Project title:	**Towards a better understanding of the biology and genetics of *Phytophthora rubi* and *Phytophthora fragariae***
Project number:	CP 173
Project leader:	Eleanor Gilroy, The James Hutton Institute (JHI), Dundee
Report:	Annual report, October 2020
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Date project commenced:	October 2017

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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.

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GROWER SUMMARY

Headline

- *Phytophthora rubi* causing raspberry root rot and *Phytophthora fragariae* causing strawberry red stele are very closely related diseases, though they infect different hosts. Understanding the biology and genetics of the diseases will help amend the current fighting strategies, such as relevance of chemical treatment, and develop new ones like resistance breeding.

Background and expected deliverables

Phytophthora rubi (raspberry root rot) and *Phytophthora fragariae* (strawberry red stele), very closely related, are currently poorly understood and understudied pathogens causing significant economic and environmental impact on soft fruit production in the UK. Currently, there are no effective chemical control measures, and the best control strategy relies on prevention, good conditions of the cultures 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 sustainable, reliable and durable fighting strategies, the pathogen first needs to be better understood so we can predict the pathogens' evolutionary potential and adapt to future control measures.

Summary and the project and main conclusions

To understand and characterise the raspberry root rot pathogen, both phenotypic and genetic aspects of the disease were studied. This comprises *P. rubi* phenotypic responses to environmental factors such as chemical pressure and changes of temperatures, its life cycle during infection and its genetic diversity.

To address the first objective, the responses of several *P. rubi* and *P. fragariae* isolates to temperatures, in terms of mycelial growth and sporulation, as well as to different doses of chemicals incorporated into the growing media were assessed. Isolates of *P. rubi* and *P. fragariae* were grown at 15°C, 18°C (control), 21°C, 25°C and 28°C. Statistical analyses showed that *P. rubi* mycelial development significantly increases from 15°C to 18°C and to 21°C but decreases at 25°C and is fully inhibited at 28°C. However, *P. rubi* sporulation was statistically similar in efficiency at all temperatures, thus demonstrating the flexibility in sporangia production across the temperature range tested.

The chemical screening study tested 7 compounds: Fluazinam, active ingredient of Shirlan and Tizca; Fluopicolide and Propamocarb, used in Infinito; Ametoctradin, active ingredient of fungicides such as Initium or Zampro; Phorce, a phosphite-based fertiliser; Dimethomorph, active ingredient of Paraat; and Metalaxyl-M, used in SL 567A. The study shows the potential for Dimethomorph (Paraat) (Figure 1a) and Fluazinam (Tizcan), which are still allowed for use on raspberries and strawberries, to control *P. rubi* and *P. fragariae* mycelial growth at a dose that is applicable in the field. Nevertheless, Metalaxyl-M (Figure 1b) incorporation in growing medium presents a variety of responses, with both inhibition under high doses of the chemical and resistance. This confirms previously reported studies on *Phytophthora* species (Nickerson, 1998; Parra and Ristaino, 2001a; Vawdrey et al., 2004; Elansky et al., 2007; Zhu et al., 2008; Pérez et al., 2009; Qi et al., 2012a; Rekanovic et al., 2012; Randall et al., 2014). Propamocarb, Ametoctradin, and Phorce display very little to no decrease of the mycelial growth with an increasing dose, indicating a lack of sufficient inhibition to growers' standards.

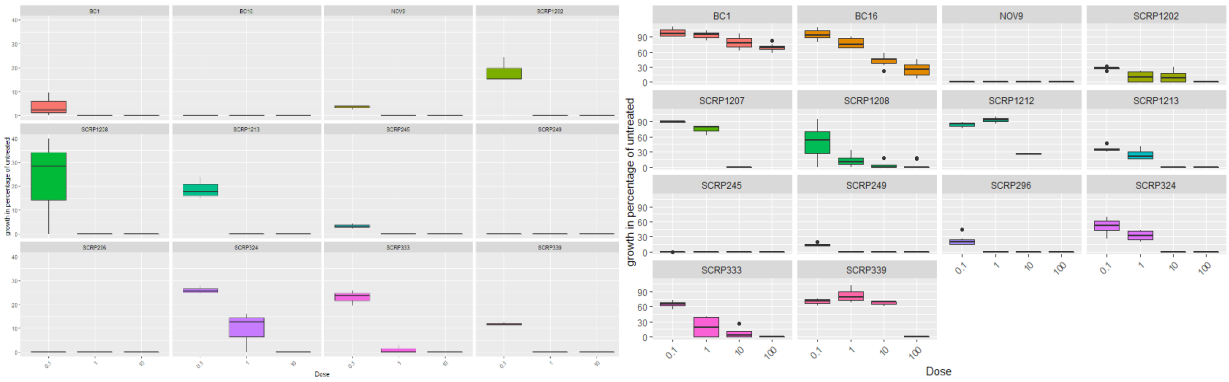


Figure 1: Boxplots of the effect of a.Dimethomorph and b. Metalaxyl-M. The x-axis represents the chemical dose (ppm) and the y-axis represents the mean mycelial diameter (in percentage of controls)

The second objective used raspberries grown in hydroponics conditions infected with a strain of *P. rubi* genetically modified to express a red fluorescent protein (see previous CP173 reports), so that infection could be followed in real-time. More insights into the pathogen behaviour were gained when the infection cycle was complete, and evidence of potential subsequent infections waves was observed (Figure 2). The hydroponic techniques first developed are being gradually optimised, assessed on a range of raspberry cultivars, as well as other berries and are now the method of choice for blackcurrant propagation at The James Hutton Institute.

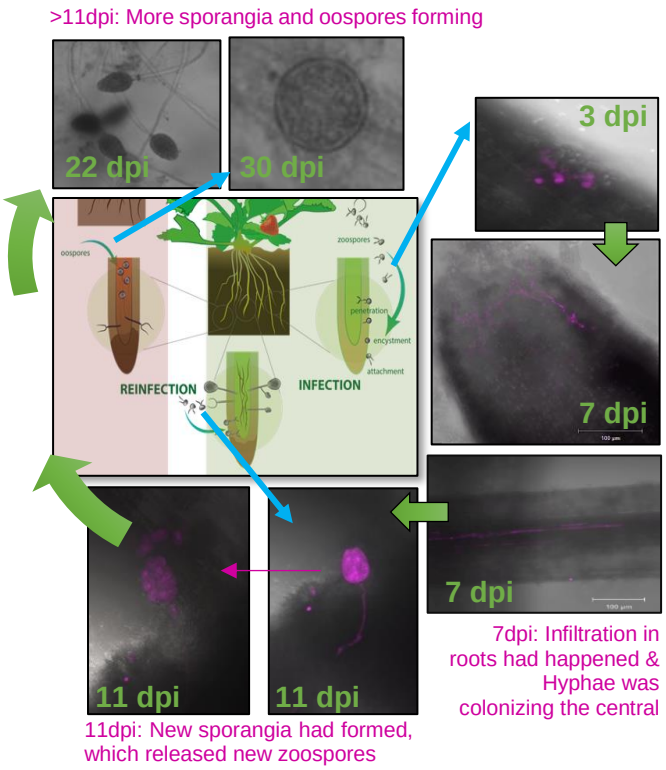


Figure 2: P. rubi infection progression observed on hydroponically grown raspberries using a red fluorescent strain (identified in magenta in confocal photos). Photos were taken with a Zeiss LSM 710 confocal microscope. Reference example cycle in centre from Penny Greaves.

Finally, genetic diversity was studies using a recently developed method, called target enrichment sequencing (PenSeq), which allows in depth sequencing of a small and select percentage of the genome (<1%) proving a cost effective and efficient way to mine multiple samples for the most significant genes of interest. This experiment first confirmed the significant distinction between the two species when comparing the genes of interest. Analyses additionally highlighted 600 genes that are completely identical between 8 isolates of *P. rubi* and 4 isolates of *P. fragariae* with no inter or intra-species polymorphism. On another hand, highly conserved and species-specific genes were also found: 142 genes were unique to *P. fragariae* and 69 genes unique to *P. rubi.* While the two pathogens are very closely related and believed to be the same species for many years, *P. rubi* infects raspberries only, while *P. fragariae* infects strawberries; and unique genes could play a key role in host choice and recognition. Furthermore, we have evidence that there is significant isolate variation amongst *P. rubi* examined thus far indicating that we are unlikely to have a single clonal lineage, but a diverse and evolving population present in our fields.

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 the field production that has been lost to the disease. This pathogen causes major economic and environmental damages 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

The project was not designated to produce immediate recommendations to growers.

SCIENCE SECTION

Introduction

Phytophthora rubi (raspberry root rot) and *Phytophthora fragariae* (strawberry red stele), very closely related, are currently poorly understood and understudied pathogens causing significant economic and environmental impact on soft fruit production in the UK. *Phytophthora* root rot (PRR) is a major raspberry disease, primarily caused by *Phytophthora rubi*, an oomycete pathogen related to the infamous potato blight agent. Although the raspberry pathogen first appeared in the 1930s, serious outbreaks only occurred in Europe and in the UK in the 1980s. Once established, the disease could be locally spread to other growers through infested soil or water, and oospores could remain dormant in the soil for up to 10 years. As field tolerance to PRR is only seen in a few raspberry cultivars, most of which are not used commercially, highly susceptible cultivars are planted, contributing to the rapid progress of the disease across fields, regions and ultimately countries. Presently, more than 70% of the UK's soil-based raspberry production is affected by PRR, forcing growers to adopt different growing systems, such as pot-based culture. Unfortunately, there is a lack of effective chemical and control treatments for PRR. Most treatments pose the risk of being overcome by resistance from *P. rubi*, as they only rely on a single active compound with a specific mode of action. Thus, *P. rubi* poses a major economic and environmental problem for the soft fruit industry. Although it appeared in the UK decades ago, there are still knowledge gaps regarding the biology, genetics and infection lifecycle of *P. rubi*. This project aims to investigate *P. rubi* phenotype and genotype. The main aim of this study is to characterise *P. rubi,* causing raspberry root rot, as there are still large gaps in our understanding of the disease. Characterising a pathogen means studying its phenotype and genotype, and when possible, drawing a parallel analysis between the two. In order to achieve this, several key objectives are addressed in this project:

- *P. rubi* and *P. fragariae* phenotypes have been studied for resistance to chemical treatments and against different temperatures, by conducting *in vitro* assays.
- *P. rubi* and *P. fragariae* genetic diversity is studied using bioinformatics tools to identify potential effectors encoded and expressed by their genomes. This work will assess the differences between the two very closely related species but also differences within each species, at the isolate level. This could indicate whether there are specific proteins responsible for host choice or give an indication about the variance between isolates of one species, comparing strains isolated from different countries over ~70 years.
- Raspberry plants have been grown in a hydroponic system and a rotation has been put in place, where new plants can be grown regularly. The hydroponic techniques

first developed is constantly improved and assessed on raspberries as well as other berries.

- Transformation has been carried out on several isolates of *P. rubi* using green and red fluorescent proteins (eGFP and tdTomato) to allow detailed study of infection in roots in real time. A successful *P. rubi* expressing tdTomato was used in infection assays.
- Real-time hydroponic infections have been carried out using a transgenic isolate of *P. rubi* expressing a red fluorescent protein on hydroponically grown raspberries. The progression of the fluorescent strain in the root was followed and regularly captured using a Zeiss LSM 710 confocal microscope. The infection assay included collection at 3, 7, 11 and 14 days after infection for further testing of gene expression through qRT-PCR.

This work will feed back into further studies on raspberry root rot (*P. rubi*) and strawberry red stele (*P. fragariae*) diseases. It will provide scientific knowledge to underpin the horticultural industry through pre-breeding and development of novel pathogen control strategies.

Materials and methods

Cultures of Phytophthora rubi and Phytophthora fragariae

Various *P. rubi* and *P. fragariae* isolates were used throughout this study (Table 1). Culturing and storage methods have been described previously in CP 173 2018 and 2019 reports. The most recent isolates (from 2017 and 2018) were obtained from infected Glen Dee raspberries, via a cane isolation method, also described in previous CP 173 reports. Isolates were kept on plates of rye agar with 100 µg/mL of ampicillin, at 18°C in the dark, before being used for the study.

P. rubi phenotypic diversity study: responses to temperatures and chemicals

P. rubi and P. fragariae isolates

Table 1 summarizes the isolates used in the phenotypic study, screening for responses to temperatures and chemicals.

Table 1: *P. rubi* and *P. fragariae* isolates used for the chemical screening and temperature assay.

Sample species	Sample name	Country	Year	Race	For chemical screening assay	For temperature assay	
						Mycelial growth	Sporulation
P. rubi	SCRP333	Scotland	1985	Race 3	Yes	Yes	Yes
	SCRP1202	The Netherlands	2010	Unknown	Yes	Yes	No
	SCRP1208	Scotland	2017	Unknown	Yes	Yes	No
	SCRP1213	Scotland	2018	Unknown	Yes	Yes	Yes
	SCRP324	Scotland	1991	Race 1	Yes	Yes	Yes
	SCRP249	Germany	1985	Unknown	Yes	Yes	No
	SCRP296	Scotland	1993	Unknown	Yes	Yes	No
	SCRP339	France	1985	Race 3	Yes	Yes	No
	SCRP1212	Scotland	2018	Unknown	Yes (Metalaxyl-M & Fluopicolide)	No	Yes
P. fragariae	BC-1	Canada	2007	Race 1 (CA1)	Yes	Yes	No
	BC-16	Canada	2007	Race 2 (CA3)	Yes	Yes	No
	NOV-9	Canada	2007	Race 3 (CA2)	Yes	Yes	No
	SCRP245	England	1945	Unknown	Yes	Yes	No

Chemical incorporation and media

Seven different treatments were used in this study: Fluazinam, active ingredient of Shirlan and Tizca; Fluopicolide and Propamocarb, used in Infinito; Ametoctradin, active ingredient of fungicides such as Initium or Zampro; Phorce, a phosphite-based fertiliser; Dimethomorph, active ingredient of Paraat; and Metalaxyl-M, used in SL 567A. Ametoctradin, a chemical impacting the zoospores and sporangia life stage of Oomycetes, was screened on four *P. rubi* isolates as part of an initial mycelial growth test. This selection provided preliminary insights into the effect of Ametoctradin on mycelia of *P. rubi* isolated over a long period of time.

Fluazinam, Dimethomorph, Fluopicolide and Metalaxyl-M (Mefenoxam), Propamocarb and Ametoctradin were sourced from Sigma Aldrich, and Phorce was obtained from Nutriphite. Rye agar with 100 µg/mL of ampicillin was used as a base medium for growing *P. rubi* and *P. fragariae* isolates for both assays. Stock solutions of 100 000 ppm made for each chemical, using dimethyl sulfoxide (DMSO) as a solvent, was incorporated into this base medium, similarly to previously reported methods (Lee et al., 1999; Groves and Ristaino, 2000; Randall et al., 2014; Saville et al., 2015). An equivalent dose of DMSO was added to the controls of the chemical study. Four doses were used for each chemical: 0 ppm (controls), 0.1 ppm, 1 ppm and 10 ppm. To further investigate resistance to Metalaxyl-M that had been previously reported in *Phytophthora spp.*, an additional dose of 100 ppm was included in the screening.

Inoculation and growth conditions

Agar plugs of 9 mm diameter of *P. rubi* and *P. fragariae* were taken from the actively growing colony margin and placed onto agar media in 50 mm diameter Petri dishes. Three replicates were used for each study (chemical and temperature screening). For chemicals with results showing potential resistance (Fluazinam and Metalaxyl-M), screening was carried out three times. Plates were incubated in the dark, at 18°C for the chemical study and 15°C, 18°C (control), 21°C, 25°C and 28°C for the temperature assay.

Mycelial measurements

Following a week's growth at the appropriate temperature in the dark, the colony diameter of *P. rubi* and *P. fragariae* isolates was measured along two perpendicular axes as per Figure 1. The average of the two measurements was calculated and results were expressed in percentage of control growth, where no chemical was incorporated into the base medium and the incubation temperature was set at 18°C. After a week of incubation, plates showing no growth at all at 25°C and 28°C were placed back at 18°C for a further 10 days and re-assessed.

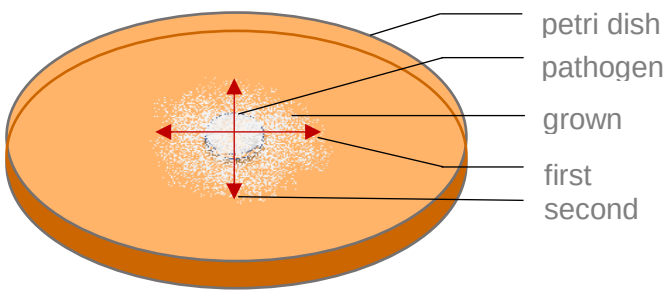


Figure 1: Diametral measurements of P. rubi mycelial growth for the phenotypic diversity assay

Chemical target genes sequences alignment

Chemical target genes for fungicides that showed diversity in mycelial growth response were retrieved using both the NCBI website and the PenSeq data. Cellulose synthase (CesA) genes CesA1, CesA3 and CesA4 were retrieved from *P. sojae* sequences found on NCBI and similar proteins and nucleotide sequences were found for *P. rubi* isolates of interest using BLASTx and BLASTp. Cellulose synthase genes CesA2 sequences were extracted from PenSeq data. Genes encoding subunits of RNA polymerase (RNAPolI, RPA190, RPA135, RPAC19, RPA12.2) in *P. infestans* as listed in Randall et al. (2014) were likewise retrieved from NCBI for *P. rubi* and *P. fragariae* isolates of interest, using the BLASTx and BLASTp tools (NCBI). Sequences for RNAPol genes RPABC23 and RPABC27 were retrieved from the PenSeq data. Nucleotide sequences were aligned using Clustal alignment in Geneious v2020.2.1. If SNP (Single Nucleotide Polymorphism) were identified between isolates sequences, protein sequences were similarly aligned to assess amino acid changes.

Sporangia production and sporangia count

Sporulation was tested under temperatures of 15°C, 18°C and 21°C, for four *P. rubi* isolates chosen to represent a variety of old and new isolates: SCRP1212, SCRP1213, SCRP296, SCRP324 and SCRP333. Sporangia were obtained following the same protocol described in previous reports. Total sporangia per field of view were counted.

Statistical analysis

Statistical analyses (ANOVA and Tukey's HSD tests) and boxplots were performed using R Studio v1.1.383. Statistical differences were considered significant if the p-value was lower than 0.05 (using a 95 % confidence interval). Three replicates were used for each isolate / chemical / dose and for each isolate / temperature when looking at mycelial growth and 20 replicates were used per isolate and temperature for the sporulation assay – two plates with 10 plugs each. Twelve isolates were used for the chemical testing and the temperature effects on mycelia, two more recently isolated *P. rubi* (SCRP1207 and SCRP1212) were added for Metalaxyl-M and Fluopicolide screening and five *P. rubi* isolates were assessed for sporulation under different temperatures. Experiments were performed once, except for the Metalaxyl-M screening, which was repeated three times.

P. rubi and *P. fragariae* genotypic diversity study: Pathogen Enrichment Sequencing (PenSeq)

Bio-informatics technologies are constantly evolving. Target Enrichment Sequencing was used in this project to study the genetic diversity of *P. rubi* and *P. fragariae*, both inter and

intra-specifically, as described in previous CP173 reports. This method enables the 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). This technique is particularly useful when looking at a subset of genes, in our case, pathogenicity genes, which represent less than 1% of *P. rubi's* total genome.

Baits library

A bait library for PenSeq was designed by gathering sequences, or partial sequences, of genes of interest for the two species *P. rubi* and *P. fragariae*. This includes RXLR, CRN and apoplastic effectors, as well as housekeeping genes. This sequences list was compiled using data from NIAB-EMR, NCBI website and various literature (Blair et al., 2008, Schena et al., 2007, Peters and Woodhall, 2014, Loos et al., 2006). The list was sent to Arbor Bioscience to design and produce baits. More than 185,000 sequences, covering a gene space of approximately 5 million nucleotides per isolate were provided for bait design, which produced 50,234 filtered baits.

Isolates collection

A first batch of twelve isolates were chosen for the first run of PenSeq, including 8 *P. rubi* and 4 *P. fragariae*: SCRP1202, SCRP1208, SCRP1213, SCRP249, SCRP296, SCRP324, SCRP333, SCRP339, BC-1, BC-16, NOV-9 and SCRP245, mixing locations, years of isolations and races (details in Table 1). DNA was extracted using a method combining a phenol-chloroform DNA extraction with a QIAGEN DNeasy Plant Mini Kit (see previous CP173 reports).

Target Enrichment Sequencing

For the Target Enrichment Sequencing of *P. rubi,* 500ng DNA was used per isolate. Library preparation was carried out using the NEBNext Ultra II, FS DNA Library Prep Kit for Illumina protocol (Biolabs). Hybridization and the rest of the enrichment was carried out using the SureSelect Hybridization protocol (Agilent).

Validation of PenSeq enrichment

The enrichment was verified prior to sequencing using betatubulin, Cox I, and an RxLR effector gene in a qPCR assay, described in the 2019 CP 173 report.

Computational analyses

Reads resulting from Pathogen Enrichment Sequencing of the 12 isolates were filtered to get rid of overlapping, duplication and adaptor sequences (trimming). The filtered reads were mapped to reference genomes, sequenced by NIAB-EMR (*P. fragariae* BC-16, GenBank accession GCA_009729455.1 and *P. rubi* SCRP333, GenBank accession GCA_009733145.1) using Bowtie2 at 0%, 3%, 5% and 10% mismatch mapping rates for each isolate. Coverage (percentage of nucleotides covered on total number of nucleotides per gene) of mapped genes (resulted from overlay of reads mapped to the reference genes) was calculated at each mismatch mapping rate for each isolate. Overall SNP (Single Nucleotide Polymorphism) calling was performed using the filtered reads and VarScan.v2.3.7 with a frequency of SNP between 40% and 60% for a minimum of 30 reads, to allow SNP call for heterozygous genes. Potential SNP for specific genes were identified using the 0% mismatch mapping rate coverage files. For those polymorphic genes, sequences for all isolates were retrieved from the 3% mismatch mapping rate mapping and aligned using MultAlin tool (Corpet, 1988) and Clustal alignment in Geneious v2020.2.1. Nucleotide sequences were translated into amino acid from the start codon with Geneious v2020.2.1 translation tool, in order to investigate whether SNPs resulted in changes in the amino acid sequences. Intra-species presence / absence of genes was identified by screening for genes showing 0% coverage at the 5% mismatch mapping rate. Those absent genes were subsequently assessed at other mismatch mapping rates for coverage differences and presence of SNPs. Similarly, inter-species presence / absence of genes was identified by screening for genes showing 0% coverage at the highest mismatch mapping rate (5%) when mapping *P. fragariae* isolates to *P. rubi* SCRP333 reference genome and *P. rubi* isolates to *P. fragariae* BC-16 reference genome. The absent genes were additionally assessed at other mismatch mapping rates.

<u>*P. rubi* infection of hydroponically grown raspberries</u>

Hydroponics raspberries

Raspberry cultivars of Glen Dee, Glen Moy and Latham have been successfully grown using the Nutrient Film Technique (NFT) as described in the previous annual reports for CP173 (2018 and 2019). Cutting are now produced on a regular basis and several other options to

optimize the method are being investigated. For instance, Glen Dee has been shown to survive in hydroponics conditions without using nutrient-soaked rockwool plugs. Fresh cuttings were dipped in rooting hormone Clonex and moved to the hydroponic tank straight away (Figure 2).

Figure 2: Raspberry cultivar Glen Dee grown in hydroponics conditions. Cuttings were taken and dipped into rooting hormone (Clonex) and directly placed into the hydroponic tank. Roots developed well after 4 weeks.

P. rubi transformation to express fluorescent proteins

When looking into woody tissues such as roots, using fluorescing strains should be easier and more detailed than staining methods, as such methods are proven difficult when working with roots and are preferably used to stain specific structures (sporangia, oospores) and should only be used in conjunction with fluorescence to provide a more detailed insight into the infection process. In this study, we combine fluorescent hyphal progression with other staining techniques.

P. rubi and *P. fragariae* were successfully transformed with fluorescent proteins, specifically eGFP (enhanced Green Fluorescent Protein) and tdTomato (Tandem Dimer Tomato or tdT), and plasmid vector pTOR (Genbank Accession EU257520), using a technique described in previous reports.

Hydroponic infection set up

In order to image *P. rubi's* life cycle in real-time in raspberry roots, genetically modified isolates expressing a fluorescent protein were used in a hydroponic infection assay, where clean intact roots were sampled regularly.

Raspberry were grown in a glasshouse with the NFT method described previously. Sporangia of a transgenic *P. rubi* strain expressing a red fluorescent protein (tdTomato) were successfully produced following previously described methods (previous annual CP173 report) and their fluorescence was checked prior to infection using the Zeiss LSM710 microscope. A 30 minute incubation at 4°C was carried out to induce a cold shock and help in the release of zoospores. Shocked sporangia were then placed in a 4L tray and Petri's solution was poured in to induce zoospore release. Roots were slightly 'damaged' by pressure applied onto the root, to aid the infection process, prior to being dipped in the zoospore solution for 6 hours. After the 6 hours-inoculation window, plants were transferred to a tank

of 25L distilled water (Deep-Water Culture hydroponic method) with an air pump and air stone to provide oxygen to the water, which was turned on after 5 to 7 days – to avoid encystment of zoospores. A control tank with non-infected plants was set up using 2 replicates of each cultivar. Samples were collected regularly: at 1-day post-inoculation (dpi), 3dpi, 7dpi, 11dpi and14dpi and confocal Z-stack images using the Zeiss LSM710 microscope were taken. Root samples for each cultivar were collected as well: they were snap-frozen in liquid nitrogen and stored at -80°C for future RNA extraction.

Root staining

As described above, using multiple microscopy-observation tools enables us to maximize the details of the infection and the *Phytophthora* structures observed (hyphae, sporangia, oospores). Furthermore, there is no selection (geneticin) in the hydroponic tanks during infection, due to concerns on raspberry plants not reacting well to such a strong chemical compound (possible extra damages to the roots, not due to the pathogen itself, would make the phenotyping and observations of infection more difficult). Therefore, it was found that the transgenic *P. rubi* (SCRP333_tdT) was losing its fluorescence under hydroponics-selection-free conditions, after 10 to 20 dpi. In order to verify that the infection occurred and that reproduction structures were indeed produced; other staining methods were carried out. Roots samples were regularly collected through the infection course and were stained using a Trypan Blue protocol adapted from Koske and Gemma (1989). Stained roots were observed under the microscope.
Additionally, some roots were stained using calcofluor, which help locating the fluorescent *P. rubi* within the roots. Staining was performed using standard manufacturer protocol.

<u>RNA extraction from infected raspberry roots</u>

RNA of samples collected during the time-course of the infection assays will be used to give us information about *P. rubi's* transcriptome, and the expression of specific genes, determining key stages of the infection and life cycle (such as haustoria formation and biotrophic *vs* necrotrophic phases). Three methods were tested to extract sufficient yields of RNA from raspberry roots: a method adapted from the Qiagen Rneasy Plant Mini Kit, using β-mercaptoethanol and Ambion Plant RNA Isolation Aid; a TriReagent method with chloroform and isopropanol; and a CTAB method from Yu et al. (2012). Roots were collected and immediately frozen in liquid nitrogen before being stored at -80°C. Prior to extraction, mortar and pestle were autoclaved and treated with RNase Zap (from Sigma Aldrich, UK). Root samples were ground in liquid nitrogen to make 100 mg of starting material, maintaining a constant frozen state, and thus avoiding thawing, before using in the various RNA extraction

methods. Extracted RNA were assayed by NanoDrop (NanoDrop 1000 Thermo Fisher Scientific).

TriReagent RNA extraction method

The TriReagent protocol used for RNA extraction from raspberry roots was as per the Thermo Fisher Scientific protocol, using the extra steps described in “Troubleshooting: Proteoglycan and polysaccharide contamination”. Chloroform (200 µL) was added instead of BCP (1–bromo–3–chloropropane), and a mix of salt solution (0.8 M sodium citrate and 1.2 M NaCl in a total volume of 250 µL) and isopropanol (250 µL, Sigma Aldrich, UK) were used, as per instructions. The RNA pellet was resuspended in 20 µL of molecular biology grade (nuclease-free) water (Sigma Aldrich, UK).

Qiagen Rneasy Plant Mini Kit RNA extraction method

The second RNA extraction method tested was adapted from RNeasy Plant Mini Kit (QIAGEN, UK) starting with 80 to 200 mg of frozen raspberry roots and using β-mercaptoethanol (Sigma Aldrich, UK) in buffer RLT (provided in the kit), to which Ambion Plant RNA Isolation Aid (Thermo Fisher Scientific) containing polyvinylpyrrolidone (PVP) was added to remove common plant contaminants (polyphenolics and polysaccharides). The addition of buffer RW1 was split to allow the incorporation of a DNase treatment. RNA extraction was performed as per manufacturer’s instructions (RNeasy Plant Mini Kit, QIAGEN, UK) and final RNA was eluted twice in 30 µL of RNase free water.

CTAB RNA extraction (Yu et al., 2012)

A CTAB RNA extraction method from Yu et al. (2012) was tested. Frozen raspberry roots (approx. 100 to 200 mg) were ground in liquid nitrogen with 0.01 g of polyvinylpolypyrrolidone (PVPP). The frozen powder was carefully transferred to an Eppendorf (Eppendorf, UK) tube containing 700 µL of extraction buffer pre-warmed at 65 °C, comprising 3 % CTAB, 100 mM Tris-HCl at pH 8.0, 1.4 M NaCl, 20 mM EDTA, 5 % PVP and 2 µL/mL of β-mercaptoethanol that was added just before use. All following steps were carried out as described in the Yu et al. (2012) protocol. Lithium chloride (LiCl) was provided by Sigma Aldrich, UK. Ethanol washes consisted in adding 75 % ethanol and centrifuging 5 minutes at 7500 x g at room temperature twice. Final RNA was re-suspended in 30 µL of DEPC-treated (Diethyl Pyrocarbonate-treated) water.

Out of the three methods tested to extract RNA from raspberry roots, only the Yu et al. (2012) gave satisfactory yields and Nanodrop results. Quality and yields of RNA extracted using this method were considerably higher than those obtained from the two previous techniques. Consequently, RNA was extracted as per Yu et al. (2012) from infected roots at several time

points (3, 7, 11 and 14 dpi) for 3 separate time-courses (see section below on RNA extraction methods) and converted into cDNA using the Takara cDNA EcoDry Premix kit, as per manufacturer's protocol. cDNA was diluted 1:3 prior to be used in qRT-PCR reaction. Housekeeping genes Cox I and betatubulin were used as endogenous controls to normalize the CT data and obtain relative expression. cDNA from SCRP333_tdT mycelia constituted the control sample to compare levels of expression to.

qRT-PCR for expression of life markers during infection

Life markers for *Phytophthora* sporulation (CDC14) and haustoria (HMP1) were retrieved from NCBI through a BLASTp search in *P. rubi* SCRP333 genome of equivalent proteins to those of *P. infestans* (for CDC14 accession AY204881.1 and for HMP1 – haustoria membrane protein - accession EU680858.1). Corresponding genes were retrieved, and primers designed using Primer 3 and Netprimer, using criteria described in previous annual reports. Primers (Table 2) were tested *in silico* first using Geneious v2020.2.1. for amplifying only the region of interest in SCRP333 and not amplifying regions of the raspberry genome, which was then confirmed through conventional PCR where they were tested against SCRP333, non-infected raspberry cDNA and water control, along with 2% agarose gels (ran at 80V for 1 hour). Primers were used in a final concentration of 300nM for a final reaction volume of 25µL in a qRT-PCR assay.

Table 2: Primers used in the qRT-PCR assay screening for expression of life markers during infection

Gene of Interest	Reason for interest	primers names	primers sequences 5'-3'	product size (bp)
CDC14	sporulation marker	qRT_Pr_CDC14_F1	GCACGTTTAATCTGACCATCTTG	86
		qRT_Pr_CDC14_R1	GTCGAACGTCTTGATGGAGATG	
HMP1	haustoria marker	SCRP333_HMP1.2_F	GGTTGGTCAGCGTCTTCATC	196
		SCRP333_HMP1.2_R	GTTGTGTCCGCCATTGTCAT	
CoxI	Endogenous controls	coxIAB_F	GGGCGCATCACATGTTTACT	127
		coxIAB_R	CCTCCCCATAAAGTTGCTAACC	
betatubulin		BetatubAB_F	AGCACGAAGGAGGTTGATGA	215
		BetatubAB_R	GCCTTACGACGGAACATAGC	

Results

P. rubi phenotypic diversity study: responses to temperatures and chemicals

Effect of temperature on in vitro P. rubi life cycle stages: mycelial development and sporulation

Isolates of *P. rubi* and *P. fragariae* were grown at several temperatures, 15°C, 18°C (control), 21°C, 25°C and 28°C.

Statistical analyses showed that the *P. rubi* mycelial development increased from 15°C to 18°C and to 21°C but decreases at 25°C and is fully inhibited at 28°C (Figure 3).

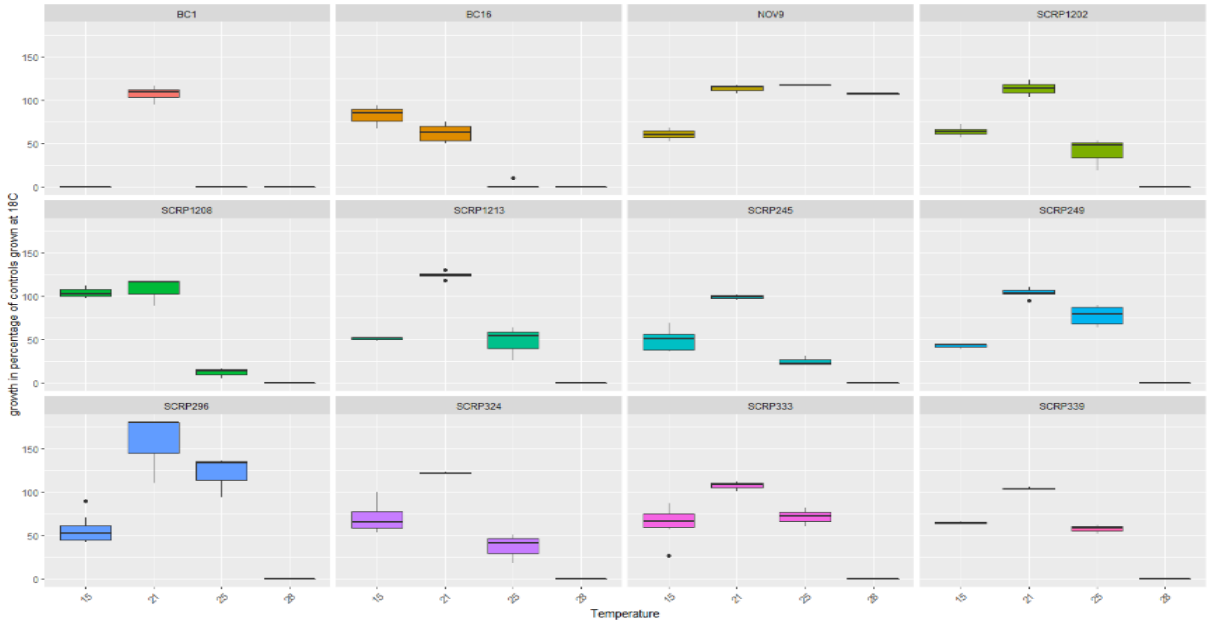


Figure 1: Boxplot representation of the effect of temperatures (x axis: 15°C, 21°C, 25°C and 28°C) on the mycelial growth of 12 isolates: four *P. fragariae* and eight *P. rubi* isolates, expressed in percentage of controls (y axis: grown at 18°C). Three replicates per isolate and temperature were used. Each temperature experiment was performed once. Total samples number = 180.

P. rubi sporulation was successful and ANOVA and Tukey's HSD tests ($p<0.05$) showed that isolate sporulation was statistically similar in efficiency at all temperatures checked (Figure 4).

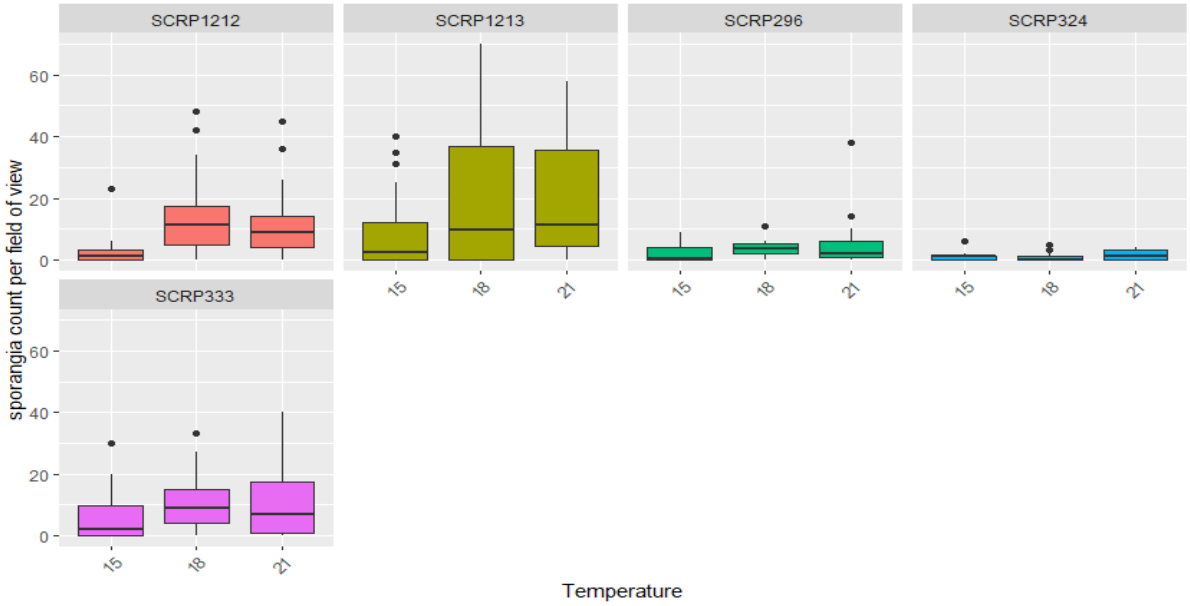


Figure 2: Boxplot representation of the effect of temperatures (x axis: 15°C, 18°C and 21°C) on the sporulation of five *P. rubi* isolates. The vertical axis represents the number of sporangia per field of view. Twenty replicates per isolate and temperature were used, as 10 mycelial plugs in two plates of sporulation solution. Each temperature experiment was performed once. Total samples number = 300.

Even though the effect of the temperature is significant on the development of *P. rubi* mycelia, it was not the case for the sporangia development across the temperature range tested. Wilcox and Latorre (Wilcox and Latorre, 2002) found similar patterns for *P. rubi* mycelial growth rising at temperatures up to 25°C and reducing at higher temperatures, though no differences amongst isolates were examined.

In vitro inhibition of P. rubi and P. fragariae mycelial growth greatly depends on the chemical incorporated

The chemical screening study highlights both inter and intra-species diversity. Seven chemicals were tested: Fluazinam, active ingredient of Shirlan and Tizca; Fluopicolide and Propamocarb, used in Infinito; Ametoctradin, active ingredient of fungicides such as Initium or Zampro; Phorce, a phosphite-based fertiliser; Dimethomorph, active ingredient of Paraat; and Metalaxyl-M, used in SL 567A. Smaller dose of newer compounds is required to achieve satisfactory inhibition. Figure 5 and 6 illustrates differences in mycelial growth inhibition of *P. rubi* and *P. fragariae* isolates between all the chemicals. Fluazinam (Figure 7) and Fluopicolide (Figure 8) treatments lead to a decreased mycelial growth with an increasing dose, showing sensitivity of the *P. rubi* and *P. fragariae* isolates to the chemicals, with *P. fragariae* generally more sensitive to Fluopicolide than *P. rubi*.

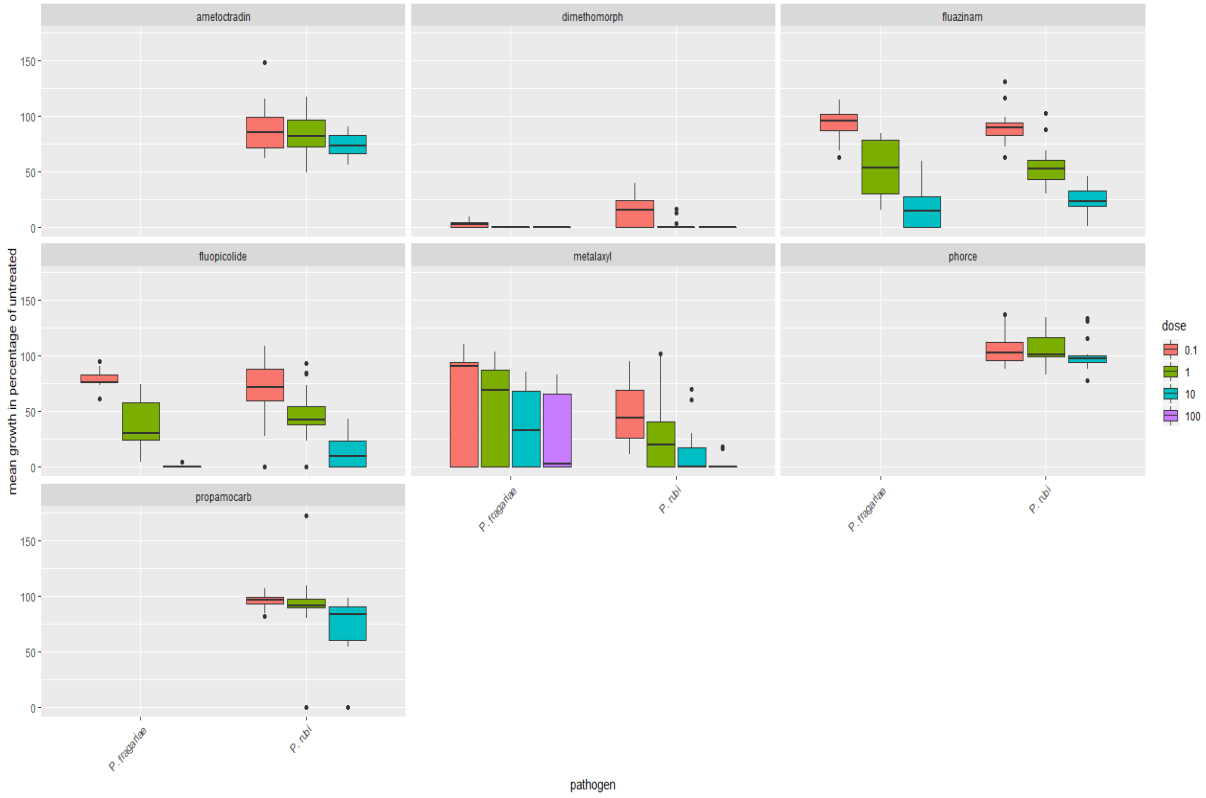


Figure 3: Boxplot representation of the effect of incorporated chemicals on the mycelial diameter (expressed in percentage of controls, y axis) of all isolates of *P. rubi* and *P. fragariae* (x axis). Three replicates per isolate and chemical were used. Each chemical experiment was performed once except for Metalaxyl-M, which was performed three times. Total samples number = 324. Results are presented per chemical and dose (in ppm).

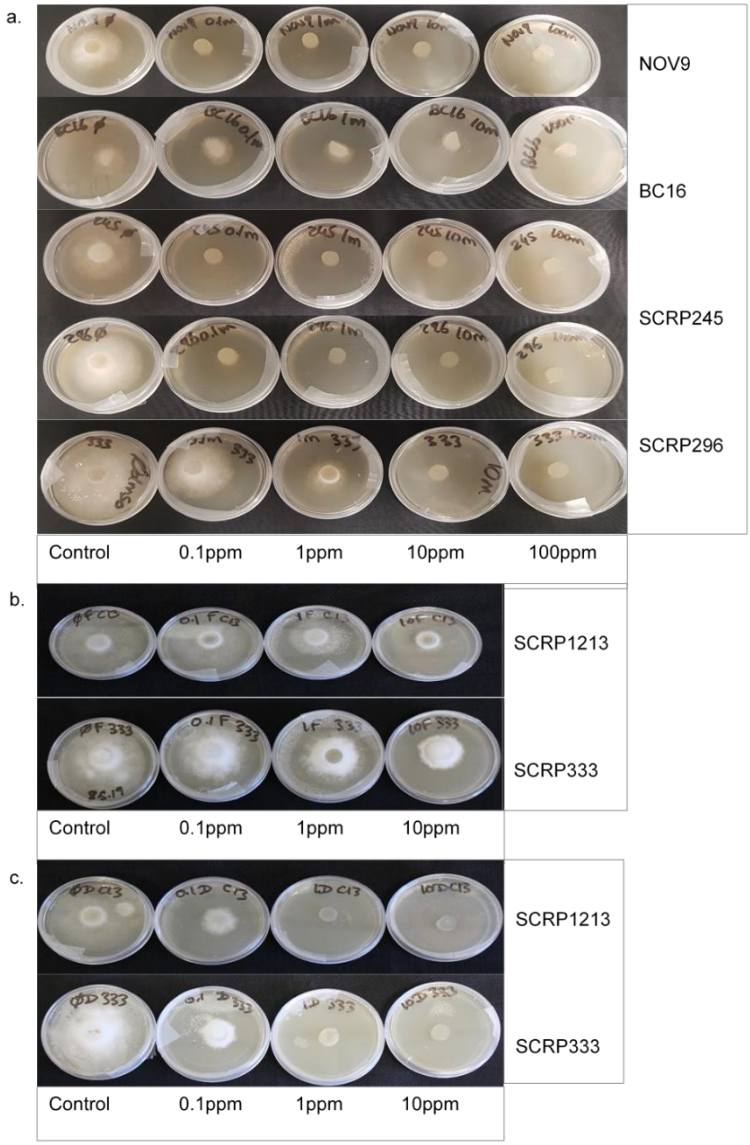


Figure 4: Photos of plates incorporating a. Metalaxyl-M, b. Fluazinam and c. Dimethomorph for several isolates at 0 ppm (controls), 0.1 ppm, 1 ppm, 10 ppm and 100 ppm (Metalaxyl-M).

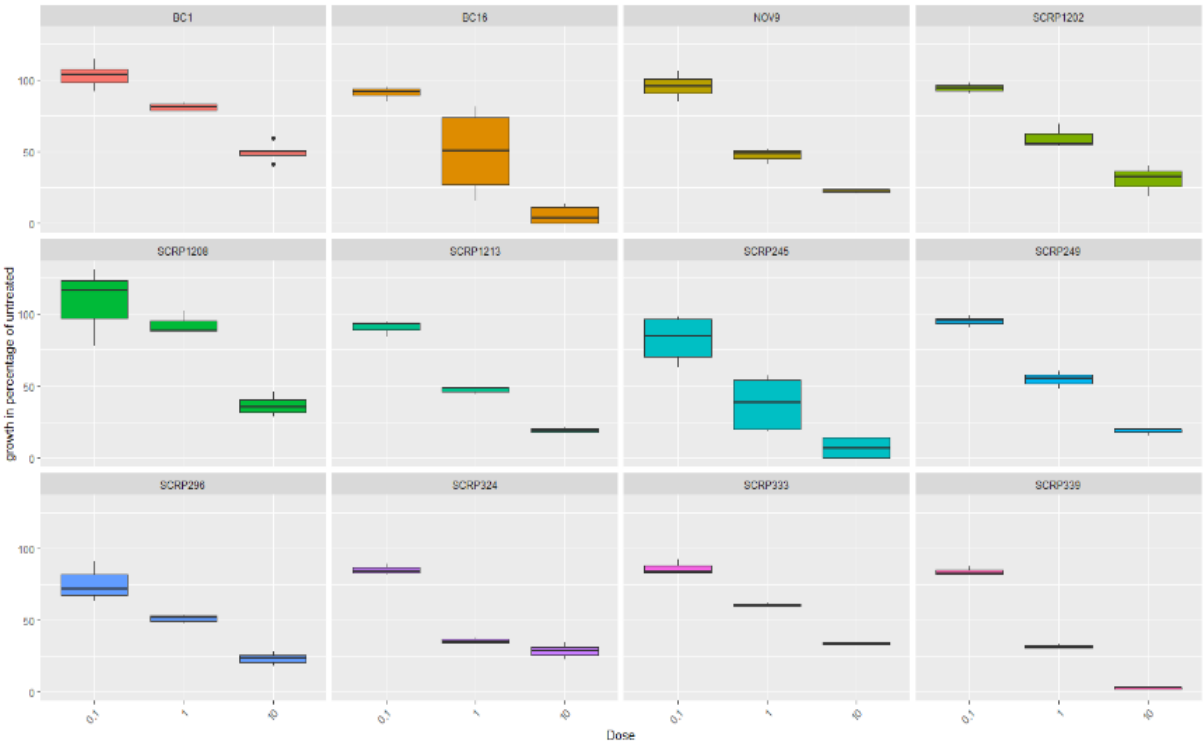


Figure 5: Boxplot representation of the effect of Fluazinam incorporated at different doses (0.1ppm, 1ppm and 10ppm, x axis) on the mycelial diameter (expressed in percentage of controls, y axis) of eight P. rubi and four P. fragariae isolates. Three replicates per isolate and chemical were used and experiment was performed once. Total samples number = 36.

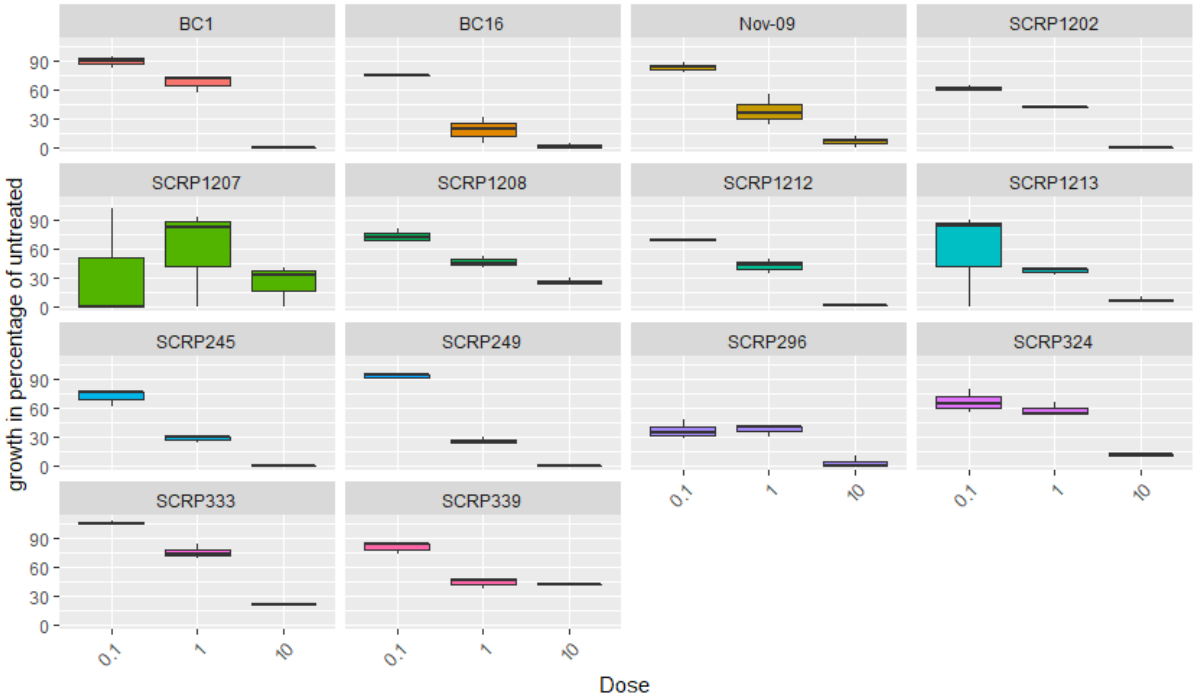


Figure 6: Boxplot representation of the effect of Fluopicolide incorporated at different doses (0.1ppm, 1ppm and 10ppm, x axis) on the mycelial diameter (expressed in percentage of controls, y axis) of eight P. rubi and four P. fragariae isolates. Three replicates per isolate and chemical were used and experiment was performed once. Total samples number = 42.

Propamocarb (Figure 9), Ametoctradin (Figure 10), and Phorce (Figure 11) display very little to no decrease of the mycelial growth with an increasing dose, indicating a lack of sufficient inhibition to growers' standards. Perhaps higher doses, as those used in experiments by Löchel and Birchmore (1990) or Samoucha and Cohen (1990), of the chemicals displaying resistance amongst *P. rubi* and *P. fragariae* isolates could lead to reduced mycelial development, although most of these fungicides field application doses are set around 1 ppm, except for Phorce, used as a fertiliser.

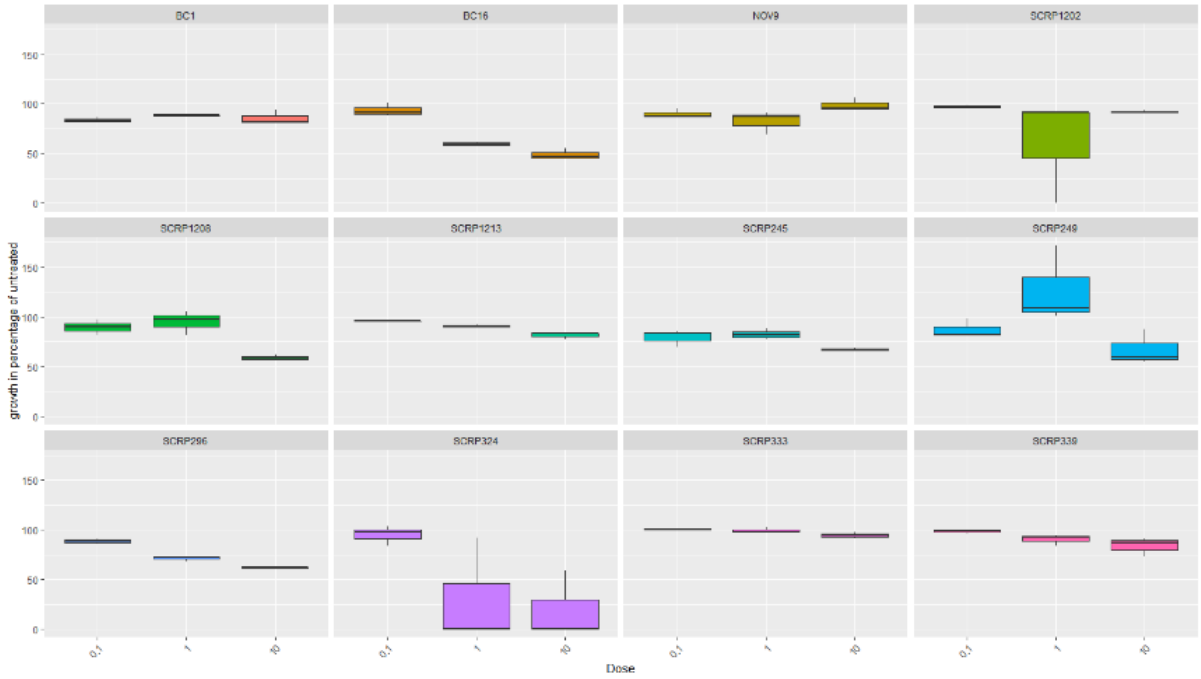


Figure 7: Boxplot representation of the effect of Propamocarb incorporated at different doses (0.1ppm, 1ppm and 10ppm, x axis) on the mycelial diameter (expressed in percentage of controls, y axis) of eight P. rubi and four P. fragariae isolates. Three replicates per isolate and chemical were used and experiment was performed once. Total samples number = 36.

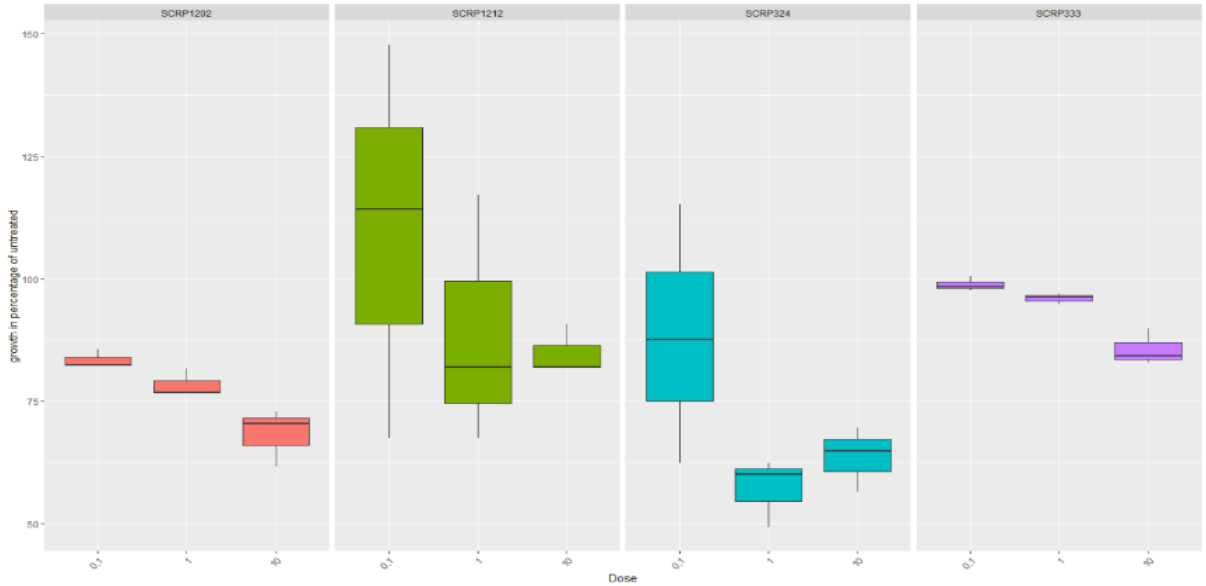


Figure 9: Boxplot representation of the effect of Ametoctradin incorporated at different doses (0.1ppm, 1ppm and 10ppm, x axis) on the mycelial diameter (expressed in percentage of controls, y axis) of four P. rubi isolates. Three replicates per isolate and chemical were used and experiment was performed once. Total samples number = 12.

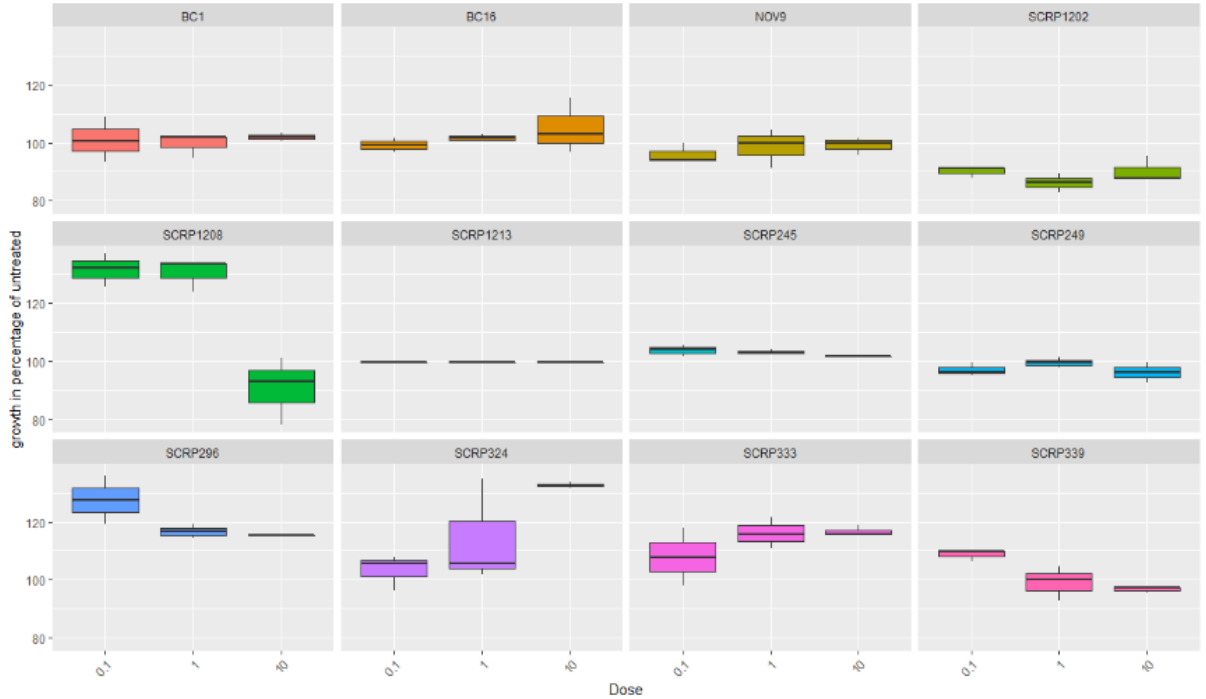


Figure 8: Boxplot representation of the effect of Phorce incorporated at different doses (0.1ppm, 1ppm and 10ppm, x axis) on the mycelial diameter (expressed in percentage of controls, y axis) of eight P. rubi and four P. fragariae isolates. Three replicates per isolate and chemical were used and

Dimethomorph (Figure 12) exhibits the best inhibitory effect on the mycelial growth of the isolates tested, to an adequate level as the average EC50 was close to the field application dose.

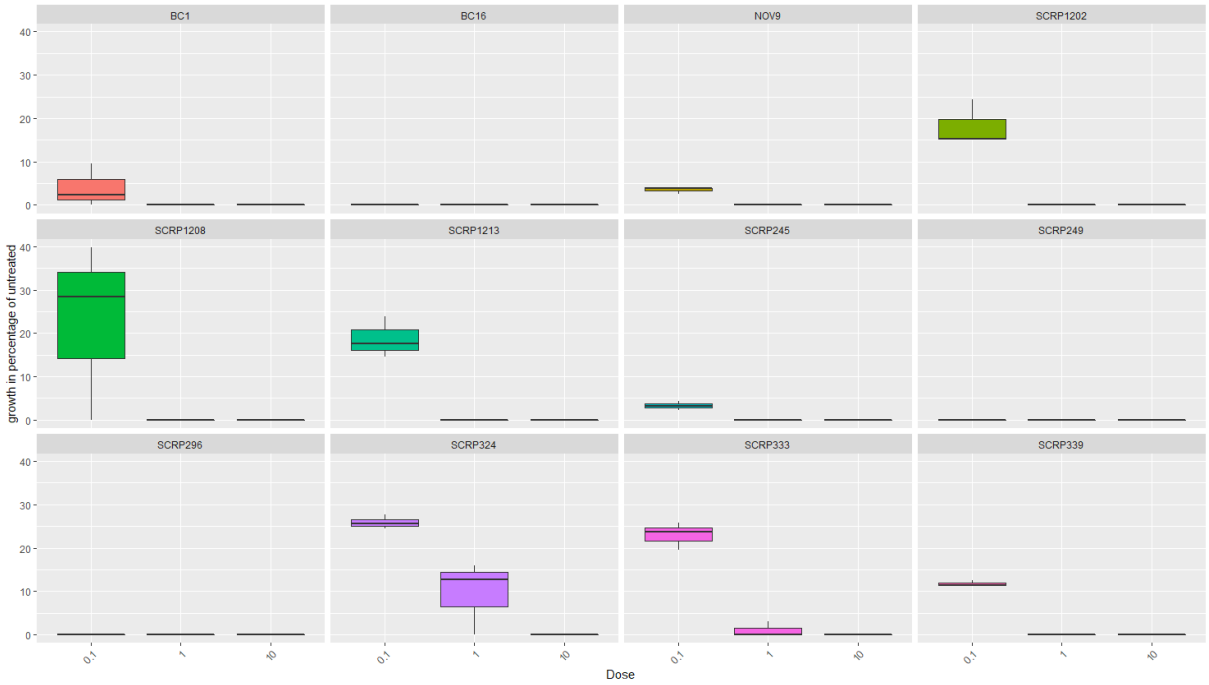


Figure 102: Boxplot representation of the effect of Dimethomorph incorporated at different doses (0.1ppm, 1ppm and 10ppm, x axis) on the mycelial diameter (expressed in percentage of controls, y axis) of eight P. rubi and four P. fragariae isolates. Three replicates per isolate and chemical were used and experiment was performed once. Total samples number = 36.

Metalaxyl-M (Figure 13) incorporation in growing medium presents a variety of responses depending on the isolate screened, with both inhibition under high doses of the chemical and resistance, where isolates grow with no statistical difference compared to controls, as previously reported by numerous studies on *Phytophthora* species, such as *P. fragariae* (Nickerson, 1998), *P. infestans* (Elansky et al., 2007; Zhu et al., 2008; Pérez et al., 2009; Rekanovic et al., 2012; Randall et al., 2014), *P. capsici* (Parra and Ristaino, 2001; Qi et al., 2012) and *P. palmivora* (Vawdrey et al., 2004).

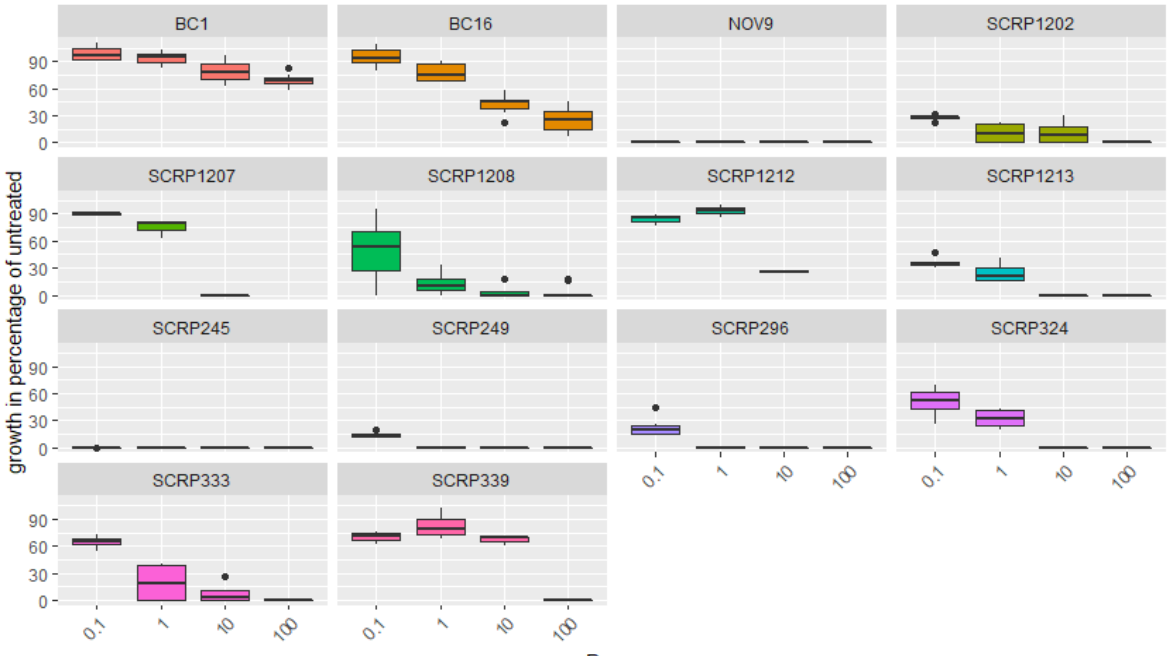


Figure 113: Boxplot representation of the effect of Metalaxyl-M incorporated at different doses (0.1ppm, 1ppm and 10ppm, x axis) on the mycelial diameter (expressed in percentage of controls, y axis) of eight P. rubi and four P. fragariae isolates (x axis). Three replicates per isolate and chemical were used and experiment was performed once. Total samples number = 126.

Elansky et al. (2007), Zhu et al. (2008) and Rekanovic et al. (2012) also observed very little or no resistance to Dimethomorph, further confirming these findings. Pesticides usage surveys validate those observations, showing a decrease in use of Fluazinam (from 44 % in 2012 to 10 % in 2016), a low use of Metalaxyl-M (1 % in 2012 and 2 % in 2018) and an increased in use of Dimethomorph (2 % in 2012 and 28 % in 2018). This chemical study emphasizes on the potential for Dimethomorph (Paraat) and Fluazinam (Tizcan), which are still allowed for use on raspberries and strawberries, to control *P. rubi* and *P. fragariae* mycelial growth at a dose that is applicable in the field. While pesticides use is decreasing, biocontrol and integrated management are strategies that are additionally investigated (Wilcox et al., 1999; Anandhakumar and Zeller, 2008).

Dimethomorph and Metalaxyl-M incorporation in growing media lead to different responses and sensitivity, hence those chemical target genes were investigated for SNPs and resulting amino acid changes which could potentially explain resistance. During the Dimethomorph screening, diversity in *P. rubi* responses showed that SCRP324 and SCRP333 could grow at 1ppm, whereas no other isolate did. Cellulose synthase (CesA) nucleotide sequences were retrieved for SCRP249, SCRP324 and SCRP333. CesA1 sequences showed one amino acid difference, however this was between SCRP324 and SCRP249/SCRP333, thus not clustering the isolates with resistance observed to the chemical. CesA2 and CesA4 sequences alignment did not show any amino acid differences between the sequences of those *P. rubi* isolates. CesA3 sequences alignment showed one amino acid change, but this clustered SCRP324 with SCRP249, and did not explain the phenotypic difference in

resistance. Similarly, Metalaxyl-M screening lead to resistance for two *P. fragariae* isolates: BC-1 and BC-16, which grew at 100ppm; while isolates SCRP245 and NOV-9 did not. RPABC23, RPABC27, RPA190, RPA135, RPAC19 and RPA12.2 sequences were retrieved for those four *P. fragariae* isolates, translated into proteins and aligned. Only RPAC19 showed amino acid differences between the sequences, though resulting in a different clustering than the resistance observed.

Consequently, the phenotypic resistances and diverse responses of isolates to dimethomorph and Metalaxyl-M could not be explained with the genes tested in this study. Most chemicals for these pathogens are not well understood, making conclusions from this data difficult at present.

P. rubi and *P. fragariae* genotypic diversity study: Pathogen Enrichment Sequencing (PenSeq)

Same-species mapping and coverage analyses

- Same-species mapping & coverage tables for several mismatch mapping rate

Filtering resulted in only 2% of reads filtered out and in a final number of 13 615 457 reads, of which 9 343 998 were for *P. rubi* and 4 271 459 for *P. fragariae*, corresponding respectively to ~8/12th and 4/12th of the total reads, as expected. Mapping of baits to the reference genomes resulted in 14958 contigs for *P. fragariae* and 14295 contigs for *P. rubi.* Average mapping for all eight *P. rubi* isolates at 0% mismatch mapping rate was 64%, meaning that on average for *P. rubi* isolates screened in this study, 64% of the reads pulled with baits were successfully mapped to a reference *P. rubi* genome (SCRP333) when no polymorphism was allowed (Table 3). Similarly, average mapping for the four *P. fragariae* isolates at 0% mismatch rate was 74%, a higher percentage due to less isolates screened, and thus less polymorphism leading to more reads mapped to the reference genome (BC-16). At 0% mismatch mapping rate, these mapping rates are expected, and similar to previous studies (Thilliez et al., 2019). At 3% mismatch mapping rate, which allows SNPs to be picked up and thus corresponding nucleotide to be successfully mapped to the reference genomes, *P. fragariae* isolates were mapped on average at 92% and *P. rubi* at 90%. While percentages of mapping will depend on the isolates screened and the reference genome, as the more different they are expected to be, the less reads from the PenSeq sequencing of the isolate of interest will be mapped to the reference genome; our PenSeq analysis (baits library) mostly included effectors, which are fast evolving genes, therefore usually diverse and showing polymorphism between isolates. Consequently, the percentages observed, still leaving reads unmapped, are to be expected.

Although isolates that were used for reference, SCRP333 and BC-16, showed the highest mapping percentages at the different mismatch rates, those were surprisingly below 100% mapping against genomes obtained from sequencing of the same strains. This has previously been observed for *P. infestans* (Thilliez et al., 2019), and it was validated by PCR that the missing genes were unable to be amplified, confirming their absence in the strain sequenced with PenSeq. This suggests that our reference isolates SCRP333 and BC-16 have lost some genes during permanent culturing on Rye agar. Reference isolate SCRP333 was provided by the James Hutton Institute to NIAB-EMR for whole genome sequencing; the genome sequence could therefore represent multiple strains of SCRP333 from different labs.

Table 3: Percentage and total reads mapped to reference genomes for 12 isolates, 8 *P. rubi* and 4 *P. fragariae* at several Bowtie mismatch mapping rates

Species	Isolate	Reads count	Reference mapped to	Bowtie mismatch mapping rate (%)	Total reads mapped	Percentage of reads mapped (%)
P. rubi	SCRP1208	874620	*P. rubi*	0	505104	57.75
P. rubi	SCRP1208	874620	SCRP333	3	787600	90.05
P. rubi	SCRP1208	874620		5	806453	92.21
P. rubi	SCRP1208	874620		10	827587	94.62
P. rubi	SCRP1213	1131707		0	756624	66.86
P. rubi	SCRP1213	1131707		3	1017021	89.87
P. rubi	SCRP1213	1131707		5	1038063	91.73
P. rubi	SCRP1213	1131707		10	1062812	93.91
P. rubi	SCRP1202	1121686		0	767144	68.39
P. rubi	SCRP1202	1121686		3	1016184	90.59
P. rubi	SCRP1202	1121686		5	1037037	92.45
P. rubi	SCRP1202	1121686		10	1060915	94.58
P. rubi	SCRP249	1499947		0	912265	60.82
P. rubi	SCRP249	1499947		3	1327752	88.52
P. rubi	SCRP249	1499947		5	1361592	90.78
P. rubi	SCRP249	1499947		10	1399576	93.31
P. rubi	SCRP296	1117692		0	686270	61.40
P. rubi	SCRP296	1117692		3	995634	89.08
P. rubi	SCRP296	1117692		5	1020467	91.30
P. rubi	SCRP296	1117692		10	1049132	93.87
P. rubi	SCRP324	1021039		0	597245	58.49
P. rubi	SCRP324	1021039		3	910019	89.13
P. rubi	SCRP324	1021039		5	933517	91.43
P. rubi	SCRP324	1021039		10	959583	93.98
P. rubi	SCRP333	1095654		0	762182	69.56

Species	Isolate	Reads count	Reference mapped to	Bowtie mismatch mapping rate (%)	Total reads mapped	Percentage of reads mapped (%)
P. rubi	SCRP333	1095654		3	991193	90.47
P. rubi	SCRP333	1095654		5	1010260	92.21
P. rubi	SCRP333	1095654		10	1032560	94.24
P. rubi	SCRP339	1481653		0	1015305	68.53
P. rubi	SCRP339	1481653		3	1334224	90.05
P. rubi	SCRP339	1481653		5	1359914	91.78
P. rubi	SCRP339	1481653		10	1390533	93.85
P. rubi total		9343998		0	6002137	64.24
				3	8379625	89.68
				5	8567302	91.69
				10	8782697	93.99
P. fragariae	BC-16	1120168	*P.*	0	864193	77.15
P. fragariae	BC-16	1120168	*fragariae*	3	1038335	92.69
P. fragariae	BC-16	1120168	*BC-16*	5	1047243	93.49
P. fragariae	BC-16	1120168		10	1056198	94.29
P. fragariae	BC-1	1182473		0	886729	74.99
P. fragariae	BC-1	1182473		3	1077333	91.11
P. fragariae	BC-1	1182473		5	1086799	91.91
P. fragariae	BC-1	1182473		10	1095851	92.67
P. fragariae	NOV-9	865044		0	667024	77.11
P. fragariae	NOV-9	865044		3	784500	90.69
P. fragariae	NOV-9	865044		5	790774	91.41
P. fragariae	NOV-9	865044		10	797659	92.21
P. fragariae	SCRP245	1103774		0	753190	68.24
P. fragariae	SCRP245	1103774		3	1017155	92.15
P. fragariae	SCRP245	1103774		5	1029044	93.23
P. fragariae	SCRP245	1103774		10	1040461	94.26
P. fragariae total		4271459		0	3171136	74.24
				3	3917322	91.71
				5	3953860	92.56
				10	3990169	93.41

Reads were mapped to the genes (here in contigs) of the reference genomes at different mismatch mapping rates, with several reads mapping per gene (overlapping reads), and the overlap of the reads gave consensus sequences for each of the ~ 15 000 genes. This essential step translates our reads for each isolate into genes from the reference genomes. The number of nucleotides covered from the consensus sequence - out of the total number

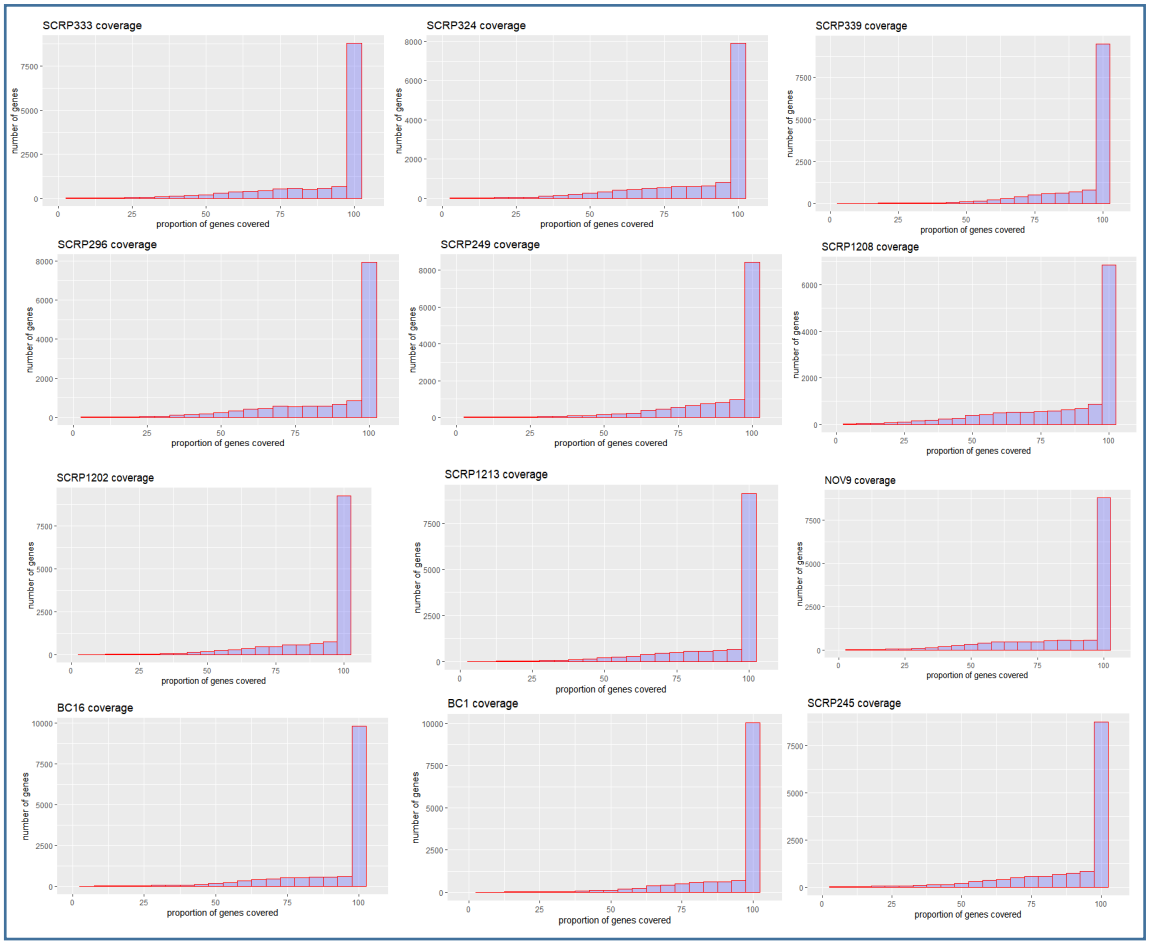
of nucleotides from the reference gene were assessed, and percentages were calculated (Table 4 and Figure 14).

Mapping and coverage are essential steps for PenSeq analyses, not only to assess the bait library design quality and the annotation of the genomes used for references (Thilliez et al., 2019), but they allow us to carry out further diversity analyses, both inter and intra-species.

Table 4: Coverage (in percentage) of the ~15 000 genes mapped at several Bowtie mismatch mapping rates

Species	**Isolate**	**Reference mapped to**	**Percentage of coverage (average for all contigs)**			
			0%mm	**3%mm**	**5%mm**	**Average per isolate**
P. fragariae	BC-16	*P. fragariae* BC-16	90.87	91.42	91.5	91.26
P. fragariae	BC-1		89.58	92.73	92.81	91.71
P. fragariae	NOV-9		85.32	87.15	87.23	86.57
P. fragariae	SCRP245		87.16	92.01	92.17	90.44
P. fragariae	**average per mm rate**		**88.23**	**90.83**	**90.93**	**90.00**
P. rubi	SCRP1208	*P. rubi* SCRP333	88.93	87.79	87.96	88.23
P. rubi	SCRP1213		85.52	91.80	91.89	89.74
P. rubi	SCRP1202		84.91	92.28	92.35	89.85
P. rubi	SCRP249		87.69	93.81	93.96	91.82
P. rubi	SCRP296		91.71	90.46	90.61	90.93
P. rubi	SCRP324		90.05	90.79	90.9	90.58
P. rubi	SCRP333		80.64	90.24	90.34	87.07
P. rubi	SCRP339		89.23	93.77	93.85	92.28
P. rubi	**average per mm rate**		**87.34**	**91.37**	**91.48**	**90.06**

a.



b.

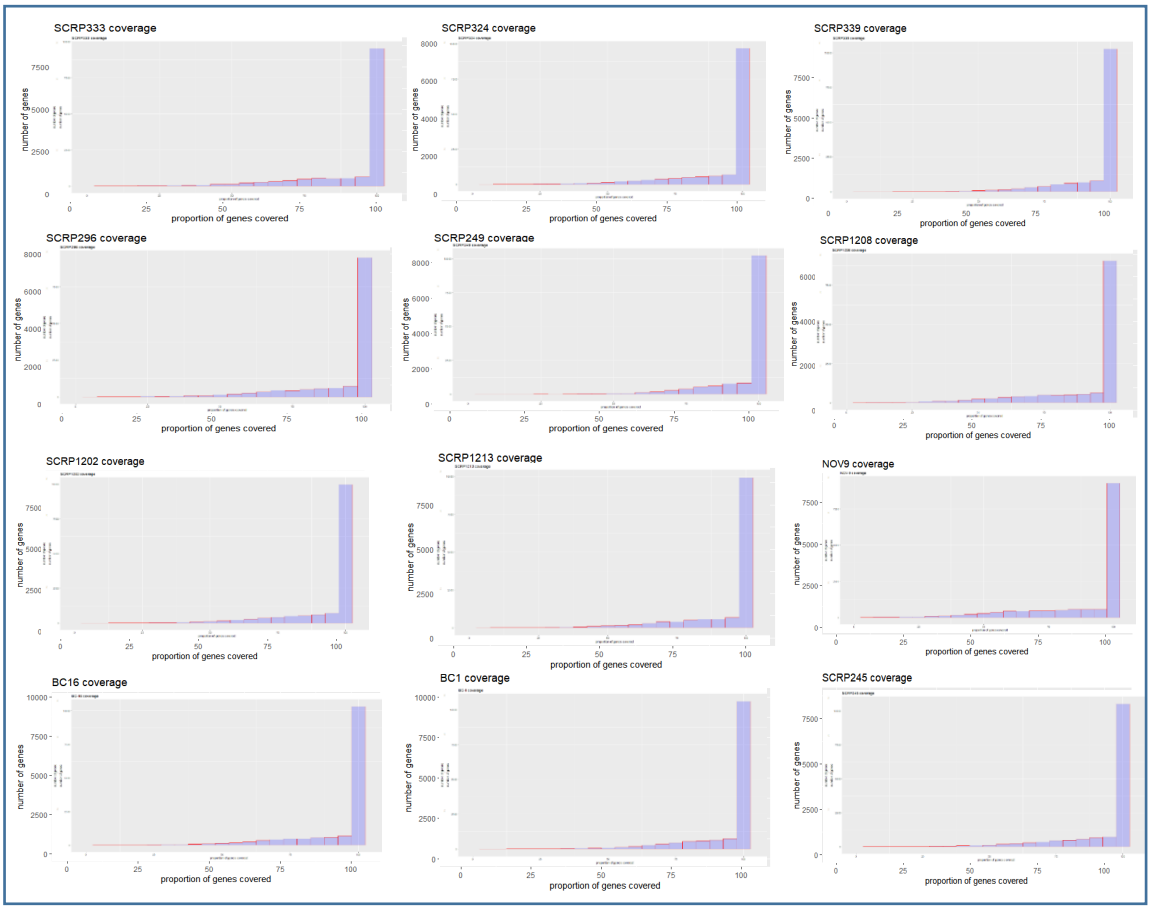


Figure 14: Coverage graphs for the 12 isolates used in the PenSeq study at a. 0% mismatch mapping rate and b. 3% mismatch mapping rate. X-axis represents the proportion of gene covered and the y-axis represents the number of genes showing that coverage

The coverage analysis allowed us to screen for genes with SNPs, as genes showing a coverage below 100% indicate a gap in the consensus sequence made by overlapping reads, due to some reads not mapping to the reference genome because of too many different nucleotides (at 0% mismatch mapping rate, any nucleotide different than the reference would result in the read not being successfully mapped; at 3% mismatch mapping rate, 3 or more nucleotide out of 100 different than the reference would result in the read not being successfully mapped to that reference).

Therefore, looking at genes coverage at 0% mismatch mapping rate show potential SNPs for isolates with less than 100% coverage. This allowed various analyses to be carried out: focussing on housekeeping genes, usually conserved, and screening for SNPs (<100% coverage) as well as resulting amino acid changes per species; very conserved genes inter and intra-species (genes showing 100% coverage at 0% mismatch mapping rate); an overall SNP calling, looking at all SNPs on all the ~15 000 genes for all isolates, designating most diverse genes within species; as well as any diversity in selected genes of interest (looking at coverage at different mismatch mapping rate of the genes of interest for all isolates, identifying SNPs and resulting amino acid changes). Results of such analyses are detailed below.

- Housekeeping / Drug target genes diversity

Twenty-two housekeeping genes were studied and included in the PenSeq study (Table 5).

Table 5: Housekeeping genes selected for the PenSeq genetic diversity study

Genes names/codes	Genes
RPABC27	RNA polymerase subunits
RPABC23	
CesA2	cellulose synthase A2
betaglucosidase	betaglucosidase
EPIC3	cystatin-like cysteine protease inhibitor
GIP2	glucanase inhibitor protein 2
INF1	elicitin INF1
AE1.1	acidic elicitin
MFT	major facilitator transporter
G3PDH	glyceraldehyde-3-phosphate dehydrogenase
60S_L10	60S ribosomal protein L10
28S.1	28S ribosomal DNA
betatubulin	betatubulin
elongation_factor1_alpha	elongation factor1 alpha
enolase	enolase
HSP90	heat shock protein 90
ras-like_ypt1	ras-like ypt1

coxI	cytochrome oxidase I
antisilencing_factor	antisilencing factor
GPA1	G-protein alpha
TRP1	indole-3-glycerol-phosphate synthase N-5'-phosphoribosyl anthranilate isomerase
nad9	NAD9 NADH dehydrogenase subunit 9

Figures 15 and 16 show coverage graphs for each of the housekeeping genes for *P. fragariae* and *P. rubi* respectively, at 0% mismatch mapping rates. Genes selected (in blue on Figures 15 and 16) show potential SNPs, by absence of mapping against the reference genome at 0% mismatch mapping rate (identified by a “dip”).

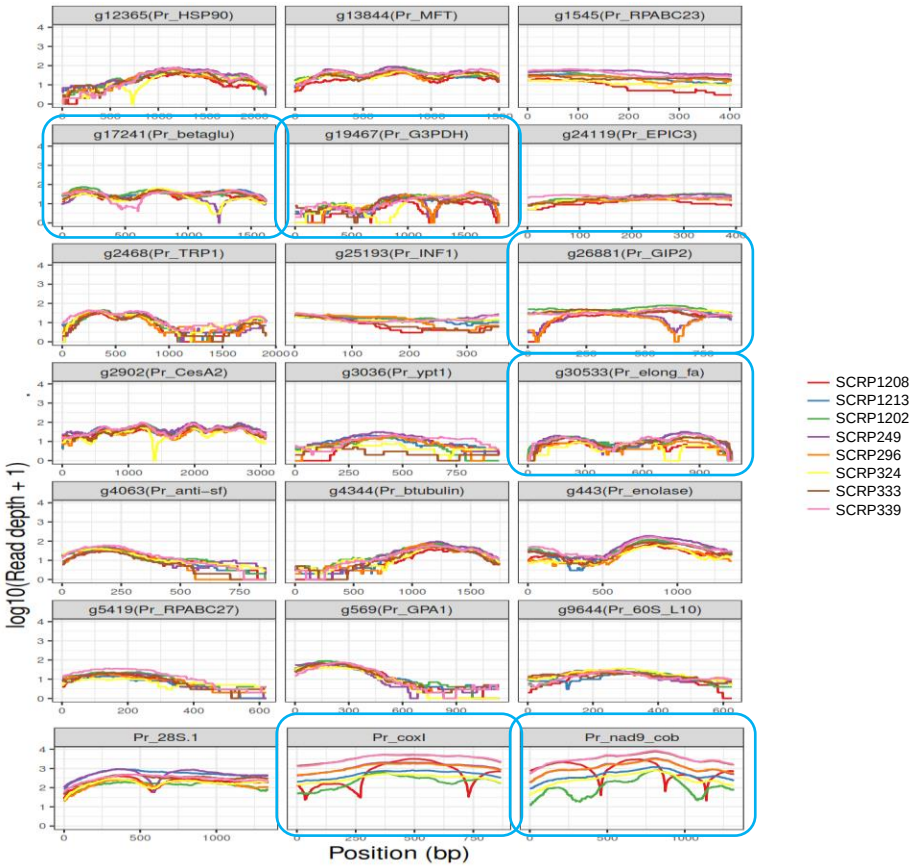


Figure 15: Coverage graphs for housekeeping genes for the 8 P. rubi isolates used in the PenSeq study at 0% mismatch mapping rate. X-axis represents the gene length and position (bp) covered and the y-axis represents the read depth (in log scale). Generally, these graphs show the mapping of each nucleotide position on the gene of interest.

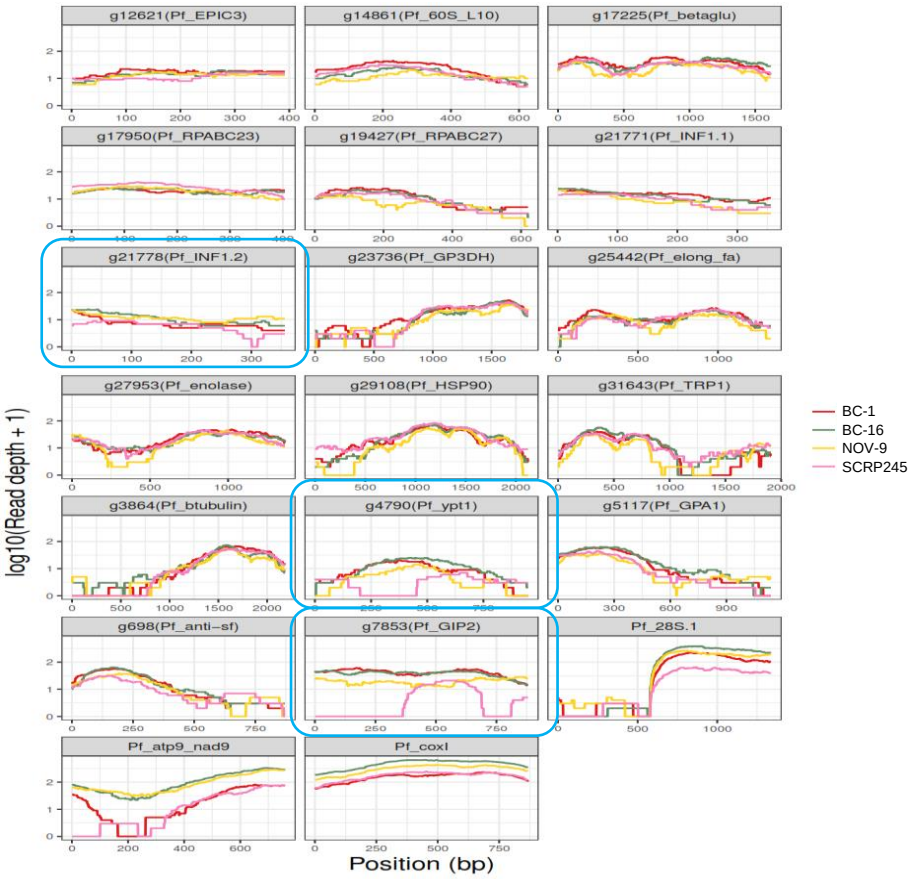


Figure 16: Coverage graphs for housekeeping genes for the 4 P. fragariae isolates used in the PenSeq study at 0% mismatch mapping rate. X-axis represents the gene length and position (bp) covered and the y-axis represents the read depth (in log scale). Generally, these graphs show the mapping of each nucleotide position on the gene of interest.

Those SNPs were further investigated as described in the Materials and methods section, by extracting the corresponding gene sequences and aligning them. *P. fragariae* housekeeping genes coverage graphs identified potential SNPs for 7 genes: Ypt1, GIP2, INF1.2, G3PDH, TRP1, HSP90, INF1.1; and nucleotide polymorphism was confirmed for 3 of these genes: Ypt1, GIP2 and INF1.2 with the corresponding extracted sequences. SNPs were always found in SCRP245, the only English isolate. Nucleotide sequences were translated into proteins as described in the Materials and methods section: Ypt1 gene showed 2 SNPs with 1 change in amino acid, INF1.2 gene showed 1 SNP with no amino acid change and GIP2 showed 7 SNPs, leading to at least 4 amino acid changes (Figure 17).

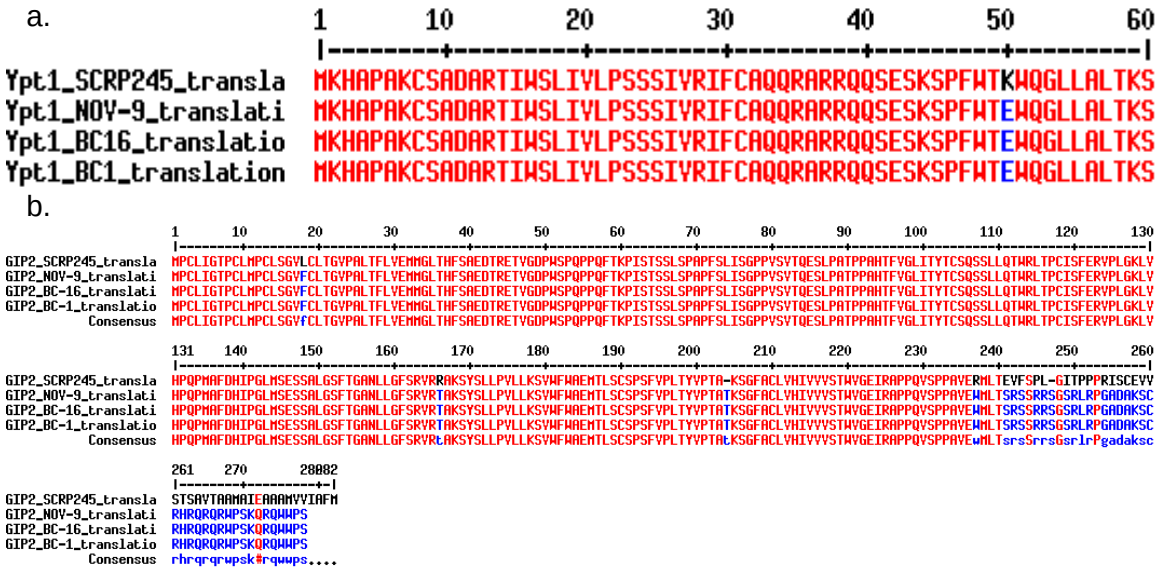


Figure 17: Alignment of proteins a. Ypt1 and b. GIP2 (glucanase inhibitor protein 2) for 4 P. fragariae isolates, showing amino acid changes and polymorphism amongst isolates for these 2 housekeeping genes. Alignments were carried out using the MultAlin tool (Corpet, 1988).

P. rubi housekeeping genes coverage graphs identified potential SNPs for 8 genes: betaglucosidase, HSP90 (heat shock protein 90), G3PDH (glyceraldehyde-3-phosphate dehydrogenase), GIP2 (glucanase inhibitor protein 2), CesA2 (cellulose synthase A2), elongation factor 1 alpha, Cox I (cytochrome oxidase I) and nad9 (NAD9 NADH dehydrogenase subunit 9) and polymorphism was confirmed for 6 of those: betaglucosidase, G3PDH, GIP2, elongation factor 1 alpha, Cox I and nad9. Betaglucosidase gene showed 1 SNP, not leading to changes in amino acid sequences; G3PDH was found to be a heterozygous gene, with 2 version of the gene, differing with 11 SNPs; GIP2 gene showed 1 SNP with 6 nucleotide added for isolate SCRP249 and SCRP296, resulting in 4 amino acid added to the protein sequences; the elongation factor 1 alpha gene was heterozygous for isolate SCRP324; the Cox I gene for isolate SCRP1208 differed at 3 SNPs, none of which resulted in an amino acid change; finally the Nad9 gene showed 3 SNPs leading to 3 amino acid changes (Figure 18).

a.

```
                1       10        20        30        40        50        60        70        80
                |--------+---------+---------+---------+---------+---------+---------+---------+-----
GIP2_SCRP249_transla  LAARRARHALLNRNSLLDALLVRSALLDRGARIDLLGRDDGVNPLRRGHTRVDGRGSLVTAAIAPVHQTDQHVVLVSRAISLDQR
GIP2_SCRP296_transla  LAARRARHALLNRNSLLDALLVRSALLDRGARIDLLGRDDGVNPLRRGHTRVDGRGSLVTAAIAPVHQTDQHVVLVSRAISLDQR
GIP2_SCRP333_transla  LAARRARHALLNRNSLLDALLVRSALLDRGARIDLLGRDDGVNPLRRGHTRVDGRGSLVTAAIAPVHQTDQHVVLVSRAIFLDQR
          Consensus   LAARRARHALLNRNSLLDALLVRSALLDRGARIDLLGRDDGVNPLRRGHTRVDGRGSLVTAAIAPVHQTDQHVVLVSRAIsLDQR

                131     140       150       160       170       180       190       200       210
                |--------+---------+---------+---------+---------+---------+---------+---------+-----
GIP2_SCRP249_transla  EGAVGEAGPSPAHGFRPHTRLDVRVVSLGQLHGGELTGLLQSQKSEVILTLAGLVEVGVVLGRDDLELLAVLRAVDIVRADRNEV
GIP2_SCRP296_transla  EGAVGEAGPSPAHGFRPHTRLDVRVVSLGQLHGGELTGLLQSQKSEVILTLAGLVEVGVVLGRDDLELLAVLRAVDIVRADRNEV
GIP2_SCRP333_transla  EGAVGEAGPSPAHGFRPHTRLDVRVVSLGQLHGGELTGLLQSQKSEVILTLAGLVEVGVVLGRDDLELLAVLRAVDIVRADRNEV
          Consensus   EGAVGEAGPSPAHGFRPHTRLDVRVVSLGQLHGGELTGLLQSQKSEVILTLAGLVEVGVVLGRDDLELLAVLRAVDIVRADRNEV

                261     270       280       290
                |--------+---------+---------|
GIP2_SCRP249_transla  ASAQDKLTRSRVDIGSDGSDGHRSSGNDGD
GIP2_SCRP296_transla  ASAQDKLTRSRVDIGSDGSDGHRSSGNDGD
GIP2_SCRP333_transla  ASAQDKLTRSRVDIGSDG---HRSSGNDGD
          Consensus   ASAQDKLTRSRVDIGSDGsdgHRSSGNDGD
```

b.

```
                1       10        20        30        40        50        60        70        80        90        100       110       120       130
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
    nad9_SCRP1213  MNYNLILLLLFIKQQIGVKEKYGICLVFFFLIIQIEEFPIMVLKDILYVKTFHVVFKYFIMNKKELFMNLLIYHNNIDYLNLIILGIKKMHNSFRLFLFLGYFSFQIDGIKNHYLQFLIIIIILPRLIII
    nad9_SCRP1202  MNYNLILLLLFIKQQIGVKEKYGICLVFFFLIIQIEEFPIMVLKDILYVKTFHVVFKYFIMNKKELFMNLLIYHNNIDYLNLIILGIKKMHNSFRLFLFLGYFSFQIDGIKNHYLQFLIIIIILPRLIII
     nad9_SCRP324  MNYNLILLLLFIKQQIGVKEKYGICLVFFFLIIQIEEFPIMVLKDILYVKTFHVVFKYFIMNKKELFMNLLIYHNNIDYLNLIILGIKKMHNSFRLFLFLGYFSFQIDGIKNHYLQFLIIIIILPRLIII
     nad9_SCRP249  MNYNLILLLLFIKQQIGVKEKYGICLVFFFLIIQIEEFPIMVLKDILYVKTFHVVFKYFIMNKKELFMNLLIYHNNIDYLNLIILGIKKMHNSFRLFLFLGYFSFQIDGIKNHYLQFLIIIIILPRLIII
     nad9_SCRP296  MNYNLILLLLFIKQQIGVKEKYGICLVFFFLIIQIEEFPIMVLKDILYVKTFHVVFKYFIMNKKELFMNLLIYHNNIDYLNLIILGIKKMHNSFRLFLFLGYFSFQIDGIKNHYLQFLIIIIILPRLIII
     nad9_SCRP339  MNYNLILLLLFIKQQIGVKEKYGICLVFFFLIIQIEEFPIMVLKDILYVKTFHVVFKYFIMNKKELFMNLLIYHNNIDYLNLIILGIKKMHNSFRLFLFLGYFSFQIDGIKNHYLQFLIIIIILPRLIII
    nad9_SCRP1208  MNYNLILLLLFIKQQIGVKEKYGICLVFFFLIIQIEEFPIMVLKDILYVKTFHVVFKYFIMNKKELFMNLLIYHNNIDYLNLIILGIKKMHNSFRLFLFLGYFSFQIDGIKNHYLQFLIIIIILLRLIII
     nad9_SCRP333  MNYNLILLLLFIKQQIGVKEKYGICLVFFFLIIQIEEFPIMVLKDILYVKTFHVVFKYFIMNKKELFMNLLIYHNNIDYLNLIILGIKKMHNSFRLFLFLGYFSFQIDGIKNHYLQFLIIIIILPRLIII
          Consensus  MNYNLILLLLFIKQQIGVKEKYGICLVFFFLIIQIEEFPIMVLKDILYVKTFHVVFKYFIMNKKELFMNLLIYHNNIDYLNLIILGIKKMHNSFRLFLFLGYFSFQIDGIKNHYLQFLIIIIILpRLIII

                131     140       150       160       170       180       190       200       210       220       230       240       250       260
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
    nad9_SCRP1213  FLDLVRPVLCYKFLVFFQCIIHLILILILIVLNILEMLIMVGLDILMPMGLLFFLLLYMYIFLEVYITVHILHQEKLYGVLVLSLFWLQLLWVMYYLGDKVFGVQLLLLIYFQQYLVKILLIGYGVVLLII
    nad9_SCRP1202  FLDLVRPVLCYKFLVFFQCIIHLILILILIVLNILEMLIMVGLDILMPMGLLFFLLLYMYIFLEVYITVHILHQEKLYGVLVLSLFWLQLLWVMYYLGDKVFGVQLLLLIYFQQYLVKILLIGYGVVLLII
     nad9_SCRP324  FLDLVRPVLCYKFLVFFQCIIHLILILILIVLNILEMLIMVGLDILMPMGLLFFLLLYMYIFLEVYITVHILHQEKLYGVLVLSLFWLQLLWVMYYLGDKVFGVQLLLLIYFQQYLVKILLIGYGVVLLII
     nad9_SCRP249  FLDLVRPVLCYKFLVFFQCIIHLILILILIVLNILEMLIMVGLDILMPMGLLFFLLLYMYIFLEVYITVHILHQEKLYGVLVLSLFWLQLLWVMYYLGDKVFGVQLLLLIYFQQYLVKILLIGYGVVLLII
     nad9_SCRP296  FLDLVRPVLCYKFLVFFQCIIHLILILILIVLNILEMLIMVGLDILMPMGLLFFLLLYMYIFLEVYITVHILHQEKLYGVLVLSLFWLQLLWVMYYLGDKVFGVQLLLLIYFQQYLVKILLIGYGVVLLII
     nad9_SCRP339  FLDLVRPVLCYKFLVFFQCIIHLILILILIVLNILEMLIMVGLDILMPMGLLFFLLLYMYIFLEVYITVHILHQEKLYGVLVLSLFWLQLLWVMYYLGDKVFGVQLLLLIYFQQYLVKILLIGYGVVLLII
    nad9_SCRP1208  FLDLVRPVLCYKFLVFFQCIIHLILILILIVLNILEMLIMVGLDILMPMGLLFFLLLYMYIFLEVYITVHILHQEKLYGVLVLSLFWLQLLWVMYYLGDKVFGVQLLLLIYFQQYLVKMLLIGYGVVLLII
     nad9_SCRP333  FLDLVRPVLCYKFLVFFQCIIHLILILILIVLNILEMLIMVGLDILMPMGLLFFLLLYMYIFLEVYITVHILHQEKLYGVLVLSLFWLQLLWVMYYLGDKVFGVQLLLLIYFQQYLVKILLIGYGVVLLII
          Consensus  FLDLVRPVLCYKFLVFFQCIIHLILILILIVLNILEMLIMVGLDILMPMGLLFFLLLYMYIFLEVYITVHILHQEKLYGVLVLSLFWLQLLWVMYYLGDKVFGVQLLLLIYFQQYLVKiLLIGYGVVLLII

                261     270       280       290       300       310       320       330       340       350       360       370       380
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
    nad9_SCRP1213  LLIVFLVYILLSLLLVLFFIFYYMKLVQIILVLLKQKTYRFILIFIQKIYLVWYYFYFLFLSFIIPILGIQIIILKLIQKHLYILYLNGIFYRFMRFDLFQIKLVVLLLCLVHLFYQYL
    nad9_SCRP1202  LLIVFLVYILLSLLLVLFFIFYYMKLVQIILVLLKQKTYRFILIFIQKIYLVWYYFYFLFLSFIIPILGIQIIILKLIQKHLYILYLNGIFYRFMRFDLFQIKLVVLLLCLVHLFYQYLL
     nad9_SCRP324  LLIVFLVYILLSLLLVLFFIFYYMKLVQIILVLLKQKTYRFILIFIQKIYLVWYYFYFLFLSFIIPILGIQIIILKLIQKHLYILYLNGIFYRFMRFDLFQIKLVVLLLCLVHLFYQ
     nad9_SCRP249  LLIVFLVYILLSLLLVLFFIFYYMKLVQIILVLLKQKTYRFILIFIQKIYLVWYYFYFLFLSFIIPILGIQIIILKLIQKHLYILYLNGIFYRFMRFDLFQIKLVVLLLCLVHL
     nad9_SCRP296  LLIVFLVYILLSLLLVLFFIFYYMKLVQIILVLLKQKTYRFILIFIQKIYLVWYYFYFLFLSFIIPILGIQIIILKLIQKHLYILYLNGIFYRFMRFDLFQIKLVVLLLCLVH
     nad9_SCRP339  LLIVFLVYILLSLLLVLFFIFYYMKLVQIILVLLKQKTYRFILIFIQKIYLVWYYFYFLFLSFIIPILGIQIIILKLIQKH
    nad9_SCRP1208  LLIVFLVYILLSLLLVLFFIFYYMKLVQIILVLLKQKTYRFILIFIQKIYLVWYYFYFLFLSFIIPTLGIQIIILKLIQKHL
     nad9_SCRP333  LLIVFLVYILLSLLLVLFFIFYYMKLVQIILVLLKQKTYRFILIFIQKIYLVWYYFYFLF
          Consensus  LLIVFLVYILLSLLLVLFFIFYYMKLVQIILVLLKQKTYRFILIFIQKIYLVWYYFYFLFlsfiipilgiqiiilkliqkhl.....................................
```

Figure 18: Alignment of proteins a. GIP2 (glucanase inhibitor protein 2) and b. nad9 (NAD9 NADH dehydrogenase subunit 9) for P. rubi isolates, showing amino acid changes and polymorphism amongst isolates for these 2 housekeeping genes. GIP2 protein sequences of non-represented P. rubi isolates were identical to the SCRP333 reference isolate one. Alignments were carried out using the MultAlin tool (Corpet, 1988).

Similarly, housekeeping genes were compared between all isolates to identify genes showing inter-species polymorphism (differences between the two species), at the nucleotide and protein level, and Figures 19 to 23 show the protein alignments where, indeed, SNPs resulted in amino acid changes leading to differences between *P. rubi* and *P. fragariae*: G3PDH (glyceraldehyde-3-phosphate dehydrogenase), GIP2 (glucanase inhibitor protein 2), betaglucosidase, Cox I (cytochrome oxidase I) and elongation factor 1 alpha.

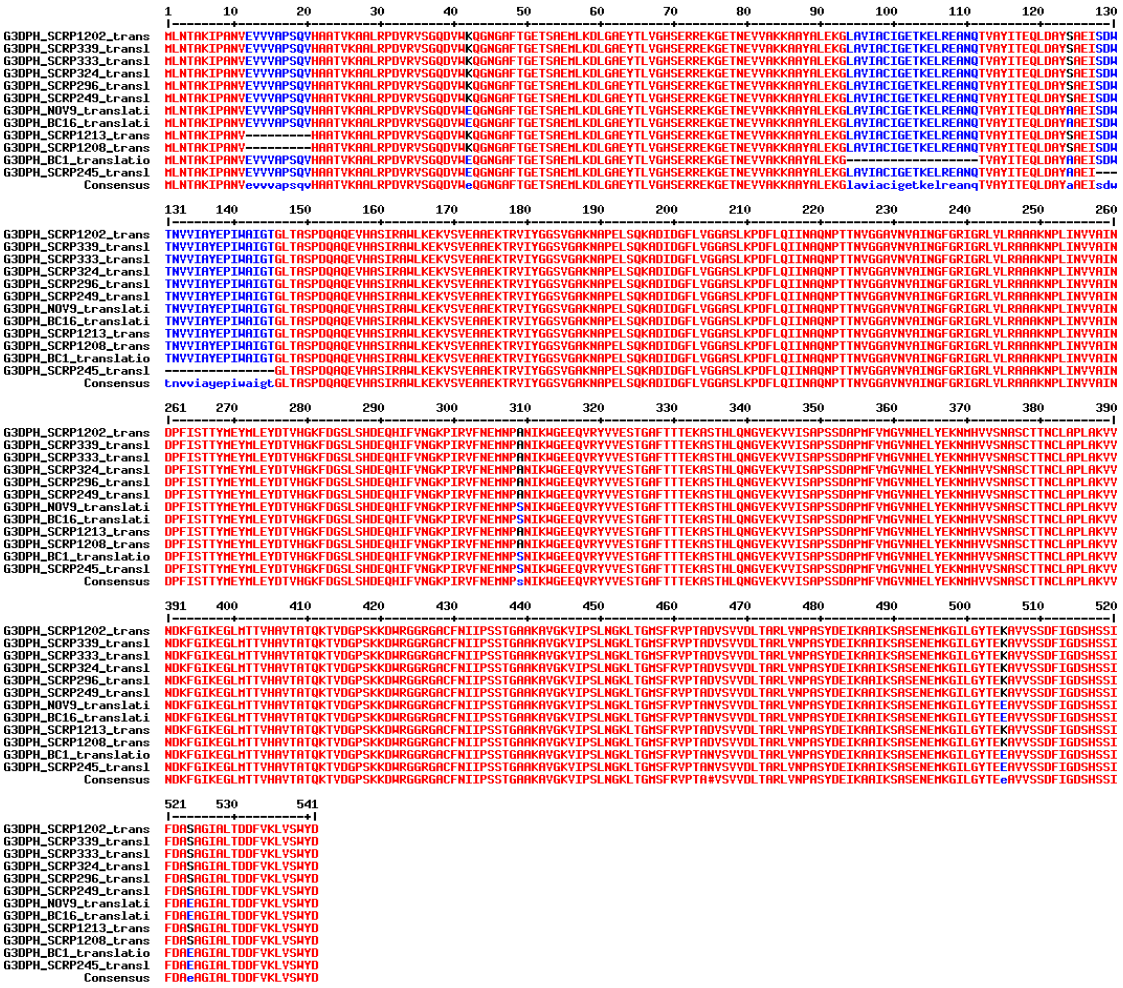


Figure 19: Alignment of G3PDH (glyceraldehyde-3-phosphate dehydrogenase) for all isolates screened in the PenSeq study, showing amino acid changes and polymorphism amongst isolates. Alignments were carried out using the MultAlin tool (Corpet, 1988).

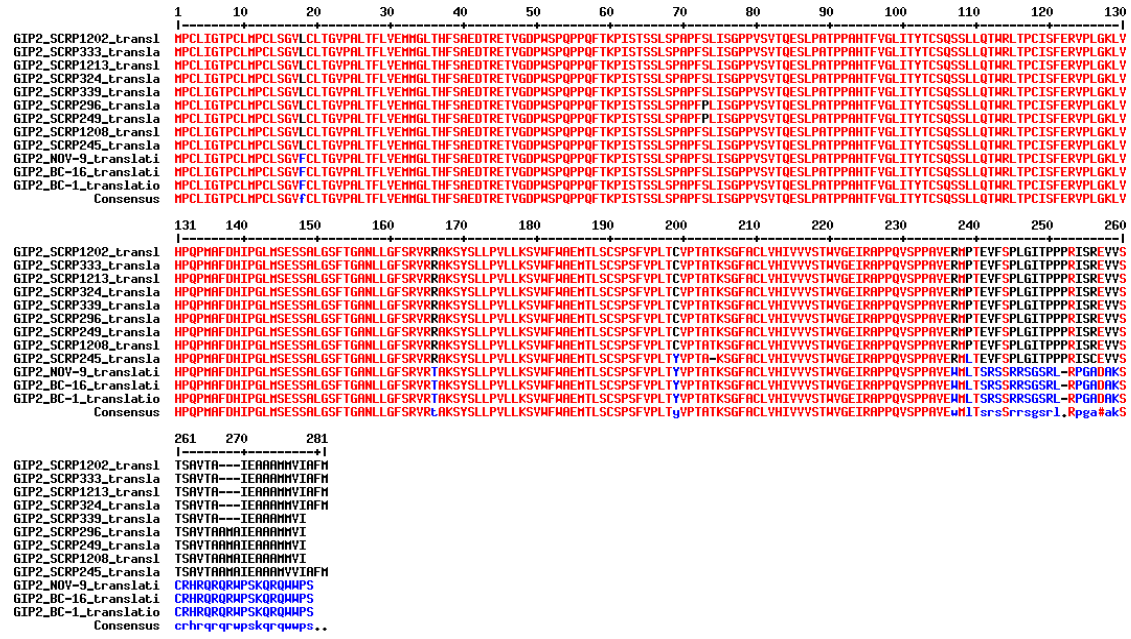


Figure 20: Alignment of GIP2 (glucanase inhibitor protein 2) for all isolates screened in the PenSeq study, showing amino acid changes and polymorphism amongst isolates. Alignments were carried out using the MultAlin tool (Corpet, 1988).

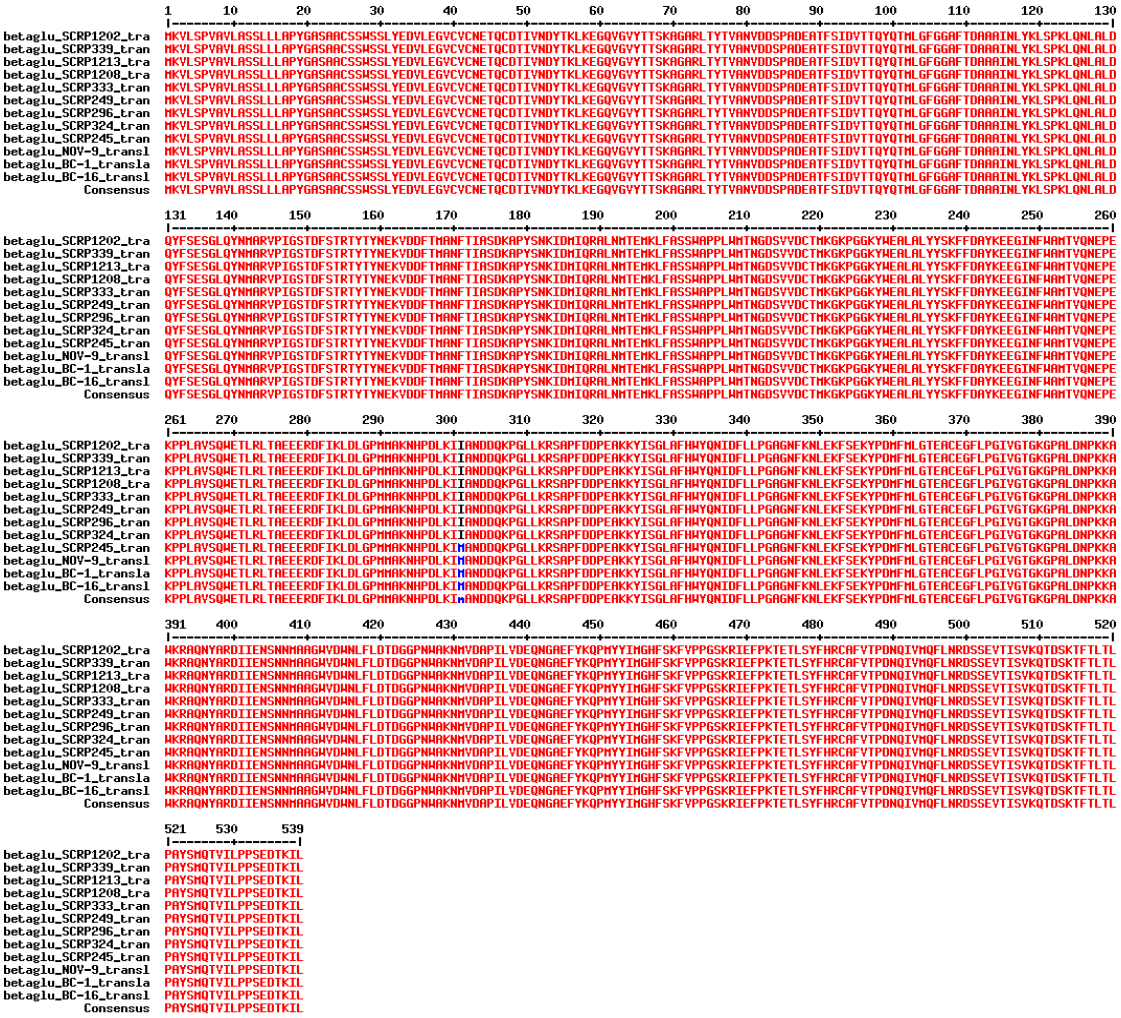


Figure 21: Alignment of betaglucosidase proteins for all isolates screened in the PenSeq study, showing amino acid changes and polymorphism amongst isolates. Alignments were carried out using the MultAlin tool (Corpet, 1988).

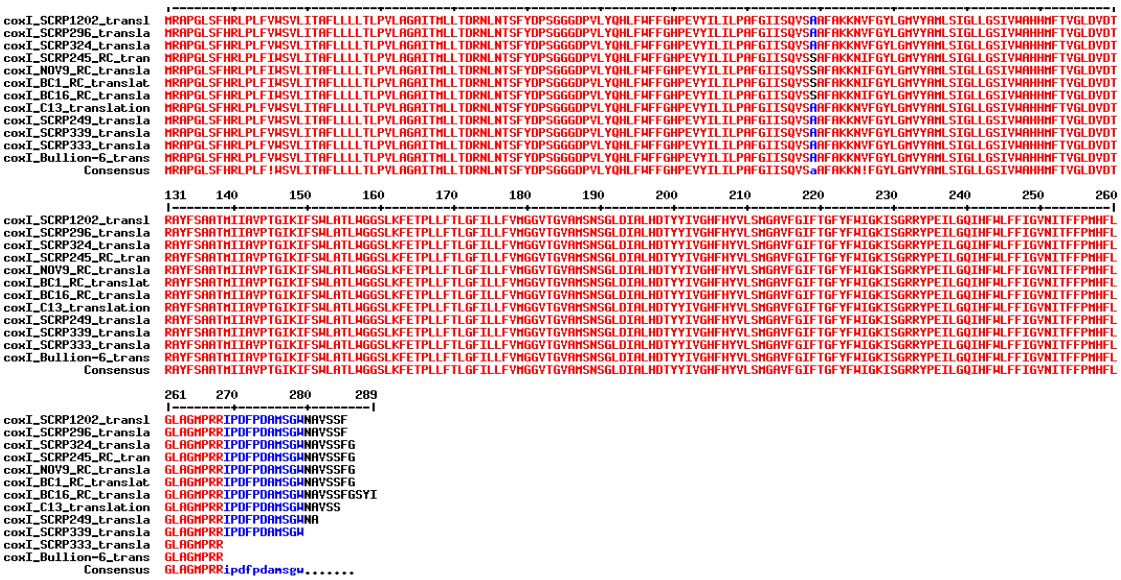


Figure 22: Alignment of Cox I (cytochrome oxidase I) proteins for all isolates screened in the PenSeq study, showing amino acid changes and polymorphism amongst isolates. Alignments were carried out using the MultAlin tool (Corpet, 1988).

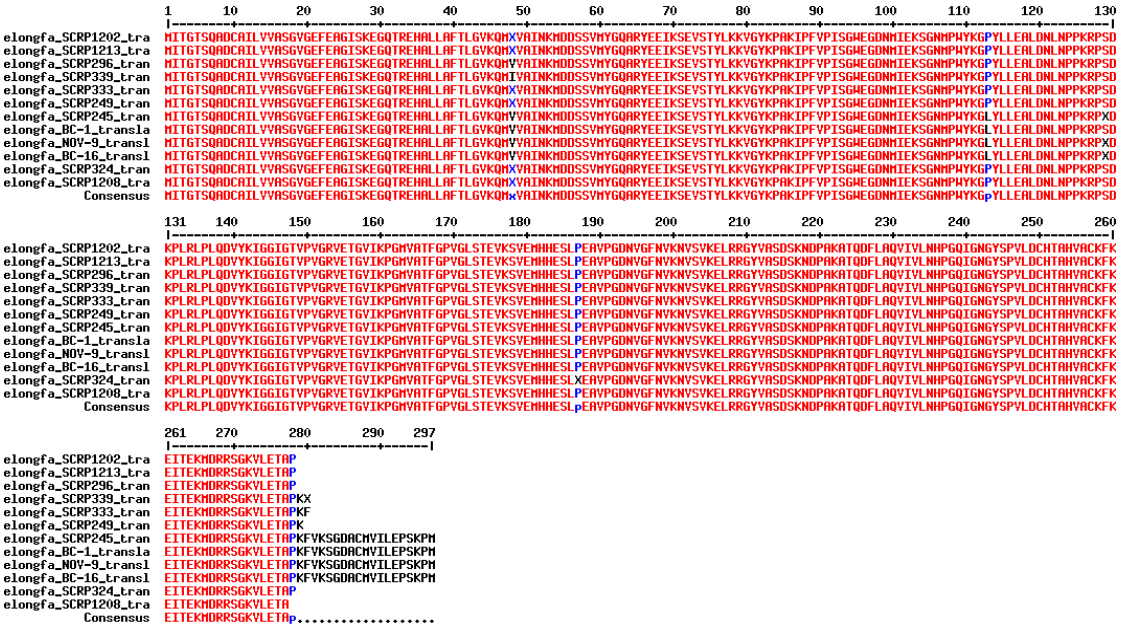


Figure 23: Alignment of elongation factor 1 alpha proteins for all isolates screened in the PenSeq study, showing amino acid changes and polymorphism amongst isolates. Alignments were carried out using the MultAlin tool (Corpet, 1988).

- Intra-species effectors diversity (presence/absence analysis)

Identical genes in each species were identified by analysing the 0% mismatch mapping rate coverage table for the ~15 000 genes, looking at genes covered at 100%, i.e. where each nucleotide got successfully mapped against the reference genomes, allowing no mismatch. This showed a total of 6448 genes that are identical between all 4 isolates of *P. fragariae* and 4350 identical between all 8 isolates of *P. rubi*.

Overall SNP calling analysis showed that for *P. fragariae,* SCRP245 had the highest number of genes where polymorphism (SNPs) was found and the highest total number of SNPs (188) on all genes screened, making it the most genetically diverse isolate amongst *P. fragariae* and thus showing either a source-location or a temporal effect, as SCRP245 was isolated from England in 1945 and the 3 other *P. fragariae* were isolated from Canada in 2007. The same gene, g6744 (unknown function), showed the most SNPs in all *P. fragariae* isolates. Similarly, *P. rubi* SCRP249 had the highest number of genes where polymorphism (SNPs) was found and highest total number of SNPs (512) on all genes screened, making it the most genetically diverse *P. rubi* amongst the 8 screened isolates. Gene g26662 showed the most SNPs in all *P. rubi* isolates.

Coverage analysis using both 0% and 3% mismatch mapping rates allowed the identification of genes that are only present in some isolates. For instance, isolate-unique genes were identified for *P. fragariae* (Table 6), while none were identified in *P. rubi*, undoubtedly due to an increased number of isolates screened.

Table 6: Number of isolate-unique genes identified through PenSeq study for *P. fragariae* isolates

Bowtie2 mismatch mapping rates / Isolates	0%mm				3%mm				5%mm			
	BC-1	BC-16	NOV-9	SCR P245	BC-1	BC-16	NOV-9	SCR P245	BC-1	BC-16	NOV-9	SCR P245
number of genes 100% covered only in isolate of interest	6	1	0	2	4	2	1	0	4	1	0	0
number of genes covered (> 0%) only in isolate of interest	13	6	3	6	5	4	3	1	5	2	3	1

This analysis additionally identified that only 1 gene was present only in *P. fragariae* isolate SCRP245 and absent in all 3 other *P. fragariae* at the highest mismatch rates; while 23 genes were absent from SCRP245 and 100% covered and identical in all 3 other *P. fragariae.* Similarly, 2 genes were found to be only present in the reference isolate BC-16 and absent in the other *P. fragariae* (at the highest mismatch rate). *P. rubi* investigation of presence / absence found 13 genes absent from the 2 most recent isolates (SCRP1208 and SCRP1213) and covered in all 6 others isolates (absence at 5% mismatch mapping rate), of which 1 gene was covered at 100% in all 6 others *P. rubi* at 0% mismatch mapping rate, thus being an identical gene between these isolates. Four genes were also found to be absent from the 2 confirmed race 3 isolates (SCRP333, SCRP339) and present with SNPs in all 6 others *P. rubi*; and 5 genes were found absent from SCRP324, a confirmed race 1 *P. rubi* isolate, and present in the other 6 isolates, and 2 of those genes were identical between isolates.

Finally, 178 *P. rubi* genes successfully mapped with PenSeq were predicted as RxLR effector proteins according to the Whisson et al. (2007) prediction model (prediction ran with Galaxy). Of these, 51 are completely identical between all *P. rubi* isolates (100% covered at 0% mismatch mapping rate) and the average coverage for the partially covered ones was 93.9% at 0% mismatch mapping rate and 99.2% at 3%.

Cross-species mapping and coverage analyses

- Cross-species mapping & coverage tables for several mismatch mapping rate

For many years, *P. rubi* and *P. fragariae* were thought to be the same species, infecting two different hosts (raspberry and strawberry). In 2007 however, Manin't Veld demonstrated that

it was in fact two different diseases, one *Phytophthora* infecting raspberries and the other strawberries (Man in't Veld, 2007). The cross-species analysis in this study uses reads from the *P. rubi* isolates mapped to the *P. fragariae* reference genome, and vice versa, informing us about the inter-species diversity: if the two species share identical genes, the corresponding reads should map to the other species genome, with or without SNPs (determined by using different mismatch mapping rates); on the other hand, if at the highest mismatch mapping rate no reads are mapped for any of the isolate of one species to the reference genome of the other, these would be species-unique genes.

In this cross-species diversity analysis, the 8 *P. rubi* isolates were mapped to the reference genome for *P. fragariae* (BC-16) and the 4 *P. fragariae* mapped to the reference genome for *P. rubi* (SCRP333). Table 7 shows the total and percentage of reads mapped at 0%, 3% and 5% mismatch mapping rates. When *P. rubi* isolates were mapped to *P. fragariae* reference genome, only 16.9% was successfully mapped at 0% mismatch and the ratio went up to 70% when using 3% mismatch mapping rate, with similar percentages when *P. fragariae* were mapped to *P. rubi*. Those percentages are much lower than those from the same-species mapping at 0% mismatch rate, with 64% of reads mapped for *P. rubi* and 74% for *P. fragariae,* thus confirming the distinction between the two species.

Table 7: Percentage and total reads mapped to reference genomes for 12 isolates, 8 *P. rubi* and 4 *P. fragariae* at several Bowtie mismatch mapping rates, for the cross-species mapping analysis, where *P. rubi* isolates were mapped to *P. fragariae* reference genome and *P. fragariae* isolates mapped to *P. rubi* reference genome.

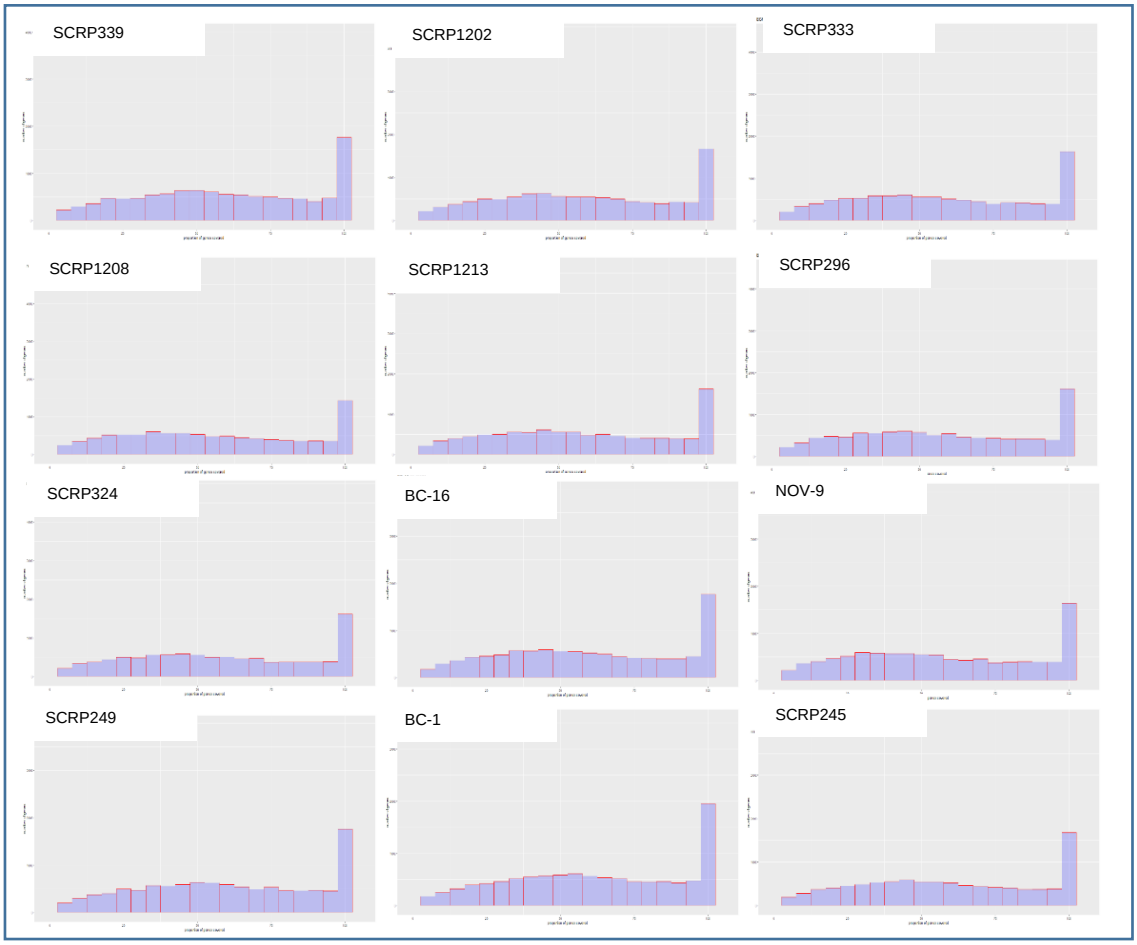
Species	Isolate	Reads count	Reference mapped to	Bowtie mismatch mapping rate (%)	Total reads mapped	Percentage of reads mapped (%)
P. rubi	SCRP1208	874620	*P. fragariae BC-16*	0	142237	16.26
P. rubi	SCRP1208	874620		3	622062.5	71.12
P. rubi	SCRP1208	874620		5	688026.5	78.67
P. rubi	SCRP1213	1131707		0	181953.5	16.08
P. rubi	SCRP1213	1131707		3	791736	69.96
P. rubi	SCRP1213	1131707		5	874463	77.27
P. rubi	SCRP1202	1121686		0	200766.5	17.9
P. rubi	SCRP1202	1121686		3	805736.5	71.83
P. rubi	SCRP1202	1121686		5	889843	79.33
P. rubi	SCRP249	1499947		0	260956	17.4
P. rubi	SCRP249	1499947		3	1046631.5	69.78
P. rubi	SCRP249	1499947		5	1155613	77.04
P. rubi	SCRP296	1117692		0	188394.5	16.86
P. rubi	SCRP296	1117692		3	782351.5	70
P. rubi	SCRP296	1117692		5	865189.5	77.41
P. rubi	SCRP324	1021039		0	164156.5	16.08
P. rubi	SCRP324	1021039		3	726116.5	71.12
P. rubi	SCRP324	1021039		5	802085	78.56
P. rubi	SCRP333	1095654		0	186389	17.01
P. rubi	SCRP333	1095654		3	776601.5	70.88
P. rubi	SCRP333	1095654		5	857883	78.3
P. rubi	SCRP339	1481653		0	256627	17.32
P. rubi	SCRP339	1481653		3	1050964	70.93
P. rubi	SCRP339	1481653		5	1161014.5	78.36
P. fragariae	BC-16	1120168	*P. rubi SCRP333*	0	176975.5	15.8
P. fragariae	BC-16	1120168		3	796996.5	71.15
P. fragariae	BC-16	1120168		5	890234	79.47
P. fragariae	BC-1	1182473		0	187769	15.88
P. fragariae	BC-1	1182473		3	827780	70
P. fragariae	BC-1	1182473		5	922443.5	78.01
P. fragariae	NOV-9	865044		0	155388	17.96
P. fragariae	NOV-9	865044		3	609740.5	70.49
P. fragariae	NOV-9	865044		5	678999	78.49
P. fragariae	SCRP245	1103774		0	162618	14.73
P. fragariae	SCRP245	1103774		3	777110.5	70.4
P. fragariae	SCRP245	1103774		5	869709	78.79

Similarly to same-species mapping, coverage was calculated for each gene at several mismatch mapping rates (Table 8 and Figure 24).

Table 8: Coverage (in percentage) of the ~15 000 genes mapped at several Bowtie mismatch mapping rates for the cross-species mapping analysis

Species	**Isolate**	**Reference mapped to**	**Average Coverage mismatch mapping rate**		
			0%	**3%**	**5%**
P. rubi	SCRP1208	*P. fragariae BC-16*	37.48	79.07	83.31621
P. rubi	SCRP1213		40.22	82.31	86.47801
P. rubi	SCRP1202		41.74	82.90	87.03287
P. rubi	SCRP249		44.29	85.17	89.20419
P. rubi	SCRP296		40.70	81.69	85.85976
P. rubi	SCRP324		39.38	81.77	86.007
P. rubi	SCRP333		40.81	80.78	84.88438
P. rubi	SCRP339		43.99	84.33	88.41232
P. rubi	**average**		**41.08**	**82.25**	**86.39934**
P. fragariae	BC-16	*P. rubi SCRP333*	43.30	81.42	85.45231
P. fragariae	BC-1		46.43	83.39	87.24787
P. fragariae	NOV-9		41.59	77.28	81.37201
P. fragariae	SCRP245		41.49	82.13	86.44214
P. fragariae	**average**		**43.21**	**81.05**	**85.12858**

a.



b.

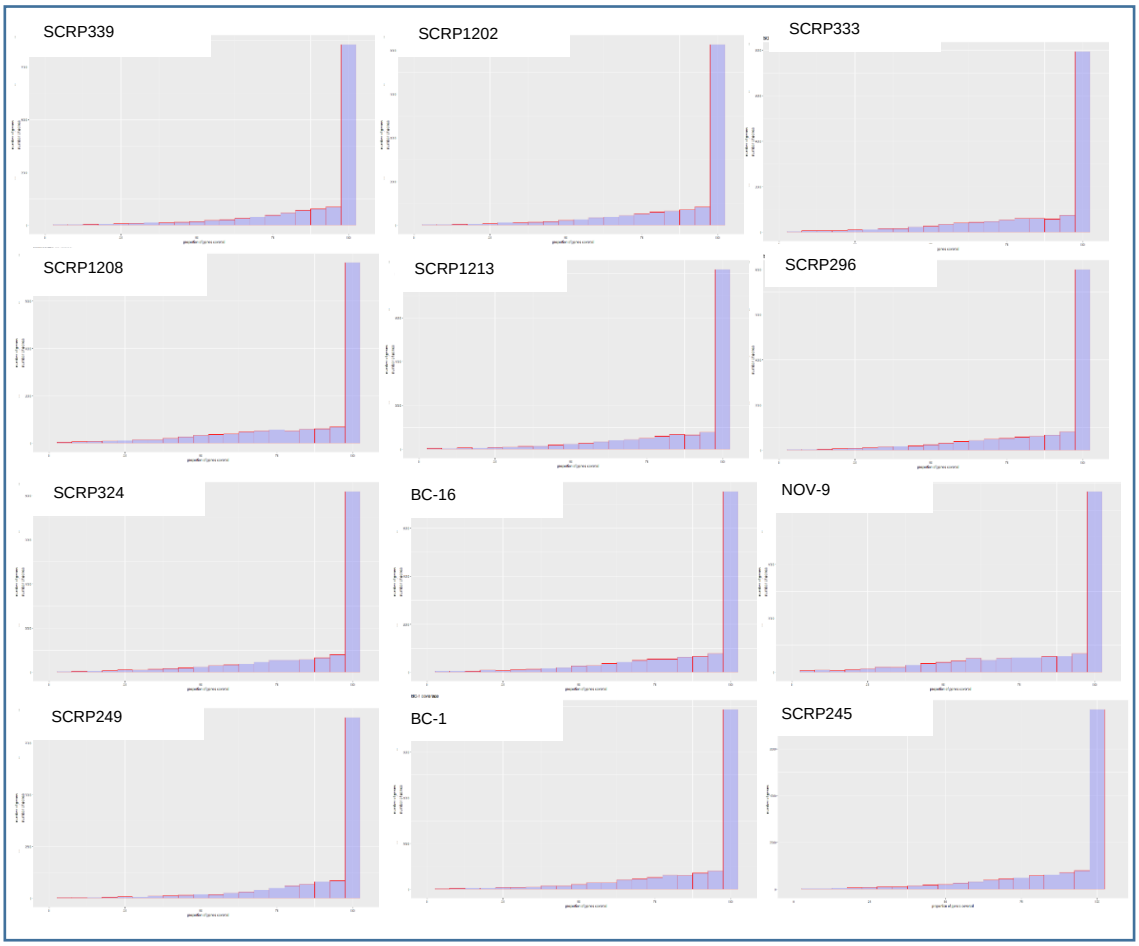


Figure 24: Coverage graphs for the 12 isolates used in the PenSeq study at a. 0% mismatch mapping rate and b. 3% mismatch mapping rate where *P. rubi* isolates were mapped to *P. fragariae* reference genome and *P. fragariae* isolates mapped to *P. rubi* reference genome (cross-species mapping). X-axis represents the proportion of gene covered (gridlines every 12.5%) and the y-axis represents the number of genes showing that coverage proportion (gridlines every 1000 genes)

- Inter-species diversity (presence/absence between *P. rubi* and *P. fragariae*)

The cross-species mapping allowed the identification of most conserved genes between the two species, genes that were completely identical, with no polymorphism, between all 12 isolates, as well as the discovery of genes unique to one species. As a result, 600 genes were found to be completely identical between all isolates of both species; while 142 genes were found to be identical between all 4 isolates of *P. fragariae* but completely absent from any *P. rubi* (genes unique to *P. fragariae*); and 69 genes were identical between the 8 *P. rubi* isolates and absent from all *P. fragariae* (genes unique to *P. rubi*). Although the two pathogens are very closely related, and believed to be the same species for many years, *P. rubi* infects raspberries only, while *P. fragariae* infects strawberries; and unique genes could play a key role in host choice and recognition.

Furthermore, 8 *P. fragariae* RxLR of interest, identified by Adams (2019) as always expressed (FPKM > 1000) at all *in planta* time points during infection with the 3 Canadian isolates (BC-1, BC-16 and NOV-9), were investigated for presence in *P. rubi* isolates and polymorphism amongst all isolates studied. Table 9 shows the coverage at 0% and 3% mismatch mapping rates for these 8 RxLR in all 12 isolates.

Table 91: Coverage of P. fragariae RxLRs of interest at 0% and 3% mismatch mapping rates for all 12 isolates used in the PenSeq study

NIAB ID		g23640		g40916		g20420		g16234		g6480		g35418		g27513		g21231	
Equivalent ID from PenSeq		g21368		g36900		g18452		g14629		g5824		g32018		g24882		g19167	
% mismatch mapping rate		0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3
P. Rubi coverage (from cross-mapping)	SCRP1202	0	0	0	88.86	0.70	52.44	67.31	98.53	0	93.51	0	52.51	0	3.15	16.35	100
	SCRP1208	0	0	38.86	90.45	0	48.49	64.62	99.30	0	99.68	0	51.93	0	0.48	30.14	100
	SCRP1213	0	61.96	22.95	79.77	0	51.28	71.52	98.72	52.69	95.73	0	49.03	0	3.39	29.22	100
	SCRP249	0	0	25	89.55	0	51.51	78.67	100	49.05	100	0	52.32	0	37.53	29.77	100
	SCRP296	91.30	100	0	81.14	24.59	51.05	70.43	100	0	92.56	0	51.35	0	35.59	29.95	100
	SCRP324	0	4.62	21.36	87.05	0	49.66	73.70	100	0	94.62	0	51.93	0	5.81	62.19	100
	SCRP333	0	0	0	76.14	0	50.58	74.65	99.11	0	93.51	0	51.35	0	36.56	71.51	100
	SCRP339	0	0	0	80.91	6.26	52.90	79.31	98.21	0	94.15	0	51.16	0	47.94	41.92	100
P. Fragariae coverage	BC-1	100	100	100	100	100	100	100	100	100	100	100	100	100	100	98.90	100
	BC-16	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	NOV-9	100	100	100	100	100	100	100	100	100	100	100	100	100	100	99.82	100
	SCRP245	100	100	100	100	96.98	100	100	100	95.73	100	52.32	100	96.85	100	97.17	100

Particularly, 4 of these RxLR are showing interest for presence / absence in *P. rubi:* indeed, gene g21368 is identical amongst *P. fragariae* but is only found in 2 *P. rubi* isolates, only one with 100% coverage at 3%mismatch. Gene g14629 shows high coverage in *P. rubi* isolates, from 98.21% to 100% at 3%mismatch, while being identical amongst *P. fragariae* isolates. Gene g5824 also shows relatively high coverage in *P. rubi*, from 92.56% to 100%, and shows SNPs in SCRP245 compared to other *P. fragariae*. Finally, g19167, identical in all *P. fragariae*, has 100% coverage in all *P. rubi* with a 3%mismatch mapping rate. However, 19

SNPs were found between isolates, resulting in different proteins between *P. rubi* and *P. fragariae*.

P. rubi infection of hydroponically grown raspberries

Confocal images of P. rubi infection in raspberry roots

Hydroponics infections using a transgenic isolate of *P. rubi* expressing a red fluorescent protein (tdTomato) on raspberries grown in hydroponics demonstrated the feasibility of the assay: *P. rubi* completed its life cycle in hydroponic conditions and roots samples were easily collected at 3, 7, 11 and 14 days after inoculation (dpi) for further testing of gene expression (on-going analyses). The progression of the fluorescent strain in the root was captured using a Zeiss LSM 710 confocal microscope.

At 3dpi, encystment, germination and penetration of the root had happened, as shown in Figure 25.

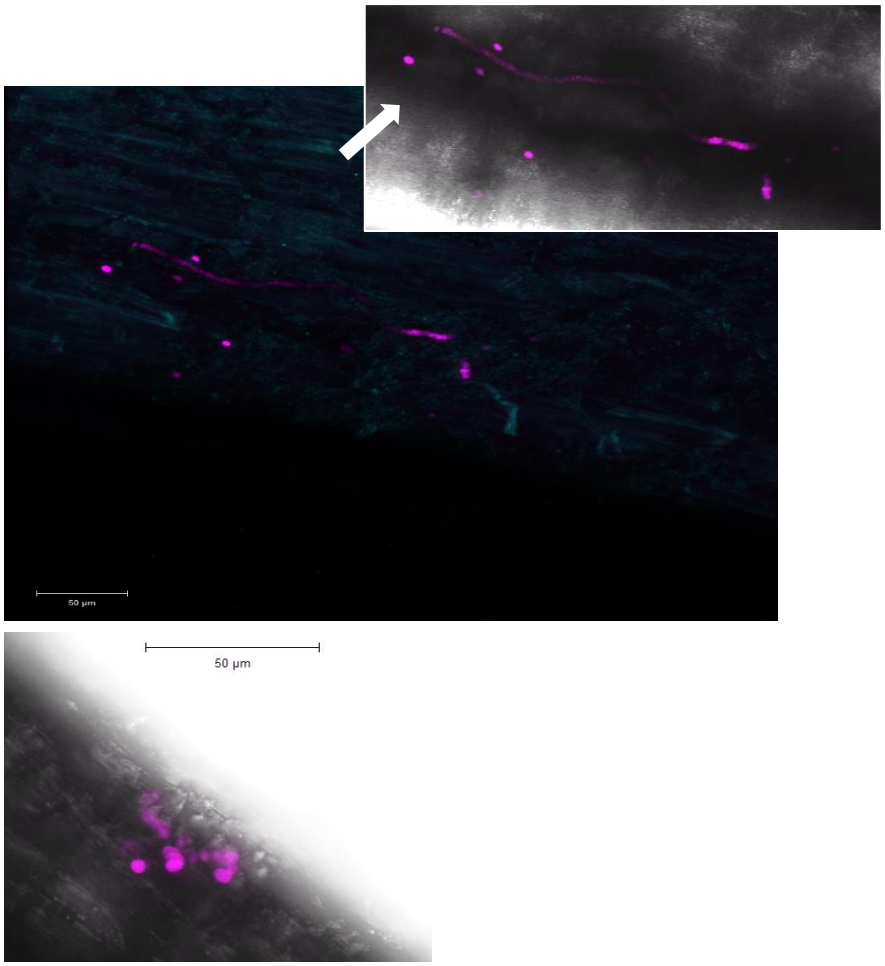


Figure 25: Confocal photos of raspberry root infected with *P. rubi* SCRP333 expressing a red fluorescent protein (tdTomato) in magenta in the pictures. Photos were taken from an infected Glen Moy root at 3 days post-inoculation and show encystement, germination and beginning of hyphal progression within the root. Root from the top photo were stained with calcofluor. Photos were taken with a Zeiss LSM 710 confocal microscope.

At 7 and 11dpi, hyphal progression inside the root was clearly observed (Figure 26).

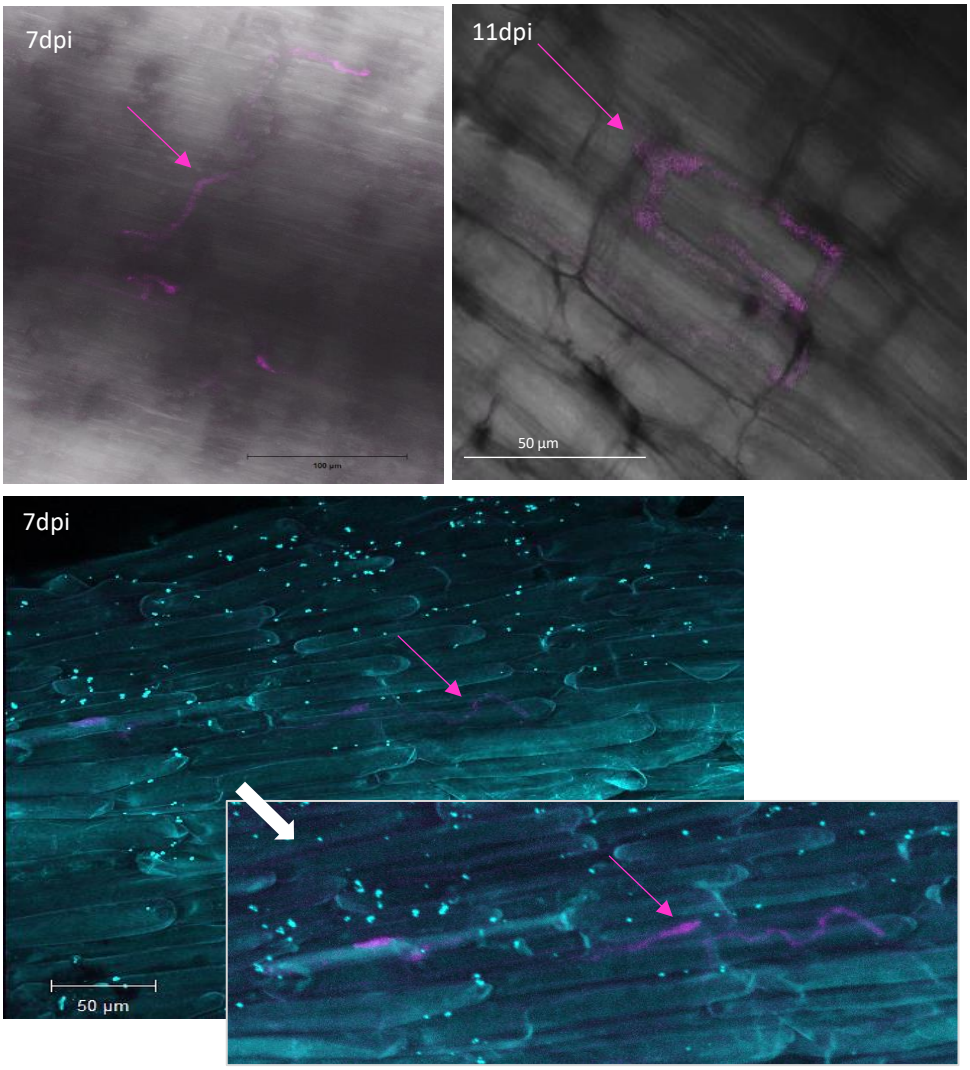


Figure 26: Confocal photos of raspberry root infected with *P. rubi* SCRP333 expressing a red fluorescent protein (tdTomato) in magenta in the pictures. Photos were taken from an infected Glen Dee root at 7- and 11-days post-inoculation and show hyphal progression within the root. Pink arrows point at the fluorescent *P. rubi* hyphae. Roots from the last photo were stained with calcofluor. Photos were taken with a Zeiss LSM 710 confocal microscope.

Confocal photos at 11dpi also showed re-infection with the formation of sporangia releasing zoospores, as displayed in Figure 27.

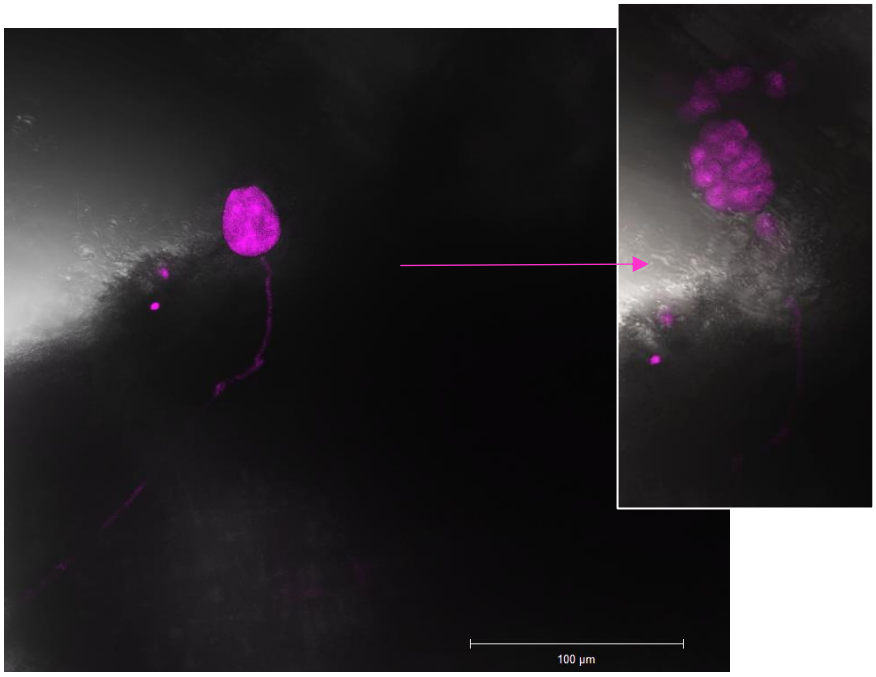


Figure 27: Confocal photos of raspberry root infected with *P. rubi* SCRP333 expressing a red fluorescent protein (tdTomato) in magenta in the pictures. Photos were taken from an infected Glen Dee root at 11-days post-inoculation and show hyphal progression within the root. Photo on the left shows fluorescent sporangia produced on infected root, while the photo on the right was taken a few minutes later and shows the sporangia releasing zoospores, for re-infection. Photos were taken with a Zeiss LSM 710 confocal microscope.

At 7, 9 and 14 dpi, oospores were observed in the roots, and at 22dpi, more sporangia had colonized the tissue (Figures 28 and 29).

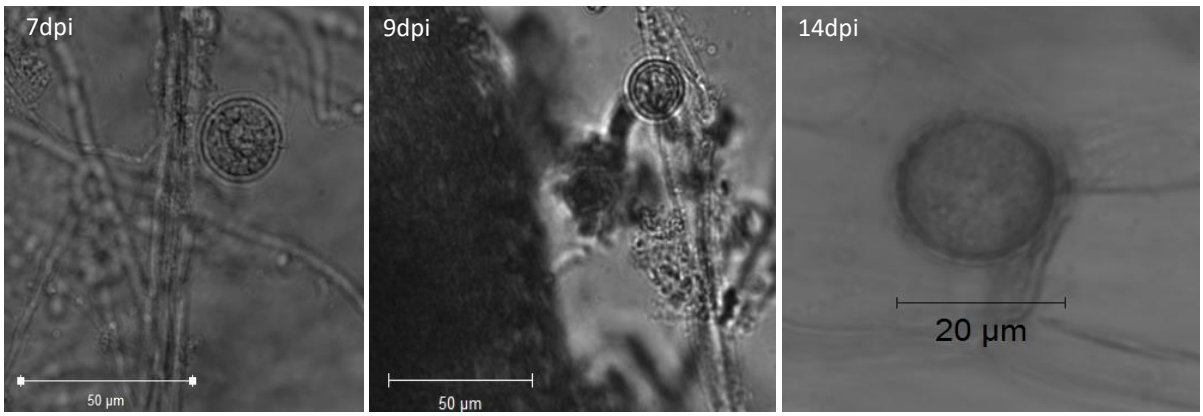


Figure 28: Confocal photos of raspberry roots infected with *P. rubi* SCRP333_tdT at 7dpi, 9dpi and 14dpi. Left and central photos were from an infected Glen Moy while photo on the right was from an infected Glen Dee raspberry root. Photos show that oospores of ~ 20-30µm diameter were formed within the root during infection. Photos were taken with a Zeiss LSM 710 confocal microscope.

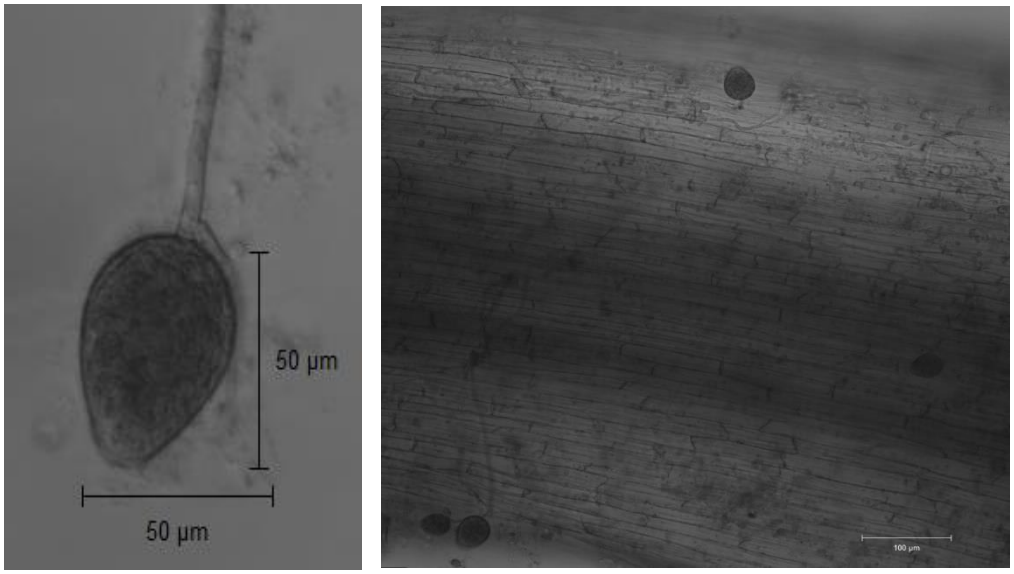


Figure 29: Confocal photos of raspberry roots infected with *P. rubi* SCRP333_tdT at 22dpi. Left photo from an infected Glen Dee while photo on the right was from an infected Glen Moy raspberry root. Photos show that more sporangia were formed within the root at later infection stages. Photos were taken with a Zeiss LSM 710 confocal microscope.

qRT-PCR assay for expression of life markers

- Primers testing

Conventional PCR and 2% agarose gels validated CDC14 and HMP1 primers, amplifying products of appropriate sizes for SCRP333 and not for uninfected raspberry cDNA (Figure 30). Two dilutions of SCRP333 gDNA were loaded in wells 5 and 6 on the CDC14 gel and 4 and 5 on the HMP1 gel; diluted cDNA from uninfected raspberry root (R) was loaded in the next well, 7 on the CDC14 gel and 6 on the HMP1 gel. Negative controls (-ve) using nucleases-free water were loaded in well 4 on the CDC14 gel and in well 7 on the HMP1 gel. Ladder of 1kb+ (L) confirmed size of products to be of those expected for CDC14 and HMP1 using the primers described in Table 4.

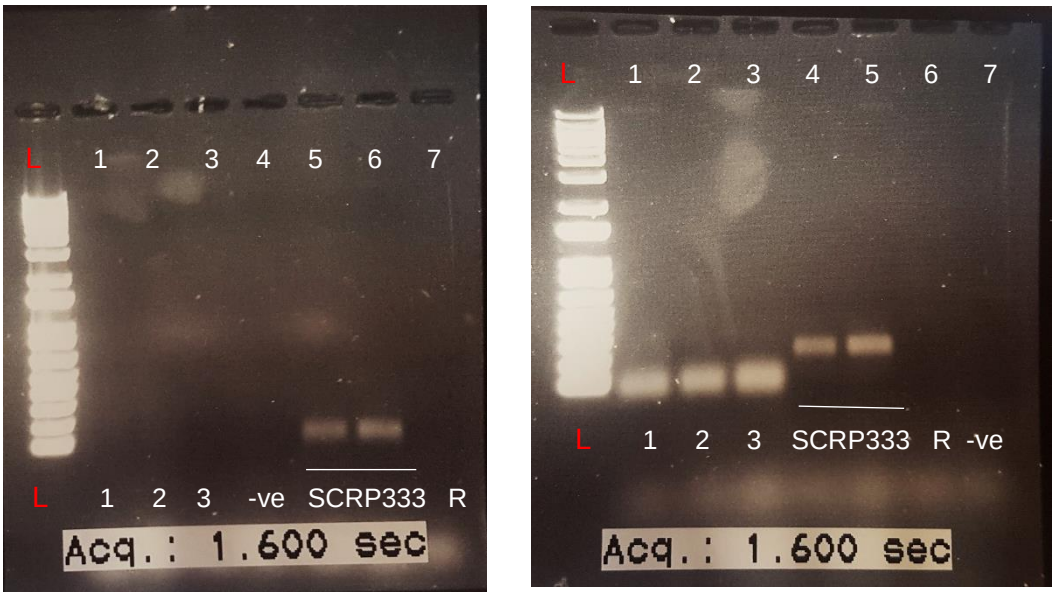


Figure 30: Agarose gels (2%) electrophoreses of PCR to validate the life marker primers CDC14 (left) and HMP1 (right). Agarose gels were running at 80V for 1 hour. L: 1kb+ ladder

Cox I and betatubulin primers have already been tested and validated in previous CP173 reports.

- Expression levels of HMP1 confirms re-infection

cDNA samples from 3 time-courses were used in a qRT-PCR assay of control genes (Cox I and betatubulin) first to investigate CT levels and thus presence and levels of *P. rubi* cDNA in infected raspberry roots. Unfortunately, CT were too high for one of the time-course, and thus only two were retained for the following analysis: February 2019 and September 2019. For the February 2019 time-course, cDNA was diluted 1:3, while for the September 2019, cDNA was used neat in the reaction. Figures 31 and 32 show relative expression of HMP1 during the September 2019 and February 2019 infections respectively, using Cox I as an endogenous control gene and SCRP333_tdT mycelia as a control sample.

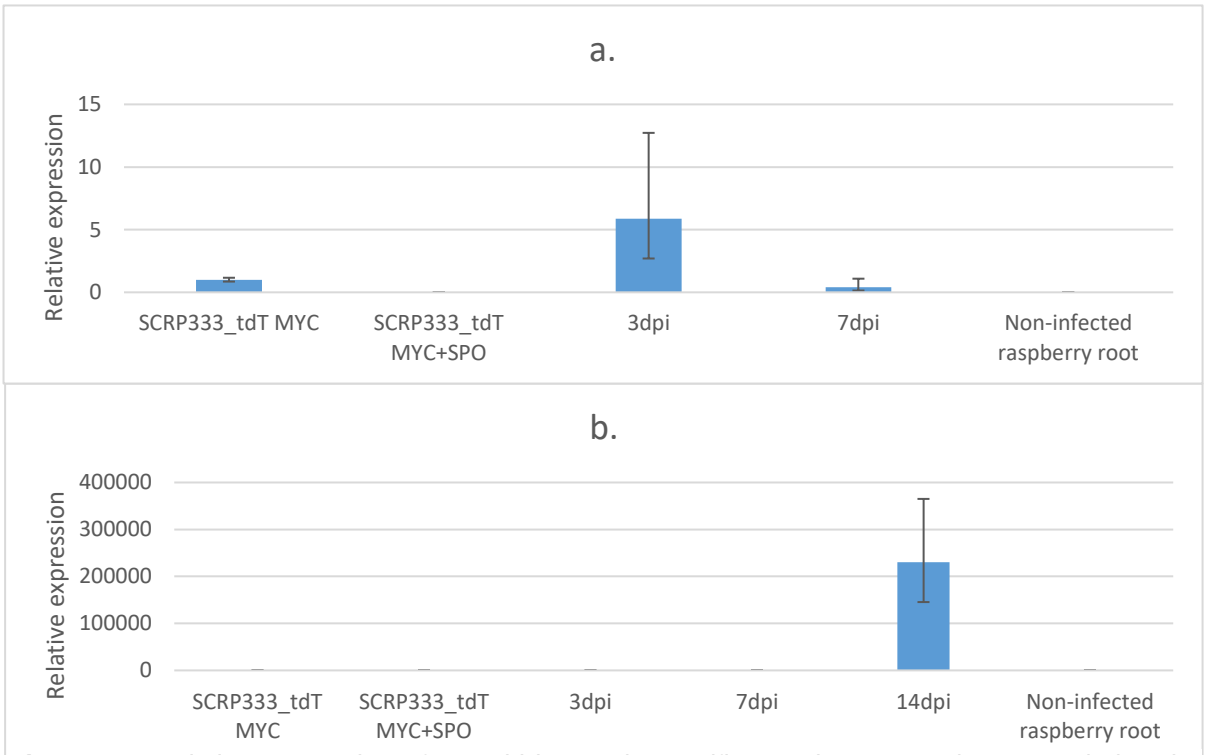


Figure 31: Relative expression of *P. rubi* haustoria-specific membrane protein HMP1 during the September 2019 infection time course at several time points, compared to SCRP333_tdT (used for infection) mycelia control. Cox I was used as an endogenous control. 14 dpi is represented in a separate graph (b.) for easy visualisation of high expression.

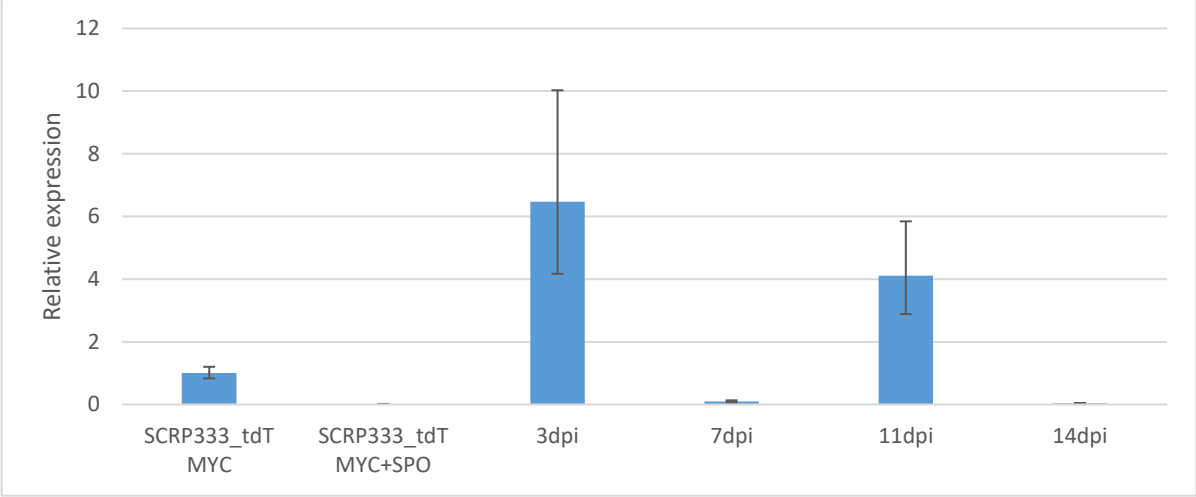


Figure 32: Relative expression of *P. rubi* haustoria-specific membrane protein HMP1 during the February 2019 infection time course at several time points, compared to SCRP333_tdT (used for infection) mycelia control. Cox I was used as an endogenous control.

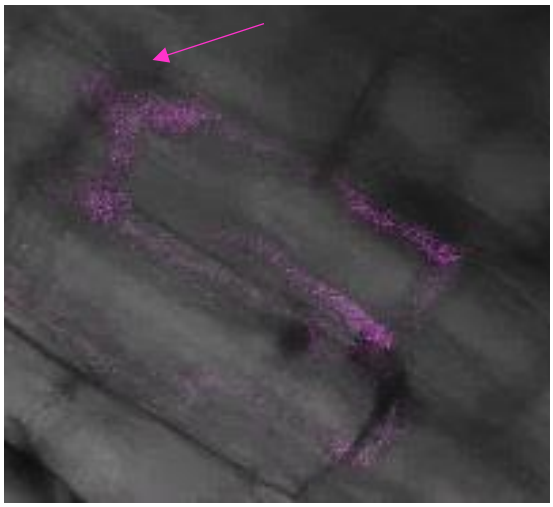


Figure 33: Confocal photo of a Glen Dee raspberry root infected with *P. rubi* SCRP333 expressing a red fluorescent protein (tdTomato) in magenta in the pictures. Photos were taken at 11 days post-inoculation during the February 2019 time-course. Pink arrow points at potential haustoria-like structure. Photos were taken with a Zeiss LSM 710 confocal microscope.

The same pattern was observed between the 2 infections (February 2019 and September 2019) with the expression level of HMP1 first going up (3dpi) then down (7dpi) then back up again (at 11dpi for February 2019 infection and 14 dpi for September 2019). This second spike in haustoria membrane protein expression level could be confirming re-infection from newly released second-generation zoospores that encysted again on the infected root (Figure 33). Validation of the expression of CDC14 is still on-going.

Discussion

P. rubi phenotypic diversity study

Phenotypic studies are important for characterizing an under-studied species and assessing the diversity of isolates from various countries and years and gives information on the

phenotypic diversity of that pathogen. This study investigates the phenotypic behaviour of several isolates of *P. rubi* and *P. fragariae,* in terms of adaptation to rising temperatures and the relevance of certain control chemicals, thus informing us on the behaviour of these pathogens under current field conditions, and guiding growers and future research. Testing both old and recent isolates adds to the applicability of this study, to look for adaptation, evolution or response of isolates that are presently found in the field, to chemicals and temperature changes.

The temperature screening study indicates that *P. rubi* and *P. fragariae* mycelial growth increased from 15°C to 18°C and to 21°C but decreases at 25°C and is fully inhibited at 28°C. Even though the effect of the temperature is significant on the development of *P. rubi* mycelia, it is not the case for sporulation, which demonstrates the flexibility in sporangia production across the temperature range tested. Wilcox and Latorre (2002) found similar patterns for *P. rubi* mycelial growth rising at temperatures up to 25 °C and reducing at higher temperatures, though no differences amongst isolates were examined. Bain and Demaree (1945) and Leonian (1934) observed the same temperature responses for *P. fragariae*, with an optimum between 18°C and 22°C and a declined rate of growth at 25°C. Comparing the two closely related species, *P. rubi* and *P. fragariae*, shows that the latter does not consistently thrive under temperatures higher than 21°C. Indeed, the mycelial growth of two *P. fragariae* isolates, BC-1 and BC-16, is inhibited at 25°C, while isolates NOV-9 and SCRP245, as well as all eight *P. rubi* isolates showed mycelial development at 25°C. Although a non-significant increase in sporangia numbers was observed at 18°C and 21°C compared to 15°C, previous studies have used low temperatures for the production of sporangia and zoospores (Bain and Demaree, 1945; Goode, 1956; Converse, 1962; Felix, 1962; Mussel and Fay, 1973). These studies show that both pathogens have a flexible range of temperatures to grow and sporulate in, from 15°C to 21°C.

The chemical screening study in this chapter highlights different responses showing both inter and intra-species diversity. Fluazinam and Fluopicolide treatments lead to a decreased mycelial growth with an increasing dose, showing sensitivity of the *P. rubi* and *P. fragariae* isolates to the chemicals. Dimethomorph had the best inhibitory effect on the mycelial growth of the isolates tested, to an adequate level. Metalaxyl-M incorporation in growing medium presents a variety of responses depending on the isolate screened, with both inhibition under high doses of the chemical and resistance, where isolates grow with no statistical difference compared to controls, as previously reported by numerous studies on *Phytophthora* species, such as *P. fragariae* (Nickerson, 1998), *P. infestans* (Elansky et al., 2007; Zhu et al., 2008; Pérez et al., 2009; Rekanovic et al., 2012; Randall et al., 2014), *P. capsici* (Parra and Ristaino, 2001; Qi et al., 2012) and *P. palmivora* (Vawdrey et al., 2004). Elansky et al. (2007), Zhu et al. (2008) and Rekanovic et al. (2012) also observed very little or no resistance to Dimethomorph, further confirming these findings. This chemical study emphasizes on the

potential for Dimethomorph (Paraat) and Fluazinam (Tizcan), which are still allowed for use on raspberries and strawberries, to control *P. rubi* and *P. fragariae* mycelial growth at a dose that is applicable in the field.

Dimethomorph and Metalaxyl-M incorporation in growing media lead to different responses and sensitivity; however, screening of corresponding chemical target genes (cellulose synthase genes for dimethomorph and RNA polymerase genes for Metalaxyl-M) did not lead to polymorphism that could explain resistance to those chemicals. The scientific evidence of how chemical treatments for *P. rubi* work is lacking and most are not well understood, which made conclusions from this data difficult at present.

P. rubi and *P. fragariae* genotypic diversity study: Pathogen Enrichment Sequencing (PenSeq)

PenSeq was proven to be a very useful tool in the assessment of inter and intra-species diversity. This technique was successfully adapted from Thilliez et al. (2019), where it was used on *P. infestans* and *P. capsici*, two *Phytophthora* species from distinct clades (clade 1 for the former and clade 2 for the latter). This study is the first time that this enrichment sequencing technique was used on such closely related *Phytophthora* species, allowing comparison of selected genes (here effectors and housekeeping genes). Enrichment validation (detailed in previous CP173 report) as well as mapping and coverage analyses prove that this PenSeq method worked, enriching the genes of interest for all 12 isolates screened. Around 15 000 genes could therefore be analysed in terms of presence / absence and polymorphism, giving great insight into the two pathogens genetics. Polymorphism was found in usually conserved housekeeping genes, though only showing a few SNPs leading to amino acid changes. For instance, the GIP2 (glucanase inhibitor protein 2) gene showed SNPs amongst isolates of both species, *P. rubi* and *P. fragariae,* with resulting amino acid changes in the protein. Glucanase inhibitor proteins (GIPs) are secreted in order to inhibit the activity of plant extracellular enzymes, such as β(1,3) and β(1,6) endoglucanases (EGases) that are involved in plant defenses responses. Polymorphism in such genes would allow for more adaptation of the isolates response to the plant defenses system. PenSeq analyses found 6448 genes identical between all 4 isolates of *P. fragariae*, corresponding to 43% of all genes screened; and 4350 genes identical between all 8 isolates of *P. rubi*, 30% of all genes screened (a smaller proportion due to a higher number of isolates assessed). Most diverse isolates and gene per species were identified: SCRP245 for *P. fragariae* (English isolate from 1945) and SCRP249 for *P. rubi* (German isolate from 1985). Further analyses focussing on inter-species diversity (i.e. differences between the two species on the same genes) confirmed the distinction between the two species; and found 600 genes that were identical between the 12 isolates (both species). It also highlighted species-unique genes, with 142

genes identical between all 4 isolates of *P. fragariae* but completely absent from any *P. rubi* (genes unique to *P. fragariae*) and 69 genes identical between the 8 *P. rubi* isolates and absent from all *P. fragariae* (genes unique to *P. rubi*). Although the two pathogens are very closely related, and believed to be the same species for many years, *P. rubi* infects raspberries only, while *P. fragariae* infects strawberries; consequently species-unique genes could play a key role in host choice and recognition.

Overall, PenSeq has proven to be an excellent method for identifying diversity within and between species for genes of interest, such as key infection proteins: effectors. The diversity evaluated in this analysis shows potential to evolve, which will depend on factors such as crop resistance and chemical applications.

Hydroponic cultures of raspberry

While raspberries are important and are becoming more and more popular fruits due to their numerous health benefits; growing techniques such as hydroponics are also used more often because of the associated environmental benefits. There is very little research carried out on growing raspberries hydroponically. The objective of hydroponics in this study was to provide easy access to the roots, with minimum damage, to follow disease progression. This can be transposed to numerous studies (root pathogens/nematodes; root colonization or morphology etc.), where roots of hydroponically grown raspberries can be assessed and monitored easily. The hydroponic method used in this project was extended to other berries at The James Hutton Institute and greatly extended our knowledge of berries in hydroponics with techniques that are constantly updated and investigated to find the optimum conditions.

P. rubi infection and life cycle

This study shows that infection assays using transgenic *P. rubi* in hydroponic were effective and constitute a good method to study root pathogens in conditions allowing clear access and assessment of roots. Transformation did not impact the ability of *P. rubi* to form reproduction and infection structures such as sporangia and zoospores and these were used to infect roots of raspberry plants grown under the nutrient film technique. Confocal photos of infected roots showed progression of *P. rubi* at different time points, in two susceptible cultivars: Glen Dee and Glen Moy.

Conclusions

This study provides novel information on *P. rubi* and *P. fragariae* isolates.

- In this project, raspberries have successfully been grown in hydroponics, and have successfully undergone and recovered from induced dormancy. Several berries are now

growing under this method at The James Hutton Institute. Details on which fertiliser, methods and cultivars could be used for NFT were narrowed down.

- *In vitro* responses to agriculturally important factors such as chemical application and effects of temperature on mycelial growth and sporulation were assessed. It shows the difference in efficacy of controlling products and the presence of resistance amongst specific isolates. Adequate and maximum temperatures for mycelial growth and sporulation are discussed.
- Genetic differences amongst isolates of one species and between the two closely related pathogens were highlighted using Pathogen Enrichment Sequencing (PenSeq), identifying very conserved genes, as well as unique ones.
- More insights into the progression of *P. rubi* in raspberry roots during infection were gained, using a transgenic *P. rubi* expressing a red fluorescent protein. At day 7, infiltration of the roots had occurred, and hyphae were colonizing the central vascular cylinder. By day 11, new sporangia had formed, which released new zoospores or a second wave of infection (re-infection) of the tissue which was confirmed by expression levels of hautoria-specific membrane protein HMP1.

Future work

- Hydroponics techniques are regularly being reviewed and tested on other raspberry cultivars as well as other berries.
- More chemical target genes will be assessed to look for resistance SNPs.
- A 2nd PenSeq run screening a further 12 isolates of *P. rubi* has been carried out and analysis is on-going to confirm previous observations. Additionally, validation through conventional PCR of some presence/ absence of genes of interest observed with PenSeq computational analyses is also on-going. Interesting genes will also be assessed with models such as Whisson et al. (2007) and Win et al. (2007), in order to gather a list of candidate RxLRs effectors, which could be used in various future studies and / or the life cycle qRT-PCR analysis, for expression levels during infection.
- More infections will be set up, with very concentrated *P. rubi* inoculum, in order to maximize the levels of *P. rubi* RNA present in the infected roots for qRT-PCR analysis (expression levels) of candidate RxLRs effectors, identified through PenSeq, and additionally confirm the expression levels of life markers.

Knowledge & Technology Transfer and Public Engagement

- **The James Hutton Institute seminars and retreats**
- **AHDB Crops PhD Conference 2020**, Nottingham – Presentation

Title: My raspberry root rot quest: the story behind infection

- **Scottish Microscopy Society Symposium**, Edinburgh, December 2019 – Poster

Title: Who are you rooting for? Investigating *Phytophthora rubi* infection of raspberries – focus on imaging the infection cycle

- **IS-MPMI XVIII Congress** (International Society for Molecular Plant-Microbe Interactions), Glasgow, July 2019 – Poster

Title: Who are you rooting for? Investigating *Phytophthora rubi* infection of raspberries – overview of the project. **BSPP Travel Award** for MPMI

- **SoapBox Science Edinburgh, June 2019 – Street presenter**

Pitch: “Small matters! Have you ever wanted to know what lies beneath your feet when you walk in a field? Come and talk to us for a "zoom-in" on pathogens! You'll learn that even -& especially- the smallest things matter in plant science & how we study them to fight them.” Concept based on Russian dolls prop, representing layers from field, to whole plant, to cells and finally to genes and specific DNA, with explanations on the project studies at each level.

- **SEFARI PhD Conference, June 2019** – Presentation - **won 2nd prize**

Title: My raspberry root rot quest: the story behind infection

- **Plant Power Day,** Dundee, May 2019
- **The James Hutton Institute Students Symposium, March 2019** – Presentation – **won 1st prize**

Title: My raspberry root rot quest: the story behind infection

- **University of Dundee Life Sciences Symposium 2019**, Crieff – Poster

Title: Who are you rooting for? Investigating *Phytophthora rubi* infection of raspberries – focus on imaging the infection cycle

- **SSCR (Scottish Society for Crop Research) – Soft Fruits Winter meeting**, JHI, Invergowrie, February 2019 – Poster

Title: Raspberry Root Rot: Investigation of the story behind the infection: Towards a better understanding of *Phytophthora rubi*

- **AHDB Crops PhD Conference 2018**, Solihull – Poster

Title: Raspberry Root Rot: Investigation of the story behind the infection: Towards a better understanding of *Phytophthora rubi*

- **BCPC (British Crop Protection Conference),** Cambridge, October 2018

Title: Raspberry Root Rot: Investigation of the story behind the infection: Towards a better understanding of *Phytophthora rubi*

- **Pupils visit at The James Hutton Institute,** August 2018
- **University of Dundee Public Engagement visit**, June 2018
- **Cell-ebration of science,** University of Dundee, May 2018
- **SFAM (Society For Applied Microbiology): Plant Pathology 'from field to clinic'**, London, April 2018 – Poster

Title: Raspberry Root Rot: Investigation of the story behind the infection: Towards a better understanding of *Phytophthora rubi* (raspberry root rot) and *Phytophthora fragariae* (strawberry red core)

- **The James Hutton Institute Students Symposium, March 2018** – Poster Presentation – **won 1st prize**

Title: Raspberry Root Rot: Investigation of the story behind the infection

- **University of Dundee Life Sciences Symposium 2018**, Crieff – Poster

Title: Raspberry Root Rot: Investigation of the story behind the infection: Towards a better understanding of *Phytophthora rubi* (raspberry root rot) and *Phytophthora fragariae* (strawberry red core)

- **AHDB Crops PhD Conference 2017**, Stratford upon Avon
- Exchange of protocols with ADAS for cane isolation, infection, reproduction structures identification; videos of zoospores release and infection

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