Project report for SSCR-Potatoes

Project title

Translating qPCR data into grower-focussed potato cyst nematode soil distribution figures.

Applicant

James Price (James Hutton Institute)

Background to the project

The GB potato industry is worth ca. £928 M farmgate value. However, this industry is under an existential threat from potato cyst nematodes (PCN) which have been spreading across many potato growing areas. Predictions suggest that the Scottish seed industry could collapse by 2050 due to this threat, potentially bringing down the entire GB potato production system soon afterwards (Toth *et al.* 2020). SASA currently provides both routine and statutory PCN testing. This ensures that land used for growing seed potatoes is clear from PCN, preventing further spread of the disease. Ware land is tested to inform grower and agronomist variety choice, including PCN resistant varieties where necessary. This testing includes soil sampling, extraction of PCN cysts, identification of species by PCR and quantification by qPCR. While this process is quick and efficient, the data output does not translate to a field environment as a translational standard curve has not been produced for the qPCR data.

References:

Toth *et al*. 2020. https://www.planthealthcentre.scot/publications/pcn-workinggroup-final-report

Aims and objectives

The James Hutton Institute and SASA have a long history of research partnerships, especially for PCN diagnostics. We will aim to undertake joint research that will allow translation of qPCR CT values from SASA into absolute values for PCN eggs/juveniles per gram of soil. Providing data as eggs/juveniles per gram of soil produces a useable figure that directly informs both the grower and their agronomist.

Research results

SASA extracts DNA from floated field samples and uses this as a template for TaqMan based qPCR assays. The complete methods including primers, probes and reagents are already published (Reid, A. *et al.* 2015). This qPCR analysis produces DNA amplification data as cycle threshold (CT) values. Lower CT values suggest higher quantities of PCN DNA and therefore a greater number of PCN in the original soil sample. CT values do not translate to a field scenario other than to provide an indication of relative PCN abundance, usually disseminated as low, medium or high level of infection. These categories are extremely broad and PCN management techniques could vary significantly within and between these classifications. This project will seek to create a standard curve to be used with SASA qPCR data allowing CT values to be directly translated to an equivalent eggs/juveniles per gram of soil.

For visual examination of PCN infections, results are usually recorded in eggs and juveniles per gram of soil. When using DNA-based methods there is no differentiation between eggs and juveniles. Considering the second stage juvenile results from the first moult within the eggshell both hatched juvenile and egg contain the same quantity of DNA. To identify how much DNA this is and to produce a standard curve, the ITS target of the SASA TaqMan qPCR assay was cloned into the pgem-T-easy vector, amplified in *E. coli* and sequenced for both *G. pallida* and *G. rostochiensis*. The standard curve based on copy number is available in appendix figure 1. Generally, the *G. pallida* probe being used by SASA has a greater affinity for the target DNA than the one used for *G. rostochiensis*.

Once the standard curve was produced the DNA target copy number per juvenile needed to be identified. However, inconsistent data was seen between assays carried out at JHI and SASA, this is due to differences in DNA extraction techniques. SASA uses an automated process to extract DNA from samples, this is fast, relatively cheap and provides consistent DNA extraction. It is currently the best system to use for this process. However, as it uses a column-based technology, there is naturally loss of DNA during the process. At JHI, a column-less technique for DNA recovery was applied, while this is closer to total DNA extraction it is not feasible on an industrial scale. Furthermore, due to cyst floatation, there should never be free juveniles and eggs in DNA extraction samples in the SASA system. Therefore, a different approach was taken to create a new logarithmic trend based on DNA recovery and amplification using the practically applied system at SASA.

Samples of known numbers of juveniles and cysts were submitted to SASA for DNA extraction and qPCR. Average CT values for both *G. pallida* and *G. rostochiensis* were taken per number of juveniles and fitted to a logarithmic trend (appendix figure 2). The equation for this trend can be used to calculate either X (second-stage juvenile per sample) or Y (CT value). Consistency of the sample DNA extraction and qPCR was confirmed using the target DNA ladder. A simplified Microsoft Excel-based tool was produced that uses the log trend equation to either calculate the number of juveniles per 400 mL sample from the SASA CT value or the expected number of juveniles and expected SASA CT value from an SRUC-type egg/juvenile per sample diagnostic test. This tool is centred on the CT values for *G. rostochiensis* and not *G. pallida* due to data consistency. Given the greater affinity of the *G. pallida* probe for the target DNA it is possible that the model will slightly underestimate *G. pallida* CT values.

This work was presented at the SSCR winter meeting (2024) with a demonstration of the conversion tool in an excel spreadsheet format.

References:

Reid A. *et al.* 2015. https://link.springer.com/protocol/10.1007/978-1-4939-2620-6_11

Outcomes

This project aimed to produce a conversion tool for SASA CT values provided during diagnostic PCN testing into grower interpretable figures represented by juveniles per gram of soil/ sample taken. These outcomes were achieved, and a conversion tool has been produced and demonstrated.

The tool has already been used to provide a conversion to supply a link between current SASA data and old figures provided by SRUC to a landowner who rents out land for potato production. This has helped the landowner to identify where their PCN control has been effective and where they need to apply morse treatment (chitin-based soil amendment in this instance). Further enquiries about using the conversion tool have also been made.

This tool could be further refined by repeating the *G. pallida* submissions to SASA. Identifying the number of ITS copies per second-stage juvenile would assist small scale PCN quantification using the standard curves produced here. However, due to DNA extraction efficiency, these would be system specific. Further refining could include incorporating test DNA in different soils. Soils with higher organic matter will naturally compete with target DNA during the DNA clean up.

This project has instigated conversations with members of SASA about reclassifying what a low, medium and high PCN infection actually represents and how these should be consequently managed. The system currently used by SASA in the PCN diagnostics lab remains to be one of the best in Europe and hopefully this conversion tool will help growers directly with data interpretation.

Next steps

We are investigating the possibility of uploading the tool with a new user interface to the PCN Action Scotland webpage (www.pcnhub.ac.uk). The tool will be slightly modified to allow users to input sample size. Once uploaded, it will be available free of charge and will be in an environment where growers/landowners will be able to find helpful information assisting with PCN control. If uploaded to our website there will be a link and information highlighting that this was an SSCR funded project and not originally covered by PCN Action Scotland.

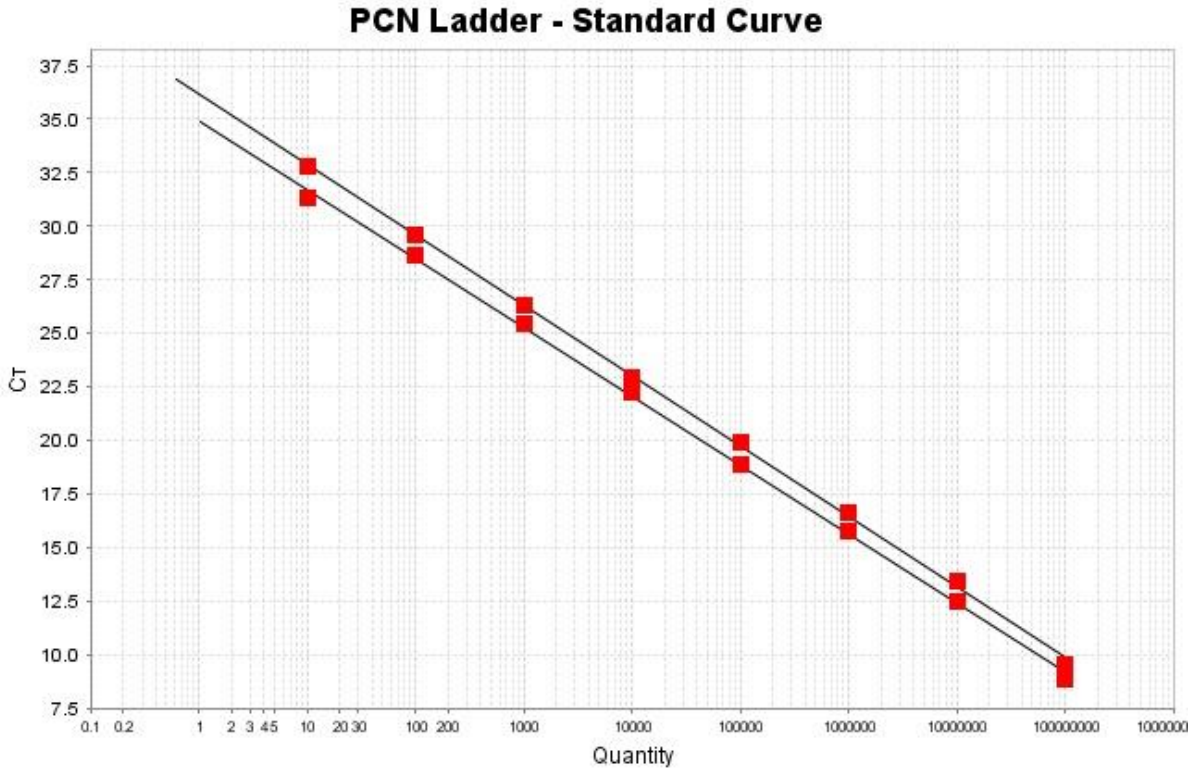

Figure 1 – Standard curves built on ITS copy number from cloned target DNA. Top line = G. rostochiensis probe, bottom line = G. pallida probe. Although copy number of DNA from both species of PCN was consistent there is a higher affinity of the G. pallida probe for the target DNA.

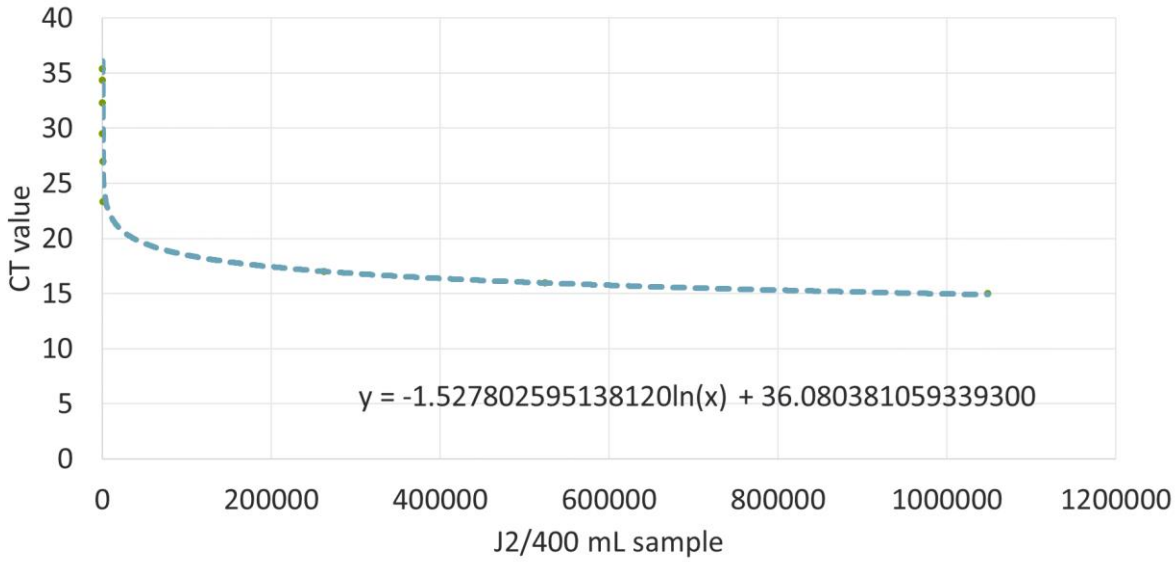

Figure 2 – Logarithmic trend of CT value to number of juveniles per standard 400 mL sample. The equation for the trend is displayed and given values for Y allows conversion of SASA CT values into juveniles per sample.