Grower Summary

FV 415

Molecular methods for detection of stem nematode (*Ditylenchus dipsaci*) in soil and predicting risk of damage to onions and leeks

Annual, 2014

Disclaimer

AHDB, operating through its HDC division seeks to ensure that the information contained within this document is accurate at the time of printing. No warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

The results and conclusions in this report may be based on an investigation conducted over one year. Therefore, care must be taken with the interpretation of the results.

Use of pesticides

Only officially approved pesticides may be used in the UK. Approvals are normally granted only in relation to individual products and for specified uses. It is an offence to use non-approved products or to use approved products in a manner that does not comply with the statutory conditions of use, except where the crop or situation is the subject of an off-label extension of use.

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

Read the label before use: use pesticides safely.

HDC is a division of the Agriculture and Horticulture Development Board.

Project Number:	FV 415
Project Title:	Molecular methods for detection of stem nematode (*Ditylenchus dipsaci*) in soil and predicting risk of damage to onions and leeks
Project Leader:	Dr Steve Ellis
Contractor/(s):	ADAS
Industry Representative:	Robert Brown, E C Brown Farms
Report:	Annual report, March 2014
Publication Date:	12/05/2014
Previous report/(s):	None
Start Date:	1 April 2013
End Date:	31 March 2015
HDC Cost (Total cost):	£48,900.00

Further information

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

HDC,
AHDB
Stoneleigh Park
Kenilworth
Warwickshire
CV8 2TL

Tel – 0247 669 2051

Headline

The PCR analysis developed by ClearDetections is effective at detecting stem nematode either on its own or in the presence of other free-living nematode species from a limited range of UK soils.

Background

Stem nematode (*Ditylenchus dipsaci*) is potentially a very destructive pest of bulb onions and leeks. Quantifying soil infestation prior to drilling is recommended as a tool to determine the suitability of land for growing onions or leeks. In general, if stem nematode is present at moderate or high levels the land is rejected as a site for a following onion or leek crop. At low levels the onion crop is sometimes grown but treated with a nematicide. However, a lack of confidence in the ability of some laboratories to identify stem nematode means that fields may be unnecessarily rejected or treated. HDC Project FV 327 identified the optimum sampling scheme and soil extraction method to give the best chance of detecting stem nematode in soil. However, identification of stem nematode by microscopy is very difficult and there are few nematologists in the UK who are confident of doing this. There are a number of *Ditylenchus* species in soil and it is important that these can be differentiated to prevent unnecessary use of nematicides or rejection of land wrongly identified as being unsuitable for onions or leeks.

As presence or absence of stem nematode is usually considered sufficient to predict the risk of pest attack it is ideally suited to PCR analysis. This has the advantage of being rapid and does not rely on a limited number of individuals with the necessary nematological expertise. A PCR assay for stem nematode has been developed by a Dutch based company (ClearDetections, a recent start up) and ADAS is already working with this group to determine whether the technique is capable of detecting a UK isolate of stem nematode either in isolation or, more practically, in extracts containing a range of nematode species. Preliminary studies with ClearDetections investigated if the stem nematode PCR was able to detect a single stem nematode, a single stem nematode amongst other nematode species and also if the other nematode species produced any false positive results in the absence of stem nematode. There were five replicates of each treatment. The results were very promising and showed that the test was able to detect a single stem nematode in 100% of cases. In view of the success of these preliminary tests there is potential for an HDC project to validate the technique from a range of sites across the UK onion and leek growing areas. In future, under the Sustainable Use Directive (SUD), protecting crops from free-living nematode damage will become increasingly reliant on integrated strategies that combine

cultural and chemical control. Robust risk assessment in which growers can be confident will be fundamental to the success of such IPM programmes.

The overall aim of the project is to validate a PCR technique for detection of stem nematode (*Ditylenchus dipsaci*) in soil as a basis for predicting risk of damage to onions and leeks.

Specific project objectives are as listed below:

1. To validate the effectiveness and specificity of qualitative PCR analysis in detecting stem nematode in extracts of free-living nematodes from UK soil samples.
2. To determine the effects of sample pre-treatment and DNA extraction on the PCR analysis for detecting stem nematode in a range of soil types from different locations throughout the UK.
3. To investigate the potential of PCR analysis to distinguish between UK populations of the oat-onion race and giant bean race of stem nematode.
4. To communicate project results to deadline via annual and final project reports, an article in HDC News and dissemination of the sampling protocol.

Summary

Year 1 of the project concentrated on Objective 1. Onion plants showing symptoms of stem nematode infestation were collected from the field and extracted by cutting them open and immersion in water for 24 hours. The identity of the nematodes was confirmed by microscopy by ADAS.

The PCR analysis was undertaken by ClearDetections in the Netherlands. The PCR tests have been developed for routine use on DNA extracts originating from nematode suspensions and utilise a SYBRGreen based detection system for ‘real time’ visualisation of PCR product.

A total of 50 Eppendorf tubes, each containing a mix of free-living nematode (FLN) species (*Trichodorus* spp., *Tylenchorhynchus* spp., *Pratylenchus* spp. *Globodera* spp (juveniles) Heerodera spp (juveniles)) but no stem nematode, a single tube containing stem nematodes extracted from plant material, and six tubes with FLN from typical English onion soils were transported from ADAS to ClearDetections.

At ClearDetections single stem nematodes were manually extracted from the sample of stem nematodes using a mounted eye lash. A single stem nematode was added to 25 of the 50 tubes with a mix of FLN.

Nematode suspensions from certain soil types, especially those with a high organic matter content, may result in high levels of PCR inhibiting substances in the final nematode DNA extracts. These inhibitory substances need to be removed before PCR. To establish whether

the ClearDetections nematode DNA extraction and purification kit is suitable for removing these substances from samples originating from English soil types, nematode suspensions from a typical English onion soil (sandy loam) were spiked with four stem nematodes (of Dutch origin) and nematode DNA was extracted and purified according to the standard protocol.
In total the following 81 nematode samples were analysed:

- 25 tubes with a single stem nematode
- 25 tubes with a single stem nematode among other FLN species
- 25 tubes with other FLN species and no stem nematode
- Six tubes with FLN from a typical English onion soil spiked with four stem nematodes

In 55 out of the 56 samples (98.2%) containing stem nematodes the pest was detected (positive result) either on its own or in combination with other free-living nematode species. All 25 free living nematode samples without a stem nematode were found to be negative. Results to date suggest that the PCR analysis developed by ClearDetections is effective at detecting stem nematode either on its own or in the presence of other free-living nematode species from a limited range of UK soils.
Where no stem nematode was present the analysis always produced a negative result and did not result in any false positives.

Financial Benefits

A validated PCR assay for stem nematode will provide the industry with a rapid, standardised and validated method of assessing the risk of nematode damage to leeks and onions. In addition, a PCR assay has the potential to provide a more reliable and cost-effective risk assessment than current labour-intensive microscope examination which is heavily reliant on a restricted number of skilled nematologists who are able to identify the pest with confidence and consistency.

Action Points

As this is only the first year of the project there are no action points for growers to date. In Year 2 of the project, work will continue to validate the PCR analysis over a wider range of soil types and also to determine if it is capable of differentiating between the oat onion and giant bean race of stem nematode.