Grower Summary

CP173

Towards a better understanding of the biology and genetics of *Phytophthora rubi* and *Phytophthora fragariae*

Annual report, October 2018

Project title:	**Towards a better understanding of the biology and genetics of *Phytophthora rubi* and *Phytophthora fragariae***
Project number:	CP173
Project leader:	Eleanor Gilroy, The James Hutton Institute (JHI), Dundee
Report:	Annual report, October 2018
Previous report:	N/A
Key staff:	Aurelia Bezanger (PhD student , author of the report), Steve Whisson, Lydia Welsh, JHI
Location of project:	The James Hutton Institute, Dundee
Industry Representative:	Ross Mitchell, Castleton Fruit Ltd, Laurencekirk
Date project commenced:	October 2017

DISCLAIMER

While the Agriculture and Horticulture Development Board seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

GROWER SUMMARY

Headline

- New insights into the biology of raspberry root rot

Background and expected deliverables

Phytophthora rubi (raspberry root rot) and *Phytophthora fragariae* (strawberry red core/stele) are currently poorly understood and understudied pathogens causing significant economic and environmental impact on soft fruit production in the UK. At the moment, there are no effective chemical control measures. The best control strategy relies on prevention and destruction of infected plants on which the pathogen depends for reproduction. However, this leaves the soil contaminated and unusable for future crop production. Consequently, the industry has been forced into pot-based annual or short-term production in substrate, but this only reduces, rather than solves the problem. In order to find suitable, reliable and durable fighting strategies, the pathogen first needs to be understood. This project aims at understanding the biology and genetics of *P. rubi* and *P. fragariae.*

Summary of the project and main conclusions

Hydroponic raspberries

In this project, raspberries have successfully been grown in hydroponics, using a Nutrient Film Technique (NFT). After dipping raspberry cuttings in a rooting hormone, they were stuck in rockwool plugs and soaked in nutrient solution at a correct pH (5.2 – 5.8). Once roots appeared and grew long enough, the plugs were transferred onto the NFT channel. Healthy roots and shoots continued to grow, and the root mat developed shoots that were placed inside rockwool media before being re-introduced into the hydroponics rotation once they grew sufficiently. Raspberry plants will be left to grow over the winter and fruiting will be assessed. Cuttings are produced on a regular basis.

P. rubi isolation from canes

In work to isolate *P. rubi* from cane material rather than roots, a protocol adapted from Stewart et al., 2014, used Italian selection media with antibiotics (PCNB, pimaricin, rifampicin, nystatin, hymexazol and ampicillin) rather than CMA-PARP media (Stewart et al., 2014; Cooke, personal communication). Once hyphal growth was isolated from canes, it was morphologically checked to narrow it down to the *Phytophthora* genus. After being sub-

cultured on Rye agar, DNA was extracted and the cox I region was sequenced along positive controls to confirm that the isolate was indeed *P. rubi*. This method was proven successful when field isolates of *P. rubi* were taken from a field in Dundee. The method was repeated twice using samples collected in East Scotland. Preliminary morphological assessment suggested *P. rubi* and molecular confirmation is on-going.

P. rubi phenotype study

Effect of temperature

Several lab isolates and field isolates of *P. rubi* and *P. fragariae* were grown at 15˚C and 18˚C. Statistical analysis showed that the *P. rubi* lab isolates grew significantly better at 18˚C whereas the *P. rubi* field isolates grew similarly under both temperatures. *P. fragariae* isolates also grew significantly better at 18˚C.

Sporangia and zoospores production

Of the 14 different types of sporulation solution tested, a soil water solution worked best in producing *P. rubi* sporangia and zoospores. The less sterile the solution, the more sporangia were produced, implying that this bacterial metabolite is highly related to the success of *P. rubi* reproduction. Swimming zoospores were successfully released. *P fragariae* kept producing full sporangia and releasing zoospores over a course of 20 days, demonstrating the extended period during which the pathogen can produce reproduction and infection structures.

Petiole inoculation for rapid resistance screening

A method adapted from Li et al., 2017 was tested for a rapid screening for resistance in raspberry cultivars. The method used raspberry petioles (rather than setting infection on roots which can be more difficult and time consuming) from Glen Moy and Latham cultivars. Petioles were set in Eppendorf tubes containing *P. rubi* mycelia slurry and put in a sealed box where the humidity was kept at an optimum level. Petioles and leaves were monitored for 3 weeks to assess symptoms. Eleven days post inoculation was found to be best to observe symptom differences, where Glen Moy leaves showed yellowing and decay sooner than the Latham ones. This method needs to be further explored in order to test for other potential *P. rubi* hosts (tayberry, blueberry etc.)

Bio-informatics and baits library design

New bio-informatics technologies are more and more popular. The Target Enrichment Sequencing will be used in this project and aims to study the diversity of *P. rubi* and *P. fragariae*, both inter and intra-specifically. This method enables the massively parallel

identification of presence/absence and sequence polymorphisms in avirulence genes, which is a prerequisite for predicting host resistance durability. The Target Enrichment Sequencing relies on amino acid sequences, called “baits”, used to target predicted effectors (pathogenic proteins). In this study, a bait library was designed for RXLR effectors, Crinkler effectors, pathogenicity genes, and fungicide targets genes. This library will help in assessing the pathogen’s diversity.

Main conclusions

So far in this project:

- Raspberries have successfully been grown in hydroponics
- A method to isolate *P. rubi* from canes, rather than roots, has been successfully developed
- More insights into the phenotype and behaviour of *P. rubi* were gained by *in vitro* experiments (effects of temperature and reproduction structures of *P. rubi* and *P. fragariae*)
- A method has been tested for a rapid screening for resistance in raspberry cultivars and potential *P. rubi* hosts
- A baits library was designed to be used in a bio-informatics assay to study *P. rubi* and *P. fragariae* effectors

Financial benefits

In some crops, the loss due to *Phytophthora* species is estimated at 40% of production and valued at $300 billion worldwide. *P. rubi*, causing raspberry root rot, has devastated the UK raspberry production, with over 80% of field production having been lost to the disease. This pathogen causes major economic and environmental damage but is very poorly understood. This project aims to gain further insights into the pathogen biology and genetics, as a foundation for further research on raspberry root rot. There are no immediate financial benefits.

Action points for growers

This project seeks to inform longer-term research of *Phytophthora rubi* and is unlikely to deliver direct action points for growers.

References

LI, Y., SUN, S., ZHONG, C. & ZHU, Z. (2017). Detached-petiole inoculation method to evaluate *Phytophthora* root rot resistance in soybean plants. Crop and Pasture Science, 68, 555.

STEWART, J. E., KROESE, D., TABIMA, J. F., LARSEN, M. M., FIELAND, V. J., PRESS, C. M., ZASADA, I. A. & GRÜNWALD, N. J. (2014). Pathogenicity, fungicide resistance, and genetic variability of *Phytophthora rubi* isolates from raspberry (*Rubus idaeus*) in the western United States. Plant Disease, 98, 1702-1708