Grower Summary

FV 349

Brassicas: Further Development of “in field” tests for resting spores of clubroot and the development of clubroot based on detection

Annual 2012

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Before using all pesticides check the approval status and conditions of use.

Read the label before use: use pesticides safely.

Further information

If you would like a copy of the full report, please email the HDC office (hdc@hdc.ahdb.org.uk), quoting your HDC number, alternatively contact the HDC at the address below.

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Project Number:	FV 349
Project Title:	Brassicas: Further Development of “in filed” tests for resting spores of clubroot and the development of clubroot based on detection.
Project Leader:	Dr Roy Kennedy
Contractor:	University of Worcester
Industry Representative:	Alistair Ewen
Report:	Year 2 + Year 3 Report 2012
Publication Date:	20 August 2012
Previous report/(s):	Annual Report 2010
Start Date:	01 April 2009
End Date:	31 May 2013
Project Cost (total project cost):	£74,190 (£82,458)

Headline

A detection test developed for the detection and measurement of clubroot spores in soil has been evaluated for use by UK growers.

A Brassica disease forecast is under development to evaluate the potential to generate risk assessments for clubroot disease based on soil type, crop and clubroot resting spore numbers.

Background and expected deliverables

Brassica crops are of high economic importance in the United Kingdom. One of the main diseases affecting Brassica crops is clubroot, caused by the soil-borne organism *Plasmodiophora brassicae*. Mild clubroot infections lead to slowed growth and delayed harvesting. Severe infections result in total crop failure. Infection is easily recognisable by swelling of root tissue causing galls and club shaped structures. Clubroot resting spores are capable of inducing disease in vegetable Brassica crops years after initial infestation of the soil.

Once soil has been contaminated, clubroot spores remain viable for up to 18 years. Information on the presence or absence of clubroot in soils has been difficult to obtain because the traditional methods cannot detect low levels of clubroot in soils. These methods are based on using the reaction of bait plants, however, large numbers of plants are required in these tests if small amounts of clubroot were to be detected. With the development of new detection methods based on molecular approaches the presence or absence of clubroot can be determined in most soil samples. These tests are laboratory based but require a high degree of precision by the operator.

In this project, a competitive lateral flow device has been developed and is under validation for use in UK commercial soils for the rapid testing and detection of the clubroot resting spores. This test can identify very low levels of clubroot in the soil (10000 spores/gram of soil).

The lateral flow device can be used in soil (field growers) and the potential for use in water based systems such as reservoirs and irrigation lines (vegetable Brassica propagators). A quantitative measurement of clubroot resting spore infestation can be made using the lateral flow test device when used in conjunction with a lateral flow reader device and standard curve data. This means that a prediction on whether the crop is at risk can be determined and at what level i.e low, medium and high risk.

Determining the clubroot resting spore number in soils using either a molecular or lateral flow test is an essential component in the development of an integrated disease management programme. Currently only two chemicals (cyazofamid – Ranman and fluazinam – Shirlan) approved for control of disease in potato crops have been demonstrated to have any potential for controlling clubroot in the field. However both these chemicals do not hold approval for clubroot control in vegetable Brassicas as their efficacy against clubroot has not yet been demonstrated. Alternative control measures are still urgently needed. Within the current project alternative products are assessed for application to clubroot infested soils to assist in the control of the disease. A Brassica disease forecast is under development to evaluate the potential to generate risk assessments for clubroot based on soil type, crop and clubroot resting spore numbers.

The expected deliverables from this project are:

- Better detection of clubroot in the field before planting the crop.
- Detection tests which can be used “in field” to determine the level of risk to the vegetable Brassica crop posed by clubroot.
- Investigation of alternative products for clubroot control in the field.
- Investigation of the economics of Brassica production under different levels of clubroot risk.

Summary of results and main conclusions

Years 2 and 3

Quantitative measurement of plant pathogens by molecular (DNA based) and immunological (antibody based) methods have in the past decade become an established procedure in the quantification of disease in many horticultural systems. In this project and using these methods, advances have been made to develop tests which can estimate disease potential of the clubroot pathogen in UK field soils. The ability to measure disease potential in field soils has useful applications in not only forecasting the risk of clubroot disease ahead of planting Brassica crops but in the management of the disease throughout the growing season.

The current study has evaluated two diagnostic tests for the measurement of clubroot in soil. The molecular test has provided the measure/yardstick to evaluate the antibody based test against (lateral flow test). Throughout the process the lateral flow test prototype has evolved

to provide a field test which shows potential for the semi-quantitative estimation of clubroot resting spores in UK commercial soils. In Year 3 of the project, 53 commercial soils were assessed by each test process and there was statistical agreement between 30 of the soils. Of those soils which fell outside the confidence limit of the statistical analysis the majority were as a result of the lateral flow device over estimating the disease risk when compared to the molecular (qPCR) test. Ranking both sets of results into low, medium and high disease risk categories however improved the correlation further.

Variation in the level of clubroot infestation in soils between the qPCR test and the lateral flow may result from the storage process of the soils ahead of testing. Dr Robert Faggian (DPI, Melbourne, Victoria: personal communication) reported that storage conditions effect clubroot resting spore DNA extraction level. Within one month at 4□C storage, the DNA test signals of clubroot infested soils were seen to decline and the resultant qPCR value generated was significantly below that originally observed. This was observed in soils collected in 2007 and 2010 (series 7 and 10) which were initially stored at 4° and then -20°C, prior to re evaluation in 2011. Soil type is also important in the context of test disease estimation. It is known that the soil type can influence DNA extraction and subsequently the outcome of PCR amplification due to the presence or absence of inhibitory substances (Lloyd-Jones and Hunter, 2001). This has also been noted for immunological tests (antibody tests i.e lateral flow device) and with particular reference to soils high in humic and fulvic acid.

Soil textural parameters could also be linked to differences in test results. Soils collected in 2007 were assessed for soil textural type and approximate proportion of sand, silt and clay composition. Following analysis by qPCR it was determined that the silt content was significant in reducing test sensitivity in naturally infested soils. This study was extended in Year 3 to newly collected soils which were identified as clubroot disease free. These soils were then artificially inoculated with clubroot disease over a concentration range of high to low. The study revealed that although silt was significant in reducing the qPCR signal in naturally infested soils the effect of sand and clay and, their interaction, was significant. Further analysis of the soils from these two data sets will provide greater information on the significance of these factors for the accurate estimation of clubroot resting spores in the soil. Future studies should address this area not only with qPCR but inclusion of the lateral flow device. Peat based soils found in the low lying sea areas of West Lancashire and Lincolnshire should also be included within the study. This may prove important in the performance of the lateral flow test and should not be limited to the effect of soil textural type but include analysis of humic and fulvic acid content along with pH. Each of which could have a significant effect on the lateral flow assay when predicting the risk of clubroot disease

occurrence at or below the generally accepted disease threshold spore load of 100000 spores gram of soil.

The development of the two test systems (laboratory test: *molecular qPCR* and field based grower test: *lateral flow device*) has provided the UK horticultural industry with the ability to assess fields for clubroot disease and measure disease potential i.e the number of disease propagules (resting spores) in the soil. Ultimately however the development and expression of the disease will be dependent on a number of factors : the resting spore concentration, the conducive or suppressive nature of the soil type, the environmental conditions over the growing season and the Brassica cultivar planted. For this part the project has in Years 2 and 3 examined the potential for development of an integrated clubroot field risk assessment which is based on knowledge of the soil resting spore concentration and the environmental parameters to predict crop response.

The prototype clubroot disease forecast model considers known crop responses to environmental parameters, within additional factors incorporated to cover spore responses. The basis for the clubroot model is derived from N_ABLE. This was initially developed by Greenwood and Draycott in 1989 and has since been well researched and documented, and further developed into an integrated N,P,K model by combination with other Potassium and Phosphorus crop response models. In the development of the clubroot disease forecast model the nitrogen component was considered to be the most important, as inclusion of other parameters would have added unnecessary complexity at this stage (and possibly not been that important to clubroot disease development). The inclusion of pH, Calcium and Magnesium however are included given their significance in the development of the disease to gall formation. Initial investigations have generated a model capable of predicting environmental parameters important to the development of clubroot disease. Further trials are required to establish whether the significant factors have a direct or indirect effect on clubroot disease potential. The ability of the model to predict clubroot disease is at this point limited by the ability to predict weather patterns. Nevertheless the model is applicable to different soils so it will be possible to work quickly towards generating risk assessments for clubroot disease based on soil type, crop and pre planting clubroot resting spore levels

In terms of clubroot control, the project also investigated the use of Limex as a potential control measure. The results confirmed the findings carried out in 2009 in Scotland that Limex could be used to control clubroot in heavily infested land while maintaining marketable yields of Broccoli crops. The optimal application rate was between 7.5–10 tons Limex/ha. The results demonstrate that the application rate could be reduced without a detrimental effect on

broccoli yields. The results show that the highest levels of clubroot were recorded in plots which were treated with standard lime or were untreated. Standard lime and Limex did not have a significant effect on the pH of the plots although high rainfall was recorded at the time of application. The pattern of clubroot development in the plots differed between 2009 and 2010 reflected in differences in rainfall between the two seasons.

Anticipated practical and financial benefit

- The usage of the detection tests for risk assessment for clubroot will improve the control of this pathogen.
- Generation of a clubroot disease forecast model based on soil type, crop and soil disease level will assist knowledge on planting risk and subsequent disease management strategies.
- New information will be available on an integrated management programme for predicting disease risk and strategies for clubroot control

Action points for growers

Specific action points for growers at this stage in the project include:

- Growers can have their soils tested for clubroot disease inoculum concentration ahead of testing.
- Limex can be used to control clubroot in affected land however it will not reduce clubroot risk in subsequent seasons.
- The optimal level of Limex required for clubroot control is 7.5 – 10 tons Limex/ha.