

Grower Summary

FV 415

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

Final 2015

Disclaimer

While the Agriculture and Horticulture Development Board 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.

©Agriculture and Horticulture Development Board 2015. No part of this publication may be reproduced in any material form (including by photocopy or storage in any medium by electronic mean) or any copy or adaptation stored, published or distributed (by physical, electronic or other means) without prior permission in writing of the Agriculture and Horticulture Development Board, other than by reproduction in an unmodified form for the sole purpose of use as an information resource when the Agriculture and Horticulture Development Board or AHDB Horticulture is clearly acknowledged as the source, or in accordance with the provisions of the Copyright, Designs and Patents Act 1988. All rights reserved.

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.

Further information

If you would like a copy of this report, please email the AHDB Horticulture office (hort.info.ahdb.org.uk), quoting your AHDB Horticulture number, alternatively contact AHDB Horticulture at the address below.

AHDB Horticulture,
AHDB
Stoneleigh Park
Kenilworth
Warwickshire
CV8 2TL

Tel – 0247 669 2051

AHDB Horticulture 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, ADAS, High Mowthorpe, Duggleby, Malton, North Yorkshire, YO17 8BP

Contractor: ADAS UK Ltd

Industry Representative: Robert Brown, E C Brown Farms

Report: Final report, 2015

Publication Date: 09 July 2015

Previous report/(s): Annual report 2014

Start Date: 01 April 2013

End Date: 30 April 2015

Project Cost: £53,850

GROWER SUMMARY

Headline

Real-time Polymerase Chain Reaction (PCR analysis) developed by ClearDetections is effective at detecting stem nematode either on its own or in the presence of other free-living nematodes and is effective in a wide range of UK soil types. There was good agreement between microscopy and PCR analysis for detection of stem nematode.

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. AHDB Horticulture 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.

With the declining availability of nematicides and with the imposition of the Sustainable Use Directive (SUD), protection of crops from free-living nematode damage in the future will become increasingly reliant on integrated pest management (IPM) 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. Detecting the presence of stem nematode is a crucial component of any risk assessment and is the main subject of this project.

As presence or absence of stem nematode is usually considered sufficient to predict the risk of pest attack it is ideally suited to Polymerase Chain Reaction (PCR) analysis. This has the advantage of being rapid and does not rely on morphological identification by a limited number of technicians 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 in this project ADAS has collaborated 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 whether the PCR analysis was able to detect 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. The results 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 the current project was commissioned to validate the PCR analysis from a range of sites across the UK onion and leek growing areas.

The overall aim of the project was 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 AHDB Grower 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 immersing 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 detection system for 'real time' visualisation of the 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) *Heterodera* 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 tube containing this pest 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 therefore needed to be removed before PCR testing. 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.

Work in year 2 concentrated on Objectives 2 and 3. Work on Objective 2 was specifically designed to investigate the potential for PCR analysis to be inhibited by substances found in nematode suspensions from different UK soil types and consisted of two experiments as below:

- Experiment 1. Validating PCR analysis for stem nematode extracted from different soil types.
- Experiment 2. Validating PCR analysis for stem nematode inoculated into representative soil types

Nematode suspensions isolated from different soil types may have pronounced effects on the PCR efficiency as components of the soil samples co-purifying with the nematode DNA may be inhibitory to the PCR reaction (sample matrix effects). To test this in Experiment 1 nematode suspensions were extracted from a range of soil types using the Seinhorst two-flask technique. These samples were examined by microscopy. Some of the samples contained stem nematode and some did not.

These samples were submitted to ClearDetections for PCR analysis. In total 170 samples were submitted in two batches for analysis (24 clay, 43 loam, 24 organic, 39 sand, 40 silt). Results showed a 99% agreement between the results of microscopy and PCR analysis.

To ensure that a full range of UK soil types was studied (alliums may not be grown in all UK soil types) it was also decided to inoculate known numbers of stem nematodes into nematode suspensions extracted from a range of different UK soil types before being submitted for PCR analysis (Experiment 2). The soil types selected were a sand, a silt, a clay, an organic soil and a loam. There were twenty five replicates of each soil type inoculated with stem nematodes (125 samples in total). Microscopy was used to confirm that none of the selected soils were infested with stem nematode.

The stem nematodes for inoculation were collected by extracting infested plant material. These plants were extracted by cutting open the infested material and immersing in water in a Baermann funnel. The extracted nematodes were collected after 24 hours and four were inoculated into each of the 25 replicate nematode suspension samples from each of the five soil types. The identity of the nematodes was confirmed by microscopy.

Across all soil types (clay, loam, organic, sand, silt) 122 out of 125 samples were positive for stem nematode (97.6%). There were three unexpected negative results; one from loam and two from sand soils. For these samples an additional Real-Time PCR was performed using a general nematode DNA assay. The results of this troubleshooting analysis confirmed the presence of nematode DNA and absence of *D.dipsaci*. The reason for this result is unclear but it is possible that the stem nematodes were lost during sample handling or transport; they may have been missed during the DNA extraction, they may have stuck to the lid of the tube or they might have been lost during the volume reduction before DNA extraction.

Objective 3 concentrated on determining whether the ClearDetections PCR analysis was capable of differentiating between *D. gigas* (giant bean race) and the more common oat-onion race. This was not possible primarily because *D. gigas* is very difficult to find in soil. In the UK ADAS has failed to detect this species in 10 years of data collected from soils from all over the UK and only recorded it in infested samples of field beans. This suggests that *D. gigas* poses a limited threat to onions and leeks in the UK. ADAS will continue to look for *D. gigas* and if it can be found, most probably following extraction of bean samples, material will be made available to ClearDetections with which to test their PCR analysis.

In conclusion the results of this project show that the PCR analysis developed by ClearDetections is effective at detecting stem nematode from a wide range of UK soil types either on its own or in the presence of other free-living nematode species. PCR analysis could become a vital component of an integrated pest management strategy for *D. dipsaci* to

help growers assess the risk from the pest. Detection of stem nematode in soil in the UK is labour-intensive and dependent on a dwindling level of expertise able to identify the pest using traditional microscopic examination. Molecular assays provides an opportunity for a rapid, standardised and validated detection test for stem nematode for UK onion and leek growers.

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. The benefit of a molecular assay is that it can be run by any molecular technician, analysing 96 samples in one run. The main costs incurred are for the PCR machine, disposables and reagents. Microscopic examination of samples can only be performed by a skilled technician one sample at the time. Real cost benefits therefore depend mostly on the laboratory under consideration and its potential sample throughput. Industry representative Robert Brown (E. C. Brown Farms) commented that the project 'is taking the next step towards operating a more adaptable method of detection, which helps alleviate the requirements of trained nematode identifying expertise. Whilst still offering a high detection test that is reliable.'

Action Points

There is nothing that an individual grower can do immediately. The output of the research suggests that a commercial sampling and testing scheme could be established. The benefits are from gaining a more reliable and rapid test for stem nematode with less reliance on a dwindling group of nematologists. A reliable and cost effective predictive test is more likely to be used by growers to allow them to avoid nematode damage and subsequent crop loss. There would also be more confidence in growing the crops without nematicide treatment.