Grower Summary

SF 130

Raspberry: Detection and quantification of Phytophthora rubi in soil and plant tissue

Final 2014

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Further information

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

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GROWER SUMMARY

Headline

- A new real-time species specific PCR assay has been designed for *Phytophthora rubi* in plant material and field soils, which is specific for the target pathogen and does not detect *P. fragariae* or other related Phytophthora species.

Background and expected deliverables

Soil-borne *Phytophthora rubi* (previously known as *Phytophthora fragariae* var. *rubi*) can infect raspberry and cause wilting, leading to the death of otherwise long-lived plants. Other species of *Phytophthora* can also cause root rot in raspberry, but *P. rubi* causes the most common and serious form of rot (Kennedy and Duncan, 1991). Raspberry root infection by *Phytophthora rubi* leads to root rot and cane death. In the absence of effective host resistance, control is focused on cultural practices and agrochemical use. The pathogen is spread either via infected planting material or through contaminated soil. Therefore, ability to rapidly detect pathogen at low levels would be a key component of a management strategy.

It is currently possible to detect *P. rubi* in plant material using conventional and molecular methods. However, there is no direct soil test to quantify the pathogen because existing PCR assays for *P. rubi* also detect other *Phytophthora* species (i.e. *P. fragariae*). Recent HDC-funded research (Project SF 97) developed quantitative polymerase chain reaction (abbreviated to qPCR) tests that enabled the detection and quantification of *V. dahliae* DNA in soil. This project will build on the knowledge gained from SF 97 to develop a method for quantifying DNA levels of *P. rubi* in sample material.

The aim of the current work is to develop a real-time quantitative PCR assay for *P. rubi* to allow the rapid detection in planting material and soil. The outcome would be a rapid specific assay for the detection of *P. rubi* in soil and plant material.

Summary of the project and main conclusions

Objective 1 – To develop and validate a new molecular assay for the quantification of *Phytophthora rubi*

Sequences for rDNA ITS, rDNA IGS, *cox* I, *cox* II and the ras-related *Ypt*-1 genes were either directly sequenced or obtained from GenBank for a range of *Phytophthora* isolates. All sequences, with the exception of those originating from *cox* I, were either identical or highly similar, with too few sequence differences present to enable the design of species specific qPCR assays for *P. rubi*. A real-time PCR assay was therefore designed to the *cox* I sequence. Despite relatively few sequence differences being present the assay was highly specific and did not cross react with a range of other *Phytophthora* species. The technical limit of quantification of the assay was 6.6 pg/µl, which is approximately 10 to 100 times less sensitive than assays designed to multi-copy genes.

Objective 2 – To investigate detection thresholds for *P. rubi* in host tissues and soils

The assay was able to detect *P. rubi* in both artificially (spiked) and naturally infested soil and plant material. In artificially infested material, the assay could detect as little as 4 mg of *P. rubi* hyphae in 50g soil but was not able to detect *P. rubi* in one 50 g soil sample spiked with 1.5 mg of hyphae. For plant material, the assay was able to detect 15 mg of *P. rubi* hyphae in a 2 g sample of raspberry crown and root material. In both cases there was a linear relationship between the weight of spiked hyphae of *P. rubi* and amount of *P. rubi* DNA detected using the assay. The assay successfully detected *P. rubi* in seven from 11 plant samples tested and two from nine soil samples tested, demonstrating detection with naturally infested samples.

Objective 3 – To promote to growers and breeders the proposed commercial availability of a rapid quantitative DNA assay for *Phytophthora rubi* in soils and plant tissue and to highlight the potential application of soil thresholds for grower selection of planting material

The assay can detect *P. rubi* in plant material and highly infested soil material. The assay will be used in parallel with established conventional testing for *P. rubi* in plant material at Fera over the next 12 months to ensure consistency with existing conventional tests, with the aim of launching a commercial service in 2015 if the validation is successful.

Financial benefits

With a *P. rubi* specific assay the presence of the pathogen in plant and soil material can be accurately determined. Accurate knowledge of the presence of *P. rubi* will inform decisions about selection of plant material and suitable planting sites. The qPCR assay can also be used reactively to rapidly confirm the presence of *P. rubi* in symptomatic plants in outbreak situations. qPCR could give a result in several days as opposed to isolation which can take several weeks.

Potential further work

- Sequence data from next generation sequencing work initiated at Fera with Defra funding will be analyzed to identify species specific multi-copy loci that can be used to design more sensitive nested qPCR assays for *P. rubi*.
- The assays developed in this project will be used in parallel with conventional testing for *P. rubi* at Fera for 12 months prior to full launch of a molecular screening service for soils of soft fruit crops. This will be launched with assays for other soft fruit pathogens including *V. dahliae* and *Phytophthora cactorum* for a comprehensive soil screening package.
- Droplet digital PCR approaches will be examined at Fera. This has the potential for much greater levels of sensitivity than qPCR or nested qPCR.
- No knowledge exists on the levels of inoculum of *P. rubi* required for disease to occur (thresholds). Field and/or glasshouse experiments are required to determine this and also to find if it varies with different ages of planting material, soil type, plant variety and environmental conditions.
- Knowledge on how *P. rubi* survives in the soil is also required. Does it survive as hyphae, spore or in organic matter? This knowledge will help determine an accurate unit to be used in the threshold discussed above but also could be used to improve assay sensitivity. For example if the pathogen survives in organic matter, organic matter could be separated from the soil, effectively concentrating the pathogen in the organic matter subsample, enabling higher levels of *P. rubi* DNA to be recovered.

Action points for growers

- Growers submitting plant material for testing for *Phytophthora* can ask for additional molecular testing for *P. rubi* using the new assay.
- Growers wishing to provide soil material for testing should contact James Woodhall by email (james.woodhall@fera.gsi.gov.uk). Soil samples should consist of at least 500 g. Ideally soil samples should consist of at least 20 sub-samples taken over one hectare.