The ecological genetics of *Pseudomonas syringae* residing on the kiwifruit leaf surface

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RUNNING TITLE (50 CHARACTERS)

Ecological genetics of *Pseudomonas syringae*

ORIGINALITY-SIGNIFICANT STATEMENT

Bacterial pathogen populations are often studied with little consideration of co-occurring microbes and yet interactions between pathogens and commensals can affect both population structure and disease progression. A fine-scale sampling of commensals present on kiwifruit leaves during an outbreak of bleeding canker disease caused by *P. syringae* pv. *actinidiae* reveals a clonal population structure. A new clade of non-pathogenic *P. syringae* (PG3a) appears to be associated with kiwifruit on a global scale. The presence of PG3a on kiwifruit has significant effects on the outcome of infection by *P. syringae* pv. *actinidiae*. This emphasises the value of studying the effect of co-occurring bacteria on pathogen-plant interactions.

SUMMARY

Interactions between commensal microbes and invading pathogens are understudied, despite their possible impact on pathogen population structure and infection processes. We describe the population structure and genetic diversity of a broad range of co-occurring *Pseudomonas syringae* isolated from infected and uninfected kiwifruit during an outbreak of bleeding canker disease caused by *P. syringae* pv. *actinidiae* (*Psa*) in New Zealand. Overall population structure was clonal and affected by ecological factors including infection status and cultivar. Most isolates are members of a new clade in phylogroup 3 (PG3a), also present on kiwifruit leaves in China and Japan. Stability of the polymorphism between pathogenic *Psa* and commensal *P. syringae* PG3a isolates from the same host was tested using reciprocal invasion from rare assays *in vitro* and *in planta*. *P. syringae*

G33C (PG3a) inhibited *Psa* NZ54, while the presence of *Psa* NZ54 enhanced the growth of *P. syringae* G33C. This effect could not be attributed to virulence activity encoded by the Type 3 secretion system of *Psa*. Together our data contribute toward the development of an ecological perspective on the genetic structure of pathogen populations.

INTRODUCTION

Kiwifruit (*Actinidia* spp.) cultivation is challenged by outbreaks of the bacterial pathogen *Pseudomonas syringae* pv. *actinidiae (Psa)* – the causative agent of bleeding canker disease. The latest outbreak was first reported in Italy in 2008 (Balestra *et al.*, 2008) before spreading rapidly through most kiwifruit growing regions of the world (Abelleira *et al.*, 2011; Everett *et al.*, 2011; Koh *et al.*, 2012; Zhao *et al.*, 2013; Sawada, 2015), arriving in New Zealand in 2010 (Everett *et al.*, 2011).

As a pathogen, *Psa* faces the challenge of colonising diverse environments before proliferating in the apoplast and vascular tissues. Colonisation of leaf surfaces prior to invasion is a key infection stage (Wilson and Lindow, 1994; Wilson *et al.*, 1999; Monier and Lindow, 2003; Pfeilmeier *et al.*, 2016), and *Psa* is likely to encounter and interact with a diverse range of plant-colonising bacteria (Hirano and Upper, 2000; Lindow and Brandl, 2003). Physical proximity increases the likelihood of competitive interactions affecting disease outcomes (Lindow and Brandl, 2003; Hibbing *et al.*, 2010) and increases the probability of horizontal gene transfer (Sawada *et al.*, 1999; Polz *et al.*, 2013; Colombi *et al.*, 2017).

Istock *et al.* (1992) made a particularly persuasive case for recognition of and incorporation of local context in the study of bacterial population biology drawing attention to effects on genetic structure (Souza *et al.*, 1992; Haubold and Rainey, 1996; Spratt and Maiden, 1999). The impact of studying only focal pathogen populations (Spratt and Maiden, 1999; Cordero *et al.*, 2012; Shapiro *et al.*, 2012; Shapiro and Polz, 2014; Rosen *et al.*, 2015) is particularly apparent in the case of *Neisseria meningitidis* (Maynard Smith *et al.*, 1993; Caugant and Maiden, 2009; Bratcher *et al.*, 2014). Biased sampling of symptomatic *N. meningitidis* produces evidence of linkage disequilibrium, yet the inclusion of *N. meningitidis* from asymptomatic meningococcal infections reveals the population structure of *N. meningitidis* to be non-clonal (Maynard Smith *et al.*, 1993; Fraser *et al.*, 2005).

Bacterial interactions are context-dependent, ranging from synergistic to antagonistic, and may have both local and global effects on the plant host (Stubbendieck *et al.*, 2016). Antagonistic or competitive interactions between microbes may be direct or indirect, resulting in the inhibition of growth or even killing (Lindow, 1986; Völksch and May, 2001; Berlec, 2012; Hockett *et al.*, 2015; Nakahara *et al.*, 2016). Synergistic interactions occur when multiple types cooperate to cause disease (Singer, 2010; Lamichhane and Venturi, 2015). For example, *P. savastanoi* pv. *savastanoi,* causative agent of olive knot disease, interacts with non-pathogenic endophytes *Erwinia sp.* and *Pantoea sp.* in cankers, enhancing the severity of disease (Marchi *et al.*, 2006; Moretti *et al.*, 2011; Buonaurio *et al.*, 2015). Synergistic interactions can also be exploitative: bacteria lacking virulence factors

can reap benefits from co-existing pathogenic isolates (Young, 1974; Hirano *et al.*, 1999; Macho *et al.*, 2007; Rufián *et al.*, 2017).

P. syringae is a member of the phyllosphere and engages in both commensal and pathogenic interactions with plants (Hirano and Upper, 2000; Mohr *et al.*, 2008). The diversity and population structure of *P. syringae* has been investigated using both multilocus sequence typing (MLST) and genome sequence analysis of pathogenic isolates collected from diseased plants (Sarkar and Guttman, 2004; Hwang *et al.*, 2005; Baltrus *et al.*, 2011; McCann *et al.*, 2013, 2017; Fujikawa and Sawada, 2016; Nowell *et al.*, 2016), but the broader context of *P. syringae* inhabiting the phyllosphere and its impact on pathogen population structure is largely unknown.

Here we describe the population structure of the *P. syringae* species complex inhabiting the kiwifruit phyllosphere during an outbreak of bleeding canker disease in New Zealand. Using an MLST scheme, we reveal a largely clonal population structure, but show that genetic diversity is significantly affected by ecological factors such as infection status and cultivar. We identified members of four *P. syringae* phylogroups (PG1, PG2, PG3 and PG5) and recovered a new monophyletic clade within PG3 (PG3a) associated with kiwifruit on a global scale. Investigations into the ecological interactions between a representative of this new clade and *Psa* reveal PG3a inhibits *Psa* proliferation, while *Psa* in turn has a beneficial effect on PG3a growth in kiwifruit.

RESULTS

Phyllosphere diversity of *Pseudomonas syringae*

Four housekeeping genes (*gapA, gyrB, gltA, rpoD*) were sequenced for each of 148 *P. syringae* isolated from two varieties of kiwifruit ('Hayward' and 'Hort16A') in both uninfected and *Psa*-infected orchards. Rarefaction analysis indicates saturation of the sampling effort (Figure S1). The infected 'Hayward' orchard displayed the highest α-diversity (D=0.904), while the uninfected 'Hort16A' orchard displayed the least α-diversity (D=0.737). There was low evenness (ED) among all sampling sites (0.136 to 0.290). Similarly, the four different sampling sites shared few species (Sørensen's index of dissimilarity = 0.847).

Multilocus sequence typing

45 unique sequence types (ST) were discovered among the 148 sequenced strains. All STs were novel, except for ST904 (*Psa*), and not described in the Plant Associated and Environmental Microbes Database (PAMDB). For a more global analysis, *P. syringae* allelic profiles were sourced from PAMDB, Tomihama *et al.* (2016) and Visnovsky *et al.* (2016).

Infected orchards (both 'Hayward' and 'Hort16A') harboured the highest number of unique STs, sharing only three STs between them (Figure S2). No STs were present in all four orchards, but two STs were found in three orchards (ST1 and ST3). From the perspective of clonal complexes (CC), the predominant ST (predicted founder) was present along with several SLVs (single locus variants). Two clonal complexes (CC) (21 strains), 5 doubletons (32 strains) and 28 singletons (95 strains)

were identified (Figure S3). CC1 and CC2 are comprised of 11 and 10 strains, respectively. ST904 (*Psa*, 15/148) and ST1 (PG3, 24/148) made up 25% of the sample (Figure S3). Strikingly, ST3 (PG3a) was isolated from three out of four orchards and was also sampled from uninfected gold (*A. chinensis* var. *chinensis*) and green (*A. chinensis* var. *deliciosa*) kiwifruit in NZ (2010) (Visnovsky *et al.*, 2016) and Japan (2015) (Tomihama *et al.*, 2016), respectively (Figure 1). ST16 was also recovered from uninfected kiwifruit leaves in NZ in both 1991 and 2013. Other Japanese kiwifruit STs group closely with *P. syringae* originating from kiwifruit in NZ.

Sequence diversity

The total concatenated alignment length was 2010 bp with no insertions or deletions detected for either of the four loci. The number of alleles ranged from 25 (*gapA*) to 35 (*rpoD*) (Table 1). There were a total of 412 polymorphic sites, ranging from 80 (16.81%, *gapA*) to 145 (28.6%, *gyrB*). The nucleotide diversity index π and Watterson's θ were highly consistent among loci, varying from 0.040 to 0.055 and 0.024 to 0.041 respectively. The average GC content of 57.99% is similar to that found in other *P. syringae* studies (59- 61%), however *gyrB* displayed an unusually low GC content of 53.15%. The pairwise genetic difference within phylogroups (PGs) was not greater than 2.7%, whereas among PGs the variability ranged from 6-11% (Table 2), consistent with previous accounts of genetic variability for *P. syringae* (Sarkar and Guttman, 2004; Morris *et al.*, 2010; Berge *et al.*, 2014).

Genetic diversity varies by host cultivar and infection status

To test whether genetic diversity was influenced by the presence of ecological structure, multivariate analyses (PERMANOVA) were performed. A highly significant difference in genetic diversity was observed among sampled orchards (Pseudo-F = 5.99, $P < 0.0001$); with pairwise Permanova tests revealing that the uninfected green orchard differed significantly from every other orchard ($P<0.003$). The cultivar ('Hayward' vs. 'Hort16A') (Pseudo-F = 5.62, $P<0.001$) and infection status of an orchard (Pseudo-F = 11.72, $P <0.001$) also had a significant effect on genetic diversity, whereas no temporal effect was found (Pseudo-F = 1.10, $P >0.34$). When testing the nested effect of all three factors, only the infection status (Pseudo-F = 6.42, $P <0.01$) had a significant impact on genetic diversity (Table S3).

Recombination among *P. syringae*

Intragenic recombination rates (ρ) ranged from 0.012 (*rpoD*) to 0.038 (*gyrB*) and 0.006 for the concatenated dataset. The ratio ε (recombination rate/mutation rate) ranged from 0.187 (concatenated*)* to 0.931 (*gyrB*) suggesting that any single nucleotide polymorphism is up to five times more likely to have arisen from a mutation than recombination (Table 3).

Clustering sequences by PG revealed no evidence of recombination within PG1 and PG5 (ρ=0), however for these phylogroups the sample size was low. There was evidence of recombination in PG3, more specifically for *gltA* (ε=1.18) and *rpoD* (ε=4.07), whereas in PG2 recombination was evident in *rpoD* (ε=1.734) alone. This level of recombination is consistent with earlier reports (Sarkar and Guttman, 2004).

Recombination was neither affected by the host cultivar or infection status (Table S4). The analysis was repeated with the inclusion of non-redundant global strains. Overall, intergenic recombination rates were low among PGs, ranging from 0.005 to 0.012 for the concatenated dataset (Table S5). In order to pinpoint any effects of recombination on phylogenetic reconstruction, single gene trees were constructed and compared. Tree topologies were significantly different from each other and from the concatenated dataset (SH test, P<0.0.5) (Figure S4).

A kiwifruit-associated clade of *P. syringae*

Maximum likelihood trees built using the concatenated alignment of unique STs revealed that nearly all NZ *P. syringae* kiwifruit isolates fell within four PGs: PG1 (13%), PG2 (29%) and PG3 (56%), with only a few isolates falling into PG5 (2%, Figure 2). Strikingly, within PG3 all NZ kiwifruit-associated isolates grouped within a new clade of PG3, hereafter referred to as PG3a. The uninfected orchards showed a higher number of PG3a isolates compared to infected orchards, although no influence of infection status was reflected in the number of unique sequence types (Table S1). This new subclade of PG3 was briefly described from a small-scale sampling of Japanese kiwifruit (clade KID0001, Tomihama *et al*. 2016). We also found that two strains isolated from kiwifruit in NZ in 2010 and 2011 (Visnovsky *et al.*, 2016) belong to this subclade.

This discovery led us to question whether the PG3a subclade might be prevalent on kiwifruit vines in other countries. To this end we interrogated an unpublished set of *P. syringae* strains collected from kiwifruit leaves in China (sampling as described in McCann *et al.* (2017), GenBank accession numbers: MG674624 – MG674645). A

phylogenetic tree based on *gltA* for NZ, Japanese and Chinese kiwifruit isolates revealed that isolates obtained from Chinese kiwifruit also clustered within PG3a (Figure 3). Interestingly, included in this group is isolate 47L9, which was collected from tea leaves (*Camellia* sp.) growing in a former kiwifruit orchard in China. No other *P. syringae* strain from the PAMDB database grouped with PG3a, suggesting PG3a is persistently associated with kiwifruit on a global scale.

Ecological interactions between PG3a and PG1

To assess whether kiwifruit-associated PG3a strains are stably maintained with *Psa* (PG1), co-inoculation experiments were performed *in vitro* and *in planta*. Two isolates sampled from the same leaf were chosen for these experiments as they were isolated from the same leaf: *P. syringae* G33C (ST1, PG3a) and *Psa* NZ54 (ST904, PG1).

In vitro *dynamics*

Psa NZ54 and *P. syringae* G33C showed similar growth dynamics when grown individually *in vitro* (Figure 4). However, when co-inoculated in liquid King's B (KB) media at an equal starting ratio, *Psa* NZ54 growth was significantly reduced (up to 100-fold) at 24 h (P <0.001, paired t-tests). This effect was amplified in shaken liquid minimal M9 medium, which mimics the nutrient-poor conditions encountered on the leaf surface (Hernández-Morales *et al.*, 2009): *Psa* NZ54 population density collapsed by 20 h in shaken M9 media (relative fitness -12.1 ±0.09, Figure 4).

In order to establish whether the instability of the interaction was influenced by the ratio of founder cells, we investigated whether *P. syringae* G33C could invade

from rare initial frequency. *P. syringae* G33C successfully invaded from rare after only 24 h in both rich KB and minimal M9 media (10:1 *Psa NZ54* : *P. syringae* G33C) and reached a similar population size as when cultured on its own in M9 (Figure 5A). Conversely, *Psa* NZ54 also invaded *P. syringae* G33C from rare (*1:10 Psa* NZ54 : *P. syringae* G33C), though it established a 100-fold reduced population size of 10^5 - 10^6 cfu ml^{-1} compared to growth alone. The population collapse of *Psa* NZ54 in shaken M9, as observed for the 1:1 competition experiments, was once again observed (Figure 5B). The striking suppression of *Psa* NZ54 by *P. syringae* G33C was unambiguously repeated across three experiments. *P. syringae* G33C outcompetes *Psa* NZ54, though both isolates can invade from rare *in vitro* (with the exception of *Psa* NZ54 in shaken M9), which suggests that in a controlled environment the polymorphism is stable.

In planta *dynamics*

In planta experiments were performed on two gold cultivars, 'Hort16A' and 'SunGold', to determine whether *Psa* NZ54 and *P. syringae* G33C also form a stable polymorphism on kiwifruit leaves. *P. syringae* G33C established an epiphytic and endophytic population size in both cultivars (Figure 6) and did not produce any visible symptoms in 'Hort16A' and 'SunGold' (Figure S5, Figure S6). *Psa* NZ54 attained a population size at least 10,000-fold greater than *P. syringae* G33C in both hosts. However, endophytic and epiphytic growth were reduced 10-fold in 'SunGold' compared to 'Hort16A' (P <0.05, Mann-Whitney U test). Plants inoculated with *Psa* NZ54 developed the first leaf spots at 4 dpi and exhibited severe symptoms at 7 dpi

237 in the more susceptible 'Hort16A', whereas in 'SunGold' leaves displayed only minor
238 symptoms at 7 dpi.

239 In 1:1 competition experiments non-pathogenic *P. syringae* G33C maintained
240 a stable population size. The presence of *Psa* NZ54 had a highly significant positive
241 effect on the growth of *P. syringae* G33C in both plant hosts (Figure 6A&B). *P.*
242 *syringae* G33C established up to 1000-fold higher epiphytic population densities in
243 'Hort16A' (P <0.01, paired *t*-tests) and 10-fold higher epiphytic and endophytic
244 population densities in 'SunGold' plants (P <0.05, paired *t*-test) compared to its
245 individual growth. Co-inoculated *Psa* NZ54 exhibited a significant reduction (P <0.05,
246 paired *t*-test) in epiphytic and endophytic growth on 'Hort16A' in the presence of *P.*
247 *syringae* G33C, but only in the early stages of the experiment. On 'SunGold' the
248 diminished growth of *Psa* NZ54 was more pronounced, with a 100-fold decrease for
249 the endophytic population at 7 dpi (P <0.05, paired *t*-test, Figure 6B). Co-inoculated
250 'Hort16A' plants exhibited a notable delay in symptom onset compared to singly
251 inoculated plants (Figure S5), whereas there was no difference for 'SunGold' (Figure
252 S6). The increased fitness of *Psa* NZ54 relative to *P. syringae* G33C in 'Hort16A'
253 competition experiments was reflected in the relative fitness parameters (0.7± 0.1*
254 for epiphytic and 4.9± 0.8* for endophytic, * P <0.05, *t*-test), whereas in 'SunGold'
255 plants *P. syringae* G33C performed better in the epiphytic environment (-1.6 ± 0.2;
256 4.7 ± 0.1* for endophytic growth).

257 To assess whether the heightened growth of *P. syringae* G33C in the
258 presence of *Psa* NZ54 was due to the virulence activity of the pathogen elicited by
259 the Type 3 Secretion System (T3SS), the competition experiment was performed

using a T3SS deficient mutant (*Psa* NZ13 Δ*hrcC*). Epiphytic growth of *P. syringae* G33C on 'SunGold' remained elevated when co-inoculated with *Psa* NZ13 Δ*hrcC*, indicating the virulence activity encoded by the T3SS was not responsible for the advantage conferred to the non-pathogenic strain (P <0.05, paired t-test, Figure 6C).

A rarity threshold for *P. syringae* G33C determines the ability to establish a stable population *in planta*. Upon co-inoculation in a 100:1 (*Psa* NZ54 : *P. syringae* G33C) ratio on 'Hort16A', *P. syringae* G33C was able to invade from rare over the first 4 days, but was then excluded by *Psa* NZ54 (Figure 7B). An initial increase in growth of *P. syringae* G33C from 0 dpi to 4 dpi was followed by a population collapse at 7 dpi with no endophytic growth detected and minimal epiphytic growth in environments dominated by *Psa* NZ54. Conversely, *Psa* NZ54 grew to the same population size in the presence of *P. syringae* G33C, as when inoculated individually (P >0.1, paired t-tests).

In the reciprocal experiment (1:100 *Psa* NZ54 : *P. syringae* G33C), *Psa* NZ54 successfully invaded from rare in both the endophytic and epiphytic environment, although the rate of invasion was reduced on the leaf surface . However, both epi- and endophytic population sizes were significantly reduced compared to single inoculations (P <0.01, paired t-tests) (Figure 7). Despite the growing population of *Psa* NZ54, *P. syringae* G33C maintained the same epiphytic population size as when inoculated individually (P >0.2, paired t-tests). The endophytic population size of *P. syringae* G33C increased (P <0.01, 7dpi, paired t-test), which mirrored the results from the 1:1 competition experiments, where the presence of *Psa* NZ54 also had a positive effect on growth of *P. syringae* G33C.

283 In order to establish whether there was an advantage to being an early
284 colonist, a time-stagger experiment was performed to see whether immigration
285 history influences the interaction (Fukami *et al.*, 2007). 'Hort16A' plants were pre-
286 inoculated with either of the two strains and followed by a subsequent inoculation
287 of the other strain after three days. Early colonization provided no advantage to *Psa*
288 NZ54 (Figure 8A), as *P. syringae* G33C maintained and established a stable
289 population by 7 dpi and exhibited no significant difference in growth compared to
290 individual growth (P >0.3, paired t-tests). Reducing the secondary inoculation density
291 resulted in a reduced initial population size at 3 and 7 dpi for *P. syringae* G33C (P
292 <0.05, paired t-tests), but by 10 dpi the level was the same as when the two strains
293 were grown individually (Figure S7C).

294 When *P. syringae* G33C was the first colonist (Figure 8B) *Psa* NZ54 grew to
295 the same population size by 7 dpi as compared to individually inoculated plants (P
296 >0.5, paired t-tests). The growth of *Psa* NZ54 was initially lower compared to
297 individual growth when inoculated at a lower density (P <0.05, paired t-tests), but
298 this difference was no longer evident for the epiphytic population by 10 dpi (Figure
299 S7B).

300 **DISCUSSION**

301 Studies of pathogen populations rarely take into consideration co-occurring
302 commensal types and yet such types are likely to be important contributors to
303 population structure and infection progress (Lindow and Brandl, 2003; Demba Diallo
304 *et al.*, 2012; Bartoli *et al.*, 2015; Buonaurio *et al.*, 2015; Rufián *et al.*, 2017). Here,

with focus on *P. syringae*, we have combined traditional population genetic approaches with experiments designed to investigate interactions among members of an ecologically cohesive population. The most significant findings include (i) a clonal population structure for commensal kiwifruit *P. syringae* (ii) strong association of genetic diversity with ecological factors, (iii) discovery of a new clade of kiwifruit-associated kiwifruit *P. syringae* within PG3 (PG3a) (Figure 2, Figure 3), (iv) complex interactions between the pathogenic *Psa* isolate and PG3a with evidence of a stable polymorphism under some *in vitro* conditions, but not *in planta* (Figure 4, Figure 6).

Overall, we found that *P. syringae* from kiwifruit display a clonal population structure, comprised of two clonal complexes and a small number of abundant STs. This sits in accord with earlier reports of clonal population structure for *P. syringae*, despite focus on pathogenic isolates which tend to undergo clonal expansion upon host specialisation (Sarkar and Guttman, 2004). Homologous recombination events are few and limited to within phylogroups for *P. syringae*, which is also supported by well-defined phylogenetic clades (Baltrus *et al.*, 2011; Bull *et al.*, 2011; Berge *et al.*, 2014; Nowell *et al.*, 2016). A more fine-scale analysis of a collection of *Pseudomonas viridiflava* (now *P. syringae* PG7 and PG8 (Bartoli *et al.*, 2014; Berge *et al.*, 2014)) isolated from *Arabidopsis thaliana* suggests that recombination at the phylogroup level is primarily within-clade rather than between clade (Goss *et al.*, 2005).

Genetic diversity varied according to ecological factors, most strikingly for *P. syringae* collected from infected orchards, where genetic diversity was highest. This may reflect effects of *Psa* on the kiwifruit immune response, which may facilitate migration of leaf colonists into the apoplast and vascular tissues and thus allow

328 access to water and nutrients. Such effects have been reported for infection of
329 potatoes by *Pectobacterium atrosepticum* (Kõiv *et al.*, 2015) and herbivore-damaged
330 bitter cress leaves (*Cardamine cordifolia*) (Humphrey *et al.*, 2014), where in both
331 instances *Pseudomonas* population densities and diversity increased following plant
332 damage.

333 We observed differences in *P. syringae* genetic diversity that appear to be
334 attributable to differences in plant genotypes. Host species and cultivar identity is
335 known to significantly affect the composition of phyllosphere bacterial communities
336 (Adams and Kloepper, 2002; Van Overbeek and Van Elsas, 2008; Whipps *et al.*, 2008;
337 Bodenhausen *et al.*, 2014; Laforest-Lapointe *et al.*, 2016; Wagner *et al.*, 2016).
338 Differences in phyllosphere *P. syringae* diversity may also be influenced by
339 environmental factors (such as humidity, nutrient availability or UV radiation) and
340 orchard management practices. Different fertilizer and spray regimes (copper,
341 antibiotics, Actigard™ and biological agents) are employed by growers to prevent or
342 manage *Psa* infection throughout the growing season
343 (http://www.kvh.org.nz/vdb/document/99346). These practices may have selected
344 for copper and streptomycin resistance in *Psa* and kiwifruit epiphytes in NZ and
345 elsewhere (Han *et al.*, 2003; Colombi *et al.*, 2017; Petriccione *et al.*, 2017).

346 Strains grouping with four major phylogroups (PG1, PG2, PG3, PG5) were
347 recovered. This level of diversity in a cultivated environment is not surprising (Goss
348 *et al.*, 2005; Bull *et al.*, 2011; Kniskern *et al.*, 2011; Beiki *et al.*, 2016; Hall *et al.*,
349 2016). Two clades of endophytic *P. syringae* pv. *syringae* were recovered from
350 symptomatic grapevines in Australia with pathogenic and non-pathogenic isolates

clustering together (Hall *et al.*, 2016). Samples obtained from citrus orchards suffering from citrus blast caused by *P. syringae* pv. *syringae* revealed isolates associated with PG2, PG7 and an unknown clade (Beiki *et al.*, 2016). Two distinct and highly divergent subclades of *Pseudomonas viridiflava* (*P. syringae* PG7) were recovered from a global sampling of wild *A. thaliana* (Goss *et al.*, 2005).

The newly recognised PG3a subclade of *P. syringae* appear to colonise kiwifruit leaves not only in NZ (dating back to 2010 (Visnovsky *et al.*, 2016)), but also in other kiwifruit growing regions of the world, including Japan (Tomihama *et al.*, 2016) and China. Data from leaf samples indicate that PG3a is not displaced by *Psa*, but the total number of PG3a isolates collected is reduced in infected orchards. Interestingly the diversity of PG3a does not seem to be affected by infection status. Strains clustering with PG3a formed the majority (>50%) of kiwifruit isolates, and these have not yet been isolated from any other plant hosts recorded in PAMDB, with the exception of isolate 47L9, collected from tea leaves (*Camellia* sp.) growing in a former kiwifruit orchard. This indicates that PG3a forms a persistent association with kiwifruit plants – an observation that is further supported by the repeated isolation of PG3a from kiwifruit across large geographic distances, suggests that PG3a may have been coevolving with its host for some time. PG3a is thus also likely to be disseminated with the exchange of plant material (such as pollen or plant cuttings) between kiwifruit growing countries. The prevalence of PG3a in other kiwifruit growing countries (e.g. Korea, France or Italy) is at present unknown. The preferential occurrence of PG3 with woody hosts (Bartoli *et al.*, 2015; Nowell *et al.*, 2016) could explain the particular grouping of the kiwifruit resident clade within

374 PG3. A similarly intriguing signal of host association was found in a collection of *P.*
375 *syringae* isolates from *A. thaliana*, where PG2 representatives dominated (Kniskern
376 *et al.*, 2011; Karasov *et al.*, 2017). Distinct lineages of non-pathogenic isolates have
377 also been described for other plant pathogens such as *Xanthomonas arboricola,*
378 where non-pathogenic strains are distant relatives of pathogenic lineages, despite
379 being isolated from the same host (Essakhi *et al.*, 2015; Triplett *et al.*, 2015).

380 The kiwifruit commensal *P. syringae* G33C (representative of the PG3a
381 subclade) successfully colonized the leaf surface and apoplast of kiwifruit without
382 production of visible disease symptoms. This is reflected in the population size,
383 which was reduced by 4-logs compared to pathogenic population size of *Psa* at 3 dpi.
384 Similar population sizes have been reported for *P. syringae* pv. *phaseolicola*, which
385 grows to a four-log higher population size on its host plant *Phaseolus vulgaris*
386 compared to a non-pathogenic isolate; a 4-log reduction was also observed in
387 resistant vs susceptible hosts (Omer and Wood, 1969; Young, 1974). Similar
388 observations have been made for other plant pathogens, for example non-
389 pathogenic *Xanthomonas* sp. displays a 4-log reduced growth compared to the
390 disease-causing *X. oryzae* pv. *oryzae* (Triplett *et al.*, 2015). Bacterial population
391 density appears to be directly related to the production of disease symptoms, as was
392 demonstrated for environmental *P. syringae* strains inoculated in kiwifruit, which
393 grew to near pathogen population size levels, but induced symptoms of disease
394 (Bartoli *et al.*, 2015).

395 When grown in competition with *Psa* NZ54, *P. syringae* G33C population
396 size increased. Studies exploring the dynamics of mixed infections have

397 demonstrated inoculum density-dependent effects on colonization (Young, 1974;
398 Macho *et al.*, 2007). The ability of non-pathogenic *P. syringae* to colonize wider
399 territories in the presence of a pathogenic strain was nicely demonstrated in a
400 confocal microscopy study (Rufián *et al.*, 2017). It is possible that *P. syringae* G33C
401 benefits from the virulence activity of *Psa* NZ54. T3SS-dependent hitch-hiking effects
402 have been observed for *P. syringae* pv. *syringae* (Hirano *et al.*, 1999), however the
403 increase *in P.* syringae G33C growth persists even in the absence of a functional T3SS
404 in the pathogenic *Psa* strain. Virulence activities not encoded by the T3SS, such as
405 phytotoxin production, may be responsible for this outcome.

406 Epiphytic and *in vitro* growth of *Psa* NZ54 was significantly reduced when
407 co-inoculated with *P. syringae* G33C. Similarly, *Psa* growth may be suppressed by co-
408 inoculation with environmental isolates of *P. syringae* (Bartoli *et al.*, 2015). Epiphytes
409 may suppress pathogen growth either as direct antagonists or indirectly via resource
410 competition (Wilson and Lindow, 1994). The specific mechanism by which *P.*
411 *syringae* G33C suppresses *Psa* remains undetermined. The *Psa* NZ54 population
412 collapse was delayed at 1:10 and 10:1 inoculation ratios, which suggests that this
413 effect was due to the accumulation of antimicrobial compounds produced by *P.*
414 *syringae* G33C. Phytotoxin production is widespread among fluorescent
415 pseudomonads and some toxins have antimicrobial activity (Bender *et al.*, 1999),
416 though contact-dependant growth inhibition (CDI) via Type 5 and 6 secretion
417 systems or bacteriocins may also mediate *P. syringae* interactions (Hayes *et al.*,
418 2010; Haapalainen *et al.*, 2012; Ruhe *et al.*, 2013; Hockett *et al.*, 2015).

Our in-depth localised sampling has revealed a global association of PG3a with kiwifruit. Additionally we have shown that this clade of non-pathogenic *P. syringae* engage in complex interactions with pathogenic *Psa*. This highlights the value of understanding genotypic diversity and ecological interactions among pathogens and non-pathogens in field settings. Clarifying how commensals persist in association with specific hosts over long periods without causing disease and the mechanism by which they modulate pathogen invasion and proliferation will contribute to a fuller understanding of plant-microbe interactions.

EXPERIMENTAL PROCEDURES

Plant tissue collection and bacterial isolation

The sampling scheme was designed to obtain strains from *Psa* infected and uninfected hosts, irrespective of disease stage (symptom development) or pathogenicity potential of the isolate. *P. syringae* was isolated from the leaf surfaces of two different cultivars of *Actinidia chinensis*: *A. chinensis* var. *chinensis* Hort16A (gold) and *A. chinensis* var. *deliciosa* Hayward (green), which vary in their susceptibility to *Psa*: 'Hort16A' is more susceptible than the green 'Hayward' (Ferrante and Scortichini, 2010; Cameron and Sarojini, 2014). One infected and one uninfected orchard of each variety was sampled by collecting three leaves from six separate vines along a diagonal path of ~400m (Table S1). Sampling occurred at three intervals during the growing season: spring (after bud break), summer and autumn (prior to harvest). Vine trunks and canes (secondary branches) were tagged to ensure resampling of the same spot. Some 'Hort16A' canes were removed during

441 routine disease management; neighbouring canes on the same vine were then
442 sampled and tagged. All uninfected Hayward vines were cut down prior to the last
443 sampling day so the adjoining block of Hayward was sampled instead. The location of
444 each sampled orchard is listed in Table S2.

445 Leaves were individually placed in 50 mL conical centrifuge tubes and washed
446 with 40 mL 10mM $MgSO_4$ buffer supplemented with 0.2 % Tween (Invitrogen, US) by
447 alternately shaking and vortexing at slow speed for 3 min. After removing the leaf,
448 the leaf wash was centrifuged at 4600 rpm for 10 min. The supernatant was
449 removed and the pellet was resuspended in 200 µL 10mM $MgSO_4$. 100 µL of the
450 resuspension was stored at -80°C and 100 µL was plated on *Pseudomonas* agar base
451 (Oxoid, UK) supplemented with 10 mg/L cetrimide, 10 mg/L fucidin and 50 mg/L
452 cephalosporin (CFC supplement, Oxoid, UK). For each leaf two isolates exhibiting *P.*
453 *syringae* colony morphology (round, creamy white) were selected randomly from
454 the plate and restreaked, then used to inoculate liquid overnight cultures for storage
455 at -80˚C. Isolates were tested for the absence of cytochrome *C* oxidase using
456 Bactident Oxidase strips (Merck KgaA, Germany), characteristic of *P. syringae*. A total
457 of 148 *P. syringae* isolates were obtained from the four orchards (Table S1).

458 **PCR amplification & sequencing**

459 A lysate was prepared for each isolate by resuspending a colony in 100 µL ddH_20 and
460 lysing the cells at 96°C for 10 min. Strains were sequenced using the Hwang *et al.*
461 (2005) MLST scheme for four housekeeping genes *gapA*, *gyrB*, *gltA (=cts)* and *rpoD*
462 (reverse). Due to amplification problems, the forward primer for *rpoD* from Sarkar
463 and Guttman (2004) was used. PCR amplification was performed with a BIO-RAD

T100 Thermal Cycler following an adapted protocol of Hwang et al. (2005): a total reaction volume of 50 µl with a final concentration of 1x PCR buffer (Invitrogen, US), 1 µM for each primer, 0.2 mM dNTP's (Bioline, UK), 1 U Taq Polymerase (Invitrogen, US), 1 µl lysed bacterial cells, 2% DMSO (Sigma-Aldrich, US) and 1.5 mM $MgCl_2$. Initial denaturation was at 94°C for 2 min, followed by 30 cycles of amplification with denaturation at 94°C for 30 s, annealing at 63°C for 30 s and elongation at 72°C for 1 min. Final elongation was for 3 min at 72°C. Samples were purified using the Exo-CIP method and sequenced by Macrogen Inc (South Korea). Sequence analysis was performed with Geneious v7.1.7 (Kearse *et al.*, 2012). Sequences were trimmed to the same length (476 bp *gap1*, 507 bp *gyrB*, 529 bp *gltA*, 498 bp *rpoD*) and concatenated (2010 bp) (GenBank accession numbers: *gapA* MG642149 - MG642296; *gyrB* MG642297 - MG642444; *gltA* MG642445 - MG642592; *rpoD* MG642593 - MG642740).

Population genetics

Sequence diversity indices

A rarefaction analysis was performed using MOTHUR v.1.34.4 by subsampling using 1,000 iterations (Schloss *et al.*, 2009). Pairwise genetic distances between isolates were calculated and sequences assigned to Operational Taxonomic Units (OTUs) based on the corresponding average pairwise genetic distance of each group.

Simpson's index of diversity (D) and evenness (ED) (α-diversity) and Sørensen's index of dissimilarity (β-diversity) were calculated using the *vegan* package (Oksanen *et al.*, 2016) in R v3.3.1 (R.Core.Team, 2016). Simpson's D was

converted to the effective number of species (D_c) in order to account for the non-linear properties of Simpson's index of diversity (Jost, 2006).

Multilocus Sequence Typing

Sequence types (STs) sharing three out of four alleles (SLV, single locus variants) were grouped using eBURST v3 (bootstrapped with 1,000,000 resamplings) (Feil *et al.*, 2004; Spratt *et al.*, 2004). A Minimum Spanning Tree providing an overview of triple locus variants was constructed using Phyloviz v2.0 (Francisco *et al.*, 2012).

165 non-redundant ST profiles of *P. syringae* strains were downloaded from the Plant Associated and Environmental Microbes Database (PAMDB, http://genome.ppws.vt.edu/cgi-bin/MLST/home.pl) (Almeida *et al.*, 2010). *P. syringae* sequences isolated recently from kiwifruit and air in Japan (Tomihama *et al.*, 2016) and kiwifruit isolates from NZ, France and the United States (Visnovsky *et al.*, 2016) were also included. A reduced set of 37 *P. syringae* isolates representing the different monophyletic groups of *P. syringae*, as well as the Japanese kiwifruit strains and the US, France and NZ kiwifruit isolates from previous years were used to provide better resolution in the phylogenies displayed in Figure 2 and Figure S4.

Sequence diversity and recombination

START2 (v0.9.0 beta) was employed to calculate parameters of genetic diversity, number of alleles and polymorphic sites, GC content and the ratio of non-synonymous to synonymous substitutions (d_N/d_S ratio) (Jolley *et al.*, 2001). The number of mutations and amino acid changes and nucleotide diversity parameter π

were calculated with DnaSP v. 5.10.1 (Rozas and Rozas, 1995). Jmodeltest 2.1.7 (Guindon and Gascuel, 2003; Darriba *et al.*, 2012) was used with default parameter settings to find the best-fitting evolutionary model. Pairwise genetic variability among and between phylogroups was calculated using MEGA7 (Kumar *et al.*, 2016).

To test whether genetic diversity varied by sampling location, time of sampling, orchard infection status and/or cultivar, a permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001; McArdle and Anderson, 2001) was performed using PRIMER v 6.1.12 (PRIMER-E Ltd., Plymouth, UK, PERMANOVA+ add-on v. 1.0.2.). Pairwise distances among unique STs were used as input and tests were run with 9999 permutations.

LDHAT v2.2a (Auton and McVean, 2007) was used to estimate the rate of mutation (Watterson's θ) and recombination (ρ) using the composite likelihood method of Hudson (Hudson, 2001) with an adaption to finite-site models. Only polymorphic sites with two alleles were included and the frequency cut-off for missing data was set to 0.2.

Phylogenetic reconstruction

Trees were built using single representatives of each unique ST from this study to improve readability of the tree. TREEPUZZLE v5.3 (Schmidt *et al.*, 2002) was used to construct maximum likelihood (ML) trees using the best-fitting evolutionary model (jModeltest) for individual genes and the concatenated alignment (100,000 puzzling steps). Dnaml (PHYLIP v3.695, Felsenstein 1989) was used to test for

529 congruence between single trees (SH-test) using default parameters, providing ML
530 trees as input and a random number seed of 333.

531 **Strains and culture conditions**

532 A list of all bacterial strains used in this study can be found in Table S2.
533 *Pseudomonas* strains were cultured in King's B or minimal M9 media at 28˚C and *E.*
534 *coli* was cultured in Luria Bertani medium at 37˚C. Liquid overnight cultures were
535 inoculated from single colonies and shaken at 250 rpm for 16 hrs. The antibiotics
536 kanamycin (kan) and nitrofurantoin (nf) were used at a concentration of 50 µg/ml.
537 Kanamycin resistant *Psa* NZ54 and *Psa* NZ13 Δ*hrcC* were employed in all *in vitro* and
538 *in planta* experiments.

539 **Mutant development**

540 *Psa* NZ13Δ*hrcC* was constructed by in-frame deletion of *hrcC* via marker
541 exchange mutagenesis. Knockout construct was generated by overlap extension PCR
542 (Ho *et al.*, 1989) using the primers listed in Table S6. DNA was amplified from *Psa*
543 NZ13 with Phusion® High-Fidelity DNA polymerase. The deletion construct was
544 inserted into pK18mobsacB (Schäfer *et al.*, 1994). The recombinant vector was
545 transferred into *Psa* NZ13 via triparental mating, using as helper *E. coli* DH5α strain
546 containing pRK2013. Mutants were selected by plating on KB kanamycin (50 µg/mL)
547 and subsequently on KB containing 5% sucrose. Mutants were screened by PCR using
548 external primers (Table S6) and the deletion was then confirmed by sequencing.

549 Triparental matings were performed to introduce a kanamycin resistant Tn*5*
550 transposon into *Psa* NZ54 and *Psa* NZ13 Δ*hrcC*. *E. coli* S17-1 Tn*5hah Sgid1* (donor)

(Zhang *et al.*, 2015), *E. coli pRK2013* (helper) (Ditta *et al.*, 1980) and *Psa* NZ54 or *Psa* NZ13 Δ*hrcC* (recipient) were grown in shaken liquid media overnight. 200 µl of donor and helper and 2mL of recipient were individually washed, pelleted and combined in 30 µl 10 mM $MgCl_2$. The mixture was plated on a pre-warmed LB agar plate and incubated at 28˚C for 24 hrs. The cells were scraped off and resuspended in 1 mL 10 mM $MgCl_2$ and plated on KB plates supplemented with kanamycin and nitrofurantoin. Bacterial growth was compared to the wild type recipient in both KB and M9 media to ensure marker introduction did not result in a loss of fitness.

Competition assays

In vitro competition assays

Competition experiments were performed *in vitro* using rich (King's B) and minimal (M9) media in a shaken and static environment. Competition experiments were performed in 1:1, 1:10 and 10:1 ratios for each of the four assay conditions. Liquid overnight cultures of each strain in KB were established from single colony inoculations. 30 mL vials with 4 mL of the appropriate media were inoculated with each strain, adjusted to a founding density of either $5x10^6$ cfu ml^{-1} (OD_{600} 0.006) or $4x10^4$ cfu ml^{-1} (OD_{600} 0.0004). Control vials were inoculated with a single strain, adjusted to $5x10^6$ cfu ml^{-1}. Cultures were incubated at 28˚C and grown over a period of 72 hrs, either still or shaken at 250rpm. Bacterial density was calculated at 0, 24, 48 and 72 hrs by plating dilutions on KB kan and M9 agar plates to distinguish between competing strains. The experiment was performed using three replicates and repeated three times.

573 *In planta competition and pathogenicity assays*

574 Epiphytic and endophytic growth of *Psa* NZ54, *Psa* NZ13 Δ*hrcC* and *P.*
575 *syringae* G33C was evaluated on 4-week old kiwifruit plantlets using single and
576 mixed-culture inoculation. Clonally propagated *A. chinensis* var. *chinensis* 'Hort16A'
577 and 'SunGold' were grown for a minimum of one month in a Conviron CMP6010
578 growth cabinet at 21˚C with a 14/10 hr light/dark cycle and 70% humidity. Bacterial
579 strains were incubated for two days at 28˚C on KB plates, after which they were
580 resuspended in 10 mM $MgSO_4$ buffer. Mixed inoculum (1:1, 1:100 and 100:1) was
581 prepared in 50 mL 10 mM $MgSO_4$ buffer and 0.002% Silwet-70 (surfactant), with
582 strains adjusted to $8x10^7$ cfu ml^{-1} (OD_{600} 0.1) or $8x10^5$ cfu ml^{-1} (OD_{600} 0.001). Single
583 strain plant inoculations were also performed using an initial $8x10^7$ cfu ml^{-1} (OD_{600}
584 0.1).

585 Plants were inoculated by submerging leaves in the inoculum for 5 s and
586 allowing to air-dry. Plants were returned to the growth cabinet and watered every
587 second day. Bacterial density was assessed at either 0, 2, 4, 7 and 10 days post
588 inoculation (dpi) or 0, 3, and 7 dpi (Δ*hrcC* competition experiments). Epiphytic
589 growth was assessed by placing inoculated leaves in separate sterile plastic bags
590 with 35 mL 10 mM $MgSO_4$ buffer and shaking manually for 3 minutes. The leaf wash
591 was centrifuged at 4600 rpm for 3 min and the supernatant discarded. Bacteria were
592 resuspended in 200 µl buffer and serial dilutions plated on M9 and KB+kan agar
593 plates.

594 Endophytic growth was assessed by removing one $1cm^2$ leaf disk per plant
595 (including the midrib), surface sterilizing in 70% EtOH for 30 sec, drying and

596 homogenising for 1 minute in a 1.5 mL Eppendorf tube containing 200 µl buffer and
597 two metal beads with the TissueLyser II (QIAGEN). The plant homogenate was
598 serially diluted and plated on M9 and KB+kan agar plates. All experiments were
599 performed in duplicate, with at least 4 replicates per experiment.

600 **Statistical analysis**

601 A Student's *t*-test was used to verify the statistical difference where
602 applicable. For non-normally distributed data with unequal variance, the Mann
603 Whitney U test was performed.

604 The fitness of each strain in the competition experiments is expressed as the
605 Malthusian parameter (Lenski *et al.*, 1991). The Malthusian parameter was
606 calculated as $M = (ln(N1_{f1}/N1_i))/(ln(N2_f/N2_i))$, where $N1_i$ is initial number of
607 cfu of strain 1 at 0h and $N1_f$ cfu after 24 hrs (*in vitro*) or 2/3 dpi (*in planta,*
608 'Hort16A'/'SunGold').

609 **BIOSECURITY AND APPROVAL**

610 All worked was performed in approved facilities and in accord with APP201675,
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REFERENCES

Abelleira, A., López, M.M., Peñalver, J., Aguín, O., Mansilla, J.P., Picoaga, A., and García, M.J. (2011) First report of bacterial canker of kiwifruit Caused by Pseudomonas syringae pv. actinidiae in Spain. *Plant Dis.* **95**: 1583.

Adams, P.D. and Kloepper, J.W. (2002) Effect of host genotype on indigenous bacterial endophytes of cotton (Gossypium hirsutum L.). *Plant Soil* **240**: 181–189.

Almeida, N.F., Yan, S., Cai, R., Clarke, C.R., Morris, C.E., Schaad, N.W., et al. (2010) PAMDB, a multilocus sequence typing and analysis database and website for plant-associated microbes. *Phytopathology* **100**: 208–215.

Anderson, M.J. (2001) A new method for non parametric multivariate analysis of variance. *Austral Ecol.* **26**: 32–46.

Auton, A. and McVean, G. (2007) Recombination rate estimation in the presence of hotspots. *Genome Res.* **17**: 1219–1227.

Balestra, G.M., Mazzaglia, A., Spinelli, R., Graziani, S., Quattrucci, A., and Rossetti, A. (2008) Cancro batterico su Actinidia chinensis. *L'informatore Agrar.* **38**: 75–76.

Baltrus, D.A., Nishimura, M.T., Romanchuk, A., Chang, J.H., Mukhtar, M.S., Cherkis, K., et al. (2011) Dynamic evolution of pathogenicity revealed by sequencing and comparative genomics of 19 Pseudomonas syringae isolates. *PLoS Pathog.* **7**: e1002132.

Bartoli, C., Berge, O., Monteil, C.L., Guilbaud, C., Balestra, G.M., Varvaro, L., et al. (2014) The Pseudomonas viridiflava phylogroups in the P. syringae species complex are characterized by genetic variability and phenotypic plasticity of pathogenicity-related traits. *Environ. Microbiol.* **16**: 2301–2315.

Bartoli, C., Lamichhane, J.R., Berge, O., Guilbaud, C., Varvaro, L., Balestra, G.M., et al. (2015) A framework to gauge the epidemic potential of plant pathogens in environmental reservoirs: the example of kiwifruit canker. *Mol. Plant Pathol.* **16**: 137–49.

Beiki, F., Busquets, A., Gomila, M., Rahimian, H., Lalucat, J., and García-Valdés, E. (2016) New Pseudomonas spp. are pathogenic to citrus. *PLoS One* **11**: e0148796.

Bender, C.L., Alarcon-Chaidez, F., Gross, D.C., Alarcón-Chaidez, F., and Gross, D.C. (1999) Pseudomonas syringae phytotoxins: Mode of action, regulation, and biosynthesis by peptide and polyketide synthetases. *Microbiol. Mol. Biol. Rev.* **63**: 266–292.

Berge, O., Monteil, C.L., Bartoli, C., Chandeysson, C., Guilbaud, C., Sands, D.C., and Morris, C.E. (2014) A user's guide to a data base of the diversity of Pseudomonas syringae and its application to classifying strains in this phylogenetic complex. *PLoS One* **9**: e105547.

Berlec, A. (2012) Novel techniques and findings in the study of plant microbiota:

Search for plant probiotics. *Plant Sci.* **193–194**: 96–102.

Bodenhausen, N., Bortfeld-Miller, M., Ackermann, M., and Vorholt, J.A. (2014) A Synthetic Community Approach Reveals Plant Genotypes Affecting the Phyllosphere Microbiota. *PLoS Genet.* **10**: e1004283.

Bratcher, H.B., Corton, C., Jolley, K.A., Parkhill, J., and Maiden, M.C. (2014) A gene-by-gene population genomics platform: de novo assembly, annotation and genealogical analysis of 108 representative Neisseria meningitidis genomes. *BMC Genomics* **15**: 1138.

Bull, C.T., Clarke, C.R., Cai, R., Vinatzer, B.A., Jardini, T.M., and Koike, S.T. (2011) Multilocus sequence typing of Pseudomonas syringae sensu lato confirms previously described genomospecies and permits rapid identification of P. syringae pv. coriandricola and P. syringae pv. apii causing bacterial leaf spot on parsley. *Phytopathology* **101**: 847–858.

Buonaurio, R., Moretti, C., da Silva, D.P., Cortese, C., Ramos, C., and Venturi, V. (2015) The olive knot disease as a model to study the role of interspecies bacterial communities in plant disease. *Front. Plant Sci.* **6**: 1–12.

Cameron, A. and Sarojini, V. (2014) Pseudomonas syringae pv. actinidiae: chemical control, resistance mechanisms and possible alternatives. *Plant Pathol.* **63**:.

Caugant, D.A. and Maiden, M.C.J. (2009) Meningococcal carriage and disease—Population biology and evolution. *Vaccine* **27**: B64–B70.

Colombi, E., Straub, C., Künzel, S., Templeton, M.D., McCann, H.C., and Rainey, P.B. (2017) Evolution of copper resistance in the kiwifruit pathogen Pseudomonas syringae pv. actinidiae through acquisition of integrative conjugative elements and plasmids. *Environ. Microbiol.* **19**: 819–832.

Cordero, O.X., Wildschutte, H., Kirkup, B., Proehl, S., Ngo, L., Hussain, F., et al. (2012) Ecological Populations of Bacteria Act as Socially Cohesive Units of Antibiotic Production and Resistance. *Science (80-.).* **337**: 1228–1231.

Darriba, D., Taboada, G.L., Doallo, R., and Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* **9**: 772–772.

Demba Diallo, M., Monteil, C.L., Vinatzer, B.A., Clarke, C.R., Glaux, C., Guilbaud, C., et al. (2012) Pseudomonas syringae naturally lacking the canonical type III secretion system are ubiquitous in nonagricultural habitats, are phylogenetically diverse and can be pathogenic. *ISME J.* **6**: 1325–1335.

Ditta, G., Stanfield, S., Corbin, D., and Helinski, D.R. (1980) Broad host range DNA cloning system for gram-negative bacteria: construction of a gene bank of Rhizobium meliloti. *Proc. Natl. Acad. Sci.* **77**: 7347–7351.

Essakhi, S., Cesbron, S., Fischer-Le Saux, M., Bonneau, S., Jacques, M.A., and Manceau, C. (2015) Phylogenetic and variable-number tandem-repeat analyses identify nonpathogenic Xanthomonas arboricola lineages lacking the canonical type III secretion system. *Appl. Environ. Microbiol.* **81**: 5395–5410.

Everett, K.R., Taylor, R.K., Romberg, M.K., Rees-George, J., Fullerton, R.A., Vanneste, J.L., and Manning, M.A. (2011) First report of Pseudomonas syringae pv. actinidiae causing kiwifruit bacterial canker in New Zealand. *Australas. Plant Dis. Notes* **6**: 67–71.

Feil, E.J., Li, B.C., Aanensen, D.M., Hanage, W.P., and Spratt, B.G. (2004) eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from Multilocus Sequence Typing Data. *J. Bacteriol.* **186**: 1518–1530.

Felsenstein, J. (1989) Phylip: phylogeny inference package (version 3.2). *Cladistics* **5**: 164–166.

Ferrante, P. and Scortichini, M. (2010) Molecular and phenotypic features of Pseudomonas syringae pv. actinidiae isolated during recent epidemics of bacterial canker on yellow kiwifruit (Actinidia chinensis) in central Italy. *Plant Pathol.* **59**: 954–962.

Francisco, A.P., Vaz, C., Monteiro, P.T., Melo-Cristino, J., Ramirez, M., and Carriço, J.A. (2012) PHYLOViZ: phylogenetic inference and data visualization for sequence based typing methods. *BMC Bioinformatics* **13**: 87.

Fraser, C., Hanage, W.P., and Spratt, B.G. (2005) Neutral microepidemic evolution of bacterial pathogens. *Proc. Natl. Acad. Sci.* **102**: 1968–1973.

Fujikawa, T. and Sawada, H. (2016) Genome analysis of the kiwifruit canker pathogen Pseudomonas syringae pv. actinidiae biovar 5. *Sci. Rep.* **6**:.

Fukami, T., Beaumont, H.J.E., Zhang, X.-X., and Rainey, P.B. (2007) Immigration history controls diversification in experimental adaptive radiation. *Nature* **446**: 436–439.

Goss, E.M., Kreitman, M., and Bergelson, J. (2005) Genetic diversity, recombination and cryptic clades in Pseudomonas viridiflava infecting natural populations of Arabidopsis thaliana. *Genetics* **169**: 21–35.

Guindon, S. and Gascuel, O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* **52**: 696–704.

Haapalainen, M., Mosorin, H., Dorati, F., Wu, R.F., Roine, E., Taira, S., et al. (2012) Hcp2, a secreted protein of the phytopathogen Pseudomonas syringae pv. tomato DC3000, is required for fitness for competition against bacteria and yeasts. *J. Bacteriol.* **194**: 4810–4822.

Hall, S.J., Dry, I.B., Blanchard, C.L., and Whitelaw-Weckert, M.A. (2016) Phylogenetic relationships of Pseudomonas syringae pv. syringae isolates associated with bacterial iInflorescence rot in grapevine. *Plant Dis.* **100**: 607–616.

Han, H.S., Nam, H.Y., Koh, Y.J., Hur, J.-S., and Jung, J.S. (2003) Molecular bases of high-level streptomycin resistance in Pseudomonas marginalis and Pseudomonas syringae pv. actinidiae. *J. Microbiol.* **41**: 16–21.

Haubold, B. and Rainey, P.B. (1996) Genetic and ecotypic structure of a fluorescent Pseudomonas population. *Mol. Ecol.* **5**: 747–761.

Hayes, C.S., Aoki, S.K., and Low, D.A. (2010) Bacterial contact-dependent delivery systems. *Annu. Rev. Genet.* **44**: 71–90.

Hernández-Morales, A., De la Torre-Zavala, S., Ibarra-Laclette, E., Hernández-Flores, J., Jofre-Garfias, A., Martínez-Antonio, A., and Álvarez-Morales, A. (2009) Transcriptional profile of Pseudomonas syringae pv. phaseolicola NPS3121 in response to tissue extracts from a susceptible Phaseolus vulgaris L. cultivar. *BMC Microbiol.* **9**: 257.

Hibbing, M.E., Fuqua, C., Parsek, M.R., and Peterson, S.B. (2010) Bacterial competition: surviving and thriving in the microbial jungle. *Nat. Rev. Microbiol.* **8**: 15–25.

Hirano, S.S., Charkowski, A.O., Collmer, A., Willis, D.K., and Upper, C.D. (1999) Role of the Hrp type III protein secretion system in growth of Pseudomonas syringae pv. syringae B728a on host plants in the field. *Proc. Natl. Acad. Sci.* **96**: 9851–9856.

752 Hirano, S.S. and Upper, C.D. (2000) Bacteria in the leaf ecosystem with emphasis on
753 Pseudomonas syringae - a pathogen, ice nucleus, and epiphyte. *Microbiol. Mol.*
754 *Biol. Rev.* **64**: 624–653.
755 Ho, S.N., Hunt, H.D., Horton, R.M., Pullen, J.K., and Pease, L.R. (1989) Site-directed
756 mutagenesis by overlap extension using the polymerase chain reaction. *Gene*
757 **77**: 51–59.
758 Hockett, K.L., Renner, T., and Baltrus, D.A. (2015) Independent co-option of a tailed
759 bacteriophage into a killing complex in Pseudomonas. *MBio* **6**: 1–11.
760 Hudson, R.R. (2001) Two-locus sampling distributions and their application. *Genetics*
761 **159**: 1805–1817.
762 Humphrey, P.T., Nguyen, T.T., Villalobos, M.M., and Whiteman, N.K. (2014) Diversity
763 and abundance of phyllosphere bacteria are linked to insect herbivory. *Mol.*
764 *Ecol.* **23**: 1497–1515.
765 Hwang, M.S.H., Morgan, R.L., Sarkar, S.F., Wang, P.W., and Guttman, D.S. (2005)
766 Phylogenetic characterization of virulence and resistance phenotypes of
767 Pseudomonas syringae. *Appl. Environ. Microbiol.* **71**: 5182–5191.
768 Istock, C.A., Duncan, K.E., Ferguson, N., and Zhou, X. (1992) Sexuality in a natural
769 population of bacteria - Bacillus subtilis challenges the clonal paradigm. *Mol.*
770 *Ecol.* **1**: 95–103.
771 Jolley, K.A., Feil, E.J., Chan, M.S., and Maiden, M.C. (2001) Sequence type analysis
772 and recombinational tests (START). *Bioinformatics* **17**: 1230–1231.
773 Jost, L. (2006) Entropy and diversity. *Oikos* **113**: 363–375.
774 Karasov, T.L., Barrett, L., Hershberg, R., and Bergelson, J. (2017) Similar levels of gene
775 content variation observed for Pseudomonas syringae populations extracted
776 from single and multiple host species. *PLoS One* **12**: 1–18.
777 Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., et al.
778 (2012) Geneious Basic: An integrated and extendable desktop software
779 platform for the organization and analysis of sequence data. *Bioinformatics* **28**:
780 1647–1649.
781 Kniskern, J.M., Barrett, L.G., and Bergelson, J. (2011) Maladaptation in wild
782 populations of the generalist plant pathogen Pseudomonas syringae. *Evolution*
783 *(N. Y).* **65**: 818–830.
784 Koh, Y.J., Kim, G.H., Koh, H.S., Lee, Y.S., Kim, S.C., and Jung, J.S. (2012) Occurrence of
785 a new type of Pseudomonas syringae pv. actinidiae strain of bacterial canker on
786 kiwifruit in Korea. *Plant Pathol.* **28**: 423–427.
787 Kõiv, V., Roosaare, M., Vedler, E., Ann Kivistik, P., Toppi, K., Schryer, D.W., et al.
788 (2015) Microbial population dynamics in response to Pectobacterium
789 atrosepticum infection in potato tubers. *Sci. Rep.* **5**: 11106.
790 Kumar, S., Stecher, G., and Tamura, K. (2016) MEGA7: Molecular Evolutionary
791 Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **33**: 1870–1874.
792 Laforest-Lapointe, I., Messier, C., Kembel, S.W., Lindow, S., Brandl, M., Herre, E., et
793 al. (2016) Host species identity, site and time drive temperate tree phyllosphere
794 bacterial community structure. *Microbiome* **4**: 27.
795 Lamichhane, J.R. and Venturi, V. (2015) Synergisms between microbial pathogens in
796 plant disease complexes: a growing trend. *Front. Plant Sci.* **6**: 385.
797 Lenski, R.E., Rose, M.R., Simpson, S.C., and Tadler, S.C. (1991) Long-term
798 experimental evolution in Escherichia coli . I . Adaptation and divergence during

2,000 generations. *Am. Nat.* **138**: 1315–1341.

Lindow, S.E. (1986) Construction of isogenic Ice strains of Pseudomonas syringae for evaluation of specificity of competition on leaf surfaces. *Perspect. Microb. Ecol. Slov. Soc. Microbiol. Ljubljana* 509–515.

Lindow, S.E. and Brandl, M.T. (2003) Microbiology of the phyllosphere. *Appl. Environ. Microbiol.* **69**: 1875–1883.

Macho, A.P., Zumaquero, A., Ortiz-Martín, I., and Beuzón, C.R. (2007) Competitive index in mixed infections: A sensitive and accurate assay for the genetic analysis of Pseudomonas syringae-plant interactions. *Mol. Plant Pathol.* **8**: 437–450.

Marchi, G., Sisto, A., Cimmino, A., Andolfi, A., Cipriani, M.G., Evidente, A., and Surico, G. (2006) Interaction between Pseudomonas savastanoi pv. savastanoi and Pantoea agglomerans in olive knots. *Plant Pathol.* **55**: 614–624.

Maynard Smith, J., Smith, N.H., O'Rourke, M., and Spratt, B.G. (1993) How clonal are bacteria? *Proc. Natl. Acad. Sci.* **90**: 4384–4388.

McArdle, B.H. and Anderson, M.J. (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology* **82**: 290–297.

McCann, H.C., Li, L.L., Liu, Y., Li, D., Pan, H., Zhong, C., et al. (2017) Origin and evolution of the kiwifruit canker pandemic. *Genome Biol. Evol.* **9**: 932–944.

McCann, H.C., Rikkerink, E.H.A., Bertels, F., Fiers, M., Lu, A., Rees-George, J., et al. (2013) Genomic analysis of the kiwifruit pathogen Pseudomonas syringae pv. actinidiae provides insight into the origins of an emergent plant disease. *PLoS Pathog.* **9**: e1003503.

Mohr, T.J., Liu, H., Yan, S., Morris, C.E., Castillo, J.A., Jelenska, J., and Vinatzer, B.A. (2008) Naturally occurring nonpathogenic isolates of the plant pathogen Pseudomonas syringae lack a type III secretion system and effector gene orthologues. *J. Bacteriol.* **190**: 2858–2870.

Monier, J.-M. and Lindow, S.E. (2003) Differential survival of solitary and aggregated bacterial cells promotes aggregate formation on leaf surfaces. *Proc. Natl. Acad. Sci.* **100**: 15977–15982.

Moretti, C., Hosni, T., Vandemeulebroecke, K., Brady, C., de Vos, P., Buonaurio, R., and Cleenwerck, I. (2011) Erwinia oleae sp. nov., isolated from olive knots caused by Pseudomonas savastanoi pv. savastanoi. *Int. J. Syst. Evol. Microbiol.* **61**: 2745–2752.

Morris, C.E., Sands, D.C., Vanneste, J.L., Montarry, J., Oakley, B., Guilbaud, C., and Glaux, C. (2010) Inferring the evolutionary history of the plant pathogen Pseudomonas syringae from its biogeography in headwaters of rivers in North America, Europe, and New Zealand. *MBio* **1**: e00107.

Nakahara, H., Mori, T., Sadakari, N., Matsusaki, H., and Matsuzoe, N. (2016) Selection of effective non-pathogenic Ralstonia solanacearum as biocontrol agents against bacterial wilt in eggplant. *J. Plant Dis. Prot.* **123**: 119–124.

Nowell, R.W., Laue, B.E., Sharp, P.M., and Green, S. (2016) Comparative genomics reveals genes significantly associated with woody hosts in the plant pathogen Pseudomonas syringae. *Mol. Plant Pathol.* **17**: 1409–1424.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Dan McGlinn, Peter R. Minchin, R.B.O., et al. (2016) vegan: Community Ecology Package.

Omer, M.E.H. and Wood, R.K.S. (1969) Growth of Pseudomonas phaseolicola in susceptible and in resistant bean plants. *Ann. Appl. Biol.* **63**: 103–116.

Van Overbeek, L. and Van Elsas, J.D. (2008) Effects of plant genotype and growth stage on the structure of bacterial communities associated with potato (Solanum tuberosum L.). *FEMS Microbiol. Ecol.* **64**: 283–296.

Petriccione, M., Zampella, L., Mastrobuoni, F., and Scortichini, M. (2017) Occurrence of copper-resistant Pseudomonas syringae pv. syringae strains isolated from rain and kiwifruit orchards also infected by P. s. pv. actinidiae. *Eur. J. Plant Pathol.* 1–16.

Pfeilmeier, S., Caly, D.L., and Malone, J.G. (2016) Bacterial pathogenesis of plants: future challenges from a microbial perspective: Challenges in Bacterial Molecular Plant Pathology. *Mol. Plant Pathol.* **17**: 1298–1313.

Polz, M.F., Alm, E.J., and Hanage, W.P. (2013) Horizontal gene transfer and the evolution of bacterial and archaeal population structure. *Trends Genet.* **29**: 170–175.

R.Core.Team (2016) R: A language and environment for statistical computing.

Rosen, M.J., Davison, M., Bhaya, D., and Fisher, D.S. (2015) Fine-scale diversity and extensive recombination in a quasisexual bacterial population occupying a broad niche. *Science (80-.).* **348**: 1019–1024.

Rozas, J. and Rozas, R. (1995) DnaSP, DNA sequence polymorphism: an interactive program for estimating population genetics parameters from DNA sequence data. *CABIOS* **11**: 621–625.

Rufián, J.S., Macho, A.P., Corry, D.S., Mansfield, J., Arnold, D., and Beuzón, C.R. (2017) Confocal microscopy reveals in planta dynamic interactions between pathogenic, avirulent and non-pathogenic Pseudomonas syringae strains. *Mol. Plant Pathol.* DOI: 10.1111/mpp.12539.

Ruhe, Z.C., Low, D.A., and Hayes, C.S. (2013) Bacterial contact-dependent growth inhibition. *Trends Microbiol.* **21**: 230–237.

Sarkar, S.F. and Guttman, D.S. (2004) Evolution of the core genome of Pseudomonas syringae, a highly clonal, endemic plant pathogen. *Appl. Environ. Microbiol.* **70**: 1999–2012.

Sawada, H. (2015) Characterization of biovar 3 strains of Pseudomonas syringae pv. actinidiae isolated in Japan. *Japanese J. Phyopathology* **81**: 111–126.

Sawada, H.H., Suzuki, F.F., Matsuda, I.I., and Saitou, N.N. (1999) Phylogenetic analysis of Pseudomonas syringae pathovars suggests the horizontal gene transfer of argK and the evolutionary stability of hrp gene cluster. *J. Mol. Evol.* **49**: 627–644.

Schäfer, A., Tauch, A., Jäger, W., Kalinowski, J., Thierbach, G., and Pühler, A. (1994) Small mobilizable multi-purpose cloning vectors derived from the Escherichia coli plasmids pK18 and pK19: selection of defined deletions in the chromosome of Corynebacterium glutamicum. *Gene* **145**: 69–73.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., et al. (2009) Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **75**: 7537–7541.

Schmidt, H.A., Strimmer, K., Vingron, M., and von Haeseler, A. (2002) TREE-PUZZLE: maximum likelihood phylogenetic analysis using quartets and parallel computing. *Bioinformatics* **18**: 502–504.

Shapiro, B.J., Friedman, J., Cordero, O.X., Preheim, S.P., Timberlake, S.C., Szabo, G.,

et al. (2012) Population Genomics of Early Events in the Ecological Differentiation of Bacteria. *Science (80-.).* **336**: 48–51.

Shapiro, B.J. and Polz, M.F. (2014) Ordering microbial diversity into ecologically and genetically cohesive units. *Trends Microbiol.* **22**: 235–247.

Singer, M. (2010) Pathogen-pathogen interaction. *Virulence* **1**: 10–18.

Souza, V., Nguyen, T.T., Hudson, R.R., Piñero, D., and Lenski, R.E. (1992) Hierarchical analysis of linkage disequilibrium in Rhizobium populations: evidence for sex? *Proc. Natl. Acad. Sci. U. S. A.* **89**: 8389–93.

Spratt, B.G., Hanage, W.P., Li, B., Aanensen, D.M., and Feil, E.J. (2004) Displaying the relatedness among isolates of bacterial species -- the eBURST approach. *FEMS Microbiol. Lett.* **241**: 129–134.

Spratt, B.G. and Maiden, M.C.J. (1999) Bacterial population genetics, evolution and epidemiology. *Philos. Trans. R. Soc. B Biol. Sci.* **354**: 701–710.

Stubbendieck, R.M., Vargas-Bautista, C., and Straight, P.D. (2016) Bacterial communities: Interactions to scale. *Front. Microbiol.* **7**: 1234.

Tomihama, T., Sekita, T., and Takikawa, Y. (2016) Wide distribution of airborne ice-nucleation active Pseudomonas syringae in agricultural environments. *Phytopathology* **5159**: 1–49.

Triplett, L.R., Verdier, V., Campillo, T., Van Malderghem, C., Cleenwerck, I., Maes, M., et al. (2015) Characterization of a novel clade of Xanthomonas isolated from rice leaves in Mali and proposal of Xanthomonas maliensis sp. nov. *Antonie van Leeuwenhoek, Int. J. Gen. Mol. Microbiol.* **107**: 869–881.

Visnovsky, S.B., Fiers, M., Lu, A., Panda, P., Taylor, R., and Pitman, A.R. (2016) Draft genome sequences of 18 strains of Pseudomonas isolated from kiwifruit plants in New Zealand and overseas. *Genome Announc.* **4**: e00061-16.

Völksch, B. and May, R. (2001) Biological Control of Pseudomonas syringae pv. glycinea by Epiphytic Bacteria under Field Conditions. *Microb. Ecol.* **41**: 132–139.

Wagner, M.R., Lundberg, D.S., del Rio, T.G., Tringe, S.G., Dangl, J.L., and Mitchell-Olds, T. (2016) Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. *Nat. Commun.* **7**: 12151.

Whipps, J.M., Hand, P., Pink, D., and Bending, G.D. (2008) Phyllosphere microbiology with special reference to diversity and plant genotype. *J. Appl. Microbiol.* **105**: 1744–1755.

Wilson, M., Hirano, S.S., and Lindow, S.E. (1999) Location and survival of leaf-associated bacteria in relation to pathogenicity and potential for growth within the leaf. *Appl. Environ. Microbiol.* **65**: 1435–1443.

Wilson, M. and Lindow, S.E. (1994) Coexistence among epiphytic bacterial Populations mediated through nutritional resource partitioning. *Appl. Environ. Microbiol.* **60**: 4468–4477.

Young, J.M. (1974) Development of bacterial populations in vivo in relation to plant pathogenicity. *New Zeal. J. Agric. Res.* **17**: 105–113.

Zhang, X.X., Gauntlett, J.C., Oldenburg, D.G., Cook, G.M., and Rainey, P.B. (2015) Role of the transporter-like sensor kinase CbrA in histidine uptake and signal transduction. *J. Bacteriol.* **197**: 2867–2878.

Zhao, Z.B., Gao, X.N., Huang, Q.L., Huang, L.L., Qin, H.Q., and Kang, Z.S. (2013) Identification and characerization of the causal agent of bacterial canker of

kiwifruit in the Shaanxi province of China. *J. Plant Pathol.* **95**: 155–162.

FIGURES

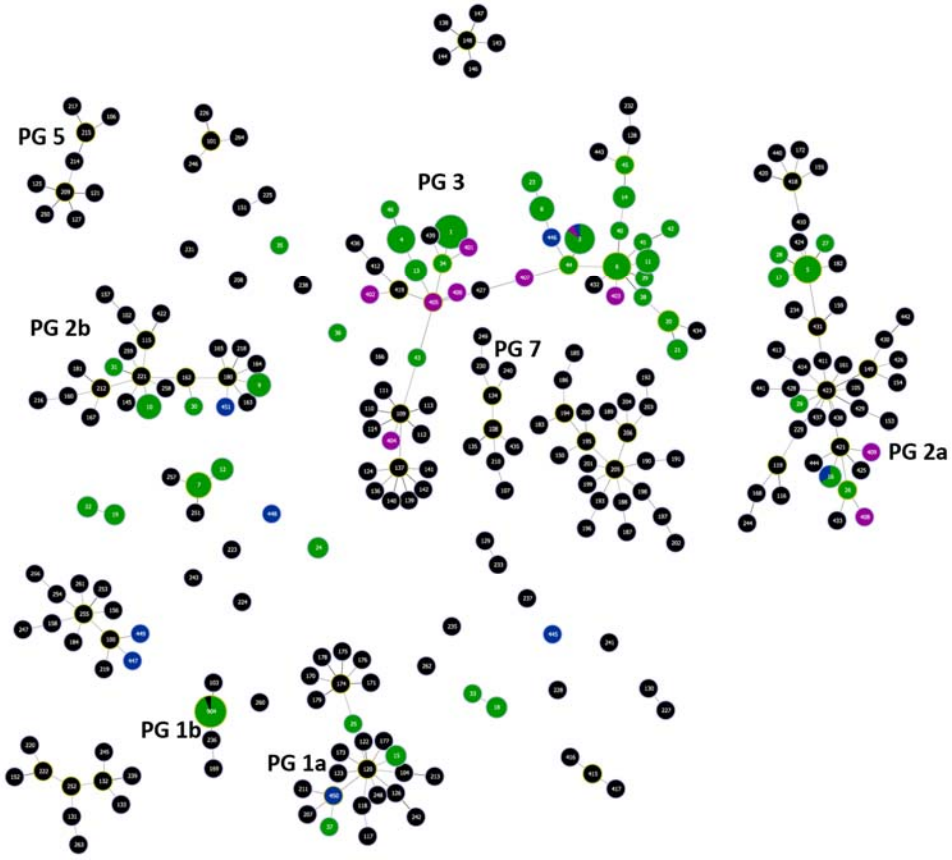
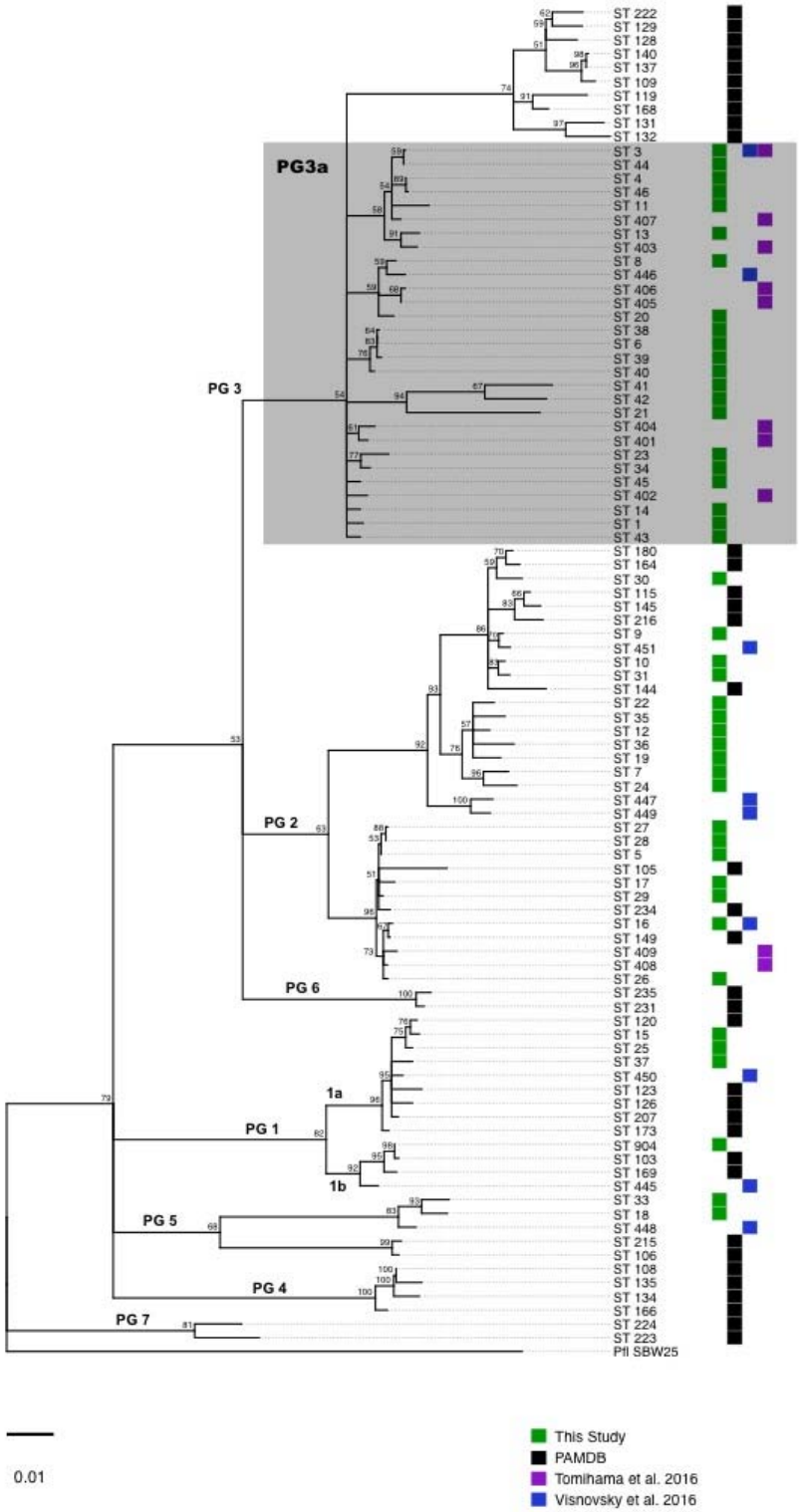


Figure 1. Global Minimum Spanning Tree (MST). Displaying the relationships between STs at the triple-locus-variant level, illustrated using PHYLOViZ (Francisco *et al.*, 2012). The size of the circle correlates with the frequency of the ST. Color-coded accorded to origin: green = this study, black = PAMDB, blue = Visnovsky *et al.* (2016), purple = Tomihama *et al.* (2016).



951

952 **Figure 2. Maximum Likelihood tree based on the concatenated alignment (2010**
953 **bp) of four housekeeping genes: *gapA*, *gyrB*, *gltA* and *rpoD*.** Maximum Likelihood
954 tree reconstructed using TREEPUZZLE based on the Tamura-Nei model using 100,000
955 puzzling steps. Single representative sequences for each ST were used to improve
956 readability (frequency of each ST and corresponding strain names listed in Table S2).
957 Values indicated at nodes are bootstrap values. The corresponding phylogroups (PG)
958 are indicated, eg. PG1 = phylogroup 1 with clades 1a and 1b. Origin of isolates is
959 illustrated in colour coded boxes, green = this study, black = PAMDB, blue =
960 Visnovsky et al. 2016, purple = Tomihama et al. 2016

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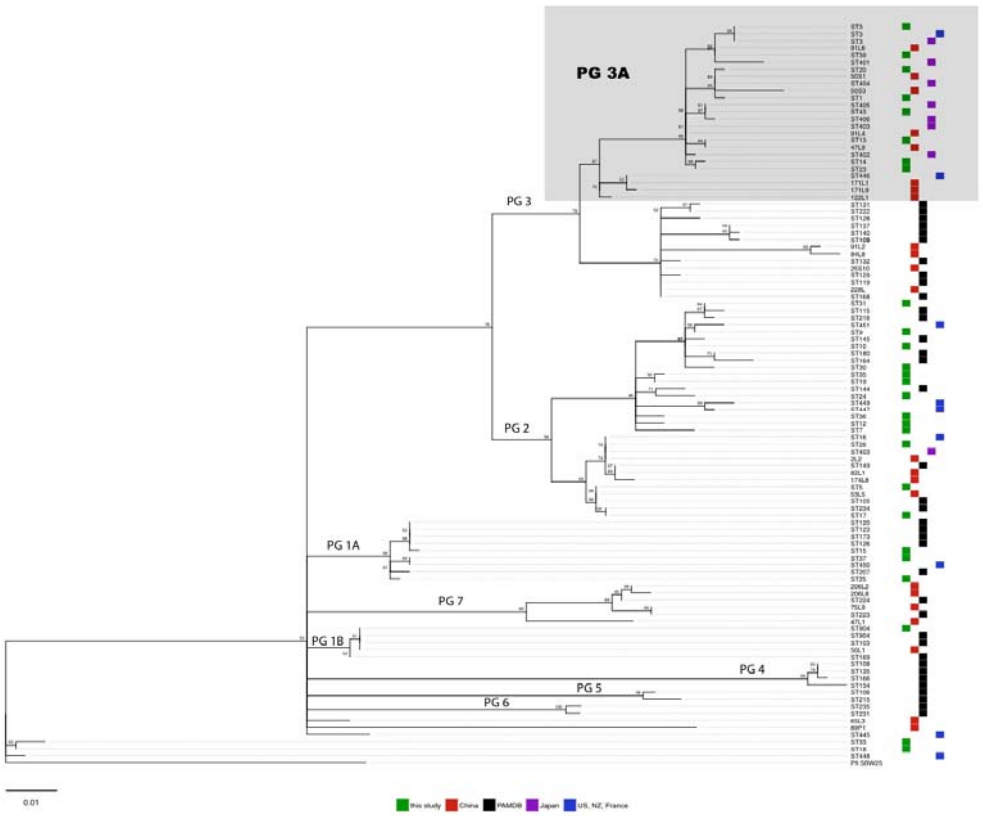


962

963 **Figure 3. Global ML tree reconstructed from *gltA* sequences highlighting the**

964 **particularity of PG3a, which includes kiwifruit isolates from NZ, China, Japan, the**

965 **US and France.** The tree was built on a 529 bp alignment using TREEPUZZLE (HKY

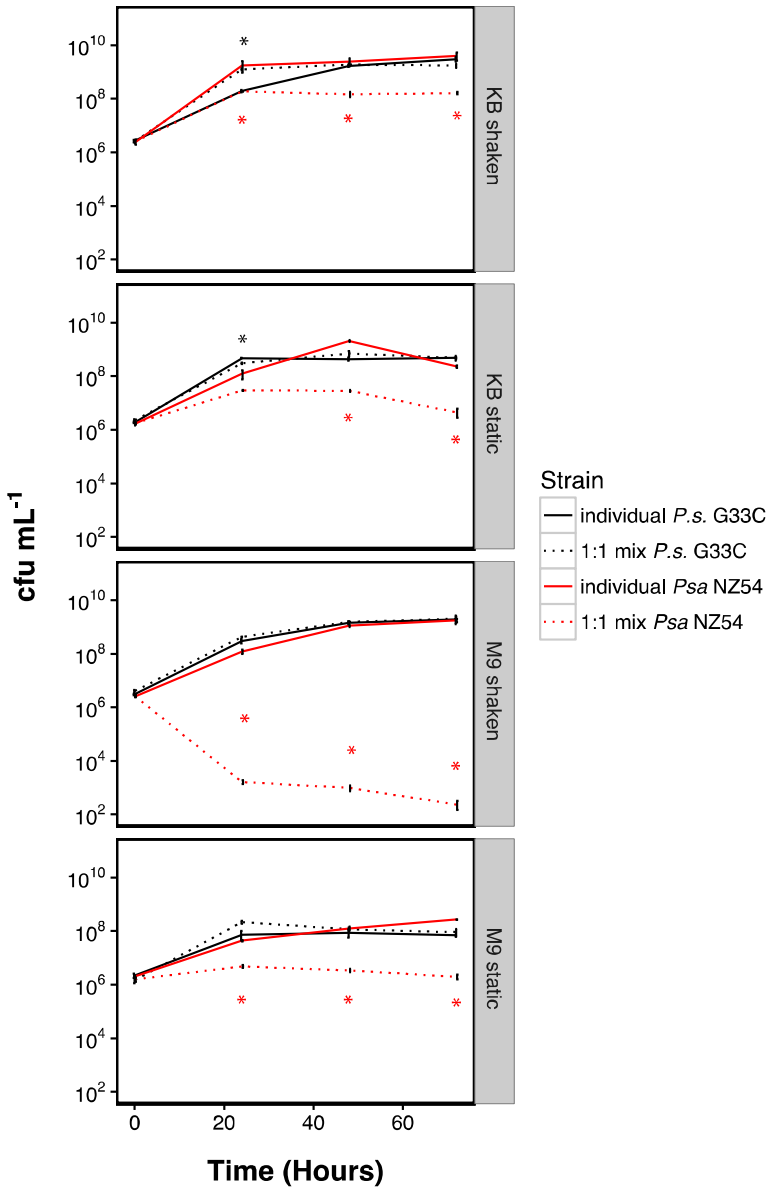
966 model; 100,000 puzzling steps), using *Pseudomonas fluorescens* SBW25 as outgroup.

967 Values indicated at nodes are bootstrap values. The source of each isolate is

968 highlighted in colour-coded boxes, green = this study, red = China, black = PAMDB,

969 purple = Japan, blue = US, NZ and France.

970



971

972 **Figure 4. Individual growth dynamics of *Psa* NZ54 and *P. syringae* G33C compared**

973 **with co-inoculation (1:1 ratio) *in vitro*.** Competition experiments were performed in

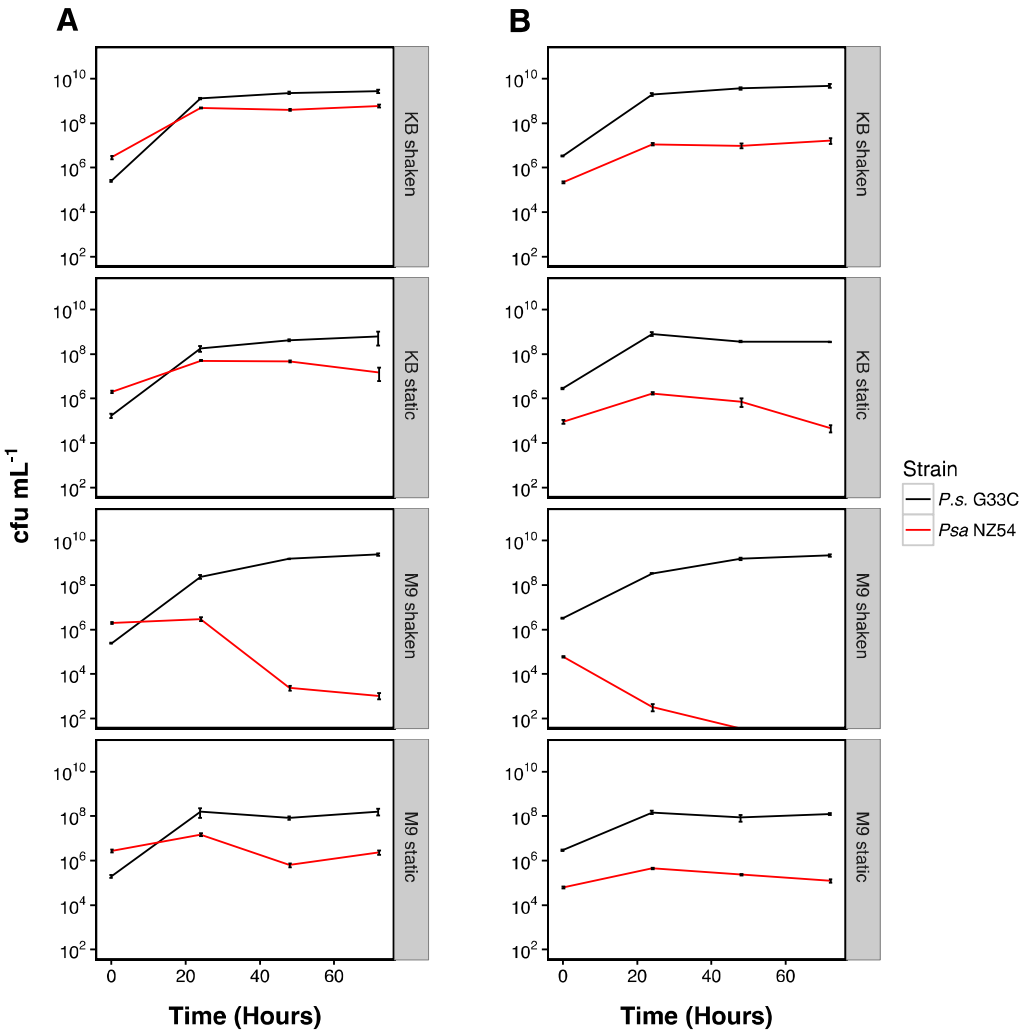
974 a 1:1 ratio (founding ratio $5x10^6$ cfu ml^{-1} each), with individual inoculations as

975 reference. Solid lines represent individual growth and dashed lines represent growth

976 in competition. The presented mean and standard error were calculated from three

977 replicates. Asterisk indicate significance between individual and co-cultured growth

978 at the 5% level (paired *t*-test).



979

980 **Figure 5. *In vitro* growth curves from invasion from rare experiments for *Psa* NZ54 :**

981 ***P. syringae* G33C and vice versa.** Vials were inoculated with a (A) 10:1 ratio and (B)

982 1:10 ratio for *Psa* NZ54 : *P. syringae* G33C. The presented mean and standard error

983 were calculated from three replicates. Parameters of relative fitness of *Psa* NZ54

984 relative to *P. syringae* G33C calculated as ln difference *Psa* NZ54 – *P. syringae* G33C

985 using the Malthusian parameters at 24hrs were -2.1* ±0.02 (KB shaken), -1.9*±0.05

986 (KB static), -12.1*±0.09 (M9 shaken) and -3.9*±0.00 (M9 static). Asterisks indicate

987 significance at the 1% level (Students *t*-test).

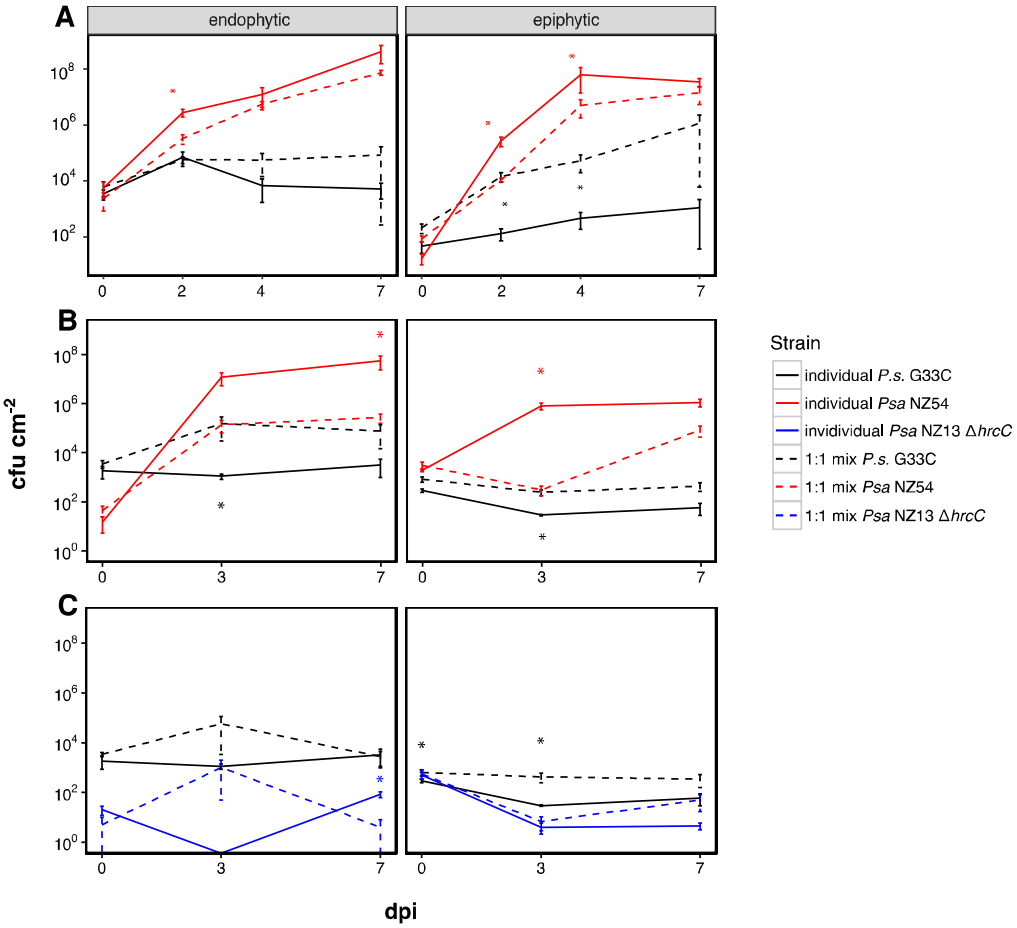
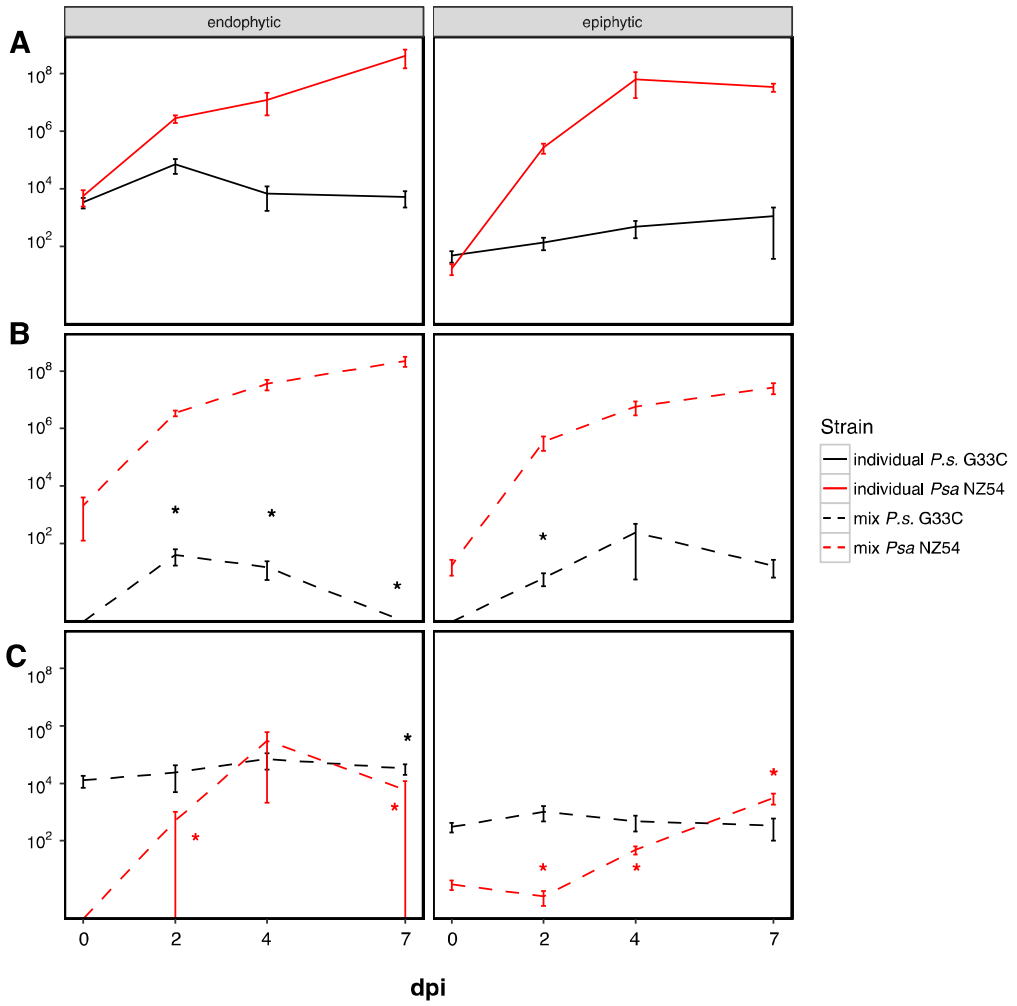


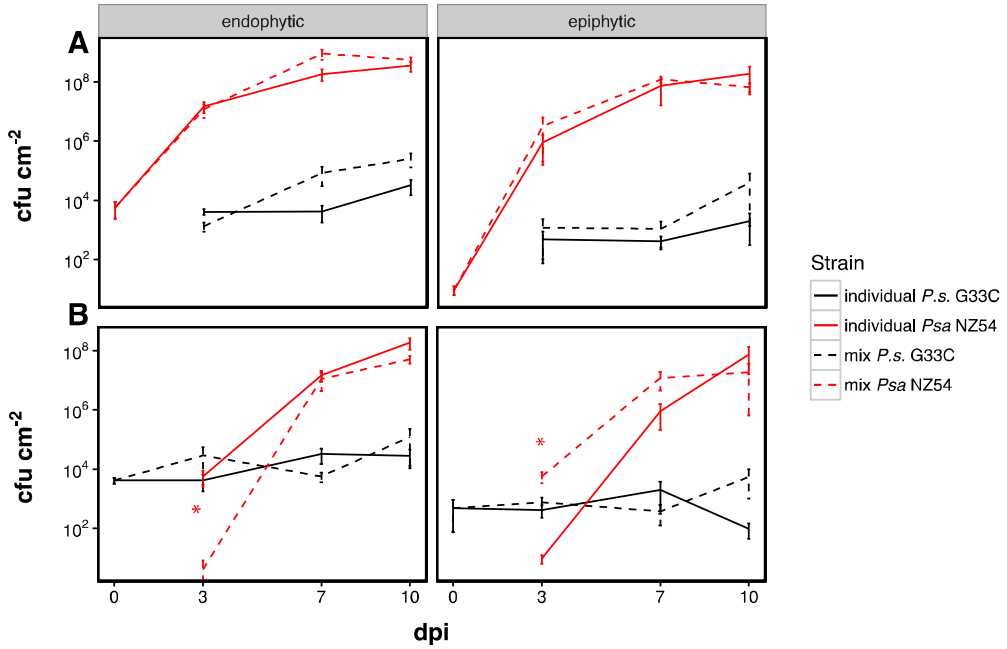
Figure 6. 1:1 competition growth assays of *Psa* NZ54 vs. *P. syringae* G33C *in planta*. 'Hort16A' plantlets (A) and 'SunGold' plantlets (B) were inoculated with a 1:1 mix of *P. syringae* G33C : *Psa* NZ54 (founding density $8x10^7$ cfu ml^{-1}). (C) 'SunGold' plants were inoculated with 1:1 mix of *P. syringae* G33C : *Psa* NZ13 Δ*hrcC* (founding density $8x10^7$ cfu ml^{-1}). Solid lines represent individual growth and dashed lines represent growth in competition. The presented mean and standard error were calculated from the mean of four ('Hort16A') and five ('SunGold') individual measurements. Asterisk indicate significance between individual and co-cultured growth at the 5% level (paired *t*-test).



998
999 **Figure 7. Invasion from rare experiments for *Psa* NZ54 : *P. syringae* G33C *in planta*.**

1000 'Hort16A' plantlets were inoculated with different ratios of strains *Psa* NZ54 : *P.*
1001 *syringae* G33C. A) Individual growth. B) Invasion from rare 100:1 and C) invasion
1002 from rare 1:100. Solid lines represent individual growth and dashed lines represent
1003 growth in competition. The presented mean and standard error were calculated
1004 from the mean of four individual measurements. Asterisks indicate significance
1005 between individual and co-cultured growth at the 5% level (paired *t*-test).

1006



1007

1008 **Figure 8. *In planta* priority effect of *Psa* NZ54 or *P. syingae* G33C with subsequent**

1009 **inoculation of the respective second strain with the same founding density.** (A) *In*

1010 *planta* growth assay of *P. syringae* G33C using 'Hort16A' plantlets pre-inoculated for

1011 three days with *Psa* NZ54 ($8x10^7$ cfu ml^{-1}). (B) *in planta* growth assay of *Psa* NZ54

1012 using 'Hort16A' plantlets pre-inoculated for three days with *P. syringae* G33C ($8x10^7$

1013 cfu ml^{-1}). Solid lines represent individual growth and dashed lines represent growth

1014 in competition. The presented mean and standard error were calculated from the

1015 mean of five individual measurements. Asterisks indicate significance between

1016 individual and co-cultured growth at the 5% level (paired *t*-test).

TABLES

Table 1: Nucleotide and amino acid diversity. L = length in bp, AA = amino acid, GC = average GC content in %, N_A = number of alleles, P = number of polymorphic sites, d_N/d_S ration, mut = mutations, π = nucleotide diversity indices, θ = Watterson's theta.

Locus	L (bp)	AA length	GC	N_A	P*	d_N/d_S	mut	π	θ
gapA	476	158	60.81	25	80 (16.81)	3.365	97	0.055	0.024
gyrB	507	169	53.15	28	145 (28.60)	0.018	184	0.054	0.041
gltA	529	176	58.45	27	88 (16.64)	0.011	102	0.040	0.025
rpoD	495	166	59.56	35	99 (20)	2.022	118	0.042	0.030
Mean	502	167	57.99	29	105 (20.51)	1.354	125	0.048	0.030

Table 2: Average pairwise genetic diversity between and among phylogroups. Analyses were conducted on the concatenated alignment (2006 bp, gaps removed) using the Maximum Composite Likelihood model with a gamma distribution of 1. N= number of strains.

N	Phylogroup	1	2	5	3
19	**1**	0.010			
43	**2**	0.098	0.027		
3	**5**	0.111	0.106	0.008	
83	**3**	0.099	0.063	0.107	0.014

1031 **Table 3: LDhat recombination analysis.** Showing the length of the alignment in bp, N

1032 = number of sequences, mutation rate θ (=2Neμ) per site, recombination rate ρ

1033 (=2Ner) per site, ratio ε = ρ/ θ and Tajima's D.

1034

Gene	Length (bp)	N	Segregating sites	θ	ρ	ε = ρ/θ	Tajima's *D*
All *P. syringae*:							
concatenated	2010	148	335	0.030	0.006	0.187	0.513
gapA	476	148	63	0.024	0.021	0.902	2.204
gyrB	507	148	116	0.041	0.038	0.931	-0.204
gltA	529	148	74	0.025	0.012	0.461	0.678
rpoD	498	148	82	0.030	0.012	0.416	0.03
Phylogroup 1							
concat	2010	19	66	0.009	0.000	0.000	0.207
gapA	476	19	8	0.005	0.000	0.000	0.407
gyrB	507	19	20	0.011	0.000	0.000	0.611
gltA	529	19	19	0.010	0.000	0.000	0.026
rpoD	498	19	19	0.011	0.000	0.000	-0.188
Phylogroup 2							
concat	2010	43	147	0.017	0.002	0.120	1.754
gapA	476	43	29	0.014	0.006	0.457	2.307
gyrB	507	43	63	0.029	0.000	0.000	2.131
gltA	529	43	27	0.012	0.008	0.654	0.993
rpoD	498	43	28	0.013	0.023	1.734	0.621
Phylogroup 3							
concat	2010	83	163	0.016	0.015	0.937	-0.746
gapA	476	83	34	0.014	0.013	0.898	2.096
gyrB	507	83	96	0.038	0.024	0.636	-2.434
gltA	529	83	13	0.005	0.006	1.175	1.991
rpoD	498	83	20	0.008	0.033	4.073	0.3
Phylogroup 5							
concat	2010	3	23	0.008	0.000	0.000	-
gapA	476	3	-	-	-	-	-
gyrB	507	3	13	0.017	0.000	0.000	-
gltA	529	3	3	0.004	0.000	0.000	-
rpoD	498	3	7	0.009	0.000	0.000	-

1035
1036

SUPPORTING FIGURES

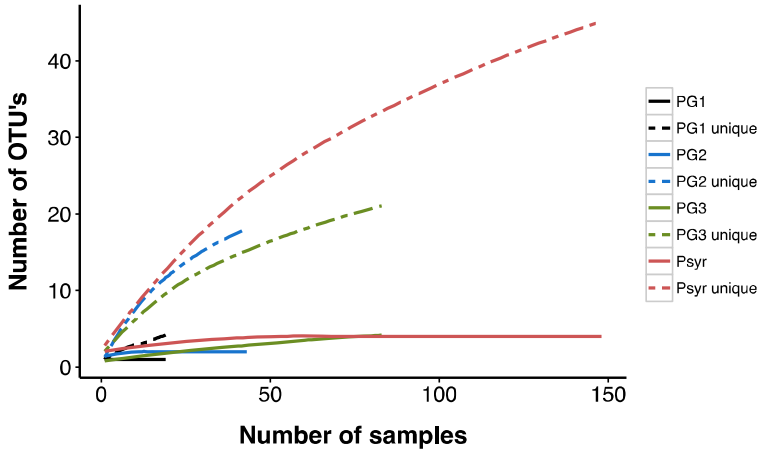


Figure S1. Rarefaction curves based on the concatenated sequences. Two curves each are shown for *P. syringae* (n=148) and the sequences grouped according to phylogroups (PG): solid lines represent grouping based on unique STs and dashed lines according to a cut-off equal to the average pairwise genetic distance of the group: PG1, PG2 & PG3 = 0.02 cut-off, Psyr all = 0.05 cut-off.

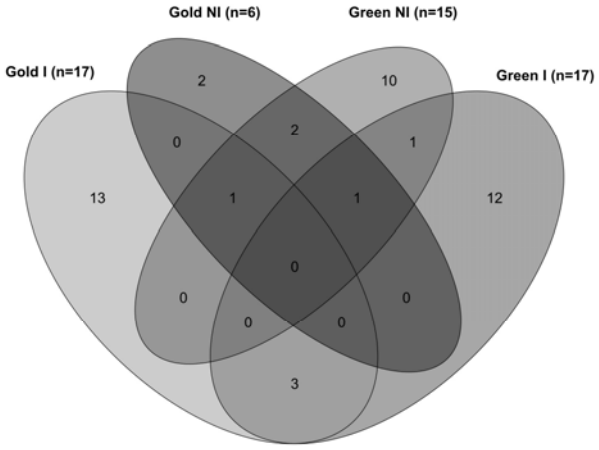


Figure S2. Shared and unique STs among orchards. Gold I = infected 'Hort16A'; Gold NI= uninfected 'Hort16A'; Green NI = uninfected 'Hayward'; Green I = infected 'Hayward' orchard; n= number of STs found in orchard.

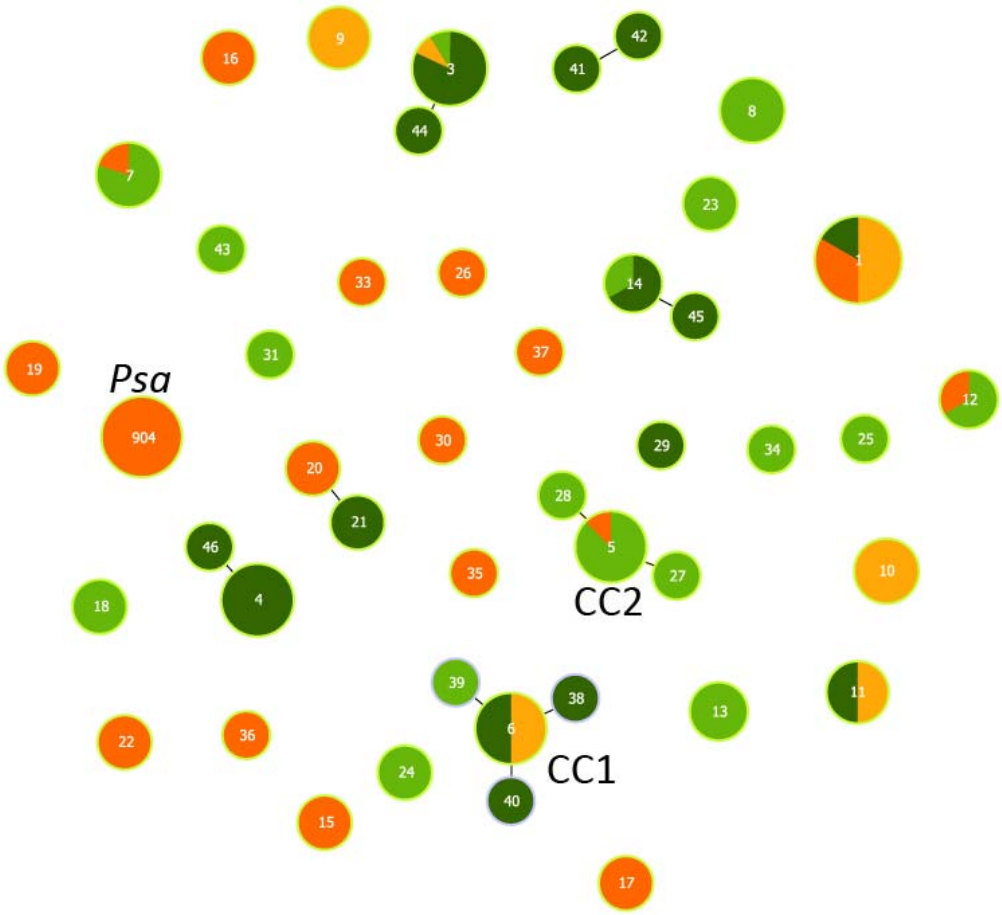


Figure S3. eBurst snapshot of STs at the single locus variant level. The size of the circles correlates with the frequency of the respective ST found in the dataset. Colours correspond to the different orchards, orange = infected 'Hort16A', yellow = uninfected 'Hort16A, dark green = infected 'Hayward', light green = uninfected 'Hayward'. CC = clonal complex.

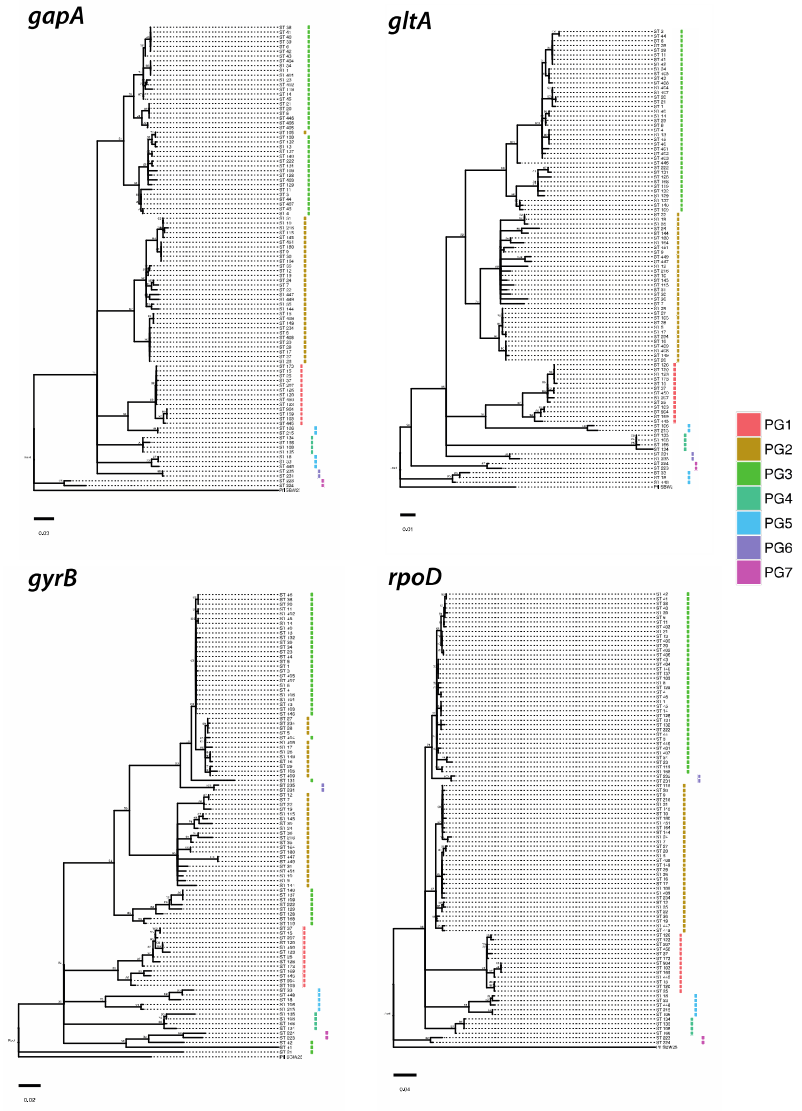
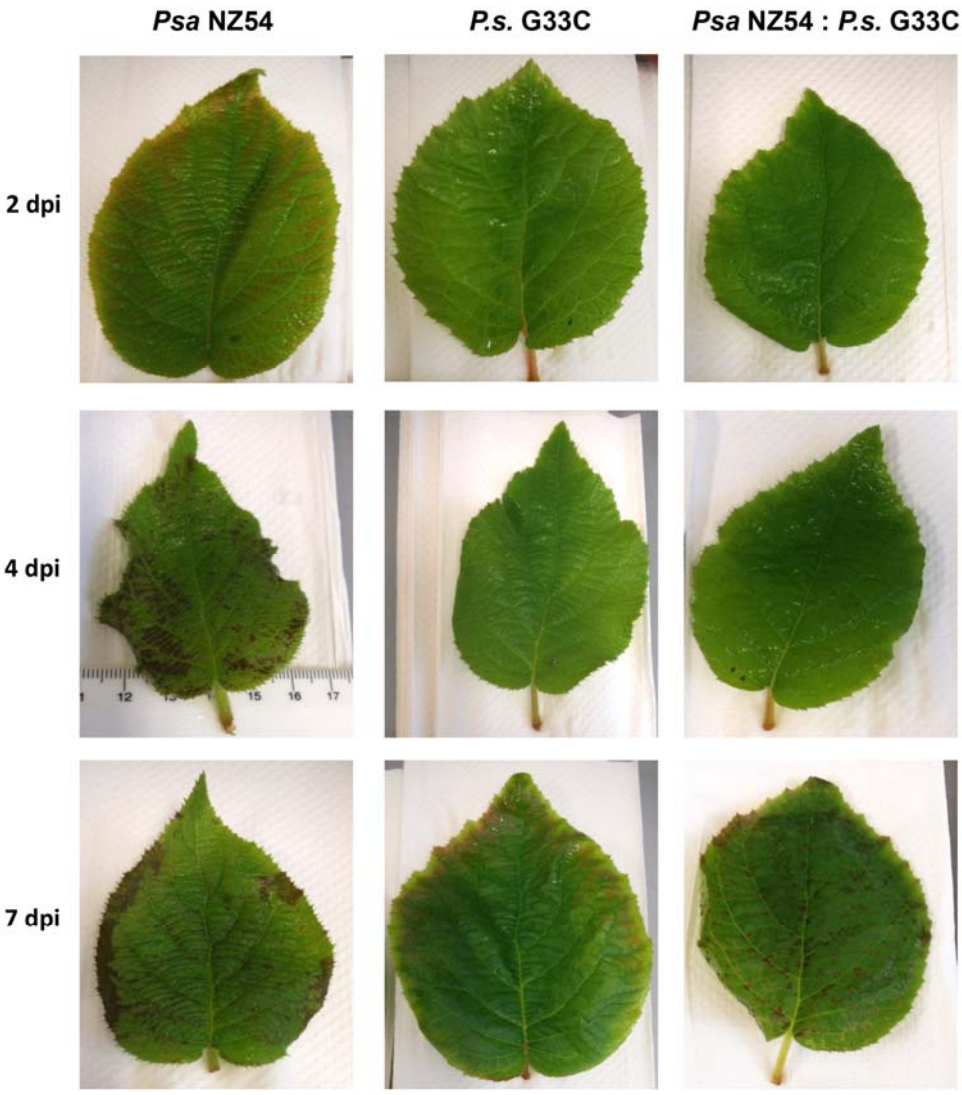


Figure S4. Maximum Likelihood trees based on single genes. Each Maximum Likelihood tree is rooted on *Pseudomonas fluorescens* SBW 25 and was reconstructed using TREEPUZZLE based on the Tamura-Nei model using 100,000 puzzling steps. Trees were built using single representatives of each unique ST to improve readability of the tree. Values indicated at nodes are bootstrap values. The corresponding phylogroup distinctions based on the concatenated ML tree are indicated with the coloured squares.



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1063 **Figure S5. Leaves of 'Hort16A' plants inoculated with *Psa* NZ54, *P. syringae* G33C**

1064 **and a 1:1 mix of *Psa* NZ54 : *P. syringae* G33C at 2, 4, and 7 days post inoculation.**

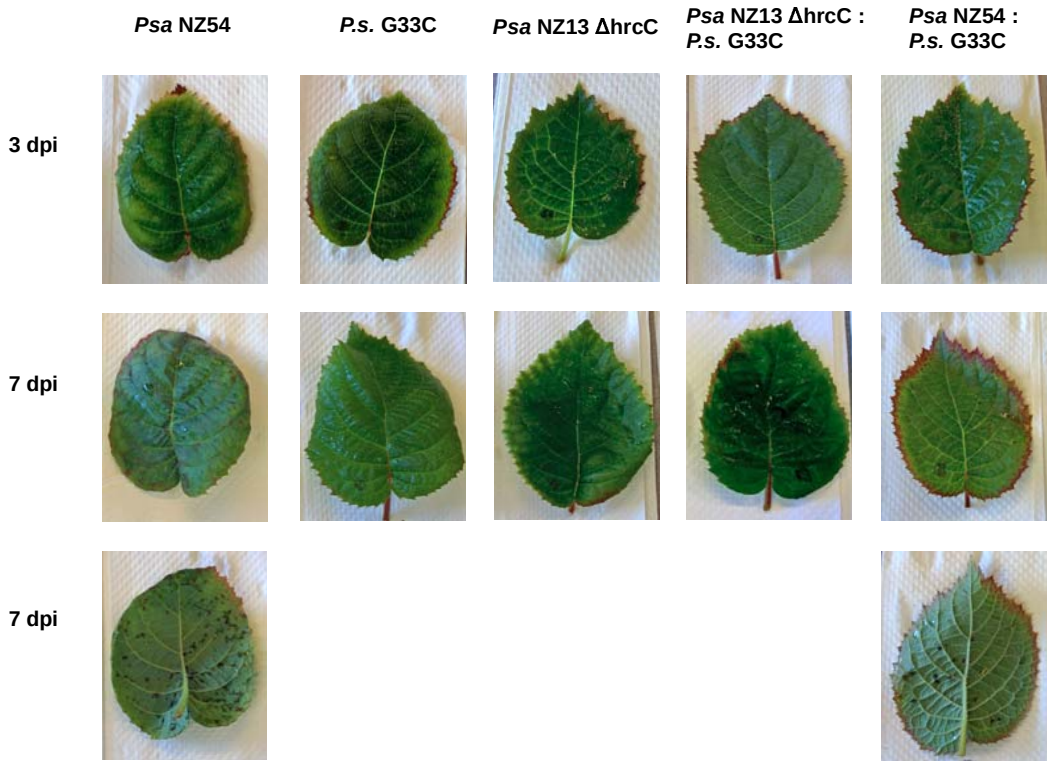
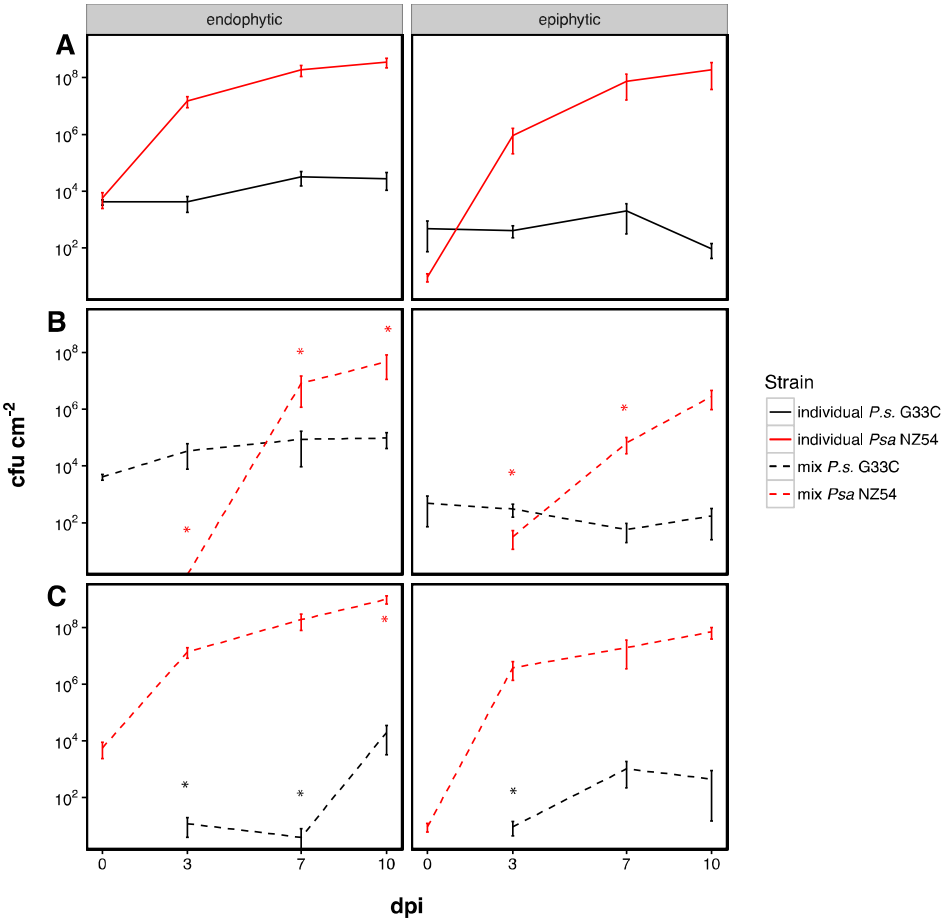


Figure S6. Leaves of 'SunGold' plants inoculated with *Psa* NZ54, *P. syringae* G33C, *Psa* NZ13 ΔhrcC and 1:1 mix of the respective strain combinations at 3 and 7 days post inoculation. For leaves showing minor leaf spots, the lower side of the leaf is also shown for easier detection of symptoms.



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1070 **Figure S7. *In planta* priority effect of *Psa* NZ54 or *P. syringae* G33C with subsequent**

1071 **inoculation of the second strain with 100-fold lower concentration.** *In planta*

1072 growth assay using 'Hort16A' plantlets pre-inoculated ($8x10^7$ cfu ml^{-1}) with one strain

1073 followed by inoculation of the second strain at ($8x10^5$ cfu ml^{-1}). The two panels

1074 display growth curves for endo- and epiphytic growth respectively. A) Individual

1075 growth, B) inoculation of *Psa* NZ 54 at day 3 and C) inoculation of *P. syringae* G33C at

1076 3 dpi. Solid lines represent individual growth and dashed lines represent growth in

1077 competition. The presented mean and standard error were calculated from the

1078 mean of five individual measurements. Asterisks indicate significance between

1079 individual and co-cultured growth at the 5% level (paired *t*-test).

SUPPORTING TABLES

Table S1. Geographic location of orchards, strain summaries and diversity indices per orchard. Specification of cultivar and infection status at the time according to KVH (Kiwifruit Vine Health), orchard ID, GPS coordinates, location and month of sampling, N = number of collected *P. syringae* strains, N PG3a = number of PG3a strains in total sample, N STs = number of unique STs, N STs PG3a = number of unique STs grouping with PG3a, D = Simpsons index of diversity, D_c = converted to effective number of species, ED = Simpsons evenness.

Table S2. List of all strains. Strain information and assigned sequence type of strains used for MLST study (all) and strains used for phylogenetic analysis (highlighted in grey). Phylogroup association only provided for isolates used for phylogenetic analysis. Alias provides the name used for competition experiments.

Table S3. PERMANOVA results of 3-factor nested analysis for differences in genetic diversity.

Table S4. LDhat recombination analysis for host and disease status. Length of alignment in bp, number of sequences, number of segregating sites, mutation rate θ, recombination rate ρ and ratio ε (ρ/ θ).

Table S5. LDhat recombination analysis for global data sorted according to phylogroup (PG). Length of alignment in bp, N = number of sequences, number of segregating sites, mutation rate θ, recombination rate ρ and ratio ε (ρ/ θ).

Table S6. List of primers used for construction of the deletion mutant *Psa* NZ13 Δ*hrcC*.