



New Project Summary Report for CP 099a - Validation of Clubroot lateral flow in Brassica cropping systems

Project Number	31100991
Title	Validation of the Clubroot lateral flow in UK commercial Brassica cropping systems
Short Title	CP 099a
Lead Contractor	University of Worcester
Other Contractors	
Start & End Dates	31 March 2013 - 30 March 2015
Industry Representative	Mr Andy Richardson – Allium and Brassica Centre
Project Budget	£66,962
AHDB Contribution	£66,962

The Problem

Economic losses resulting from disease development in agricultural cropping systems can be reduced by accurate and early detection of plant pathogens. Early detection can provide the grower with useful information on optimal crop rotation patterns, varietal selections, appropriate control measures, harvest date and post-harvest handling. Classical methods for the isolation of pathogens are commonly used only after disease symptoms are apparent. This frequently results in a delay in application of control measures at potentially important periods in crop production.

In contaminated soils isolation of the pathogen can be slow, require expertise and prove difficult

depending on the growth characteristics of the organism involved. Where a pathogenicity or baiting test is used the process may require an expensive glass house facility and take up to two months for disease expression to be visible.

Brassica crops are of high economic importance within the UK horticultural industry. One of the main diseases affecting Brassica crops is Clubroot which is caused by the soil borne organism *Plasmodiophora brassicae*. Clubroot resting spores are capable of inducing disease in Brassica crops years after initial infestation. Once soil has been contaminated clubroot spores can remain viable for up to eighteen years. The current industry method of soil assessment for diagnosis of the disease relies on a seven week plant bait test. In 1983, it was reported that clubroot infestation exceeded 10% of land on which crucifers were grown. A more recent survey funded by the Home Grown Cereals Association (HGCA, UK) established levels of 53% of Scottish fields were infested by the disease.

With the development of new detection methods based on molecular approaches a semi-quantitative measurement of clubroot in soil can now be determined in most soil samples. This test however is laboratory based, requires a high degree of precision by the operator, and expensive when compared to crop acreage. The sample size of 0.25g soil is also a limiting factor. HDC funding (FV349) has provided assessment of the q PCR test in UK soils and utilised it to validate a developed competitive lateral flow test strip device for the rapid testing and detection of the clubroot resting spores in field soils. This lateral flow is able to detect clubroot spores at close to epidemiological significant levels (10000 spores gram of soil) and has the potential to be used in water based systems such as reservoirs and irrigation lines (propagators). Nevertheless the sample volume of 0.25 g soil / test remains a limiting issue for commercial usage.

Lateral flow technology provides the platform to take a test for clubroot disease to the field which is robust, rapid, inexpensive and can be used by non-scientific staff. The challenge is to increase the soil sample size and so make to test applicable to bigger cropping acreages. Studies at the National Pollen and Aerobiology Research Unit, Worcester (NPARU) have shown that *P. brassicae* specific antibody coated magnetic beads can label and isolate resting clubroot spores directly from a range of soil types. This process enables the concentration of resting spores from the collected soil sample and to date up to 50 g lots have been tested. The

potential exists to significantly increase this soil sample size. The magnetic extraction phase provides the resting spores in a 'clean state', free of soil and inhibitors, and in a volume that can be applied in total and directly to a lateral flow field test strip. With the use of a hand held magnetic reader device this process would provide a platform for integration with the existing clubroot lateral flow to provide an 'in field' soil test for the clubroot pathogen. This process could be applied to other soil borne diseases and form the basis of a multiplex field soil test.

The ability to diagnose pathogen levels accurately, rapidly and at a low cost will assist in the prediction of plant disease occurrences in field setting. This will enable the appropriate disease control measures to be put in place and only when required. In addition, the use of resistant and susceptible varieties could be strategically deployed depending on pathogen infestation levels. Where fields are rented on an annual basis for commercial crop production the ability to map disease potential ahead of rental agreements would prove useful and assist in containment of further disease spread.

Aims and Objectives

Project aims

To provide a quick, inexpensive and reliable test that can be used to identify soil concentrations of clubroot disease in horticultural soils.

1. Offer UK horticultural growers a supported clubroot soil testing.

2. Further validate the clubroot lateral flow test (in its final format) using commercial soils (from all infested areas of the UK) and comparing results generated by quantitative molecular quantitative PCR (q PCR).

3. Utilise immunomagnetic extraction to:
 - Isolate and concentrate clubroot resting spores from soil into a low volume solution.

 - Reduce soil preparation time and increase the soil weight currently tested from 0.25g to 50g.

 - Optimise the extraction and isolation process in Year 1 and incorporate in to both the q PCR

and lateral flow process

- Validate the system in Year two soils testing

Objectives of the Club Root programme (long term)

1. Simplify the current soil preparation procedure and increase the volume of soil that can be assessed for estimation of clubroot contamination in soils.
2. Effectively market the clubroot soil tests within the UK horticultural industry. Provide a supported period of time for uptake and build confidence in the system for long term usage
3. To produce commercially available lateral flow test devices which can be used to sample increased soil volumes and provide information on clubroot soil concentration.
4. Grower Awareness of the tests available and develop confidence in these during the supported period.
5. Provide supported National clubroot soils testing to be rolled out as a standalone product from 2015.

Approach

Methods, Work plan and approaches to be taken

Year 1

HDC Grower Assisted Clubroot Soil Assessment:

Testing of soils for measurement of clubroot resting spore concentration will be immediately made available to HDC Brassica grower members. Advertisement of these services and co-ordination of soils for sampling will be through the Brassica Growers Association. Andy Richardson, Technical advisor to the BGA, will provide organisational lead for this process and co-ordinate the process with NPARU. Field soil collection protocols will be electronically distributed to participating members. Growers would be requested to supply information relating to the soils provided i.e. cropping history, previous clubroot problems in neighbouring fields and whether clubroot had previously been observed in the soils for testing.

On receipt of soils at the NPARU the samples will be air dried, milled and ground. This process

will enable resting spore populations to be evenly dispersed within the sample ahead of testing. For each soil, replicate 0.25g samples will be processed by lateral flow and q PCR to determine quantitative measurement of clubroot resting spore concentrations. Results of these tests will be reported to the grower along with information on soil pH. If appropriate the grower can discuss the results with staff members at the NPARU. The growers would be contacted at the end of the season to determine whether clubroot had been observed in soils planted with Brassica crops.

The soils provided in Year 1 would be stored at -80⁰ C and later assessed by immunomagnetic fishing.

Immunomagnetic Fishing – soil protocol optimization

Development and optimisation of the Immunomagnetic fishing process would continue in artificially infested clubroot soils to increase the volume of soil that could be assayed. After isolation and concentration, protocols would be further developed and evaluated to quantify the 'fished' resting spores by both lateral flow and q PCR. Commercial soils in Year 1 would then be fished for clubroot resting spores and captured concentrations determined by q PCR and lateral flow. These results would be compared with the initial soil forecasts for clubroot soil resting spore concentrations. Evaluation and development of this process would if necessary continue in to Year 2 of the study.

Year 2

The above process would be repeated in Year 2 to include the immunomagnetic fishing protocol at the initial soils testing stage where possible. Using the process of immunomagnetic fishing for collection and concentration the lateral flow test would then be validated by comparing results with the q PCR test. The developed process would be evaluated for use by growers as a DIY format and compared with the existing lateral flow soil assay (0.25 g analysed)