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# Identifying resistance in wild and ornamental cherry towards bacterial canker caused by *Pseudomonas syringae*

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**Abstract**

Bacterial canker is a major disease of stone fruits and is a critical limiting factor to sweet cherry (*Prunus avium* L.) production worldwide. One important strategy for disease control is the development of resistant varieties. Partial varietal resistance in sweet cherry is discernible using shoot or whole tree inoculations, however these quantitative differences in resistance are not evident in detached leaf assays. To identify novel sources of resistance to canker, we used a rapid leaf pathogenicity test to screen a range of wild cherry, ornamental *Prunus* species and sweet cherry x ornamental cherry hybrids with the canker pathogens, *Pseudomonas syringae* pvs. *syringae*, *morsprunorum* races 1 and 2, and *avii*. Several *Prunus* accessions exhibited limited symptom development following inoculation with each of the pathogens, and this resistance extended to 16 *P. syringae* strains pathogenic on sweet cherry and plum. Resistance was associated with reduced bacterial multiplication after inoculation, a phenotype similar to that of commercial sweet cherry towards non-host strains of *P. syringae*. Progeny resulting from a cross of a resistant ornamental species *P. incisa* with susceptible sweet cherry (*P. avium*) exhibited resistance indicating it is an

inherited trait. Identification of accessions with resistance to the major bacterial canker pathogens is the first step towards characterising the underlying genetic mechanisms of resistance and introducing these traits into commercial germplasm.

## Introduction

Plant diseases caused by bacteria remain problematic for the global horticultural industry due to a lack of effective control measures (Sundin *et al.*, 2016). The genus *Prunus* contains over 400 species, a selection of which are grown for top fruit, ornamental use and timber production (Bortiri *et al.*, 2001). Bacterial canker, caused by members of the *Pseudomonas syringae* species complex, can be a major limiting factor in the cultivation of *Prunus* spp. (Vicente *et al.*, 2004; Omrani *et al.*, 2019). The disease is primarily characterised by necrosis, gummosis and/or dieback of woody plant tissues. In addition, the pathogens colonise other plant tissues where they exist epiphytically or invade to cause leaf and fruit spots, and blossom blight. These tissues can act as reservoirs for later woody tissue infection (Crosse, 1966). At least five phylogenetically distinct clades of *P. syringae* are known to cause bacterial canker on *Prunus*. These include *P. syringae* pv. *morsprunorum* race 1 (*Psm* R1), *P. syringae* pv. *morsprunorum* race *2 (Psm* R2), *P. syringae* pv. *syrin*gae (*Pss*), *P. syringae* pv. *persicae* and the more recently discovered *P. syringae* pv. *avii* (Psa) and *P. cerasi* (Ménard *et al.*, 2003; Kałużna *et al.*, 2016; Parisi *et al.*, 2019). *Psm* R1 and *Psm* R2 are genetically distinct, belonging to different phylogroups within the species complex and can be alternatively referred to as within the species *P. amygdali* and *P. avellanae*, respectively (Gomilla *et al*. 2017). Bacterial strains differ in host range and aggressiveness towards particular species in the genus (reviewed in Bultreys & Kałużna, 2010) . A recent study identified a range of factors that contributed to bacterial virulence, but also found knockout of genes encoding possible avirulence proteins, including the effector *HopAU1*, led to hypervirulent bacterial phenotypes, suggesting a quantitative level of resistance exists even in susceptible cultivars (Neale et al., 2021)

Control measures available for bacterial canker are limited. The genotypically diverse *P. syringae* clades causing the disease may vary in sensitivity to control measures and can rapidly evolve and transfer genes conferring resistance to chemicals such as copper-based biocides and antibiotics (Sundin *et al.*, 2016). The genetic diversity of bacterial canker pathogens poses a challenge in the generation of novel controls because responses to all potential pathogens must be tested. Progress is being made in the development of specific biological controls such as the use of bacteriophages

(Rabiey *et al.*, 2020) that could be utilised in combinations effective against all clades. A complementary approach is to breed for resistance, a strategy particularly important in forestry, where spraying control is impractical (Vicente *et al.*, 2004). Ideally, resistance against multiple clades would be most beneficial or an alternative strategy would be to stack resistance-associated loci effective against the different clades into new varieties.

The molecular mechanisms involved in plant resistance towards bacterial pathogens, such as *Pseudomonas syringae,* have been extensively characterised in model plant species such as *Arabidopsis thaliana*. Resistance involves heightened immunity that occurs at the plant cell surface through receptor detection of pathogen associated molecular patterns, as well as the intracellular detection of pathogen virulence proteins (effectors) injected into plant cells. These two components of resistance are now known to be intrinsically linked (Ngou *et al.*, 2021).

There is limited knowledge of resistance in *Prunus* towards bacterial canker pathogens. Cherry and apricot varieties with partial resistance to one or more of the pathogens have been identified using methods such as laboratory-based shoot inoculations and field tree inoculations (Santi *et al.*, 2004; Farhadfar *et al.*, 2016; Hulin *et al.*, 2018a; Omrani *et al.*, 2019). In our previous study, we found that the partial resistance seen in woody tissue of certain cherry cultivars was not differentiated using detached leaf syringe-infiltration assays (Hulin *et al.*, 2018a). This partial resistance seen in woody tissues is likely quantitative, involving multiple alleles having small effects, with the most resistant varieties still succumbing to disease under favourable conditions. Although only partial, such resistance could be highly useful for *Prunus* breeding as it could reduce overall pathogen load in orchards as part of an integrated disease management approach (Sundin *et al.*, 2016). In addition, it is arguably more durable than single resistance (*R*)-gene based immunity, which is theoretically more frequently overcome during pathogen evolution (Pilet-Nayel *et al.*, 2017). Progress towards understanding the genetic factors involved in bacterial canker resistance has been made by Omrani *et al.* (2019) who identified quantitative trait loci (QTL) involved in partial resistance in apricot. These loci contained genes involved in phytohormone signalling, a process known to play a pivotal role during the plant immune response.

Studies reporting the screening of *Prunus* for canker resistance have focused on established commercial varieties. However, wild relatives can provide robust sources of disease resistance not found in crop genotypes and may be introduced during crop breeding. Non-host resistance is defined as the ability of all genotypes of a plant species to resist all genotypes of a pathogen (Heath,

2000). Such resistance traits can be transferred into crops. For example, relatives of apple such as *Malus* x *robusta* 5 and *Malus floribunda* have been utilised extensively to introduce complete resistance towards the fireblight pathogen *Erwinia amylovora* both through breeding and transgenic strategies (Campa *et al.*, 2019). In addition, wild accessions of kiwifruit have been identified with resistance towards the canker pathogen *P. syringae* pv. *actinidiae* using large scale *in vitro* assays (Wang *et al.*, 2020). *Prunus* is a diverse genus that includes five subgenera: *Amygdalus*, *Cerasus*, *Prunus*, *Laurocerasus* and *Padus* (Chin *et al.*, 2014), with many natural and artificial inter-specific hybrids. The subgenus Cerasus includes *P. avium* (sweet and wild cherry), *P. cerasus* (sour cherry) and *P. mahaleb*. Wild cherry is native to Europe, Africa and Western Asia (Miljković *et al.*, 2019) and exhibits greater genetic diversity than sweet cherry (Avramidou *et al.*, 2010), potentially including diversity in genes conferring resistance to pathogens.

Studies have already shown wild *Prunus* to be important sources of resistance to pathogens such as plum pox virus (Decroocq *et al.*, 2005). Therefore, in this study we aimed to identify resistance in accessions of wild cherry. Sweet cherry cultivars are known to vary in their resistance towards bacterial canker disease under field conditions (Farhadfar *et al.*, 2016; Mgbechi-Ezeri *et al.*, 2017), but no complete resistance has been reported. We screened a wide variety of wild cherry accessions and *Prunus* species related to cherry for resistance to the bacterial canker pathogens. We also screened several hybrids of susceptible sweet cherry crossed with ornamental species. Our results have identified potential sources of resistance to members of each of the pathogenic clades of *P. syringae*.

## Methods

### Plant material

The *Prunus* germplasm utilised in this study (Ta**ble 1 )** was propagated at National Institute of Agricultural Botany East Malling Research (NIAB EMR), in East Malling, UK . The experiments conducted with each accession are listed in Table 1. Samples from mature trees, grown in fields at East Malling, were used for large screens including the sweet cherry shoot tests and leaf symptom screens of all wild, ornamental and hybrid *Prunus*. For tests of *in planta* bacterial multiplication in which material was needed for multiple repetition of experiments, selected accessions (*P. incisa*, Groton A, Groton B, Penny and Sweetheart), were grafted onto Gisela 5 rootstocks and actively growing four-month-old trees were grown in polytunnels, to obtain leaves over an extended period.

Due to limited leaf availability, either cultivars Penny or Sweetheart were used as sweet cherry susceptible controls in population counts.

Sixteen sweet cherry cultivars were examined in cut-shoot inoculation tests and a subset were also used for detached leaf assays. Fifty-two genotypes of wild cherry (*P. avium*) were screened with detached leaf assays. These included trees originally propagated from woodland across the UK (GPS coordinates are listed in Table S1), intentionally representing the nationwide diversity of this species, and focusing on accessions of interest for the forestry industry. In addition, 37 relatives of sweet cherry were included in the detached leaf screening programme. These relatives included 16 ornamental species/known hybrids within the subgenus *Cerasus*, nine inter-specific hybrids (susceptible sweet cherry cv. Napoleon crossed with the ornamental species *P. canescens*, *P. incisa*, *P. nipponica*, *P. kurilensis* and *P. mahaleb*), as well as 11 accessions of additional *Prunus* species from different subgenera (*Amygdalus*, *Prunus* and *Padus*).

**Bacterial strains**

Strains of *Pseudomonas syringae* utilised and the experiments they were included in are listed in Table 2. The most used strains were: *Psm* R1-C (R1-5244) originally isolated from sweet cherry, *Psm* R1-P, (R1-5300) isolated from plum with low virulence on sweet cherry, *Psm* R2 (PsmR2-leaf, renamed MH001) isolated from sweet cherry and *Pss* (Pss-9644) also isolated from sweet cherry. For the wild cherry screening, the pathogen *P. syringae* pv. *avii* (avii5271) was also included. Screening was later extended to a diverse range of strains on selected *Prunus* accessions. The pathogenicity of the strains was extensively characterised in Hulin *et al.* (2018a). Culturing and inoculum preparation were as in this previous work (Hulin *et al.*, 2018a). Briefly, strains were grown from long-term 20% glycerol stocks at -80$^{o}$C on Kings B agar (King *et al.* 1984) for 2-3 days. Single colonies were then inoculated into lysogeny broth and grown overnight at 28°C with orbital shaking at 180 rpm. Cultures were centrifuged at 3500 x g for 10 min before resuspending in 10 mM $MgCl_2$ to an optical density (OD) of 0.2 ($OD_{600}$) which corresponds to approximately 2 x $10^8$ CFU/ml. This inoculum was then diluted to generate the different inoculum concentrations required for each experiment.

**Pathogenicity assays**

Shoots were collected from mature trees and inoculated using the dip inoculation method described in Hulin *et al*. (2018a). Briefly, 12 cm one-year old shoots were collected when field-grown trees were dormant (December-February). Before inoculation, shoots were surface sterilised with 70% ethanol and allowed to air dry. The apical end was cut with secateurs (removing 1 cm) and dipped in

bacterial inoculum of 2 x $10^7$ CFU/ml) for 5 min. Shoots were blotted dry on paper towel and sealed with parafilm. The basal end of the shoot was then cut (removing 1 cm) and kept in water for one week at 16°C with 16:8hr light dark cycles. Shoots were then randomised using a fully randomised design, in oasis foam and kept at 16°C in a controlled environment room for a further 5 weeks with 16:8hr light dark cycles. They were routinely watered to keep the foam constantly moist. Shoots were assessed by peeling away the top layer of tissue and measuring the length of underlying necrosis. This experiment was repeated five times.

Detached leaf pathogenicity assays were conducted in spring 2018 and 2020, utilising 2 to 3 - week old leaves from field-grown mature trees. For population counts, leaves from actively growing 4 month-old grafted trees in polytunnels were used to allow multiple repetitions of these experiments. The top three fully expanded leaves were chosen for experiments, due to their expected similar susceptibility (Mgbechi-Ezeri *et al.*, 2017).

Leaf pathogenicity assays and population counts were conducted as in Hulin *et al*. (2018a). Leaves were infiltrated using a blunt-ended syringe and usually at an inoculum concentration of 2x$10^6$ CFU/ ml (100-fold dilution of a 0.2 $OD_{600}$ suspension). After incubation for ten days at 22°C, this inoculum concentration allowed clear differentiation between responses to strains pathogenic to cherry and to other hosts (Hulin *et al.,* 2018a). Each leaf received a mock inoculation as a control and where appropriate, different strains were compared on the same leaves (up to six inoculation sites) to reduce plant variability. Symptoms were scored on a scale of 0-5 (0; none, 1; limited browning, 2; browning < 50% inoculated area, 3; browning >50% inoculated area, 4; complete browning, 5; complete browning with spread away from initial lesion. Experiments were repeated at least three times. Population counts of bacteria within disease lesions were conducted as previously described (Hulin *et al.,* 2018a): Leaves were surface sterilised with 70% ethanol before excision of leaf disks from the inoculated area with a 0.5 cm cork borer and ground in 10mM $MgCl_2$. Serial dilutions were plated onto Kings B agar with cephalexin (80 mg/L) and cycloheximide (200 mg/L).

**Statistical analyses**

All statistical analyses and graph generation were performed using R software (R Core Team, 2012), and the packages ggplot2, lmerTest, lme4, emmeans, ordinal and multcomp (Hothorn *et al.*, 2008; Wickham, 2009; Bates et al. 2015; Christensen, 2019; Lenth *et al.*, 2020). For population counts and necrosis data from shoot experiments Analysis of Variance (ANOVA) was used to determine

statistical differences between treatments. Where datasets were unbalanced due to the grouping of multiple experiments with one or more treatments missing, REML was utilised to generate a linear mixed model. Means were extracted from the model using the program emmeans and *post-hoc* comparisons generated using the cld function within the multcomp package. Where residuals from the linear model/ANOVA were not normally distributed the data were log transformed and the model run again and residuals checked with qqnorm. To analyse the symptom score data from pathogenicity assays, the ordinal package was utilised, specifically the function clmm, which is optimised for ordinal data.

## Results

### Partial resistance is seen in woody tissue but not leaf tissue of sweet cherry cultivars

Varietal resistance has been reported in sweet cherry under field conditions (Hulin *et al.* 2018a). To extend the range of sweet cherry cultivars screened for differences in resistance, detached shoot assays were conducted using representative strains from the three major canker-causing clades *Psm* R1, *Psm* R2 and *Pss* as shown in Fig. 1. The strain *Psm*R1-P, recognised as virulent on plum but not cherry (Hulin et al, 2018a), was also included (see full data Fig. S1). Statistical analysis revealed significant differences in necrosis length between cultivars ($p < 0.01$, d.f = 15), strains ($p < 0.01$, d.f = 4) and an interaction between them ($p < 0.01$, d.f = 60). Overall, cultivars showed a large degree of variability in the length of necrotic lesion produced, which meant that apparent differences in susceptibility of many cultivars were deemed not significantly different. However, cultivars such as Merton Glory and Colney showed partial resistance to all three of the major canker pathogens, with necrosis lengths significantly lower than in the most susceptible varieties such as Van and Roundel. We previously reported that the cultivar Merton Glory exhibited partial resistance to bacterial canker (Hulin *et al.*, 2018a). All cultivars showed very limited susceptibility to *Psm*R1-P, the strain virulent on plum but less virulent on cherry (Fig. S1).

In an earlier study, detached leaf syringe-infiltration assays did not reproduce the quantitative differences seen in woody tissues of cherry varieties (Hulin *et al.*, 2018a). To further examine the use of leaf inoculation to differentiate varietal resistance within sweet cherry, leaves of three cultivars which had varied in their response in the shoot assays (Fig. 1), ranging from partially resistant to susceptible and highly susceptible, (Colney, Sweetheart and Van), were inoculated with progressively lower bacterial concentrations than $10^6$ CFU/ml as used in earlier work. Bacterial population counts were determined after 10 days (Fig. 2). There were significant differences

between strains ($p < 0.01$, d.f = 2), and concentrations ($p < 0.01$, d.f = 15), and an interaction between them ($p < 0.01$, d.f = 4). However, even from the lowest inoculum level, the different cultivars did not vary significantly in final bacterial populations ten days post-inoculation ($p = 0.055$, d.f = 2). The cultivar Colney which had exhibited reduced susceptibility in the shoot assay, did not show any reduction in bacterial populations compared to Sweetheart and Van at any of the concentrations, although at the lowest, *Psm* R1 and R2 grew to higher levels in Van compared to the other cultivars. These experiments confirmed that, in these sweet cherry cultivars, leaf infiltration inoculations did not reproduce the differential susceptibility to canker scored using cut shoots.

**Wild cherry and other *Prunus* species exhibit leaf-based resistance to *Pseudomonas syringae***

Although leaf inoculation assays did not reproduce the differential susceptibility observed in cut shoots of sweet cherry cultivars, in previous work the more tractable leaf tests did clearly demonstrate non-host resistance to strains of *P. syringae* pathogenic on other plants (Hulin *et al.* 2018a and b). We therefore examined if any leaf-based resistance could be found in the wider germplasm that would give levels of resistance to the cherry pathogens comparable to non-host resistance.

Fifty-two wild cherry accessions, and four susceptible sweet cherry accessions for comparison, were screened using young leaves from mature trees (Fig. 3). In initial experiments, *Psm* R1, *Psm* R2 from cherry and plum, and *Pss* were used for inoculation at $10^6$ CFU/ml, and in the final screen *P. syringae* pv. *avii (Psa*) was also included as this has been reported to be a pathogen of wild cherry (Ménard *et al.*, 2003). The wild cherries exhibited a wide range of responses to the bacterial canker pathogens, from no, or very limited symptoms to complete necrosis of the inoculated region (see representative images of scores in Fig. 3b). Results are presented in Figure 3a in order of the increasing severity of symptoms observed (mean overall symptom score per cultivar). Several accessions produced limited or no symptoms during this screening. In particular, the wild cherries *P.a.* Groton B, *P. a.* FD1-57-4/122, *P. a*. Deadmans Wood and *P. a*. Thruxton Vallets (numbered 23, 19, 16 and 48 respectively in Fig. 3a) were scored as highly resistant.

Ordinal statistical analysis confirmed that there were significant differences between accessions ($p < 0.01$, d.f = 55), and strains ($p < 0.01$, d.f = 4). However, an interaction model could not be fitted due to complete separation of the response factor preventing model convergence (e.g., where in selected cases all scores were the same for a particular strain x cultivar combination) as discussed in Allison (2008). Nevertheless, in some genotypes there were clear differential reactions to the

pathogenic strains (listed in Table S2). For example, genotypes 15, Coed-y-Stig and 25, Howley Wood showed resistance to *Psa* and *Psm*R1-P, respectively, but were susceptible to other strains. Sweet cherry cultivars were resistant to the plum strain *Psm*R1-P (graphs shaded in red in Fig. 3a), but several wild cherries were susceptible e.g. 31, Marlow Common 1902 and 21, Frydd Wood 1908, the latter recording very little symptom development by the other strains. Another pattern to emerge was lesion formation following inoculation with *Psa* and *Psm* R1-C from cherry, but resistance to other strains as recorded in accessions - 1, Arger Fen A; 7, Bunny Old Wood B; 27, Lowdham lane and 50,Tyn-y-Bryn. The statistical analysis indicated that accessions 23, *P.a.* Groton B; 19, *P. a.* FD1-57-4/122 and 48, *P. a*. Thruxton Vallets were significantly reduced compared to sweet cherry controls. Other possibly resistant accessions such as 16, *P. a*. Deadmans Wood were not deemed significantly different (based on Tukey posthoc groupings) which may have been due to reduced data for this accession.

Screening by leaf inoculation was then extended to a range of other *Prunus* species using *Psm* R1-C, *Psm* R1-P, *Psm* R2 and *Pss* (Fig. 4) which are the main pathogens of cherry. Species tested included members of the subgenus *Cerasus* (Fig. 4a), sweet cherry inter-specific hybrids with other *Cerasus* species (Fig. 4b), subgenus *Prunus* (Fig. 4c), subgenus *Amygdalus* (Fig. 4d), and subgenus *Padus* (Fig. 4e). Statistical analysis again indicated that there were significant differences between accessions ($p < 0.01$, d.f = 34), and strains ($p < 0.01$, d.f = 3). Those with significantly less symptom development overall, compared to sweet cherry (cv. Napoleon, as this was a parent of most of the interspecific hybrids) are marked by asterisks in Fig. 4. Accessions of *P. dulcis*, *P. cerasifera*, *P. padus*, *P. pensylvanica*, *Prunus* x *gondouinii* and *P. incisa* all exhibited very limited to no symptom development when inoculated with the major cherry pathogens. Inter-specific hybrids of sweet cherry with other species within the Cerasus subgenus (Fig. 4b), included three progeny from a *P. incisa* x *P. avium* sweet cherry cross and all failed to develop significant lesions.

Leaves of several accessions of wild cherry and other *Prunus* species developed limited symptoms after inoculation with the major cherry pathogens. To determine if this resistance operated against a wider range of isolates from each pathogenic clade, two of the most resistant accessions (wild cherry Groton B and ornamental species *Prunus incisa*), as well as susceptible sweet (Penny and Sweetheart) and wild (Groton A) cherry cultivars for comparison, were screened with 16 previously characterised *P. syringae* strains pathogenic on cherry and plum (Fig. 5). The wild cherry Groton B generally recorded low levels of symptom development, but a tree from the same woodland, Groton A, was highly susceptible and comparable to the sweet cherry varieties (see Fig. 3). This test with

307 further strains confirmed that Groton B exhibited resistance, although some strains of *Pss* were able
308 to cause lesions. Inoculation with each of the 16 strains tested failed to cause symptoms in the
309 ornamental species *P. incisa*. Statistical analysis confirmed differences between cultivars ($p<0.01$,
310 df=4), with Groton B and *P. incisa* recording significantly lower symptom scores to all pathogenic
311 strains.
312
313 **The more resistant varieties of wild and ornamental cherry support lower *in planta* bacterial**
314 **multiplication**
315 The wild cherry Groton B and ornamental species *Prunus incisa* had shown a high level of resistance.
316 To establish if bacterial multiplication was reduced within the leaves of these cultivars, populations
317 were counted 10 days after inoculation (Fig. 6a). Two susceptible sweet cherries and a susceptible
318 wild cherry from the same forest as Groton B (Groton A) were included for comparison.
319 Representative images of symptoms taken during initial screens of these accessions are displayed in
320 Fig. 6b. Statistical analysis revealed that there were significant differences between strains ($p < 0.01$,
321 d.f = 2) and accessions ($p < 0.01$, d.f = 4) as well as an interaction between them ($p < 0.01$, d.f = 8).
322 The more resistant genotypes Groton B and *P. incisa* supported lower bacterial populations of both
323 *Psm* R1 and *Psm* R2 10 dpi and showed limited or no symptom development compared to
324 susceptible cultivars. Multiplication of *Pss* was not significantly lower in Groton B than in the
325 susceptible sweet cherry cultivars (Penny and Sweetheart) in this experiment, but *P. incisa* again
326 proved to be resistant.
327
328
329 **Relationship between resistance response and bacterial inoculum dose**
330 To see if the observed resistance in certain accessions was robust to increasing bacterial inoculum
331 concentrations, Groton B, *P. incisa* and the susceptible cultivar Penny, were inoculated using
332 increasing doses ranging from $10^6$ CFU/ml to $10^8$ CFU/ml (Fig. 7). At day 0 (Fig. 7a), there was no
333 significant difference between bacterial numbers in accessions ($p=0.32$, df=2). After 10 dpi, the wild
334 cherry Groton B supported high bacterial populations of all pathogens when inoculated at $10^7$
335 CFU/ml and $10^8$ CFU/ml, with resistance only apparent at the lower inoculum concentration (Fig.
336 7b). By contrast, the ornamental species *P. incisa* recorded significantly reduced bacterial
337 populations even when inoculated at $10^8$ CFU/ml for *Psm* R1 and *Psm* R2, although *Pss* appeared to
338 overcome any resistance using the highest inoculum concentration. Symptom scoring in these
339 experiments revealed that at the lower concentration ($10^6$ CFU/ml) Groton B and *P. incisa* recorded
340 very limited symptom formation after 10 days (Fig. 7c), confirming the results presented in Fig. 3 and

4. By contrast, at the higher inoculum concentrations, symptoms were more apparent and similar to those observed in sweet cherry cv. Penny, particularly for the more virulent *Pss*.

The restriction of bacterial populations in *P. incisa,* particularly towards *Psm* R1 and *Psm* R2 at higher inoculum concentrations was similar to a non-host resistance response as seen previously in cherry towards plum and *Aquilegia* pathogens (Hulin *et al.*, 2018a). To examine if the multiplication of the sweet cherry pathogen *Psm* R1-C was similar to non-pathogens of cherry in *P. incisa*, several strains were inoculated at the highest inoculum concentration ($2x10^8$ CFU/ml) on *P. incisa* and compared with a susceptible cherry four days after infiltration (Fig. 8). The non-pathogens *Psm*R1-P from plum and RMA1 (a pathogen of *Aquilegia*) reached levels between $1x10^5$-$1x10^6$ CFU/leaf disk in cherry cv. Sweetheart, whilst the pathogenic strain *Psm* R1-C grew a log higher. *Psm* R1-C did not grow as well in *P. incisa* where it reached levels of $1x10^5$-$1x10^6$ CFU/leaf disk. However, the non-pathogens of cherry multiplied even less in *P. incisa* than they did in the sweet cherry. These results indicated that *Psm* R1-C may be more adapted to *P. incisa* than strains originating from unrelated plant hosts even though the ornamental cherry species still appears to have significant resistance.

Finally, to confirm if the resistance response of Groton B and *P. incisa* seen in leaves was reflected in woody tissue, a cut shoot assay was performed (Fig. 9). Unfortunately, the *P. incisa* shoots were not amenable to this assay and dried out, likely due to their thinness. However, the assay confirmed Groton B like the more resistant sweet cherry cultivars Merton Glory and Colney showed much reduced necrosis compared to the susceptible sweet cherry Penny.

**Discussion**

The development of rapid laboratory-based tests to allow screening for resistance in trees is a major challenge that underpins the rapid development of new cultivars that resist pests and diseases. Hulin *et al.* (2018a) addressed this issue in relation to cherry canker and found that cut shoot assays most closely reflected canker disease development in whole tree tests in the field. Although the more tractable leaf inoculation failed to differentiate sweet cherry cultivar resistance levels, it did allow clear differentiation between the canker pathogens and pathogens of other plants. Non-host resistance was well defined in leaves and reflected the failure of the non-pathogens to cause symptoms in woody tissues. In this study, we describe further analysis of partial resistance in sweet cherry cultivars and use a leaf infection-based screen of wild cherry and related *Prunus* spp. to identify potential new sources of resistance to all clades of *P. syringae* that cause cherry canker. Arguably, assays on woody tissues, such as shoots or whole trees, are required to fully determine

bacterial canker resistance in breeding programmes. However, the use of non-woody material for screening provided a rapid way to search for strong resistance phenotypes and has been utilised in other studies, including detached leaves (Mgbechi-Ezeri *et al.*, 2017) and micro-propagated plantlets (Vicente & Roberts, 2003).

In our first experiments we inoculated a range of sweet cherry cultivars with *P. syringae*, and detected variation in susceptibility to *Psm*R1, *Psm*R2 and *Pss* in the woody tissue (cut shoots) but not in leaf tissue, even at low inoculum concentrations. This suggested that perhaps leaf assays are not sensitive enough to pick up small differences in cultivar susceptibility, or perhaps tissue-specific differences in immune responses may occur. Further studies using less mechanical methods, that do not bypass surface-based immunity, such as spray or dip inoculations of leaves, might reveal subtle differences between cultivars (Liu *et al.*, 2015). We do not know what mechanisms of partial resistance are operating in woody shoots of the less susceptible cultivars such as Colney and Merton Glory. The differences in lesion formation observed could be due to the physical structure of the woody tissues rather than some differential biochemical defence response. The more susceptible varieties might have larger intercellular spaces between cambial tissues that allow more rapid unrestricted bacterial colonisation from the cut end of the shoot. Such a tissue-based difference would explain the lack of expression of resistance in leaves where a dynamic, cellular response may be the key to prevention of colonisation. These hypotheses remain to be tested. Although woody tissues are, arguably, the main sites of infection by *P. syringae* causing canker disease, other tissues such as leaves and blossom can be colonised and harbour the pathogen (Crosse, 1966) and resistance in these tissues is of use for breeding programmes.

Although, the responses of sweet cherry cultivars tested could not be differentiated on leaves, we reasoned that relatives of sweet cherry might exhibit resistance in non-woody tissues as seen in previous work (Vicente & Roberts, 2003). A large screen of diverse wild cherry revealed several accessions, notably Groton B and FD1-57-4/122, that exhibited resistance to strains from all the canker-producing *P. syringae* clades. These data support previous observations during projects focused on wild cherry. Groton B was identified as being significantly more resistant in cut shoot tests in 1996 and 1998 at EMR (K. Russell pers. comms). Similarly, FD1-57-4/122 is a seedling selection bred at East Malling from a wild mazzard seedling F1/3a, originally introduced in 1914. F1/3a was shown to have resistance when screened in a clonal rootstock breeding programme at East Malling (Garrett, 1979). A sibling of FD1-57-4/122, FD1-57-4/166 was also found to be more resistant in plantlet assays (Vicente & Roberts, 2003).

Differential symptom development in some accessions also suggests the existence of a pattern of resistance and susceptibility, as observed in examples of race and cultivar specific resistance in other plant/bacterium interactions, for example in bean halo blight disease (Arnold *et al.,* 2011). Differentials observed are listed in Table S2, but no simple model based on the presence of *R* genes matching each clade could be fitted to the data. The reactions observed to the plum strain *Psm* R1-P are of particular interest. Resistance to *Psm* R1-P in sweet cherry could be due to resistance triggered by the intracellular detection of pathogen effectors such as HopAB1 by the plant immune system. Genomic analysis revealed the *hopAB1* effector gene is present in this strain but not its cherry pathogenic relatives (Hulin *et al*., 2018a and b). Several wild *P. avium* accessions were susceptible to infection by the plum strain, developing distinct lesions, and presumably these accessions could lack a receptor recognising HopAB1, such as Pto in tomato species (Chien *et al.*, 2013). The role of HopAB1 as an inducer of effector triggered immunity and/or a virulence determinant should be tested by genetic dissection through deletion of *hopAB1* from *Psm* R1-P.

The study was the extended to other *Prunus* species and sweet cherry hybrids. In particular, some *Prunus* species also displayed resistance to the major pathogen strains, and the Fuji cherry accession *P. incisa* proved resistant to all 16 canker pathogens tested. The resistance suggested by lack of symptom development in wild cherry and related *Prunus* spp. was confirmed through analysis of bacterial multiplication in leaves. Bacterial populations reached in *P. incisa* were lower than those recorded in the selected wild cherry accession Groton B. The dynamics of population growth in *P. incisa* were similar to those recorded for non-pathogens in sweet cherry. The similarly reduced multiplication of the non-host plum and *Aquilegia* pathogens in *P. incisa* compared with sweet cherry indicates that there may be a more rapid deployment of resistance, perhaps mediated through an enhanced level of cell surface-based immunity and/or effector-mediated intracellular responses. Whatever the biochemical nature of resistance, the lack of symptoms found in the hybrids between *P. incisa* and the sweet cherry cv. Napoleon after challenge with the major pathogens suggests that the resistance from *P. incisa* is probably inherited as a dominant trait.

The resistant wild cherry and *Prunus* accessions selected, Groton B and *P. incisa*, respectively, have now been incorporated into breeding programmes to introgress resistance into commercial sweet cherry genotypes and generate more resistant varieties for growers. Progeny of Groton B have also been selected for wild cherry breeding programmes to improve canker resistance in the forestry industry (K. Russell pers comm). Such work can take up to 15 years. The routine testing of progeny

performance against the main canker pathogens during these projects and future genetic research will provide further insights into the genetic controls underlying the outcome of the *Prunus*/*P. syringae* interaction.

**Figure legends**

Figure 1: Susceptibility of sweet cherry cultivars to *Pseudomonas syringae* infection. Boxplots show length of disease symptoms in cut shoots inoculated with *P. syringae Psm* R1-C 5244, *Psm* R2 MH001 or *Pss* 9644 six weeks after inoculation. The boxplots are ordered by estimated marginal means derived from the linear model to visualise the range of responses, although the graphs are of raw data. Individual data points are included and coloured for each separate experiment and the arithmetic mean is shown with a black diamond. This experiment was repeated up to five times per cultivar x strain combination. This figure shows the results for the three main pathogens, whilst the full data including results using *Psm*R1-Plum and mock inoculated controls (neither of which caused significant symptoms) are presented in Figure S1. REML analysis indicated a significant difference between cultivars ($p < 0.01$, d.f = 15), strains ($p < 0.01$, d.f = 4) and an interaction between them ($p < 0.01$, d.f = 60). Tukey-HSD (P = 0.05, confidence level: 0.95) significance groups obtained from the estimated marginal model emmeans are presented separately for each bacterial strain as letters under the graph.

Figure 2: Bacterial population counts for three cherry pathogens after their inoculation at different concentrations into leaves of three sweet cherry cultivars. Boxplots show the day 10 population counts for cultivars that showed differential responses in the cut shoot assay (Fig. 1). Individual data points are included and the arithmetic mean is shown with a black diamond. This experiment was performed once. There were significant differences between strains ($p < 0.01$, d.f = 2), and concentrations ($p < 0.01$, d.f = 15), and an interaction between them ($p < 0.01$, d.f = 4), whilst cultivars were not significantly different in this analysis ($p = 0.055$, d.f = 2). Tukey-HSD (P = 0.05,

confidence level: 0.95) significance groups for the different strains at particular concentrations are shown.

Figure 3: Use of leaf inoculation to screen wild cherry accessions for susceptibility to the canker pathogens. a: Boxplots of symptom scores from 52 wild cherry accessions and four sweet cherry cultivars (shaded in red) 10 days after inoculation with five *Pseudomonas syringae* strains. The strains *Psa* (black)*, Psm* R1-C (white), *Psm* R1-P (light grey), *Psm* R2 (dark grey) and *Pss* (mid grey) are coloured in different shades. Individual data points are included and coloured for each separate experiment. This experiment was performed up to five times for each strain x accession. The accessions are ordered according to their resistance to infection (blue box contains accession number): 1, *Prunus avium* (P. a.) Arger Fen A; 2, Arger Fen E; 3, Barming Lane; 4, Beardown Wood; 5, Buckland Wood 8; 6, Bunny Old Wood A; 7, Bunny Old Wood B; 8, Burghley Wood; 9, Chalky Road; 10, Charger; 11, Cherryhill Copse A; 12, Chisbury Wood 1905; 13, Cobtree; 14, Coed-Felin-Gat; 15, Coed-y-Stig; 16, Deadmans Wood; 17, Dean Wood 1918; 18, Everdon Stubbs B; 19, FD1-57-4/122; 20, Ffynone; 21, Frydd Wood 1908; 22, Groton A; 23, Groton B; 24, Hamlet Wood C; 25, Howley Wood; 26, Lockeridge B; 27, Lowdham Lane; 28, Lower Broxford Wood A; 29, Lower Broxford Wood B; 30, Malvern Hills; 31, Marlow Common 1902; 32, Narth A; 33, Orleans-141; 34, Pencelli Wood B; 35, Penley Wood A; 36, Postlebury B; 37, Poulton Wood A; 38, Primrose Wood; 39, Prospect Cottage; 40, Roundhill Wood; 41, Saxtens Wood B; 42, SC 311-33 (S27,S28); 43, Snarkhurst; 44, South Wood; 45, Stoke Row 1903; 46, Tank Wood; 47, Thornes Wood; 48, Thruxton Vallets; 49, Thundersley Wood; 50, Tyn-y-Bryn; 51, Wepre Park; 52, Wilmay Copse; and the sweet cherry cultivars - 53, Penny; 54, Sweetheart; 55, Van; 56, Colney. Ordinal regression analysis indicated that there were significant differences between cultivars ($p < 0.01$, d.f = 55), and strains ($p < 0.01$, d.f = 4). Those cultivars which showed significantly reduced symptoms across the strains compared to the least susceptible sweet cherry cultivar (55, Van) are marked with an asterisk.

b: Representative pictures of symptoms in each score category. Symptoms were scored as 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. Infiltration sites were inside the four black pen marks.

Figure 4: Leaf inoculation-based screen of a range of *Prunu*s species and hybrids (see Table 1 for full descriptions) for susceptibility to the cherry canker pathogens. The boxplots show symptom scores 10 days after inoculation with four *Pseudomonas syringae* strains. The strains *Psm* R1-C, *Psm* R1-P,

*Psm* R2 and *Pss* are coloured in shades of grey. Individual data points are included and coloured for each separate experiment. This experiment was performed up to two times for each strain x accession. a: *Prunus* subgenus *Cerasus*, b: *Prunus avium* hybrids, c: *Prunus* subgenus *Prunus*, d: *Prunus* subgenus *Amygdalus*, e: *Prunus* subgenus *Padus*. Where the hybrids in b were also screened the plot is shaded to show this (e.g. *P. incisa* E621 in **a** is the parent of three hybrids coloured in blue). *P. avium* cv. Napoleon (highlighted in red) was a parent of most hybrids (see Table 1 for more details). Ordinal regression analysis indicated that there were significant differences between cultivars ($p < 0.01$, d.f = 34), and strains ($p < 0.01$, d.f = 3). The accessions that showed significantly reduced symptoms across the strains compared to cherry cultivar Napoleon (*P. av* Nap) are marked with an asterisk. Symptom scoring was as shown in Figure 3.

Figure 5: Screening of several accessions with multiple strains of the cherry canker pathogens. The boxplots show symptom scores 10 days after inoculation with sixteen *P. syringae* strains. The strains are coloured by clade *Psa*, *Psm* R1-C, *Psm* R1-P, *Psm* R2 and *Pss,* in shades of grey. Individual datapoints are included and the experiment was performed only once. Ordinal analysis confirmed differences between cultivars ($p<0.01$, df=4). Symptom scoring was as shown in Figure 3.

Figure 6: Bacterial population counts of cherry pathogens inoculated into leaves of sweet, wild and ornamental cherry cultivars. Sweet cherry (Penny, Sweetheart), wild cherry (Groton A and Groton B) and ornamental cherry (*P. incisa*) a: Boxplots show the day 10 population counts for each strain on each cultivar after inoculation with $2x10^6$ CFU/ml of each strain. Individual data points are included and the arithmetic mean is shown with a black diamond. This experiment was performed once. ANOVA revealed there were significant differences between strains ($p < 0.01$, d.f = 2) and cultivars ($p < 0.01$, d.f = 4) as well as an interaction between them ($p < 0.01$, d.f = 8). Tukey-HSD (P = 0.05, confidence level: 0.95) significance groups for the whole data set comparison are labelled (a, b or c). b: Representative pictures of disease symptoms for each strain x cultivar combination (images taken during initial screens documented in Fig. 3,4); infiltration sites were inside the four black pen marks. Note the lack of macroscopic lesions in *P. incisa*.

Figure 7: Bacterial population counts of cherry pathogens inoculated into leaves of three genotypes at different inoculum concentrations.
a: Boxplots show the day 0 population counts for cultivars. Individual data points are included and coloured for each separate experiment and the arithmetic mean is shown with a black diamond. This experiment was repeated up to four times per cultivar x strain combination. ANOVA revealed a

significant difference between strains ($p<0.01$, df=2), concentrations ($p<0.01$, df=2) and an interaction between them ($p<0.01$, df=4). There was no significant difference in bacterial populations between cultivars ($p=0.32$, df=2). Tukey-HSD (P = 0.05, confidence level: 0.95) significance groups for the different strains at particular concentrations are presented.
b: Boxplots show the day 10 population counts for cultivars. The layout is the same as in a. ANOVA revealed a significant difference between strains ($p<0.01$, df=2), cultivars ($p<0.01$, df=2), concentrations ($p<0.01$, df=2) and a cultivar: strain interaction ($p<0.01$, df=4), cultivar: concentration interaction ($p<0.01$, df=4) and strain: concentration interaction ($p=0.03$, df=4).
c: Symptom scores at day 10 using the same scoring system as in Figure 3. Data are presented as in a and b. Ordinal analysis revealed a significant difference between strains ($p<0.01$, df=2), concentrations ($p<0.01$, df=2), cultivars ($p<0.01$, df=2) and a cultivar: concentration interaction ($p<0.01$, df=4).

Figure 8: Bacterial populations of a cherry pathogen (*Psm*R1-C) and two strains originating from different plants (plum, *Psm* R1-P and *Aquilegia vulgaris*, RMA1) that are non-pathogenic to cherry following inoculation into leaves of *P. incisa* and sweet cherry cv. Sweetheart at $10^8$ CFU/ml. Boxplots show the day four population counts for cultivars. Individual data points are included and the arithmetic mean is shown with a black diamond. This experiment was performed once. ANOVA revealed a significant difference between strains ($p<0.01$, df=2), cultivars ($p<0.01$, df=1) and an interaction between them ($p<0.01$, df=2). Tukey-HSD (P = 0.05, confidence level: 0.95) significance groups comparing all cultivar x strain combinations are presented.

Figure 9: Susceptibility of sweet and wild cherry cultivars to *Pseudomonas syringae* infection using cut shoots. Boxplots show length of disease symptoms in cut shoots inoculated with *Psm* R1-C, *Psm* R2 or *Pss* six weeks after inoculation. Individual data points are included and the arithmetic mean is shown with a black diamond. This experiment was performed once. ANOVA revealed a significant interaction between strains ($p<0.01$, df=4) and cultivars ($p=0.01$, df=3). Note the resistance of Groton B to all strains.

Figure S1: Susceptibility of sweet cherry cultivars to *Pseudomonas syringae* infection (full results from Figure 1). Boxplots show length of disease symptoms of cut shoots inoculated with a control (10mM $MgCl_2$), *P. syringae Psm* R1-P, *Psm* R1-C, *Psm* R2 or *Pss* six weeks after inoculation. The

boxplots are ordered by estimated marginal means derived from the linear model to visualise the range of responses, but the graphs are of raw data. Individual data points are included and coloured for each separate experiment and the arithmetic mean is shown with a black diamond. This experiment was repeated up to five times per cultivar x strain combination.

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691

Table 1 *Prunus* accessions screened in this study. Information includes Subgenus, species and accession. [a]: Abbreviation used on Figures 3 and 4. [b] Accessions taken to further tests. [c] *Prunus* subgenus *Cerasus* interspecific hybrids from crosses with *P. avium*. # Accessions showing significantly reduced symptom development compared to susceptible sweet cherry controls. Experiment each accession is included in: a: sweet cherry cut-shoot (Fig. 1, Fig. S1), b: sweet cherry leaf populations with different inoculum concentrations (Fig. 2), c: wild cherry leaf symptom screen (Fig. 3), d: other *Prunus* species leaf screen (Fig. 4), e: Selected accessions large leaf symptom screen with sixteen bacterial strains (Fig. 5), f: Selected accessions leaf population counts (Fig. 6), g: Selected accession leaf population counts at different inoculum concentrations (Fig. 7), h: Leaf population count with non-host *P. syringae* strains (Fig. 8), i: Cut shoot inoculation with selected accessions (Fig. 9).

| **Subgenus** | **Species** | **Accession** | **Group** | **Abbreviation[a]** | **Experiment** |
|---|---|---|---|---|---|
| *Cerasus* | *P. avium* (sweet) | Penny | Sweet | 53 | acefgi |
| *Cerasus* | *P. avium* (sweet) | Sweetheart | Sweet | 54 | abcefh |
| *Cerasus* | *P. avium* (sweet) | Van | Sweet | 55 | abc |
| *Cerasus* | *P. avium* (sweet) | Colney | Sweet | 56 | abci |
| *Cerasus* | *P. avium* (sweet) | Kordia | Sweet | | a |
| *Cerasus* | *P. avium* (sweet) | Merchant | Sweet | | a |
| *Cerasus* | *P. avium* (sweet) | Stella | Sweet | | a |
| *Cerasus* | *P. avium* (sweet) | Merton Glory | Sweet | | ai |
| *Cerasus* | *P. avium* (sweet) | Regina | Sweet | | a |
| *Cerasus* | *P. avium* (sweet) | Lapins | Sweet | | a |
| *Cerasus* | *P. avium* (sweet) | Roundel | Sweet | | a |
| *Cerasus* | *P. avium* (sweet) | Newstar | Sweet | | a |
| *Cerasus* | *P. avium* (sweet) | Summersun | Sweet | | a |
| *Cerasus* | *P. avium* (sweet) | Korvic | Sweet | | a |
| *Cerasus* | *P. avium* (sweet) | Inge | Sweet | | a |
| *Cerasus* | *P. avium* (sweet) | Napoleon | Sweet | P. av Nap | ad |
| *Cerasus* | *P. avium* (wild) | P. a. Arger Fen A | Wild | 1 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Arger Fen E | Wild | 2 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Barming Lane | Wild | 3 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Beardown Wood | Wild | 4 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Buckland Wood 8 | Wild | 5 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Bunny Old Wood A | Wild | 6 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Bunny Old Wood B | Wild | 7 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Burghley Wood | Wild | 8 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Chalky Road | Wild | 9 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Charger | Wild | 10 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Cherryhill Copse A | Wild | 11 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Chisbury Wood 1905 | Wild | 12 | c |

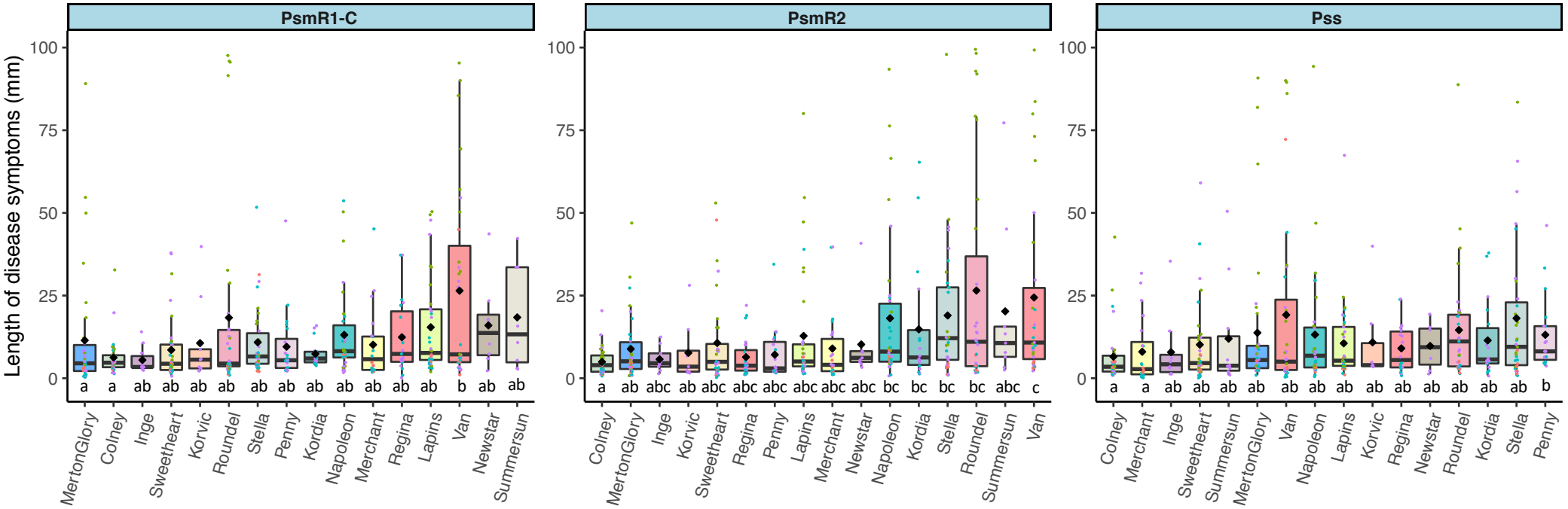
| | | | | | |
|---|---|---|---|---|---|
| *Cerasus* | *P. avium* (wild) | P. a. Cobtree | Wild | 13 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Coed-Felin-Gat | Wild | 14 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Coed-y-Stig | Wild | 15 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Deadmans Wood | Wild | 16 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Dean Wood 1918 | Wild | 17 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Everdon Stubbs B | Wild | 18 | c |
| *Cerasus* | *P. avium* (wild) | P. a. FD1-57-4/122 | Wild | 19[#] | c |
| *Cerasus* | *P. avium* (wild) | P. a. Ffynone | Wild | 20 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Frydd Wood 1908 | Wild | 21 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Groton A | Wild | 22 [b] | cef |
| *Cerasus* | *P. avium* (wild) | P. a. Groton B | Wild | 23 [b#] | cefgi |
| *Cerasus* | *P. avium* (wild) | P. a. Hamlet Wood C | Wild | 24 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Howley Wood | Wild | 25 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Lockeridge B | Wild | 26 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Lowdham Lane | Wild | 27 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Lower Broxford Wood A | Wild | 28 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Lower Broxford Wood B | Wild | 29 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Malvern Hills | Wild | 30 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Marlow Common 1902 | Wild | 31 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Narth A | Wild | 32 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Orleans-141 | Wild | 33 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Pencelli Wood B | Wild | 34 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Penley Wood A | Wild | 35 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Postlebury B | Wild | 36 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Poulton Wood A | Wild | 37 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Primrose Wood | Wild | 38 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Prospect Cottage | Wild | 39 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Roundhill Wood | Wild | 40 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Saxtens Wood B | Wild | 41 | c |
| *Cerasus* | *P. avium* (wild) | P. a. SC 311-33 (S27,S28) | Wild | 42 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Snarkhurst | Wild | 43 | c |
| *Cerasus* | *P. avium* (wild) | P. a. South Wood | Wild | 44 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Stoke Row 1903 | Wild | 45 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Tank Wood | Wild | 46 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Thornes Wood | Wild | 47 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Thruxton Vallets | Wild | 48[#] | c |
| *Cerasus* | *P. avium* (wild) | P. a. Thundersley Wood | Wild | 49 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Tyn-y-Bryn | Wild | 50 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Wepre Park | Wild | 51 | c |
| *Cerasus* | *P. avium* (wild) | P. a. Wilmay Copse | Wild | 52 | c |

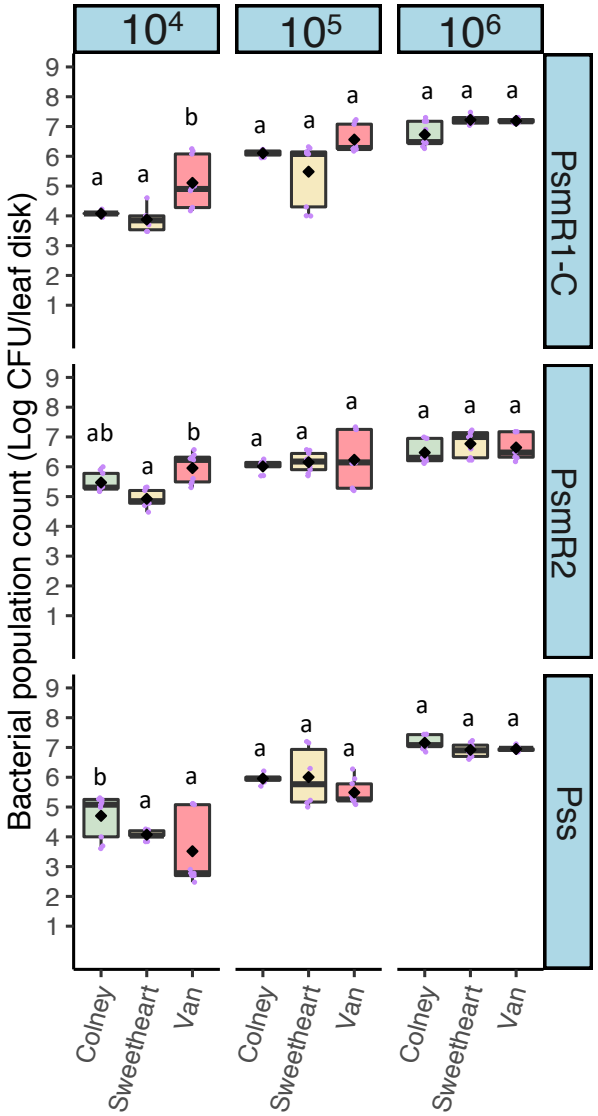
| | | | | | |
|---|---|---|---|---|---|
| *Cerasus* | *P. avium* tetraploid | | Ornamental | P. av 4x | d |
| *Cerasus* | *P. canescens* | F1296 | Ornamental | P. cn F1 | d |
| *Cerasus* | *P. canescens* | F1327 | Ornamental | P. cn F2 | d |
| *Cerasus* | *P. cerasus* | Kelleris 16 | Ornamental | P. ce K16 | d |
| *Cerasus* | *P. cerasus* | Ujfehertoi Furtos | Ornamental | P. ce UF | d |
| *Cerasus* | *P. dawyckensis* | GM61 | Ornamental | P. da GM61 | d |
| *Cerasus* | *P. incisa* | E621 | Ornamental | P. in E621[b #] | defgh |
| *Cerasus* | *P. maackii* | G280 | Ornamental | P. mc G280 | d |
| *Cerasus* | *P. mahaleb* | SL64 | Ornamental | P. mh SL64 | d |
| *Cerasus* | *P. maximoriczii* | | Ornamental | P. mx | d |
| *Cerasus* | *P. pennsylvanica* | | Ornamental | P. pen[#] | d |
| *Cerasus* | *Prunus* sp. | Ingram Dwarf | Ornamental | P. sp. ID | d |
| *Cerasus* | *P.* x *gondouinii* | Kanzas Sweet | Ornamental | P x g KS | d |
| *Cerasus* | *P.* x *gondouinii* | Marvel Duke | Ornamental | P x g MD[#] | d |
| *Cerasus* | *P. cerasus* | Elmer | Ornamental | P. ce Elmer | d |
| | | | | | |
| *Cerasus* | *P. avium* x *P. canescens* | Napoleon x P. canescens F1327 | Hybrid | Nap x P. cn F2[c] | d |
| *Cerasus* | *P. avium* x *P. kurilensis* | Napoleon x P. kurilensis (1) | Hybrid | Nap x P. ku(1) [c] | d |
| *Cerasus* | *P. avium* x *P. kurilensis* | Napoleon x P. kurilensis (2) | Hybrid | Nap x P. ku(2) [c] | d |
| *Cerasus* | *P. avium* x *P. nipponica* | Napoleon x P. nipponica | Hybrid | Nap x P. ni [c] | d |
| *Cerasus* | *P. avium* x *P. incisa* | Napoleon x P.incisa E621 (1) | Hybrid | Nap x P. in(1) [c#] | d |
| *Cerasus* | *P. avium* x *P. incisa* | Napoleon x P.incisa E621 (2) | Hybrid | Nap x P. in(2) [c#] | d |
| *Cerasus* | *P. avium* x *P. incisa* | Napoleon x P.incisa E621 (3) | Hybrid | Nap x P. in(3) [c#] | d |
| *Cerasus* | *P. canescens* x *P. avium* | P. canescens F1296 x Napoleon | Hybrid | P. cn F1 x Nap [c] | d |
| *Cerasus* | *P. mahaleb* x *P. avium* | | Hybrid | P. mh x P. av [c] | d |
| | | | | | |
| *Prunus* | *P. armeniaca* | Tomcot | *Prunus* sp. | P. ar | d |
| *Prunus* | *P. cerasifera* | M3 | *Prunus* sp. | P. cf M3[#] | d |
| *Prunus* | *P. cerasifera* | M5 | *Prunus* sp. | P. cf M5 | d |
| *Prunus* | *P. cerasifera* | M7 | *Prunus* sp. | P. cf M7 | d |
| *Prunus* | *P. domestica* | Seneca | *Prunus* sp. | P. do Se | d |
| *Prunus* | *P. domestica* | Victoria | *Prunus* sp. | P. do Vic | d |
| | | | | | |
| *Amygdalus* | *P. amygdalo-persica* | MB137 2817 | *Prunus* sp. | P. a-p | d |
| *Amygdalus* | *P. dulcis* Redwood | Redwood | *Prunus* sp. | P. du RW[#] | d |
| *Amygdalus* | *P. persica* Hiu Hun Tao | Hiu Hun Tao | *Prunus* sp. | P. per | d |

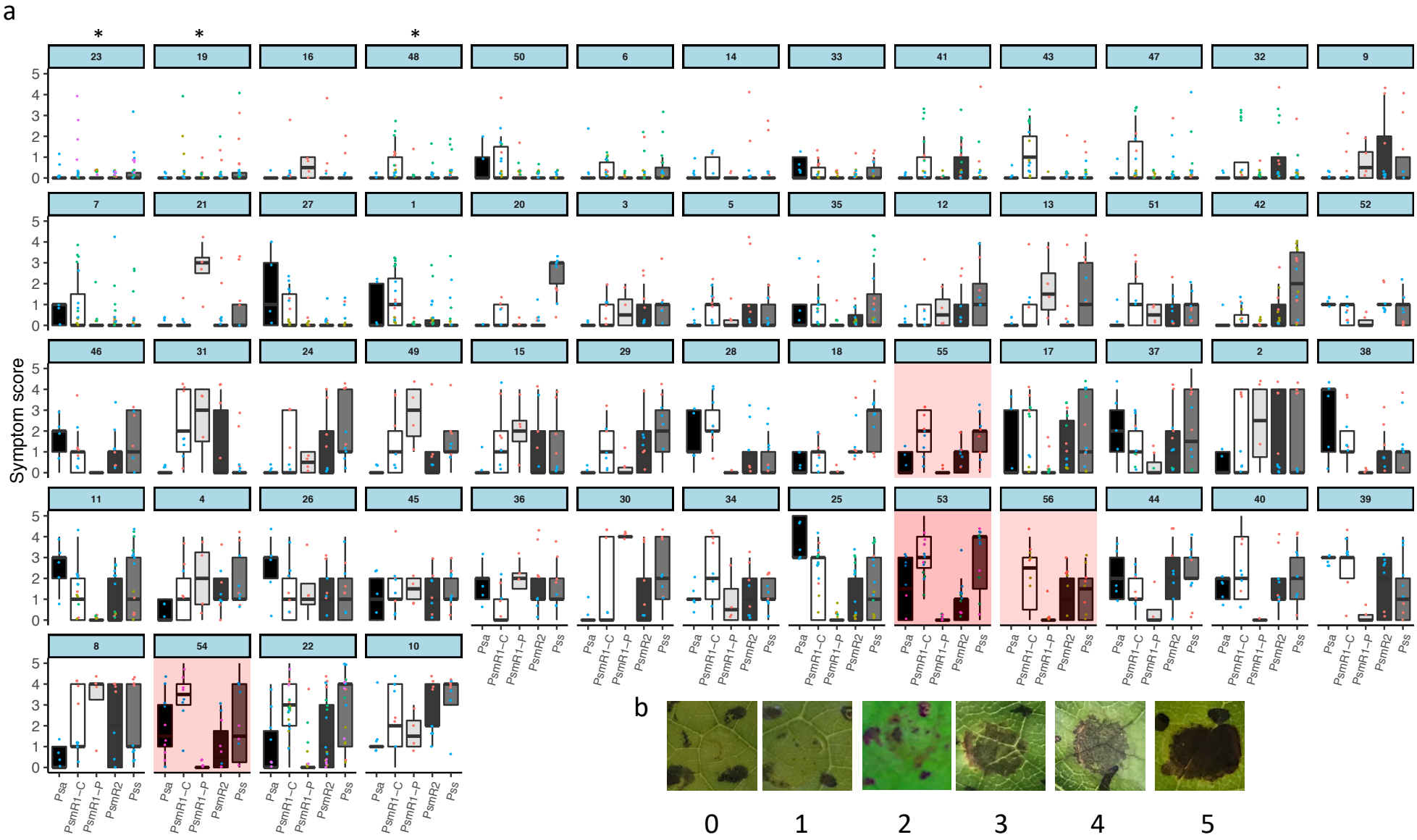
| | | | | | |
|---|---|---|---|---|---|
| *Padus* | *P. Padus* x Virginia | C292-2 | *Prunus* sp. | P. pad x Vir[#] | d |

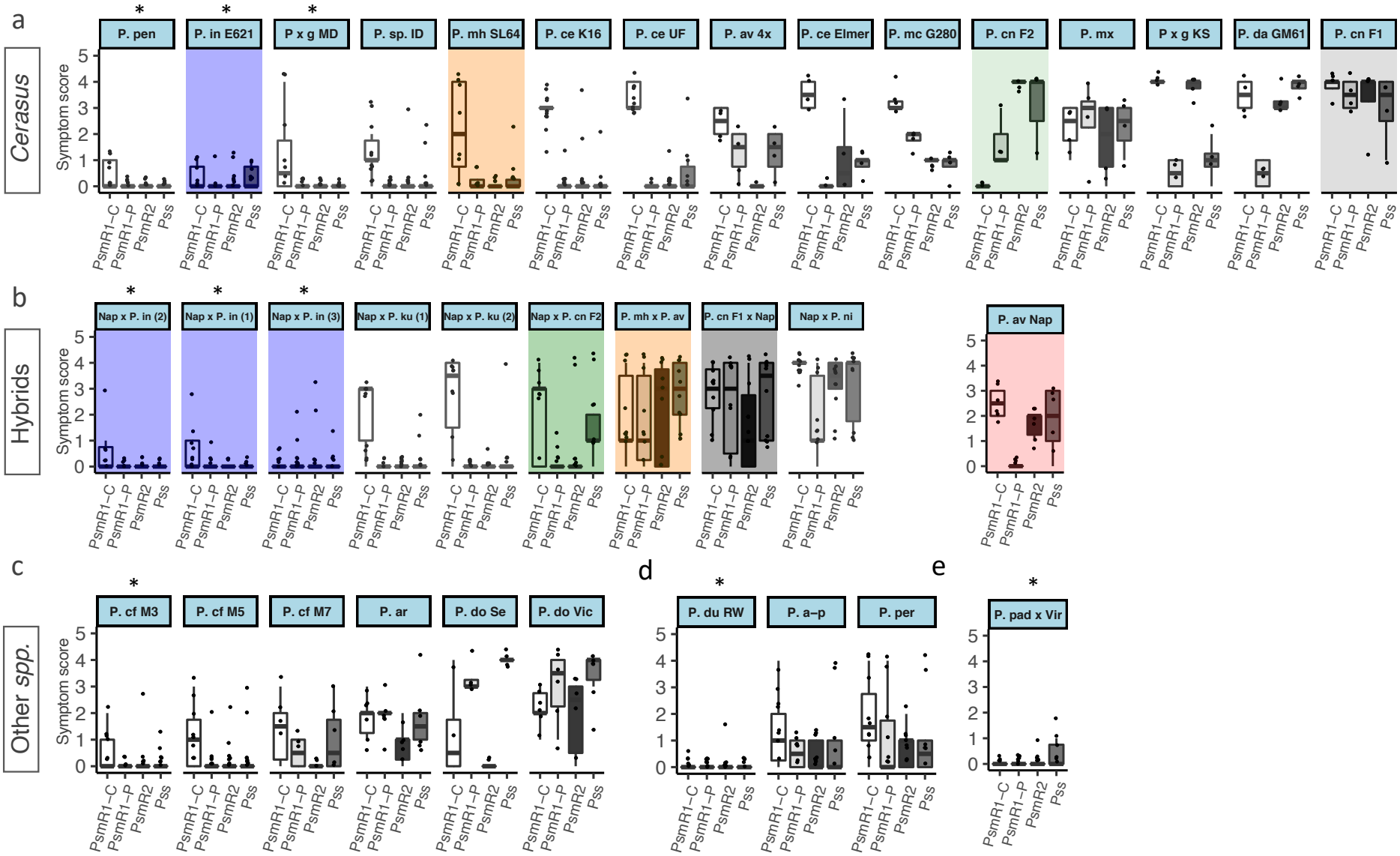
Table 2: Strains of *Pseudomonas syringae* used in this study with host of origin and original isolator. Experiment lists which experiments each strain was used for; a: sweet cherry cut-shoot (Fig. 1, Fig. S1), b: sweet cherry leaf populations with different inoculum concentrations (Fig. 2), c: wild cherry leaf symptom screen (Fig. 3), d: other *Prunus* species leaf screen (Fig. 4), e: Selected accessions large leaf symptom screen with sixteen bacterial strains (Fig. 5), f: Selected accessions leaf population counts (Fig. 6), g: Selected accession leaf population counts at different inoculum concentrations (Fig. 7), h: Leaf population count with non-host *P. syringae* strains (Fig. 8), i: Cut shoot inoculation with selected accessions (Fig. 9). * Strains not pathogenic on sweet cherry in previous study (Hulin et al. 2018a), all other strains are pathogenic on cherry.

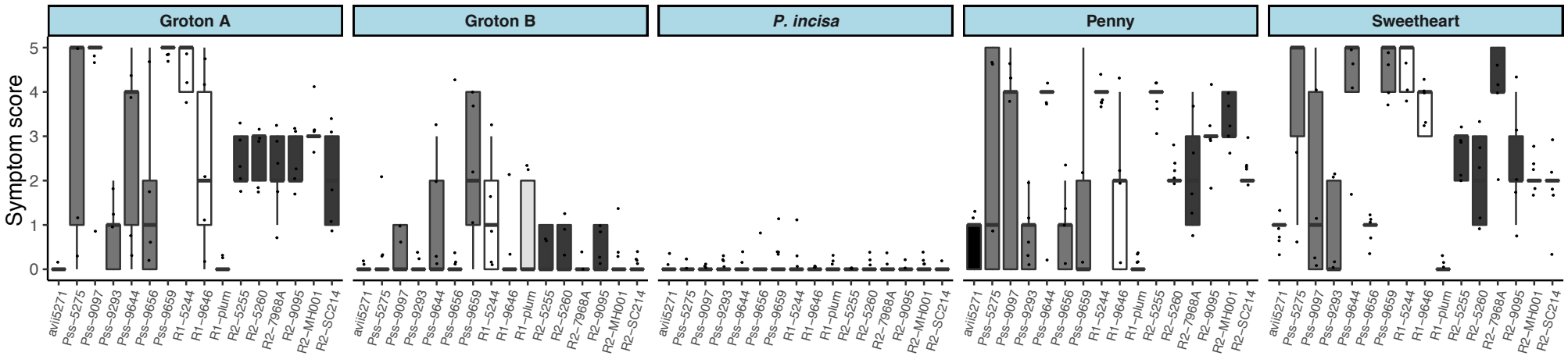
| **Strain** | **Clade** | **Plant host** | **Isolator** | **Experiment** |
|---|---|---|---|---|
| R1-5244 | *P. syringae* pv *morsprunorum* R1 | *Prunus avium* | Garrett, 1990 | abcdefghi |
| R1-plum/R1-5300* | *P. syringae* pv *morsprunorum* R1 | *Prunus domestica* | Garrett, 1990 | acdehi |
| R1-9646 | *P. syringae* pv *morsprunorum* R1 | *Prunus avium* | Roberts, 2012 | e |
| R2-leaf/MH001 | *P. syringae* pv *morsprunorum* R2 | *Prunus avium* | Hulin, 2014 | abcdefgi |
| R2-5255 | *P. syringae* pv *morsprunorum* R2 | *Prunus avium* | Prunier, n.d. | e |
| R2-5260 | *P. syringae* pv *morsprunorum* R2 | *Prunus avium* | Garrett, n.d. | e |
| R2-7968A | *P. syringae* pv *morsprunorum* R2 | *Prunus avium* (wild) | Vicente, 2000 | e |
| R2-9095 | *P. syringae* pv *morsprunorum* R2 | *Prunus avium* (wild) | Roberts, 2010 | e |
| R2-SC214 | *P. syringae* pv *morsprunorum* R2 | *Prunus avium* | Roberts, 1983 | e |
| avii5271 | *P. syringae* pv *avii* | *Prunus avium* (wild) | Garrett, 1990 | ce |
| Pss-5275 | *P. syringae* pv *syringae* PG:2d | *Prunus avium* (wild) | Garrett, 1990 | e |
| Pss-9097 | *P. syringae* pv *syringae* PG:2d | *Prunus avium* | Roberts, 2010 | e |
| Pss-9293 | *P. syringae* pv *syringae* PG:2b | *Prunus domestica* | Roberts, 2011 | e |
| Pss-9644 | *P. syringae* pv *syringae* PG:2d | *Prunus avium* | Roberts, 2012 | abcdefgi |
| Pss 9656 | *P. syringae* pv *syringae* PG:2b | *Prunus avium* | Roberts, 2012 | e |
| Pss 9659 | *P. syringae* pv *syringae* PG:2d | *Prunus avium* | Roberts, 2012 | e |
| RMA1* | *P. syringae* sp. | *Aquilegia vulgaris* | Jackson, 2012 | i |


PsmR1-C
PsmR2
Pss
Length of disease symptoms (mm)
100
75
50
25
0
a a ab ab ab ab ab ab ab ab ab ab ab b ab ab
MertonGlory Colney Inge Sweetheart Korvic Roundel Stella Penny Kordia Napoleon Merchant Regina Lapins Van Newstar Summersun
a ab abc ab abc abc abc abc abc abc bc bc bc bc abc c
Colney MertonGlory Inge Korvic Sweetheart Regina Penny Lapins Merchant Newstar Napoleon Kordia Stella Roundel Summersun Van
a a ab ab ab ab ab ab ab ab ab ab ab ab ab b
Colney Merchant Inge Sweetheart Summersun MertonGlory Van Napoleon Lapins Korvic Regina Newstar Roundel Kordia Stella Penny

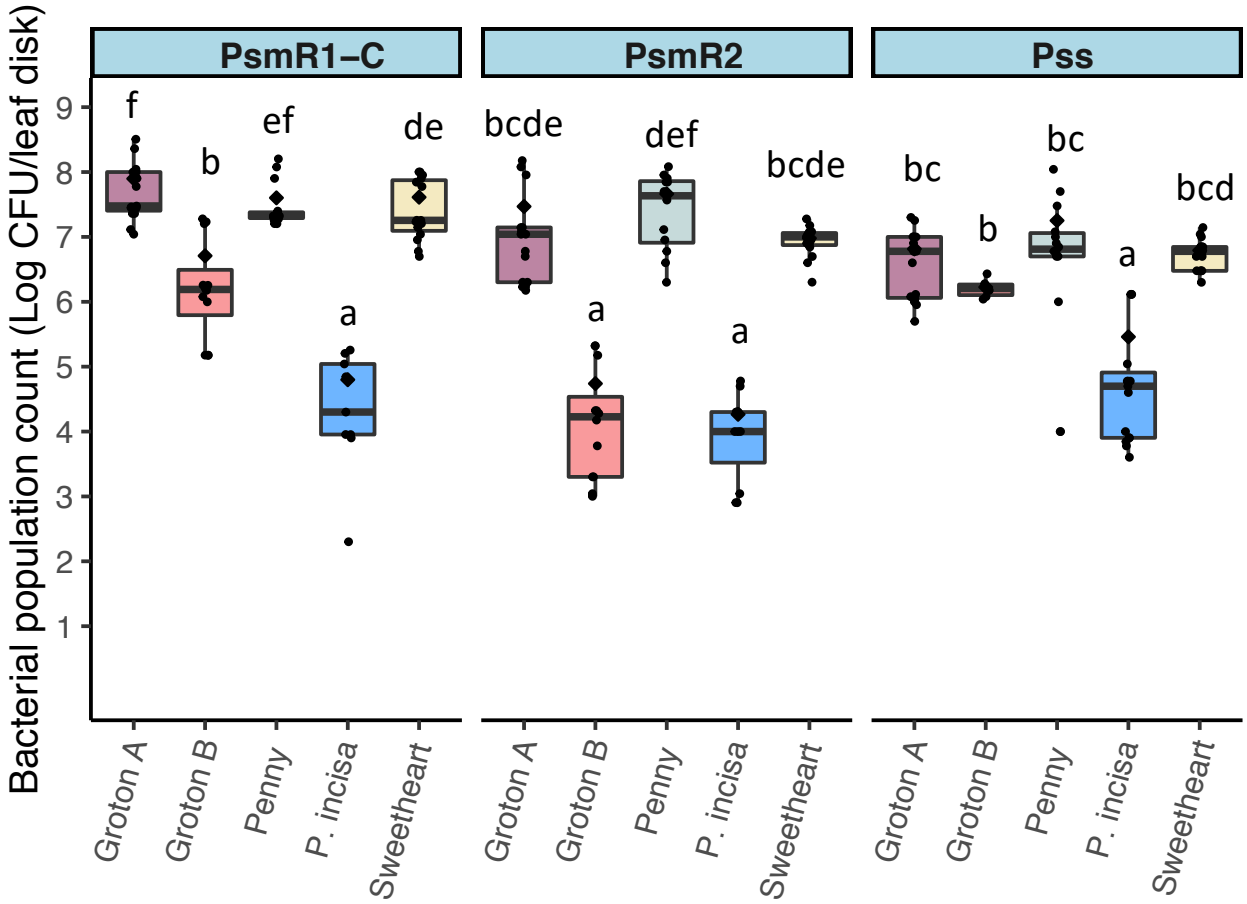

10^4
10^5
10^6
PsmR1-C
PsmR2
Pss
Bacterial population count (Log CFU/leaf disk)
Colney
Sweetheart
Van


a
b
Symptom score
0
1
2
3
4
5
Psa
PsmR1–C
PsmR1–P
PsmR2
Pss

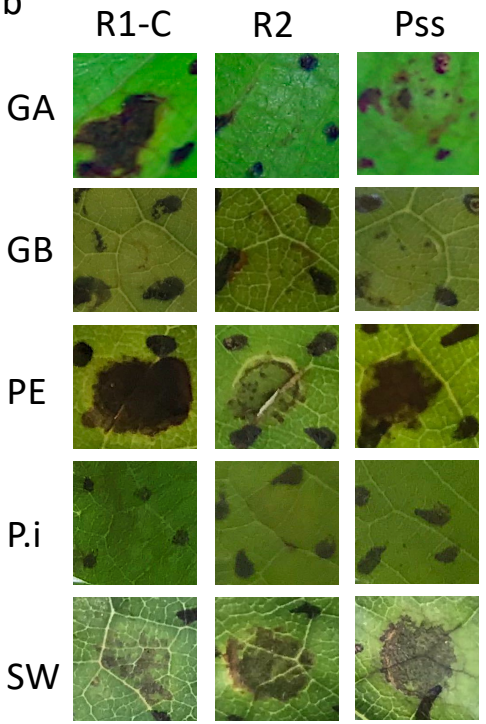

a
Cerasus
P. pen
P. in E621
P x g MD
P. sp. ID
P. mh SL64
P. ce K16
P. ce UF
P. av 4x
P. ce Elmer
P. mc G280
P. cn F2
P. mx
P x g KS
P. da GM61
P. cn F1
b
Hybrids
Nap x P. in (2)
Nap x P. in (1)
Nap x P. in (3)
Nap x P. ku (1)
Nap x P. ku (2)
Nap x P. cn F2
P. mh x P. av
P. cn F1 x Nap
Nap x P. ni
P. av Nap
c
Other spp.
P. cf M3
P. cf M5
P. cf M7
P. ar
P. do Se
P. do Vic
d
P. du RW
P. a–p
P. per
e
P. pad x Vir
Symptom score
PsmR1–C
PsmR1–P
PsmR2
Pss

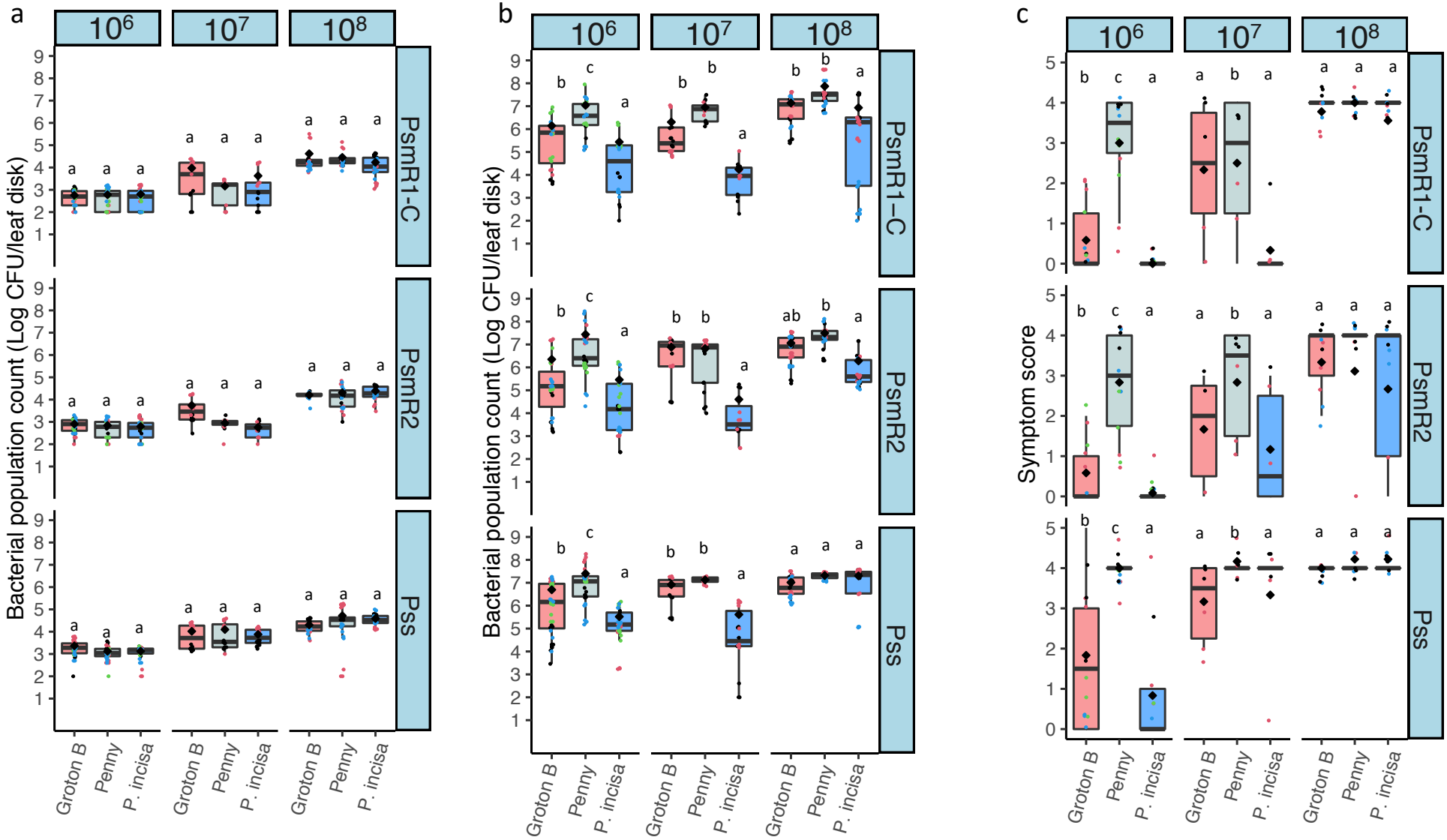

Groton A
Groton B
P. incisa
Penny
Sweetheart
Symptom score
0
1
2
3
4
5
avii5271
Pss−5275
Pss−9097
Pss−9293
Pss−9644
Pss−9656
Pss−9659
R1−5244
R1−9646
R1−plum
R2−5255
R2−5260
R2−7968A
R2−9095
R2−MH001
R2−SC214

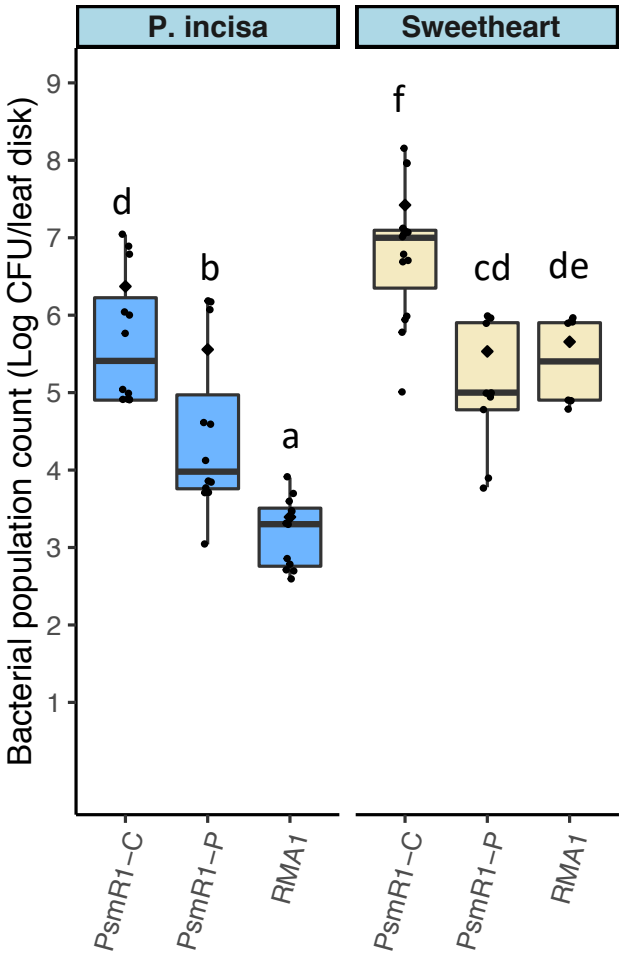
a

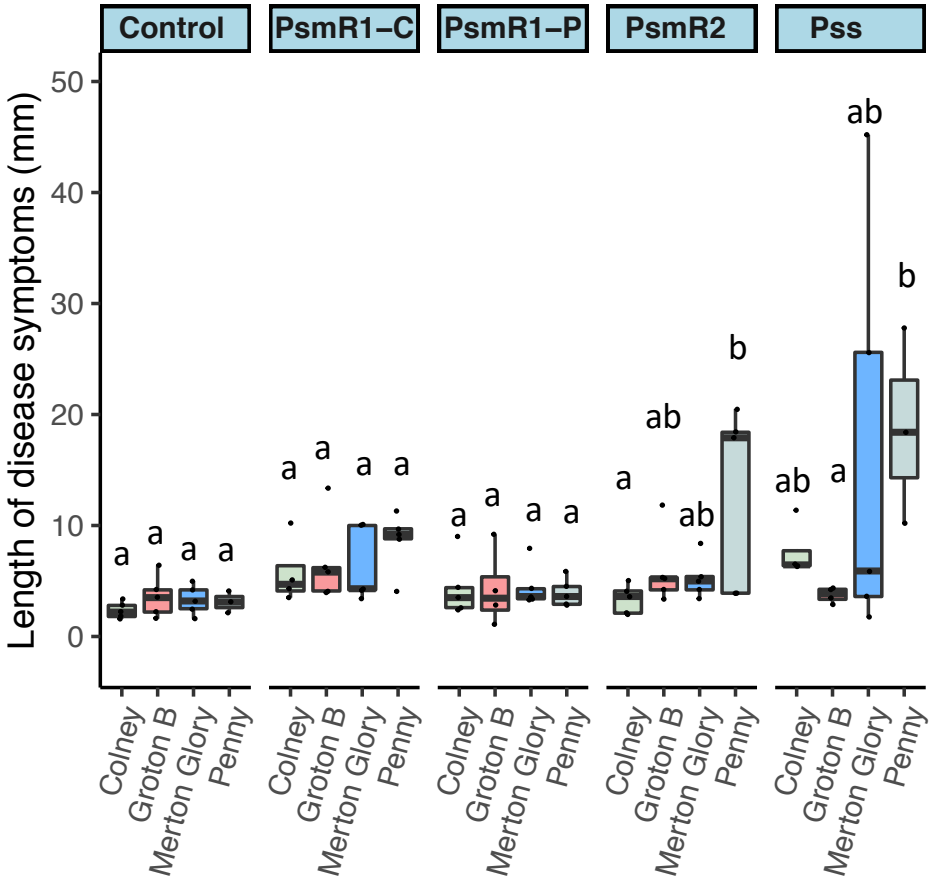

PsmR1–C
PsmR2
Pss
Bacterial population count (Log CFU/leaf disk)
1
2
3
4
5
6
7
8
9
f
b
ef
a
de
bcde
a
def
a
bcde
bc
b
bc
a
bcd
Groton A
Groton B
Penny
P. incisa
Sweetheart

b


R1-C
R2
Pss
GA
GB
PE
P.i
SW


a
b
c
$10^6$
$10^7$
$10^8$
PsmR1-C
PsmR2
Pss
Bacterial population count (Log CFU/leaf disk)
Symptom score
Groton B
Penny
P. incisa


P. incisa
Sweetheart
Bacterial population count (Log CFU/leaf disk)
9
8
7
6
5
4
3
2
1
d
b
a
f
cd
de
PsmR1-C
PsmR1-P
RMA1
PsmR1-C
PsmR1-P
RMA1


Control
PsmR1–C
PsmR1–P
PsmR2
Pss
Length of disease symptoms (mm)
0
10
20
30
40
50
a
a
a
a
a
a
a
a
a
a
a
a
a
ab
ab
b
ab
a
b
Colney
Groton B
Merton Glory
Penny

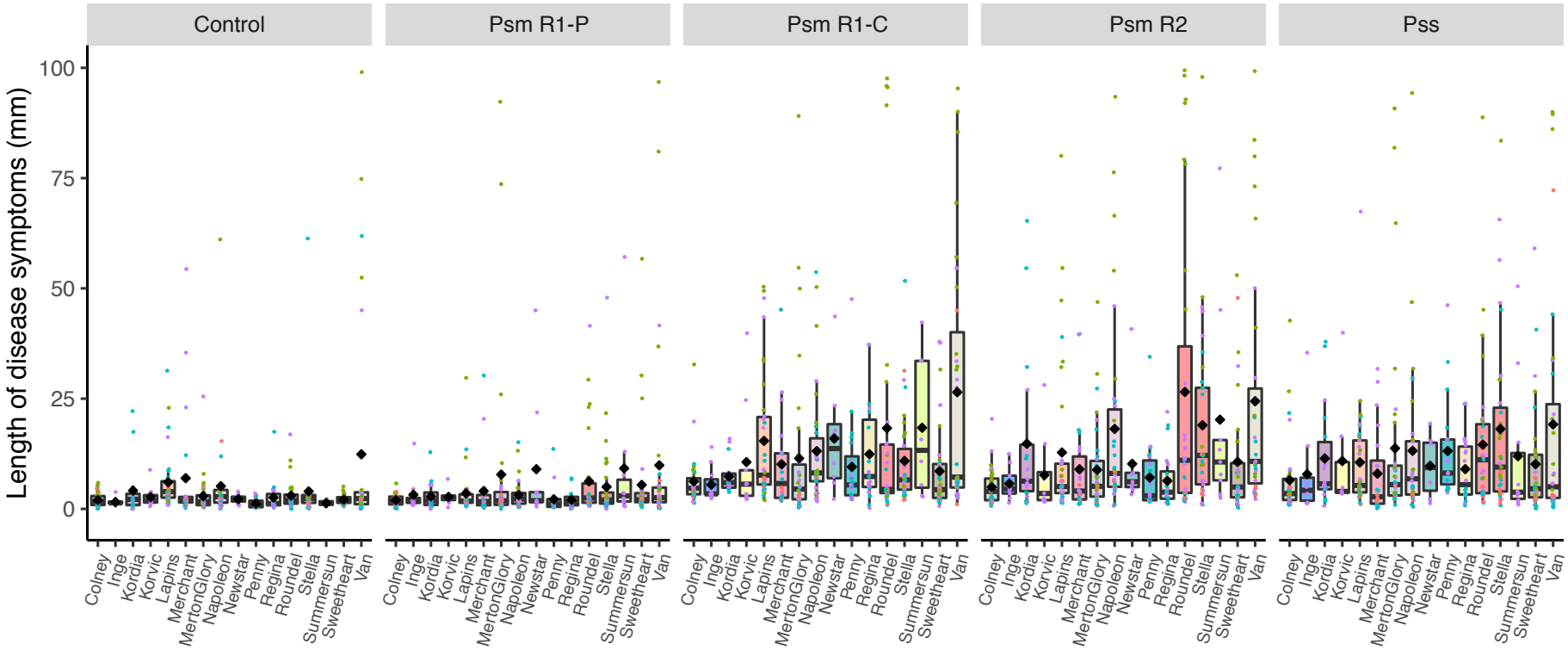

Control
Psm R1-P
Psm R1-C
Psm R2
Pss
Length of disease symptoms (mm)
100
75
50
25
0
Colney
Inge
Kordia
Korvic
Lapins
Merchant
MertonGlory
Napoleon
Newstar
Penny
Regina
Roundel
Stella
Summersun
Sweetheart
Van

Table S1. Origins of *Prunus avium* wild cherry accessions screened in this study. [a]: Bred at NIAB EMR in Kent. Blank fields are unknown. Grid ref refers to ordnance survey grid reference.

| Accession | No. | County | Grid ref | Longitude | Latitude |
|---|---|---|---|---|---|
| P. a. Arger Fen A | 1 | Suffolk | TL934355 | 51.98428 | 0.81496 |
| P. a. Arger Fen E | 2 | Suffolk | TL933355 | 51.98432 | 0.813506 |
| P. a. Barming Lane | 3 | Kent | TQ716550 | 51.26832 | 0.458425 |
| P. a. Beardown Wood | 4 | Devon | SS780073 | 50.85231 | -3.73452 |
| P. a. Buckland Wood 8 | 5 | Buckinghamshire | SP909078 | 51.76152 | -0.6843 |
| P. a. Bunny Old Wood A | 6 | Nottinghamshire | SK581282 | 52.84818 | -1.13874 |
| P. a. Bunny Old Wood B | 7 | Nottinghamshire | SK583283 | 52.84905 | -1.13576 |
| P. a. Burghley Wood | 8 | Lincolnshire | TF022048 | 52.63134 | -0.4914 |
| P. a. Chalky Road | 9 | Kent | | | |
| P. a. Charger [a] | 10 | Kent | TQ710568 | 51.28467 | 0.450693 |
| P. a. Cherryhill Copse A | 11 | Hampshire | SU641128 | 50.91112 | -1.08963 |
| P. a. Chisbury Wood 1905 | 12 | Wiltshire | SU269652 | 51.38521 | -1.61483 |
| P. a. Cobtree | 13 | Kent | TQ744588 | 51.30161 | 0.500375 |
| P. a. Coed-Felin-Gat | 14 | Carmarthenshire | SN525188 | 51.84826 | -4.14279 |
| P. a. Coed-y-Stig | 15 | Denbighshire | SJ087611 | 53.1392 | -3.36629 |
| P. a. Deadmans Wood | 16 | Kent | TQ723568 | 51.28428 | 0.469316 |
| P. a. Dean Wood 1918 | 17 | Buckinghamshire | SU972909 | 51.60855 | -0.59775 |
| P. a. Everdon Stubbs B | 18 | Northamptonshire | SP606564 | 52.20251 | -1.1147 |
| P. a. FD1-57-4/122 [a] | 19 | Kent | TQ710568 | 51.28467 | 0.450693 |
| P. a. Ffynone | 20 | Pembrokeshire | SN239385 | 52.0169 | -4.56768 |
| P. a. Frydd Wood 1908 | 21 | Powys | SO075901 | 52.50093 | -3.36409 |
| P. a. Groton A | 22 | Suffolk | TL976432 | 52.05195 | 0.88048 |
| P. a. Groton B | 23 | Suffolk | TL976432 | 52.05195 | 0.88048 |
| P. a. Hamlet Wood C | 24 | Kent | TQ745526 | 51.24588 | 0.498783 |
| P. a. Howley Wood | 25 | Gloucestershire | SO666210 | 51.88655 | -2.48669 |
| P. a. Lockeridge B | 26 | Devon | SX438665 | 50.47745 | -4.2028 |
| P. a. Lowdham Lane | 27 | Nottinghamshire | SK646477 | 53.0227 | -1.03837 |
| P. a. Lower Broxford Wood A | 28 | Devon | SS847032 | 50.81683 | -3.63809 |
| P. a. Lower Broxford Wood B | 29 | Devon | SS844031 | 50.81588 | -3.64232 |
| P. a. Malvern Hills | 30 | Worcestershire | SO771430 | 52.08487 | -2.33561 |
| P. a. Marlow Common 1902 | 31 | Buckinghamshire | SU827864 | 51.57042 | -0.80815 |
| P. a. Narth A | 32 | Monmouthshire | SO528061 | 51.75159 | -2.68515 |
| P. a. Orleans-141 | 33 | Pas de Calais | | | |
| P. a. Pencelli Wood B | 34 | Powys | SO085252 | 51.91778 | -3.3318 |
| P. a. Penley Wood A | 35 | Wrexham | SJ419407 | 52.96051 | -2.86638 |
| P. a. Postlebury B | 36 | Somerset | ST741433 | 51.18833 | -2.37198 |
| P. a. Poulton Wood A | 37 | Kent | TR058365 | 51.09088 | 0.937445 |
| P. a. Primrose Wood | 38 | East Sussex | TQ545325 | 51.07104 | 0.20385 |
| P. a. Prospect Cottage | 39 | Gloucestershire | SO531040 | 51.73274 | -2.68052 |
| P. a. Roundhill Wood | 40 | Hertfordshire | SP939086 | 51.76821 | -0.64063 |

| | | | | | |
|---|---|---|---|---|---|
| P. a. Saxtens Wood B | 41 | Kent | TQ584647 | 51.35929 | 0.27368 |
| P. a. SC 311-33 (S27,S28) | 42 | Kent | TQ588651 | 51.36277 | 0.279599 |
| P. a. Snarkhurst | 43 | Kent | TQ825556 | 51.27033 | 0.614808 |
| P. a. South Wood | 44 | Surrey | TQ077345 | 51.0997 | -0.46323 |
| P. a. Stoke Row 1903 | 45 | Oxfordshire | SU666849 | 51.55906 | -1.04069 |
| P. a. Tank Wood | 46 | Kent | TQ906326 | 51.0611 | 0.718607 |
| P. a. Thornes Wood | 47 | Devon | SS985105 | 50.88504 | -3.44429 |
| P. a. Thruxton Vallets | 48 | Hertfordshire | SO439335 | 51.9971 | -2.81852 |
| P. a. Thundersley Wood | 49 | Essex | TQ785881 | 51.56353 | 0.573882 |
| P. a. Tyn-y-Bryn | 50 | Powys | SJ053062 | 52.64524 | -3.40109 |
| P. a. Wepre Park | 51 | Flintshire | SJ297682 | 53.2062 | -3.05399 |
| P. a. Wilmay Copse | 52 | Kent | TQ579655 | 51.36662 | 0.26686 |

**Table S2**. Differential reactions recorded in leaves, grouped on resistance or susceptibility to strains based on the upper box plot line being greater than 1.0. The tabulated scores are for 0, box plot quartile less than 1; 1, 1-2; 2, 2-3 and 3 more than 3. Examples of clear differentials are highlighted in red. Note accession 21 which is resistant to all strains except *Psm*R1-P from plum.

| **Accession** | ***Psa*** | ***Psm* R1 -C** | ***Psm* R1-P** | ***Psm* R2** | ***Pss*** |
|---|---|---|---|---|---|
| **Resistant to *Psm R1*-C** | | | | | |
| 9 | 0 | 0 | 1 | 2 | 1 |
| 21 | 0 | 0 | 3 | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 | 2 |
| 42 | 0 | 0 | 0 | 1 | 3 |
| | | | | | |
| **Resistant to *Pss*** | | | | | |
| 50 | 1 | 1 | 0 | 0 | 0 |
| 43 | 0 | 1 | 0 | 0 | 0 |
| 47 | 0 | 1 | 0 | 0 | 0 |
| 7 | 1 | 1 | 0 | 0 | 0 |
| 21 | 0 | 0 | 3 | 0 | 0 |
| 27 | 3 | 1 | 0 | 0 | 0 |
| 1 | 1 | 2 | 0 | 0 | 0 |
| 48 | 1 | 1 | 0 | 0 | 0 |
| 31 | 0 | 3 | 3 | 3 | 0 |
| | | | | | |
| **Resistant to *Psm* R2** | | | | | |
| 50 | 1 | 1 | 0 | 0 | 0 |
| 43 | 0 | 1 | 0 | 0 | 0 |
| 47 | 0 | 1 | 0 | 0 | 0 |
| 7 | 1 | 1 | 0 | 0 | 0 |
| 21 | 0 | 0 | 3 | 0 | 0 |
| 27 | 3 | 1 | 0 | 0 | 0 |
| 1 | 1 | 2 | 0 | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 | 2 |
| 13 | 0 | 1 | 2 | 0 | 3 |
| 48 | 1 | 1 | 0 | 0 | 0 |
| | | | | | |
| **Resistant to *Psa*** | | | | | |
| 43 | 0 | 1 | 0 | 0 | 0 |
| 47 | 0 | 1 | 0 | 0 | 0 |
| 9 | 0 | 0 | 1 | 2 | 1 |
| 21 | 0 | 0 | 3 | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 | 2 |
| 12 | 0 | 1 | 1 | 1 | 2 |
| 13 | 0 | 1 | 2 | 0 | 3 |
| 51 | 0 | 2 | 1 | 1 | 1 |
| 42 | 0 | 0 | 0 | 1 | 3 |

| | | | | | |
|---|---|---|---|---|---|
| 31 | 0 | 3 | 3 | 3 | 0 |
| 24 | 0 | 3 | 0 | 2 | 3 |
| 49 | 0 | 2 | 3 | 1 | 2 |
| 15 | 0 | 2 | 2 | 2 | 2 |
| 29 | 0 | 2 | 0 | 2 | 3 |
| 2 | 0 | 3 | 3 | 3 | 3 |
| 30 | 0 | 3 | 3 | 2 | 3 |
| | | | | | |
| **Resistant to *Psm* R1-plum** | | | | | |
| 50 | 1 | 1 | 0 | 0 | 0 |
| 43 | 0 | 1 | 0 | 0 | 0 |
| 47 | 0 | 1 | 0 | 0 | 0 |
| 7 | 1 | 1 | 0 | 0 | 0 |
| 27 | 3 | 1 | 0 | 0 | 0 |
| 1 | 1 | 2 | 0 | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 | 2 |
| 42 | 0 | 0 | 0 | 1 | 3 |
| 48 | 1 | 1 | 0 | 0 | 0 |
| 24 | 0 | 3 | 0 | 2 | 3 |
| 29 | 0 | 2 | 0 | 2 | 3 |
| 28 | 2 | 2 | 0 | 1 | 1 |
| 18 | 1 | 1 | 0 | 1 | 3 |
| 17 | 2 | 2 | 0 | 2 | 3 |
| 37 | 2 | 2 | 0 | 2 | 3 |
| 38 | 3 | 2 | 0 | 1 | 1 |
| 11 | 3 | 2 | 0 | 3 | 3 |
| 25 | 3 | 3 | 0 | 2 | 3 |
| 44 | 2 | 1 | 0 | 2 | 2 |
| 40 | 1 | 3 | 0 | 1 | 2 |
| 39 | 2 | 2 | 0 | 2 | 1 |
| 22 | 1 | 3 | 0 | 3 | 3 |
| | | | | | |
| **Susceptible to *Psm* R1-plum** | | | | | |
| 21 | 0 | 0 | 3 | 0 | 0 |
| 12 | 0 | 1 | 1 | 1 | 2 |
| 13 | 0 | 1 | 2 | 0 | 3 |
| 51 | 0 | 2 | 1 | 1 | 1 |
| 31 | 0 | 3 | 3 | 3 | 0 |
| 49 | 0 | 2 | 3 | 1 | 2 |
| 15 | 0 | 2 | 2 | 2 | 2 |
| 2 | 0 | 3 | 3 | 3 | 3 |
| 4 | 1 | 2 | 3 | 2 | 3 |
| 30 | 0 | 3 | 3 | 2 | 3 |
| 8 | 1 | 3 | 3 | 3 | 3 |
| | | | | | |