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BRIEF REPORT

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The plant host environment influences competitive interactions between bacterial pathogens

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Abstract

Bacteria compete for resources in diverse environments using an array of antagonistic strategies, including the production of narrow-spectrum protein antibacterials termed bacteriocins. Although significant research has focused on bacteriocin-mediated dynamics in culture environments, little research has explored bacteriocin-mediated dynamics within a host context, particularly in plant environments. Here, we show that a bacterial plant pathogen, *Pseudomonas syringae* pv. *syringae* (*Psy*), expresses a bacteriocin both in culture and in leaf apoplast when co-inoculated with a bacteriocin-sensitive competitor, *P. syringae* pv. *phaseolicola* (*Pph*). Although there is an observable negative effect of the bacteriocin on the *Pph* population at most time points both in culture and in the leaf apoplast, a bacteriocin-mediated benefit to *Psy* was only observed when the producing strain was co-infiltrated at a low population frequency (1:9) into the leaf apoplast. At 6 days post-infiltration, *Psy* achieved an eightfold population increase compared to a bacteriocin-deficient mutant in the apoplast. No bacteriocin-mediated benefit for *Psy* was observed under the culture conditions tested. Additionally, we found that the bacteriocin-mediated benefit for *Psy* was dependent on the Type III Secretion System. Taken together, our results demonstrate that the fitness benefit of bacteriocin-mediated antagonism is influenced by interactions within the host plant.

INTRODUCTION

Microbial competition is pervasive throughout the microbiological world. Populations with overlapping niche requirements often engage in both direct (interference) and indirect (exploitation) competition (Ghoul & Mitri, 2016; Granato et al., 2019). Outcomes of microbial competition have numerous consequences at the population and community levels and can also be harmful for plant and animal hosts by causing disease. For bacteria, one of the most intensively studied forms of competition is interference competition mediated by the production of antimicrobials, including protein toxins called bacteriocins (Ghequire & De Mot, 2015; Kommineni et al., 2015; Majeed et al., 2011; Riley & Wertz, 2002). Most bacteriocins are narrow spectrum within an individual species, where they target strains closely related to the producer (Mills et al., 2017; Riley & Chavan, 2007). A group of bacteriocins known as tailocins are multi-protein complex bacteriocins that are morphologically and evolutionarily related to the tails of Caudovirales bacteriophages (Ghequire & De Mot, 2014; Hockett et al., 2015; Scholl, 2017). Due to their large size, tailocins particles must be released via cell lysis resulting in a cost to the individual producing bacterium (Scholl, 2017).

In silico and in vitro work has demonstrated the benefit of bacteriocin production is dependent on the environmental context. Initial studies examined competition with two populations of a producer and a sensitive

strain in a physically unstructured environment such as liquid broth (Chao & Levin, 1981). When both populations are at equal frequencies, they can reach equilibrium as the sensitive population is able to exploit the resources made available from the killing activity by the producer (Durrett & Levin, 1997). In this scenario, there is no fitness benefit for the producer as its population remains the same. However, when the environment is spatially structured (e.g. agar plate) the two species form microcolonies, which results in local interactions where bacteriocins only affect cells that are physically close to the producing cells, as well as resources made available by killing (Chao & Levin, 1981; Kerr, 2007). This allows the producer to preferentially gain a fitness benefit, an increase in population size, from the available resources and space.

Competition within host environments can add an additional level of complexity compared to a static lab culture setting, given the host's potential to sense and respond to microbial invaders. In mice models, bacteriocin production by the human pathogen *Salmonella enterica* in the gut provides a competitive advantage against *Escherichia coli*, but only if *S. enterica* is able to induce inflammation (Nedialkova et al., 2014). The change in the gut environment results in both increased bacteriocin production by *S. enterica* as well as expression of the bacteriocin surface receptor in *E. coli*. Other in vivo studies in animal systems have also shown that bacteriocin production can result in the reduction or elimination of targeted populations (Corr et al., 2007; Kommineni et al., 2015; Sassone-Corsi et al., 2016; Yu et al., 2020), though it is not clear to what extent interaction with the animal host was important for the competitive outcomes in these studies. Indeed, there have been very few studies that have explicitly assessed the role of the host in affecting bacteriocin-mediated interactions.

Bacteriocins have also been shown to promote invasion of the producer into another population. Invasion into a sensitive population by six *E. coli* colicin producers was positive-frequency dependent (Riley & Gordon, 1999). When the number of *E. coli* producing cells increased, resulting in a higher toxin particle number, the time needed for invasion decreased. Whereas the growth of another *E. coli* bacteriocin producing population was negative-frequent dependent, in that bacteriocin production was beneficial when the producer's population was small relative to the competitor (Chao & Levin, 1981). Furthermore, higher bacteriocin production rates aided invasion into a bacteriocin-sensitive population, especially at low initial cell frequencies (Ghazaryan et al., 2019). Contrary to these findings, modelling predicts a different outcome where the benefits for the producer at low frequency are reduced as resources liberated by bacteriocin killing will be as likely to benefit the sensitive population as it will the bacteriocin producing population (Inglis et al., 2009; Weber et al., 2014).

Beyond basic ecological questions, understanding the fitness benefit of bacteriocins has implications for the creation of biological control agents. For plant health, we have relied on antagonist mechanisms such as toxins and antibiotics; however, it could be useful to also think about when and where it is beneficial for the agent to antagonize a target pathogen. Bacteriocins are of increasing interest as they could reduce non-target effects as observed with chemicals (McEvoy, 1996; Montesinos, 2007). On the surface of plant cells within the apoplast, bacteria can form microcolonies of single or multiple species where they can interact and compete (Bogino et al., 2013; Morris & Monier, 2003). In addition, bacteria have to evade host defences to successfully populate using virulence factors or by *in trans* effector-mediated plant defence suppression for example (Dodds & Rathjen, 2010; Rufian et al., 2018; Singh & Singh, 2018; Xin et al., 2018). To date, there are few studies that have investigated the role of bacteriocin-mediated antagonism in a plant context, let alone in the apoplast, thus it is not clear how much dynamic interaction there was with the host (Dorosky et al., 2018; Godino et al., 2016; Hert et al., 2005; Li et al., 2020).

To investigate plant-associated bacteriocin-mediated competition, we used *Pseudomonas syringae* as a model, as it is possible for multiple distantly related strains of this species to infect the same plant host and many can produce bacteriocins to antagonize competitors (Hirano & Upper, 2000; Holtsmark et al., 2008). Here, we performed a series of in vitro co-inoculations and *in planta* co-infiltrations over an 8-day period with *P. syringae* pv. *syringae* (*Psy*) and *P. syringae* pv. *phaseolicola* (*Pph*), both virulent plant pathogens that cause bacterial brown spot and halo blight in *Phaseolus vulgaris* (Common bean), respectively (Burkholder, 1926; van Hall, 1902). *Psy* is a generalist pathogen that can infect multiple hosts, whereas *Pph* is a specialist with a narrow host range of legume species (Baltrus et al., 2011; Morris et al., 2019). Key to this interaction is that *Psy* encodes a bacteriocin that targets *Pph* (Hockett et al., 2015). In this study, we sought to answer two related questions. First, under what ecological conditions is bacteriocin production beneficial for the producer? Second, how do host interactions influence the fitness benefits of bacteriocin production (i.e. an increased population size when in competition)? This work highlights the importance of understanding how host structure and activity influence microbial competition and is a critical step to improve disease suppression in plant and animal hosts.

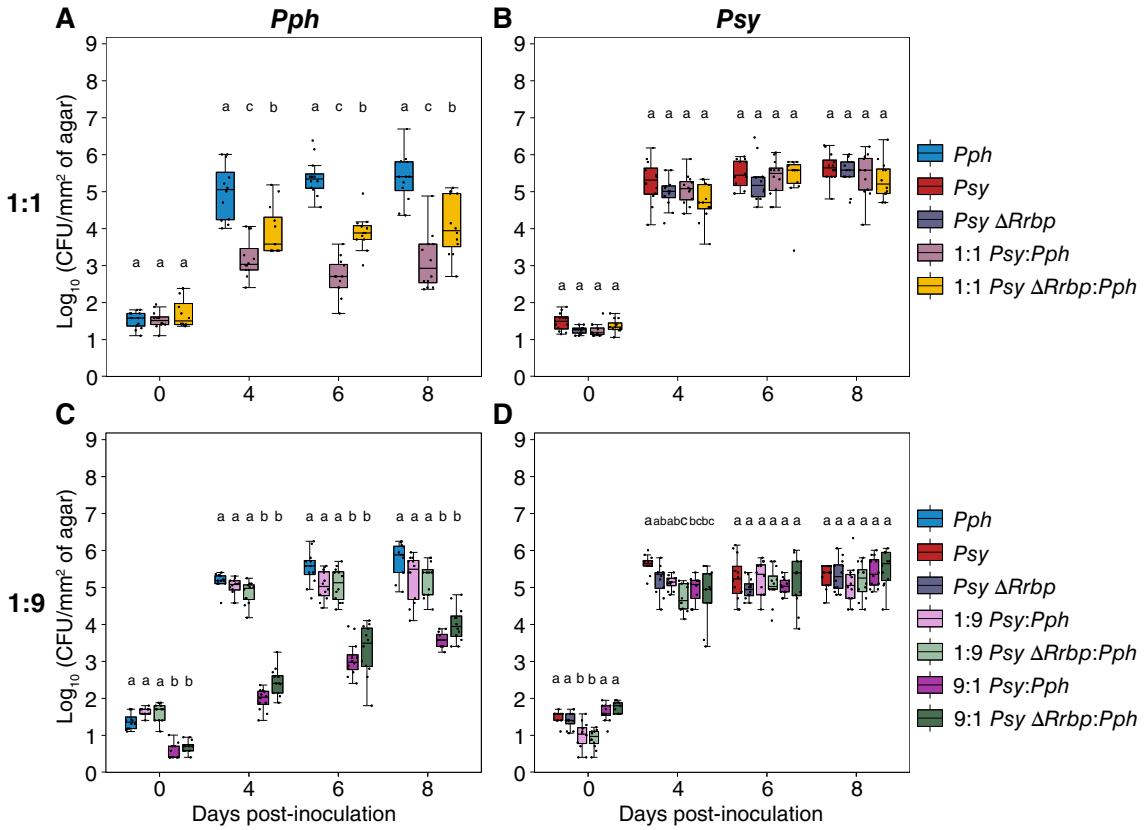

FIGURE 1 Bacteriocin-mediated competition in vitro detrimental for sensitive strain, yet no fitness benefit for producer. Bacterial populations of *Pph* in (A) 1:1 and (C) 1:9 initial starting frequencies (*Psy*:*Pph*), and *Psy* strains in (B) and (D), respectively, are shown across 8 days post-inoculation. Data points represent four replicates from three independent experiments and error bars indicate standard error of the mean. Average values with the same letter are not significantly different by Tukey's HSD test ($p \leq 0.05$)

RESULTS

Bacteriocins are expressed in vitro but there is no detectable fitness benefit for *Psy* at 1:1 and different co-inoculation ratios

To investigate whether bacteriocin-mediated antagonism provides a fitness benefit within an agar environment, we spotted individual or mixed strains (1:1) of either *Psy* (bacteriocin-producer) or *Psy* Δ*Rrbp* (bacteriocin-deficient mutant; Hockett et al., 2015), and *Pph* (bacteriocin-sensitive) on KB agar. At several time points post-inoculation, the growing culture was sampled, and strains were enumerated (Table S1). *Pph* populations were reduced in co-inoculation with either *Psy* or *Psy* Δ*Rrbp* at all time points in comparison to *Pph*-only [Figure 1(A)]. The population reduction was greater for *Pph* co-inoculated with *Psy* compared to *Psy* Δ*Rrbp*, suggesting the bacteriocin was expressed and active under these culture conditions. Bacteriocin production did not provide a detectable fitness benefit in 1:1 co-inoculation in an agar setting due to no significant fitness differences between *Psy* or *Psy* Δ*Rrbp* populations in individual or co-inoculations [Figure 1(B)]. Individual inoculation of the bacteriocin-deficient complement strain *Psy* Δ*Rrbp*:*Rrbp* at 4 dpi seemed to be greater by threefold compared to *Psy* Δ*Rrbp*; however, the statistical difference is only true in one of three independent experiments [$p \leq 0.04$; Figure S2(B)].

As bacteriocin production in 1:1 co-inoculation did not result in a fitness benefit for *Psy*, we sought to assess whether the population frequency influenced competition, as has been previously shown in other systems (Chao & Levin, 1981; Gordon & Riley, 1999; Inglis et al., 2009; Kerr, 2007). We altered the inoculation frequency between *Psy* strains and *Pph* to either 1:9 or 9:1. For example, '*Psy* minority' would represent 1:9 (*Psy*:*Pph*), and vice versa for '*Psy* majority' at 9:1 (*Psy*:*Pph*). In vitro *Pph* minority populations were reduced in co-inoculation with *Psy* or *Psy* Δ*Rrbp* compared to *Pph*-only across all time points [$p \leq 0.0001$; Figure 1(C)]. In *Pph* majority competitions, *Pph* growth with *Psy* and *Psy* Δ*Rrbp* was not different to *Pph*-only [Figure 1(C)]. Somewhat unexpectedly, *Psy* minority reached a population equivalent to the *Psy* majority treatments by 4 dpi [$p \leq 0.0001$; Figure 1(D)]. This

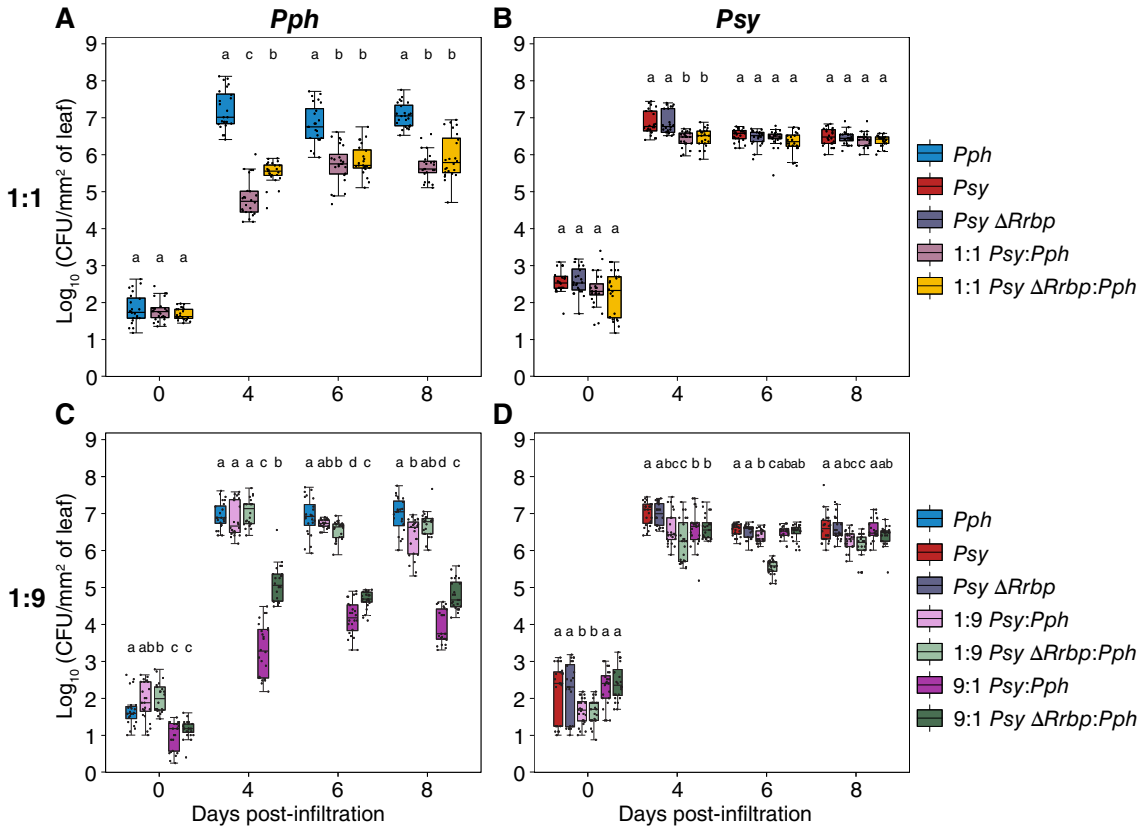

FIGURE 2 Initial low frequency provides fitness benefit for bacteriocin producer at select timepoint. Initial inoculation frequencies of 1:1 are shown in (A) and (B) and 1:9 in (C) and (D) for both *Pph* and *Psy* strain populations (*Psy*:*Pph*). Black dots represent eight replicates across three independent assays over 8 days. Statistically, differences from Tukey's HSD test ($p \leq 0.05$) represented by letters and error bars indicate standard error of the mean

trend was maintained at 6 and 8 dpi. As expected, the *Psy* Δ*Rrbp*:*Rrbp* population behaved similarly to *Psy* and *Psy* Δ*Rrbp* in both individual and mixed inoculations [Figure S2(D)]. Taken together, in vitro, there was no detectable benefit to bacteriocin production for *Psy*, yet a negative effect of bacteriocins on *Pph* is clearly observed.

Psy is competitively superior to *Pph* in 1:1 plant co-infiltration regardless of bacteriocin production

To determine whether the leaf apoplast environment affects the competitive interactions between *Psy* and *Pph*, we infiltrated common bean leaves with either individual or 1:1 mixed inocula of the same strains used in the in vitro assay. Similar to our in vitro results, we found that co-infiltration with *Psy* resulted in a 10-fold greater reduction of the *Pph* population than co-infiltration with *Psy* Δ*Rrbp* [$p \leq 0.0001$; Figure 2(A)]. This effect, however, was only observed at 4 dpi. There were no statistical differences after this timepoint, where the *Pph* population was suppressed by a similar amount by both *Psy* and *Psy* Δ*Rrbp*. The complement strain *Psy* Δ*Rrbp*:*Rrbp* showed a similar reduction of *Pph* at 4 dpi, suggesting the *Pph* reduction in co-infiltrations is due to bacteriocin production [$p \leq 0.0001$: Figure S3(A)].

For *Psy*, there was no difference in population growth for *Psy*-only compared to *Psy* Δ*Rrbp*-only at all dpi [Figure 2(B)]. There was, however, a sevenfold reduction in population size of *Psy* and *Psy* Δ*Rrbp* in 1:1 co-infiltration at 4 dpi compared to *Psy*-only and *Psy* Δ*Rrbp*-only, respectively ($p \leq 0.0001$). Therefore, 1:1 co-infiltration with a sensitive strain shows that bacteriocin production provided no fitness benefit for *Psy*.

At low frequency, bacteriocin production provides *Psy* a fitness benefit when competing with *Pph* in the apoplast

The negative effect of the bacteriocin production on *Pph* minority was apparent at all time points, with no effects on *Pph*-majority populations [Figure 2(C)]. Notably, there was an 80-fold reduction for co-infiltrated *Pph*

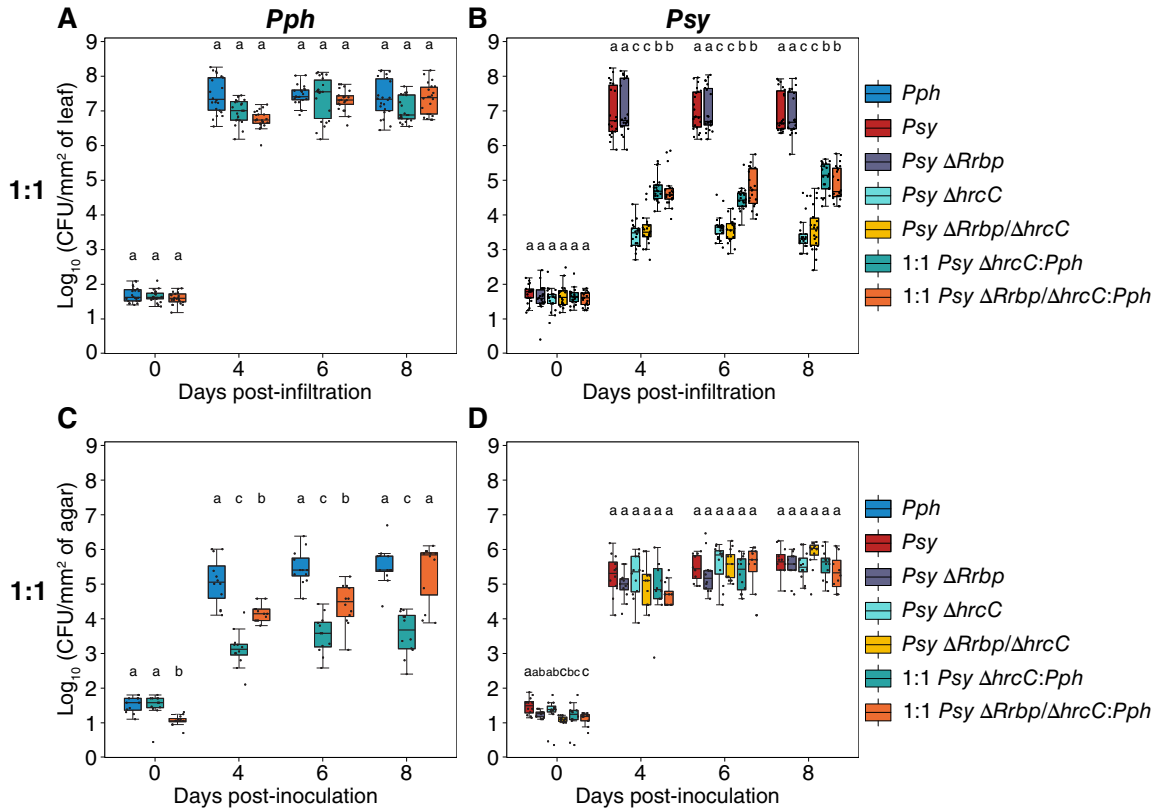

FIGURE 3 Avirulent *Psy* Δ*hrcC* cannot reduce sensitive population 1:1 *in planta* competition. Bacterial growth of *Pph* strains is presented (A) in culture and (C) *in planta*, along with Pph (C) in vitro and (D) *in planta* from initial 1:1 frequency (*Psy*:*Pph*). Data points across 8 days post-inoculation represent eight and four replicates, for *in planta* and in vitro respectively, from three independent experiments and error bars indicate standard error of the mean. Average values with the same letter are not significantly different by Tukey's HSD test ($p \leq 0.05$)

minority with *Psy* at 4 dpi compared to *Pph* minority co-infiltration with *Psy* Δ*Rrbp* ($p \leq 0.0001$). The differences in population to *Pph*-only were greater for *Pph* minority with *Psy* (6000-fold) in comparison to co-infiltration with *Psy* Δ*Rrbp* (80-fold; $p \leq 0.0001$). The differences between co-infiltrated *Pph* minority populations decrease at 6 and 8 dpi, yet the population sizes of co-infiltrated compared to *Pph*-only remained fairly similar to levels at 4 dpi ($p \leq 0.0001$).

When *Psy* minority is co-infiltrated with *Pph* there is a statistical eightfold increase compared to co-infiltrated *Psy* Δ*Rrbp* minority at 6 dpi [Figure 2(D); $p \leq 0.0001$]. Both *Psy* minority and *Psy* Δ*Rrbp* minority performed equivalently at 4 or 8 dpi, indicating this was the first observation that bacteriocin production is beneficial when faced with a dominant sensitive population. The *Psy* Δ*Rrbp* minority presented between a 5- to 10-fold reduction at all time points to *Psy* Δ*Rrbp*-only ($p \leq 0.0001$ for all comparisons). The complement *Psy* Δ*Rrbp*:*Rrbp* minority population level was similar to *Psy* minority indicating the population increase is due to bacteriocin production [Figure S3(B)]. There were no differences between *Psy* majority and *Psy* Δ*Rrbp* majority when co-inoculated with *Pph* [Figure 2(D)]. Additionally, *Psy* majority strains exhibited a decrease in population relative to the *Psy*-only infiltration at 4 dpi but maintained roughly equivalent populations at 6 and 8 dpi. Overall, *Psy* starting at a low cell frequency (in the minority) provided a bacteriocin-mediated fitness benefit.

Psy virulence is required for *in planta* bacteriocin-mediated effects

To investigate the role of virulence in pathogen–host interactions, a mutation of the *hrcC* gene, a structural component of the Type III Secretion System (T3SS), was introduced into *Psy*. Δ*hrcC* mutants are impaired in their ability to suppress plant defences, and thus incapable of causing disease (Deng et al., 1998; Hirano et al., 1999). The *Pph* population *in planta* was not affected during 1:1 co-infiltration with either *Psy* Δ*hrcC* or *Psy* Δ*Rrbp*/Δ*hrcC* and was able to maintain populations comparable to *Pph*-only infiltration for all dpi [Figure 3(A)]. This was opposite to in vitro

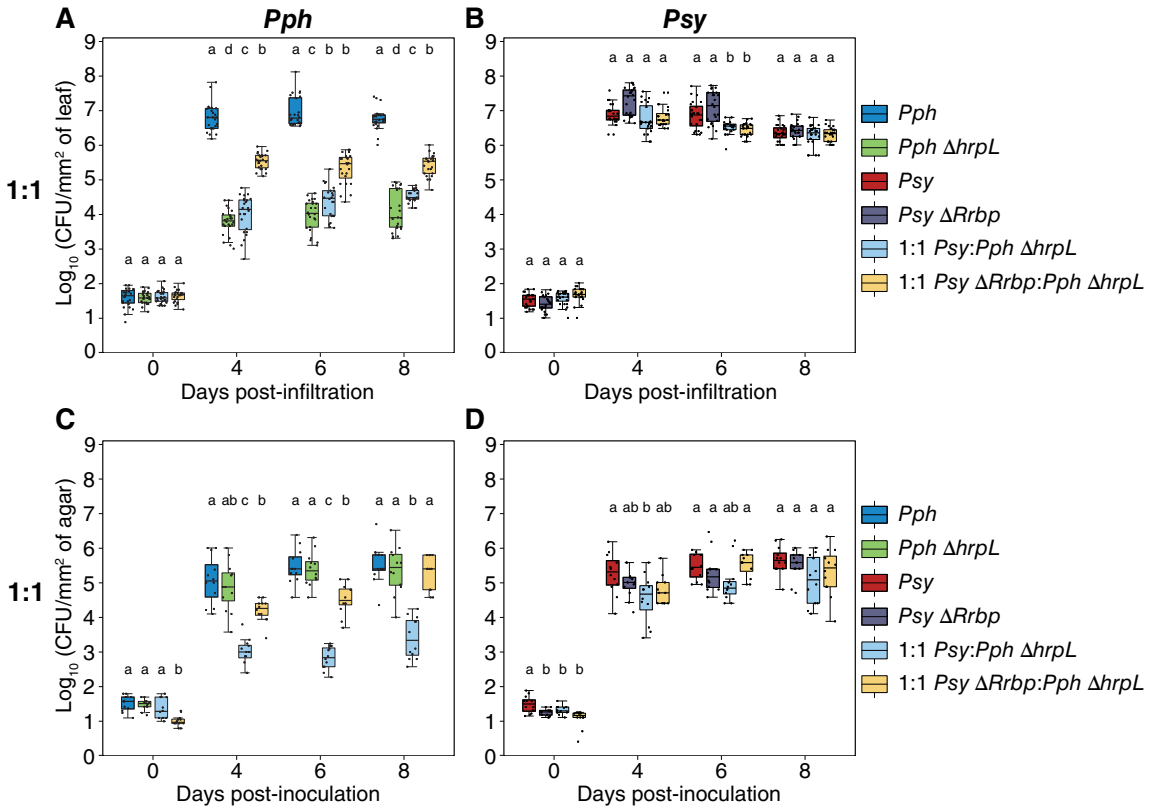

FIGURE 4 No difference in populations of *Psy* strains with avirulent *Pph* Δ*hrpL* populations. In 1:1 competition across 8 dpi bacterial growth is shown for (A) *Pph* and (B) *Psy in planta*, and (C) *Pph* and (D) *Psy* in culture (*Psy*:*Pph*). Data points represent eight and four replicates, for *in planta* and in vitro, respectively, from three independent experiments over 8 days and error bars indicate standard error of the mean. All treatments were compared by a Tukey's post hoc test (ANOVA) and significant differences are represented by letters ($p \leq 0.05$)

competition, where *Pph* co-inoculated with *Psy* Δ*hrcC* at 4 and 6 dpi was reduced by 10-fold compared to the 100-fold reduction at 8 dpi with *Psy* Δ*Rrbp*/Δ*hrcC* [-Figure 3(C)]. In comparison to *Pph*-only, a 100-fold and 10-fold detriment occurred for *Pph* in co-inoculation with *Psy* Δ*hrcC* and *Psy* Δ*Rrbp*/Δ*hrcC*, respectively.

No differences were observed between *Psy* Δ*hrcC* and *Psy* Δ*Rrbp*/Δ*hrcC* populations *in planta* during 1:1 co-infiltration with *Pph* [Figure 3(B)]. Yet, these populations were partially rescued when co-infiltrated with *Pph* by an average increase of 8- to 20-fold at 6 and 8 dpi compared to *Psy* Δ*hrcC*-only and *Psy* Δ*Rrbp*/Δ*hrcC*-only ($p \leq 0.0001$). Both individual and co-infiltrations of *Psy* Δ*hrcC* and *Psy* Δ*Rrbp*/Δ*hrcC* were reduced by 50- to 100-fold from 4 to 8 dpi compared to *Psy*-only infiltration ($p \leq 0.0001$). Whereas in vitro competition showed no differences between any individual and mixed *Psy* strains [Figure 3(D)]. These results show that virulence is required for *Psy* to dominate the co-infection environment, as well as to gain a fitness benefit from bacteriocin production.

No bacteriocin-mediated fitness benefit for *Psy* in co-infiltration with avirulent sensitive strain

To identify if *Psy* could gain a fitness benefit in co-inoculations with an avirulent sensitive strain, the *hrpL* gene, required for the activation of the *hrp*/*hrc* locus responsible for T3SS expression, was knocked-out in *Pph* (Hockett et al., 2015; Ortiz-Martín et al., 2010). The *Pph* Δ*hrpL* populations *in planta* were reduced in both individual (1000-fold) and co-infiltrations (between 10- to 100-fold) compared to *Pph*-only from 4 dpi [$p \leq 0.0001$; Figure 4(A)]. For all timepoints the co-infiltration of *Pph* Δ*hrpL* with *Psy* resulted in a 9- to 30-fold increase to *Pph* Δ*hrpL*-only ($p \leq 0.0001$). However, the population of *Pph* Δ*hrpL* co-infiltration with *Psy* was lower than co-infiltration with *Psy* Δ*Rrbp* at 4 and 8 dpi ($p \leq 0.0302$). In vitro competition resulted in a similar trend with co-inoculated *Pph* Δ*hrpL* presenting a 20-fold reduction with *Psy* compared to *Psy* Δ*Rrbp* at 4 dpi and reduced by 70- to 80-fold at 6 and 8 dpi [$p \leq 0.0001$; Figure 4(C)]. By 8 dpi, the population

of *Pph* Δ*hrpL* co-inoculated with *Psy* Δ*Rrbp* is equal to *Pph* Δ*hrpL*-only.

The co-infiltration of *Psy* with *Pph* Δ*hrpL* was not statistically different *in planta* to co-infiltrated population of *Psy* Δ*Rrbp*, and both *Psy*-only and *Psy* Δ*Rrbp*-only [Figure 4(B)]. However, in vitro co-inoculated *Psy* was statistically reduced by threefold compared to *Psy*-only [$p \leq 0.033$; Figure 4(D)]. Together these results suggest a virulent producer does not benefit from bacteriocin-mediated competition with an avirulence sensitive population.

DISCUSSION

In this study, we sought to understand how interactions with a host plant affect bacteriocin-mediated competition between two bacterial plant pathogens. Overall, our in vitro results did not show a bacteriocin-mediated fitness benefit (i.e. an increased population size) for the bacteriocin producer, *Psy*, at any starting frequency when competing with the sensitive strain, *Pph*. Conversely, *in planta* co-infiltrations did show a bacteriocin-mediated fitness benefit for *Psy* when at an initially low frequency. *Pph*, however, suffered from bacteriocin-mediated inhibition during both in vitro and *in planta* co-inoculation. Intriguingly, the *in planta* benefit to *Psy* and detriment to *Pph* occurred at specific time points and were not maintained consistently across all time points. Additionally, virulence aided *Psy* bacteriocin-mediated suppression of *Pph* in the plant environment. These results indicate that bacteriocin-mediated interactions within a host plant are influenced by host physiology and pathogen virulence over the course of an infection.

Previous bacteriocin antagonism studies have been performed using computer models or laboratory systems, showing that a fitness benefit for the toxin-producing population is dependent on the environment (Chao & Levin, 1981; Kerr, 2007; Majeed et al., 2011). Our results suggest that when at parity or in the majority there was no bacteriocin-mediated fitness benefit in vitro for *Psy* compared to *Psy* Δ*Rrbp* across 8 dpi. It is likely that the sampling of the entire colony is a global measurement of the cumulative effects of local interactions between cells that might mask a fitness benefit that is localized to the colony periphery. The detriment was not a complete elimination of *Pph* as it is hypothesized that primarily sensitive cells at the edges are affected, with the cells near the centre of the microcolony being able to persist (Kerr, 2007). Both *Psy* minority strains are also able to overcome the initial low frequency (regardless of bacteriocin killing) compared to the *Pph* minority indicating *Psy* possesses some additional method of competitive advantage over *Pph*.

Differences in apoplast spatial structure, available resources and host compatibility create a dynamic host environment for two pathogens to compete that is more complex than an agar plate (Dangl & Jones, 2001; Farvardin et al., 2020; O'Leary et al., 2016; Rico & Preston, 2008). In 1:1 co-infiltration, the outcome of *in planta* competition was similar to the in vitro competition where there was no benefit for the bacteriocin producer but there was a bacteriocin mediated detriment to *Pph*, similar to the effects Li et al. (2020) showed for competition between *P. syringae* pv. *tomato* and *P. syringae* pv. *lachrymans* (Li et al., 2020). However, the detriment was not maintained from 6 dpi onwards, potentially indicating the effect of bacteriocin production in the apoplast is limited either by changes in behaviour of *Pph* or changes in the apoplast environment, or both. Previous work has suggested that both sensitive and producing populations are able to coexist through spatial partitioning (Czárán & Hoekstra, 2003; Kerr, 2007), which may occur in the leaf apoplast.

Our results indicate that bacteriocin production is beneficial in a negative-frequency dependent manner, where production is favoured when the population is low (Kerr, 2007; Müller et al., 2019). In our case, this fitness benefit was not observed immediately post-inoculation and occurred once the initial *Psy* minority had a high population level. Importantly, our results showed that the *Pph* population was significantly reduced by bacteriocin-mediated killing at 4 dpi and that the *Psy* population is suppressed when co-inoculated with *Pph* compared to *Psy*-only at the same time point. Taken together, these results indicate that it should have been possible to observe a bacteriocin-mediated benefit for *Psy* at 4 dpi. We also considered the use of the competitive index (CI) to present the fitness benefit, since we have paired populations of *Pph* and *Psy* strains for each treatment. We believe, however, that such calculations would be misleading as the CI will certainly show *Psy* performing better compared to *Psy* Δ*Rrbp*, but this difference would, in nearly all cases, result from less killing of *Pph* by *Psy* Δ*Rrbp* rather than any increase in the *Psy* population compared to *Psy* Δ*Rrbp*. This observation also occurs about the same time that disease symptoms for *Pph* were distinctly identifiable in individual infiltration (e.g. water-soaking and yellowing). Therefore, we hypothesize that during co-infiltration *Pph* can gain greater access to host nutrients at the height of disease progression increasing its population, and the bacteriocin production of *Psy* is able to overcome this growth whereas *Psy* Δ*Rrbp* cannot resulting in the reduction of *Psy* Δ*Rrbp* population at 6 dpi.

Bacterial pathogens must contend with the host plant defences to enable establishment and proliferation of their populations. Pathogenic bacteria can use the T3SS encoded by the *hrp* and *hrc* genes to suppress the plants response in nearby plant cells (Alfano et al., 2000; Arnold et al., 2003). During *in planta* infiltrations both *Psy* Δ*hrcC* and *Psy* Δ*Rrbp*/Δ*hrcC* populations were greatly reduced compared to *Psy*.

When co-infiltrated, however, T3SS mutant strains received an *in trans* benefit from the virulent *Pph* strain. This has been observed in other work with avirulent strains of *P. syringae* which relied on the proximity of virulent strains to reduce the plant cells effector-triggered immunity (Macho et al., 2007; Omer & Wood, 1969; Rufian et al., 2018). Similarly, *Pph* Δ*hrpL* benefitted from co-infiltration with virulent *Psy*. There was also no detriment for *Pph* in the presence of *Psy* Δ*hrcC*, indicating that *Psy* virulence is required for a bacteriocin-mediated effect on *Pph*.

While previous research has focused mainly on the outcomes of bacteriocin-mediated antagonism in vitro, we show that such outcomes may not be directly translated into a host plant environment. Our findings show that under certain frequency and temporal conditions bacteriocin production can promote *Psy* fitness while targeting the sensitive strain population. Further research is needed to elucidate the exact spatial distribution of the infiltrated bacteria, such as the use of fluorescent microscopy, alongside measuring the rates of bacteriocin production in the apoplast. Bacteriocin-mediated killing does not necessarily equate to a bacteriocin-mediated fitness benefit. We suggest that this dichotomy applies to past and current biological control research, where the objective typically is to limit the effects of the pathogen but there was no examination of the benefit of the agent to proliferate and maintain present in the field (Fravel, 1988).

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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