Final Report

May 2023

Student Project No. CTP-FCR-2020-9

Title: Towards the characterisation of the raspberry root rot complex

Investigating raspberry root rot

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Report No: [AHDB Use only]

This is the final report of a PhD project that ran from May 2020 to April 2023. The work was funded by AHDB, Harper Adams University and the British Society for Plant Pathology (BSPP).

CONTENTS

1. Industry Summary

Raspberry root rot (RRR) caused by a consortium of oomycete species is an economically important disease of raspberries. Current management practices rely on an integrated program of chemical control via fungicides and cultural methods such as irrigation system sterilisation and planting resistant genotypes. The increasing deauthorization of fungicides with activity against *Phytophthora* species coupled with improving diagnostic technologies have prompted further research into the consortia of oomycete species involved in plant diseases like RRR.

The aim of this study was to investigate the *Phytophthora* species present in the roots of plants exhibiting symptoms of root rot on nine commercial raspberry farms in the UK through traditional methods of isolation and molecular diagnostics such as lateral flow devices, polymerase chain reaction (PCR), and high throughput sequencing (HTS). Furthermore, the pathogenicity of the isolates obtained was assessed on a panel of commercially relevant raspberry varieties through detached leaf, root, and whole plant pathogenicity assays.

Four isolates of the *Phytophthora* species *P. citrophthora* and one isolate of *P. erythroseptica*, *P. cryptogea* and *P. pseudocryptogea* were recovered from symptomatic raspberry roots via isolation. Six isolates of the *Phytopythium* species *Pp. litorale* and one isolate of *Pp. vexans* were also recovered, a first report of these species in raspberry and the UK. High throughput Illumina sequencing revealed 41 distinct sequences amplified across sites corresponding to nine *Phytophthora* species, four *Globisporangium* species, three *Peronospora* species and three *Phytopythium* species. *Phytophthora rubi*, *Phytophthora cactorum*, *Phytophthora citrophthora,* and *Phytophthora bishii* - a relatively new introduction to the UK, were detected in 100% of samples. *Peronospora sparsa*, the causal agent of downy mildew in roses, and *P. rubi* were the two species with the highest abundance across all samples. Farm location was the most significant factor affecting the diversity and abundance of the species detected.

Subsequent pathogenicity testing on detached leaves and roots revealed plant genotype has a significant effect on the virulence of the isolates obtained in this study. The *Phytopythium* species *Pp. litorale* and *Pp. vexans* exhibited high and moderate pathogenicity on raspberry, respectively, resulting in fast-growing lesions on detached raspberry leaves and roots. Additionally, crown and root rot were observed in whole raspberry plants eight weeks after inoculation with zoospores of *Pp. litorale* and *Pp. vexans*.

This study has demonstrated the diversity of root rot-causing species associated with RRR in the UK, adding to our understanding of the disease. Furthermore, two new pathogens of raspberry

have been identified which are targets for further research into breeding varieties with a more broad resistance.

2. Introduction

Root rot of the European red raspberry (*Rubus idaeus*), caused by a yet-unknown consortium of *Phytophthora* species, is a recurring and destructive disease of this commodity fruit. Raspberry root rot (RRR) was first noted in the United Kingdom in 1980 (Duncan et al., 1987). The disease is most frequently observed during persistent periods of high rainfall and humidity and when the crop is in high productivity. While RRR begins as an infection of the plant root system, symptoms observed above-ground include premature chlorosis, leaf wilt, red-brown cane necrosis, floricane death and stunted primocane growth (Wilcox, 1989). Infected plants have sparse foliage with few emerging primocanes. Leaves of infected canes are bronzed and striped with scorching at margins.

Below ground, fine lateral roots of infected plants are characteristically red/brown and are soft and easily crushed. These plants can produce feeder roots, but they are weak and cannot absorb the nutrients needed to sustain growth and fruiting (Stewart et al., 2014). This timing corresponds with the most economically important stage of raspberry growing, thus severely impacting a grower's ability to profit from this work-intensive crop. As such, RRR is a significantly limiting factor in UK raspberry production.

Historically, *Phytophthora* root rot has been attributed to more species than just *P. rubi*. Duncan *et al*. (1987) reported the pathogenicity of *Phytophthora megasperma*, *Phytophthora erythroseptica* and *Phytophthora dreschleri* (considered synonymous with *Phytophthora cryptogea*) on red raspberry in the UK (Cline *et al.*, 2008). Wilcox (1989) also investigated the pathogenicity of *P. megasperma*, *P. cryptogea*, *Phytophthora cactorum, P. citricola and Phytophthora fragariae* var. *rubi* (now known as *Phytophthora rubi*) on raspberry in New York, US. Wilcox and Latorre (2002) observed *P. cryptogea*, *P. citricola*, *P. rubi*, *P. megasperma* and *Phytophthora gonopodyides* in Chilean raspberry plants. Additionally, *Phytophthora bisheria* (now known *as Phytophthora bishii*) was reported in red raspberry in Australia (Abad *et al.*, 2008). Recent reports indicate more *Peronosporales* species are aossciated with raspberry root rot than previously thought.

Due to deauthorisation of many chemical control agents, current disease management relies on prevention through cultural practice and growing resistant cultivars. Infection prevention is employed through securing clean planting material, maintenance of freely draining soil and growing resistant cultivars. We hypothesise that there are more species than *P. rubi* responsible for RRR in

the UK and that *P. rubi* is less prevalent than previously reported. Breeding programmes typically use *P. rubi* to test plant susceptibility to *Phytophthora*, however, changing species diversity in the RRR complex may impact the reliability of these genotypes in the field.

This project will employ traditional methods of *Phytophthora* identification such as isolation with selective media, and morphology, in addition to molecular methods such as PCR, Sanger sequencing and High Throughput Sequencing (HTS) to determine *Phytophthora* species diversity. The pathogenicity of the resultant isolates will also be determined via detached leaf and whole plant trials. The findings from this project will improve our understanding of RRR in UK raspberry production and inform growers and breeders on how best to reduce the disease.

Through improving our understanding of the prevalence of these pathogens and their associated risk, mitigation and treatment systems can be developed to reduce their impact on UK production and their spread throughout the global raspberry growing network.

3. Materials and methods

3.1. Sampling and isolation

The roots of raspberry plants exhibiting symptoms of RRR were sampled in September 2020 and September 2021. Samples were taken from 13 farms, four of which were sampled in 2020 (I-IV) and nine in 2021 (1-9) plants of 11 different varieties (A-K), see Figure 1 for approximate sampling locations. Isolation was performed from the diseased roots according to a modified version of the method outlined in Stewart *et al.* (2014). DNA from the resultant isolates was extracted using the Sigma-Aldrich Extract-N-Amp™ Plant extraction and dilution buffers (Sigma-Aldrich, UK), following the manufacturer's protocol. PCR using the ITS5 and ITS4 primer set and Sanger sequencing was performed on the DNA from these cultures (White et al., 1990). The sequences were run through the BLASTN database to identify the species (Altschul et al., 1990).

Figure 1: Approximate location of farms sampled as part of this study.

3.2. Molecular analysis

One hundred milligrams of roots from each sample was freeze dried prior to DNA extraction using the Qiagen PowerSoil Pro Kit (Qiagen) as per the manufacturer's instructions. The DNA was amplified using the *Phytophthora* genus-specific primer pair YPh1F and YPh2R, which amplified a portion of the *Ypt1* gene. The amplicons were then sent for Sanger sequencing and identified using the BLASTN database (Schena *et al*., 2008).

To determine the diversity and abundance of *Phytophthora* and *Phytopythium* species present in UK raspberry roots, freeze-dried samples were sent to Novogene for metabarcoding using the Illumina platform. The taxonomic α diversity indexes Simpson and Shannon were assessed, and β diversity was assessed using principal components (PC) and non-metric multidimensional scaling (NDMS) using Bray-Curtis distances. All bioinformatic and statistical analysis was performed in R.

3.3. Pathogenicity testing

To assess the risk of the species isolated in this study to commercial raspberry varieties grown in the UK, detached leaf, root and whole plant pathogenicity testing was conducted. The pathogenicity of the isolates obtained and the susceptibility of five raspberry varieties were assessed; Tulameen (susceptible to root rot), Valentina, Latham (resistant to root rot), and two proprietary varieties coded Variety 4 and Variety 5. Sterilised leaves (four biological replicates per variety) were floated in a 10% (w/v) soil extract solution into which six plugs taken from the growing edge of 10-day old isolates recovered from farm sampling has been transferred. The floats were incubated at 20 °C and observed daily for signs of infection. The leaves were incubated for seven days. Lesion area and disease percentage for each leaf was calculated using the American Phytopathological Society (APS) Assess 2.0 software (Lamari, 2002). The percentage disease of each leaf was analysed using a one-way analysis of variance (ANOVA) and Tukey's HSD (honestly significant difference) test using R statistics software. Seven days after inoculation, leaves exhibiting lesions were removed from the soil extract, sterilised, and placed on *Phytophthora*-specific media. Re-isolation from the diseased leaf lesions was performed according to the method outlined in Stewart et al. (2014).

To determine if the isolates obtained can cause disease on raspberry roots, detached root inoculation was performed according to a modified version of the method outlined in Pathrose et al. (2010). Sterilised roots of the same varieties used in the leaf assay were placed on a moist sterile filter paper in a 90 x 15 mm Petri dish. The cut end of the root was suspended in 500 µL sterile water in a 1.5 mL Eppendorf tube sealed with Parafilm™ to maintain hydration throughout the course of the experiment. The apical portion of the root was wounded using a sterile inoculation needle and 5 µL of a $1x10^4$ zoospores/mL suspension from each isolate was pipetted onto the wound. The Petri dish was sealed with Parafilm™ to maintain humidity and incubated at 20 °C in the dark for 14 days. Each isolate:variety treatment was replicated three times.

To re-isolate the oomycetes, the Petri dish was split into four sections (Figure 1), each zone represented the spread of the infection via tissue necrosis through the root. Zone 1 included the inoculation point at the apical end of the root, Zone 2 was mid-apical zone of the root, Zone 3 was the mid-basal zone of the root and Zone 4 was the basal end of the root segment i.e., the end growing closest to the plant crown, see Figure 2. This zonation was to assess the spread of each isolate i.e., how far it had travelled through the root. The roots were assessed every day for 14 days and necrotic zones were recorded.

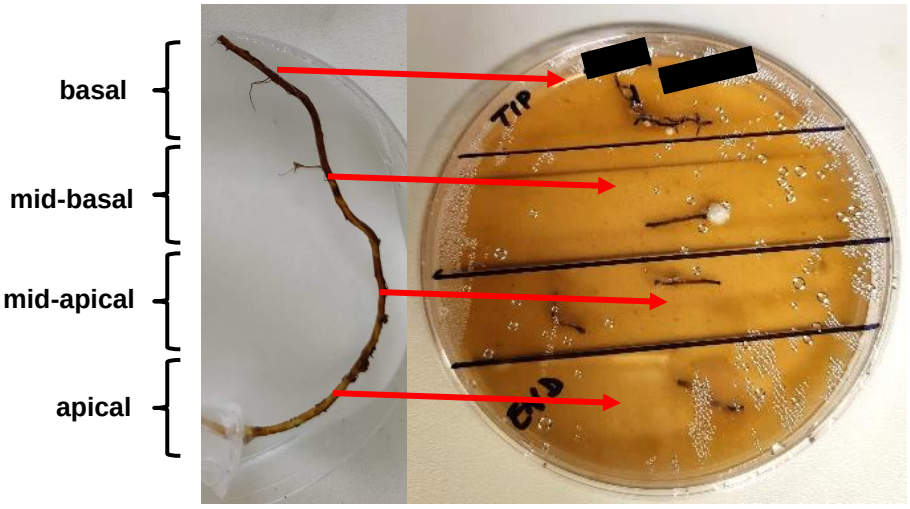

Figure 2: Example of zonation of roots which had been inoculated with the zoospores of isolates of *Phytophthora* and *Phytopythium* isolates and incubated for 14 days. The areas noted on the left; basal, mid-basal, mid-apical and apical indicates where the root was subsectioned for re-isolation.

Five root sections per sample were aseptically transferred to 9 cm Petri dishes containing V8-PARP. The diseased root tissue were sterilized in 70% ethanol four pieces of root per variety-isolate pairing were carefully submerged in V8-PARP media. The plates were incubated in the dark at 20°C until mycelial growth was observed. The hyphal tips of growing colonies were transferred onto fresh V8-PARP and their zone of origin was noted. A rapid DNA extraction, PCR with ITS4 and ITS 5 primers and Sanger sequencing to confirm isolate identity was performed.

To assess the ability of the isolates to infect whole raspberry plants, a trial was established wherein 160 one-year old primocane plants of varieties Tulameen, Valentina, Latham and Variety 4 and 5 (32 plants per variety) were inoculated with 40 mL of a $1x10^4$ zoospores per mL suspension of each isolate. The experiment was run for 8 weeks or until all inoculated plants exhibited symptoms of disease. At the end of the experiment, each root system was scored according to disease percentage from 0-100%. Analysis of variance (ANOVA) of the overall root disease scores was performed in R Studio v1.4.05.5. Re-isolation, rapid DNA extraction, PCR with ITS4 and ITS 5 primers and Sanger sequencing to confirm isolate identity was performed according to the method outlined above.

4. Results

4.1. Isolation and molecular analysis

Due to variation in cultivar susceptibility to RRR, and some growers having a primary cultivar with an additional smaller crop of a secondary cultivar, cultivars were not sampled equally. The isolation of symptomatic tissue onto selective agar resulted in 29 isolates which produced *Phytophthora*-like sporangia. All isolates were recovered from four farms in the West Midlands and Scotland (Table 1).

Table 1: Locations of farms in which *Phytophthora* and *Phytopythium* isolates were recovered.

Code	Farm location
4	Herefordshire, England
5	Perthshire, Scotland
6	Perthshire, Scotland
7	Perthshire, Scotland

Phytopythium litorale was the most frequently isolated species, with six isolates obtained, four of which came from cane material and two were from roots. Out of the nine farms sampled in 2021, isolates came from only four sites which showed high RRR disease incidence (over 70% incidence reported by the grower).

Sanger sequencing confirmed the identity of 12 isolates as five species of *Phytophthora* and seven isolates of two *Phytopythium* species by sequencing of the ribosomal *Ypt* gene region (Table 2).

Table 2: Sanger sequencing results from the amplification of the ITS region of DNA extracted from isolates obtained from UK raspberry farms using the ITS4 and ITS5 primer pair.

Species	**Similarity (%)**	**Number of isolates**	**Variety code**	**Farm code**
Phytophthora citrophthora	99.5	2	A	4
Phytophthora cryptogea	100	1	C	7
Phytophthora pseudocryptogea	98.7	1	C	4
Phytophthora erythroseptica	98.2	1	B	6
Phytopythium litorale	99.8	6	A,C,E	4,5,6,7
Phytopythium vexans	99.6	1	D	6

Molecular analysis via amplification and sequencing of the ITS region directly from diseased plant samples using the Yph1F and Yph2R primer pair detected nine *Phytophthora* species in 16 samples from seven farms (Table 3). *Pp. litorale* was the most frequently detected species.

Notably, *P. rubi* was detected in just one raspberry and this site had very low reported occurrence of RRR. No *Phytopythium* species were detected in raspberry roots as *Phytophthora*-specific primers were used for these samples.

Table 3: Sanger sequencing results from amplification of the *Ypt* gene region of DNA extracted from symptomatic raspberry roots using the Yph1F and Yph2R primer pair.

***Phytophthora* species**	**Number of samples**	**Variety code**	**Farm code**	**Country**
P. citrophthora	4	A	4	Scotland
P. plurivora	3	A,D	2,4	England
P. idaei	2	B	2,6	England
P. rubi	2	B, E	1,5	Scotland
P. pseudocryptogea	1	D	4	Scotland
P. hedraiandra	1	C	7	Scotland
P. meadii	1	A	5	England
P. ilicis	1	B	6	Scotland

4.2. Metabarcoding analysis

From a total of 134 root samples taken, 87 yielded a PCR product with the *Ypt1* primers. Sixty two out of these 87 samples sent to Novogene for DNA extraction and amplicon sequencing yielded ITS PCR products. Samples from Farm I and Farm II did not produce any *Phytophthora* PCR product using the 18Ph2F/5.8S-1R and ITS6/5.8S-1R primer set, however, these samples did produce a PCR product with the *YpT1* gene primers (Table 4).

Table 4: Number of samples per farm which produced a PCR product using the YpT1 primers and were sent for sequencing, and those which produced high quality clustered reads through metabarcoding using ITS primers.

Farm	**Location**	**# of samples taken**	**# of samples positive for *ypt1***	**# of samples positive for ITS**
I	Kent, England	7	2	0
II	Kent, England	7	2	0
III	Kent, England	5	2	3
IV	Kent, England	5	4	4
1	Stafford, England	15	10	10*
2	Ludlow, England	15	10	4*
3	Hereford, England	15	5	1
4	Stafford, England	17	10	9*
5	Perth, Scotland	10	10	7
6	Aberdeen, Scotland	15	16	13
7	Perth, Scotland	15	10	5
8	Kent, England	4	2	2
9	Kent, England	4	4	4
Total number of samples		**134**	**87**	**62**

*Two asymptomatic samples from Farms 1, 2 and 4 were sent for amplicon sequencing to assess the effect of plant health on the diversity and abundance of *Peronosporales* species.

Taxonomic classification of the metabarcoding data revealed a high percentage of *Phytophthora* across all farms (Figure 3). *Peronospora* species, a genus which includes the rose downy mildew-causing pathogen *Personospora sparsa*, were high in farms II, 1 and 2. Metabarcoding revealed a high diversity of *Phytophthora*, *Phytopythium*, *Peronospora* species present in UK raspberry farms (See Table A1 in the Appendix).

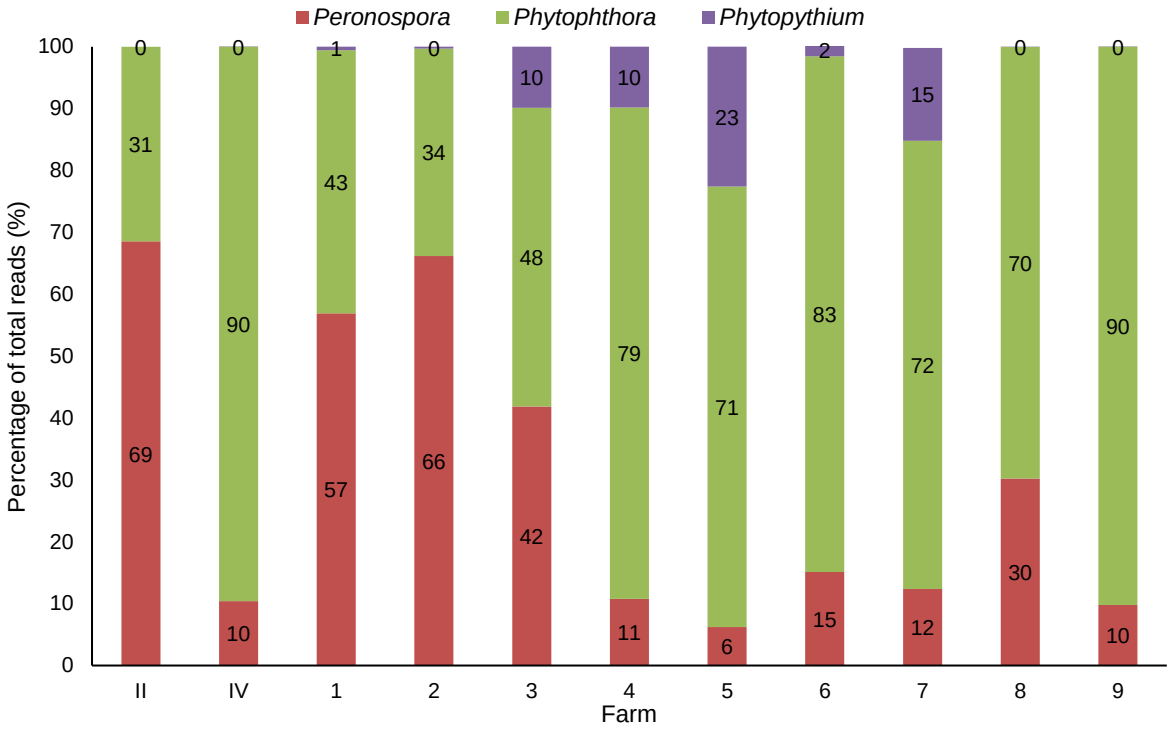

Figure 3: Percentage of total reads of the *Peronospora*, *Phytophthora* and *Phytopythium* species present on nine commercial raspberry farms in 2021.

Amplicon sequence variant analysis revealed *P. rubi* as the most frequently detected species across all farms, the species was detected in 100% of samples and comprised 32% of the total reads for all samples. Farm 1 in the West Midlands had a consortium of eight *Phytophthora* species present, while Farms 8 and 9 in Kent had just *P. rubi*, *P. cactorum* and *P. citrophthora*. *P. pini*, *P. crassamura* and *Phytophthora* sp1 were not detected in samples taken on farms in Kent. *Phytophthora cryptogea* and *P. pseudocryptogea* were detected in one sample on Farm III and one sample on Farm IV in Kent (Table 5).

P. rubi, *P. cactorum* and *P. bishii* were detected on all varieties sampled. *P. citrophthora* was only detected on samples from Variety A, B, D and E. *P. crassamura* was detected on samples from Variety A, B and D. *Phytophthora* sp1 was detected on Variety B and F only (Table 5).
Samples from Farm 7 and Farm IV had the highest percentage of *P. rubi* with 61.8% and 50.5% of reads in each farm, respectively. Sequencing revealed the presence of a phylotype corresponding to *Phytophthora crassamura* in 15 samples and 0.006% of total reads, this species had the highest read in a sample taken from a soil-grown raspberry plant in Farm 1. A phylotype corresponding with a *Phytophthora* species collected from Holm Oak in Spain by Català et al. in 2017 was detected in 14 samples and 0.1% of total reads (Table 5).

Table 5: *Phytophthora* species detected on 11 commercial raspberry farms via metabarcoding.

Farm	Region	Total # of samples	Number of samples with reads								
			P. rubi	*P. cactorum*	*P. citrophthora*	*p. pini*	*P. bishii*	*P. cryptogea*	*P. pseudocryptogea*	*P. crassamura*	*Phytophthora sp. 1*
III	Kent	3	3	3	2	0	3	0	1	0	0
IV	Kent	4	4	4	1	0	4	1	0	0	0
1	W. Midlands	10	10	10	7	7	10	6	4	7	6
2	W. Midlands	4	4	4	3	3	4	3	2	2	1
3	W. Midlands	1	1	1	1	1	1	1	1	0	0
4	Scotland	9	9	9	8	8	9	4	6	2	0
5	Scotland	7	7	7	7	7	7	4	3	1	1
6	Scotland	13	13	13	13	8	13	9	9	2	0
7	Scotland	5	5	5	1	0	5	5	4	0	0
8	Kent	2	2	2	1	0	2	0	0	0	0
9	Kent	4	4	4	1	0	4	0	0	0	0
Total # of samples		**62**	**62**	**62**	**45**	**34**	**62**	**33**	**30**	**14**	**8**

Three *Phytopythium* species were detected; *Pp. litorale*, *Pp. citrinium* and *Pp. vexans* and Farm 6 had the highest number of reads for all three *Phytopythium* species; 2.1%, 1.7% and 0.4% of total farm reads, respectively. Samples from Scottish farms had the highest detection rates for all three *Phytopythium* species. *Pp. citrinium* and *Pp. vexans* were not detected in Kent and were most frequently detected on samples from Scottish farms (Table 6).

Table 6: *Phytopythium* species detected on 11 commercial raspberry farms via metabarcoding.

Farm	Region	Number of samples with reads			
		Total	***Pp. litorale***	***Pp. citrinium***	***Pp. vexans***
II	Kent	3	0	0	0
IV	Kent	4	1	0	0
1	West Midlands	10	4	5	2
2	West Midlands	4	1	1	0
3	West Midlands	1	1	1	1
4	Scotland	9	7	7	2
5	Scotland	7	6	5	5
6	Scotland	13	12	8	9
7	Scotland	5	5	0	0
8	Kent	2	1	0	0
9	Kent	4	1	0	0
Total number of samples		**61**	**39**	**27**	**19**

The α-diversity of a sample indicates the richness and the evenness of species in a sample. The Chao1 index was used as this accounts for species of a low abundance in a sample. The Simpson index accounts for the number of taxa and the abundance and typically gives more weight to dominant taxa. These α-diversity indices were used to assess the effects of location and variety on *Phytophthora* diversity within samples. Farm and plant variety had the largest effect on α-diversity on the Chao1 index, $p<0.002$ and $p< 2x10^{-16}$, respectively, indicating the species richness and evenness of *Phytophthora* communities differ between site and plant variety (Table 7).

Table 7: Permutation-based ANOVA on the alpha diversity index variability accounted for by farm location, plant variety, the interaction between location and variety. Values in bold indicate a *p*-value <0.05.

Indices	*P*-value			
	Farm location	**Variety**	**Interaction**	**Residuals**
Chao1	**0.002**	**$<2x10^{-16}$**	0.073	672
Simpson	0.8824	0.7562	0.8824	13757.2

ADONIS analysis of the Bray-Curtis index also highlighted the significant effect of farm location on β-diversity ($p = 0.001$). There did not appear to be a high diversity of *Phytophthora* between varieties. This farm effect can be seen in the NMDS analysis in ADONIS analysis of the β-diversity using the Bray-Curtis distance similarity index showed farm location had a significant effect on the β-diversity of samples. Samples from the same farm cluster together on the NMDS plot (Figure 3). Samples did not cluster by the substrate type, suggesting this factor does not affect the β-diversity of *Phytophthora* species. However, a clustering was visible in farms in Perthshire and Kent, suggesting samples from these regions have a similar β-diversity on the Bray-Curtis index.

Samples from farms in Herefordshire had similar β-diversity to the other samples, with no apparent clustering (Figure 4).

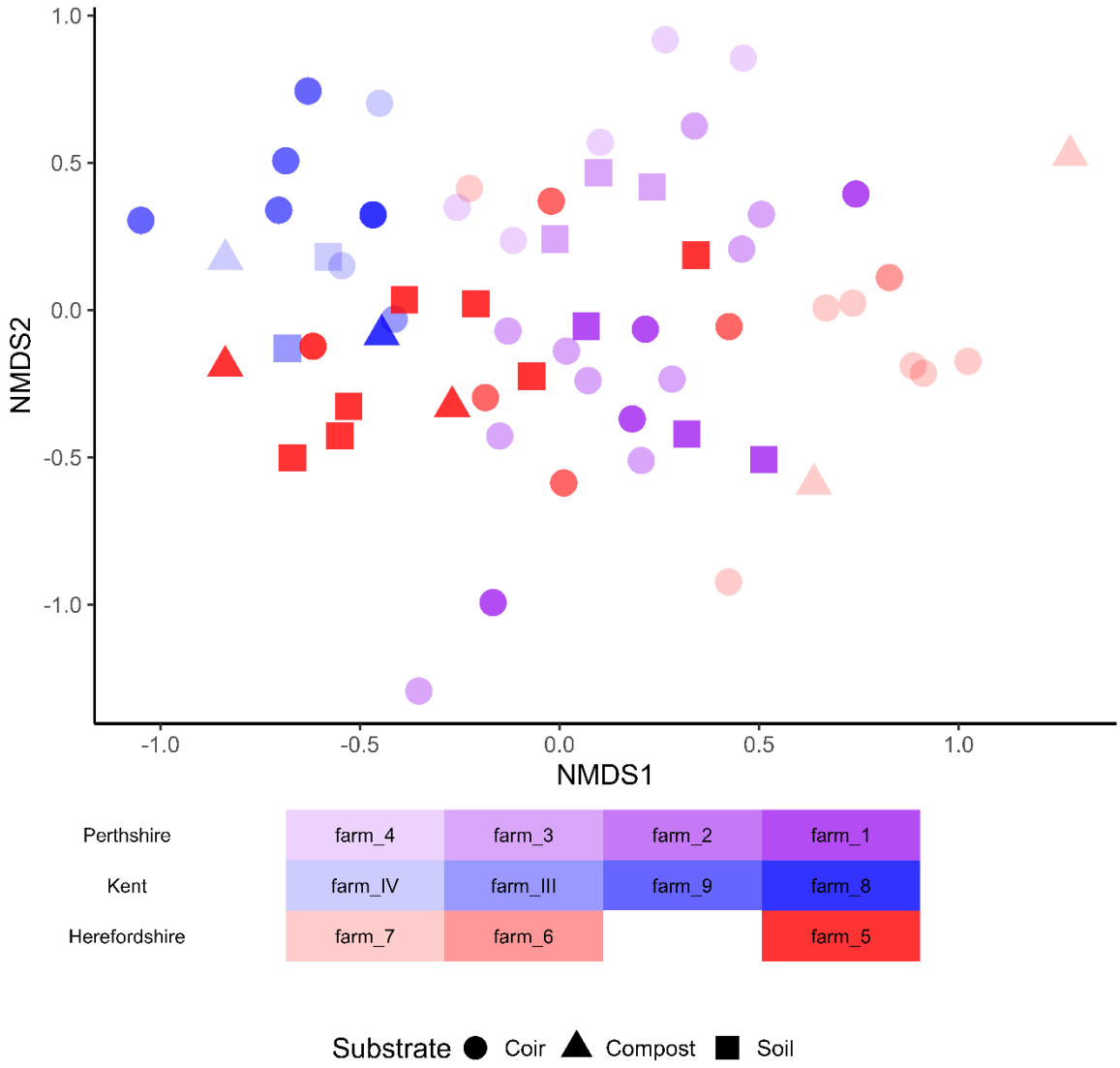

Figure 4: Non-metric multidimensional scaling (NMDS) analysis of the Bray-Curtis similarity index showing β-diversity of *Phytophthora* in each sample. Distance between points equates to dissimilarity. The effect of factors farm, location and substrate type on diversity are shown. Farm regions are separated by colour, different shapes indicate the three substrate types assessed.

4.3. Pathogenicity testing

The *Phytophthora* species *P. citrophthora*, *P. pini*, and *P. rubi* and the *Phytopythium* species *Pp. litorale* and *Pp. vexans* were chosen for pathogenicity testing on a wider panel of isolates as they displayed reliable pathogenicity across three replicates of the pilot trial.

Visual analysis of raspberry leaves after seven days incubation indicated *P. citrophthora*, *P. pini*, *Pp. litorale* and *Pp. vexans* had the highest lesion areas compared to the control treatment. Leaves of Valentina and Variety 4 floated in soil extract infected with *P. rubi* had large lesion areas,

whereas the lesion areas of the other varieties were low, and some leaves had no lesions and appeared healthy (Figure 5).

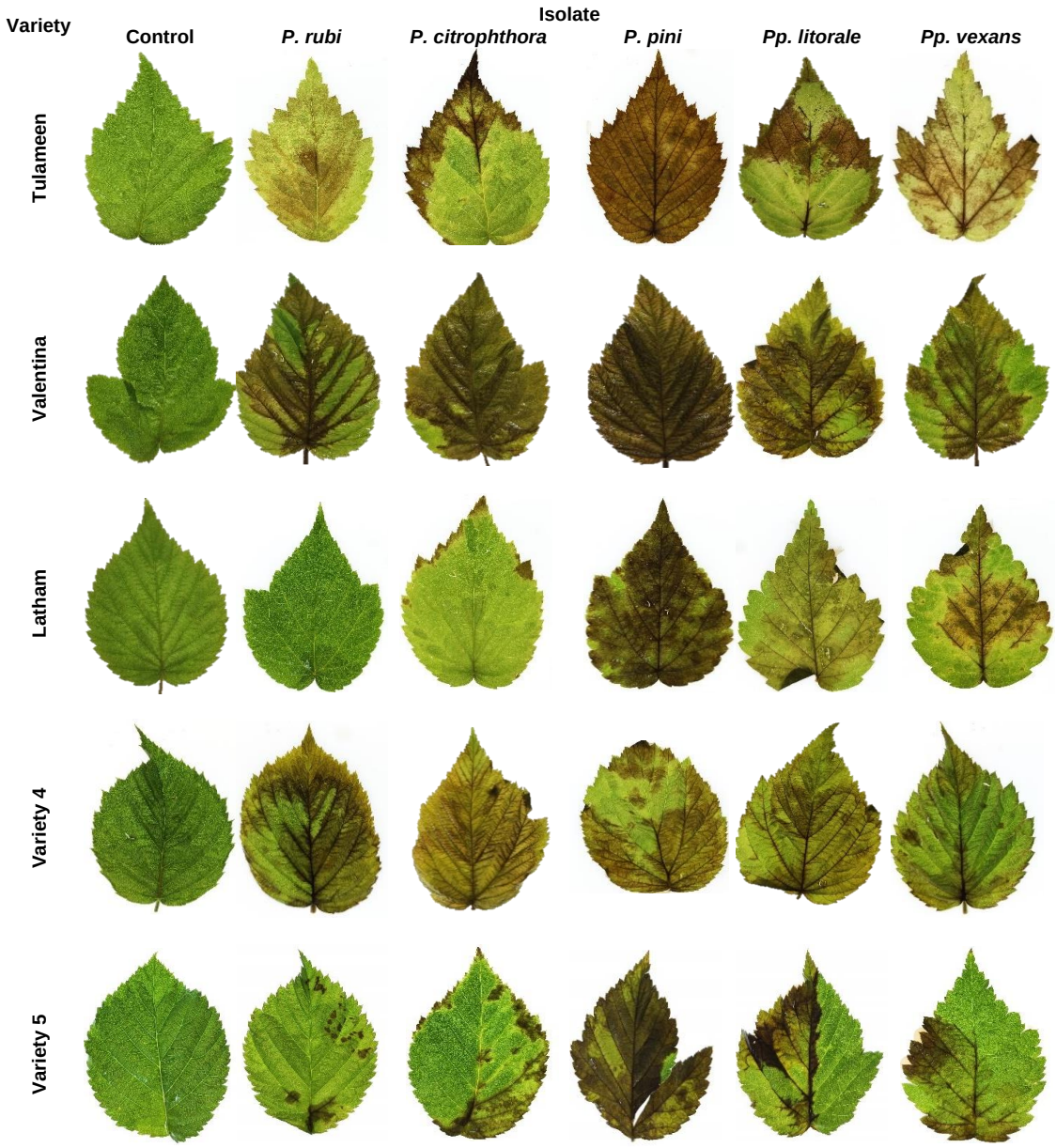

Figure 5: Representative raspberry leaves of five varieties floated in soil extract infested with *Phytophthora* and *Phytopythium* isolates after seven days incubation at 20°C.

ANOVA and Tukey's HSD test on the percentage disease on each leaf confirmed this observation on the isolates tested. Leaves infected with *P. citrophthora* and *P. pini* showed significantly higher disease percentage than the control leaves across all varieties (**Error! Reference source not found.**). *Pp. litorale* showed significantly higher disease percentage than the control leaves on all

varieties tested. No significant difference in the disease percentage of leaves infected with *P. rubi* and *Pp. vexans* compared to the control was observed on all varieties tested.

The high humidity in the Petri dish of the detached root assay caused some opportunistic root fungi such as *Botrytis cinerea* and yeast to grow on some of the roots during the incubation step, including the control roots. Re-isolation from many of the treatment plates was not possible due to excessive contamination of PARP plates with yeasts and other fungi. The species that were successfully re-isolated and their identities confirmed via PCR and Sanger sequencing are presented in Table 8.

Table 8: *Phytophthora* and *Phytopythium* species re-isolated from detached raspberry roots.

Isolate	Variety	Area of root
P. citrophthora	Tulameen, Latham	Basal end
P. pini	Tulameen, Latham, Variety 5	Apical end and Basal end
Pp. vexans	Tulameen, Latham	Apical end and Basal end

The glasshouse housing the whole plants was heavily infested with two-spotted spider mite, a destructive pest of raspberry leaves, in the sixth week of the trial. All of the above-ground portion of the plants were affected; therefore, the above-ground assessments could not be completed, and the symptoms noted cannot be reliably related to oomycete infection. As two-spotted spider mites do not affect plant roots, the trial was continued for two weeks to allow the oomycetes to infect the roots. After eight weeks, the above ground sections of the plants were removed, and the root balls were assessed and given an overall disease score.

The four replicate control plants of Tulameen, Valentina and Latham all showed some disease. No oomycete species were re-isolated from the roots of Latham plants, indicating the root rot disease symptoms noted may come from another pathogen. No statistically significant differences between the overall disease percentage of treated vs control plants were noted (Figure 6).

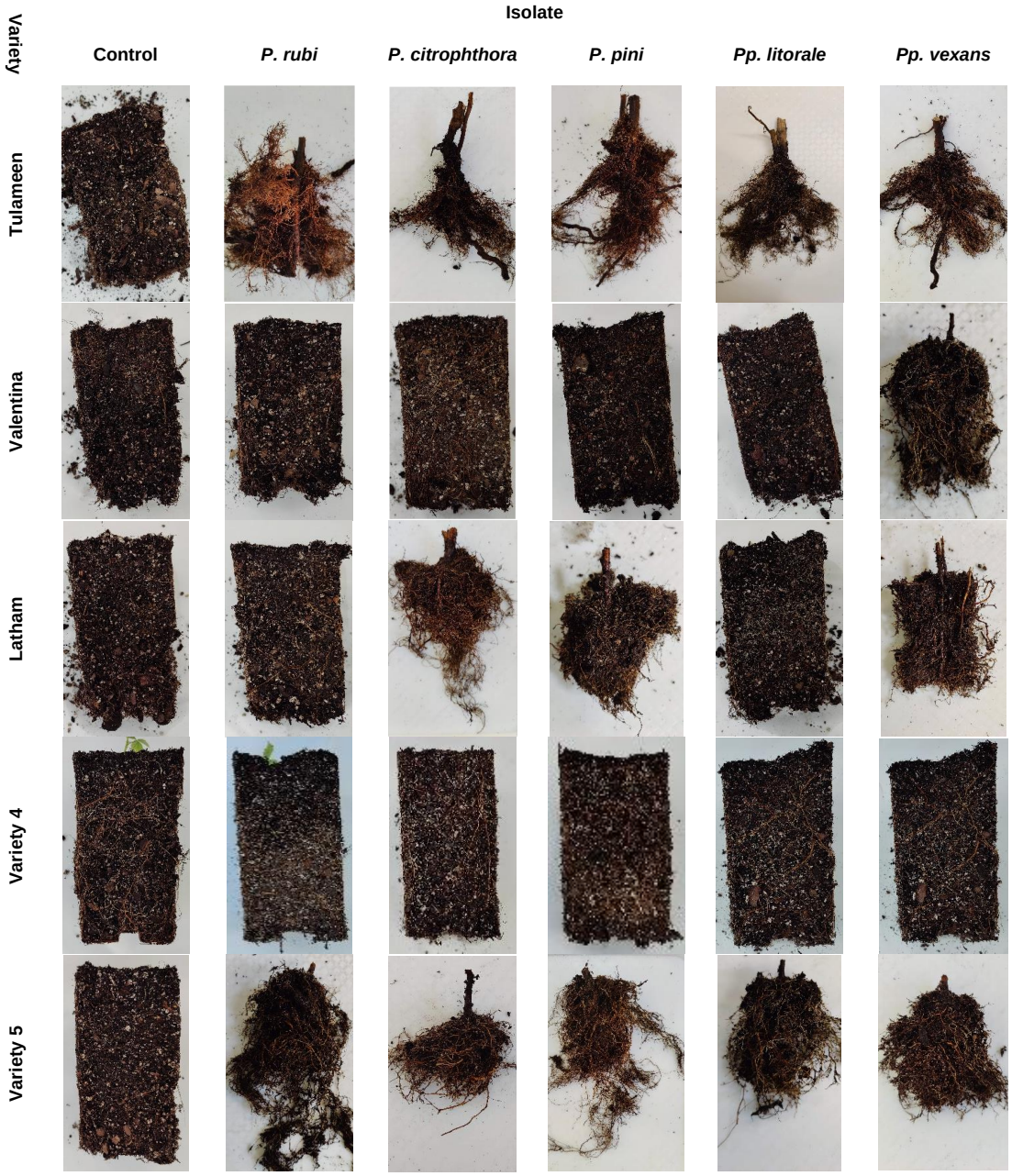

Figure 6: The root balls of raspberry plants of five varieties eight weeks post inoculation with zoospores of *Phytophthora* and *Phytopythium.*

Root and cane dissections of the plants showing root rot symptoms revealed black/brown lesions consistent with *Phytophthora* infection. *Pp. vexans*, *P.pini*, *P. citrophthora*, *Pp. litorale* were successfully re-isolated from these root and cane lesions (Figure 7). No oomycetes were isolated from control plants.

Variety	Isolate	Lesions	Number of reps
1	*P. rubi*		1
1	*Pp. vexans*		2
1	*Pp. litorale*		1
1	*P. pini*		1
3	*P. citrophthora*		2
2	*Pp. vexans*		1
5	*P. citrophthora*		1
5	*Pp. vexans*		2

Figure 7: *Phytophthora* and *Phytopythium* species re-isolated from inoculated raspberry plants of five varieties. The lesions which were isolated from are shown on longitudinal and lateral sections of the root base and cane. The number of plants from which each species could be re-isolated from out of a total of four replicates per variety are given.

5. Discussion

Phytophthora root rot is the most destructive disease of raspberry in the UK, and the majority of previous reports indicate *Phytophthora rubi* as the primary causal agent. This work aimed to assess if colloquial reports of a more diverse *Phytophthora* species composition in raspberry were correct. Together the results of these studies aim to improve our knowledge of raspberry root rot, the species involved in the disease, and factors which can affect its occurrence and severity.

Isolation of species from symptomatic root tissue onto *Phytophthora*-specific media revealed the presence of *P. citrophthora*, *P. pini*, *P. cryptogea*, *P. pseudocryptogea* and *P. erythroseptica* in the raspberry samples. The apparent absence of *P. rubi* in isolations was notable, but not unexpected. *P. rubi* is notoriously difficult to isolate from diseased tissue, potentially owing to its slow growth in comparison to the other species isolated. A notable find of this study was the isolation of *Phytopythium* species *Pp. litorale* and *Pp. vexans* and subsequent pathogen of raspberry (Browne et al., 2023). Methods are currently in production to better detect *Phytopythium* in crops, which may prevent its spread through propagation networks and ultimately mitigate its effects on growers. The results outlined in this project show *Pp. litorale* can be detected using the *Phytophthora* genus-specific LFD. While *Pp. vexans* was not detected using this method, a rapid Loop-Mediated Isothermal Amplification Method (LAMP) has been developed for detection of this species by Wang et al. (2021) which can be used for screening plant material from propagation stock and by plant pathologists surveying for this species.

Similarities and some stark differences were observed in the pathogen populations detected via isolation and PCR, and those detected through metabarcoding. *Phytophthora citrophthora*, *P. pini*, *P. cryptogea*, *P. pseudocryptogea* and *Pp. litorale* and *Pp. vexans* were detected across all three methods. *P. erythroseptica* was only detected through direct isolation, and *P. ilicis* and *P. plurivora* were only detected through PCR and Sanger sequencing with *Ypt1* primers. Metabarcoding revealed a much more diverse *Phytophthora* and *Phytopythium* community than predicted. Notably, *P. cactorum* and *P. bishii* were detected in all samples analysed. *P. cactorum* is not reported as a pathogen of raspberry, however, all the farms sampled also grew strawberry, a well-reported host of *P. cactorum* (Pánek et al., 2022). Cross-contamination may have occurred through poorly sanitized irrigation lines or run-off of excess water from strawberry growth tunnels to raspberry tunnels. A slow-growing isolate from raspberry plants in Australia in 1996 was formally designated as *P. bishii* in 2008 by Abad et al. (2008). The species was detected in raspberry in the UK by Wedgewood et al. (2020) in 2020 and by Stewart et al. (2014) in Washington in 2014. There are no reports of this species as a pathogen of raspberry, however pathogenicity was confirmed in strawberry (Abad et al., 2008). This analysis identified an additional *Phytopythium* species, *Pp.*

citrinium in 44% of samples. The risk of these species is unknown, both *P. bishii* and *Pp. citrinium* may be potential future targets for pathogenicity and host resistance studies.

This work presents an insight into the community of oomycete species associated with raspberry root rot, and the effect of key factors such as farm location and variety on their diversity and abundance. This analysis presents new potential target species for resistance testing and adds to our knowledge of oomycete pathogens of soft fruit. Furthermore, this work highlights the potential risk of these species to UK raspberry production through pathogenicity testing on commercial raspberry varieties grown by UK producers. The limitations of this work include the use of just one isolate of each species in the pathogenicity assessments as just one isolate of *P. pini* and *Pp. vexans* were available. A larger panel of multiple isolates of each species may aid in addressing the intra-species pathogenicity variation observed in previous studies of *Phytophthora* and *Phytopythium* isolates. Due to time and financial limitations of the project, it was only possible to sample the farms in one season (Autumn), however sampling the plants on the grower sites prior to planting and exposure to the potential oomycete community in the planting substrate/irrigation system of the grower may shed some light on the source of these diverse *Phytophthora* species. Future study is needed in order to gain a more comprehensive understanding of the raspberry root rot complex. Further research recommendations include:

- Field pathogenicity trials of *P. citrophthora*, *P. pini*, *P. cryptogea*, *Pp. litorale* and *Pp. vexans.*
- Assessing the effect of biocontrols on *P. citrophthora*, *P. pini*, *P. cryptogea*, *Pp. litorale* and *Pp. vexans.*
- Fungicide and biofungicide sensitivity testing of all species testing in this study.
- Testing the pathogenicity of *P. bishii* and *Pp. citrinium* on UK plants to assess their risk to UK raspberry production.

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7. Appendix

Table A1: Summary of the 41 operational taxonomic units (OTUs) generated using a 97% sequence similarity. OTUs sequences were compared to the GenBank nt database using the BLASTN+ for species identification. Species in bold are those with the highest reads per OTU.

Species	Similarity (%)	Clade (*Phytophthora* only)	Number of reads/OTU	% of total reads	Fragment size (bp)
Globisporangium intermedium	99.56	–	214	0.0	225
Globisporangium perplexum	100	–	84	0.0	220
Globisporangium rostratum	100	–	1085	0.0	295
Globisporangium ultimum	100	–	176	0.0	217
Hyaloperonospora parasitica	99.54	–	3195	0.1	219
Phytophthora bishii	**100**	**Subclade 2d**	**293,780**	**4.3**	**221**
Phytophthora cactorum	**99.54**	**Subclade 1a**	**775,951**	**19.9**	**216**
Phytophthora cactorum	**100**	**Subclade 1a**	**441,299**	**5.8**	**216**
Phytophthora citricola/pini	**100**	**Subclade 2c**	**2,888,842**	**10.2**	**182**
Phytophthora. citrophthora	**100**	**Subclade 2a**	**510,950**	**28.8**	**195**
Phytophthora. citrophthora	**100**	**Subclade 2a**	**59,089**	**3.3**	**193**
Phytophthora cryptogea	**100**	**Subclade 8a**	**14,657**	**0.3**	**205**
Uncultured Phytophthora clone sp1	95.58	Clade 1	323	0.0	212
Phytophthora crassamura	100	Subclade 6b	6,140	0.1	225
Phytophthora pseudocryptogea	100	Subclade 8a	13,540	0.2	206
Phytophthora pseudocryptogea	100	Subclade 8a	5,603	0.1	206
Phytophthora rubi	**100**	**Subclade 7**	**1,389,673**	**32.0**	**230**
Peronospora sparsa	**100**	**–**	**1,235,657**	**28.9**	**214**
Peronospora medicaginis-minimae	100	–	48,296	0.9	219
Peronospora cf. fagopyri	100	–	22	0.0	218
Phytopythium citrinium	97.6	–	13,003	0.2	250
Phytopythium citrinium	98.4	–	11,054	0.2	251
Phytopythium citrinium	98.8	–	486	0.0	249
Phytopythium citrinium	98.8	–	263	0.0	249
Phytopythium citrinium	**98.4**	**–**	**61,856**	**1.8**	**250**
Phytopythium citrinium	98.4	–	32,233	0.6	250
Phytopythium citrinium	100	–	65	0.0	249
Phytopythium citrinium	100	–	127	0.0	251
Phytopythium citrinium	100	–	85	0.0	251
Phytopythium citrinium	100	–	29	0.0	251
Phytopythium citrinium	100	–	30	0.0	251

Phytopythium litorale	**100**	**–**	**166260**	**12.9**	**235**
Phytopythium litorale	100	–	2626	0.0	235
Phytopythium vexans	100	–	30364	0.6	237
Phytopythium vexans	99.16	–	3211	0.1	237
Phytopythium vexans	99.58	–	1250	0.0	237
Phytopythium vexans	100	–	202	0.0	237
Pseudoperonospora urticae	100	–	487	0.0	216
Pythium heterothallicum	97.56	–	8911	0.2	205
Pythium sylvaticum	100	–	363	0.0	288
Pythium anandrum	100	–	523	0.0	162