New Project Summary Report for FV 415: Detecting and predicting damage stem nematode affecting Onions and Leeks

Project Number	31304150	
Title	Molecular methods for detection of stem nematode (Ditylenchus dipsaci) in soil and predicting risk of damage to onions and leeks	
Short Title	FV 415: Detecting and predicting damage stem nematode affecting Onions and Leeks	
Lead Contrator	ADAS UK Ltd	
Other Contractors		
Start & End Dates	31 March 2013	30 March 2015
Industry Representative	Robert Brown	
Project Budget	£53,850	
AHDB Contribution	£48,900	

The Problem

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 crop is sometimes grown but treated with a nematicide. However, a lack of confidence in the ability of some labs to be able 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 joint venture between Wageningen University and BLGG Agroxpertus) 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. Results showed that test was able to detect single stem nematode in 100% of cases and a single stem nematode among other free-living species in 80% of cases. In the one test where no stem nematode was detected among other nematode species it is suspected that the stem nematode was not successfully transferred to the PCR tube. 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.

Aims and Objectives

(i) Project aim(s):

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.

(ii) Project objective(s):

o To validate the effectiveness and specificity of qualitative PCR analysis in detecting stem nematode in extracts of free-living nematodes from UK soil samples.

o 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.

o To investigate the potential of PCR analysis to distinguish between UK populations of the oat-onion race and giant bean race of stem nematode.

o To communicate project results to deadline via annual and final project reports, an article in HDC

News and dissemination of the sampling protocol.

Interdependence of objectives

Objectives 1and 2 are interdependent in that they will be achieved by analysis of the same samples. Objective 3 is only dependent on objective 1 for locating sites which are infested with stem nematode. Objective 4 involves communication of results so is interdependent upon completion of all other objectives.

Risk of objectives not being met

The main risk to this project is the inability to find sites infested with stem nematode of both the oat-onion and giant bean races. ADAS will use grower/agronomist contacts to ensure that samples are taken from sites known to be infested with stem nematode from a range of soil types. The identity of the nematodes will be confirmed by in-house ADAS experts using soil extraction followed by microscopy. This method has been very successful at locating sites with stem nematode over a number of years.

Approach

All objectives will continue over both years of the project with the exception of Objective 1. This is to ensure a robust test of the PCR assay over the widest range of genetic variability, soil types and geographical regions.

(i) Methods

Objective 1. Validating the effectiveness and specificity of PCR for stem nematode (Year 1)

Stem nematodes will be extracted by ADAS from both plants and soil. Extraction from soil will involve the use of the Seinhorst two-flask technique which was shown to be most effective for stem nematode in FV 327 Onions: Improving risk assessment for stem nematode. Extraction from plant material will involve cutting open the infested material and immersion in water in a Baermann funnel. The extracted nematodes are collected after 24 hours. The identity of the nematodes will be confirmed by microscopy.

The PCR analysis will be undertaken by ClearDetections. The PCR tests have been developed for routine use on DNA extracts originating from nematode suspensions (containing DNA of approximately 10,000 individual unknown nematodes). The real-time PCR tests use a SYBRGreen based detection and enable the user to monitor the amplification of the nematode PCR product without the requirement for analysis on agarose gels, and can allow the further development of effective quantitative PCR based assays. The specificity of these tests is

demonstrated by routine analysis of both the cycle threshold (Ct) value and the melting temperature of the PCR products detected. The standard operating procedure of the SYBRGreen based test prescribes analysing the Ct value and Tm of any qPCR product formed and a test result can only be positive if these are found to be within the assay parameters. This confirmatory analysis of the amplified product is especially important when the test is performed on DNA extracts with unknown contents, which is often the case when testing soil samples.

Objective 2. Validating the sensitivity of PCR analysis in detecting stem nematode from a range of soil types (Years 1 & 2)

Stem nematodes isolated from different soil types and locations across the UK may amplify differently due to possible soil matrix effects dictated by the local soil composition. Nematodes will be isolated from soil using the same methods as described in Objective 1. These samples will be submitted to ClearDetections for PCR analysis. Different nematode suspensions isolated from soil 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 investigate this, known numbers of stem nematodes will be inoculated into a range of different UK soil types before being extracted as described in objective 1, and submitted for PCR analysis (see also Objective 4). Variation in PCR Ct value against a standard curve would be indicative of sample- to sample variation due to matrix effects of the soil extract and would need to be taken into consideration when interpreting any nematode PCR results.

Objective 3. To investigate the potential of PCR analysis to distinguish between oat-onion and giant bean races of stem nematode (Years 1 & 2)

In the UK both the oat-onion and giant bean race of stem nematode are present in soil. The giant bean race is not a threat to onions and so it is important to be able to distinguish races so that land is not wrongly rejected as being unfit for onions and leeks. Both races of stem nematode will be extracted from soil or from plant material using the methods described at Objective 1, and the specificity of the PCR will be evaluated.

Objective 4. Communication of results and knowledge transfer (Years 1 & 2)

The HDC Research Manager will be regularly updated of progress with the project. Annual and final project reports will be submitted to deadline and the date for article to be submitted to HDC News will be agreed with the Knowledge Transfer Manager. The potential for a paper in a scientific journal will be considered but has not been costed into the proposed project.