Grower Summary

CP 136

Development and testing of single and multiplex diagnostic devices for rapid and precise early detection of oomycete root and collar rot pathogens for disease avoidance, management and control

Annual 2017

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Further information

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Date project commenced:	01/06/2015
Expected completion date:	31/06/2018

GROWER SUMMARY

Headline

- A representative cross-sector collection of identified isolates of *Phytophthora* and *Pythium* species from plant roots, collars and crowns as well as compost and water samples has been assembled. More than 30 isolates of commonly seen non-target species have also been identified and collected. These collections are important for two reasons: firstly to make sure antibodies are raised to isolates/species that represent current disease threats to the UK industry and secondly for testing the specificity and efficacy of the antibody tests developed in this project. Although a good collection has been assembled it will continue to be expanded with ongoing nursery visits and incoming clinic samples.
- Progress with antibody development is very promising with antibodies already assessed with potential to discern +/- oomycete, +/- *Phytophthora,* and combinations that can discern *Phytophthora* clade 7 (includes *P. fragariae* and *P. rubi*) and clade 8 (includes *P. cryptogea* and *P. ramorum*). A further two *Phytophthora* antibodies are still to be assessed plus 10 raised to *Pythium* species. Four *Phytophthora* antibodies have been selected for the first series of field tests using the LFD format.

Background

Oomycetes and crop disease. Worldwide, oomycete diseases cause significant losses across a range of agricultural and horticultural commodities. The diseases they cause include seedling blights, damping-off, crown and root rots, foliar blights and downy mildew. Of the Oomycetes (a group of fungus-like organisms), *Pythium* species are well known for causing damping-off and seed rot diseases. Often occurring just after planting, as young seedlings emerge. *Pythium* related disease epidemics are also synonymous with root rots on newly emerged or more mature plants and soft rots of fleshy fruit. Likewise, the aptly named *Phytophthora* genus (*Phyto* (plant) *phthora* (destroyer)) cause significant damage worldwide on a range of crop types. Often associated with above ground plant parts i.e. shoot apex, leaf, stem and fruit they are also responsible for root and crown rots.

Detection and diagnosis. Reliable and affordable detection and diagnosis are key to effective oomycete disease management. With increasing globalization, travel and the international trade in plants the risk of disease through inadvertent introduction is exacerbated. A classic example of this was reported by White (HDC PC 97) with widespread dissemination of *Pythium* species across UK nurseries via Danish trolleys. Early diagnosis can provide growers with vital information regarding the effectiveness of nursery sanitization processes, source contaminants, control measures to prevent spread, disease containment or

eradication, varietal selection, harvest date and post-harvest handling. Information on pathogen presence prior to the possibility of infection can be used to highlight where and when treatments are needed, potentially thereby reducing disease epidemics significantly. Classical methods for the isolation and identification of oomycete crop pathogens however are commonly used only after disease symptoms are observed and take valuable time to implement. Current best practice diagnostic tests for *Pythium* and *Phytophthora* take upwards of 24 hrs with bait tests and between 3 and 10 days by conventional agar methods.

Even with identification to genus, the choice is still between taking further time to carry out a pathogenicity test if the potential pathogen has been isolated or the application of immediate control measures. Immediate control measures would likely be recommended in the event of detecting *Phytophthora* sp., whereas the immediate response to a *Pythium* sp.-positive test would be more ambivalent unless this was clearly linked to plants showing unequivocal symptoms. This is because most *Phytophthora* species currently known are plant pathogens, and whilst different species have different host preferences and host ranges, it is assumed that mere presence of detectable inoculum is an indication of potential trouble. On the other hand, a large proportion of the 200 or so species of *Pythium* are saprophytic or certainly not known to be pathogenic to any horticultural crop, and at least four species are even mycophagous, some with the capacity to elicit disease resistance mechanisms in plants (Vallance *et al*., 2009) and therefore even potentially beneficial. Rapid *in situ* diagnosis to genus level is currently possible using commercially-available ELISA-based LFD test kits (e.g. Alert LF™ kits, Adgen Phytodiagnostics and Pocket Diagnostic® kits, Forsite Diagnostics). Whilst of some help, these tests are unfortunately limited by their lack of specificity and the potential cross-reaction of the antibodies used with some non-target species of closely related oomycete genera leading to some 'false-positives'.

This AHDB-funded project (CP 136), now in its final year, is aimed at improving this situation by developing new monoclonal antibodies for LFD kits, raised to selected specific species (or groups within species known as clades) of *Phytophthora* and *Pythium*. With these more specific kits it is hoped that a better idea of potential disease threats can be quickly obtained on site. In addition to greater specificity of on-site test kits, this project has the ambitious aim of developing a reliable test for pathogen viability in an on-site kit format. Such a test would be very useful in monitoring the efficacy of treatment systems for the elimination of plant pathogens from irrigation water. More details about the types of test being developed are provided in the year 1 (2016) Grower Summary for this project.

Summary

***Nursery visits and continued isolate sourcing, identification and collection across each of the sectors*:**

Regular site visits have continued throughout the second year of the project and, together with isolates taken from plant clinic samples sent to Worcester, and isolates kindly supplied by James Townsend at Stockbridge Technology Centre, isolates collected from visits have been assembled into a representative culture collection containing more than 110 cleaned and identified isolates of *Pythium, Phytophthora*, and a wide range of background non-pathogen species.

Although at a slower pace, collections continue and isolates are being identified based on their morphology, with key isolates having their identifications confirmed by PCR and ITS DNA sequencing. The culture collection is important for its use in the extensive cross-reactivity testing needed to make sure that the antibodies raised in the project are 'doing what it says on the tin' and detecting specific pathogens and NOT common background species of oomycetes and fungi.

***Continued antibody and molecular probe development and testing*:**

The second year of this project has seen the major raising, purifying and testing of antibodies for the later development of detection tests. The bulk of the antibodies raised to Phytophthora species have passed through their first level of tests, although there are still some important fusions to complete and cell lines to test.

Cross-reactivity testing so far has revealed that we have a good general 'oomycete-specific' polyclonal antibody. This will be useful for the development of water tests where, in combination with a viability marker, it could form the basis for an effective test for the efficacy of irrigation water treatments. Key to this test will be a suitable viability marker, and for this a series of monoclonal antibodies have been raised to live zoospore preparations as well as a polyclonal antibody to a specific glycoprotein (CBEL) that is involved in the zoospore germination process. These antibodies will be tested against live (infectious) zoospore cysts and cysts killed by different sterilisation treatments (UV, heat, and chlorination - each of which can have quite different effects of the cysts' structures and contents), in the hope that they will be able to clearly discern the 'live' from 'killed' cysts.

In addition to these antibodies, a monoclonal antibody (3H7 H3) has been found to be nearly genus-specific – detecting only *Phytophthora* species plus the pathogenic species *Pythium ultimum*. Several other monoclonal antibodies have shown promising levels of specificity, detecting *Phytophthora cryptogea* only or a combination of *P. cinnamomi, P. rubi*, and *P. cryptogea*. We are now exploring whether it will be possible to deploy a combination of these antibodies in a test to identify, (a) *Phytophthora* sp., (b) *P. cryptogea* (*Phytophthora* clade 8),

and (c) *P. rubi/P. cinnamomi* (*Phytophthora* clade 7). The first experimental lateral flow test strips are due to be ready for laboratory evaluations with field samples in August 2017.

Two more antibodies raised to *Phytophthora* are still to be tested for cross-reactivity and further final batch will be ready by the end of September 2017.

With the Pythium antibodies, the first batch of 10 antibodies are ready for cross-reactivity testing in July 2017, but there are also still fusions being set up with the potential to raise a further 16 or so antibody cell lines for testing later in year 3.

An important part of this project has focussed on developing a multiplexing platform for rapid clinic-based testing of field samples. This would allow the rapid, simultaneous testing of samples for a number of different possible pathogen species in a single sample at the same time. In year 1 of this project, some promising results were obtained following an oligonucleotide (DNA-based) approach. This involved attaching oligonucleotide probes to coloured magnetic beads that allowed capture and quantitation of species-specific amplified pathogen DNA via a MAGPIX Luminex detection system (this system is described in more detail in the year 1 annual report {Wakeham *et al.,* 2016}). However, despite successful amplifications of pathogen DNA and successful coupling of capture oligonucleotides to magnetic beads, it has proved impossible to adapt the oligonucleotide array already developed by other researchers (Tambong *et al*., 2006) for use on a magnetic bead array. Nevertheless, this does not mean that a multiplex system is not attainable within this project and work is currently under way to determine whether it is possible to run multiple antibody tests concurrently using the same MAGPIX testing platform and the effective antibodies raised in this project.

Financial Benefits

Reliable and affordable detection and diagnosis are key to effective oomycete disease management. With increasing globalization, travel and the international trade in plants the risk of disease spread through inadvertent introduction is increased. Pathogen detection prior to infection or the development of symptoms invariably improves the efficacy of timed control measures and can significantly reduce disease epidemics and control treatment inputs. This project provides considerable scope for benefit in terms of early detection and targeted treatments (sanitization programs, biological and / or chemical control). The introduction of tests will also assist disease certification schemes.

The use of rapid and accurate diagnostic tests will provide a significant step forward in the development of lower-input farming systems and help minimize the number and volume of fungicide interventions by detecting problems early and removing the need for prophylactic ‘insurance’ applications.

Fungicide usage is costly and can be one of the major inputs in crop production after fuel and labour. Targeted application of control measures will help delay the onset of pathogen resistance to fungicides, thus prolong their useable life. The cost of diagnostic tests must be compared with a typical spend per hectare for materials and labour for a single fungicide treatment. Ultimately, financial benefit will be gained through improved quality and improved control procedures.