AHDB Horticulture

STANDARD RESEARCH REPORT

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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.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

Phytophthora may not be the only culprit in raspberry root rot.

Background

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. The disease is most frequently observed during persistent periods of high rainfall and humidity and when the crop is in high productivity. 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, root rot is a significantly limiting factor in UK raspberry production. Current control strategies rely on cultural practices due to the lack of fungicide efficacy. Infection prevention is employed through securing clean planting material, maintenance of freely draining soil and sterilising irrigation lines. Infection risks have led to ~70% of UK raspberry growers moving from field to pot-based cultivation which involves more consumables and labour, increasing the costs involved in raspberry production.

Raspberry root rot is an understudied field of research. Much is to be gained from further understanding the species involved in the disease. This project seeks to elucidate the diversity and pathogenicity of raspberry *Phytophthora* in the UK through large scale surveying of UK grower sites, direct isolation from symptomatic plants and subsequent pathogenicity studies to determine their risk to UK production. Additionally, the project will investigate whether factors such as location, treatment regimes and variety affect the diversity of *Phytophthora* in UK raspberry using state-of-the-art genetic analysis. Findings from this project will add to our knowledge of UK raspberry *Phytophthora* and highlight the importance of the inclusion of other *Phytophthora* species into raspberry breeding programme resistance screens to produce varieties which are more resilient to changes in *Phytophthora* species dominance in raspberry root rot, ensuring clean and robust genotypes for UK growers.

Summary

Surveys and Sampling

In the first year of this project, 13 UK raspberry grower sites were sampled across England and Scotland. Root and cane tissue were taken from healthy plants and plants exhibiting root rot symptoms i.e., wilting, chlorosis, cane lesions. Additionally, a questionnaire was distributed which collected agronomy details and the Raspberry root rot experiences of individual growers.

Root and cane isolations

Diseased cane and roots taken from grower sites were plated onto *Phytophthora*-specific media using a protocol adapted from Stewart et al. (2014) which consisted of cornmeal agar amended with antibiotics (rifampicin and ampicillin) and fungicides (pimaricin PCNB and hymexazol) which reduced the growth of fast-growing fungi and bacteria. Isolates were sub-cultured onto fresh agar plates and grown in the dark at 18°C for 14 days. DNA was extracted from the isolates and from diseased raspberry and a small sample of blackberry roots after which they were sequenced to determine their identity. Twelve isolates of five species of *Phytophthora* and seven isolates of two *Phytopythium* species were collected. To our knowledge this constitutes the first report of *Phytopythium vexans*, *Phytopythium litorale*, *Phytophthora hedriandra* and *Phytophthora meadii* on red raspberry and the first report of *P. meadii* in the UK. *P. rubi* and *Phytophthora bishii* were detected in symptomatic blackberry roots, a first report of both species on blackberry. Pathogenicity testing of these isolates on a range of commercially relevant cultivars is ongoing. Further tests will be conducted using these pathogens to record symptoms of their infection and disease rate.

Detached leaf inoculations

To determine the pathogenicity of the isolates recovered on UK farms, leaves of a proprietary commercial variety were sterilised and inoculated with five species of *Phytophthora* and two species of *Phytopythium* and observed over seven days. Two methods of infection were trialled to assess which is most suitable. These trials are ongoing, with preliminary results being inconclusive. The purpose of these trials is to assess the potential of a detached leaf infection as a reliable and efficient method to test varietal susceptibility to *Phytophthora* and *Phytopythium* which could be used within breeding programmes to screen for resistance.

Financial Benefits

Over 16 thousand tonnes and 146.8 million pounds worth of raspberries were produced in the UK in 2019, a figure which is steadily growing with the popularity of the fruit (DEFRA, 2020). However, root rot can have a devastating financial impact on raspberry growers due to the cost of replacing diseased canes and lost fruit crop. This project seeks to increase our understanding of root rot in raspberry and the effects of emerging pathogens on the crop. Through extensive pathogenicity screening and sampling, we hope to reduce the financial loss associated with raspberry root rot and improve upon rapid screening processes for new robust varieties.

Action Points

At this stage of the project, recommendations to change-of-practice cannot be given.

References

DEFRA. (2020). *Latest horticulture statistics*. https://www.gov.uk/government/statistics/latest-horticulture-statistics

Stewart, J. E., Kroese, D., Tabima, J. F., Larsen, M. M., Fieland, V. J., Press, C. M., Zasada, I. A., & Grünwald, N. J. (2014). Pathogenicity, fungicide resistance, and genetic variability of Phytophthora rubi isolates from raspberry (Rubus idaeus) in the western United States. *Plant Disease*, *98*(12), 1702–1708. https://doi.org/10.1094/PDIS-11-13-1130-RE

SCIENCE SECTION

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*, which is 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).

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 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 U.K. raspberry production and inform growers and breeders on how best to reduce the disease.

Materials and methods

Raspberry plants exhibiting symptoms of RRR were sampled in September 2021. Samples were collected from nine grower sites, from five raspberry varieties and one blackberry (*Rubus* subgenus *rubus*) variety in England and Scotland. Sites are coded 1-9 and varieties coded A-D (BB for blackberry), to preserve grower confidentiality. An average of ten samples were taken per site. Five blackberry root samples were taken from site 5 only, at the grower's request. Two to three canes and roots with rhizosphere or substrate soil attached were taken and placed in 1 L plastic bags. Trowels, secateurs, and handsaws were thoroughly disinfected using 70% ethanol between samples. Canes and soil were maintained separately to minimize soil pathogen contamination. Samples were placed in coolers during transport and held in a 4°C cold store until processed.

Isolation from symptomatic tissue

Isolation was performed according to a modified version of the method outlined in Stewart *et al.,* (2014). Diseased roots, i.e., those which had signification browning or apparent lesions, were placed in a sieve and rinsed in running tap water to remove soil/substrate. The roots were cut into 10 mm sections and transferred to 70% ethanol for 1 minute and rinsed twice in sterile distilled water. After rinsing, roots were placed on sterile filter papers to dry for 30 seconds. Five root sections per sample were aseptically transferred to 9 cm Petri dishes containing CMA (Difco Cornmeal Agar) amended with pimaricin (0.4 mL/L of a 2.5% (w/v) stock), ampicillin (250 mg/L), rifampicin (0.1 mg/L) and pentachloronitrobenzene (PCNB; 100mg/L; CMA-PARP (Jeffers and Martin, 1986). Diseased canes were cut into 10 mm sections, soaked in sodium hypochlorite (1.2% available chlorine) for 2 minutes and rinsed in sterile distilled water three times. Four pieces of cane, per sample, were carefully submerged in Petri dishes

containing CMA-PARP. The plates were incubated in the dark at 20°C until mycelial growth was observed (5-7 days). The hyphal tips of growing colonies were transferred onto fresh CMA-PARP. Cultures were transferred to CMA media to ensure no contaminating fungi were present.

Optimizing DNA extraction from raspberry roots

To determine whether freezing or freeze-drying roots prior to DNA extraction yielded higher quality DNA product, both methods were trialed. The roots of 5 samples were thoroughly washed with tap water to remove residual soil or substrate. Between 1-2 g of root sample were placed into 2 mL Eppendorf tubes and either frozen at -80 °C, or frozen and freeze-dried prior to DNA extraction. Extraction was performed using the Qiagen PowerSoil Pro Kit (Qiagen) as per the manufacturer's instructions. DNA was quantified by spectrophotometry (Nanodrop, Thermo Scientific) to determine which method was most effective.

Identification of *Phytophthora* isolates

To determine if the isolates in question were oomycetes, plugs of each isolate were placed in a Petri dish containing 20 mL of sterile distilled water and incubated at room temperature on a bench top for 48 hours (h) to promote the production of sporangia and oospores. After 48 h, sections of the submerged plugs were observed under a microscope. Isolates exhibiting oospores and sporangia with morphological similarity to *Phytophthora* were selected for molecular identification via Sanger sequencing.

To determine the identity of cultures, a rapid fungal DNA extraction was performed using the Sigma-Aldrich Extract-N-Amp™ Plant extraction and dilution buffers (Sigma-Aldrich, U.K), following the manufacturer's protocol. The extracted DNA was stored at -20°C in preparation for downstream analysis. For Sanger sequencing, the internal transcribed spacer 1 (ITS1), 5.2S and ITS2 region (>900 bp) was amplified from the DNA using the ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3 ') and ITS4 (5' - TCCTCCGCTTATTGATATGC-3 ') primers (White *et al*., 1990). Sterile MilliQ water was used as a negative control, genomic DNA extracted from stock cultures of *Phytophthora fragariae* and *Cladosporium cladosporioides* were used as oomycete and fungal positive controls, respectively.

PCR amplifications were carried out in 25 µL reaction volumes. Each reaction tube contained 2.5 µL of both primers at 10 µM, 2.5 µl of sterile MilliQ water, 12.5 µl of 2x PCR MyTaq

Red Mix (containing 5 mM dNTPs and 15 mM $MgCl_2$) (Bioline), and 5 µL of DNA template or MilliQ H_2O. Thermo-cycling reaction was carried out in a BioRad C1000 Touch Thermal Cycler with the following programme; 34 cycles of denaturing at 94°C for 30 s, annealing at 55 °C for 30 s, elongation at 72 °C for 30 s, and a final step at 72 °C for 10 min. The PCR amplification products were separated by electrophoresis in 1.5% agarose gels stained with GelRed (Biotium) in 1 X TAE (40 mM Trisacetate pH 8.0, 1 mM EDTA) buffer at 100 V for 1 h and visualised under a UV trans-illuminator (Bio-Rad ChemiDoc™ MP Imaging System). Images were taken with Image Lab™ (version 5.2) image acquisition and analysis software. Amplification product size was determined by comparison with 500 bp hyper DNA ladder (Bioline). Sanger sequencing using both forward and reverse primer was performed by Eurofins Genomics, Ebersberg, Germany

Molecular analysis of *Phytophthora* species in symptomatic raspberry material

Due to a high volume of samples and the time-intensive nature of isolation, a subset of roots and canes were taken from each farm for molecular testing. The roots of each subset sample were thoroughly washed with tap water to remove residual soil or substrate. Canes were cut into 10 x10 mm sections using secateurs and scalpels which were disinfected with 70% ethanol between samples. Between 1-2 g of root sample were placed into 2 mL Eppendorf tubes and frozen at -80 °C and freeze-dried prior to DNA extraction using the Qiagen PowerSoil Pro Kit (Qiagen) as per the manufacturer's instructions. DNA was quantified by spectrophotometry (Nanodrop, Thermo Scientific) before downstream analysis. Five microliters of each DNA sample (undiluted and ten times diluted) was amplified using the *Phytophthora* genus-specific primer pair YPh1F (CGACCATKGGTGTGGACTTT) and YPh2R (ACGTTCTCMCAGGCGTATCT) which amplify a portion of the *Ypt1* gene (Schena *et al*., 2008). Amplification conditions were: 1 cycle of 95°C for 2 min; 35 cycles of 94 °C for 30 s, 58 °C for 45 s, 72 °C for 30 s; and a final cycle of 72 °C for 10 min. Sterile MilliQ water was used as a negative control and *P. cactorum* DNA as a positive control. The PCR amplification products were observed by electrophoresis on a 1.5% gel and sent for Sanger sequencing as above.

Detached leaf inoculation

Detached leaf assay was modified from (Loyd et al., 2014). A panel of *Phytophthora* and *Phytopythium* isolates, as described in Table 1 and a plain agar control were grown on CMA agar in the dark at 20°C for two weeks. Raspberry leaflets were collected from the newest,

fully expanded leaf. Briefly, the raspberry leaves were surface sterilized in sodium hypochlorite (0.25% available chlorine) for 3 minutes, briefly submerged in 70% (v/v) ethanol and then rinsed three times in sterile distilled water and dried on blotting paper in a laminar air flow cabinet.

Leaf float assay

A 10% (v/v) sterile soil extract was produced using the method outlined in Abad et al. (2019). Six agar plugs cut from the leading edge of 10-day old *Phytophthora* cultures using a sterilized 7 mm cork borer were submerged into a 40 mm Petri dish with 30 mL of the sterile soil extract. Raspberry leaves sterilised as above were then floated on top. The Petri dishes were stored at 22°C and observed daily for signs of infection. Seven days after inoculation, leaves exhibiting lesions were removed from the soil extract, sterilised, and placed on *Phytophthora*-specific media as outlined below.

Detached leaf assay

The leaves were wounded to the left of the midrib using a sterile inoculation needle. Agar plugs were cut from the leading edge of 10-day old *Phytophthora* and *Phytopythium* cultures using a sterilized 3 mm cork borer and placed on the wounded site. A positive control of the *Phytophthora rubi* isolate SCRP339 with confirmed pathogenicity on raspberry and a negative control of uncolonized CMA were included.

Four inoculated leaves per isolate were incubated at 22°C on to moist tissue paper in a 4L plastic lunchbox with a lid to maintain a humid atmosphere. The experiment was set up according to a randomised complete block design. The experiment was monitored daily and the inside of the trays were misted if leaves exhibited any signs of drying. The leaves were imaged daily for 10 days. Markers of *Phytophthora* disease such a lesion area, wilting and discolouration was recorded for each leaf. Lesion area and disease percentage for each leaf was calculated using the APS Assess 2.0 software. The percentage disease of each leaf was analysed using a one-way ANOVA and Tukey's HSD test using R statistics software.

Re-isolation from the diseased leaf lesions was performed according to the method outlined in Stewart et al. (2014). Diseased leaves, i.e., those which had signification browning or apparent lesions, were cut into 10 mm^2 sections, soaked in sodium hypochlorite (1.2% available chlorine) for 2 minutes and rinsed in sterile distilled water three times and two pieces of leaf per sample were carefully submerged in 9 cm Petri dishes containing CMA (Difco cornmeal agar, 17 g/1000 ml of deionized water) amended with pimaricin (0.2 ml), ampicillin-Na (0.250

g), rifampicin (0.4 µL of 2.5% aqueous solution), and pentachloronitrobenzene (PCNB 5 ml) (CMA-PARP). The plates were sealed with Parafilm and incubated in the dark at 18°C until mycelial growth was observed (5-7 days after isolation). The hyphal tips of growing colonies were transferred onto fresh CMA-PARP. Cultures were routinely transferred to V8 media (10 mL Campbell's V8 juice, 400 mL deionized water and 8.5 g agar) to ensure no contaminating fungi were present which could affect pathogenicity and sequencing results.

To determine the identity of cultures, a rapid fungal DNA extraction and PCR amplification was performed using ITS 4 and ITS5 and samples were sent for sequencing using the same method as outlined previously.

Table 1: *Phytophthora* and *Phytopythium* isolates used in detached raspberry leaf assays.

Code	Species	Crop/Source	Country of origin
11/11	*Phytophthora citricola*	Raspberry root	Scotland
10/08	*Phytophthora citrophthora*	Raspberry root	England
ICO45	*Phytopythium litorale*	Raspberry root	Scotland
10/07	*Phytophthora cryptogea*	Raspberry root	Scotland
14/05	*Phytophthora vexans*	Raspberry root	England
13/08	*Phytophthora erythroseptica*	Raspberry root	Scotland
13/05	*Phytophthora pseudocryptogea*	Raspberry root	Scotland
SCRP339	*Phytophthora rubi*	Raspberry/JHI	Scotland
SCRP1213	*Phytophthora rubi*	Raspberry/JHI	Scotland

Results

Grower Survey

Information obtained about U.K. grower's raspberry production are outlined in Table 2. The survey showed that 100% of growers chose a *Phytophthora*-resistant cultivar as the primary method of disease control on their farms, and 82% of the 18 growers surveyed used a fungicide regime.

Table 2: Results from the 2020 *Phytophthora* Disease Management Survey completed by 18 U.K. raspberry growers detailing the substrate type (**1**), length of plant production (**2**) and control methods (**3**) they use to prevent *Phytophthora* root rot.

Substrate Type	**Percentage of growers**
Compost	17%
Coir	47%
Soil	35%
Length of Plant Production	
4-6 years	31%
3-4 years	31%
2-3 years	37%
1-2 years	1%
Control Methods	
Fungicide (Dimethomorph/Metalaxyl)	82%
Chemical water sterilization	11%
UV water sterilization	5%
Growing resistant cultivar	100%

Optimisation of raspberry root DNA extraction

Following extraction of DNA from symptomatic raspberry roots with the Qiagen PowerSoil Pro kit, samples which has been freeze-dried prior to extraction had higher DNA concentrations than those which had been frozen only. In addition, samples

which had been freeze-dried had consistent 260/280 ratios of ca. 1.8, whilst the frozen samples had 260/280 values ranging from 1.7-2.0 (Table 3).

Table 3: Nanodrop results including DNA concentration (ng/µL), and ratios of contamination (260/280 and 260/230) of DNA extracted from raspberry roots which had either been freeze dried (FD) or frozen (FR) prior to extraction with a commercial kit.

Sample ID	ng/µl	260/280
1/11 FR	63.87	1.75
1/11 FD	79.82	1.89
1/16 FR	52.59	2.03
1/16 FD	150.44	1.88
1/35 FR	25.09	1.78
1/35 FD	89.73	1.83
4/26 FR	32.03	1.9
4/26 FD	56.97	1.82
8/40 FR	93.32	1.86
8/40 FD	61.05	1.94

Isolates obtained from grower sampling

Through plating symptomatic root tissues from sampling onto *Phytophthora*-selective agar, and subsequent isolation of growing hyphae, twenty-three cultures were obtained which had colony morphology comparable to *Phytophthora*. The ITS region of each isolate was sequenced using Sanger technology. The identities of the isolates are listed in Table 4. Samples were collected only from plants exhibiting symptoms of RRR, sites had varying levels of disease meaning 5-15 samples were taken per farm with an average of 10 samples per farm. 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 from symptomatic tissue onto selective agar resulted in 12 isolates of five species of *Phytophthora* and seven isolates of two *Phytopythium* species from raspberry roots and canes (Table 4). No isolates were recovered from blackberry material. *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, isolates came from only four sites which showed high RRR disease incidence (over 70% incidence reported by the grower).

Table 4: Sanger sequencing results from the ribosomal internal transcribed spacer (ITS) region of isolates recovered from symptomatic raspberry tissue.

Species	Number of isolates	Variety code	Farm code	Host	Plant tissue	Country
Phytophthora citricola	2	A,B	4,2	*Rubus idaeus*	Root	Scotland
Phytophthora citrophthora	3	B	1	*Rubus idaeus*	Root	England
Phytophthora cryptogea	3	B,C	1,3,2	*Rubus idaeus*	Root	Scotland
Phytophthora pseudocryptogea	2	B	1	*Rubus idaeus*	Root	Scotland
Phytophthora erythroseptica	2	A	2	*Rubus idaeus*	Root	Scotland
Phytopythium litorale	6	A,C	2,3	*Rubus idaeus*	Cane	Scotland
Phytopythium vexans	1	D	2	*Rubus idaeus*	Cane	Scotland

Molecular analysis via amplification and sequencing of the ITS region directly from diseased plant samples detected nine *Phytophthora* species in 16 samples from seven farms (Table 5). *P. citrophthora* was the most frequently detected species. Notably, *P. rubi* was detected from just one raspberry sample and this site had very low reported occurrence of RRR. *P. rubi* was also detected in one blackberry sample from a site which had severe RRR infection, a blackberry sample from the same site also contained *P. bishii*, which was not detected in any raspberry samples. No *Phytopythium* species were detected in raspberry roots as *Phytophthora*-specific primers were used for these samples, as *Phytopythium* was previously unreported in raspberry.

Table 5: Sanger sequencing results from the ribosomal internal transcribed spacer (ITS) region of DNA extracted from symptomatic raspberry roots.

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

Detached Leaf Inoculation

Screening bioassays were conducted using the detached-leaf method as described by Yokomi & Gottwald (1988). Pathogenicity screening using detached leaf assays has previously been reported as a successful technique in determining the pathogenicity of raspberry insect pests. A study by Elmekabaty et al. (2020) analysed the effect of the large raspberry aphid *Amphorophora idaei* using detached raspberry leaves and noted the technique was a reliable pathogenicity screening method. Similarly, Pettitt et al. (2011) noted the time-efficiency and reliability of detached leaf assay in determining the pathogenicity of *Pythium* isolates on Chrysanthemum leaves. For the leaf float test, analysis of variance (ANOVA) and Tukey's HSD test showed no significant difference in the disease percentage of leaves infected with the *Pp. litorale* or *P. citricola*. However, *P. citrophthora*, *P. cryptogea*, *P. erythroseptica*, *Pp. vexans* and the *P. rubi* isolates SCRP 1213 and SCRP 339 showed significantly higher disease percentage than the control leaves floated in soil extract with uncolonised CMA (Figure 1).

a

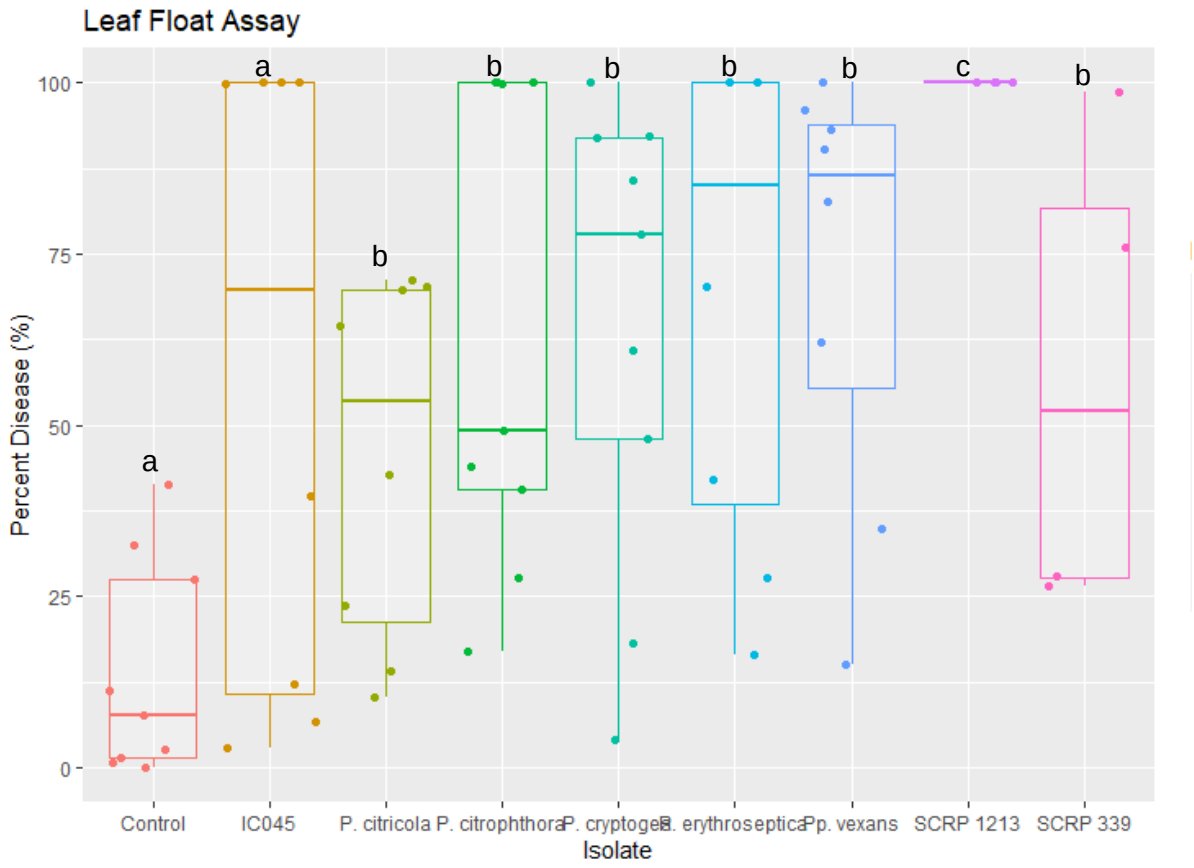

Figure 1: Disease percentage of raspberry leaves floated in soil extract infested with *Phytophthora* and *Phytopythium* isolates over three experimental replicated experiments.. ANOVA and Tukey's HSD test were performed in R Studio v1.4.05.5 and statistical differences are given as labelled letters.

Analysis of the average disease scores of the direct inoculation test revealed no significant differences between the control and any of the isolates included in the study (Figure 2).

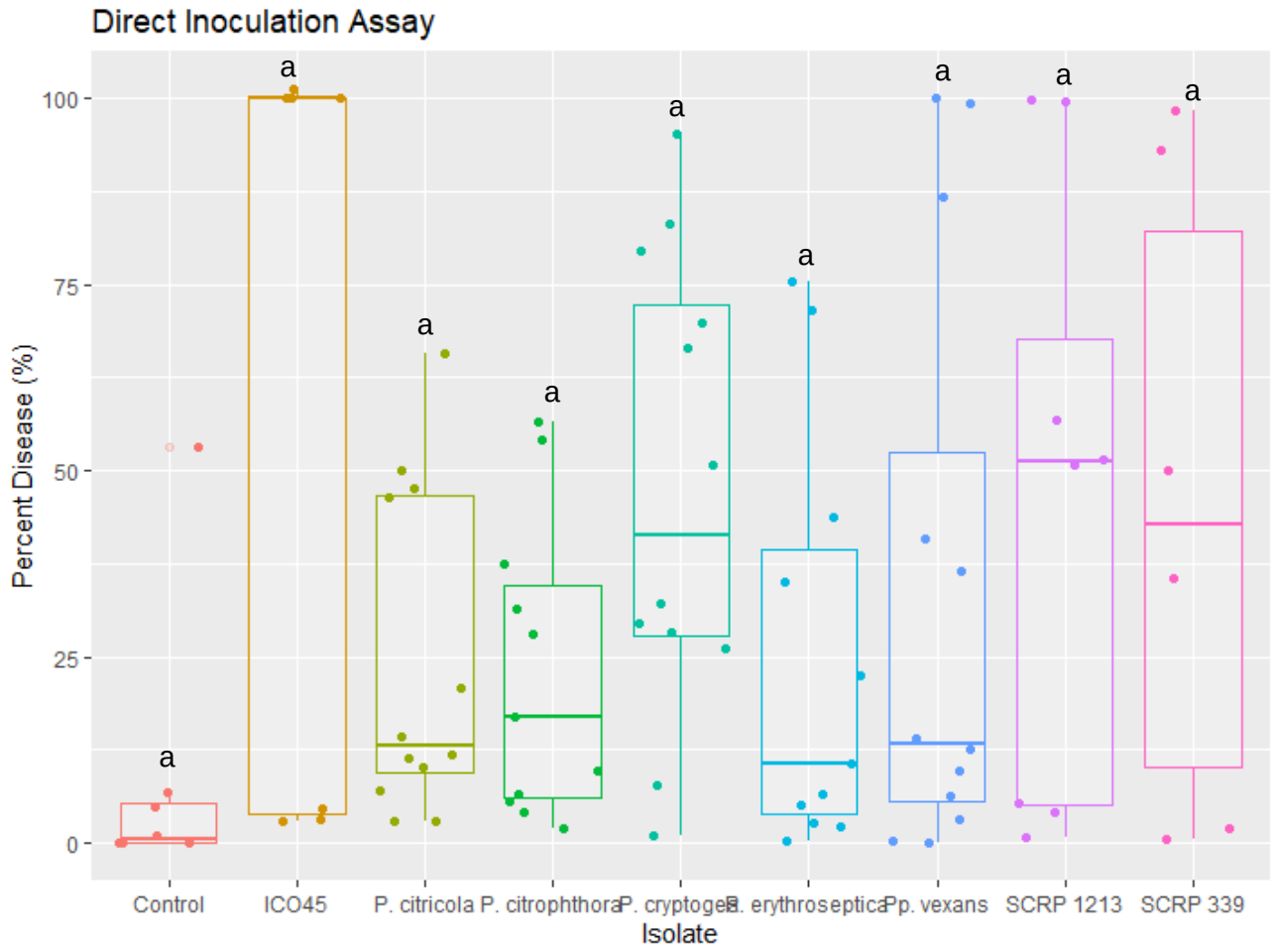

Figure 2: Disease percentage of raspberry leaves directly inoculated with *Phytophthora* and *Phytopythium* isolates from three replicated experiments. ANOVA and Tukey's HSD test were performed in R Studio v1.4.05.5 and no statistical differences were identified (denoted by letter a).

Discussion

The preliminary findings of this work, while still in development, are informative for both the project as a whole and for the industry in which it is directly involved with. Contrary to previous reports of *Phytophthora* diversity in UK red raspberry crops *(AHDB SF158 Report, 2020*), the results of this study show an apparent absence of *P. rubi* on raspberry samples with *P. rubi* detected only in samples from blackberry. Numerous reports on *Phytophthora* in red raspberry note *P. rubi* as the dominant species in various countries including the UK (Benedict et al., 2018; Gigot et al., 2013; Koprivica et al., 2009; Pattison et al., 2004; Stewart et al., 2014;

Tabima et al., 2018; Wilcox et al., 1993). However, the findings in this study hint at changing trends in raspberry *Phytophthora* diversity in the UK. A communication from industry agrees that species such as *P. citrophthora*, *P. citricola* and *P. bisheria* are increasingly being isolated from cultivated raspberry crops in the UK (Dr Kelly Ivors, Pathologist, Driscoll's Global Plant Health; Pers. comm.).

Many factors can influence the species composition of *Phytophthora* in agricultural environments. Ruiz Gómez *et al.* (2019) noted the impact of disease symptoms on the diversity of oomycete species in holm oak decline areas in the Iberian Peninsula with species diversity being significantly lower in highly defoliated zones than in areas with less severe symptoms. Changing climatic conditions, management practices, plant cultivars, growing systems, agrochemical and plant transport may influence the diversity of *Phytophthora* communities in raspberry plants. The detection of *Phytophthora* species such as *P. hedraiandra*, and *P. ilicis*, which are previously unreported in raspberry, and *P. meadii*, unreported in the UK, is of note. *P. hedraiandra* was formally described in 2004, having been isolated from *Viburnum* plants (de Cock et al., 2015). Moralejo *et al.* (2009) note the species is spreading through nursery trade, specifically ornamental nurseries in southern and northern Europe, which is a major trade route for raspberry and could explain its detection in this study. A study by Prigigallo *et al.* (2015) further reinforces the theory that plant nurseries are an important source of the introduction and spread of non-native *Phytophthora* species into environments. The same study reported *P. meadii* in the soils surrounding mint (*Menta* sp.) and mandarin (*Citrus reticulata*) plants and in the roots of several ornamental species (Prigigallo et al., 2015).

Two *Phytophthora* species, *P. rubi* and *P. bishii* were detected in blackberry samples showing symptoms similar to RRR. To our knowledge this constitutes the first report of either species in blackberry, however, further studies are needed to confirm their prevalence and pathogenicity. Aghighi *et al.* (2016) reported multiple *Phytophthora* species, including *P. bilorbang* and *P. cryptogea*, as pathogenic on European blackberry (*Rubus anglocndicans*) in Australia. A study by Duncan *et al.* (1987) reported an apparent resistance of the blackberry cultivar Aurora to five *Phytophthora* species which caused significant disease in raspberry. This may explain the apparent absence of other *Phytophthora* species in the blackberry samples analysed in this study.

Two species of *Phytopythium*, *Pp. litorale* and *Pp. vexans* were recovered from symptomatic raspberry roots. The cane and root tissue from which they were isolated exhibited near-

identical symptoms to those from which *Phytophthora* was isolated. *Phytopythium* species are noted to be an intermediate between *Phytophthora* and *Pythium*. To our knowledge, there are no published reports investigating the presence of *Phytopythium* species in red raspberry. *Phytopythium* has recently been reported as pathogenic to other members of the *Rosaceae* family such as strawberry, apples and pears (Moein et al., 2019; Pánek et al., 2022).

The site in which *Phytopythium* isolates were recovered in this study notably used water from a local river to irrigate their plants. Furthermore, *Pp. vexans* and *Pp. litorale* were isolated from sites at a low elevation and residual pooling of drip irrigation around the pots was observed. Open water systems such as lakes and rivers can be a significant source of oomycete pathogens such as *Phytopythium*, with many representatives of this genus detected in freshwater and flooded environments (Nam & Choi, 2019; Redekar et al., 2019). *Phytopythium* such as *Pp. irregulare*, *Pp. ultimum* and *Pp. sylvaticum* were reported to have higher disease severity on apple seedlings under higher irrigation regimes (Moein et al., 2019). Benfradj *et al.* (2017) and Jabiri *et al.* (2021) noted a higher prevalence of *Pp. vexans* in drip irrigation systems versus submersion irrigation in citrus trees and apple and pear orchards. Furthermore, over 30 species of *Phytophthora* have been described in water systems around the world (Redekar et al., 2019; Scibetta et al., 2012). Thus, the management of irrigation systems to prevent overflooding and regular disinfection of irrigation lines may reduce the spread of these pathogens in raspberry crops.

The results from the detached leaf assay are currently inconclusive, three experimental replicates of both the leaf float and direct inoculation assays have been completed and analysed, however, more are necessary to accurately determine the effect of each pathogen on the leaves.

Further work will be conducted to determine the pathogenicity of the *Phytophthora* species identified in this work to whole raspberry plants. As a result, dominant *Phytophthora* pathogens reported in plants with RRR can then be included in host resistance and genotype susceptibility studies which at present may focus on *P. rubi* due to its reported dominance in the RRR complex. The focus on *P. rubi*-specific screening may not be as effective with changing patterns in RRR species diversity as reported in this study. Further investigation into oomycete diversity on UK red raspberry will ensure more targeted and effective breeding programmes can be maintained.

Conclusions

The potential of *Phytopythium* species to be a major contributor to RRR has yet to be determined, and to our knowledge there have been no reports on the pathogenicity of this genus on red raspberry. The work outlined in this study describes a more diverse *Phytophthora* and oomycete species composition in red raspberry than previously reported. These findings highlight the importance of regular surveying and sampling of cultivated raspberry crops as a method of disease management. Awareness of the changing patterns of oomycete diversity in raspberry allows researchers to identify changes in life cycles, disease symptoms and pathogenicity of species of interest, which serves to inform growers and breeders. The detection of *Phytopythium* species which have not been identified in raspberry previously is of note, and their respective threat to UK production remains unknown.

Future Work

- Perform pathogenicity screening of six *Phytophthora* and *Phytopythium* isolates of interest in pots and tissue culture plants to assess symptom development and pathogenicity
- Metabarcoding of samples from grower surveying to determine *Phytophthora* species diversity in UK raspberry production

Knowledge and Technology Transfer

NIAB East Malling Trust Board - oral presentation

Fruit Focus 2020 – oral presentation

AHDB Soft Fruit Day 2020 and 2021 - poster presentation

HAU Postgraduate Student Colloquium 2020 - oral presentation, 2021 - poster presentation

Berry Gardens Research and agronomy Conference 2021 - oral presentation

CTP-FCR conference 2019-2022 - oral presentation

British Mycological Society meeting 4-7th April 2023 - poster presentation

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