



Grower Summary

CP 113

Maintaining and developing
capability in vegetable crop
pathology

Annual 2016

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Fellowship staff: ("Trainees")	Dr John Clarkson & Dr Andrew Taylor
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Industry Representative:	
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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 *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 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