Project Title: Functional peptides as new tools for the control of fire blight

Report Type: Final Project Report

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WTFRC Collaborative Costs: None

Budget 1
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Item	1-Mar-23	1-Mar-24
Salaries		
Benefits		
Wages		
Benefits		
RCA Room Rental		
Shipping	$250.00	$250.00
Supplies	$13,150.50	$13,150.00
Travel	$232.00	$232.00
Plot Fees	$1,331.50	$1,398.00
Miscellaneous		
Total	$14,964.00	$15,030.00

Footnotes:

Travel: Travel to Columbia View Research Orchard 25 mi x 16 trips at $0.58 per mile

Plot fees: 0.5 acres at $2,663 per acre 2023, $2,796 per acre 2024.

Supplies include synthesis of the experimental compound ($12,000), and field trial supplies: $500 for Personal Protective Equipment and spray supplies, $500 for laboratory supplies, $150 for sprayer services after the trial.

OBJECTIVES

1. Determine the efficacy functional peptides in controlling fire blight by means of (i) evaluating flower cluster infections weekly for three weeks starting from when symptoms become visible, and (ii) enumerating the population levels of *Erwinia amylovora* 1, 4, and 7 days after inoculation.
2. Evaluate fruit marking.
3. Compare results with the ones obtained using either streptomycin (antimicrobial activity), Actigard (induction of plant systemic acquired resistance) or water (water-treated control).

SIGNIFICANT FINDINGS

Objective 1

- Peptides applied 4 times during bloom provided moderate disease control and may be best incorporated as rotational products in an integrated program during low-risk periods.
- In both years, peptides reduced *E. amylovora* populations. While in 2023 (high-risk scenario) the reduction was only significant 1 day after inoculation, in 2024 (moderate-risk scenario) *E. amylovora* populations were significantly lower than the water-treated control at 1, 4 and 7 days after inoculation.
- No interaction with surfactants allowed for use to improve efficacy.

Objective 2

- Peptides did not show fruit skin marking in any of the 25 fruit evaluated each year. None of the other treatments resulted in commercially important fruit skin marking of 3 or greater.

Objective 3

- Streptomycin (Firewall 50WP) and Actigard applied 3 times during the bloom period significantly reduced the number of infections compared to the peptides. Blossom Protect + Buffer Protect applied twice was not significantly different than the peptides in 2023, which was of high-risk for fire blight, but it significantly reduced infections in 2024.
- The reduction of *E. amylovora* population obtained with the peptides was comparable to the one obtained with streptomycin standard (antimicrobial control) at 1 day after inoculation in 2023 and at 1 and 4 days after inoculation in 2024.

RESULTS AND DISCUSSION

In vitro trials (2024)

Population levels of *E. amylovora* strain Ea153N were significantly reduced by both peptides, BP178 and BP100, showing logarithm reductions from 2 to 6 logarithms, depending on peptide type and concentration (Fig. 1). The peptide BP100 at the lower concentration (4.5 ppm) reduced *E. amylovora* populations by 2 logarithms, while applied at the highest concentration (18 ppm), the reduction was of 6 logarithms (reaching the detection limit of the plate counting technic). The peptide BP178 showed a strong reduction in *E. amylovora* population levels already at its lowest concentration (5.5 logarithm reduction after 3 h of contact test), and a 6-logarithm reduction (reaching the detection limit of the plate counting technic) when applied at the medium and high concentrations. BP100 at the highest concentration and BP178 at the medium and high concentrations were not significantly different than the streptomycin control (Firewall 50WP at 50 ppm equivalent to streptomycin at 25 ppm). Almost all reductions were observed 30 min after the contact test started, indicating the fast activity of these peptides.

The activity of the peptides was not affected by the addition of surfactants, neither Regulaid nor NuFilm P (Fig. 2). Both peptides tested at two of the three concentrations studied in the first experiment showed a reduction of 6 logarithms after a 3 h contact test, without differences between the presence or not of surfactant. For the water-treated control, the addition of surfactants significantly impacted (although slightly) *E. amylovora*, showing a small reduction in its population levels.

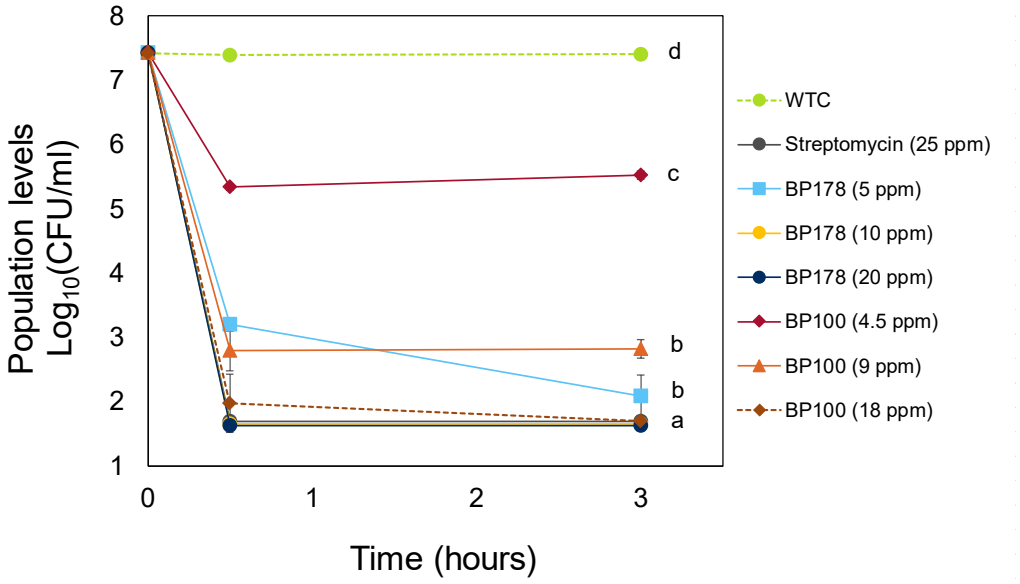

Figure 1. Effect of peptides at three different concentrations on the survival of *E. amylovora* strain Ea153N. A contact test was performed, and samples were taken after 30 min and 3 hours of incubation. Values are the means of three replicates and error bars represent the standard deviation of the mean. Different letters indicate significant differences between treatments and concentrations according to Tukey's test ($P < 0.05$).

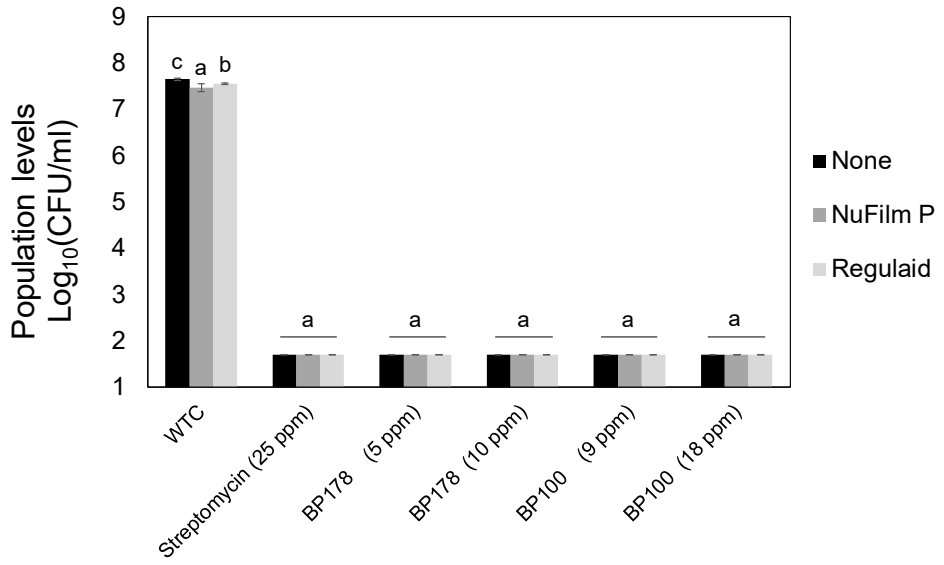

Figure 2. Effect of the surfactants on the activity of the peptides against *E. amylovora* strain Ea153N. Two different concentrations per peptide and two commonly used surfactants, Regulaid and Nufilm P, were tested. Values are the means of three replicates, and error bars represent the standard deviation of the mean. Different letters within a treatment indicate significant differences between the surfactants according to Tukey's test ($P < 0.05$).

Field trials (2023-2024)

In 2023, environmental conditions during bloom (28 Apr – 7 May 2023) were very conducive to fire blight disease (Appendix 1 and 2). Temperatures were warm and ranged from an average maximum of 78.2 °F to minimum of 51.5 °F with 47.4% average humidity. During the following week of petal fall (8 May – 14 May 2023), temperature ranged from an average maximum of 79.5 °F to a minimum of 49.6 °F with 45.5% average humidity. Three precipitation events occurred after the inoculation of *E. amylovora*, one on 5 May (0.04 in), approximately 31 h after inoculation (17 to 20 h after the full bloom + 1-day sprays), and the other two on 8 May, 20 to 23 h after petal fall sprays (0.01 in) and 28 to 31 h after petal fall sprays (0.06 in).

The peptide BP178 applied alone on the flower clusters the day before inoculation, the morning after inoculation and 4 days after inoculation (petal fall) resulted in 40.4 infections per 100 clusters, not significantly different than the water-treated control in 2023 (Table 1). The biological standard Blossom Protect + Buffer Protect, with 32 infections per 100 clusters, was also not significantly different than the water-treated control (19.2% relative control[1]). The streptomycin standard applied the morning before evening inoculation (full bloom), the morning after inoculation and 4 days after inoculation (petal fall) provided significant control with 7.2 infections per 100 clusters (81.9% relative control). The systemic acquired resistance inducer Actigard applied at first bloom (3 days before inoculation), the morning before evening inoculation (full bloom) and 4 days after inoculation (petal fall) resulted in 16.4 infections per 100 clusters (58.7% relative control), significantly different than the water-treated control and not significantly different than the streptomycin standard. No treatments resulted in commercially important fruit skin marking of 3 or greater (Table 1).

Table 1. Effect of the bifunctional peptide BP178 and controls applied to apple, cv. Gala, on infection of *E. amylovora* in apple blossoms in Wenatchee, WA, in 2023 [z]

Treatment	Amount per 100 gal	Timing [y]	Infections per 100 clusters [x]				Fruit skin marking [w]	
Streptomycin standard (Firewall 50WP) [v]	8 oz	3,4,6	7.2	±	1.6	a [u]	0.02	a
Blossom Protect + Buffer Protect	1.25 lb + 5 lb	2,3	32.0	±	2.0	b	0	a
Actigard [t]	2 oz	1,3,6	16.4	±	5.2	a	0	a
Bifunctional peptide BP178	4.3 oz (122 g)	2,4,6	40.4	±	3.8	b	0	a
Water-treated control	NA	3,4,6	39.6	±	4.9	b	0	a

[z] Inoculation was conducted on the evening of 3 May 2023 at full bloom (of king blooms) using a suspension of freeze-dried cells of *E. amylovora* strain Ea153N (streptomycin and oxytetracycline sensitive and nalidixic acid resistant strain) prepared at $5x10^6$ CFU ml^{-1} (verified at $4.2x10^6$ CFU ml^{-1}).

[y] Timings, 1: first bloom, 2: 70-90% bloom, 3: morning before evening inoculation (full bloom), 4: morning after inoculation, 5: 2 days after inoculation, 6: 4 days after inoculation (petal fall), 7: 7 days after inoculation

[x] Transformed sqrt(x + 1) prior to analysis of variance; non-transformed means are shown.

[w] Fruit skin marking is rated from an average of 25 fruit per tree. Rated on a 0 to 15 scale where ratings below 3 indicate no commercial downgrades.

[v] Amended with Regulaid: 16 fl oz per 100 gallons. Buffered to 5.6 pH.

[u] Treatments followed by the same letter are not significantly different at P=0.05 Fisher's T test (LSD).

[t] Amended with Regulaid: 16 fl oz per 100 gallons.

The peptide BP178 reduced *E. amylovora* populations at 1 and 4 days after inoculation, showing significant differences compared to the water-treated control 1 day after inoculation. The streptomycin

[1] 'relative control' (S_{rc}) $S_{rc} = (1 - I_t \div I_c) \times 100$ where I_t and I_c are incidence of diseases flower clusters for a treatment and the water-treated control respectively.

standard significantly reduced *E. amylovora* populations at all time points analyzed (1, 4 and 7 days after inoculation), while none of the other standards/treatments showed a reduction of the population size of the pathogen compared to the water-treated control (Fig. 3).

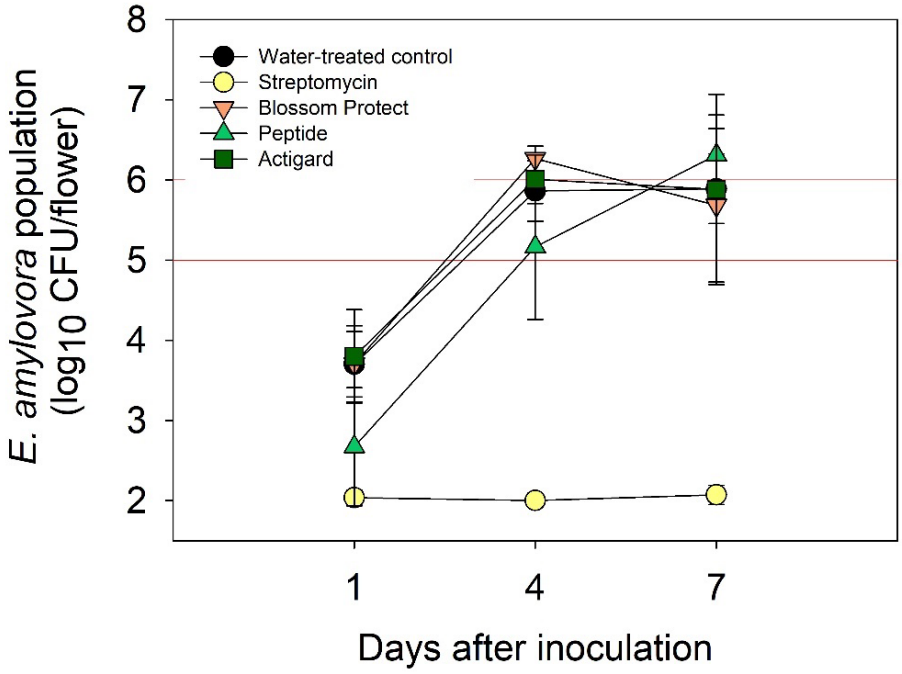

Figure 3. Effect of the peptide BP178 and controls applied to Gala apple trees on the population size of *E. amylovora* strain Ea153N on flowers 1 day after inoculation (full bloom + 1 day), at petal fall (full bloom + 4 days) and 3 days post petal fall (full bloom + 7 days).

In 2024, environmental conditions during bloom (15 Apr – 24 Apr, 2024) were cool and ranged from an average maximum temperature of 64.3 °F to minimum of 39.5 °F with 31.1% average humidity. During petal fall (April 25 – 1 May, 2024), temperature ranged from an average maximum of 63.0 °F to a minimum of 41.6 °F with 52.7% average humidity. One precipitation event occurred after the inoculation of *E. amylovora*, on 25 April (0.45 in), approximately 5 days after inoculation (26 to 30 h after the petal fall sprays) and another on 4 May (1.8 in) (Appendix 1 and 2). All applications were made under fast drying conditions.

The combination of both peptides, BP178 and BP100, resulted in 32.8 infections per 100 clusters, not significantly different than the water-treated control (38 infections per 100 clusters). The systemic acquired resistance inducer Actigard showed a significant reduction compared to the water-treated control, with 17.7 infections per 100 clusters (54% relative control). The antibiotic streptomycin significantly reduced infections (5.7 infections per 100 clusters, 81.5% relative control) compared to the water-treated control and both treatments tested. No treatments resulted in commercially important fruit marking of 3 or greater (Table 2).

The combination of both peptides significantly reduced *E. amylovora* populations at 1, 4 and 7 days after inoculation. The streptomycin standard significantly reduced *E. amylovora* populations at all time points analyzed (1, 4 and 7 days after inoculation), while none of the other standards/treatments, except Actigard at 4 days after inoculation, showed a significant reduction of the population size of the pathogen compared to the water-treated control (Fig. 4).

Table 2. Effect of the peptides and control treatments applied to apple, cv. Gala, on the infection of *E. amylovora* in apple blossoms in Wenatchee, WA, in 2024[*]

Treatment	Amount per 100 gal	Timing [z]	Infections per 100 clusters	Fruit skin marking [y]
Streptomycin standard (Firewall 50WP) [x]	8 oz	3,4,5	5.7 ± 1.3 a [w]	0.18 ± 0.05 a [v]
Blossom Protect + Buffer Protect	1.25 lb + 5 lb	1,3	21.9 ± 2.5 b	0.17 ± 0.04 a
Actigard [u]	2 oz	1,3,5	17.7 ± 2.8 b	0.27 ± 0.11 a
Peptide BP178 [t]	6 oz (170 g)	2,4		
Peptide BP100 [t]	2.8 oz (80 g)	5,6	32.8 ± 3.7 c	0.13 ± 0.03 a
Water-treated control	NA	3,4,5	38.0 ± 2.9 c	0.03 ± 0.01 a

[*] Inoculation was conducted on the evening of 20 Apr 2024 at full bloom (of king blooms) using a suspension of freeze-dried cells of *E. amylovora* strain Ea153N (streptomycin and oxytetracycline sensitive strain) prepared at $5x10^6$ CFU ml^{-1} (verified at $6.1x10^6$ CFU ml^{-1}).

[z] Timings 1: 15 Apr, 2: 17 Apr, 3: 20 Apr (full bloom), 4: 21 Apr, 5: 24 Apr (petal fall), 6: 27 Apr. All applications were conducted in the morning, and inoculation was conducted on the evening of 20 Apr.

[y] Fruit skin marking is rated from an average of 25 fruit per tree. Rated on a 0 to 15 scale where ratings below 3 indicate no commercial downgrades.

[x] Amended with Regulaid: 16 fl oz per 100 gallons. Buffered to 5.6 pH.

[w] Treatments followed by the same letter are not significantly different at P=0.05 Fisher's T test (LSD).

[v] Treatments followed by the same letter are not significantly different at P=0.05 Fisher's T test (LSD).

[u] Amended with Regulaid: 16 fl oz per 100 gallons.

[t] Amended with NuFilm P: 8 fl oz per 100 gallons. Buffered to 6.5 pH.

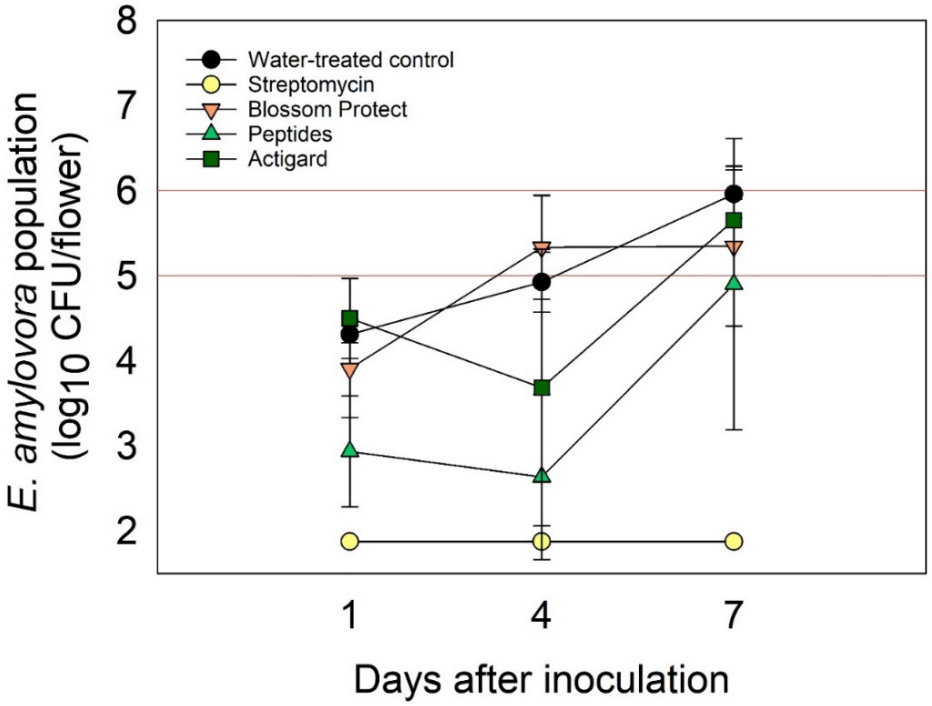

Figure 4. Effect of the peptides and control treatments applied to Gala apple trees on the population size of *E. amylovora* strain Ea153N on flowers 1 day after inoculation (full bloom + 1 day), at petal fall (full bloom + 4 days) and 3 days post petal fall (full bloom + 7 days).

Over the past decades, functional peptides have continuously been proposed as a potential tool to create both, more sustainable pesticides and disease resistant crops (Montesinos E. et al., 2012; Van Esse et al., 2020; Montesinos E., 2023), but more work is needed to study their efficacy in controlling diseases in the field. In the present grant, we studied the application of functional peptides for the control of fire blight in apple trees in Wenatchee, WA. Results from 2023, which was of exceptionally high-risk regarding fire blight conducive weather conditions, showed no control of fire blight with one of the experimental peptides applied on the flower clusters of young susceptible Gala apple trees the day before inoculation, the morning after inoculation, and 4 days after inoculation (petal fall) at a concentration of 4.3 oz/100 gal (1.22 g/3.785 L). The application of the peptide only to the area of the tree to be inoculated (flower clusters) using a manual pump 4-gallon backpack sprayer, as well as its application at a lower concentration than the one initially proposed due to reduced peptide availability for 2023 season could have been some of the reasons why we did not see control. Also, since peptides are experimental products, in 2023 the peptide was applied in the field by itself since no combinations with acidifiers or surfactants were tested.

In 2024, a couple of *in vitro* experiments were conducted prior to start the fire blight season in order to (i) confirm the bactericidal activity of the peptides against the *E. amylovora* strain Ea153N and determine their minimal bactericidal concentration, and (ii) test the effect of two commonly used surfactants on the bactericidal activity of the peptides to understand if we can combine them to obtain better results in the field. Results showed that both peptides tested have a high bactericidal activity against *E. amylovora* strain Ea153N and that their activity is mainly observed within the first 30 min of contact. Additionally, no negative effects were observed when peptides were mixed with Regulaid or NuFilm P at 8 fl oz/100 gal, indicating the possibility to include any of these surfactants in the treatment.

Results from the field trial in 2024 showed an improvement of the peptide's performance, probably due to an increase in the concentrations used, the addition of a fourth application, the application of the peptides to the whole tree using a Stihl SR420 mist blower backpack sprayer, and the combination of the peptides with the surfactant NuFilm P. Even though fire blight risk was moderate during 2024 (Appendix 2), a very similar percentage of infection was observed in the water-treated controls for both years, indicating that environmental conditions did not play a significant role in the increased performance of the peptides.

Reduction of *E. amylovora* populations was observed both years using the peptides; in 2024 the reduction was significant at all time points analyzed compared to the water-treated control, but only significant at 1 day after pathogen inoculation in 2023. This correlates with the increased control of the peptides observed in 2024, indicating that the bactericidal activity of the peptides is their main mechanism of action. The fact that in 2024 we applied the peptides to the whole tree, and not only to the flower clusters, could also have increased the activation of the defense system of apple trees by these peptides, especially the BP178 (Moll et al., 2022). However, a more in-depth study should be carried out in order to understand this mechanism, especially in the field.

Recent research suggests that the delivery of peptides using other application methods such as endotherapy are more effective in activating the plant defense system (Moll et al., 2024), and this could be an interesting idea to explore in order to achieve higher control. However, further advancement and assessment of endotherapy, considering technological aspects and social acceptance, are necessary. Another viable approach that has been proved more reliable than topical treatment could be the heterologous expression of peptides in plant crops, but its use is more restricted (genetic modified organisms are vaned in several countries), and it requires additional research in terms of food safety and environmental impact (Montesinos E., 2023).

Even though we obtained better results in 2024, the reduction in infections obtained with the peptides was not statistically significant compared to the water-treated control. Seeing that peptides are very active *in vitro*, and that the concentration used in the field is 15 to 50 times higher than their minimal bactericidal concentration, we hypothesize that clear and sunny spraying days could have impacted the activity of these peptides since proteins and peptides are generally known for being vulnerable to UV light (Gammelgaard et al., 2019). In 2024, all applications were done 1 to 2 h before sunrise, and this should have been enough time for the peptides to act against the pathogen, but would be interesting to study if evening applications improve their performance, as they are already recommended for other radiation sensitive products like the antibiotic kasugamycin (Slack S.M. et al., 2021).

Other mechanisms that could have affected the activity of the peptides against the target pathogen are (i) limited access of the peptide to the pathogen due to adsorption by envelopes or external structures (e.g., biofilm barriers, exopolysaccharides, capsules), (ii) active elimination from cells (e.g., efflux pumps, outer membrane vesicles secretion), (iii) degradation by proteases, (iv) or enzymatic chemical modification (Lima et al., 2021). To overcome these obstacles, the use of mixtures of peptides with different physicochemical characteristics and/or mechanisms of action could be of interest. Several physicochemical conditions and compounds can also reduce peptide activity (e.g., cations, pH, phenolics), especially from cationic amphipathic peptides like BP178 and BP100. Modifications of the peptide sequence with non-natural amino acids (e.g., including D-amino acids) (Ng-Choi et al., 2014), or adequate formulations (e.g., nanoencapsulation) could also be studied to minimize their impact.

METHODS

Synthesis of the bifunctional peptide (2023-2024, University of Girona)

Peptides were synthesized manually by the solid-phase procedure as previously described (Badosa et al., 2013). Briefly, a PAC-ChemMatrix resin (0.66 mmol/g) was used, and once the peptidyl sequence was completed, the resulting resins were treated with trifluoroacetic acid (TFA)/H_2O/triisopropylsilane (TIS) (95:2.5:2.5) for 2 h at room temperature. Following TFA evaporation and diethyl ether extraction, the crude peptide was dissolved in H_2O, lyophilized, analyzed by HPLC, and characterized by mass spectrometry to determine its purity.

The bifunctional peptide BP178 is a long chain peptide (29 amino acids), and its manual synthesis is still expensive, especially the large amounts needed for field trials. In 2023, only 2 g of the peptide were provided, and adjustments to the protocol were made in order to reduce the amount of peptide needed. In 2024, and with the aim of increasing the number of applications in the field and the dose of the peptide, the synthesis of 3.5 g of BP178 was combined with the synthesis of 2 g of BP100, an antimicrobial peptide that has proven to be effective against fire blight (from *in vitro* to the field). The synthesis of BP100 is most cost effective due to its shorter length (11 amino acids).

In vitro trials (2024, Washington State University)

To confirm the minimal bactericidal concentration of the peptides against *E. amylovora* strain Ea153N and assess the effect of two different surfactants on the activity of the peptides, two *in vitro* experiments were conducted. In both experiments, three replicates for each concentration, peptide, and surfactant were used, and a water-treated control and an antibiotic control (streptomycin at 25 ppm) were included.

Peptide and antibiotic preparation: Lyophilized peptides were solubilized in sterile distilled water buffered with phosphate buffered saline at 6.5 pH to a stock concentration of 1 mM. For the second

experiment, sterile distilled water buffered with phosphate buffered saline at 6.5 pH was amended with the corresponding surfactant at a concentration of 0.6 ml/L (equal 8 fl oz/100 gal). Dilutions of the stock solutions were made to obtain the desired final peptide concentrations. The antibiotic control (Firewall 50WP) was prepared in sterile deionization water and filter sterilized inside the hood using a 0.2 µmm filter.

Bactericidal activity of the peptides: The bactericidal activity of the peptides was tested against a cell suspension of *E. amylovora* strain Ea153N (nalidixic acid resistant), adjusted to 10^7 CFU/ml. A contact test was performed at final peptide concentrations of 5, 10, and 20 ppm (1.5, 3.1, and 6.25 µM) for BP178 and 4.5, 9, and 18 ppm (3.1, 6.25, and 12.5 µM) for BP100. The multi-well plate was incubated at room temperature under low shaking conditions (90 rpm) and samples were taken after 30 min and 3 h. Culturable cells were quantified using the plate counting method, consisting of plating 20 µl of 10-fold dilutions on nutrient agar amended with nalidixic acid, and incubating plates at 28°C for 2 to 3 days to determine the CFU/ml.

Effect of two surfactants on the activity of the peptides: The effect of two commonly used surfactants, Regulaid and Nufilm P, on the activity of both peptides was studied against a cell suspension of *E. amylovora* strain Ea153N (nalidixic acid resistant), adjusted to 10^7 CFU/ml. A 3 h contact test was performed at final peptide concentrations of 5 and 10 ppm for BP178 and 9 and 18 ppm for BP100 and CFU/ml were quantified using the plate counting method, as described above.

Field trials (2023-2024, Washington State University)

Site and plots: A 0.6 acre research block of 250 third to forth leaf apple trees cultivar Gala rootstock G41 planted on a 4 ft spacing at the WSU Columbia View Orchard 48 Longview Rd. East Wenatchee, WA 98802-8283 was used for the trials. Soils are Cashmont Gravely Sandy Loam with a 3-8% slope. The site has good air drainage and some wind protection.
The experiment was arranged in a randomized complete block with five single tree replicates (100+ flower clusters). Experimental blocks were spaced from one another by two buffer trees.

Treatments and inoculation: Treatments included the peptides, as well as positive and negative controls. Positive controls included the antibiotic streptomycin as a control for bactericidal activity, the systemic acquired resistance inducer acibenzolar-S-methyl (Actigard) as a control for plant defense activation, and Blossom Protect + Buffer Protect as an organic control. A water-treated control was applied as a negative control treatment. Treatments were applied to the whole tree according to manufacturer recommendations using a Stihl SR420 mist blower backpack sprayer. Products were applied to wet, near dripping previously calibrated to equal 100 gal/A. In 2023, the peptide was applied by tree to the area of the tree to be inoculated (flower clusters) using a manual pump 4-gallon backpack sprayer. Application dates in 2023 were: 30 Apr (1), 2 May (2), 3 May (3, full bloom), 4 May (4), 5 May (5), 7 May (6, petal fall), 10 May (7); in 2024 were: 15 Apr (1), 17 Apr (2), 20 Apr (3, full bloom), 21 Apr (4), 24 Apr (5, petal fall), 27 Apr (6). All applications were made under fast drying conditions. At 80-90% bloom (of the king blooms), on 3 May 2023 and 20 April 2024, *E. amylovora* strain Ea153N (streptomycin and oxytetracycline sensitive and nalidixic acid resistant strain) was applied at $5x10^6$ CFU ml^{-1} (verified at $4.2x10^6$ CFU ml^{-1} and $6.1x10^6$ CFU ml^{-1}) to lightly wet each cluster using freeze dried inoculum. A 4-gallon backpack sprayer (solo) will be used to lightly wet clusters.

Evaluation: Trees were visually evaluated for flower cluster infection weekly from when symptoms became visible, 9 to 16 days after inoculation, for 2 weeks and infection counts summed across all dates. Infected flower clusters were removed in each evaluation. *E. amylovora* was enumerated 1, 4, and 7 days after inoculation from a bulk sample of 10 flowers per replicate (2 flowers from 5 different clusters). Flowers were sonicated in sterile DI water for 3 minutes and a 10-µl sample of the wash and

two 1:100 dilutions were spread on nutrient agar amended with nalidixic acid (50 µg/ml) and cycloheximide (50 µg/ml) to selectively enumerate *E. amylovora* (Ea153N). Fruit was evaluated for fruit skin marking before fruit colored over. 25 fruit per replicate were rated. Russet ratings are on a 1 to 15 scale with individual values lower than 3 considered insignificant for commercial packing. Environmental conditions were tracked on an hourly basis including temperature, humidity, leaf wetness, solar radiation and windspeed.

Statistical analysis

Statistical analysis was performed with SAS v 9.4. For the first *in vitro* experiment, general linear mixed models (MIXED) analysis of variance ANOVA with time as repeated measure was used. General linear mixed models (GLIMMIX) analysis of variance ANOVA was used for the second *in vitro* experiment. For both experiments, multiple means comparisons were performed according to the Tukey's honestly significant difference (HSD) test at a P value ≤ 0.05. For the field trials, general linear mixed models (GLIMMIX) analysis of variance ANOVA and multiple means comparison (LSD) for infections (normal distribution) and fruit marking (negative binomial distribution) were used.

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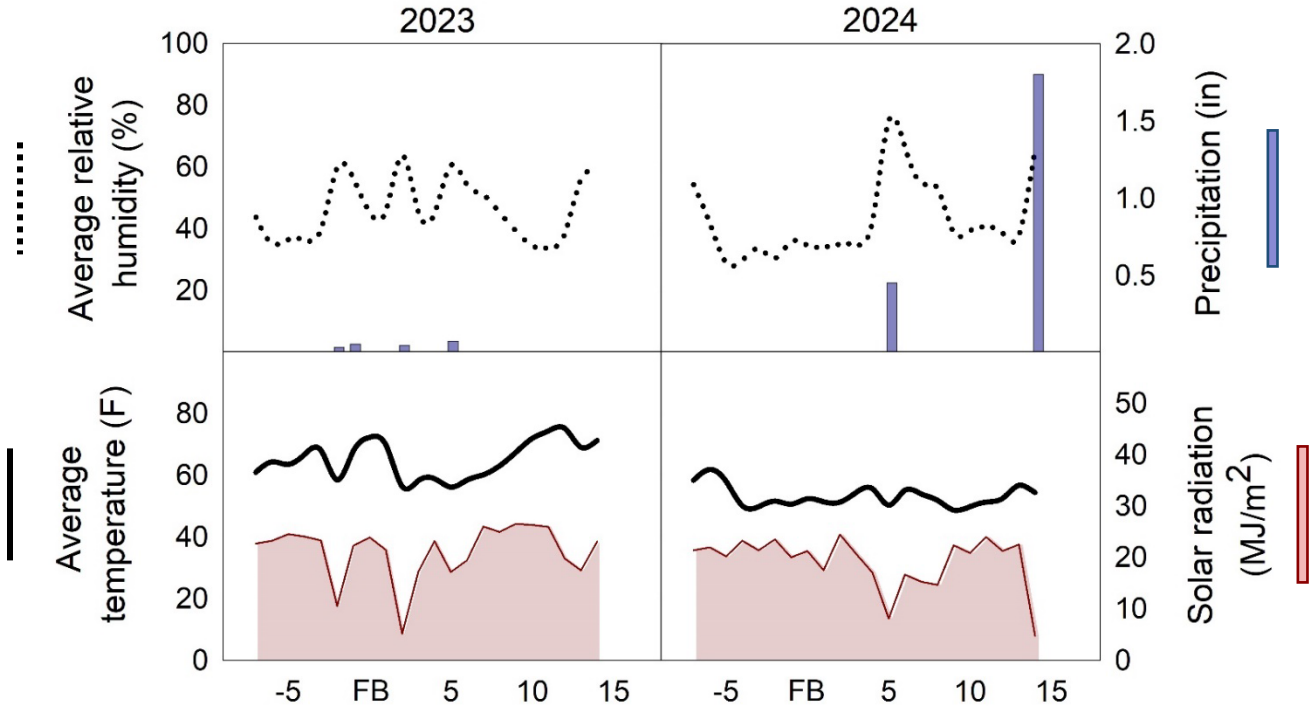
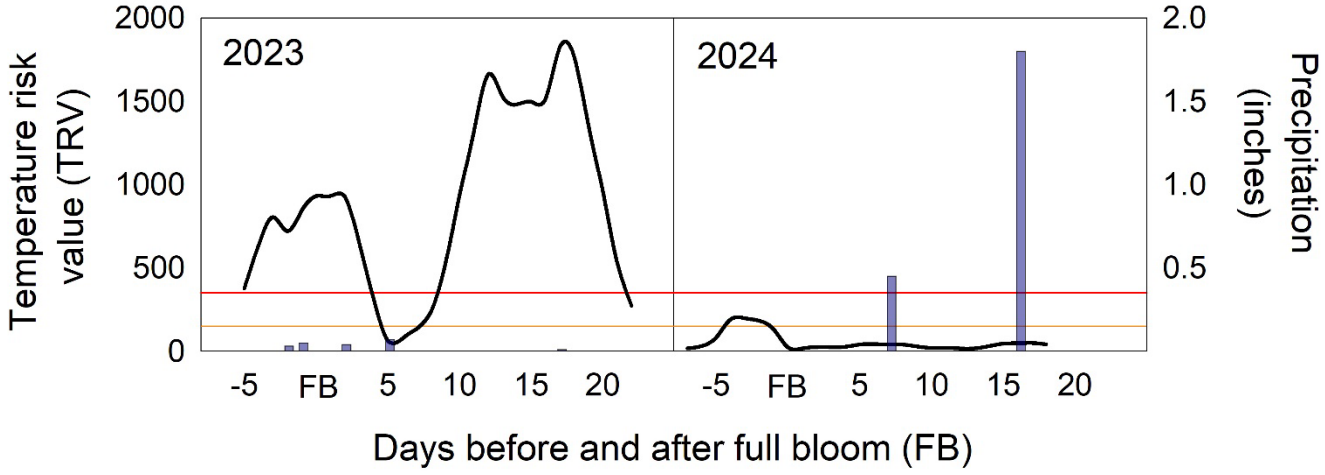
Appendix 1. Environmental conditions (temperature, humidity, precipitation and solar radiation) during bloom.



Appendix 2. Fire Blight Temperature Risk Values (TRV) During Bloom in East Wenatchee, WA. Blue bars indicate rainfall events. The orange and red lines are the risk thresholds for fire blight, created based on observations of more than 30 years of infection events in Washington and Oregon. The orange line is the high risk threshold (150 TRV), and the red line is the extreme risk threshold (350 TRV).



Project Title: Functional peptides as new tools for the control of fire blight

Key words: Fire blight, functional peptides, control

Abstract:

Functional peptides have been proposed as sustainable tools for controlling plant diseases, including fire blight in apple trees, but field efficacy remains underexplored. This study evaluated the application of peptides to manage fire blight caused by *Erwinia amylovora* in Wenatchee, WA. In 2023, under high disease-risk conditions, peptide applications on apple cv. Gala trees did not significantly reduce infection rates. Factors such as localized application (on the flower clusters), suboptimal concentrations, and the absence of surfactants likely influenced the outcome. In contrast, 2024 field trials demonstrated improved peptide performance, likely due to increased concentration, whole-tree application, and co-application with the surfactant NuFilm P. Peptide activity correlated with a reduction in bacterial populations, suggesting a primarily bactericidal mechanism of action. Laboratory assays confirmed the peptides' bactericidal activity against *E. amylovora* and compatibility with surfactants.

Despite enhanced field performance in 2024, the peptides did not achieve statistically significant infection reduction compared to the water-treated control. Environmental factors, such as UV exposure or proteolytic degradation, likely contributed to the moderate efficacy observed. Increasing peptide concentration through the use of novel methods with higher delivery efficiency or the optimization of more cost-efficient production systems, and the adoption of strategies to mitigate environmental challenges (e.g., evening applications, peptide modifications, or advanced formulations like encapsulation) could improve field performance. So overall, while peptides show promise, their successful application requires further research and optimization to address these critical challenges.