

Grower Summary

SF 130

Raspberry: Detection and
Quantification of *Phytophthora*
rubi in soil and plant tissue

Annual/Final 2013

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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 the full report, please email the HDC office (hdc@hdc.ahdb.org.uk), quoting your HDC number, alternatively contact the HDC at the address below.

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HDC is a division of the Agriculture and Horticulture Development Board.

Project Number:	SF 130
Project Title:	Raspberry: Detection and quantification of Phytophthora rubi in soil and plant tissue
Project Leader:	Dr Jeff Peters
Contractor:	Fera
Industry Representative:	Tim Place, Place UK Ltd
Report:	Annual Report 2013
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Previous report/(s):	N/A
Start Date:	01 April 2012
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Project Cost:	£37,158

Headline

- A novel real-time PCR assay has been designed for *Phytophthora rubi*.

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, 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 by planting clean canes in contaminated soil. Therefore, being able 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 real-time PCR assays for *P. rubi* cross-react with other *Phytophthora* spp (i.e. *P. fragariae*). Specific conventional PCR assays for *P. rubi* have been developed by JHI/SASA for use on plant material but are not suitable for testing soils. Recent HDC-funded research (SF 97) developed quantitative polymerase chain reaction (abbreviated to QPCR) tests that enabled the detection and quantification of *V. dahliae* and *V. albo-atrum* DNA in soil. The proposed project aims to 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 QPCR primer set for *P. rubi* to allow the rapid detection in planting material and soil. Outcomes would be a quick and inexpensive assay for *P. rubi* detection and an initial indication of how soil DNA levels affect disease development in the main raspberry varieties.

Summary of the project and main conclusions

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

A molecular, real-time PCR, test has been developed for *Phytophthora rubi*. The sequencing work required to develop the PCR test showed that *P. rubi* and *P. fragariae* are closely related and few sequence differences exist between the two species. One gene, the Cox1 gene, shows promise as a target for discriminating between the two species. Two assays

have been designed, one for *P. fragariae* and another for *P. rubi*. Initial results suggest specificity is possible. However, with few differences in the DNA sequences, the first attempt at an assay has produced assays of relatively low sensitivity which would not be suitable for use for soil diagnostics. Further primer sites within the Cox1 gene will be determined along with a nested real-time PCR approach which should increase both specificity and sensitivity.

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

The research is still on-going. So far, infected plant tissue is detected at higher levels than soils. Work is ongoing to increase the detection of *P. rubi* in soil.

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 promotion of the new real-time PCR to *P. rubi* will be done during the second year of the project.

Financial benefits

It is not possible to estimate the potential financial benefits from this work until the project is completed. Benefits may arise from:

- a) Reduced losses from *P. rubi*;
- b) Increased accuracy in the identification of *P. rubi* present in soft fruit soils;
- c) Development of a rapid pre-plant soil test for *P. rubi*:

Action points for growers

- A new molecular test has been developed that discriminates between *P. rubi* and *P. fragariae* thus suggesting that a novel diagnostic assay will be available for detecting soil-borne *P. rubi*.
- Further validation work is required in year 2 to ensure that the test is suitable for commercial testing.