Characterisation of the pathogenicity of strains of *Pseudomonas syringae* towards cherry and plum

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Running head- Pseudomonas pathogenicity

Key words- Pseudomonas, pathogenicity, avirulence, non-host

Abstract

Bacterial canker is a major disease of cherry and other stone fruits caused by several pathovars of *Pseudomonas syringae*. These are *P.s* pv. *morsprunorum* race 1 (*Psm* R1), *P.s* pv. *morsprunorum* race 2 (*Psm* R2) and *P.s* pv. *syringae* (*Pss*). *Psm* R1 and R2 were originally designated as races of the same pathovar, however phylogenetic analysis has revealed them to be distantly related. This study characterised the pathogenicity of *P. syringae* on cherry and plum, in the field and the laboratory. The field experiment identified variation in host cultivar susceptibility to the different pathogen clades. The cherry cultivar Merton Glory exhibited a broad resistance to all clades, whilst cultivar Van showed race-specific resistance. *Psm* R1 may be divided into a race structure with some strains pathogenic to both cherry and plum and others only pathogenic to plum. The results of laboratory-based pathogenicity tests were compared to results obtained on whole-trees. Only cut shoot inoculations were found to be sensitive enough to detect cultivar variation in susceptibility. Measuring population growth of bacteria in detached leaves reliably discriminated pathogens from non-pathogens. In addition, symptom appearance discriminated *Psm* races from non-pathogens which triggered a rapid hypersensitive response (HR). The pathogen *Pss* rapidly induced disease lesions and therefore may exhibit a more necrotrophic lifestyle than hemi-biotrophic *Psm* races. This in-depth study of pathogenic interactions, identification of host resistance and optimisation of laboratory assays, will provide a framework for future genetic dissection of virulence and host resistance mechanisms.

Introduction

Pseudomonas syringae is a globally important plant pathogen, and includes strains associated with plants and aquatic environments (Dudnik & Dudler, 2014). Plant pathogenic strains can be divided into pathovars, which are only able to infect particular host species. Strains within pathovars can also be further distinguished into races, which show specificity towards particular host cultivars (Joardar *et al*., 2005). *P. syringae* is referred to as a species complex due to the high level of divergence between individual clades (Berge *et al*., 2014). Currently, nine genomospecies, based on DNA-DNA hybridisation, and thirteen phylogroups, based on Multi-Locus Sequence Typing (MLST), have been described (Gardan *et al*., 1999; Parkinson *et al*., 2011).

Several distantly related pathovars of *P. syringae*, which belong to different phylogroups, are known to cause bacterial canker of *Prunus*. This genus of stone-fruit trees includes economically important species such as cherry, plum, peach and apricot. Focusing on sweet cherry (*Prunus avium*), members within three distinct phylogroups of *P. syringae* have been characterised as the main causal agents of canker. These are *P. syringae* pv. *syringae* (*Pss*), *P. syringae* pv. *morsprunorum* (*Psm*) race 1 (R1) and *P. syringae* pv. *morsprunorum* race 2 (R2) (Bultreys & Kaluzna, 2010). The two *morsprunorum* races are specifically found only on *Prunus* species, whilst *Pss* strain are more variable and able to infect various plant species (Bultreys & Kaluzna, 2010). Although distantly related, *Psm* R1 and R2 were initially distinguished based on virulence towards particular cherry cultivars, so were described as races of pv. *morsprunorum* (Garrett, 1978). Another pathovar (*P. syringae* pv. *avii*) is pathogenic on wild cherry (Ménard *et al*., 2003). *P. syringae* is able to infect throughout the year and cause necrotic lesions on all aerial plant organs, including fruit, leaves and blossom. The pathovars invade dormant woody tissues through leaf scars and wounds in winter. They occupy the cambial tissue and produce black necrotic cankers in spring. During the growing period, there is a large diverse population of epiphytic bacteria that grow on the

surface of the leaves. Bacteria may also enter the leaf and induce necrotic lesions that eventually drop out of the leaf, causing shot-hole symptoms. The asymptomatic leaf population are thought to provide the inoculum for woody tissue infections (Crosse, 1959). Bacterial canker is an annual problem for the global cherry fruit industry and is particularly devastating in young orchards, where it has been reported to cause up to 75% loss of trees (Spotts *et al*., 2010). Chemical control for this disease is currently limited to spraying with copper-based compounds, a treatment that has recently been restricted across Europe (Stone & Baker, 2010). Breeding for resistance is a desirable alternative method of control. Recent studies have identified rootstock selections and scion varieties exhibiting a degree of resistance (Santi *et al*. 2004; Spotts *et al*. 2010; Li *et al*. 2015; Farhadfar *et al*. 2016). Despite this progress, there is still a lack of totally resistant varieties available and the genetic factors underlying canker resistance remain unknown.

An understanding of how the divergent clades of *P. syringae* cause bacterial canker is crucial to breeding efforts. The epidemiology of this disease was determined through field inoculation studies at East Malling in the UK (Crosse, 1966; Crosse & Garrett, 1966; Freigoun & Crosse, 1975; Garrett, 1978). Molecular techniques such as Repetitive Element Sequence-Based (REP) PCR and Multi-Locus Sequence Typing (MLST) and various morphological methods have been used to survey the bacterial populations in orchards (Vicente & Roberts, 2007; Gilbert *et al*., 2008; Kaluzna *et al*., 2010). These studies revealed that the three pathovars co-exist within orchards, with each other and non-pathogenic Pseudomonads. To characterise pathogenicity, several laboratory and field-based assays have been developed (Crosse & Garrett 1966; Vicente & Roberts 2003; Gilbert *et al*. 2009). Improved assays are required for screening for host resistance. Gilbert *et al*. (2009) used various lab-based tests to determine the pathogenicity of strains isolated from stone-fruits in Belgium. They found that no individual laboratory assay could reliably predict pathogenicity under field conditions. Field inoculations are therefore required to fully ascertain pathogenicity and differences in host response.

The breeding of resistant cherry cultivars has been hindered due to the complex nature of this disease (Garrett, 1979). Early work reported variation in cultivar susceptibility towards the different clades of pathogenic *P. syringae*, with two cultivars Napoleon and Roundel exhibiting differential susceptibility towards the two races of *Psm*. Napoleon was found to be resistant to R2 but susceptible to R1, and vice versa for Roundel (Garrett, 1978). It may therefore be challenging to breed resistance to all three of the genetically distinct *P. syringae* clades.

Various studies have established the mechanisms of host immunity in the model *P. syringae* pathosystems of *Solanum lycopersicum* (tomato), *Arabidopsis thaliana* (thale cress) and *Phaseolus vulgaris* (bean) (Preston, 2000; Quirino & Bent, 2003; Arnold *et al*., 2011). *P. syringae* uses a range of virulence factors, including Type III secretion system effector proteins (T3Es) to suppress the plant immune system. Plant immunity can be broadly divided into two stages: PAMP-Triggered Immunity (PTI) and Effector-Triggered Immunity (ETI) (Jones & Dangl, 2006). PTI is a response towards conserved pathogen molecules and allows plants to exhibit non-host resistance to many potential pathogens. ETI occurs when host resistance proteins (R proteins) detect the presence of pathogen effectors and typically leads to a hypersensitive cell death response (HR), which prevents the spread of the pathogen (Senthil-Kumar & Mysore, 2013). ETI is associated with varietal resistance within a host species, whereby particular host cultivars have R genes that trigger the HR towards particular pathogen races. Studies have also found that ETI may play a role in non-host resistance (Gill *et al*., 2015). Initial studies of ETI-associated host resistance were focused on qualitative resistance, whereby a single R gene provides complete resistance against a particular pathogen. Although the above generalisations about PTI and ETI are valid, it is well known that in the field varietal resistance is often quantitative; whereby resistance leads to reduction but not absence of the disease. This type of resistance is often controlled by more than one gene, with different host genotypes exhibiting a range of susceptibility levels to the pathogen (Poland *et al*.,

124 2009), and even towards single T3Es (Iakovidis *et al.*, 2016). Quantitative host resistance may
125 encompass a combination of PTI and ETI (Corwin *et al.*, 2016). As no fully canker-resistant host
126 genotypes have been identified, the genetic basis of host resistance to bacterial canker is likely to be
127 quantitative. It may involve many different genes that additively contribute to more resistant
128 phenotypes.
129
130 To analyse the genetics of pathogenicity and disease resistance to canker, the pathogenicity of a
131 diverse range of *P. syringae* strains was studied. Experiments were conducted, using both lab-based
132 and field inoculations, to characterise the interaction of the strains with both cherry and plum. This
133 robust series of infection experiments provides a reliable pathogenicity framework for future
134 genetic dissection of virulence and disease resistance.
135
136

Materials and Methods

Bacterial strains

Strains of *Pseudomonas syringae* (listed in Table 1) were grown on King's B agar at 25 °C. For liquid culture, strains were grown in Lysogeny Broth (LB) shaking at 25 °C, 150 rpm.

Genome sequencing

Nineteen *P. syringae* strains were genome sequenced using the Illumina MiSeq V3. DNA was extracted using the Puregene Yeast/Bact Kit (Qiagen). DNA libraries were prepared by fragmenting the DNA using a sonicating water bath for 30 seconds. DNA was then size-selected by gel electrophoresis to obtain fragments of 400-700 bp using the Zymogen gel extraction kit (Zymo Research). Libraries were created using the NextFlex Rapid-DNA sequencing kit. Barcodes were multiplexed to allow pooling of multiple samples. Libraries were quality checked using the Fragment Analyzer (Advanced Analytical) and Qubit (Life Technologies).

Libraries were sequenced using the Illumina Mi-Seq V3 (Illumina) 300 bp paired-end reads. Raw data for each genome was quality checked and trimmed using fastqc-mcf (https://code.google.com/p/ea-utils/wiki/FastqMcf). The reads were error corrected using Quake prior to assembly (Kelley *et al*., 2010). Each genome was then assembled using SPAdes 3.7.0 (Bankevich *et al*., 2012) and summary statistics generated using Quast (Gurevich *et al*., 2013).

Phylogenetic analysis

A phylogenetic tree was created for all sequenced strains and other strains which had genome sequences available on NCBI. The nucleotide sequences of seven house-keeping genes (*acnB*, *fruK*, *gapA*, *gltA*, *gyrB*, *pgi* and *rpoD*) were extracted from all genomes and individually aligned using Geneious 7.1.9. The alignments were concatenated and trimmed to produce an overall alignment of

9393bp. A Bayesian phylogeny was created of this alignment using the Geneious plug-in of MrBayes (Huelsenbeck & Ronquist, 2001). The GTR gamma model of evolution was used with a burn-in length of 100,000 and sub-sampling frequency of 200.

Plant material

All *Prunus* material was propagated at NIAB-EMR. For whole-tree inoculations, one-year old grafted trees were used. Cherry cultivars were grafted on the rootstock Gisela 5, whilst plum was grafted on St Julian A. For detached leaf assays, 1-2 week old, fully-expanded leaves were obtained from glasshouse grown trees. Immature green cherry fruits were obtained from mature trees.

Pathogenicity assays

Bacterial inoculum was prepared from overnight LB cultures. These were spun down (3500 g, 10 minutes) and re-suspended in sterile 10 mM $MgCl_2$. A spectrophotometer was used to measure concentration, with an optical density of 0.2 (OD_{600}) being ~$2x10^8$ CFU/ml (Debener *et al*., 1991).

Whole-tree inoculations

Whole-trees were inoculated though either wounds or leaf scars (Crosse & Garrett, 1966). Field inoculations were performed in October 2015 and glasshouse wound inoculations in February 2015. Bacterial suspensions of $2x10^7$ CFU/ml were used for inoculations.

To inoculate through wounds, a sterile scalpel was used to cut a shallow wound into the trunk of the tree and 200µl of inoculum was pipetted into the wound. To inoculate leaf scars, the leaf was removed and 10µl of bacterial suspension was pipetted on the exposed scar. The inoculation sites were covered with parafilm and duct tape. Multiple inoculations were performed on the same tree, with at least 4 buds between inoculations. For the field experiment the trees were left for 6 months before assessment in May 2016, whereas the glasshouse experiment was assessed after 2 months.

To assess disease the bark was stripped back and length of necrosis was measured using a caliper (field experiment only). A disease score was determined as 1: no symptoms 2: limited browning, 3: necrosis and gumming and 4: necrosis, gumming and spreading from site of inoculation.

Cut shoot inoculations

A cut shoot assay was performed as in previous studies (Krzesinska *et al*., 1992; Santi *et al*., 2004). Eight strains were inoculated onto four cherry and two plum cultivars. Bacterial inoculum was prepared at a concentration of $2x10^7$ CFU/ml. Dormant one-year shoots (5mm diameter) were cut into 10 cm sections. They were sterilised with 0.5% hypochlorite for five minutes, rinsed in tap water and left overnight to air-dry. Next, 5 mm from the shoot tip was cut and the shoot was dip inoculated for five minutes. The wound was covered with parafilm and the shoot bases were freshly cut (5 mm) and placed in transparent-boxes immersed in 20 mm deep distilled water. The shoots were incubated at 15 °C with 16-hour light, 8-hour dark cycle for one week. Next, shoots were transferred to −2 °C for one week to simulate frost damage. Finally, the basal 10 mm of each shoot was removed and they were placed in water-soaked Oasis Foam (Oasis Floral). These were incubated for a further 4 weeks at 15 °C. The trays were covered with cling-film to maintain a high humidity.

The shoots were assessed by peeling back the bark from the top 30 mm of the shoot. Digital images were captured and analysed with software (Li *et al*., 2015) to determine the percentage area of necrosis.

Cherry fruit inoculations

To inoculate immature cherry fruits a stab-inoculation method was used (Moragrega & Llorente, 2003). Fruits were sterilised in 0.5% hypochlorite for five minutes and rinsed in distilled water. Bacteria were then scraped from 5-day old plates using a 24g needle and stabbed into the plant

material. Fruits were placed in transparent boxes lined with moist tissue paper to maintain a high humidity. The fruits were kept at 22 °C (16hr light, 8hr dark) and visually assessed over time. Two independent assays on the different cultivars were performed.

Leaf inoculations and microscopy

Inoculum concentration varied from $2x10^6$ CFU/ml to $2x10^8$ CFU/ml. Freshly picked, 1-2 week old leaves were used for leaf inoculations. The leaves were infiltrated with bacterial suspension from the abaxial surface using a blunt-ended syringe. Leaves were then placed in plastic trays, which contained a 10 mm layer of water agar (10g/L), covered in damp paper towel. The tray was sealed inside a transparent bag and incubated at 22 °C (16hr light, 8hr dark). The leaves were left for a maximum of 10 days before assessment. At least three leaves were inoculated for each isolate, with the three replicate leaves coming from different plants.

Bacterial population growth within the leaves was measured over time. Day 0 populations were always calculated to check that the inoculum concentrations were similar between treatments. Leaf discs were excised using a sterile cork borer (0.5cm). Discs were then homogenised in 10 mM $MgCl_2$. A dilution series was plated out to determine bacterial concentration (CFU/ml). Each concentration was plated out three times (pseudoreplicates). Overall, for each bacterial strain studied there were three replicate leaf inoculations and three pseudoreplicates to measure the concentration of each. Two independent experiments were performed for the leaf assays of a subset of strains inoculated on cherry and plum and the inoculations on different cherry cultivars.

Electron microscopy was performed by Dr Ian Brown (University of Kent) on infected cherry leaves. Detached leaves were infiltrated with bacteria at $2x10^6$ CFU/ml and incubated for one week at 22 °C.

Inoculated leaves were cut into 2 mm squares using a razor blade in a drop of cold fixative (2.5% glutaraldehyde in 100mM sodium cacodylate buffer pH 7.2 [CAB]) on dental wax and processed as previously described (Soylu *et al*., 2005). Sections were viewed in a Jeol 1230 TEM with an accelerating voltage of 80kV and images recorded with a Gatan Multiscan 791 digital camera.

Experimental design and statistical analysis

To randomise the glasshouse whole-tree canker assay, an incomplete block-design was used. This allowed assessment of 22 different strains on 22 trees, with five replicates of each strain. In the field experiment, the virulence of eight strains was assessed on four cherry and two plum cultivars, using two different inoculation methods. To reduce the number of trees required, the eight different strains were divided across two trees, with each tree also having one negative control. This meant that two adjacently planted trees comprised one experimental unit of all strains and controls inoculated on the same cultivar using one inoculation method. A balanced incomplete design was used to randomise strain positions onto the two trees. A balanced complete design was then used to randomise the different cultivars and inoculation methods within 10 blocks in the field. Each block contained 24 trees (16 cherry and eight plum), and the total experiment involved 240 trees.

R software (R Core Team, 2012) was used for all statistical analyses as described in detail in supplementary methods. All ANOVA tables are also presented in the supplementary data.

Results

Phylogenetics

To determine the diversity of strains isolated from cherry and plum, the genomes of 18 *P. syringae* strains were sequenced. The strains included bacteria representative of all three previously designated clades, *Psm* R1, *Psm* R2 and *Pss*. *Pss* and *Psm* R1 included strains isolated from both cherry and plum, whilst the *Psm* R2 strains all originated from cherry. A previously undescribed

267 strain that did not belong to these clades (*Ps* 9643), which had been isolated from a plum leaf wash
268 was also included. Finally, an additional strain (RMA1) isolated from the perennial species
269 *Aquilegia vulgaris*, that preliminary analysis had shown to be closely related to *Psm* R2 was
270 sequenced. The DNA sequences of seven MLST genes were then extracted from the genomes.
271 Homologous sequences from 59 genome assemblies of additional strains within the *P. syringae*
272 complex were then downloaded from NCBI. These included strains that were also isolated from
273 *Prunus* and other plant species for comparison. A Bayesian phylogenetic tree was then generated
274 based on a concatenated alignment of the seven genes.
275
276 The *P. syringae* phylogeny is presented in Figure 1, with strains isolated from *Prunus* highlighted.
277 *Psm* R1, *Psm* R2 and *Pss* were found within phylogroups 3, 1 and 2 respectively. The two *Psm*
278 races fell into discrete monophyletic clades, with individual strains being very closely related. By
279 contrast, *Prunus Pss* isolates exhibited greater diversity. Strains isolated from cherry and plum did
280 not form distinct host-specific clusters in any of the pathogenic clades, indicating that they are
281 closely related and may cross-infect the two *Prunus* species. *Ps* 9643 was closely related to the
282 *Prunus persicae* pathogen (*P.s* pv. *persicae*), whilst RMA1 was an out-group to the clade
283 containing *Psm* R2 and the pathovars *P.s.* pv. *actinidiae*, *P.s* pv. *avellanae* and *P.s.* pv. *theae*
284 (which infect kiwifruit, hazelnut and tea respectively).
285
286 **Characterising pathogenicity of a range of *P. syringae* strains on cherry and plum trees**
287
288 **Whole-tree glasshouse experiment** To determine the fundamental ability of each strain to cause
289 bacterial canker on cherry, a whole-tree wound inoculation experiment was performed. All strains
290 isolated from cherry and plum, as well as related pathogens of other plants: *P.s* pv. *phaseolicola*
291 1448A (*Pph*), *P.s* pv. *avellanae* BPIC631 (*Psav*) and RMA1, were included. Comparisons between
292 strains were made based on the level of necrosis produced in the cambial layer underneath the bark

293 at the site of inoculation after two months of incubation. Strains exhibited a wide range of virulence
294 profiles on cherry (Figure 2). Both the non-pathogens and negative control gave very limited
295 browning and callusing associated with a wound response. Pathogenicity was indicated by black,
296 necrotic lesions that sometimes spread from the inoculation site and were associated with gumming.
297 There was clear variation between members of the different *Prunus*-infecting clades. Strains of *Psm*
298 R1 and R2 showed variation in virulence, but rarely spread from the inoculation site. Meanwhile,
299 most strains of *Pss* were able to spread. Within *Psm* R1, only two cherry strains (R1-5244 and R1-
300 9646) caused gumming and necrosis, whilst R1-9657 showed reduced virulence, not significantly
301 different to the plum R1 strains. Symptoms caused by strains of *Psm* R1 isolated from plum were
302 not significantly different from those associated with the non-pathogens. Most strains of *Psm* R2
303 were pathogenic, however R2-5260 showed reduced virulence. Apart from one strain, *Pss* was
304 highly pathogenic, with symptoms typically spreading from the site of inoculation. The strain *Ps*
305 9643 isolated from a plum leaf, but found not to be closely related to the other canker pathogens
306 (Figure 1), behaved as a non-pathogen of cherry.
307
308 **Whole-tree field experiment** A set of strains with contrasting pathogenicity and host of isolation
309 was chosen for pathogenicity screening under field conditions, using leaf scar and wound
310 inoculations on cherry and plum cultivars. The strains included cherry pathogens (R1-5244, R2-
311 leaf, *Pss* 9097 and *Pss* 9293) and non-pathogens (R1-5300, *Ps* 9643, *Pph* and RMA1). Concerning
312 cherry, the cultivar Merton Glory is reported to be tolerant to canker (APS, 1966), Napoleon and
313 Roundel show race-specific differences, with Napoleon being susceptible to *Psm* R1 and tolerant to
314 R2 (and vice versa in Roundel) (Garrett, 1978). The cultivar Van is reported to be universally
315 susceptible (Long & Olsen, 2013). For plum, Victoria is reported as susceptible and Marjorie's
316 Seedling is more resistant (RHS, n.d.).
317

In cherry, data for both disease score (on an ordered categorical scale) and symptom length (mm) are presented in Figure 3. With both inoculation methods, the pathogens (R1-5244, R2-leaf, *Pss*-9097 and *Pss*-9293) caused necrosis and gumming (score ≥3), and in some cases lesions spread extensively beyond the inoculation site. In contrast to the glasshouse wound inoculations, all three pathogenic clades (*Psm* R1, *Psm* R2 and *Pss*) were able to spread from site of inoculation (previously only *Pss* appeared to spread). The non-pathogen inoculations generally induced limited browning (scores 1-2), with disease score profiles similar to the control. In the field, contamination by wild Pseudomonads may have occurred, and this explained why some control inoculations generated disease symptoms (6% of controls scored ≥3). For disease score, both inoculation methods were analysed together. The percentage of inoculations exhibiting disease symptoms (score ≥ 3) was greater in the wound inoculations than scar. Whilst, comparing cultivars, higher scores were more frequently observed in Napoleon than the other three cultivars.

Data for lesion length are also presented in Figure 3 (A2/B2). Due to differences in variance the two inoculation methods were analysed separately. REML analyses indicated there were significant differences between bacterial strains and host cultivars for both inoculation experiments. In both the length and score analyses there were no significant interactions between treatments, as pathogen and non-pathogen responses were consistent across the cultivars, with the two inoculation methods. There did appear to be variation in *Psm* R2 virulence between the cultivars, particularly after wound inoculation (Figure 3-B2), with reduced virulence compared to *Psm* R1 on Van, but a high level of virulence on Roundel. The plum strain *Psm* R1 5300 was not significantly different from the non-pathogens, indicating that it lacks pathogenicity for cherry. The two *Pss* strains varied considerably in virulence, with the cherry isolate *Pss* 9097 being associated with higher disease scores than the plum isolate *Pss* 9293. This is consistent with the results of the glasshouse inoculation, where *Pss* 9293 showed a reduced ability to cause canker. The cultivar Merton Glory appeared to be more tolerant to canker, with the lowest overall mean symptom length. Pathogenic strains were able to

cause disease symptoms on this cultivar but the length of these symptoms were not significantly greater than non-pathogens. This indicated that the pathogens could cause disease, but not spread effectively from site of inoculation.

In plum (Figure 4), symptoms produced were similar to those on cherry, with necrosis and gumming being indicative of disease. For the disease score, only strains with confirmed pathogenicity were able to spread (score = 4), however in comparison to the cherry inoculations, the R1-5300 plum isolate was pathogenic. As in cherry, infections through scars produced reduced disease scores compared to wound inoculations. The lesion length analysis showed that the only strains that were ever significantly different from the control were *Pss* 9097 and *Psm* R1 5300. Although analysis revealed there was no significant difference between the two cultivars, the plum cultivar Marjorie's Seedling did not appear to be susceptible to leaf scar infection, as no strain caused a necrosis length significantly different from the control. The plum cultivar Victoria was slightly more susceptible, with all strains of *Pss* and *Psm* R1 causing some necrosis.

Laboratory-based pathogenicity assays

The whole-tree inoculations allowed the the virulence of different *P. syringae* strains to be determined and identified non-pathogenic isolates. To rapidly screen for differences in virulence these methods are slow and involve the destruction of whole trees. To undertake large-scale resistance screens of *Prunus* mapping populations or to perform molecular studies of pathogenicity these methods are intractable. Therefore, several laboratory-based assays were assessed for their ability to reflect infection of whole trees.

Cut shoot inoculations

Several studies have documented the use of detached shoots for screening for bacterial canker resistance (Krzesinska *et al.*, 1992; Santi *et al.*, 2004; Li *et al.*, 2015). Using strains included in the

field assay, cherry and plum were screened with the cut shoot method. This involved using one-year old dormant shoots and inoculating a cut end by dipping in bacterial suspension. The extent to which necrosis spread down the shoot cambial tissue from this point could then be used to measure quantatitive differences in bacterial virulence/host resistance.

Figure 5 presents the results on both cherry and plum shoots. Strains exhibited host specificity towards the two *Prunus* species and towards particular cultivars. Focusing on cherry, pathogenic strains within *Psm* R1-5244, *Psm* R2-5255 and *Pss* 9097 were able to cause necrosis on >5% of the shoot area. The two *Psm* races varied in virulence on the different cultivars. As in the field experiment, *Psm* R2 was more virulent on Roundel, but less virulent on Van compared to *Psm* R1. The cut shoot test also confirmed that Merton Glory showed some tolerance compared to the other cultivars. On plum, the level of necrosis on Victoria was greater than that on Marjorie's Seedling. As observed in the field experiment, the plum strain of *Psm* R1 (R1-5300), was able to cause necrosis where it had failed on cherry. On cv. Victoria the *Aquilegia* pathogen RMA1 caused necrosis similar to *Pss* 9097.

Inoculation of detached immature cherry fruits

The suitability of immature cherry fruits was assessed for screening for bacterial canker resistance. Following stab inoculation, symptoms developed within a few days. Examples of the different clades that infect *Prunus* produced remarkably different symptoms on this tissue. Strains of *Pss* produced large necrotic lesions on cherry fruits within 2 days, and these expanded over time. By contrast, both *Psm* races produced water-soaked lesions within 2 days, and these did not increase in size. Most of the non-pathogens caused limited browning. Qualitative symptom assessment therefore allowed differentiation between pathogens and non-pathogens (Figures S1-S4). Measurements of lesion diameter caused by all *P. syringae* strains (Figure 6), confirmed significant differences between strains. However, diameters of the *Psm*-induced water-soaked lesions were not

397 greater than non-pathogens symptoms. The strain *Ps* 9643, although non-pathogenic on trees,
398 caused a similar level of water-soaking to the pathogenic *Psm* races (Figure S4), indicating a failure
399 of fruit to mount a resistant reaction towards this strain.
400
401 The three pathogenic clades were then used to screen different cherry cultivars. The results for
402 lesion diameter are presented in Figure S5. Lesions caused by *Pss* were smaller on Merton Glory
403 and Napoleon than on Van. However, no differences in lesion size or appearance between the two
404 *Psm* races on different host cultivars were found, in contrast to experiments on woody tissues.
405
406 **Inoculation of detached leaves**
407 A pilot experiment determined the best method of leaf inoculation was by blunt syringe-infiltration
408 (Figure S6). Bacterial multiplication was initially recorded following inoculation with a low
409 concentration of bacteria ($2x10^6$ per ml). On cherry, the pathogens (R1-5244, R2-leaf and *Pss*-
410 9097) exceeded levels of 10^6 CFU/ml within four days (Figure 7) and caused black necrosis at the
411 site of infection. The non-pathogens, including the plum isolate R1-5300 failed to reach 10^6
412 CFU/ml even after 10 days *in planta* and did not produce symptoms. These results support those
413 found on whole-trees, with only those strains capable of causing bacterial canker being able to
414 reach high levels within leaves. On plum, the pathogens also exceeded 10^6 CFU/ml after 4 days.
415 However, some of the strains that were non-pathogenic on cherry were able to grow to similar
416 levels as the pathogens. The *Psm* R1 plum isolate R1-5300 and RMA1 isolated from *Aquilegia*
417 *vulgaris* were found to be capable of multiplication. In the case of R1-5300 this result supports
418 results from inoculation of woody tissues indicating that it is a pathogen of plum but not cherry.
419 The ability of RMA1 to multiply within plum leaves did not support the field experiment where it
420 caused similar symptoms to the negative control.
421

Next, the population growth of all strains used in this study was tested. An end-point bacterial population count was taken after 10 days. Statistical analysis grouped the pathogens and non-pathogens into separate groups, validating population measurements as a method to differentiate pathogenic and non-pathogen strains (Figure S7).

The induction of the HR was also tested. To determine the best concentration to detect a HR, symptom development was scored at different concentrations (Figure S8). Scores were 0: no lesion, 1: limited browning, 2: browning <50% of inoculated area, 3: browning >50% of inoculated area, 4: complete browning, 5: browning and spread from inoculation site. Area Under the Disease Progression Curve (AUDPC) values were calculated to make comparisons based on timing of symptoms. The strains varied in their ability to cause lesions at the different concentrations, particularly the non-pathogen RMA1 which failed to induce more than limited browning (score 2), except when inoculated at the highest concentration. The *Pss* strain induced rapid lesion formation within 24 hours and on rare occassions spread slightly from the site of inoculation. At the higher concentrations the final lesions of all strains were similar in appearance, but could be differentiated by symptom timing. The timing of lesion onset was found to clearly differentiated the pathogenic *Psm* races from other strains. The non-pathogens (including the plum *Psm* R1-5300) and pathogenic strain *Pss* 9097 all induced rapid lesion formation at the highest concentration, with complete browning of the inoculation site (score 4) within the first 48 hours, which was suggestive of a HR. Pathogenic *Psm* R1 and R2 induced slower symptom development. This was indicative of a hemi-biotrophic interaction with the host. To study this interaction in more detail *Psm* R2 was inoculated onto detached leaves and electron microscopy used to examine bacteria-plant cell interactions. The bacteria were found to multiply initially in the apoplastic space without causing plant cell death, confirming hemi-biotrophic development, although some wall alterations were noted next to colonies. (Figure S9A -C).

448 To compare the host reactions on cherry and plum, the leaf population count and symptom scoring
449 experiments were extended with a group of representative strains (Figure 8). As before, population
450 counts clearly differentiated pathogens and non-pathogens (Figure 8A). On both hosts, pathogens
451 exceeded 10^7 CFU/ml and produced necrotic lesions. In comparison, non-pathogens failed to induce
452 symptoms and did not reach 10^7 CFU/ml. In the symptom scoring experiment (Figure 8B) all
453 strains gave symptoms in the leaves, however the timing of symptoms was used to differentiate
454 pathogenicity and hypersensitivity. On cherry, both *Pss* pathogens and the non-pathogens *Ps* 9643,
455 R1-5300 and RMA1 induced symptoms rapidly, R2-leaf, *Pph* and *Psav* were slightly slower and
456 R1-5244 only induced symptoms 48-72 hpi. In plum, the two *Pss* strains and *Ps* 9643 rapidly
457 induced symptoms. Other non-pathogens were slower and not significantly faster than R2-leaf.
458 Symptom development of plum *Psm* R1 5300 was not significantly different from cherry R1-5244,
459 both inducing symptoms 48 hpi, indicating that in plum the two pathogens behave similarly.
460 Representative images of symptoms on cherry and plum leaves over time are presented in Figure
461 S10.
462
463 **Suitability of leaves for resistance screening**
464 The leaf population assay clearly differentiated pathogens from non-pathogens. However, a screen
465 for canker resistance would involve discriminating subtle differences in pathogen growth on
466 different cherry genotypes. To see if detached leaves could discriminate cultivar differences, the
467 assay was tested on four cultivars with differences in susceptibility recorded on woody tissue in the
468 field. Strains representing the three cherry-infecting pathovars were tested. The three strains were
469 able to grow to exceed 10^6 CFU/ml (Figure 9) and cause disease symptoms in all cultivars. This
470 suggested that on leaves, any host-resistance to the pathogens could not be easily discriminated.
471 The leaf system, although useful for comparing strains with divergent virulence levels may not be
472 sensitive enough to detect the subtle differences between races of the pathogens found in the field.
473

Discussion

In this study, the genomes of a set of *P. syringae* isolates from different hosts were sequenced. The ability of these strains to cause canker disease on cherry and plum was characterised. Breeding for resistance towards this complex disease is particularly challenging due to the large number of divergent strains that appear to be pathogenic. Host resistance to cherry canker is likely to be multi-factorial and potential mechanisms of resistance towards the different cherry-infecting clades may operate at different stages of the disease cycle. Phylogenetic analysis confirmed that the three major canker-causing clades (*Psm* R1, *Psm* R2 and *Pss*) fall in separate phylogroups, and therefore pathogenicity towards cherry has arisen multiple times in the *P. syringae* species complex. As the different clades have convergently evolved, it is likely that host resistance mechanisms targeted towards them differ significantly.

First, the ability of individual bacterial strains to cause cherry canker was assessed using a glasshouse whole-tree inoculation. This provided a baseline to compare with the results of laboratory-based assays, that may or may not correlate with ability to cause canker. Although strains of *Psm* R1 were phylogenetically indistinct, they could be divided into pathogenic and non-pathogenic isolates, with non-pathogenic isolates failing to cause gumming and black necrosis. Non-pathogenic strains isolated from distantly related plant species were unable to cause disease, supporting the theory that individual pathovars are mostly specialised to their particular host plant (Sarkar *et al*., 2006). All *Psm* R1 isolates from plum were non-pathogenic on cherry. Their lack of cherry pathogenicity may be due to host-specific factors. By contrast, all isolates of *Pss* (from plum and cherry) caused disease on cherry, indicating that these strains exhibit a greater host range.

Strains with variable virulence levels were then pathogenicity tested under field conditions, in assays which should be representative of natural disease. The different host-specificities of *Psm* R1 strains on cherry and plum were confirmed. The cherry isolate *Psm* R1 5244 was pathogenic to both

cherry and plum, whereas *Psm* R1 5300 was only pathogenic on plum trees. This is an interesting result as phylogenetics revealed this clade to be highly homogeneous (Figure 1). As the phylogenetic analysis was based only on core house-keeping genes in the core genome, it may be missing divergence in the flexible genome that are responsible for differences in pathogenicity. Genomic analysis of these strains could reveal important differences in virulence factor repertoires that dictate host specificity. Interestingly, the results support studies done at East Malling looking at *Psm* R1 host specificity (Crosse & Garrett 1970). *Psm* R1 was originally designated as a race based on differences with *Psm* R2, however it is now known that these are two divergent clades, so should not really be designated as races of the same pathovar. However, the differences in pathogenicity of members of *Psm* R1 may indicate that, at least within the bacterial populations occupying orchards in UK, there may be a race structure within this clade, with the different groups varying in ability to infect different *Prunus* species. Members of the group containing *Psm* R1-5300 may be restricted in growth on cherry due to the expression of avirulence factors. Further sampling of a diverse range of strains from different *Prunus* species and cultivars should confirm this hypothesis.

The field inoculations were assessed using both disease score and symptom length. For disease score, cherry leaf scars appeared much less susceptible to infection. The leaf scar may act as a barrier to infection and reduce bacterial concentrations as the bacterial population is bottle-necked. Therefore, a higher percentage of trees scored highly for wound inoculations as this by-passed the barrier to infection. For symptom lengths, results were more variable, with most pathogen inoculations only spreading slightly. Only in rare cases did they cause severe necrosis, sometimes exceeding 100mm. The results revealed significant differences between cherry cultivars. In both wound and leaf scar inoculations, the cultivar Merton Glory exhibited a broad level of tolerance to all three pathogenic clades. This cultivar is therefore a candidate for further study of the mechanisms underlying resistance. Although the analysis did not show a strain by cultivar interaction, there was variation in resistance to *Psm* R2. This strain was associated with only limited

disease on Van, whilst the cultivar Roundel was highly susceptible. Van is therefore a candidate cultivar exhibiting race-specific resistance. There was no significant difference in symptom length or disease score of pathogenic *Psm* R1 and R2 on Napoleon, contrasting to previous studies that suggested Napoleon to be resistant to R2 (Garrett, 1978). In addition, previous studies reported *Pss* and *Psm* R2 to be less invasive through leaf scars inoculations than *Psm* R1 (Crosse & Garrett 1966; Freigoun & Crosse 1975), which contrasts to this study where all clades caused disease. Differences in experimental procedure could have led to variation in results. The original studies used fully mature trees which may exhibit contrasting resistance mechanisms to the young trees used in this study (Freigoun & Crosse 1975; Garrett 1978).

The field experiment on plum demonstrated significant differences between strains, but not between cultivars. The cultivar Marjorie's Seedling was not susceptible to any strains inoculated through leaf scars indicating this is unlikely to be a natural entry point for pathogens. Indeed, previous reports suggest that plum pathogens do not naturally enter through the leaf scars (Crosse, 1966). However, some pathogenic strains were found to be capable of causing disease on the cultivar Victoria when inoculated through leaf scars.

Several rapid laboratory-based assays were tested for their suitability for resistance screening. Assuming that the field wound inoculations represent the natural disease, the results of the other tests for a set of strains on cherry cv. Van were correlated against the wound results (Figure 10). The leaf scar, cut shoot and leaf population assays all correlated well with the wound results ($r > 0.70$), whilst the fruit assay did not correlate well ($r = 0.37$).

Both inoculations of woody tissues (leaf scar and cut shoots) correlated well with the wound results. The cut shoot assay provided a rapid assessment that could differentiate pathogens and non-pathogens. It was also sensitive enough to detect variation in pathogen virulence on the different

552 cultivars. For example, *Psm* R2 caused the greatest necrosis on cv. Roundel in all three woody
553 tissue inoculation tests, indicating that this cultivar is highly susceptible to *Psm* R2. In addition,
554 differential virulence of *Psm* R1 and *Psm* R2 on cv. Van was supported by both the cut shoot and
555 field experiments, suggesting this cultivar may possess some resistance to *Psm* R2. Various studies
556 have utilised cut shoot inoculations of *P. syringae* and fungal canker-causing pathogens.
557 Differences in virulence of the same isolate between field and laboratory results are sometimes
558 reported (Farhadfar *et al.*, 2016; Gomez-Cortecero *et al.*, 2016). Therefore, a combination of whole
559 tree and shoot tests could provide the most robust method of assessment. The cut shoot assay
560 provided a means to perform rapid high-throughput screening, with speed aided through automated
561 image analysis of shoots (Li *et al.*, 2015). Therefore, the results of such tests may help narrow down
562 a list of putatively resistant genotypes, before resistance testing on whole trees.
563
564 The lack of correlation of the cherry fruit test with the field experiment indicated that the lesion
565 development on fruit induced by a *P. syringae* strain may not reflect its pathogenicity in the field.
566 Nevertheless, the qualitative symptoms this assay provides are useful to rapidly differentiate the
567 different pathogenic clades. The induction of symptoms by strains non-pathogenic in the field (e.g.
568 *Ps* 9643, Figure S4), indicated that results must be considered with caution. In comparison, the leaf
569 population assay correlated well with wound inoculations and allowed discrimination of pathogens
570 and non-pathogens. However, when the three pathogenic clades were inoculated across cherry
571 cultivars (Figure 9) they all exceeded 10^6 CFU/ml *in planta* and caused symptom development. The
572 presence of similar symptoms on both susceptible and tolerant varieties means that this method
573 would not be very applicable for large-scale screening. The leaf and fruit assays were therefore not
574 sensitive enough to determine subtle differences in cultivar susceptibility seen in the field
575 experiment such as the resistance of cherry cv. Van to Psm R2. The quantitative differences in
576 resistance of the different cherry cultivars in the field and shoot experiments may be tissue-specific,

577 and therefore resistance phenotypes in fruit and leaves may differ substantially from those found in
578 dormant woody tissues.
579
580 Detached leaves provided a rapid means to assess pathogenicity through the measurement of
581 bacterial population counts over time. Cherry leaf population counts clearly discriminated
582 pathogenic and non-pathogenic strains. When inoculated at a low concentration only pathogenic
583 strains (R1-5244, R1-leaf and *Pss* 9097) were able to cause disease lesions on cherry, which
584 appeared 7-10 dpi. On Plum, the non-pathogen RMA1 and plum isolate R1-5300 were able to grow
585 to similar levels to the cherry pathogens (Figure 7). The fact that RMA1 was able to grow to high
586 levels in plum leaves does not correspond to its pathogenicity in the field assay. Interestingly, in the
587 cutshoot assay (Figure 5) RMA1 caused necrosis on plum similar to the *Pss* pathogen. The field
588 experiment showed that RMA1 is not a true pathogen of plum, however, its virulence in the lab-
589 based assays may indicate it has adaptive potential to cause disease when inoculated in unnaturally
590 high concentrations directly onto plant tissue. Its inability to cause any disease on cherry in all lab-
591 based assays indicated that cherry may exhibit a robust non-host immune response towards this
592 non-pathogen, which is different to that expressed in plum.
593
594 Symptom development on cherry leaves allowed differentiation of hypersensitive and pathogenic
595 responses. When inoculated at high concentrations all strains produced necrotic lesions, however
596 non-pathogens were found to induce symptoms earlier than pathogens of *Psm* R1 and R2. The
597 activation of the HR may mean that ETI is operating against non-pathogens in cherry leaves, and
598 differences in effector repertoires between cherry-infecting strains and non-pathogens could reveal
599 those effectors that are detected. In particular, there were clear differences in pathogenicity of the
600 two *Psm* R1 strains on cherry, which agreed with the whole-tree assay. The HR on cherry was clear
601 for non-pathogens *Psm* R1 5300, *Ps* 9643 and RMA1, whereas symptom development associated
602 with *Pph* and *Psav* was slower. This slower onset of symptoms may mean that any hypersensitive

603 response induced by these strains is weaker or that more basal resistance mechanisms such as
604 PAMP-triggered immune responses play a greater role in preventing their population growth in
605 leaves. Interestingly, although *Pss* strains reached high population levels in the leaves, they
606 triggered symptom development at a similar rate to the HR caused by non-pathogens. *P. syringae* is
607 traditionally described as a hemi-biotrophic pathogen (Lindeberg *et al.*, 2012), with delayed
608 symptom onset during the biotrophic phase followed by symptoms during a necrotrophic phase.
609 The results indicated that on leaves *Pss* may be more necrotrophic as it triggers symptoms rapidly.
610 Further study could reveal the factors inducing these rapid symptoms. The production of non-
611 ribosomal peptide toxins is common in strains of phylogroup 2, which includes *Pss* (Dudnik &
612 Dudler, 2014), and if expressed early could cause the necrotic symptoms seen. Indeed, a study of
613 *Pss* toxins (Yin-Yuan & Gross, 1991) showed that syringomycin is expressed within the first 24
614 hours of inoculation of immature cherry fruits. *Pss* could also be deliberately triggering the HR like
615 other necrotrophic pathogens to aid disease development (Govrin & Levine, 2000). Further study of
616 the immune responses occurring within plant cells would be required to test these hypotheses.
617
618 The failure of designated non-pathogenic strains to produce symptoms in woody tissues was
619 reflected by their low multiplication and induction of a HR-like response in leaves. Such clear cut
620 resistance is characteristic of *avr/R* gene interactions reflecting ETI. By contrast, where differential
621 reactions were observed between cultivars challenged with pathogenic *Psm* and *Pss*, quantitative
622 differences in symptoms were seen. The lack of clear differentials between cultivars suggests that
623 variation in susceptibility is not based simply on *avr/R* gene recognition. In field conditions, this
624 plant-pathogen interaction lasts for many months. Perhaps, factors important not just for
625 pathogenicity, but for the ability of bacterial populations to successfully colonise and persist
626 through the season, dictate the outcome of this interaction. Resistance mechanisms that reduce
627 persistence in woody tissue, e.g. responses that block bacteria spreading to new tissues and
628 acquiring nutrients, may prevent a pathogenic strain from causing severe disease.

629

630 This study has focused on the detailed analysis of pathogenicity in strains used for genome
631 sequencing. Results show that representatives of the three clades of *P. syringae* that cause bacterial
632 canker may utilise distinct mechanisms of virulence and trigger differing host resistance
633 mechanisms in cherry. A HR is putatively triggered in leaves, indicating that effector-triggered
634 immunity may be operating in cherry against pathogens of other hosts. Cherry leaves and fruit
635 failed to sufficiently reveal varietal differences to the same extent as experiments on woody tissues.
636 This suggests that some resistance mechanisms are tissue-specific. A whole range of complex
637 variable traits could be involved in these varietal differences in susceptibility. These include timing
638 of leaf drop, phellogen activity and differences in leaf-surface bacterial populations which act as
639 inocula for wood infections, as discussed by Crosse (1966). Breeding resistance to at least three
640 rather distinct groups of a pathogen remains a challenging prospect. Cultivars such as Merton Glory
641 that exhibit resistance to all three clades may be useful for determining the genetic basis of broad-
642 spectrum resistance mechanisms, independent of ETI.

643

644 **Acknowledgments**

645 We thank Steve Roberts, Helen Neale and David Guttman for providing bacterial strains used in
646 this study. Electron microscopy was performed by Dr Ian Brown (University of Kent). We also
647 thank Karen Russell and Connie Garrett for valuable advice about the pathogenicity test
648 development, as well as the East Malling Farm and Glass team for assistance in tree grafting and
649 planting. The authors declare no conflict of interest.

650

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652 **References**

653 APS, 1966. Merton cherries from England. *Journal of Fruit Varieties and Horticultural Digest*, **20**.

654 Arnold DL, Lovell HC, Jackson RW, Mansfield JW, 2011. Pathogen profile *Pseudomonas syringae*
655 pv . *phaseolicola*: From " has bean " to supermodel. *Molecular Plant Pathology* **12**, 617–
656 627.
657 Bankevich A, Nurk S, Antipov D *et al*., 2012. SPAdes: A new genome assembly algorithm and its
658 applications to single-cell sequencing. *Journal of computational biology : A journal of*
659 *computational molecular cell biology* **19**, 455–77.
660 Berge O, Monteil CL, Bartoli C *et al*., 2014. A user's guide to a data base of the diversity of
661 *Pseudomonas syringae* and its application to classifying strains in this phylogenetic
662 complex. *PLoS ONE* **9**, e105547.
663 Bultreys A & Kaluzna M, 2010. Bacterial cankers caused by *Pseudomonas syringae* on stone fruit
664 species with special emphasis on the pathovars *syringae* and *morsprunorum* Race 1 and
665 Race 2. *Journal of Plant Pathology* **92**, S1.21-S1.33.
666 Corwin JA, Copeland D, Feusier J *et al*., 2016. The quantitative basis of the *Arabidopsis* innate
667 immune system to endemic pathogens depends on pathogen genetics. *PLoS Genetics* **12**, 1–
668 29.
669 Crosse JE, 1959. Bacterial canker of stone-fruits. IV. Investigation of a method for measuring the
670 inoculum potential of cherry trees. *Annals of Applied Biology* **47**, 306–317.
671 Crosse JE, 1966. Epidemiological relations of the *Pseudomonad* pathogens of deciduous fruit trees.
672 *Annual review of phytopathology* **4**, 291–310.
673 Crosse, JE, & Garrett CME, 1966. Bacterial canker of stone-fruits. VII. Infection experiments with
674 *Pseudomonas morsprunorum* and Ps. *syringae*. *Annals of Applied Biology* **58**, 31–41.
675 Crosse, JE, & Garrett CME, 1970. Pathogenicity of *Pseudomonas morsprunorum* in relation to host
676 specificity. *Journal of General Microbiology* **62**, 315–327.
677 Debener T, Lehnackers H, Arnold M, Dangl JL, 1991. Identification and molecular mapping of a
678 single Arabidopsis thaliana locus determining resistance to a phytopathogenic *Pseudomonas*
679 *syringae* isolate. *The Plant journal : for cell and molecular biology* **1**, 289–302.

680 Dudnik A, Dudler R, 2014. Genomics-based exploration of virulence determinants and host-specific
681 adaptations of P*seudomonas syringae* strains isolated from grasses. *Pathogens* **3**, 121–148.
682 Farhadfar S, Keshavarzi M, Bouzari N, Moghadam L, Soleimani A, 2016. Susceptibility of cherries
683 to bacterial canker (*Pseudomonas syringae* pv . *syringae*) in field and laboratory.
684 *International journal of Agriculture and Forestry* **6**, 20–27.
685 Freigoun SO, & Crosse JE, 1975. Host relations and distribution of a physiological and pathological
686 variant of *Pseudomonas morsprunorum*. *Annals of Applied Biology* 81, 317–330.
687 Gardan L, Shafik H, Belouin S, Broch R, Grimont F, Grimont P, 1999. DNA relatedness among the
688 pathovars of *Pseudomonas syringae* and description of *Pseudomonas tremae* sp. nov. and
689 *Pseudomonas cannabina* sp. nov. (ex Sutic and Dowson 1959). *International Journal of*
690 *Systematic Bacteriology* **49**, 469–478.
691 Garrett CME, 1978. Pathogenic races of *Pseudomonas morsprunorum*. In: *Proceedings of the IVth*
692 *International Conference on Plant pathogenic Bacteria Vol II*. 889–890.
693 Garrett CME, 1979. Screening *Prunus* rootstocks for resistance to bacterial canker, caused by
694 *Pseudomonas morsprunorum*. *Journal of Horticultural Science* **54**, 189–193.
695 Gilbert V, Legros F, Maraite H, Bultreys A, 2008. Genetic analyses of *Pseudomonas syringae*
696 isolates from Belgian fruit orchards reveal genetic variability and isolate-host relationships
697 within the pathovar *syringae*, and help identify both races of the pathovar *morsprunorum*.
698 *European Journal of Plant Pathology* **124**, 199–218.
699 Gilbert V, Planchon V, Legros F, Maraite H, Bultreys A, 2009. Pathogenicity and aggressiveness in
700 populations of *Pseudomonas syringae* from Belgian fruit orchards. *European Journal of*
701 *Plant Pathology* **126**, 263–277.
702 Gill US, Lee S, Mysore KS, 2015. Host versus nonhost resistance: Distinct wars with similar
703 arsenals. *Phytopathology* **105**, 580–587.

704 Gomez-Cortecero A, Saville RJ, Scheper RWA *et al.*, 2016. Variation in host and pathogen in the
705 *Neonectria*/*Malus* interaction; towards an understanding of the genetic basis of resistance to
706 European canker. *Frontiers in Plant Science* **7**, 1365.
707 Govrin EM, Levine A, 2000. The hypersensitive response facilitates plant infection by the
708 necrotrophic pathogen *Botrytis cinerea*. *Current Biology* **10**, 751–757.
709 Gurevich A, Saveliev V, Vyahhi N, Tesler G, 2013. QUAST: quality assessment tool for genome
710 assemblies. *Bioinformatics* **29**, 1072–1075.
711 Huelsenbeck JP, Ronquist F, 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*
712 **17**, 1572–1574.
713 Iakovidis M, Teixeira PJPL, Exposito-Alonso M *et al.*, 2016. Effector triggered immune response in
714 *Arabidopsis thaliana* is a quantitative trait. *Genetics* **204**, 337–353.
715 Joardar V, Lindeberg M, Jackson RW *et al.*, 2005. Whole-genome sequence analysis of
716 *Pseudomonas syringae* pv. *phaseolicola* 1448A reveals divergence among pathovars in
717 genes involved in virulence and transposition. *Journal of Bacteriology* **187**, 6488–6498.
718 Jones JDG, Dangl JL, 2006. The plant immune system. *Nature* **444**, 323–329.
719 Kaluzna M, Ferrante P, Sobiczewski P, Scortichini M, 2010. Characterization and genetic diversity
720 of *Pseudomonas syringae* from stone fruits and hazelnut using repetitive-PCR and MLST.
721 *Journal of Plant Pathology* **92**, 781–787.
722 Kelley DR, Schatz MC, Salzberg SL, 2010. Quake: Quality-aware detection and correction of
723 sequencing errors. *Genome Biology* **11**, R116.
724 Krzesinska EZ, Nina A, Azarenko M, 1992. Excised twig assay to evaluate cherry rootstocks for
725 tolerance to *Pseudomonas syringae* pv. *syringae*. *HortScience* **27**, 153–155.
726 Li B, Hulin MT, Brain P, Mansfield JW, Jackson RW, Harrison RJ, 2015. Rapid, automated
727 detection of stem canker symptoms in woody perennials using artificial neural network
728 analysis. *Plant Methods* **11**, 57.

729 Lindeberg M, Cunnac S, Collmer A, 2012. *Pseudomonas syringae* type III effector repertoires: Last
730 words in endless arguments. *Trends in Microbiology* **20**, 199–208.
731 Long J, Olsen L, 2013. *Sweet cherry cultivars for brining, freezing, and canning in Oregon*.
732 Available at: https://catalog.extension.oregonstate.edu/files/pr.
733 Ménard M, Sutra L, Luisetti J, Prunier JP, Gardan L, 2003. *Pseudomonas syringae* pv. *avii* (pv.
734 nov.), the causal agent of bacterial canker of wild cherries (*Prunus avium*) in France.
735 *European Journal of Plant Pathology* **109**, 565–576.
736 Moragrega C, Llorente I, 2003. Susceptibility of European pear cultivars to *Pseudomonas syringae*
737 pv. *syringae* using immature fruit and detached leaf assays. *European Journal of Plant*
738 *Pathology* **109**, 319–326.
739 Parkinson N, Bryant R, Bew J, Elphinstone J, 2011. Rapid phylogenetic identification of members
740 of the *Pseudomonas syringae* species complex using the *rpoD* locus. *Plant Pathology* **60**,
741 338–344.
742 Poland JA, Balint-Kurti PJ, Wisser RJ, Pratt RC, Nelson RJ, 2009. Shades of gray: The world of
743 quantitative disease resistance. *Trends in Plant Science* **14**, 21–29.
744 Preston GM, 2000. *Pseudomonas syringae* pv. *tomato*: The right pathogen, of the right plant, at the
745 right time. *Molecular Plant Pathology* **1**, 263–275.
746 Quirino BF, Bent AF, 2003. Deciphering host resistance and pathogen virulence: The
747 *Arabidopsis*/*Pseudomonas* interaction as a model. *Molecular Plant Pathology* **4**, 517–530.
748 R Core Team, 2012. R: *A language and environment for statistical computing*. Vienna, Austria: R
749 Foundation for Statistical Computing.
750 RHS, *Bacterial canker*. Available at: https://www.rhs.org.uk/advice/profile?PID=86.
751 Santi F, Russell K, Menard M, Dufour J, 2004. Screening wild cherry (*Prunus avium*) for resistance
752 to bacterial canker by laboratory and field tests. *Forest Pathology* **34**, 349–362.
753 Sarkar SF, Gordon JS, Martin GB, Guttman DS, 2006. Comparative genomics of host-specific
754 virulence in *Pseudomonas syringae*. *Genetics* **174**, 1041–1056.

755 Senthil-Kumar M, Mysore KS, 2013. Nonhost resistance against bacterial pathogens: retrospectives
756 and prospects. *Annual review of phytopathology* **51**, 407–427.
757 Soylu S, Brown I, Mansfield J, 2005. Cellular reactions in *Arabidopsis* following challenge by
758 strains of *Pseudomonas syringae*: From basal resistance to compatibility. *Physiological and*
759 *Molecular Plant Pathology* **66**, 232–243.
760 Spotts RA, Wallis KM, Serdani M, Azarenko AN, 2010. Bacterial canker of sweet cherry in
761 Oregon—Infection of horticultural and natural wounds, and resistance of cultivar and
762 rootstock combinations. *Plant Disease* **94**, 345–350.
763 Stone A, Baker B, 2010. Organic management of late blight of potato and tomato with copper
764 products. Available at: http://articles.extension.org/pages/18351/organic-management-of-
765 late-blight-of-potato-and-tomato-with-copper-products
766 Vicente JG, Roberts SJ, 2003. Screening wild cherry micropropagated plantlets for resistance to
767 bacterial canker. In: Santa Lacobellis N,, Collmer A, Hutcheson S, *et al.*, eds. Pseudomonas
768 syringae *and related pathogens.* Springer Netherlands, 1–8.
769 Vicente JG, Roberts SJ, 2007. Discrimination of *Pseudomonas syringae* isolates from sweet and
770 wild cherry using rep-PCR. Eur*opean Journal of Plant Pathology* **117**, 383–392.
771 Yin-Yuan M, Gross D, 1991. Expression *in vitro* and during plant pathogenesis of the *syrB* gene
772 required for syringomycin production by *Pseudomonas syringae* pv. *syringae*. *Molecular*
773 *plant-microbe interactions : MPMI* **4**, 28–36.

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775
776

777 **Table Legends**

778

779 **Table 1**

780 Bacterial strains used in this study with host of isolation and reference/source. Strains sequenced in
781 this study are listed first, followed by the out-group strains *Pph* and *Psav* included in pathogenicity

tests and then the rest of the strains used soley for phylogenetic analysis. The Genbank accessions of genomes used for the phylogenetic analysis are included. Full genbank accessions of strains sequenced in this study will be released upon publication.

Figure Legends

Figure 1

Bayesian phylogenetic tree of *P. syringae*. The phylogeny was constructed using a concatenated alignment of seven genes (*acnB*, *fruK*, *gapA*, *gltA*, *gyrB*, *pgi* and *rpoD*). A subset of strains from the three major phylogroups were selected for analysis, with the canker-causing clades *Psm* R1, *Psm* R2 and *Pss* highlighted. Phylogroups are labelled P1-3. Strains isolated from cherry are in pink, whilst those from plum are in blue. Strains in bold were pathogenicity tested in this study. Scale bar shows substitutions per site. Bootstrap support values <99% are presented.

Figure 2

Percentage of trees in each disease score category after wound inoculation of *P. avium* cv. Van with different isolates of *P. syringae*: *Psm* R1, *Psm* R2, *Pss*, selected outgroup non-host strains and a no bacteria control. Data presented are the percentage of replicates (n=5) for each strain in each disease category. Disease symptoms were scored on a ordinal scale as illustrated: 1, no symptoms; 2, limited browning; 3, necrosis and gumming; 4, necrosis, gumming and spread from site of inoculation. Strains are ordered based on increasing disease score. Strains isolated from cherry are labelled in pink, whilst those from plum are in blue. Statistical Tukey-HSD (p=0.05, confidence level: 0.95) groupings of bacterial strains determined by a Proportional Odds Model (POM) analysis are presented above the bar.

Figure 3

Field inoculations of different cherry cultivars with selected strains of *P. syringae*. Data presented are the disease score and length of disease symptoms based on symptom observations six months after inoculation. A: Leaf scar inoculation. B: Wound inoculations. 1: Percentage of trees in each disease score category (colour-coded from light pink to red by score: no symptoms, browning, necrosis and gumming and necrosis, gumming and spreading from site of inoculation). 2: Boxplot of length of symptoms associated with each strain on the four cultivars. Boxplots are colour-coded for each strain based on clade *Psm* R1 (blue), *Psm* R2 (green), *Pss* (red), outgroup avirulent strains (orange) and no bacterial control (black). All data points (n=10) are presented. Strains on all plots are colour-coded based on host of isolation (cherry in pink, plum in blue and other hosts in black). For disease score POM analysis indicated that there was a significant difference between inoculation method ($p<0.01$, df=1), between *P. syringae* strains ($p<0.01$, df=8) and between cultivars ($p<0.01$, df=3). For symptom length, REML analyses indicated there were significant differences between strains and cultivars for both the leaf scar and wound experiments ($p<0.01$, df=8 and $p<0.01$, df=3 respectively). Tukey-HSD ($p=0.05$, confidence level: 0.95) groups are presented above each strain for each cultivar.

Figure 4

Field inoculations of different plum cultivars with selected strains of *P. syringae*. Data presented are the disease score and length of disease symptoms based on symptom observations six months after inoculation. A: Leaf scar inoculation. B: Wound inoculations. 1: Percentage of trees in each disease score category (colour-coded from light pink to red by score: No symptoms, browning, necrosis and gumming and necrosis, gumming and spreading from site of inoculation). 2: Boxplot of length of symptoms associated with each strain on the four cultivars. Boxplots are colour-coded for each strain based on clade *Psm* R1 (blue), *Psm* R2 (green), *Pss* (red), outgroup avirulent strains (orange) and no bacterial control (black). All data points (n=10) are presented. Strains on all plots

834 are colour-coded based on host of isolation (cherry in pink, plum in blue and other hosts in black).
835 For disease score POM analysis indicated there were significant differences between inoculation
836 method ($p<0.01$, df=1), strains ($p<0.01$, df=8) and cultivars ($p<0.01$, df=1). For symptom length,
837 REML analyses indicated there were significant differences between strains in both inoculation
838 experiments ($p<0.01$, df=8) but not between host cultivars ($p=0.20$, df=1 for leaf scar, $p=0.35$, df=1
839 for wound). Tukey-HSD ($p=0.05$, confidence level: 0.95) groups are presented above each strain for
840 each cultivar.
841
842 **Figure 5**
843 Lesion development on cut shoots of cherry and plum cultivars following inoculation with *P.*
844 *syringae*. A: Boxplot of percentage area of necrosis in the top 30mm associated with different *P.*
845 *syringae* strains on four cherry cultivars. All data points for each treatment (n=10) are presented.
846 The bar chart is colour-coded based on clade, *Psm* R1: blue, *Psm* R2: green, *Pss*: red, non-
847 pathogens: orange and control: black. B: The same parameters for two different plum cultivars. C:
848 Representative images of the symptoms on shoots inoculated with *Pss* 9097 on cv. Napoleon (1-4)
849 or the no bacteria control (5). Strain numbers on all plots are colour-coded based on host of
850 isolation (cherry in pink, plum in blue and other hosts in black). An ANOVA revealed there were
851 significant differences between bacterial strains ($p<0.001$, df=8), no significant difference between
852 the susceptibility of the two *Prunus* species ($p=0.57$, df=1) and there was a significant interaction
853 between *Prunus* species and *P. syringae* strain ($p<0.01$, df=8) as well as interactions between strain
854 and individual cultivars ($p<0.01$, df=36). Tukey-HSD ($p=0.05$, confidence level: 0.95) significance
855 groups for the different strains for each separate cultivar are presented above each boxplot.
856
857 **Figure 6**
858 Boxplot to show diameter of necrosis caused by different *P. syringae* strains on immature cherry
859 fruits. Strains isolated from cherry and plum are highlighted in pink and blue. The bar chart is

colour-coded, *Psm* R1: blue, *Psm* R2: green, *Pss*: red, non-pathogens: orange and control: black. All data points for each treatment (n=5) are shown. Representative images are presented. 1: *Psm* R1, 2: *Psm* R2, 3: *Pss*, 4: non-pathogens, 5: control. An ANOVA revealed significant differences between strains ($p<0.01$, df=21). Tukey-HSD ($p=0.05$, confidence level: 0.95) significance groups are presented above each bar.

Figure 7

Population counts of different strains over time on cherry cv. Van (A) and plum cv. Victoria (B) leaves. The strains isolated from cherry and plum are highlighted in pink and blue. Line colours for each strain are presented in the key. Population counts are Log CFU/ml. Data presented are the mean values (n=9), with error bars showing standard error above and below the mean. An ANOVA revealed significant differences between strains ($p<0.01$, df=8).Tukey-HSD ($p=0.05$, confidence level: 0.95) significance groups for the different strains (based on day 10 populations) are presented.

Figure 8

Pathogenicity of different strains, assessed by population counts and symptom scores, on cherry and plum leaves. A: Boxplots of day 10 population counts on cherry cv. Van and plum cv. Victoria. Strains isolated from cherry are pink whilst plum are blue. Boxplots are colour-coded by clade, with *Psm* R1: blue, *Psm* R2: green, *Pss*: red, non-pathogen: orange. Data presented are all the values for each treatment of two independent experiments (n=18). ANOVAs for both cherry and plum revealed significant differences between strains ($p<0.01$, df=8). Tukey-HSD ($p=0.05$, confidence level: 0.95) significance groups for the different strains are presented. B: Symptom development over time. Symptoms were scored, 0: no symptoms, 1: limited browning, 2: <50% inoculated area brown, 3: >50% inoculated area brown, 4: Complete browning. Strains are colour-coded as in A. Data presented are the mean values for each treatment of two independent

886 experiments (n=8). Symptom development over time was analysed using the Area Under the
887 Disease Progression Curve (AUDPC) analysis. ANOVAs for both cherry and plum revealed
888 significant differences between strains ($p<0.01$, df=8). Tukey-HSD ($p=0.05$, confidence level: 0.95)
889 significance groups are presented in the table next to the plot.
890
891 **Figure 9**
892 Bacterial multiplication recorded in different cherry cultivars. Boxplot of day 10 population counts
893 of three pathogenic *P. syringae* strains on different cherry cultivars. Strains are colour-coded by
894 clade, with *Psm* R1: blue, *Psm* R2: green, *Pss*: red. All three strains were cherry isolates so the
895 names are coloured pink. Data presented are all the values for each treatment of two independent
896 experiments (n=18). Tukey-HSD ($p=0.05$, confidence level: 0.95) significance groups for the
897 different strains on each separate cultivar are presented. An ANOVA revealed significant
898 differences between strains ($p<0.01$, df=2), cultivars ($p<0.01$, df=3) and a significant interaction
899 ($p<0.01$, df=6).Tukey-HSD groups comparing the different cultivars are also presented.
900
901 **Figure 10**
902 Correlation of different inoculation experiments with the whole-tree wound inoculations performed
903 in the field. The scatterplot was created using the mean standardised disease scores for each
904 bacterial strain on cherry cv. Van. A linear model (lm) line was plotted for each experiment. The
905 Pearson's correlation coefficients correlating the results of each experiment with the wound field
906 inoculations are presented.
907
908 **Supporting material**
909
910 **Figure S1**

911 Images of immature cherry fruits inoculated with *Psm* R1 strains. Images were taken 10dpi. Five
912 replicate cherries were inoculated per strain. Strains are colour-coded based on host of isolation as
913 pink (cherry) or blue (plum).
914
915 **Figure S2**
916 Images of immature cherry fruits inoculated with *Psm* R2 strains. Images were taken 10dpi. Five
917 replicate cherries were inoculated per strain. Strains are colour-coded based on host of isolation as
918 pink (cherry).
919
920 **Figure S3**
921 Images of immature cherry fruits inoculated with *Pss* strains. Images were taken 10dpi. Five
922 replicate cherries were inoculated per strain. Strains are colour-coded based on host of isolation as
923 pink (cherry) or blue (plum).
924
925 **Figure S4**
926 Images of immature cherry fruits inoculated with non-pathogen strains and a no-bacteria control.
927 Images were taken 10dpi. Five replicate cherries were inoculated per strain.
928
929 **Figure S5**
930 Boxplot of diameter of necrosis caused by cherry pathogens on four cherry cultivars using
931 immature green cherry fruits. Strains are colour-coded with those isolated from cherry in pink and
932 the no bacterial control in black. The boxplots are colour-coded by clade: *Psm* R1: blue, *Psm* R2:
933 green, *Pss*: red and no bacteria control: black. Data presented are all values (n=20) per treatment of
934 two independent experiments. An ANOVA revealed significant differences between strains
935 ($p<0.01$, df=3), cultivars ($p<0.01$, df=3) and a significant interaction ($p<0.01$, df=9). Tukey-HSD

(p=0.05, confidence level: 0.95) significance groups for the different strains for each separate cultivar are presented above each boxplot.

Figure S6

Symptoms observed in detached cherry leaves using different inoculation methods. Representative images of the four methods – infiltration, stab, droplet and wound + droplet. Leaves show inoculation with *Psm* R1-5244 or a 10mM MgCl2 control.

Figure S7

Boxplot of day 10 population counts of all strains used in this study on cherry cv. Van leaves. Strains are colour-coded with those isolated from cherry in pink, plum in blue and non-pathogens in black. The boxplots are coloured by clade: *Psm* R1: blue, *Psm* R2: green, *Pss*: red. The 10mM MgCl2 control is not included as no bacteria were found. The data presented are all values for each treatment (n=9). An ANOVA revealed significant differences between strains ($p<0.01$, df=20). Tukey-HSD (p=0.05, confidence level: 0.95) significance groups for the different strains are presented.

Figure S8

Symptom development over time after inoculation of various *P. syringae* strains in cherry cv. Van at different concentrations. Strains are colour-coded, with those isolated from cherry in pink and plum in blue. Symptoms were scored from 0-5. 0: no symptoms, 1: limited browning, 2: browning <50% of inoculated site, 3: browning >50% of inoculated site, 4: Complete browning, 5: Spread from site of inoculation. Data presented are the means (n=4) and error bars show the standard error above and below the mean. The lines for each strains are colour-coded with *Psm* R1: blue, *Psm* R2: green, *Pss*: red, non-pathogen RMA1: orange. Symptom development over time was analysed using AUDPC. An ANOVA revealed significant differences between strains ($p<0.01$, df=4),

963 concentrations ($p<0.01$, $df=3$) and a significant interaction ($p<0.01$, $df=12$). Tukey-HSD ($p=0.05$,
964 confidence level: 0.95) significance groups are presented.
965
966
967 **Figure S9**
968 TEM images of *Psm* R2-leaf in a detached cherry leaf one week after inoculation. Arrows point to
969 putative papilla formation in the plant cell wall next to a bacterial colony containing dead bacterial
970 cells. A: Bacteria inhabiting apoplastic space next to cells. Note that no cell wall alterations
971 appeared in the plant cells. B: Cell wall alterations (papilla formation) shown by arrows in plant
972 cells. C: A bacterial colony containing dead and alive bacteria next to plant cells.
973
974 **Figure S10**
975 Images of symptom development over time on cherry and plum. A: Cherry cv. Van, B: Plum cv.
976 Victoria. The same leaf was imaged 16, 24, 48 and 72hpi. Arrows indicate the first appearance of
977 symptoms for that particular strain. Strains are labelled: 1: *Psm* R1-5244, 2: *Psm* R1-5300, 3: *Psm*
978 R2-leaf, 4: *Ps*-9643, 5: *Pss*-9097, 6: *Pss*-9293, 7: RMA1, 8: *Psav*, 9: *Pph*, C: No bacteria control
979
980 **Table S1**
981 Proportional Odds Model (POM) analysis of the glasshouse whole-tree wound inoculations. Model
982 comparisons are first shown with the ANOVA comparing models. The summary of the final model
983 (score ~g1) is shown along with lsmeans Tukey-HSD groupings of strains (corresponds to
984 groupings on Figure 2).
985
986 **Table S2**

REML analysis of field inoculation of cherry inoculated by leaf scar. The REML model and ANOVA are presented, followed by lsmean Tukey-HSD groupings for cultivars, strains and then strains on each cultivar (corresponds to groupings on Figure 3).

Table S3

REML analysis of field inoculation of cherry inoculated by wound. The REML model and ANOVA are presented, followed by lsmean Tukey-HSD groupings for cultivars, strains and then strains on each cultivar (corresponds to groupings on Figure 3).

Table S4

REML analysis of field inoculation of plum inoculated by leaf scar. The REML model and ANOVA are presented, followed by lsmean Tukey-HSD groupings for strains and then strains on each cultivar (corresponds to groupings on Figure 4).

Table S5

REML analysis of field inoculation of plum inoculated by wound. The REML model and ANOVA are presented, followed by lsmean Tukey-HSD groupings for strains and then strains on each cultivar (corresponds to groupings on Figure 4).

Table S6

POM analysis of the cherry field inoculations. Model comparisons are first shown with the ANOVA comparing models. The summary of the final model (score~strain+cv+ino+block) is then presented.

Table S7

Lsmean Tukey-HSD groupings for different treatment combinations from the POM analysis of cherry field inoculations. Groups for strains on different cultivars are presented (corresponds to groupings on Figure 3), followed by groupings of cultivars in each inoculation method and then strains across the two inoculation methods.

Table S8

POM analysis of the plum field inoculations. Model comparisons are first shown with the ANOVA comparing models. The summary of the final model (score~strain+cv+ino+block) is then presented.

Table S9

Lsmeans Tukey-HSD groupings for different treatment combinations from the POM analysis of plum field inoculations. Groups for strains on different cultivars are presented (corresponds to groupings on Figure 3), followed by groupings of cultivars in each inoculation method and then strains across the two inoculation methods.

Table S10

ANOVA table of cut shoot inoculations followed by lsmeans Tukey-HSD groupings for the strains on each cultivar (corresponds to groupings on Figure 5).

Table S11

ANOVA table of immature cherry fruit inoculations of all isolates followed by Tukey-HSD groupings for the strains extracted using the agricolae package function HSD.test (corresponds to groupings on Figure 6).

Table S12

REML analysis of immature cherry fruit inoculations where different bacterial strains were inoculated onto different host cultivars. The REML model is presented. Lsmeans Tukey-HSD groups for strains on different cultivars are presented (corresponds to groupings on Figure S5), followed by groupings based on all possible treatments.

Table S13

ANOVA table of day 10 leaf population counts of different bacterial strains inoculated on cherry and plum. Tukey-HSD groups for strains are presented (corresponds to groupings on Figure 7).

Table S14

REML analysis of day 10 leaf population counts of reference bacterial strains inoculated on cherry leaves. The model is shown followed by ANOVA table. Lsmeans Tukey-HSD groups for strains are presented (corresponds to groupings on Figure 8).

Table S15

REML analysis of day 10 leaf population counts of reference bacterial strains inoculated on plum leaves. The model is shown followed by ANOVA table. Lsmeans Tukey-HSD groups for strains are presented (corresponds to groupings on Figure 8).

Table S16

ANOVA table of AUDPC analysis of leaf symptom score over time of different bacterial strains inoculated on cherry. Tukey-HSD groups for strains are presented (corresponds to groupings on Figure 8).

Table S17

ANOVA table of AUDPC analysis of leaf symptom score over time of different bacterial strains inoculated on cherry. Tukey-HSD groups for strains are presented (corresponds to groupings on Figure 8).

Table S18

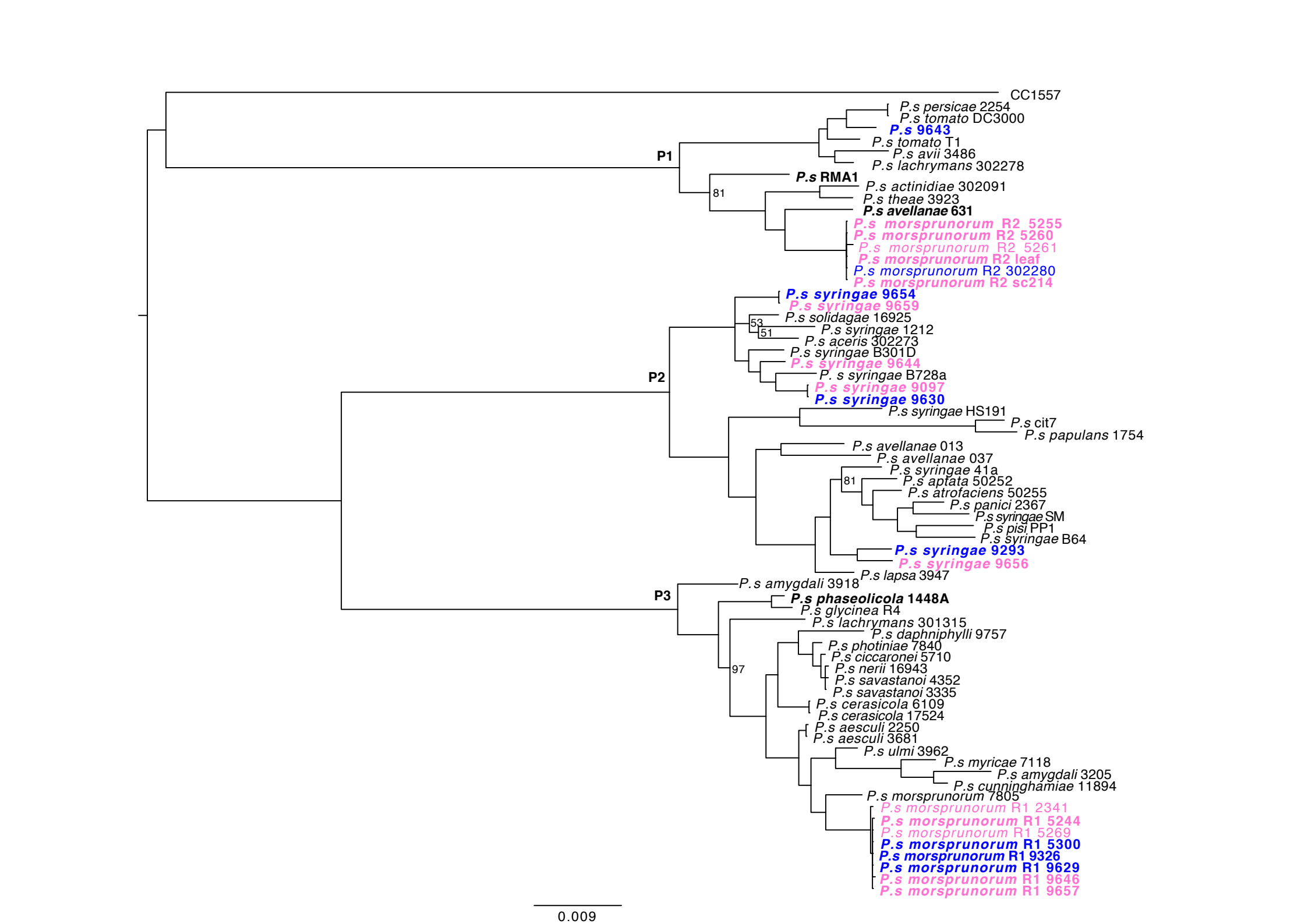
ANOVA table of day 10 leaf population counts of different bacterial strains inoculated on different cherry cultivars. Tukey-HSD groups for strains are presented (corresponds to groupings on Figure 9).

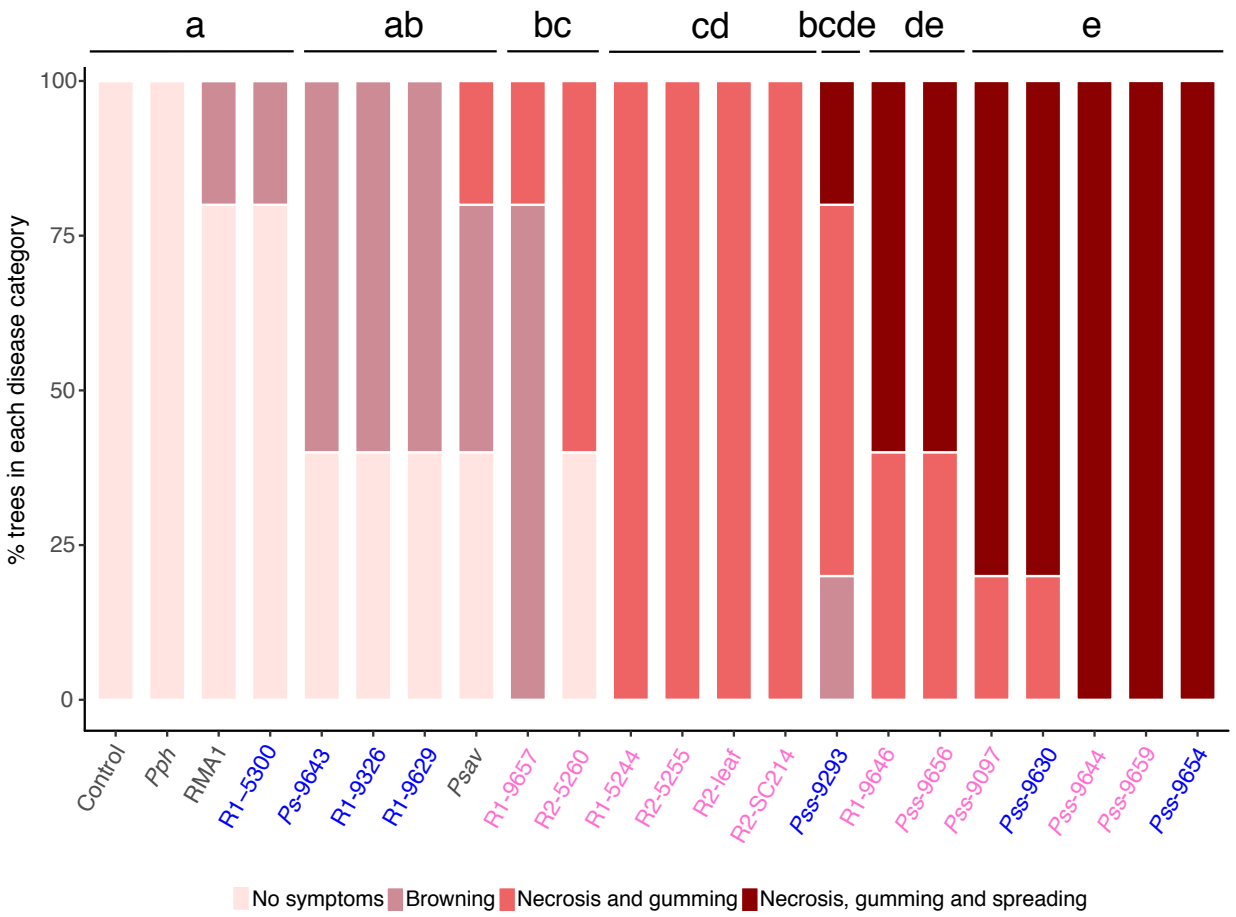
Table S19

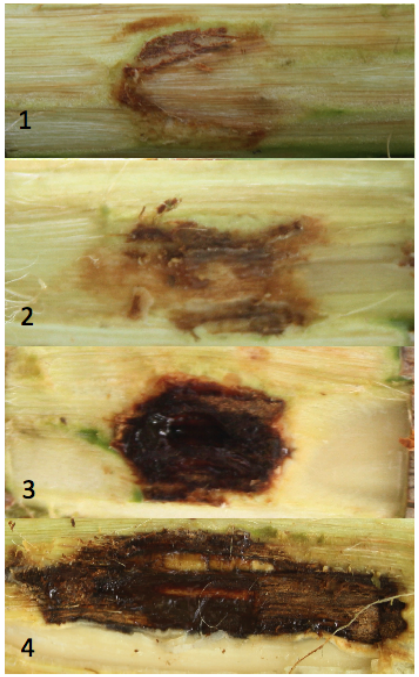
ANOVA table of leaf population counts of all isolates used in this study, followed by Tukey-HSD groupings for the strains extracted using the agricolae package function HSD.test (corresponds to groupings on Figure S7).

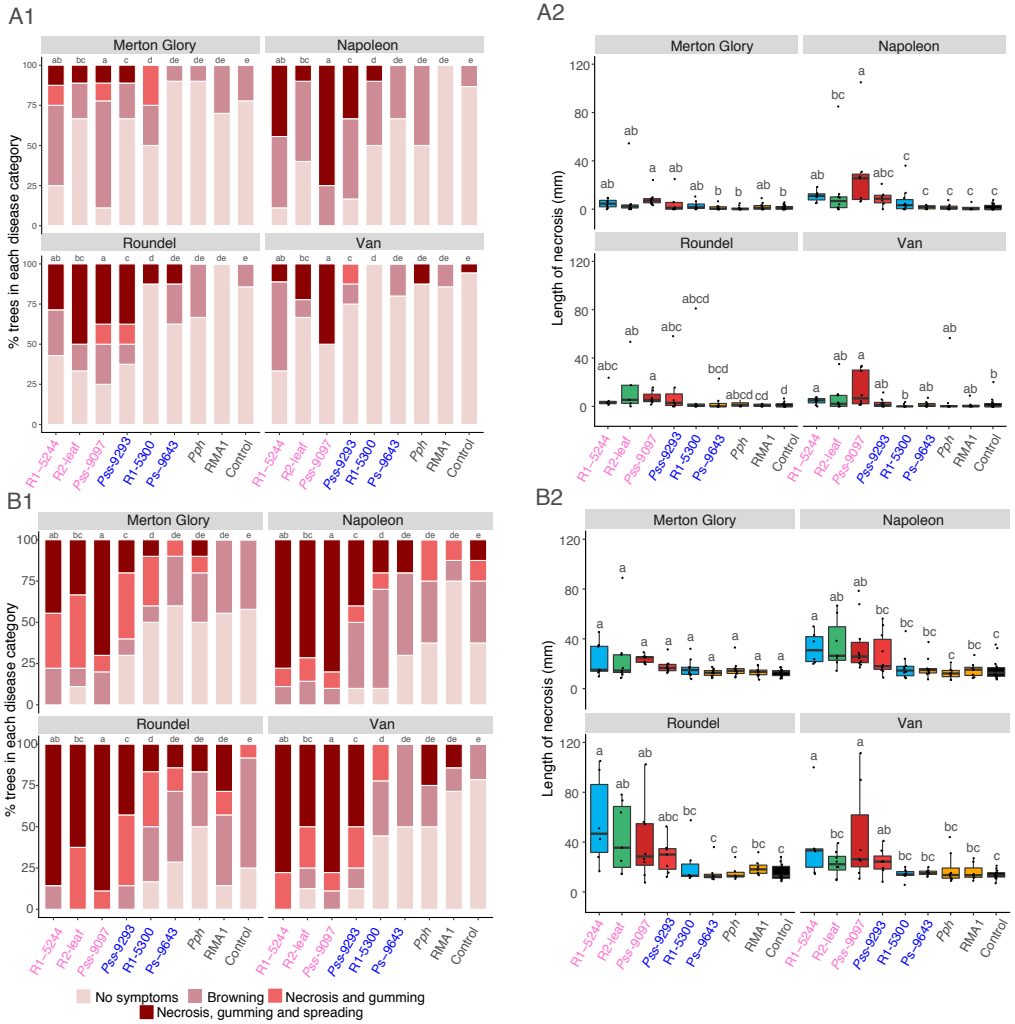
Table S20

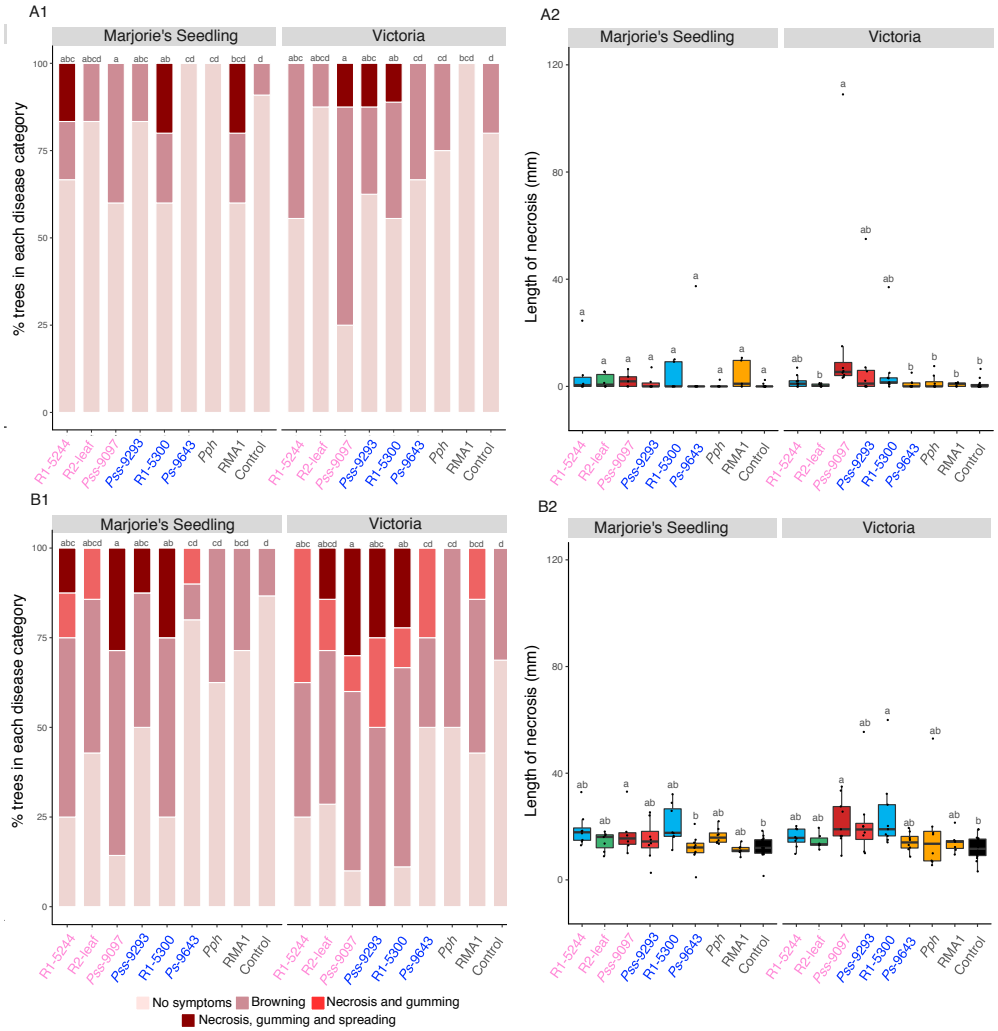
ANOVA table of AUDPC analysis of symptom score on leaves of several bacterial strains inoculated at different concentrations. This is followed by lsmeans Tukey-HSD groupings for the strains (corresponds to groupings on Figure S8).

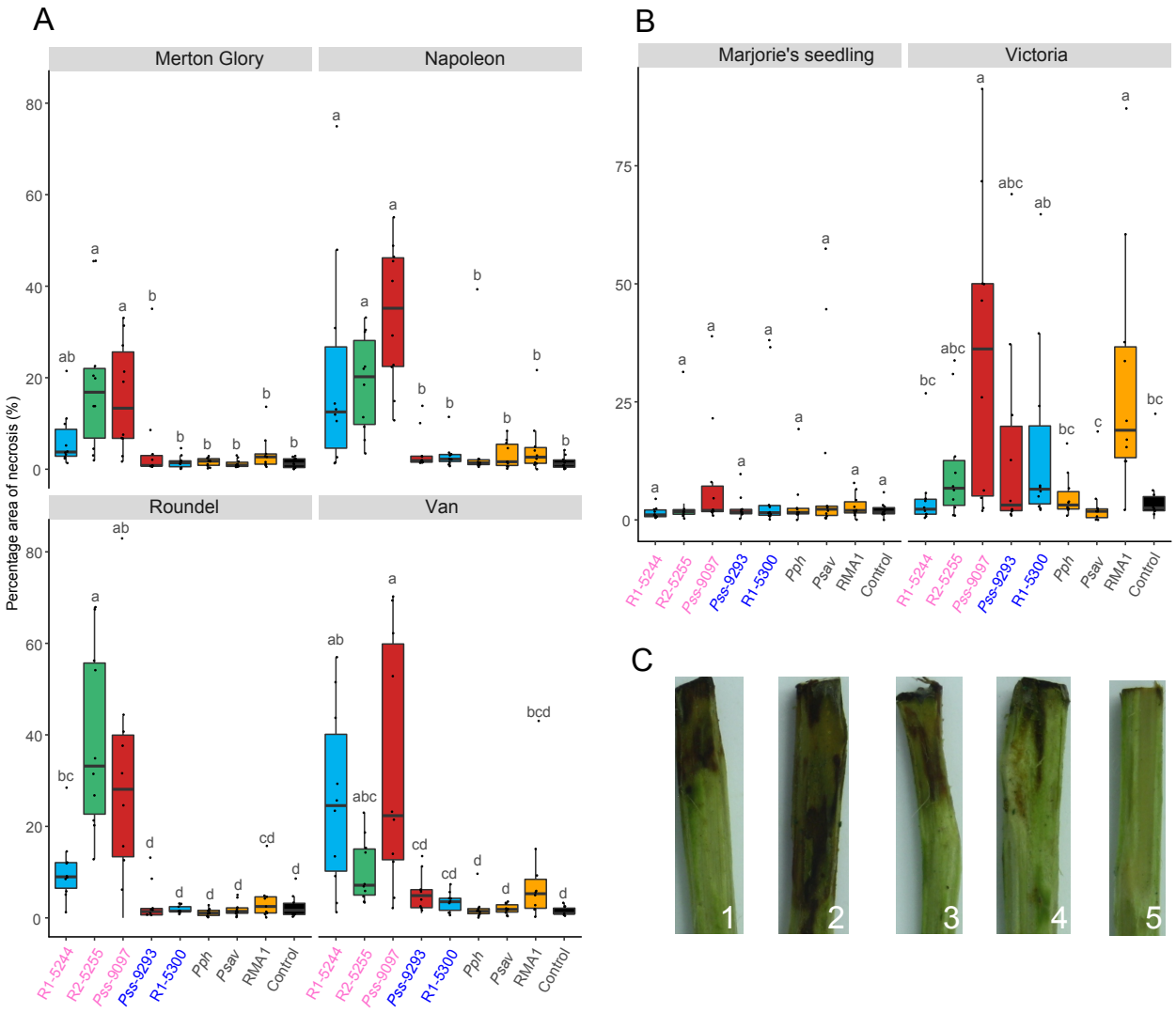

CC1557
P.s persicae 2254
P.s tomato DC3000
P.s 9643
P.s tomato T1
P.s avii 3486
P.s lachrymans 302278
P1
P.s RMA1
81
P.s actinidiae 302091
P.s theae 3923
P.s avellanae 631
P.s morsprunorum R2 5255
P.s morsprunorum R2 5260
P.s morsprunorum R2 5261
P.s morsprunorum R2 leaf
P.s morsprunorum R2 302280
P.s morsprunorum R2 sc214
P.s syringae 9654
P.s syringae 9659
P.s solidagae 16925
53
51
P.s syringae 1212
P.s aceris 302273
P.s syringae B301D
P.s syringae 9644
P2
P. s syringae B728a
P.s syringae 9097
P.s syringae 9630
P.s syringae HS191
P.s cit7
P.s papulans 1754
P.s avellanae 013
P.s avellanae 037
P.s syringae 41a
81
P.s aptata 50252
P.s atrofaciens 50255
P.s panici 2367
P.s syringae SM
P.s pisi PP1
P.s syringae B64
P.s syringae 9293
P.s syringae 9656
P.s lapsa 3947
P.s amygdali 3918
P3
P.s phaseolicola 1448A
P.s glycinea R4
P.s lachrymans 301315
P.s daphniphylli 9757
P.s photiniae 7840
P.s ciccaronei 5710
97
P.s nerii 16943
P.s savastanoi 4352
P.s savastanoi 3335
P.s cerasicola 6109
P.s cerasicola 17524
P.s aesculi 2250
P.s aesculi 3681
P.s ulmi 3962
P.s myricae 7118
P.s amygdali 3205
P.s cunninghamiae 11894
P.s morsprunorum 7805
P.s morsprunorum R1 2341
P.s morsprunorum R1 5244
P.s morsprunorum R1 5269
P.s morsprunorum R1 5300
P.s morsprunorum R1 9326
P.s morsprunorum R1 9629
P.s morsprunorum R1 9646
P.s morsprunorum R1 9657
0.009

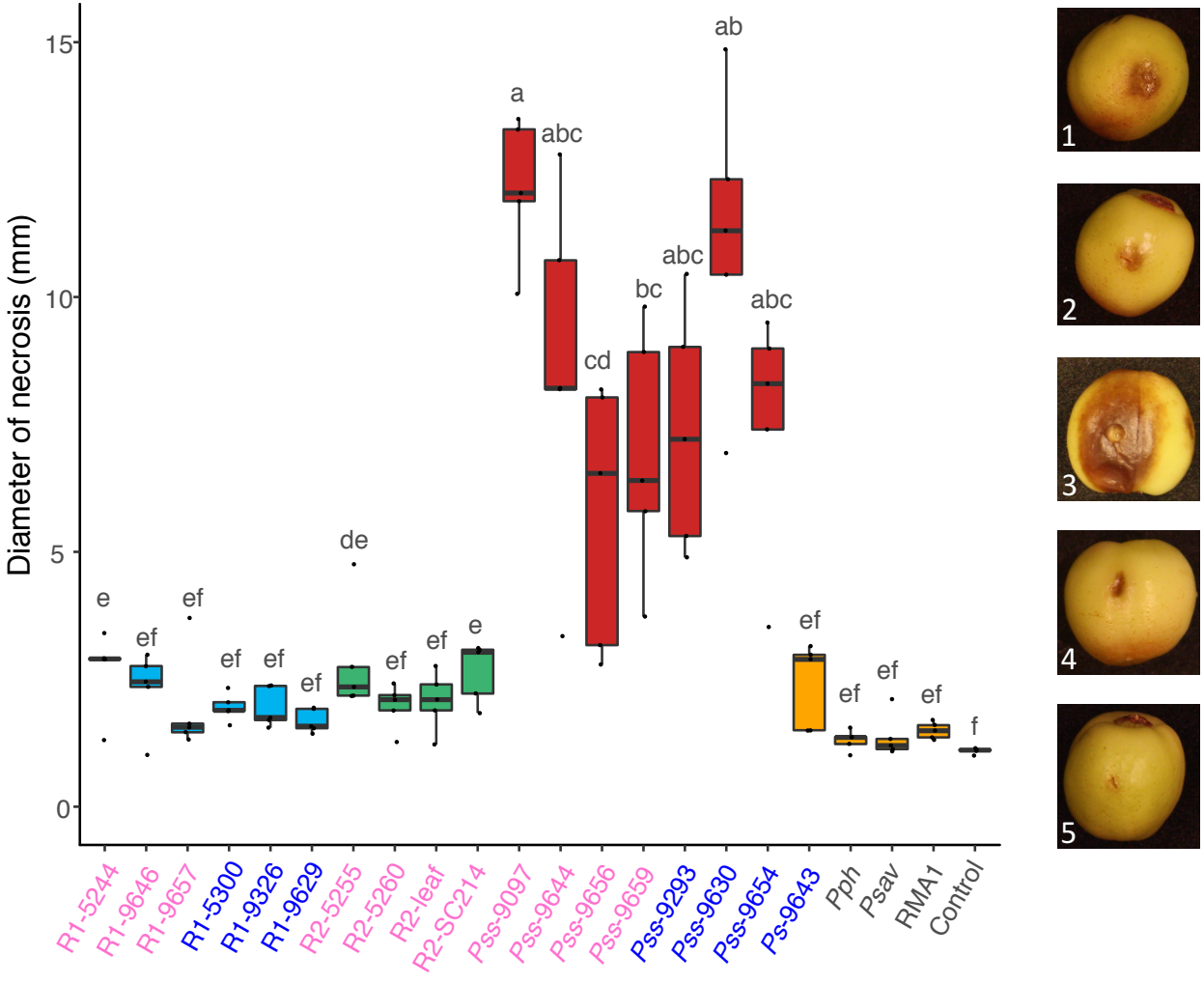

a
ab
bc
cd
bcde
de
e
100
75
50
25
0
% trees in each disease category
Control
Pph
RMA1
R1-5300
Ps-9643
R1-9326
R1-9629
Psav
R1-9657
R2-5260
R1-5244
R2-5255
R2-leaf
R2-SC214
Pss-9293
R1-9646
Pss-9656
Pss-9097
Pss-9630
Pss-9644
Pss-9659
Pss-9654
No symptoms
Browning
Necrosis and gumming
Necrosis, gumming and spreading

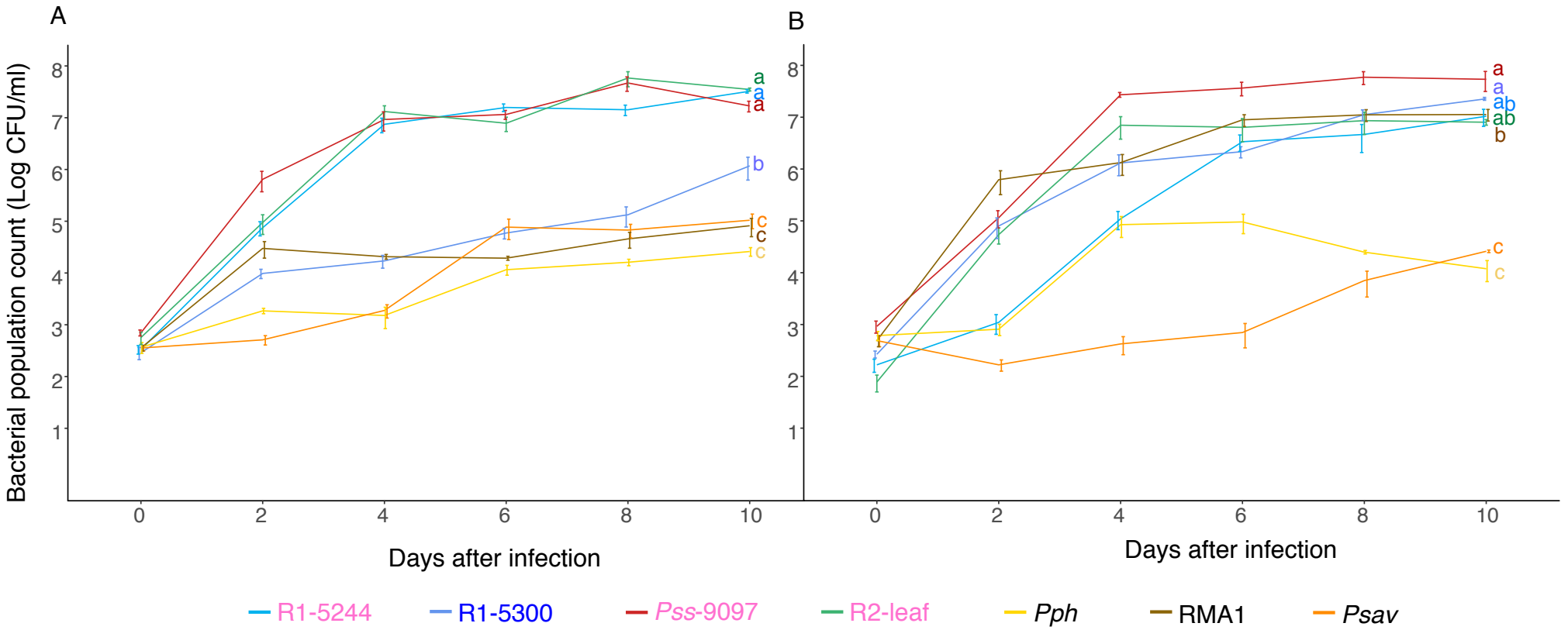

1
2
3
4

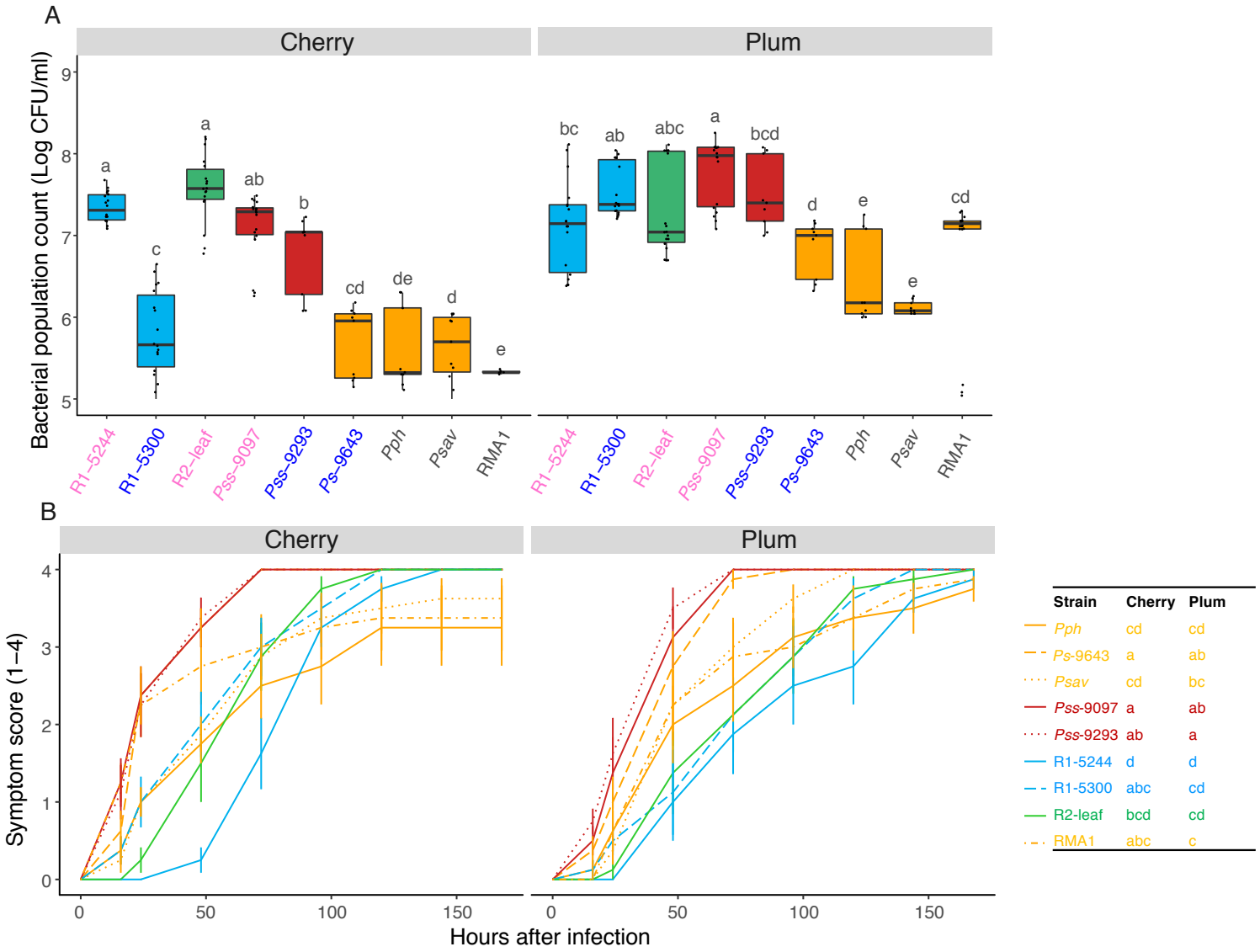

A1
Merton Glory
Napoleon
Roundel
Van
% trees in each disease category
R1–5244
R2-leaf
Pss-9097
Pss-9293
R1-5300
Ps–9643
Pph
RMA1
Control
A2
Length of necrosis (mm)
B1
B2
No symptoms
Browning
Necrosis and gumming
Necrosis, gumming and spreading

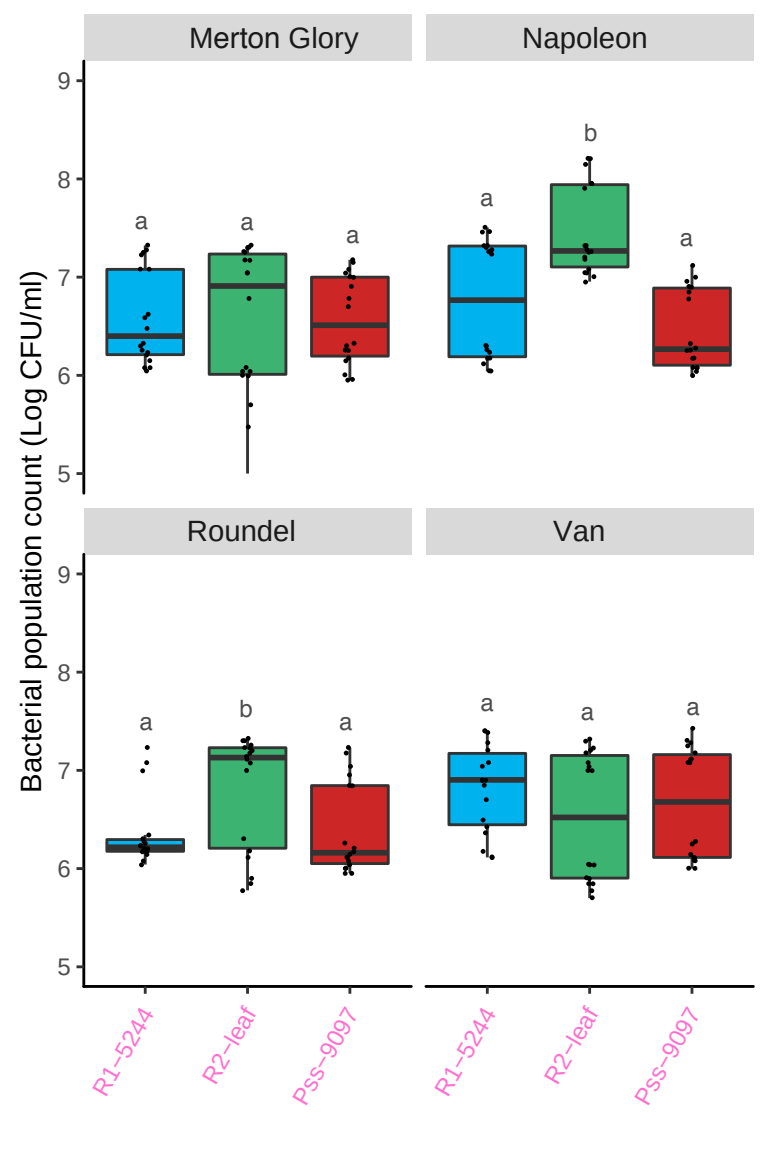

A1
Marjorie's Seedling
Victoria
% trees in each disease category
R1-5244
R2-leaf
Pss-9097
Pss-9293
R1-5300
Ps-9643
Pph
RMA1
Control
A2
Length of necrosis (mm)
B1
B2
No symptoms
Browning
Necrosis and gumming
Necrosis, gumming and spreading

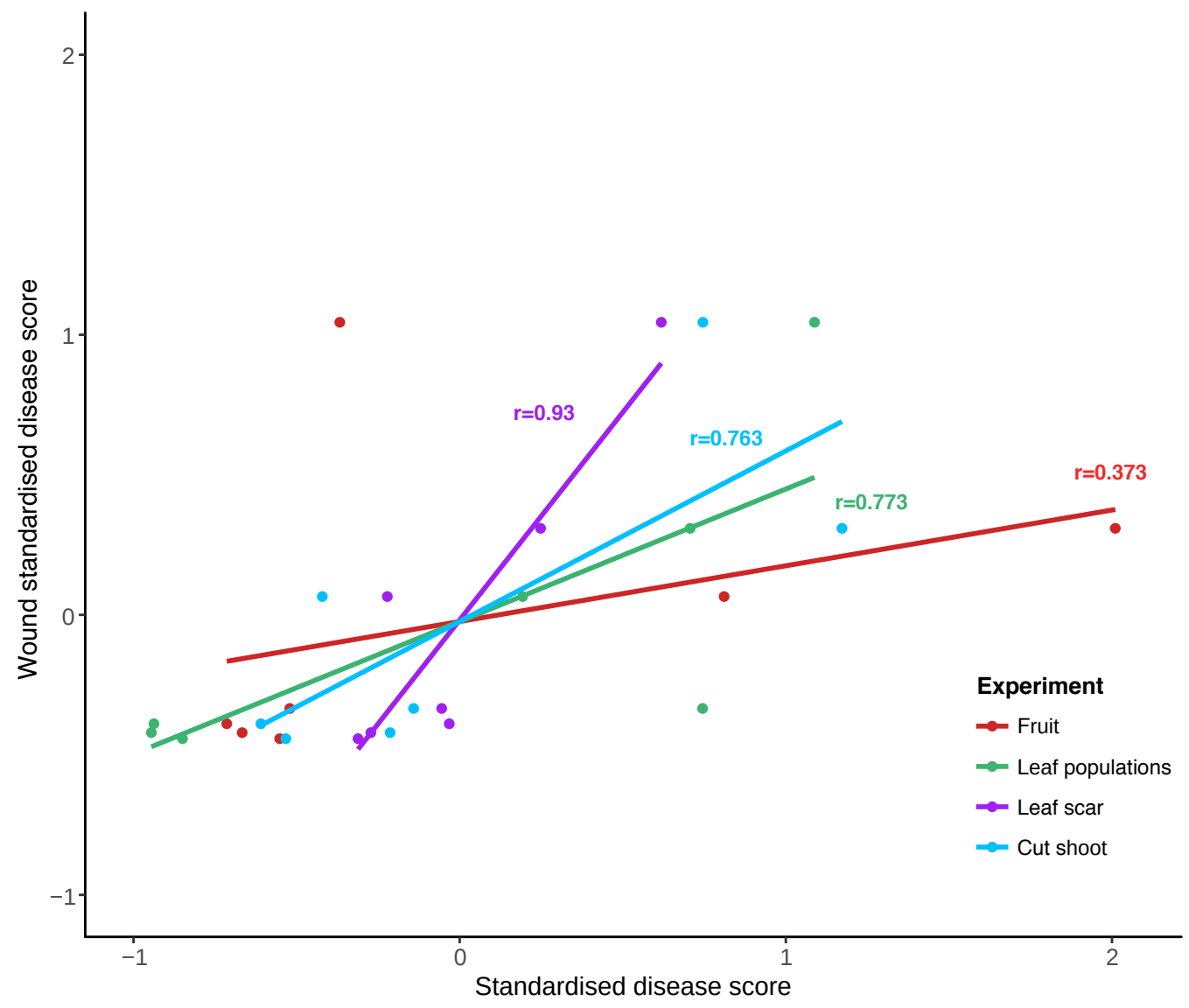

A
Merton Glory
Napoleon
Roundel
Van
Percentage area of necrosis (%)
0
20
40
60
80
R1-5244
R2-5255
Pss-9097
Pss-9293
R1-5300
Pph
Psav
RMA1
Control
B
Marjorie's seedling
Victoria
0
25
50
75
C
1
2
3
4
5


Diameter of necrosis (mm)
15
10
5
0
e
ef
ef
ef
ef
ef
de
ef
ef
e
a
abc
cd
bc
abc
ab
abc
ef
ef
ef
ef
f
R1-5244
R1-9646
R1-9657
R1-5300
R1-9326
R1-9629
R2-5255
R2-5260
R2-leaf
R2-SC214
Pss-9097
Pss-9644
Pss-9656
Pss-9659
Pss-9293
Pss-9630
Pss-9654
Ps-9643
Pph
Psav
RMA1
Control
1
2
3
4
5


A
B
Bacterial population count (Log CFU/ml)
Days after infection
Days after infection
R1-5244
R1-5300
Pss-9097
R2-leaf
Pph
RMA1
Psav


A
Cherry
Plum
Bacterial population count (Log CFU/ml)
9
8
7
6
5
a
c
a
ab
b
cd
de
d
e
bc
ab
abc
a
bcd
d
e
e
cd
R1−5244
R1−5300
R2−leaf
Pss−9097
Pss−9293
Ps−9643
Pph
Psav
RMA1
B
Cherry
Plum
Symptom score (1−4)
4
3
2
1
0
0
50
100
150
Hours after infection
Strain
Cherry
Plum
Pph
cd
cd
Ps-9643
a
ab
Psav
cd
bc
Pss-9097
a
ab
Pss-9293
ab
a
R1-5244
d
d
R1-5300
abc
cd
R2-leaf
bcd
cd
RMA1
abc
c


Merton Glory
Napoleon
Roundel
Van
Bacterial population count (Log CFU/ml)
9
8
7
6
5
a
a
a
a
b
a
a
b
a
a
a
a
R1−5244
R2−leaf
Pss−9097
R1−5244
R2−leaf
Pss−9097


Wound standardised disease score
Standardised disease score
2
1
0
−1
−1
0
1
2
r=0.93
r=0.763
r=0.773
r=0.373
Experiment
Fruit
Leaf populations
Leaf scar
Cut shoot

Strain	Pathovar	Race	Host/Isolation source	*Prunus* host cv.	Host tissue	Reference	BioProject/accession
R1-5244	*morsprunorum*	1	*Prunus avium*	unknown	Cankerous wood	This study	PRJNA345357
R1-5300	*morsprunorum*	1	*Prunus domestica*	Victoria	Unknown	This study	PRJNA345357
R1-9326	*morsprunorum*	1	*Prunus domestica*	Victoria	Leaf wash	This study	PRJNA345357
R1-9629	*morsprunorum*	1	*Prunus domestica*	Victoria	Leaf wash	This study	PRJNA345357
R1-9646	*morsprunorum*	1	*Prunus avium*	Stella	Leaf wash	This study	PRJNA345357
R1-9657	*morsprunorum*	1	*Prunus domestica*	Victoria	Leaf wash	This study	PRJNA345357
R2-5255	*morsprunorum*	2	*Prunus avium*	Napoleon	Unknown	This study	PRJNA345357
R2-5260	*morsprunorum*	2	*Prunus avium*	Roundel	Unknown	This study	PRJNA345357
R2-leaf	*morsprunorum*	2	*Prunus avium*	Napoleon	Leaf lesion	This study	PRJNA345357
R2-SC214	*morsprunorum*	2	*Prunus avium*	Wild cherry	Leaf lesion	This study	PRJNA345357
syr9097	*syringae*		*Prunus avium*	unknown	Cankerous wood	This study	PRJNA345357
syr9293	*syringae*		*Prunus domestica*	Victoria	Leaf wash	This study	PRJNA345357
syr9630	*syringae*		*Prunus domestica*	Victoria	Leaf wash	This study	PRJNA345357
syr9644	*syringae*		*Prunus avium*	Stella	Leaf wash	This study	PRJNA345357
syr9654	*syringae*		*Prunus domestica*	Victoria	Leaf wash	This study	PRJNA345357
syr9656	*syringae*		*Prunus avium*	Kiku-Shidare	Leaf wash	This study	PRJNA345357
syr9659	*syringae*		*Prunus avium*	Kiku-Shidare	Leaf wash	This study	PRJNA345357
Ps-9643	-		*Prunus domestica*	Victoria	Leaf wash	This study	PRJNA345357
RMA1	-		*Aquilegia vulgaris*	Winky	Leaf lesion	This study	PRJNA345357
PsavBP631	*avellanae*		*Corylus avellana*			O'Brien *et al.* 2012	AKBS00000000
Pph1448a	*phaseolicola*		*Phaseolus vulgaris*			Joardar *et al.* 2005	CP000058

Strain	Pathovar	Race	Host/Isolate source	*Prunus* Host cv.	Reference	BioProject/accession
acer302273	*aceris*		*Acer* sp.		Baltrus *et al.* 2012	AEAO00000000
act302091	*actinidiae*		*Actinidia deliciosa*		Baltrus *et al.* 2012	AEAL00000000
aes2250	*aesculi*		*Aesculus hippocastanum*		Green *et al.* 2010	ACXT00000000
aes3681	*aesculi*		*Aesculus hippocastanum*		Green *et al.* 2010	ACXS00000000
amy3205	*amygdali*		*Prunus dulcis*		Bartoli *et al.* 2015	JYHB00000000
amyICMP3918	*amygdali*		*Prunus dulcis*		Thakur *et al.* 2016	LJPQ00000000
atroDSM50255	*atrofaciens*		*Triticum aestivum*		Baltrus *et al.* 2014	AWUI00000000
avelVe013	*avellanae*		*Corylus avellana*		O'Brien *et al.* 2012	AKCK00000000
avelVe037	*avellanae*		*Corylus avellana*		O'Brien *et al.* 2012	AKCJ00000000
avii3846	*avii*		*Prunus avium*		Nowell *et al.* 2016	LIIJ00000000
castCFBP4217	*castaneae*		*Castanea crenata*		Nowell *et al.* 2016	LIIH00000000
CC1557	-		Snow		Hockett *et al.* 2014	AVEH00000000
cera6109	*cerasicola*		*Prunus yedoensis*		Nowell *et al.* 2016	LIIG00000000
ceraICMP17524	*cerasicola*		*Prunus yedoensis*		Thakur *et al.* 2016	LJQA00000000
ciccICMP5710	*ciccaronei*		*Ceratonia siliqua*		Thakur *et al.* 2016	LJPY00000000
cit7	-		*Citrus sinensis*		Baltrus et al. 2012	AEAJ00000000
cunnICMP11894	*cunninghamiae*		*Cunninghamia lanceolata*		Thakur *et al.* 2016	LJQE00000000
daphICMP9757	*daphniphylli*		*Daphniphyllum teijsmannii*		Thakur *et al.* 2016	LJQF00000000
glyR4	*glycinea*		*Glycine max*		Qi *et al.* 2011	AEGH00000000
lach301315	*lachrymans*		*Cucumis sativus*		Baltrus *et al.* 2012	AEAF00000000
lach302278	*lachrymans*		*Cucumis sativus*		Baltrus *et al.* 2012	AEAM00000000
lapsaICMP3947	*lapsa*		*Zea* sp.		Thakur *et al.* 2016	LJQQ00000000
mors302280	*morsprunorum*		*Prunus domestica*		Baltrus *et al.* 2012	AEAE00000000
morsU7805	*morsprunorum*		*Prunus mume*		Mott *et al.* 2016	LGLQ00000000
Strain	*Pathovar*	*Race*	*Host/Isolate source*	Prunus Host cv.	Reference	BioProject/accession

myriICMP7118	*myricae*		*Myrica rubra*		Thakur *et al.* 2016	LJQV00000000
neriiICMP16943	*savastanoi*		*Olea europea*		Thakur *et al.* 2016	LJQW00000000
paniLMG2367	*panici*		*Panicum miliaceum*		Liu *et al.* 2012	ALAC00000000
papu1754	*papulans*		*Malus sylvestris*		Nowell *et al.* 2016	JYHI00000000
persNCPPB2254	*persicae*		*Prunus persica*		Zhao *et al.* 2015	LAZV00000000
photICMP7840	*photiniae*		*Photinia glabra*		Thakur *et al.* 2016	LJQO00000000
pisiPP1	*pisi*		*Pisum sativum*		Baltrus *et al.* 2014b	AUZR00000000
R1-2341	*morsprunorum*	1	*Prunus cerasus*	unknown	Nowell *et al.* 2016	LIIB00000000
R1-5269	*morsprunorum*	1	*Prunus cerasus*	unknown	Nowell *et al.* 2016	LIHZ00000000
R2-5261	*morsprunorum*	2	*Prunus avium*	Roundel	Nowell *et al.* 2016	LIIA00000000
sava3335	*savastanoi*		*Olea europea*		Rodriguez-Palenzuela *et al.* 2010	ADMI00000000
sava4352	*savastanoi*		*Olea europea*		Thakur *et al.* 2016	LGKR00000000
soliICMP16925	*solidagae*		*Solidago altissima*		Thakur *et al.* 2016	JYHF00000000
syr1212	*syringae*		*Pisum sativum*		Baltrus *et al.* 2014	AVCR00000000
syr41a	*syringae*		*Prunus armeniaca*		Bartoli *et al.* 2015	JYHJ00000000
syrB301D	*syringae*		*Pyrus communis*		Ravindran *et al.* 2015	CP005969
syrB64	*syringae*		*Triticum aestivum*		Dudnik and Dudler 2013	ANZF00000000
syrB728a	*syringae*		*Phaseolus vulgaris*		Feil *et al.* 2005	CP000075
syrHS191	*syringae*		*Panicum miliaceum*		Ravindran *et al.* 2015	CP006256
syrSM	*syringae*		*Triticum aestivum*		Dudnik and Dudler 2013	APWT00000000
thea3923	*theae*		*Camelia sinensis*		Mazzaglia *et al.* 2012	AGNN00000000
tomDC3000	*tomato*		*Solanum lycopersicum*		Buell *et al.* 2003	AE016853
tomT1	*tomato*		*Solanum lycopersicum*		Almeida *et al.* 2009	ABSM00000000
ulmiICMP3962	*ulmi*		*Ulmus* sp.		Thakur *et al.* 2016	LJRQ00000000