PREDICTIVE DIAGNOSTICS FOR SOIL-BORNE DISEASES OF POTATO: AN ESSENTIAL COMPONENT OF IPM

J L Brierley, L Sullivan, J L Lynott and A K Lees The James Hutton Institute, Invergowrie, Dundee DD2 5DA E-mail: Jennie.Brierley@hutton.ac.uk

Summary: Molecular diagnostic methods to detect and quantify soil-borne potato pathogens have been developed. Establishing disease risk associated with soilborne inoculum informs decisions on Integrated Pest Management (IPM). In this paper we review risk predictions for black dot and powdery scab, and outline the ongoing research into establishing disease risk thresholds for *Rhizoctonia solani* AG3 and pathogenic *Streptomyces* spp.

INTRODUCTION

Integrated Pest Management (IPM) is an environmentally and economically sustainable approach to managing the impact of crop pests, pathogens and weeds. IPM strategies combine available methods (IPM tools) for pathogen and disease monitoring, risk prediction and control of pest, pathogen and weed populations into programmes where the tools operate synergistically to reduce pests and disease with minimal environmental impact and economic risk. IPM as summarised by the Scottish Government are shown in Figure 1. http://www.gov.scot/Topics/farmingrural/Agriculture/Environment/Pesticides/IntegratedPestManagement



- Appropriate cultural controls such as crop rotations and the use of resistant varieties.
- Physical and mechanical controls including the use of nets, mulches and mechanical weeding.
- Enhancement of wildlife habitats to encourage biodiversity and beneficial organisms that provide biological control.
- Monitoring of crops for pests, weeds and diseases and the use of forecasts and thresholds for treatment.
- Tailored and efficient use of chemical inputs such as fertilisers and pesticides.

Figure 1. The main components of IPM as summarised by the Scottish Government.

Soil-borne pathogens of potato cause a number of serious blemish diseases. By employing appropriate soil sampling strategies in conjunction with a method for soil DNA extraction and real-time PCR assays to detect and quantify target pathogens, we can establish the relationship between soil-borne inoculum and disease risk. Knowing the risk of disease associated with soil-borne inoculum allows informed decisions on IPM to be made. Further

information on IPM research at The James Hutton Institute can be found at <u>http://ipm.hutton.ac.uk/</u>

PREDICTING DISEASE RISK FROM SOIL-BORNE PATHOGENS

Soil sampling and pathogen quantification

An appropriate soil sampling strategy underpins the validity of pathogen detection and disease risk assessments on a field scale. A soil sample should be taken pre-planting in a W shape across a field (<4 ha) consisting of 1kg bulked from soil taken using a mini auger, narrow trowel or grass plot sampler from 100 points (0-10cm depth). After thoroughly mixing the sample, a sub-sample (60g) is taken for processing using a Planetary Ball Mill PM400 (Retsch) prior to DNA extraction (Brierley *et al.*, 2009). Inoculum levels of *Colletotrichum coccodes, Spongospora subterranea, Rhizoctonia solani* AG3 and pathogenic *Streptomyces* spp. can be determined using real-time PCR assays (Cullen *et al.*, 2002; van de Graaf *et al.*, 2003, Lees *et al.*, 2002 and Qu *et al.*, 2011, respectively). Before an estimate of disease risk can be ascribed to detectable pathogen levels, a process of validation is required.

Assessing disease risk

Through the monitoring of commercial potato crops and extensive glasshouse and field trials, the risk of black dot and powdery scab associated with levels of *C. coccodes* and *S. subterranea* respectively has been determined. Work is ongoing to establish disease risk associated with *R. solani* AG3 and pathogenic *Streptomyces* spp.

Black dot: Soil inoculum levels of *C. coccodes* can be reliably quantified and levels relate to risk of disease (Lees *et al.*, 2010). It has been demonstrated that seed-borne inoculum is relatively less important than soil-borne inoculum in causing black dot on progeny tubers at harvest. The incidence and severity of black dot at harvest were low in trials where seed was the main source of inoculum, irrespective of the level of seed-borne inoculum visibly present at planting (Lees *et al.*, 2010). The effect of soil inoculum on black dot development on tubers has been studied in conjunction with control options such as; cultivar resistance, azoxystrobin in-furrow treatment, irrigation and crop duration (time from planting to harvest) (Brierley *et al.*, 2015). Therefore, by assessing disease risk associated with soil inoculum, growers can make informed decisions regarding cultivar selection and further reduce risk using a combination of reduced irrigation, shorter crop duration and in-furrow application of azoxystrobin where appropriate.

Powdery scab: Quantifying *S. subterranea* in soil prior to planting provides an assessment of disease risk (Brierley *et al.*, 2013). With no effective chemical control options, utilizing site selection (i.e. avoiding high risk fields) and host resistance remain the most effective strategies for controlling powdery scab. Reliable alternative crop protectants and biopesticides effective against powdery scab have yet to be identified, but could potentially provide a component to IPM in the future.

Black scurf: Detection of *R. solani* AG3 in field soil indicates an increased risk of black scurf developing in a crop (33% when inoculum detected compared to 11% when not); however, disease was found in crops even when no seed or soil-borne inoculum was detected (Brierley *et al.*, 2016). Ways to improve the robustness of detection are being explored, for example, increasing sampling intensity and extraction of the fungal propagules from soil preceding DNA extraction which may increase sensitivity of detection and optimise the value of testing for this pathogen.

Common scab: The causal pathogen of common scab is a group of saprophytic bacteria described here as pathogenic *Streptomyces* spp. Difficulty with identifying a diagnostic target specific to only pathogenic species and strains of *Streptomyces* has hindered research into establishing disease risk associated with seed- and soil-borne inoculum. Validation of the quantification of seed and soil inoculum using a real-time PCR assay (Qu *et al.*, 2011) which detects txtAB genes, widely thought to be associated with the majority, if not all, of the pathogenic *Streptomyces* spp. is underway.

To date three seed stocks, 24 washed tubers per stock, have been tested. One stock was disease free, a second had a 4% incidence, and the third a 29% incidence of common scab based on visual inspection. All tubers with common scab symptoms tested positive for pathogenic *Streptomyces* spp. when tested with real-time PCR. A number of symptomless tubers also tested positive (at relatively low levels). In the seed stock with no symptoms this might indicate symptomless infection, in the other two stocks, as symptoms were present on some tubers, it is more difficult to distinguish symptomless infection from surface contamination. The quantification of pathogenic *Streptomyces* spp. in artificially inoculated soil has been demonstrated (Figure 2). Artificially inoculated soils are currently being used to determine sensitivity of inoculum detection and establish the relationship between soil inoculum and disease development in glasshouse trials. Additionally, seed stocks and field soil are being tested to determine our ability to detect inoculum and determine its relation to disease development. This research is utilising the centre for sustainable cropping (CSC), a 6 field-rotation, at The James Hutton Institute.



Figure 2. Detectable *Streptomyces* spp. (pg DNA/ g soil) in soil inoculated with increasing amounts of inoculum suspension (*Streptomyces* spp. isolated from common scab lesions on cultivar Maris Piper): 4 replicate soils per inoculum level.

CONCLUSIONS

Control options to reduce the risk of blemish diseases developing on potato crops can be employed based on knowledge of disease risk associated with individual fields. This development enables field selection to be a principal component of integrated disease control. Where a number of fields are tested, those posing a high risk to disease development can be avoided, or planted with a cultivar with some resistance. This is particularly useful on rented land where cropping history may not be known. On land which does not contain inoculum, or where there is a low risk associated with soil inoculum level, planting of infected seed should be avoided to prevent further soil contamination. By quantifying soil inoculum prior to planting, growers can exploit host resistance and target cultivars to fields. Utilizing host resistance remains the most effective strategy for controlling powdery scab, whilst for black dot other crop management options can be employed to further reduce risk; such as reduced irrigation, early harvest and where appropriate in-furrow application of azoxystrobin. Work is continuing to establish robust predictive risk assessments for black scurf and common scab.

ACKNOWLEDGEMENTS

This work was funded by AHDB potato and the Scottish Government's Rural and Environment Science and Analytical Services (RESAS) Division.

REFERENCES

- Brierley JL, Hilton AJ, Wale SJ, Peters JC, Gladders P, Bradshaw NJ, Ritchie F, MacKenzie K, Lees AK, 2015. Factors affecting the development and control of black dot on potato tubers. Plant Pathology 64,167-177.
- Brierley JL, Sullivan L, Wale SJ, Hilton AJ, Kiezebrink DT, Lees AK, 2012. Relationship between *Spongospora subterranea* soil inoculum level, host resistance and powdery scab on potato tubers in the field. Plant Pathology 62, 413-420.
- Cullen DW, Lees AK, Toth IK, Duncan JM, 2002. Detection of *Colletotrichum coccodes* from soil and potato tubers by conventional and quantitative real-time PCR. Plant Pathology 51, 281-292.
- Lees AK, Cullen DW, Sullivan L, Nicolson MJ, 2002. Development of conventional and quantitative real-time PCR assays for the detection and identification of *Rhizoctonia solani* AG-3 in potato and soil. Plant Pathology 51, 293-302.
- Lees AK, Brierley JL, Stewart JA, Hilton AJ, Wale SJ, Gladders P, Bradshaw NJ & Peters JC, 2010. Relative importance of seed-tuber and soil-borne inoculum in causing black dot disease of potato. Plant Pathology 59, 693-702.
- Brierley JL, Stewart JA, Lees AK, 2009. Quantifying potato pathogen DNA in soil. Applied soil ecology 41, 234–238.
- van de Graaf P, Lees AK, Cullen DW, Duncan JM, 2003. Detection and quantification of *Spongospora subterranea* in soil, water and plant tissue sample using real-time PCR. European Journal of Plant Pathology 109, 589-597.
- Qu XS, Wanner LA, Christ BJ, 2011. Multiplex real-time PCR (TaqMan) assay for the simultaneous detection and discrimination of potato powdery and common scab diseases and pathogens. Journal of Applied Microbiology 110, 769-777.