standard biennial growing system employed (Hanks 2002). The most economically damaging pathogen is *Fusarium oxysporum*. f.sp. *narcissi* (FON), causing 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. Pathogenic isolates of *Fusarium oxysporum* are highly host specific. The factors which determine the host specificity and pathogenicity of different *F. oxysporum* f. spp. (special forms adapted to a host) are poorly understood although recent studies have identified the role specific genes ('SIX' genes) in a tomato infecting isolate (Lievens *et al* 2009; Ma *et al* 2010). If the genetic basis for pathogenicity could be similarly 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 molecular diagnostic test for *S. cepivorum* was tested and shown to be effective and specific, allowing for field testing for this disease. This is a quantitative test based using advanced qPCR techniques and could be used to test soil for levels of *S. cepivorum* DNA.
- Potential qPCR diagnostic tests for the parsnip canker pathogens *Itersonilia* and *Mycocentrospora acerina* are also being developed and initial results are promising. These tests would be beneficial for seed, soil and root testing.
- A total of six *Botrytis* species were found to be associated with UK onions, some of which may cause leaf blights and others neck rot. The species identified were *B. allii*, *B. aclada, B. squamosa, B. byssoidea, B. cinerea* and *B. pseudocinerea*. It is thought that *B. allii, B. aclada* and *B. byssoidea* are responsible for neck rot but all of the species may be responsible for associated with leaf blights. In addition, a *Stemphylium* species, possibly *S. vesicarium* was consistently isolated from onion leaves and may cause a leaf blight if climatic conditions are optimal. This species is known to cause severe leaf blight in other countries, particularly with a warmer climate so should be monitored in the UK, especially

taking into account current climate change models. Accurate identification of *Botrytis* species is important for deployment of appropriate control measures.

- New isolates of *S. cepivorum* and *P. destructor* were obtained and characterised by sequencing several different genes. For each pathogen, isolates were 100% identical based on molecular characterisation, suggesting clonal populations and lack of diversity. In addition, sequences were often identical to isolates from other countries. Although this could imply that control measures, such as fungicides and resistant varieties, should be effective against all isolates, there may be variation in other functional genes which could result in biological variation and potential fungicide resistance or new pathotypes.
- A set of 30 FON isolates was characterised and shown to have variable effector gene compliments, strongly suggesting a race structure exists. This would impact on breeding efforts as a variety that is resistant to one race may be susceptible to another. The effector genes identified will be useful for the future development of a molecular diagnostic for FON.
- A new *Fusarium* disease of statice (*Limonium sinuatum*), caused by *F. oxysporum*, was identified and reported for the first time (Taylor *et al* 2016b). The observed symptoms were typical of a *Fusarium* wilt: wilting leaves and flower stalks, often initially progressing down one side of the stem, followed by leaf and plant death. When stems were cut open, brown staining of the vascular tissue was observed. This disease was seen to cause vary large losses in one nursery and should be closely monitored in any nursery growing stocks.

Financial Benefits

None to report

Action Points

None to report

SCIENCE SECTION

Introduction

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). *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 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.

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Downy mildew

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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. Pathogenic isolates of *Fusarium oxysporum* are highly host specific and are therefore distinguished as *formae speciales* (Leslie & Summerell 2006), of which more than 120 have been identified (Michielse & Rep 2009). 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 in xylem (SIX) genes located on mobile pathogenicity chromosomes in *F. oxysporum* f. sp. *lycopersici*, the f. sp. infecting tomato (Lievens *et al*

2009; Ma *et al* 2010). If the genetic basis for pathogenicity could be similarly 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.

Statice wilt

Fusarium wilts are known to affect a range of flower species. Statice (*Limonium sinuatum*) is commercially grown in many parts of the world as an annual cut-flower crop for use in fresh or dried markets (Taylor *et al* 2016b). Pathogen species reported on this plant include *Colletotrichum, Botrytis, Cercospora, Rhizoctonia and Peronospora*. Previously, there were no reports of *Fusarium* wilt of statice.

Materials and methods

Objective 1.6: Test published PCR diagnostic for *S. cepivorum*

A recent publication (Woodhall *et al* 2012) detailed a large-scale DNA extraction method and qPCR diagnostic for *S. cepivorum*. We set out to test this method and potentially adapt it for to use with a standard, kit-based soil DNA extraction and a Roche Lightcycler qPCR instrument. Initially, the assay was tested against a dilution series of *S. cepivorum* DNA (isolate GS1) at concentrations ranging from 10 ng/µl to 1 pg/µl. All reactions were carried out in triplicate as shown in Table 1. Primers and probes were as described by Woodhall *et al* 2012 with the exception of the 3' end of the probe which had a tetramethylrhodamine (TAMRA) quencher rather than a minor groove binder (MGB). Cycling conditions were as follows: 1 cycle of 95°C for 3 mins followed by 45 cycles of 95°C for 5s and 60°C for 30s. A second experiment was carried out to further test the specificity of the assay and included DNA from the soilborne fungi *S. sclerotiorum, S. nivalis, S. subarctica, S. minor, S. trifoliorum, Phoma* sp., *Rrizoctonia solani, Verticillium* sp., and *Fusarium oxysporum* f. sp. *cepae*. All non-target DNA samples were diluted to 10 ng/µl. Soil samples taken from the white rot quarantine field area at Wellesbourne, sampled at 3-4 week intervals over the growing season, were also included with DNA extracted using a Powersoil® DNA isolation kit (MoBIO Laboratories), following the manufacturer's guidelines.

Table 1. Reaction set up for testing a *S. cepivorum* diagnostic assay.

Reagent	Volume per reaction (µl)	Final concentration (µM)
Primer: Z996-340F (3µM)	1	0.3
Primer: Z996-450R (3µM)	1	0.3
Probe: Z996-382T (1µM)	1	0.1
DNA	1	
SensiFAST™ Probe No-ROX mix (Bioline)	5	1x
Water	1	
Total	10	

Objectives 1.8/1.9: Develop qPCR for *P. violae* using WCC Roche Lightcycler / Quantify *P. violae* in soil samples from AHDB Horticulture project FV 405 and other samples where available

This work was carried out to support the studentship of Kathryn Hales (AHDB Studentship FV 432). Previous work in this studentship had identified potential primers for a specific qPCR assay based on two genes; Ochromonas mastigoneme protein (OCM1) and Cellulose synthase (CS). These primers were tested against DNA from a range of soil samples from project FV 415 which had tested positive or negative based on a standard assay using published primers based on the ITS region of the rDNA (Klemsdal *et al* 2008). The PCR was carried out in 20µl reactions containing primers (1µM), 5µl of RedTaq (Sigma) and 1µl of DNA using the cycling conditions in Table 2. A portion (4µl) of each PCR product was run on a 1.2% agarose gel to assess amplification.

Table 2. Cycling conditions for testing *P. violae* specific primers.

	ITS primers (Klemsdal *et al* 2008)	CS primers	OCM1 primers
Initial denaturation	94°C, 2 min	94°C, 5 min	94°C, 2 min
Denaturation	94°C, 1 min	94°C, 1 min	94°C, 1 min
Annealing	61°C, 1 min	65°C, 30s	62°C, 30s
Extension	72°C, 1 min	72°C, 10s	72°C, 30s
Final extension	72°C, 5 min	72°C, 5 min	72°C, 5 min

*Shaded steps carried out for 30 cycles

Remaining soil from the samples described above were also used to test an oospore capture method described by Kathryn Hales (FV 432, annual report 2016). PCR was carried out on DNA from the captured oospores using the same primer pairs and 4µl of the PCR product run on a 1.2% agarose gel.

Due to some sensitivity issues with the existing assays, a Taqman-based qPCR assay was also developed. Primers were designed to the ITS region of *P. violae*: AT_ITS FOR: 5' TGGTGTTTTCGGACGCTGCGCTG-3' and AT_ITS REV: 5' TCCGCACACACACATTGCTG

and were used in conjunction with a Taqman probe: 5' (6FAM)CGGAGGAGGAACGAAGGTTGGTCTTGT(TAMRA)-3'. This assay was tested using DNA from a range of *Pythium* species and soil samples from project FV 405 and two samples of the DNA extracted using the oospore capture method. All non-target DNA samples were diluted to 10 ng/µl. Initially, different primer concentrations were tested and 0.2 µM was found to be optimum (data not shown). DNA of isolate *P. violae* HL was used to create a dilution series ranging from 1 ng/µl to 0.1 fg/µl. Reactions were set up in triplicate and were as described for the white rot assay with the exception of the primers of which 0.5 µl of 4 µM was used per reaction. Cycling conditions were as follows: 1 cycle of 95°C for 3 mins followed by 45 cycles of 95°C for 5s and 67°C for 30s. DNA quantities were extrapolated using a standard curve method. The correlation between quantity of DNA detected and band brightness using convention PCR (Klemsdal *et al* 2008 primers) was calculated in Excel (Pearson correlation). The correlation between DNA quantity and number of carrots with cavity spot (project FV 415) was also calculated.

Objective 1.10: Identify potential primers for *Itersonilia* diagnostics from existing gene sequences (or whole genome sequence)

Work in a BBSRC-funded PhD studentship identified a primer pair which was potentially specific to *Itersonilia* based on the tRNA methyl transferase gene. However, the amplicon was too long for qPCR. Therefore, two sets of modified primers were designed based on this gene: Iter FOR1: CGCCTGTGCTCTTTGTGACAG; Iter REV1: GATGACCTGACGACCGCTGTG; Iter FOR2: GGACCGATCTGCGACTGCTC and Iter REV2: CTGGAGAGACTGACCCATATC. Primers were initially tested using DNA from a range of *Itersonilia* isolates using conventional PCR as previously described. Cycling conditions were as follows: 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. The annealing temperatures were 64°C for FOR1/REV1 and 62°C for FOR2/REV2. PCR products were run on a 1.2% agarose gel as described. Following this, FOR2/REV2 were tested as a qPCR primer pair. A dilution series of isolate *Itersonilia* isolate IP35 was made ranging from 10 ng/µl-10 fg/µl. Cycling conditions were as follows: 1 cycle of 95°C for 3 mins followed by 45 cycles of 95°C for 5s and 62°C for 10s and 72°C for 10s. All dilutions were run in triplicate and a melt curve analysis was carried out.

Using the *Mycocentrospora acerina* genome sequence generated in a BBSRC-funded PhD project, putative specific primer pairs were also designed and tested using conventional PCR. Primers were designed to a potential pathogenicity gene cluster: PEPGAP for: TGCGACTAACACAAAGCGTTGG / PEPGAP rev: GCTACCGGGAGCCAAGAGATG and

PEP5 for: TGGTGCAGCCTCGTCAGCAA / PEP5 rev: GAGCACAGTAAGTAACAGAGG. PCR was carried out as previously described, using an existing set of *Mycocentrospora* isolates. The annealing temperatures used were 64°C for PEPGAP and 60°C for PEP5. Products were visualised on a 1.2% agarose gel.

Objectives 2.1/2.2: Collect new isolates of *Sclerotium cepivorum, Peronospora destructor* (onion downy mildew), *Botrytis squamosa* (botrytis leaf blight) and *Botrytis allii* (neck rot of onion) / Confirm identity and characterise isolates from 2.1 by gene sequencing

Following on from the 2015 annual report, some additional isolates of the various onion pathogens were collected and isolated as described. In particular, new isolates of *B. squamosa* were obtained from onions grown at Wellesbourne. Isolations were carried out by cutting out 0.5cm sections of infected leaf, surface sterilising in 70% ethanol for 1 min and plating on potato dextrose agar (PDA) containing 20 µg/ml chlortetracycline.

For *P. destructor* isolates, fresh spores were scraped off the leaf surface and placed in a 2ml tube. DNA was extracted using a method from Stephen Rehner (USDA-ARS, Beltsville, USA). 0.5g each of 0.1 mm zirconia ceramic beads and acid washed 2.5-3.5 mm glass beads were added to the tubes. Tubes were placed in a FastPrep machine (MpBio) for 20 sec at 4.5m/s. This step was repeated twice after which 300µl of filter sterilised extraction buffer (2.1g sodiummetasilicate, 0.5g citric acid, 2.64ml 2-butoxy ethanol, 13.5ml 1M Tris-HCL pH 7.0, 194ml SDW) was added. Tubes were shaken and incubated at 100°C for 10 mins before centrifuging at full speed for 10 mins. After this, 175µl of the supernatant was removed and used 1 in 10 diluted (in TE pH 8) for PCR. For white rot and *Botrytis* isolates, liquid cultures were set up by placing 3 agar plugs (5mm) in a 50ml tube containing 25ml of sterile potato dextrose broth (PDB). These were incubated at 20°C for 7 days, rinsed in SDW and freeze-dried. DNA was extracted from freeze-dried mycelium using the method described for downy mildew.

For *P. destructor* isolates, PCR was carried out for three different loci: ITS (ITS1F and ITS4 primers; Gardes & Burns, 1993), cytochrome oxidase I (oom-cox1 levup and oom-cox1 levlo; Robideau *et al*, 2011) and cytochrome oxidase II (oom-cox2-rc4 and oom-cox2-for; Choi *et al*, 2015). Additional primer pairs did not produce any PCR products (data not shown). Conditions for PCRs were carried out as described in the relevant publications.

For *Botrytis* species, PCR's were carried out using three different loci: ITS (as above), intergenic spacer (IGS) primers Chilvers 1F and BOTRY1R (Khan *et al*, 2013) and

Glyceraldehyde 3-phosphate dehydrogenase (G3PDH, Khan *et al* 2013). Conditions for PCRs were carried out as described in the relevant publications.

For *S. cepivorum* isolates, initial identification was carried out using ITS1F and ITS4 primers. Isolates were further characterised using published primers for G3PDH / heat shock protein-HSP60 (Andrew *et al*, 2012), β-Tubulin (Bt2a and Bt2b primers, Glass & Donaldson 1995), 28S (Vilgalys & Hester 1990) and 18S (Hibbett, 1996).

For all PCRs, products were visualised by running 4µl on a 1.2% agarose gel and the remaining PCR product was purified (QIAquick PCR Purification Kit, Qiagen) and sequenced by Sanger sequencing carried out at GATC Biotech. Sequences were aligned and phylogenetic trees constructed using the methods described by Taylor *et al*, (2016a).

Additional Objective: Molecular characterisation of *F. oxysporum* f. sp. *narcissi* (FON) isolates.

A set of 30 FON isolates were confirmed as pathogenic on *Narcissus* bulbs in a previous project (BOF 074). These isolates were screened for the presence of 14 SIX genes as described by Taylor *et al* (2016a). In addition, the isolates were also characterised by sequencing three housekeeping genes: translation elongation factor 1 (EF-α), β-Tubulin (TUB2) and RNA polymerase II second largest subunit (RPB2). All PCRs were carried out as described by Taylor *et al* (2016a). Products were sequenced, sequences aligned and phylogenetic trees constructed.

Additional work: First report of *Fusarium oxysporum* causing a vascular wilt of statice (*Limonium sinuatum*) in the UK (Taylor *et al*, 2016b)

Work was carried out with a BBSRC-funded summer student to investigate the cause of wilting in statice plants observed at a nursery in Lincolnshire in June 2016. The symptoms consisted of wilting leaves and flower stalks, eventually followed by leaf necrosis and plant death. Staining of the vascular tissue was also observed. Isolations were carried out by excising 2-3 cm long sections of infected stem, surface sterilising in 5% sodium hypochlorite for 2 mins, rinsing in sterile water and plating on potato dextrose agar (PDA) containing 20 µg/ml chlortetracycline. Following incubation at 20°C for 4 days, fungal colonies were sub-cultured onto PDA and grown for a further 10 days. DNA was extracted from four isolates (using the method described previously) and the translation elongation factor 1-α (EF1-α) gene amplified by PCR and sequenced (Taylor *et al*, 2016a). To test pathogenicity flowering statice plants (cv. Velvet Wings) were removed from pots, the bottom third of the root system excised and the remaining roots soaked in a conidial suspension (1×10^6 conidia/ml) for 5 min. As a negative control, a non-pathogenic *F. oxysporum* isolate (Fo47) was used to inoculate plants in the same way, while uninoculated control plants were soaked in sterile

water. All statice (six plants per isolate) were then replanted in fresh compost and pots placed in a randomised design in a temperature controlled glasshouse (25°C day, 18°C night).

Additional work: Development of diseased areas in Wellesbourne quarantine field

Fusarium basal rot area: Following the successful inoculation of an area field with FOC isolate FUS2 in 2015 (see 2015 report for details), the field was re-inoculated in April 2016. FOC inoculum was prepared in flasks as described by Taylor *et al* (2013) and mixed with F2 + S compost (Levingtons) before spreading over 18 1.83 m beds of 53 m length to achieve 6 x 10^9 cfu per cubic metre soil with incorporation by raking to a depth of 10 cm. Onion seed (cv. Red Baron) was then drilled (4 rows per bed) and above- ground symptoms of *Fusarium* disease (chlorotic leaves, wilting, plant death), monitored throughout the season. After 19 weeks, foliar symptoms were scored on all plants in 3m lengths within six randomly selected beds using the following scale: 0-no symptoms, 1- mild symptoms, 2- moderate symptoms, some leaf dieback, 3- plant death. After 23 weeks, all the bulbs were removed from 4 m lengths within six randomly selected beds. All bulbs were bisected and symptoms of basal rot recorded as 0- no symptoms, 1- mild symptoms, confined to the base; 2- moderate symptoms, spreading up the bulb scales; 3- severe symptoms/rotten bulbs.

White rot area: Following on from work in 2015 where salad onions were grown in an area (24 1.83 m beds of 50 m length) previously infested with *S. cepivorum* sclerotia, white rot symptoms were scored in this crop in November 2015 (20 weeks after drilling). All plants from five randomly selected 3m length of bed were dug up and scored for presence or absence of symptoms (collapsed roots, white fluffy mycelium, presence of sclerotia).

Sclerotinia area: In 2015 an area (23 1.83 m beds of 42 m length) was artificially infected with *Sclerotinia sclerotiorum* by inoculating 600 plants at regular intervals within a lettuce crop (6600 plants, cv. Senna) with agar plugs of mycelium. In October 2015 (16 weeks after transplanting), the number of sclerotia produced was estimated for eight randomly selected infected lettuce, following drying for 4 days at room temperature.

Results

Objective 1.6: Test published PCR diagnostic for *S. cepivorum*

The published PCR-based diagnostic for *S. cepivorum* was found to work effectively using the Roche lightcycler instrument (Fig. 1). The assay efficiency was 91.5% (an assay with 90-110% efficiency is acceptable), with an error of 0.008 (<0.1 is desirable) and a slope of -3.83 (an assay with 100% efficiency has a slope of -3.32). Using a standard curve method, the quantity of *S. cepivorum* DNA detected was extrapolated. Some weak amplification was observed for *Sclerotinia nivalis* and *Sclerotinia. trifoliorum* but the level of quantification was only 0.02 and 0.01% of an equivalent *S. cepivorum* sample (Table 3). For the soil samples from the white rot infested area of the quarantine field, the levels of *S. cepivorum* were low but detectable at 7 and 13 weeks post drilling and the amount of DNA doubled from 13 weeks to 16 weeks. A peak of DNA levels was observed 19 weeks after drilling.

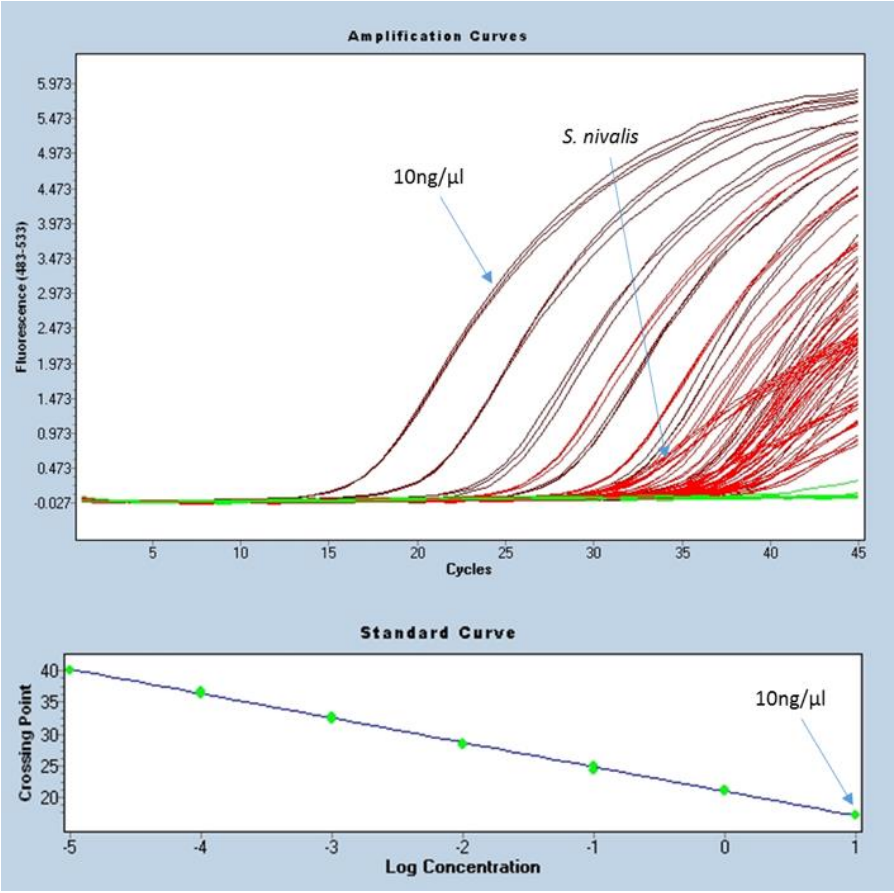

Figure 1. Amplification and standard curves for *S. cepivorum* diagnostic assay. Brown lines on the amplification curve represent the dilution series of *S. cepivorum* and red lines represent soil samples and non-target species.

Table 3. Quantification of samples from non-target species and soil using a *S. cepivorum* diagnostic assay. DNA quantities were calculated using a standard curve method. SEM=standard error of mean. All non-target DNA samples were tested at 10ng/……..

Sample	pg of DNA detected	SEM
S. nivalis 210061	2.04	0.03
S. subarctica HE1	0.31	0.01
S. sclerotiorum P7	0.10	0.00
S. minor	0.02	0.01
S. trifoliorum 316	1.38	0.09
S. sclerotiorum LL2	0.01	0.00
Phoma sp.	0.02	0.02
R. solani	0.00	0.00
Verticillium sp.	0.00	0.00
F. oxysporum FUS2	0.00	0.00
Quarantine field, 7 weeks after drilling	0.06	0.01
Quarantine field, 13 weeks after drilling	0.05	0.01
Quarantine field, 16 weeks after drilling	0.10	0.03
Quarantine field, 19 weeks after drilling	7.17	6.10

Objectives 1.8/1.9: Develop qPCR for *P. violae* using WCC Roche Lightcycler / Quantify *P. violae* in soil samples from AHDB Horticulture project FV 405 and other samples where available

The newly developed primers for *P. violae,* OCM1 and CS, were compared to the published primers (Klemsdal *et* al., 2008). The sensitivity was found to be much lower with the new primer pairs, leading to no amplification for soil samples where a strong band was observed with the Klemsdal primers (result for OCM shown in Fig. 2). New primers were therefore designed based on ITS and tested against a range of *Pythium* species. The specificity appeared to be good, with some weak amplification in other species, particularly *P. irregulare* (Fig. 3). This was converted to a quantitative Taqman assay (Fig. 4) by designing a probe in between the two primers. The assay was tested and shown to be extremely sensitive, with accurate detection of *P. violae* down to 1fg of DNA (1×10^{-6} ng). In addition, very minimal detection was observed with the other *Pythium* species tested where the highest level of amplification was from *P. irregulare* (Table 4). However, this was still equivalent to 0.00005% of a corresponding *P. violae* sample. *P. violae* was effectively detected in soil samples (Table 4). The assay efficiency was 99.5% with an error of 0.009 and a slope of -3.34.

The Taqman assay was also found to be well correlated with band brightness using the conventional PCR-based Klemsdal assay (Table 4, Fig. 5, r=0.85, P<0.001) but did not seem to correlate with the number of carrots with cavity spot (r=0.18, not significant). When DNA was extracted from the same set of soil samples following the oospore capture method, no significant amplification was observed (data not shown). The reasons for this are currently being investigated.

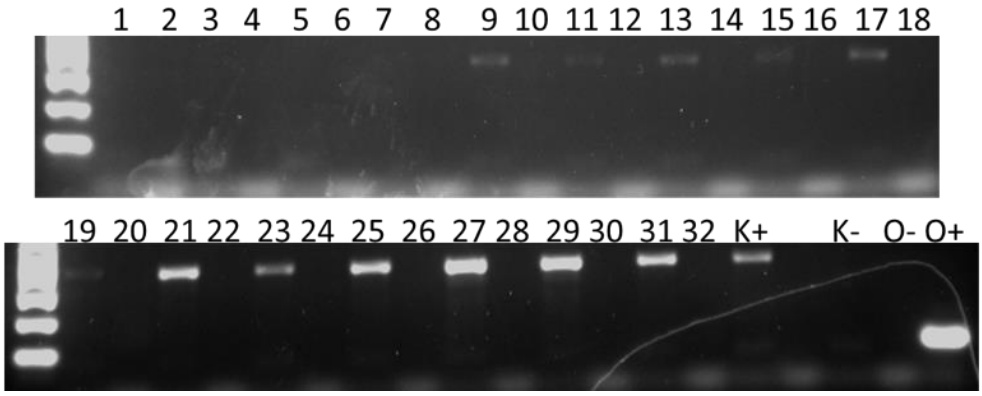

Figure 2. Comparison of OCM1 and Klemsdal primers for detection of *P. violae*. DNA from a selection of soil samples was amplified and the PCR products run in adjacent wells (e.g. 1 & 2 are the same soil sample). Odd numbers relate to products amplified by the Klemsdal primers and even numbers relate to OCM1 primer products. K+= positive control, Klemsdal primers, K-=negative control, Klemsdal primers; O+=positive control, OCM1 primers, O-=negative control, OCM1 primers.

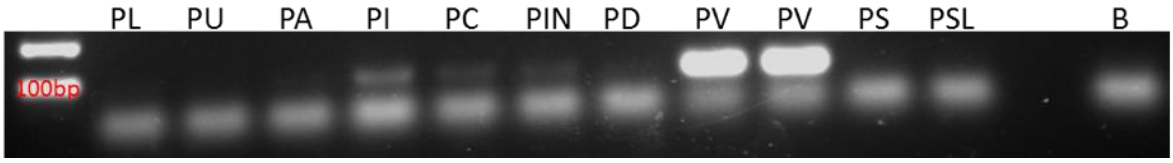

Figure 3. Testing AT_ITS primers for detection of *P. violae* using conventional PCR. DNA from different *Pythium* species was amplified and PCR products run in following lanes: PL= *P. lutarium*, PU= *P. ultimum*, PA= *P. attrantheridium*, PI= *P. irregulare*, PC= *P. cryptoirregulare*, PIN= *P. intermedium*, PD= *P. debaryanum*, PV= *P. violae*, PS= *P. sulcatum*, PSL= *P. sylvaticum*, B=blank.

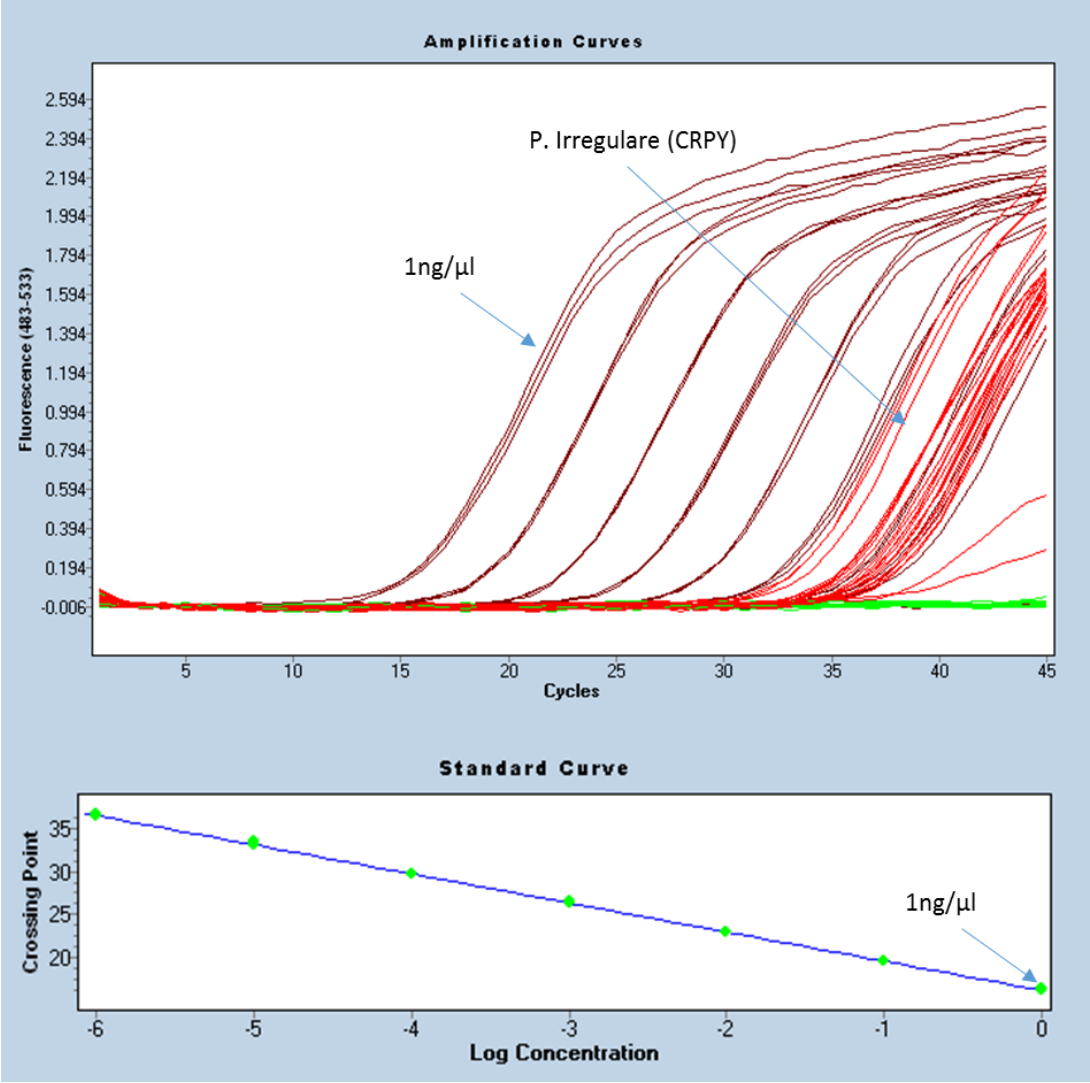

Figure 4. Taqman assay for quantification of *P. violae*. Amplification curves and dilution series from 1ng/µl to 1fg/µl are shown. Brown lines on the amplification curve represent the dilution series of *P. violae* and red lines represent soil samples and non-target species.

Table 4. Quantification of samples from non-target species and soil using a newly developed Taqman assay for *P. violae*. DNA quantities were calculated using a standard curve method. SEM=standard error of mean.

Sample	*Details*	Band brightness (Klemsdal primers)	DNA detected (fg)	SEM
173	*P. lutarium*		0.00	0.00
174	*P. ultimum*		0.35	0.18
48	*P. attrantheridium*		0.13	0.13
86	*P. intermedium*		0.62	0.05
87	*P. debaryanum*		0.33	0.17
91	*P. sulcatum*		0.15	0.08
100	*P. sylvaticum*		0.06	0.06
CRPY	*P. irregulare*		5.10	0.76
175	*P. ultimum*		1.85	0.25
24	*P. sylvaticum*		1.30	0.15
25	*P. intermedium*		0.18	0.18
197	*P. irregulare*		0.57	0.22
780	soil sample	0	0.63	0.35
781	soil sample	0	0.55	0.08
758	soil sample	0	0.37	0.22
783	soil sample	0	0.37	0.21
765	soil sample	1	6.14	0.65
774	soil sample	1	1.69	0.42
775	soil sample	1	9.71	1.34
760	soil sample	1	3.77	0.23
764	soil sample	1	13.90	1.05
757	soil sample	1	2.64	0.28
761	soil sample	2	25.63	1.23
770	soil sample	3	174.00	4.58
779	soil sample	3	84.77	2.29
776	soil sample	3	174.33	2.60
784	soil sample	3	149.33	1.33
777	soil sample	3	66.77	6.41
770T	soil sample, sucrose extraction	0	0.77	0.42
776T	soil sample, sucrose extraction	0	0.42	0.22

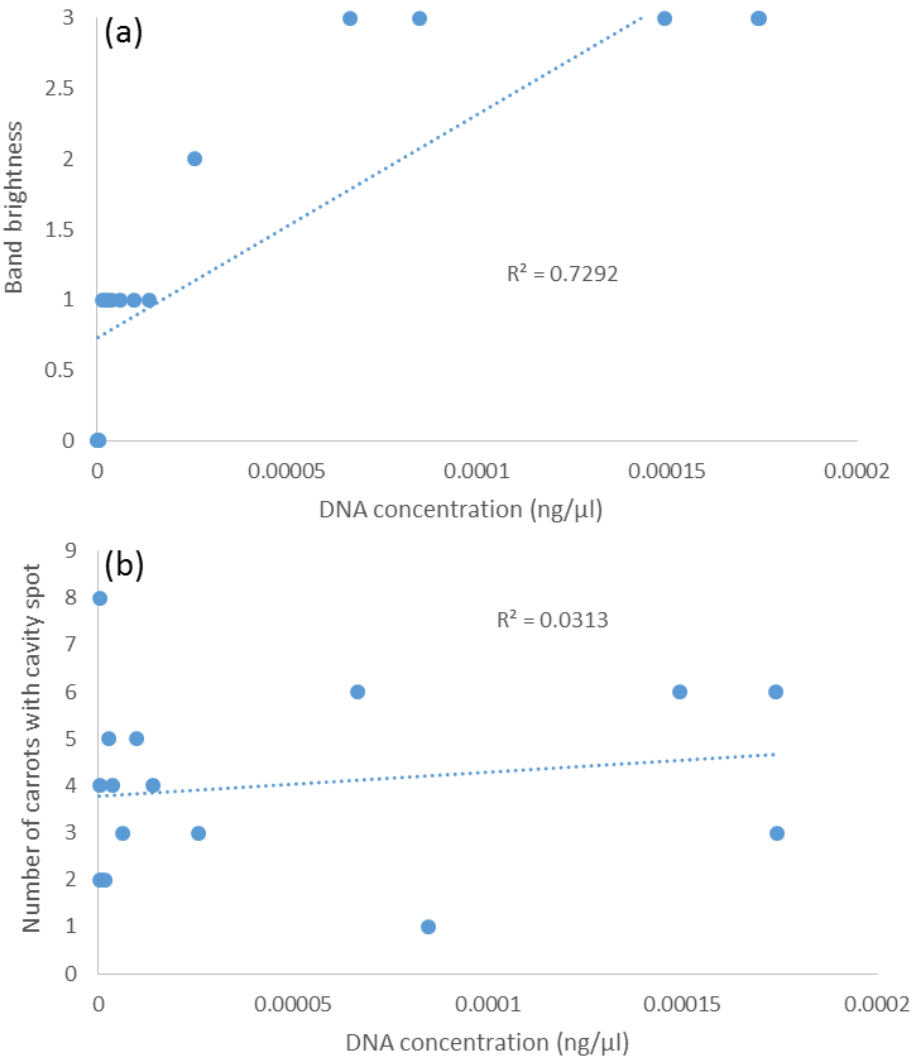

Figure 5. Correlation between *P. violae* DNA concentration detected using a new Taqman assay and (a) band brightness using a conventional PCR assay (Klemsdal primers) or (b) number of carrots with cavity spot.

Objective 1.10: Identify potential primers for *Itersonilia* diagnostics from existing gene sequences (or whole genome sequence)

New primers for use as a potential *Itersonilia* diagnostic were tested. The primer pair FOR1 and REV1 were not effective as only weak amplification was observed for two of the four *Itersonilia* isolates initially tested (Fig. 6a). Primer pair FOR2 and REV2 produced strong amplification from all seven *Itersonilia* isolates tested (Fig 6a & b). Only very weak amplification was observed against non-target species, mainly from *Fusarium oxysporum* f.sp. *pisi* (Fig. 6c). In addition, this primer pair was suitable for use in a quantitative assay as they were shown to produce efficient and reproducible results when tested (Fig. 7, assay efficiency 99.5%, error 0.017, slope -3.35).

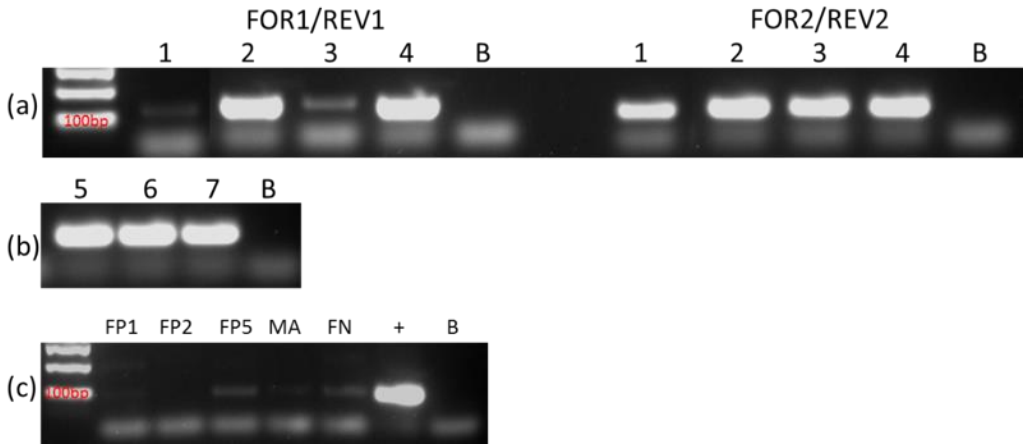

Figure 6. PCR products for primers developed as for a potential *Itersonilia* diagnostic. (a) primer pairs FOR1/REV1 and FOR2/REV2; (b) further testing of FOR2/REV2; (c) testing FOR2/REV2 for amplification of DNA from other fungi. 1-7= different I*tersonilia* isolates. FP= *Fusarium oxysporum* f. sp. *pisi* (race 1, 2 or 5); MA= *Mycocentrospora acerina*; FN= *Fusarium oxysporum* f. sp. *narcissi*. B=blank.

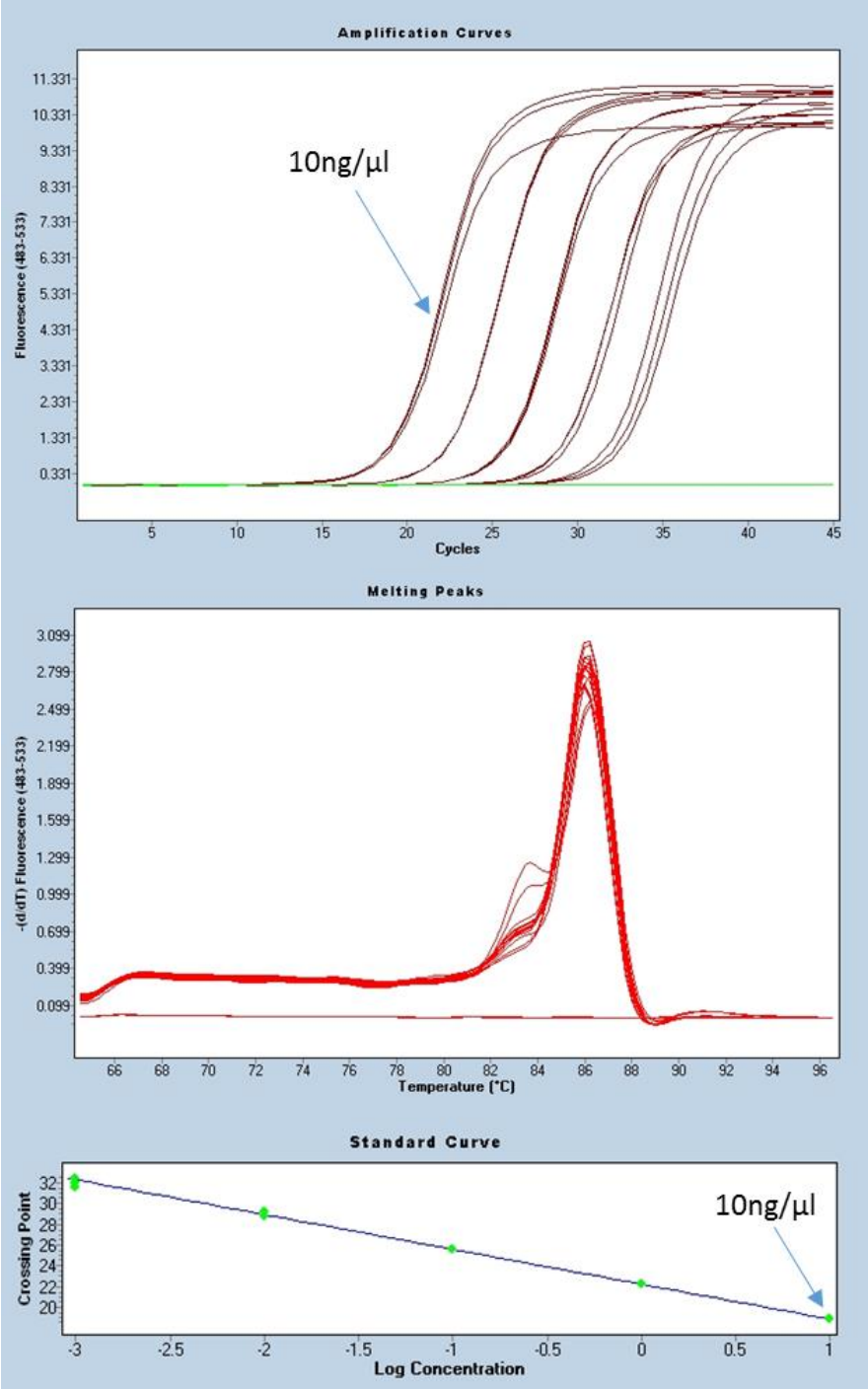

Figure 7. Testing ITER FOR2/REV2 as a quantitative assay. Amplification curve, melting peaks and standard curve are shown.

Using the *Mycocentrospora acerina* genome sequence putative specific primer pairs designed to a possible pathogenicity gene cluster resulted in strong amplification for isolate 6 but no amplification was observed for isolates 3, 5, 7 and 25 (Fig. 8). Four additional primer pairs for a *M. acerina* diagnostic were also tested with similar results (not shown). This may

be due to sequence variation or may be due to degraded DNA. No amplification was seen from *Fusarium oxysporum* f. sp. *pisi* race 1. This is significant as this fungus is very closely related to *M. acerina* based on genome comparisons.

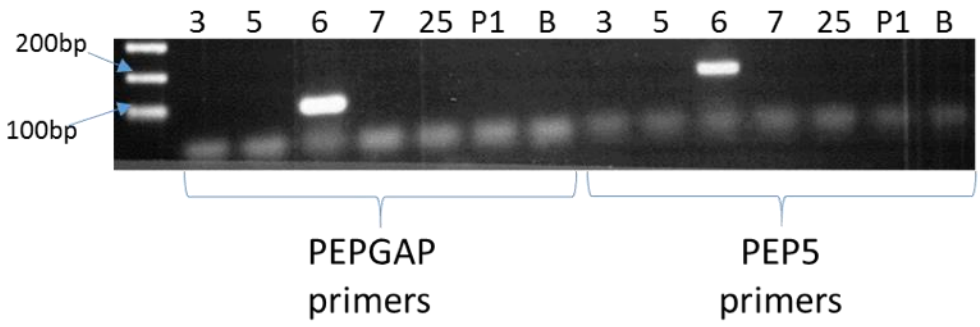

Figure 8. Testing putative *M. acerina* specific primers. 3, 5, 6, 7 and 25 are all isolates of *M. acerina* and P1 is *Fusarium oxysporum* f. sp. *pisi* race1. B=blank.

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)

Additional isolates of major onion pathogens were collected in 2015-6 (Table 5). With the exception of *P. destructor*, all isolates have been secured in long-term storage. After many attempts (see 2015 report), two isolates of *B. squamosa* were obtained from onions grown at Wellesbourne.

Table 5. Isolates of onion pathogens collected during this project.

Isolate name	Molecular ID	date collected	Location	Onion variety
RU1	*P. destructor*	15/08/2014	Rugby, Warks	Centro
RU2	*P. destructor*	15/08/2014	Rugby, Warks	Hysky
WE1	*P. destructor*	04/09/2014	Wellesbourne, Warks	Various
WE2	*P. destructor*	04/09/2014	Wellesbourne, Warks	Jaune des Cevennes
CU1	*P. destructor*	26/08/2014	Cubbington, Warks	Salad onions
Ratpit	*P. destructor*	20/05/2015	Thetford, Norfolk	Toughball
LH	*P. destructor*	20/05/2015	Thetford, Norfolk	Toughball
JD	*P. destructor*	25/08/2015	Methwold, Norfolk	Centro
FRONTIER	*P. destructor*	20/07/2016	Holbeach Bank, Lincs	Rumba
WR-LG1, LG5, LG11, LG13, LG15	*S. cepivorum*	11/06/2015	Loosegate, Lincs	Tornado
WR- AR5 AR7, AR10, AR13, AR17	*S. cepivorum*	10/06/2015	Aswick Grange, Lincs	Unknown
GS1, GS6	*S. cepivorum*	12/08/2015	Littleport, Cambs	Coded
RUG 1-1	*S. cepivorum*	20/08/2015	Rugby, Warks	Sturon
RUG 2-2	*S. cepivorum*	20/08/2015	Rugby, Warks	Sturon
JD1, JD2	*S. cepivorum*	25/08/2015	Methwold Hythe, Norfolk	Centro
WR3, WR4, WR5, WR6	*S. cepivorum*	23/09/2015	Wellesbourne, Warks	White Lisbon
FRO10, FRO11	*S. cepivorum*	20/07/2016	Holbeach Bank, Lincs	Rumba
B7 (QF07)	*B. cinerea*	27/05/2015	Wellesbourne, Warks	Red baron
WRAR4	*B. cinerea*	11/06/2015	Aswick Grange, Lincs	Unknown
BQ2	*B. squamosa*	12/15	Wellesbourne, Warks	white lisbon/*A.fistulosum*
BQ3	*B. squamosa*	12/15	Wellesbourne, Warks	white lisbon/*A.fistulosum*
AT1	*B. byssoidea*	24/05/2015	Warks/Worcs	salad onions
CH1	*B. byssoidea*	24/05/2015	Warks/Worcs	salad onions
CH2	*B. byssoidea*	24/05/2015	Warks/Worcs	salad onions
PL1	*B. pseudocinerea*	24/05/2015	Warks/Worcs	salad onions
PL2	*B. pseudocinerea*	24/05/2015	Warks/Worcs	salad onions
ELV1a	*B. allii*	06/06/2016	Elveden, Norfolk	bulb onion
ELV2	*B. allii*	06/06/2016	Elveden, Norfolk	bulb onion
ELV3	*B. allii*	06/06/2016	Elveden, Norfolk	bulb onion
ELV4	*B. allii*	06/06/2016	Elveden, Norfolk	bulb onion
GL1	*B. cinerea*	08/06/2016	Wellesbourne, Warks	Various
GL2	*B. cinerea*	08/06/2016	Wellesbourne, Warks	Various
9722B	*B. allii*	unknown	onion seed, Steve Roberts	Unknown
9732	*B. allii*	unknown	onion seed, Steve Roberts	Unknown
9736	*B. aclada*	unknown	onion seed, Steve Roberts	Unknown
9745	*B. allii*	unknown	onion seed, Steve Roberts	Unknown
9749	*B. allii*	unknown	onion seed, Steve Roberts	Unknown
9752	*B. aclada*	unknown	onion seed, Steve Roberts	Unknown

Isolate name	Molecular ID	date collected	Location	Onion variety
Baytree2	*B. cinerea*	20/07/2016	Wykeham, Lincs	Rumba
baytree3	*B. cinerea*	20/07/2016	Wykeham, Lincs	Rumba
Bucks4	*B. cinerea*	20/07/2016	Moulton Seas End, Lincs	Setton/Rumba mix
Bucks5	*B. cinerea*	20/07/2016	Moulton Seas End, Lincs	Setton/Rumba mix
Frontier1	*B. cinerea*	20/07/2016	Holbeach Bank, Lincs	Rumba
Frontier2	*B. cinerea*	20/07/2016	Holbeach Bank, Lincs	Rumba
SE1	*Stemphylium sp.*	14/10/2015	Spalding, Lincs	Unknown
SE3	*Stemphylium sp.*	14/10/2015	Spalding, Lincs	Unknown
SE6	*Stemphylium sp.*	14/10/2015	Spalding, Lincs	Unknown
SQ1	*Stemphylium sp.*	12/15	Wellesbourne, Warks	White Lisbon/A.fistulosum
SQ2	*Stemphylium sp.*	12/15	Wellesbourne, Warks	White Lisbon/A.fistulosum
SQ3	*Stemphylium sp.*	12/15	Wellesbourne, Warks	White Lisbon/A.fistulosum
Baytree1	*Stemphylium sp.*	20/07/2016	Wykeham, Lincs	Rumba
Bucks1	*Stemphylium sp.*	20/07/2016	Moulton Seas End, Lincs	Setton/Rumba mix
AT2	*Alternaria infectoria*	23/05/2016	Warks/Worcs	Unknown
BQ1	*Trichoderma sp.*	12/15	Wellesbourne, Warks	white lisbon/A.fistulosum
Bucks2	*Harzia acremonioides*	20/07/2016	Moulton Seas End, Lincs	Setton/Rumba mix
Bucks3	*Harzia acremonioides*	20/07/2016	Moulton Seas End, Lincs	Setton/Rumba mix

2.2 Confirm identity and characterise isolates from 2.1 by gene sequencing

P. destructor

The identity of nine *P. destructor* isolates was confirmed by sequencing of COX1 and COX2 Based on COX1 (Fig. 9), all the UK isolates had identical sequences to an isolate from Germany deposited on GenBank (KJ654046). *P. destructor* forms a distinct clade with the closest relatives being *P. alpicola* (from *Ranunculus aconitifolius*) and *P. pulveracea* (from *Helleborus niger* and *Helleborus foetidus*).

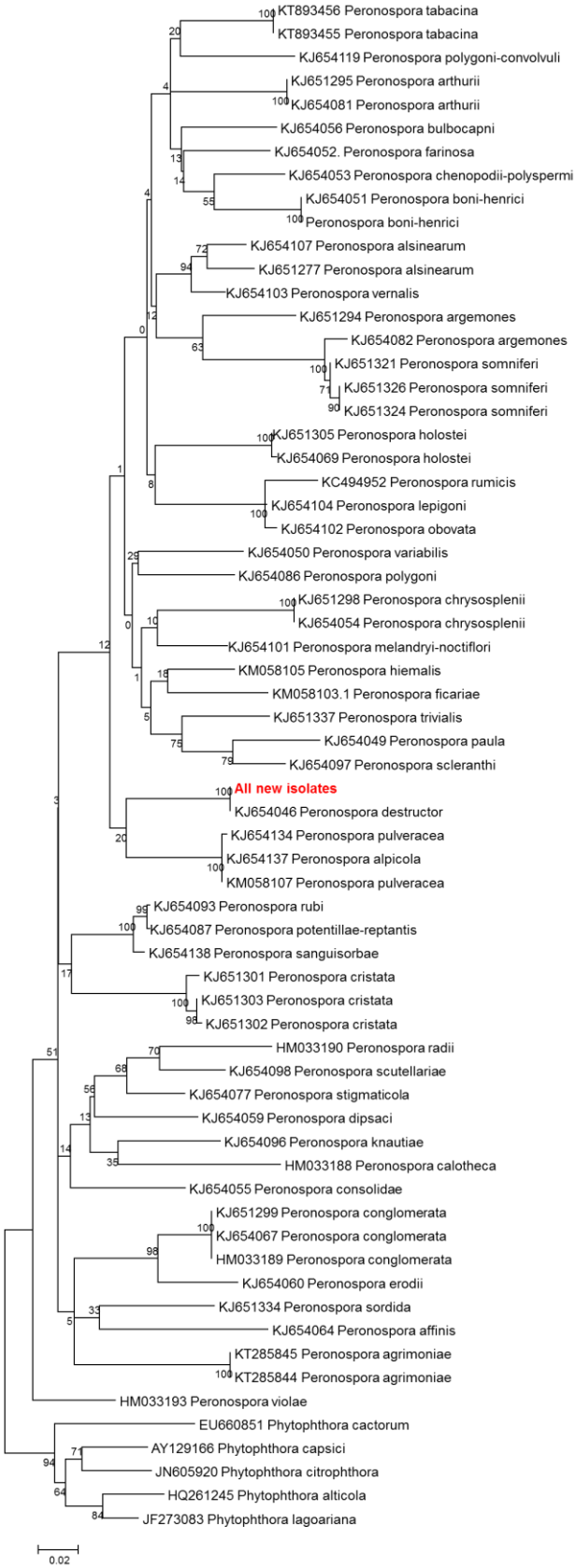

Figure 9. Maximum likelihood tree of *Peronospora* isolates from onion and other hosts based on COX1. Numbers represent bootstrap values (1000 replicates). Scale bar indicates 0.02 substitutions per site. The tree is rooted using sequences from *Phytophthora*.

Based on COX2 sequence, all the UK isolates had identical sequences to the same isolate from Germany (KJ654195), further suggesting a clonal origin (Fig. 10). *P. destructor* appears to be quite distinct from the other *Peronospora* species with the closest relative being *P. kochiae-scopariae*, an isolate obtained from the herb *Bassia scoparia.*

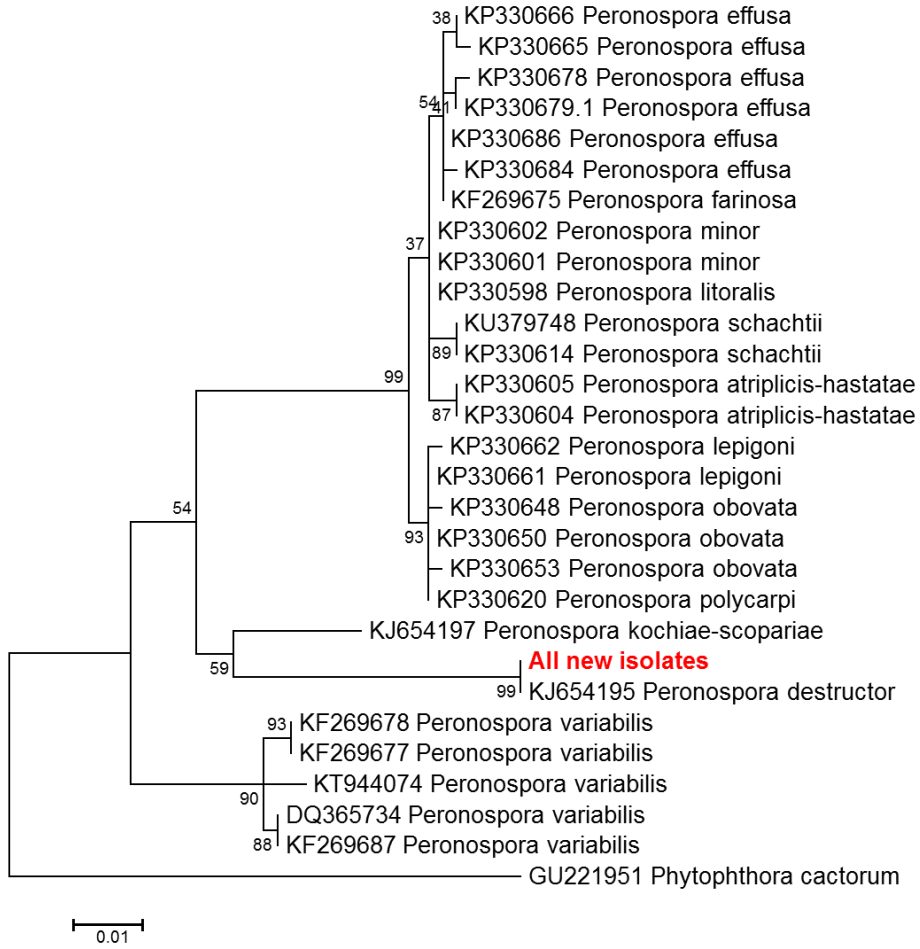

Figure 10. Maximum likelihood tree of *Peronospora* isolates from onion and other hosts based on COX2. Numbers represent bootstrap values (1000 replicates). Scale bar indicates 0.01 substitutions per site. The tree is rooted using *Phytophthora cactorum*.

ITS sequences were not analysed for all isolates as they often resulted in a mixed sequence, probably due to other organisms being present on the onion leaf. However, a single sequence was obtained for the isolate Ratpit. Based on ITS sequences, *P. destructor* isolates are clearly distinguished from other *Peronospora* species (Fig. 11). The ITS sequence for the isolate Ratpit was identical to isolates from Japan and Norway but has small differences from isolates from Australia, Italy and the USA.

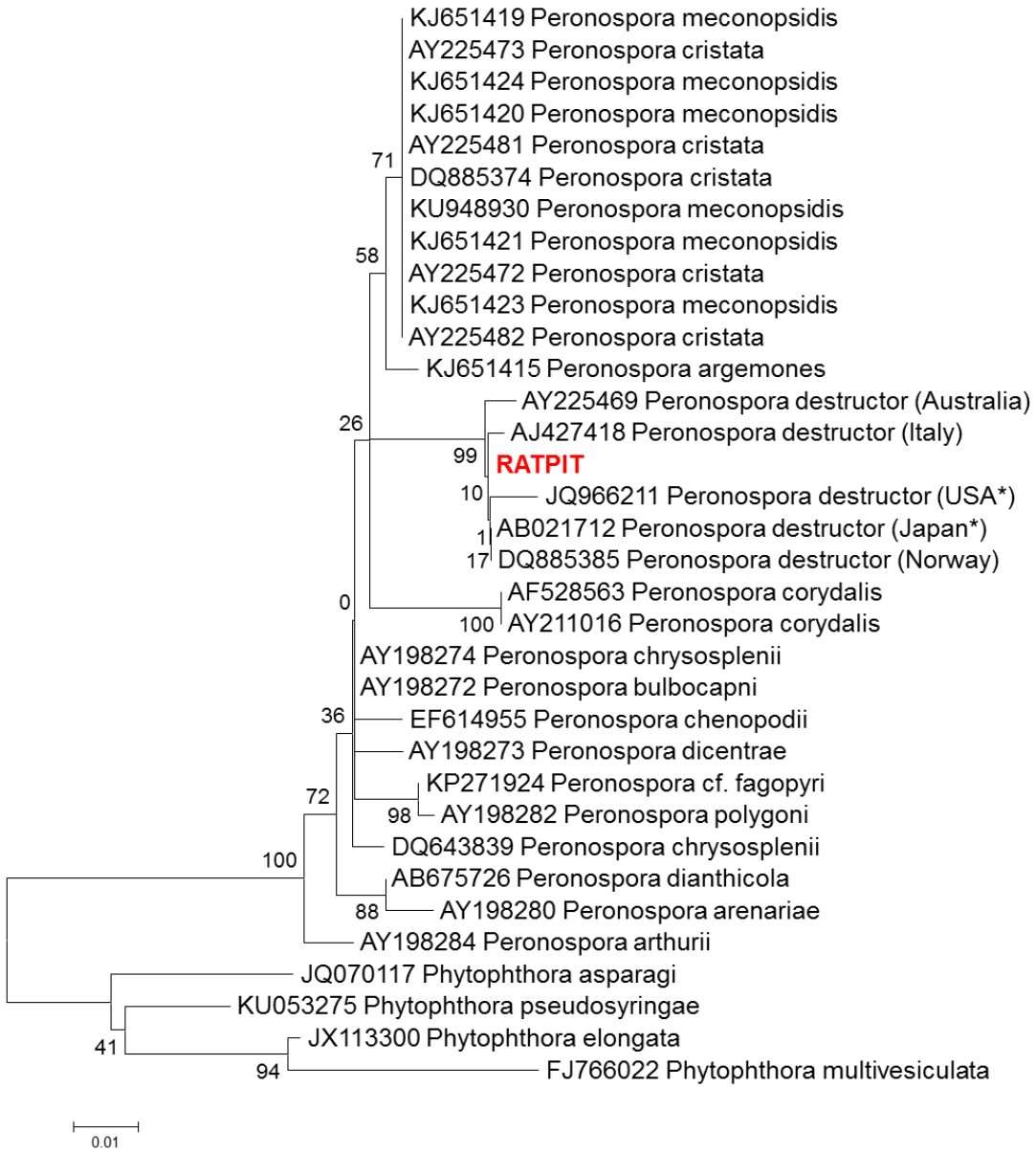

Figure 11. Maximum likelihood tree of *Peronospora* isolates from onion and other hosts based on ITS. Numbers represent bootstrap values (1000 replicates). Scale bar indicates 0.01 substitutions per site. The tree is rooted using *Phytophthora spp.* An asterisks indicates some uncertainty on the country of origin.

Botrytis spp.

A total of six different *Botrytis* species (*B. allii, B. byssoidea, B. aclada, B. cinerea, B. pseudocinerea* and *B. squamosa*) were found to be associated with diseased onion samples with foliar symptoms. Some *B. cinerea* isolates did not produce an amplicon with the IGS primers and are hence not included in the analysis. The phylogeny based on IGS sequences distinguished all the different *Botrytis* species with the exception of CH1 and CH2 which appear to fall between *B. byssoidea* and *B. allii* (Fig. 12). However, the G3PDH phylogeny identified both CH1 and CH2 as *B. byssoidea*. Greater variation was observed within *B. allii* than *B. aclada*. G3PDH sequence does not clearly separate *B. aclada* and *B. allii* isolates but does appear to distinguish *B. byssoidea* from *B. allii* (Fig. 13). Therefore, sequencing both loci is beneficial. The new isolates of *B. squamosa* (and also the isolates from the Crop Centre culture collection) did not amplify with G3PDH primers.

Additional *Botrytis* isolates from onion were sourced from a culture collection housed at Warwick Crop Centre. Based on the IGS and G3PDH sequences, some of these isolates were re-classified (Table 6).

Table 6. Corrected species identification for a range of *Botrytis* isolates from a culture collection held at Warwick Crop Centre.

Isolate	Original classification	Revised classification
113	*B. squamosa*	*B. squamosa*
118	*B. squamosa*	*B. squamosa*
119	*B. squamosa*	*B. squamosa*
236	*B. allii*	*B. allii*
237	*B. allii*	*B. aclada*
238	*B. allii*	*B. aclada*
241	*B. allii*	*B. aclada*
242	*B. allii*	*B. aclada*
252	*B. allii*	*B. aclada*
255	*B. allii*	*B. allii*
4092	*B. allii*	*B. allii*
47186	*B. allii*	*B. allii*
IMI1 (147186)	*B. allii*	*B. allii*
IMI2 (292066)	*B. allii*	*B. allii*

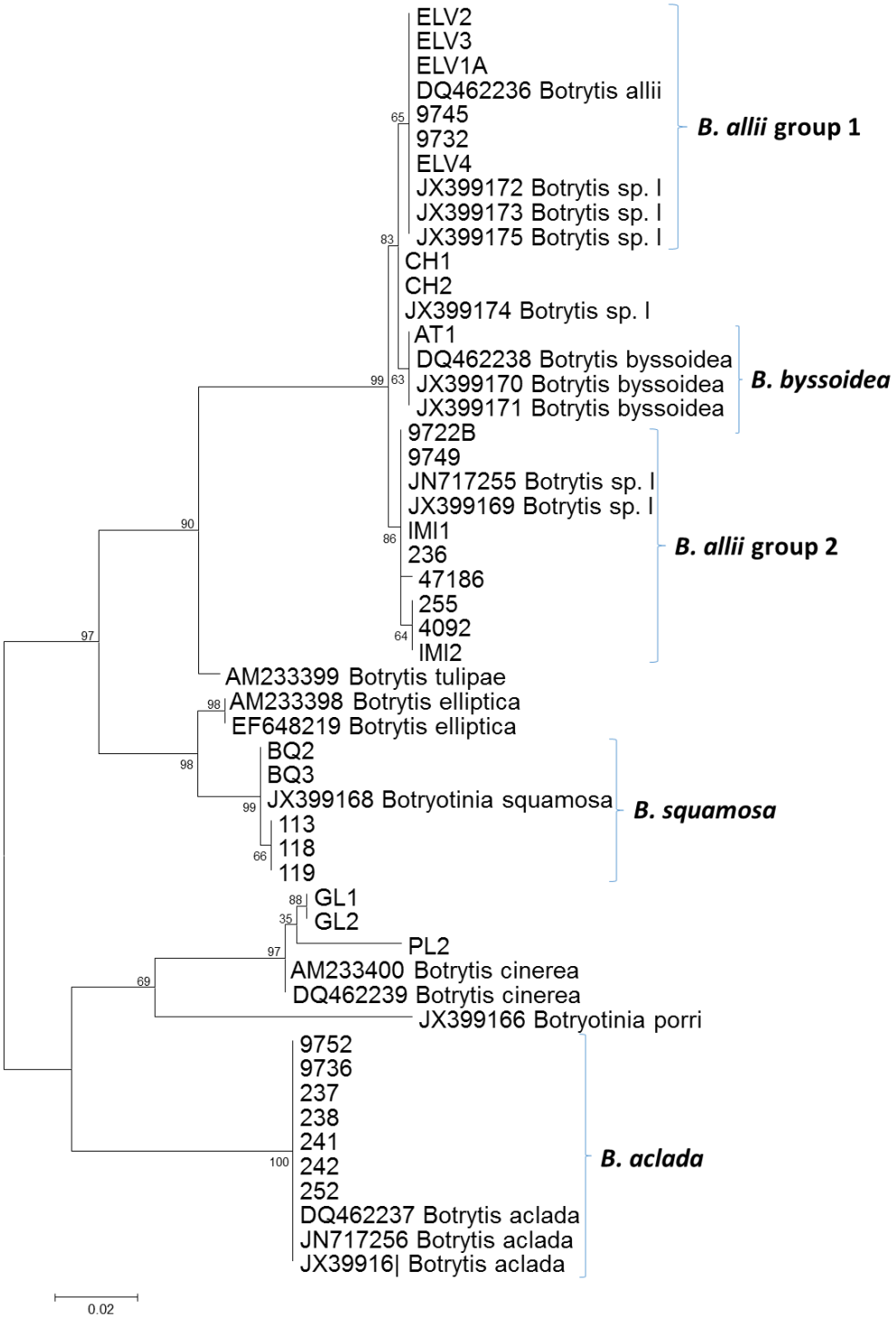

Figure 12. Maximum likelihood tree of *Botrytis* isolates from onion and other hosts based on IGS. Numbers represent bootstrap values (1000 replicates). Scale bar indicates 0.02 substitutions per site.

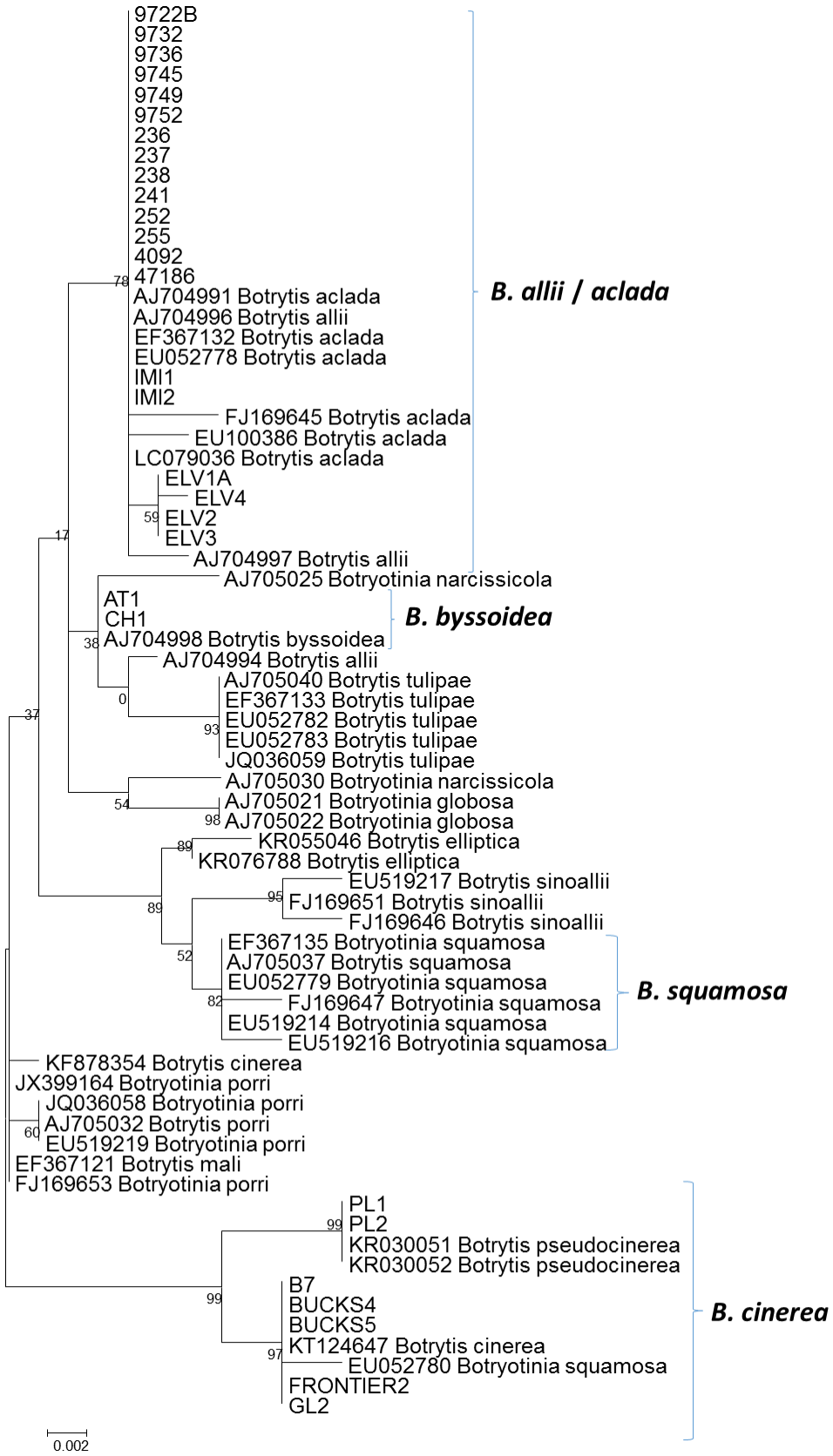

Figure 13. Maximum likelihood tree of *Botrytis* isolates from onion and other hosts based on G3PDH. Numbers represent bootstrap values (1000 replicates). Scale bar indicates 0.002 substitutions per site. The tree is rooted using *Botrytis cinerea* sequences.

S. cepivorum

A total of 22 isolates of *S. cepivorum* were assembled from eight different locations. Surprisingly, all isolates were 100% identical based on the sequences of six different loci. ITS sequence clearly separated *S. cepivorum* from other species but does not clearly resolve some of the other species within the *Sclerotinaceae* (Fig. 14). Despite their diverse origins, all isolates of *S. cepivorum* share identical ITS sequences with a single exception, a previously reported isolate from the UK (FJ231400) which has a single base change. An additional isolate (FJ231402), labelled as *S. cepivorum*, appears to be wrongly classified and is very closely related to *S. perniciosum*. There is no information available detailing the host or pathogenicity of this isolate.

The phylogenies based on 18S and 28S sequences do not resolve the species within the *Sclerotinaceae* and there are no sequences on the public repositories for *S. cepivorum* (data not shown). However BLAST searches of the *S. cepivorum* sequences revealed 99% identity with both *S. sclerotiorum* and *B. cinerea* for 18S and 28S. Based on both HSP60 (Fig. 15) and G3PDH (Fig. 16), all UK isolates had an identical sequence to an isolate from USA (JQ036104/ JQ036035), suggesting a clonal origin. Both these loci also clearly separated *S. cepivorum* from other species within the *Sclerotiniaceae*. Based on β-tubulin sequences, *S. cepivorum* could clearly be distinguished from *S. sclerotiorum* and *S. nivalis* (Fig. 17). No further *Sclerotiniaceae* β-tubulin sequences exist on public repositories.

Other fungi

Whilst attempting to isolate *B. squamosa* from onion leaves, A *Stemphylium* species was also consistently isolated. All isolates shared identical ITS sequences and were provisionally identified as *S. vesicarium* based on 100% nucleotide identity. However, these isolates also shared 100% identity with some other *Stemphylium* species. Other species isolated from onion leaves/bulbs included *Alternaria infectoria*, *Trichoderma* sp. and *Harzia acremonioides* (or a close relative of this fungus- 100% nucleotide identity based on ITS).

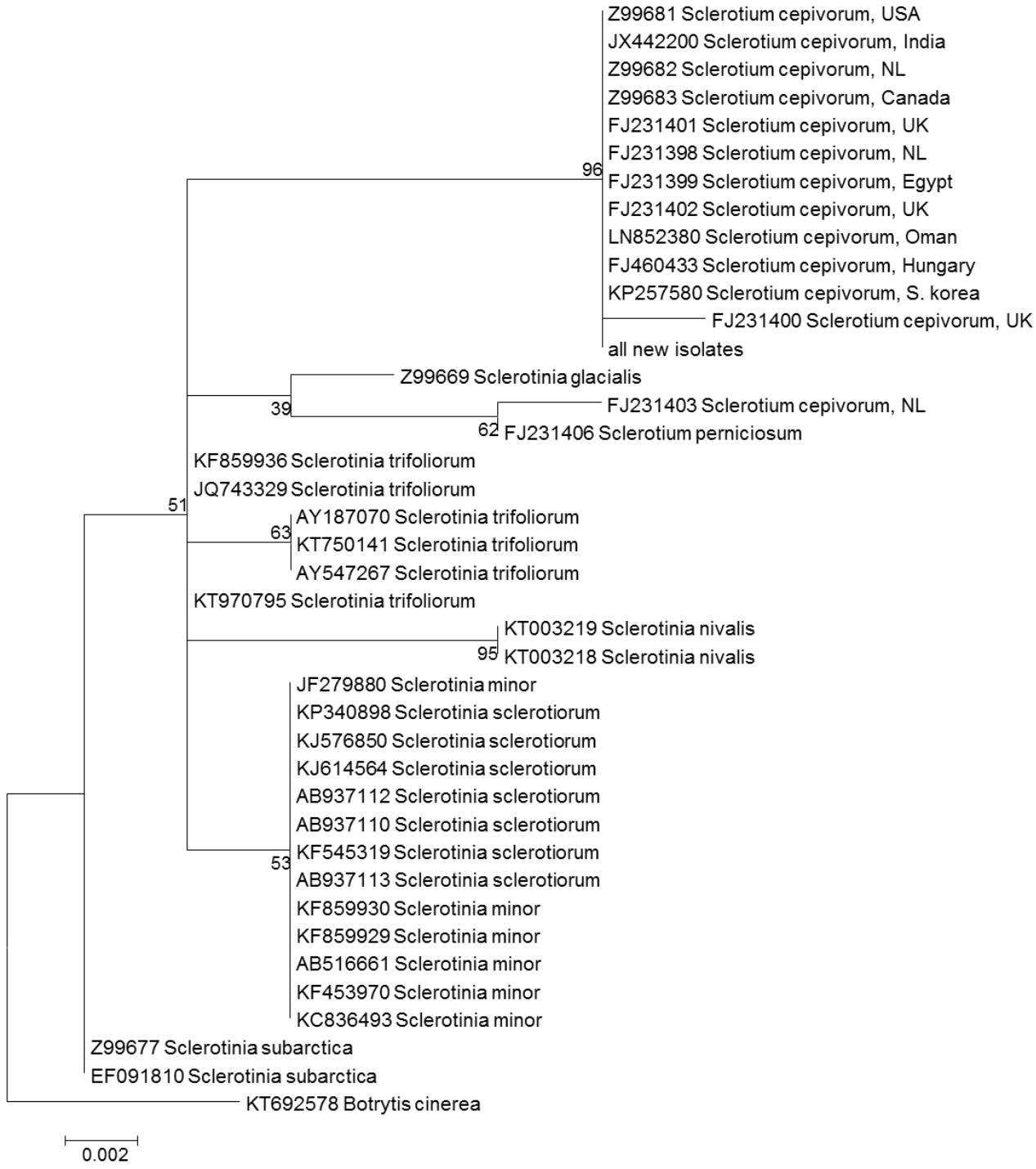

Figure 14. Maximum likelihood tree of *S. cepivorum* isolates from onion and other *Sclerotinaceae* based on ITS. Numbers represent bootstrap values (1000 replicates). Scale bar indicates 0.002 substitutions per site. The tree is rooted through *B. cinerea*.

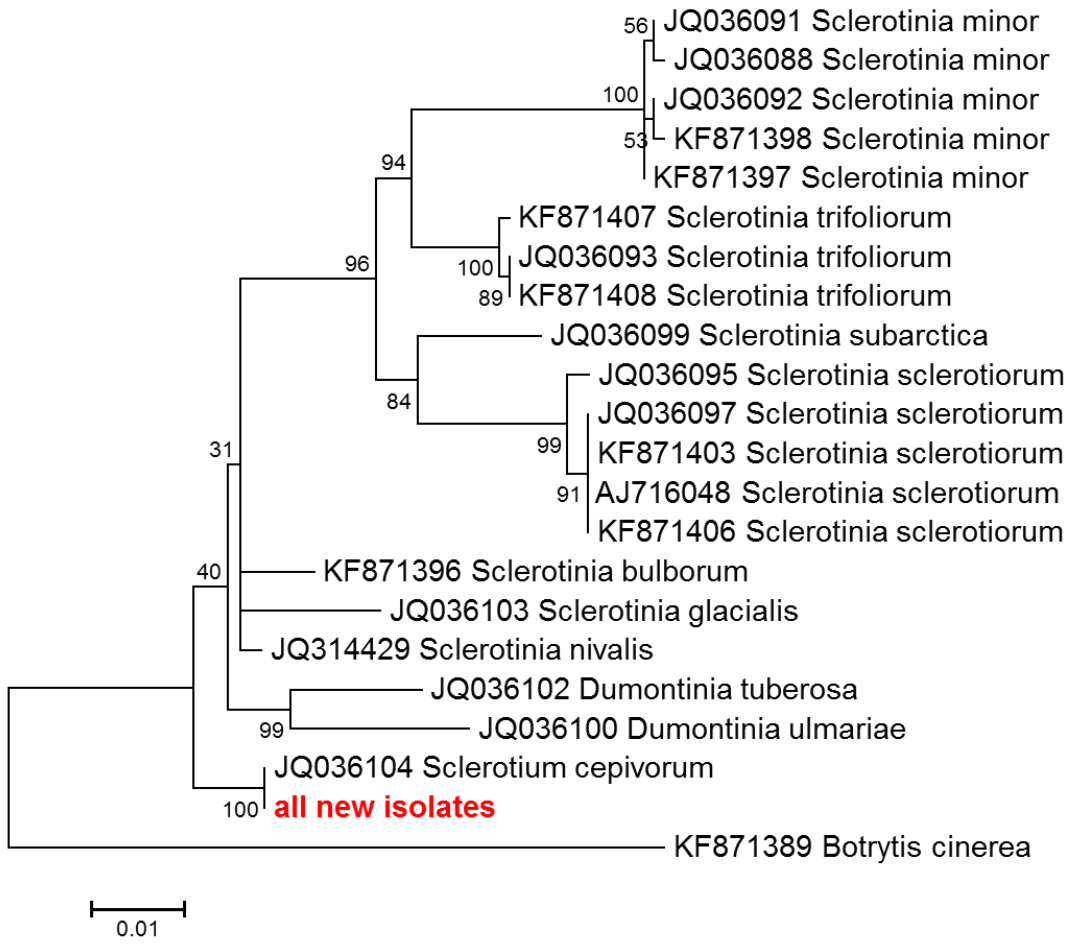

Figure 15. Maximum likelihood tree of *S. cepivorum* isolates from onion and other *Sclerotinaceae* based on HSP60. Numbers represent bootstrap values (1000 replicates). Scale bar indicates 0.01 substitutions per site. The tree is rooted through *B. cinerea*.

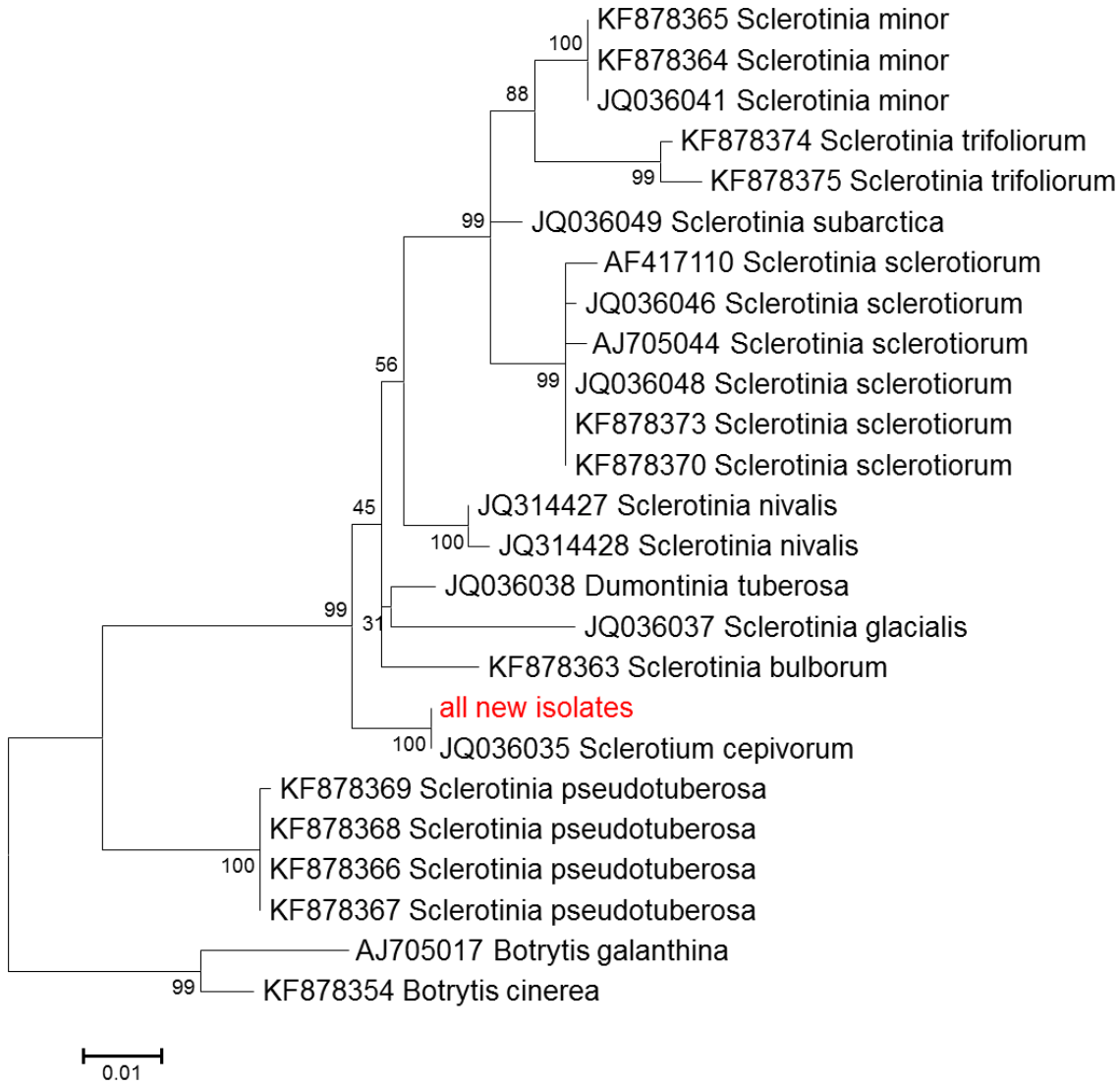

Figure 16. Maximum likelihood tree of *S. cepivorum* isolates from onion and other *Sclerotinaceae* based on G3PDH. Numbers represent bootstrap values (1000 replicates). Scale bar indicates 0.01 substitutions per site. The tree is rooted through *B. cinerea*.

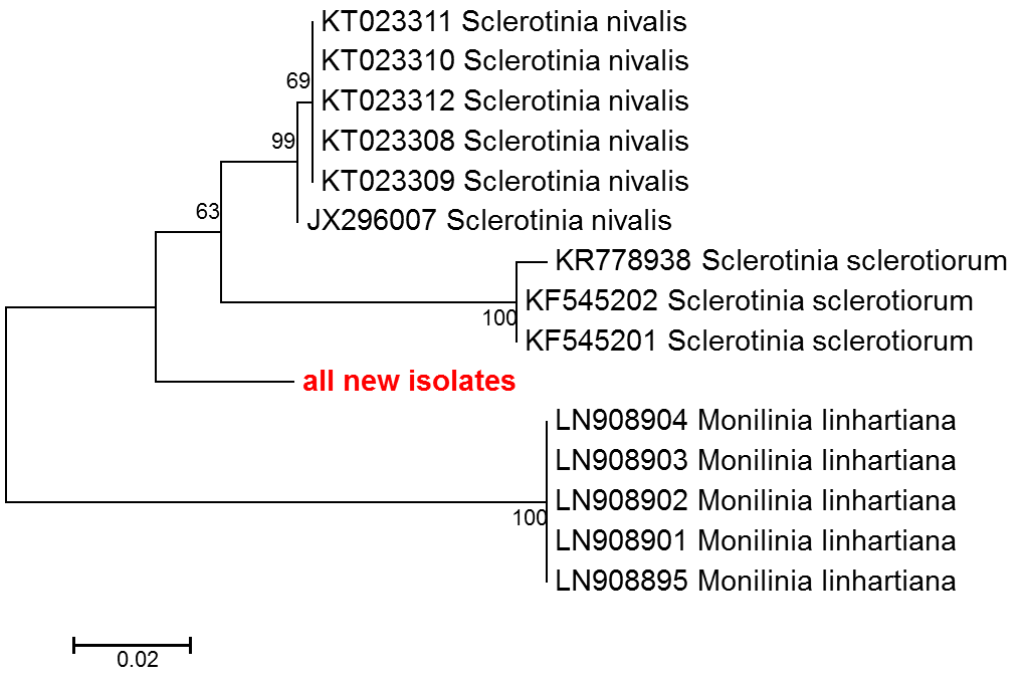

Figure 17. Maximum likelihood tree of *S. cepivorum* isolates from onion and other Sclerotinaceae based on β-Tubulin. Numbers represent bootstrap values (1000 replicates). Scale bar indicates 0.01 substitutions per site. The tree is rooted through Monilinia linhartiana.

Additional Objective: molecular characterisation of *F. oxysporum* f. sp. *narcissi* (FON) isolates

Based on the sequences of three housekeeping genes (*EF1-α, TUB2* and *RPBII*), all FON isolates were identical with a single exception; FOXN129 showed a single base change (T to A) in TUB2 causing a single amino acid change from phenylalanine to tyrosine. Differences between the isolates were also observed based on the presence / absence of SIX genes where four distinct groups of isolates were observed (Table 7) containing five, four two or one SIX gene respectively. All isolates contained SIX10, 26 of the 30 isolates contained SIX7, 27 contained SIX9 and 26 contained SIX12. A single isolate, FOXN63, contained SIX13. Where a positive PCR product was obtained, the sequences of the SIX gene amplicons were identical for all isolates. Very high levels of sequence identity with the respective genes in *F. oxysporum* fsp. *lycopersici* were also observed, with all genes having at least 84% protein identity and 93% nucleotide identity. The FON SIX9 gene was also 99% identical to FOC SIX9.

Table 7. Presence of SIX genes in *F. oxysporum* f. sp. *narcissi* isolates

Isolate	Location	Variety	Lesion area	SIX7	SIX9	SIX10	SIX12	SIX13
63	Truro, Cornwall	Magnificence	7.37					
139	Falmouth, Cornwall	Scrumpy	8.28					
115	Falmouth, Cornwall	Pinza	7.22					
7	Norfolk	White Lion	6.84					
42	Holt, Norfolk	Golden Ducat	6.81					
75	Norwich, Norfolk	Pheasant's Eye	6.79					
89	Cornwall (supplier C)	Jedna	6.54					
97	Cornwall (supplier C)	Hollywood	6.42					
122	Falmouth, Cornwall	Unique	6.40					
24	Boston, Lincs	Carlton	6.36					
29	Norfolk	Fortune	6.18					
11	E Cornwall	Carlton	6.12					
38	Spalding, Lincs	Carlton	5.86					
34	Holt, Norfolk	Sempre Avanti	5.78					
94	Cornwall (supplier C)	Hollywood	5.53					
87	Cornwall (supplier C)	Golden Ducat	5.36					
118	Falmouth, Cornwall	Orkney	4.59					
55	Penzance	White Lion	4.44					
58	Spalding, Lincs	Great Leap	4.15					
129	Falmouth, Cornwall	Whiskey Galore	4.02					
141	Falmouth, Cornwall	Mithrel	4.02					
19	Spalding, Lincs	Quirinus	3.64					
15	Spalding, Lincs	St Keverne	3.53					
152	Moulton, Lincs	Carlton	3.36					
133	Falmouth, Cornwall	Hampton Court	3.30					
68	Spalding, Lincs	Spellbinder	3.24					
77	Norwich, Norfolk	Pheasant's Eye	4.44					
81	Truro, Cornwall	Salome	7.49					
46	Truro, Cornwall	Salome	7.05					
3	Norfolk	White Lion	6.71					

Additional work: First report of *Fusarium oxysporum* causing a vascular wilt of statice (*Limonium sinuatum*) in the UK (Taylor *et al*, 2016b)

Fungal isolates obtained from the statice plants were confirmed as *Fusarium oxysporum* based on EF1-α sequences. All isolates obtained were identical. In culture, both micro-and macroconidia typical of *F. oxysporum* were observed (Fig. 18b & c). In the pathogenicity assay, 20 days after re-planting, typical wilt symptoms were observed in plants inoculated with the four *F. oxysporum* isolates from statice and after 40 days all the inoculated plants were dead (Fig. 18, Taylor *et al*, 2016b). All plants inoculated with *F. oxysporum* isolate Fo47 and uninoculated control plants remained healthy. The pathogen was successfully re-isolated from infected plants with cultures exhibiting the same morphology as described previously.

Following DNA extraction, amplification and sequencing of the EF1-α gene, all sequences were 100% identical to those obtained for the original isolates.

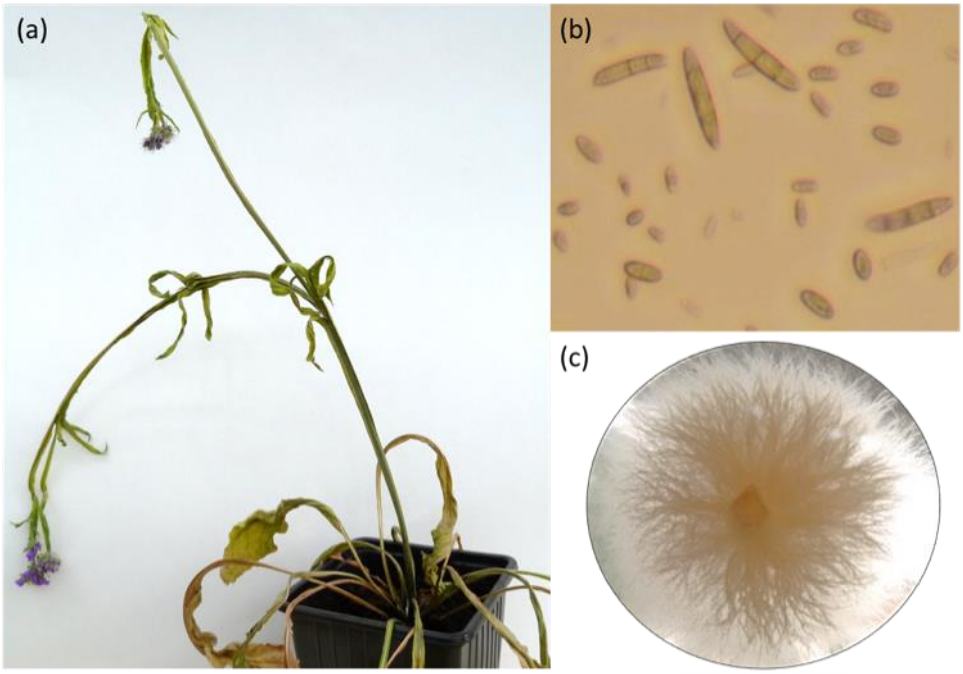

Figure 18. *Fusarium* wilt of statice (*Limonium sinuatum*). (a) typical wilt symptoms; (b) morphology of micro- and macroconidia; (c) typical colony morphology

Additional work: Development of diseased areas in Wellesbourne quarantine field

White rot area

A total of 663 plants were scored for symptoms, with 581 showing evidence of white rot (Fig. 19). The mean disease incidence was 87.7% with a standard error of 1.71. This area therefore represents a very high level of disease pressure.

Figure 19. symptoms of *Allium* white rot observed in the Warwick Crop Centre quarantine field.

***Fusarium* basal rot area:**

Severe symptoms of basal rot were observed throughout the growing season (Fig. 20). Based on foliar symptoms, the mean symptom score per plot was 2.04 (SEM=0.09) and the mean disease incidence was 92.9% (SEM=1.55). Based on bulb symptoms, the mean symptom score was 1.69 (SEM=0.11) with a disease incidence of 76.9% (SEM=3.35). However, it should be noted that this does not account for plants which have died back at an earlier stage and are no longer visible in the plot.

***Sclerotinia* area**

Where plants were inoculated, 100% infection was observed (Fig. 21). The mean number of sclerotia produced per plant was 112 (SEM=14.5). This equates to 89869 sclerotia produced in the field and 85 sclerotia per m^2.

Figure 20. Symptoms of *Fusarium* basal rot observed in the Warwick Crop Centre quarantine field.

Figure 21. Symptoms of *Sclerotinia* rot observed in the Warwick Crop Centre quarantine field.

Discussion

Objective 1.6: Test published PCR diagnostic for *S. cepivorum*

The published PCR-based diagnostic was tested and shown to be effective and specific to *S. cepivorum*. Low levels of DNA were detected in samples taken from the Wellesbourne quarantine field which is known to be infested. A peak in detection was observed later in the season, coinciding with an increase in the number of symptomatic plants. It may be more beneficial to carry out large scale DNA extractions (as described Woodhall *et al* 2012), in order to capture sclerotia which may be dispersed within the soil. This would give a more accurate prediction of disease risk in a field where low numbers of sclerotia may be sufficient to cause significant disease. A future objective of this fellowship will be to investigate possible large-scale DNA extraction methods from soil.

Objectives 1.8/1.9: Develop qPCR for *P. violae* using WCC Roche Lightcycler / Quantify *P. violae* in soil samples from AHDB Horticulture project FV 405 and other samples where available

A new quantitative PCR-based Taqman assay was developed in order to achieve increased sensitivity for detection of *P. violae*. The assay was shown to be highly sensitive and capable of accurately quantifying levels of DNA as low as 1fg and was more accurate than a conventional PCR assay using published primers (Klemsdal *et al* 2008). The assay will be very useful for monitoring *P. violae* levels in experiments and field trials and is currently being used in PhD Studentship FV 432. Soil samples from project FV 405 were tested and accurate quantification was achieved when compared with the conventional PCR assay. However, further work needs to be carried out to assess the utility of this assay in conjunction with the oospore spore capture procedure. If these two processes can be optimised then larger soil samples can be analysed for the presence of *P. violae* allowing for a more accurate assessment of pathogen load in carrot growing soils. As part of FV 432, this work will be further developed by relating soil DNA quantities to disease levels in the field.

Objective 1.10: Identify potential primers for *Itersonilia* diagnostics from existing gene sequences (or whole genome sequence).

Itersonilia primers suitable for qPCR were identified which appear to be specific and result in good amplification from a range of different isolates. There is also the possibility of converting this into a Taqman assay thus allowing an increased level of sensitivity. The PCR test will be potentially very useful for testing seeds, soil and roots for *Itersonilia*. Parsnip seed is routinely

tested by industry for *Itersonilia* using an agar based test and in the future a PCR based approach may increase accuracy and detection sensitivity. It would also be beneficial to determine what level of DNA on seeds correlates with significant disease levels in the field. Using the *M. acerina* genome sequence, potential diagnostic primers were also designed and tested. Further work is needed however to develop these primers to make them suitable as a potential diagnostic test for soil, roots and seed.

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) / 2.2 Confirm identity and characterise isolates from 2.1 by gene sequencing

Isolates of *P. destructor* were collected from various sites around the UK and were all shown to be identical to isolate from Germany, Norway and Japan based on housekeeping gene sequences. This suggests a clonal origin of these isolates and could imply that control measures such as fungicides and resistant varieties are effective against all isolates. Small differences (based on ITS) were observed between a new UK isolate and isolates from USA, Italy and Australia possibly related to local adaptation. However it should be noted that fungi can have identical housekeeping gene sequences but differ in functional and effector gene profiles. Downy mildew resistance in onion has been introgressed from *Allium roylei* (Scholten *et al* 2007) and this has been released as the resistant onion cultivar Santero F1, by Hazera seeds. This resistance is currently 100% effective although being based on single gene resistance, it is likely that the pathogen will overcome this resistance. However, at the current time, we have no evidence for significant variation within *P. destructor*, at least in the UK and Northern Europe based on housekeeping genes.

A total of six *Botrytis* species (*B. allii, B. aclada, B. byssoidea, B. squamosa, B. cinerea* and *B. pseudocinerea*) were found to be associated with diseased onions with foliar symptoms. Previous studies have shown that *B. allii* (now divided into *B. allii / B. aclada*), *B. byssoidea* and *B. squamosa* are all associated with onions in the UK (Presly, 1985). In addition, *B. cinerea* (and probably *B. pseudocinerea*) is well known to be associated with a range of plants. *B. byssoidea, B. squamosa* and *B. allii* can all cause lesions on both bulb onion and salad onion leaves (Presly 1985), but *B. squamosa* was rarely isolated in this project and it may be that other species have a greater involvement in leaf blight than was previously thought. Alternatively, *B. squamosa* may be more difficult to isolate than other species. *B. allii, B. byssoidea* and *B. aclada* all cause neck rot (Chilvers & du Toit 2006) and were all isolated. A range of *Botrytis* isolates will be tested for their pathogenicity against onion in the future. Utilising a combination of IGS and G3PDH gene sequences, *Botrytis* species can be

distinguished without the need for the examination of spore morphology. In New Zealand, variation within *B. allii* was detected based on the presence of an intron in the ITS region (Khan *et al* 2013). The description of this species (termed *Botrytis* sp, I) is circumspect, but it may be beneficial to test the UK isolates for the presence of this intron. When attempting to isolate *B. squamosa*, a *Stemphylium* species, (probably *S. vesicarium*) was consistently isolated. This species is known to cause leaf blight of onions, especially in warmer climates (Suheri & Price 2000). It is unclear whether *Stemphylium* is simply present on leaves or is actually causing some blight symptoms in the field in the UK. This disease should be monitored as it may increase due to climate change.

A total of 22 *S. cepivorum* isolates were obtained from eight locations in the UK. Despite the diversity in isolate origin, all isolates were 100% identical based on sequences from different loci. This was surprising, but was also supported by 100% identity with sequences from isolates originating from other countries. The single exception was a 1bp difference in an historic isolate from the UK. This suggests a clonal origin for *S. cepivorum* and may mean a single effective control measure will be effective against all isolates. However, control of *Allium* white rot can be very challenging, and host resistance has hitherto not been identified although there is some potential for biological control (Clarkson *et al* 2006).

The fungus *Harzia acremonioides* (or a very close relative) was also isolated from onion leaves. These leaves were examined under a microscope and seen to have structures typical of *Stemphylium* on the surface. This is an interesting finding as *Harzia acremonioides* has been reported to be a contact mycoparasite of *Stemphylium botryosum* (Urbasch 1986). It may be that *Harzia acremonioides* also acts as a mycoparasite of *S. vesicarium*, the *Stemphylium* species associated with onion leaf blight. This fungus could be beneficial for future biological control of *Stemphylium* blight on onion.

Additional Objective: molecular characterisation of *F. oxysporum* f. sp. *narcissi* (FON) isolates

FON isolates were characterised and shown to vary in their complement of pathogenicity (SIX) genes. The presence / absence of SIX genes defines races in many f. sp. (Taylor *et al* 2016a) and as such there is strong evidence for the presence of a race structure in FON. The existence of races has not been previously reported and further research would be required to confirm this theory. If a race structure is confirmed, this would impact on breeding for FON resistance. In addition, all isolates tested contained SIX10, presenting this gene as a possible target for designing specific primers for a qPCR diagnostic which could be used to predict /

monitor disease in the field. Further work would be required to determine the correlation between DNA quantities and disease in the field.

Additional work: First report of *Fusarium oxysporum* causing a vascular wilt of statice (*Limonium sinuatum*) in the UK (Taylor *et al*, 2016b)

A new disease of statice, *Fusarium* wilt, was identified and reported for the first time (Taylor *et al*, 2016b). This disease caused severe wilt, akin to many *Fusarium* wilts reported. Control is likely to be difficult due to the accumulation of long-lived chlamydospores in the soil. Statice growers should be aware of this disease as it can cause devastating losses and spread around a nursery very rapidly.

Additional work: Development of diseased areas in Wellesbourne quarantine field

Quarantine field areas for *Fusarium* basal rot and white rot of onion as well as *Sclerotinia* were established which resulted in high and consistent disease levels. These will serve as an invaluable future resource for field trials testing control options.

Conclusions

- A published PCR-based diagnostic for *S. cepivorum* was tested and shown to be effective and specific, enabling field testing for this pathogen and potential assessment of disease risk.
- Potential diagnostic tests for the parsnip canker pathogens *Itersonilia* and *M. acerina* are being developed and initial results are promising. These tests would be beneficial for seed, soil and root testing.
- A total of six *Botrytis* species were found to be associated with onion, some of which may cause leaf blights and others neck rot. Species were identified by sequencing IGS and G3PDH genes. Accurate identification of *Botrytis* species can help inform decisions on control measures.
- Isolates of *S. cepivorum* and *P. destructor* were shown to be 100% identical based on molecular characterisation, suggesting a clonal origin. Furthermore, 100% identity with isolates from other countries was often observed.
- A *Stemphylium* species, possibly *S. vesicarium* was consistently isolated from onion leaves and may cause a leaf blight if climatic conditions are optimal.
- A set of 30 FON isolates was characterised and shown to have variable effector gene compliments, strongly suggesting a race structure in this forma specialis.

- Quarantine field areas with high and consistent disease levels were developed for onion white rot, onion basal rot and Sclerotinia disease and will serve as an invaluable future resource for field trials.
- A new disease of statice, *Fusarium* wilt, was identified and reported for the first time.

Knowledge and Technology Transfer

Grower/technical visits

- Visited Hobson Farming and met with Rodger Hobson (30th Nov)
- VCS visit to Warwick Crop Centre- presented posters (14th Dec)
- Visited Hobson Farming and met with Rodger Hobson (18th Jan)
- Meeting with Enza Zaden to discuss Fusarium (15th Jan)
- Visited Whiteheads nursery in Boston, Lincs to sample stocks and statice for *Fusarium*-isolations carried out by student including from water sample (22nd June).
- Visited 3 fields in Lincolnshire (18th July) with diseased onions and collected samples with symptoms of white rot and downy mildew. Met with Carl Sharp from the *Allium* and Brassica Centre

Conferences / seminars

- Attended and presented posters on *Fusarium* research and this fellowship at the UK Carrot and Onion conference (4th-5th Nov)
- Attended, gave an oral presentation titled 'Understanding pathogenicity and resistance in the *Fusarium oxysporum*-onion pathosystem' and presented a poster on the same subject at the HAPI conference in Cambridge (1-2nd March 2016).
- Attended and gave an oral presentation titled 'Understanding pathogenicity and resistance in *Fusarium oxysporum* f.sp. *cepae*' at the *Fusarium* satellite meeting (part of the European Fungal Genetics meeting) in Paris (3rd April 2016)
- Presented a seminar titled 'Understanding the genetic control of pathogenicity and resistance for *Fusarium oxysporum* in onion' in the Warwick Crop Centre seminar series (19th May 2016)
- Attended and gave a presentation titled '*Fusarium* basal rot of onion' at the Elsoms onion conference (Elveden estate, 6th June 2016)

- Attended and presented a poster on *Fusarium* basal rot at to the New Frontiers in Crop Research event in London (20th Oct)

Publications

- Paper titled: 'Identification of pathogenicity-related genes in *Fusarium oxysporum* f. sp. *cepae*' published in Molecular Plant Pathology.
- Paper titled: 'First report of *Fusarium oxysporum* causing a vascular wilt of statice (*Limonium sinuatum*) in the UK' submitted to Plant Disease.

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