

VI1784 Cold temperature and frost effects on Psa, 2019: Project final report

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EXECUTIVE SUMMARY

VI1784 Cold temperature and frost effects on Psa, 2019: Project final report

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This is the final report on the current three-year project investigating the effect that frost has in exacerbating *Pseudomonas syringae* pv. *actinidiae* (Psa) disease development in kiwifruit vines in New Zealand. The aim of the project was to produce quantitative data on the effects of frost on Psa so that frost risk can be incorporated into the Psa risk model. Objectives included investigation of the following:

1. Low temperatures and frost in kiwifruit orchards
2. Psa infection in relation to low temperature and frost conditions using a detached cane assay
3. Modelling Psa risk in relation to low temperature and frost risk.

As the project progressed contract variations were made to cater for changes required as results were analysed and because of low frequency of field frost conditions. The overall focus was on three main experimental approaches:

1. Detached canes collected from commercial orchards using wounding with either pre-frost inoculation or post-frost inoculation with Psa (2017, 2018 and 2019)
2. Potted plants exposed to natural frost conditions in an orchard with wounding and pre- or post-frost inoculation (2018)
3. Potted plants exposed to simulated frost using ice packs, again with wounding pre- or post-frost inoculation (2019).

The planned use of green shoots in 2019 to investigate the effects of spring frost on Psa development had to be revised because field frosts did not occur after August.

All experiments included controls with no inoculation and/or no frost. The cultivars *Actinidia chinensis* var. *chinensis* 'Zesy002' (commonly known as Gold3) and *A. chinensis* var. *deliciosa* 'Hayward' were used in all the experiments, except 3), the ice pack experiment, which used only Gold3.

The project also carried out a frost climatology study and monitoring of frost events (screen temperature < 0°C) and Psa development in two kiwifruit orchards, one near Waihi and one near Maketu in the Bay of Plenty. At each site, one Gold3 and one 'Hayward' block were monitored. The climatology helped us to understand frost frequency and severity in New Zealand kiwifruit growing regions. The frosts monitored in these orchards were typical for the Bay of Plenty/Waihi kiwifruit growing area. The most severe frost recorded during the study had a minimum temperature of -5.0°C and a duration of 13 hours (55 frost °C hours). The frosts

were all relatively mild compared with those reported in Italy to be associated with severe Psa damage. There, minimum temperatures between -10 and -15°C occurred frequently during January 1985. It was concluded that winter frosts, which need to be very severe ($< -6^{\circ}\text{C}$) to damage cold-hardened cane tissues and increase Psa risk, are relatively uncommon in New Zealand kiwifruit orchards. However, frosts in spring can damage new shoot growth and pose a serious risk. Material for the detached cane assays was collected from these four orchard blocks.

The detached cane assay provided valuable information, as summarised below.

- The temperature and frost duration at which frost damage started to occur was about -6.0°C for 4 h, which, when expressed as frost degree hours (temperature below zero times duration) is 24 frost $^{\circ}\text{C}$ hours.
- Psa lesion lengths in Gold3 were 8% greater following inoculation than in 'Hayward' over all the experimental factors for 'frost' treatments other than the -6°C frost for 4 hours (24 frost $^{\circ}\text{C}$ hours) treatment. However, for the -6°C frost, which caused tissue damage in the dormant canes, the increase in lesion length was not significantly different for both cultivars (23% increase). While more severe frost conditions would presumably cause greater Psa development, there was an upper limit to the extent to which frost damage could increase Psa risk. This was shown in pilot experiments conducted in 2016, where -10.0°C for 16 h (160 frost $^{\circ}\text{C}$ hours) killed canes completely, with no Psa development.
- The detached cane experiments suggested that pre-frost inoculation (canes already infected before frost) resulted in greater Psa lesion development than post-frost inoculation (canes infected after frost), but this finding was not borne out by the potted plant experiments in 2018 and 2019. It was concluded that this finding could have been an artefact of the detached cane methodology.
- In the detached cane experiments, there was month to month variation in Psa lesion growth in inoculated canes that received tissue-damaging frost treatment during autumn, winter and spring. However, unexpectedly, Psa development was greatest in the winter months (June and July) when cold hardening should have been greatest. This does not appear to be consistent with previously published data on 'Hayward' where frost damage (without Psa) was least during the winter months when vines are fully cold hardened.
- The 2019 detached cane experiment revealed that this technique may produce some spurious results because of time-dependent effects on cane physiology resulting from detachment of the cane pieces from the vine. These effects are not fully understood, but results generated using this assay need to be interpreted with caution.

The 2018 potted plant experiment investigated the effect of natural field frost conditions on potted Gold3 and 'Hayward'. Unfortunately only one short frost event occurred at the study site (-0.5°C for 40 minutes; 0.33 frost $^{\circ}\text{C}$ hours). Psa-inoculated canes exposed to field frost developed significantly longer lesions than those when kept under a shelter for Gold3, but not for 'Hayward'. This probably reflects Gold3's greater susceptibility to Psa cane infection and may indicate lower frost tolerance in Gold3 than 'Hayward'. A comparison of pre-frost versus post-frost Psa inoculation timing showed that Psa development was the same whether the canes are already infected at the time of frost exposure or whether they became infected after frost exposure. This was in contrast to the detached cane assay, as indicated above.

The ice pack method used in the 2019 potted plant experiment on Gold3 added a different and valuable perspective on the effects of frost on Psa development. The frost event achieved with the ice packs (-5.1°C for 8.0 hours; 41 frost °C hours) was sufficient to increase Psa lesion development and this was consistent with the detached cane assay results over the three years, where 24 frost °C hours appeared to be a threshold for frost damage leading to greater Psa development. In this experiment, pre-frost inoculation resulted in significantly greater lesion length in 'Hayward' than in Gold3 compared with post-frost inoculation.

The overall pre-frost versus post-frost differences across the three experimental approaches are somewhat difficult to interpret. Given the artificial nature of the detached cane assay and the possibility of experimental artefacts as a result of using detached canes, there may be no real difference in the effect on Psa development of pre-frost inoculation versus post-frost inoculation.

Preliminary work to incorporate frost risk into the Psa risk model has been done using the data from the project. However, there are key aspects about the effect of frost on Psa development that still lack suitable quantitative data. A preliminary function has been developed to describe the increase in Psa infection risk due to increasing frost °C hours (function F), taking into account the 24 frost °C hours required before tissue damage starts to occur and hypothetically proposing that Psa infection risk is doubled once 80 to 100 frost °C hours are reached. To determine whether the assumptions are correct, further quantitative data are needed to confirm the upper threshold for frost damage for different cultivars. The ice pack with potted plant experimental approach may be the best way to achieve this.

The ultimate goal of this work is to provide new information that can contribute to an update of the frost protection manual, however, current data are not sufficient to allow this. An article for the NZ kiwifruit Journal is planned to summarise the new information gained from this report.

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1 INTRODUCTION

Frost conditions in kiwifruit orchards can damage kiwifruit vines and increase the risk of bacterial canker, caused by *Pseudomonas syringae* pv. *actinidiae* (Psa). Freezing temperatures damage cane tissues through the formation of ice crystals and this allows immediate colonisation of the damaged tissues by Psa bacteria (Ferrante & Scortichini 2014).

The Kiwifruit Vine Health (KVH) Psa-V Risk Model (referred to herein as the Psa risk model) was developed by Plant & Food Research in 2011/12 for use by kiwifruit growers and industry managers to assist with Psa management by identifying when weather conditions within a region are suitable for Psa infection. However, this model does not currently use frost as an input into the risk calculation. This three-year project has sought to understand relationships between frost occurrence and Psa development through experiments conducted in selected orchards in Waihi and Bay of Plenty. A combined approach was used that included monitoring of frosts and Psa disease in the orchard, controlled temperature experiments in the laboratory using field-collected detached canes, and field frost experiments using potted plants. It was originally planned to investigate how natural frost conditions during spring increase Psa risk in emergent green shoots. However, warm spring conditions in 2018 and 2019 meant that no frosts were recorded after August.

This report describes the third year (winter 2019) of experimental data and summarises the overall findings from the three years. In the final section a concept is proposed for how frost could be incorporated into the Psa risk model and identifies further work required before this can be done. As previously recognised, a separate project will be required to code an upgraded risk algorithm and this will involve a subcontract to HortPlus Ltd to implement the upgraded model on the KVH website. We recommend reading this report in conjunction with the two previous reports on the project (Beresford et. al 2018 and Beresford et. al 2019).

Abbreviations

The following abbreviations are used throughout this report for the names of the bacterial canker pathogen and the kiwifruit cultivars used in the study:

Psa:	<i>Pseudomonas syringae</i> pv. <i>actinidiae</i>
Gold3:	<i>Actinidia chinensis</i> var. <i>chinensis</i> 'Zesy002'
'Hayward':	<i>A. chinensis</i> var. <i>deliciosa</i> 'Hayward'

2 ORCHARD FROST MONITORING, 2019

2.1 Methods

Frost occurrence was monitored during 2019 in the same two study orchards as in 2017, one near Waihi and one near Maketu, using one Gold3 and one 'Hayward' block at each site. The Waihi site (altitude 120 m) was more frost prone than the Maketu site (altitude 11 m). The weather monitoring equipment set up in the blocks in 2017 was used again for a third year. Note that all frost temperatures referred to in this report are screen temperatures relevant to air frost and not grass minimum temperatures relevant to ground frost.

2.2 Results

In 2019, there were generally fewer frosts than in the previous two years and the minimum temperatures were not as low (Table 1 and Figures 1 and 2), reflecting the more mild winter conditions during 2019 than 2017 or 2018. The greatest number of frosts (Figure 1) and the lowest temperatures (Figure 2) over the three years occurred in 2017. Appendix 1 provides details of frost event durations, minimum temperatures and frost degree hours for each frost event in each of the four orchard blocks during 2019.

Table 1. A, Number of frost events (temperature $\leq 0^{\circ}\text{C}$ for ≥ 1 hour) and the lowest recorded temperature in each month between April and September 2019 in *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) and *A. chinensis* var. *deliciosa* 'Hayward' kiwifruit blocks at the Waihi and Maketu study orchards. B, Numbers of frost events and lowest temperatures between April and September in the study orchards in 2017, 2018 and 2019.

A.		Gold3 2019		'Hayward' 2019	
Location	Month	Number frosts	Lowest temp ($^{\circ}\text{C}$)	Number frosts	Lowest temp ($^{\circ}\text{C}$)
Waihi	April	0	-	0	-
	May	2	-1.9	1	-1.3
	June	3	-1.6	9	-4.0
	July	1	-1.9	2	-1.7
	August	1	-0.0	1	-0.1
	September	1	-0.2	0	-
Total/average		8	-1.3	13	-1.8
Maketu	April	0	-	0	-
	May	0	-	1	-0.4
	June	3	-2.0	8	-2.8
	July	1	-0.0	3	-0.5
	August	5	-2.1	5	-2.3
	September	-	-	-	-
Total/average		9	-1.1	17	-1.5

B.		Gold3		Hayward	
Year	Location	Number frosts	Lowest temp. ($^{\circ}\text{C}$)	Number frosts	Lowest temp. ($^{\circ}\text{C}$)
2017	Waihi	19	-5.0	17	-4.8
	Maketu	15	-2.6	16	-3.2
2018	Waihi	24	-3.6	23	-3.3
	Maketu	15	-3.0	19	-3.5
2019	Waihi	8	-1.9	13	-4.0
	Maketu	9	-2.1	17	-2.8

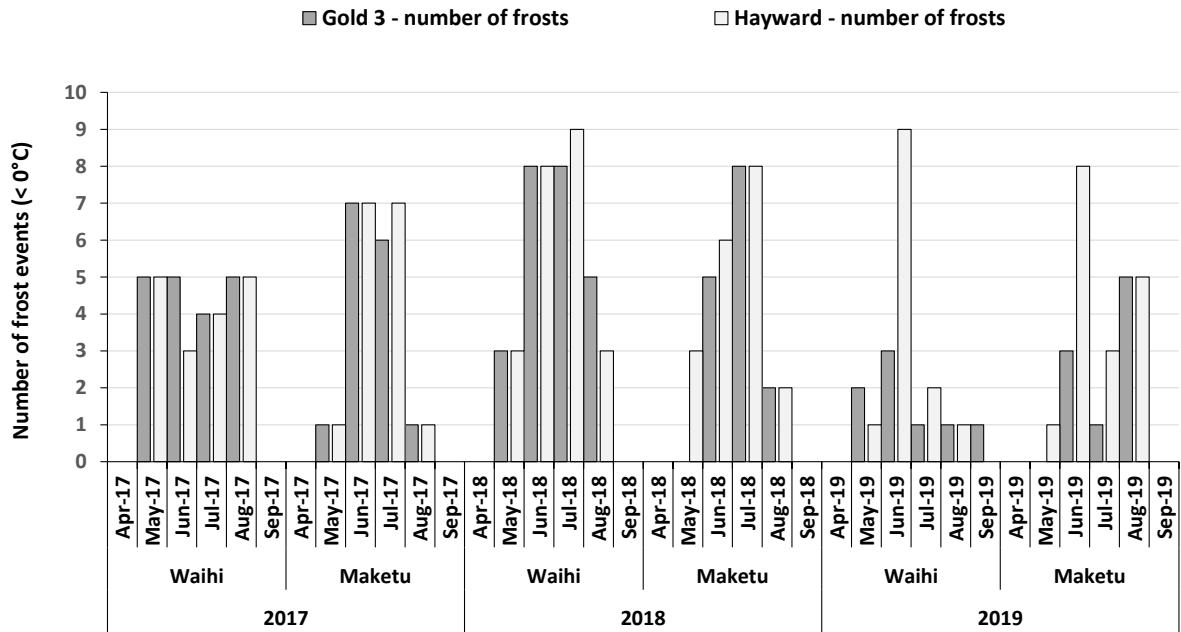


Figure 1. Number of frost events (temperature $\leq 0^{\circ}\text{C}$ for ≥ 1 hour) recorded each month (April to September) in 2017, 2018 and 2019 in the *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) and *A. chinensis* var. *deliciosa* 'Hayward' study blocks in the Waihi and Maketu kiwifruit orchards.

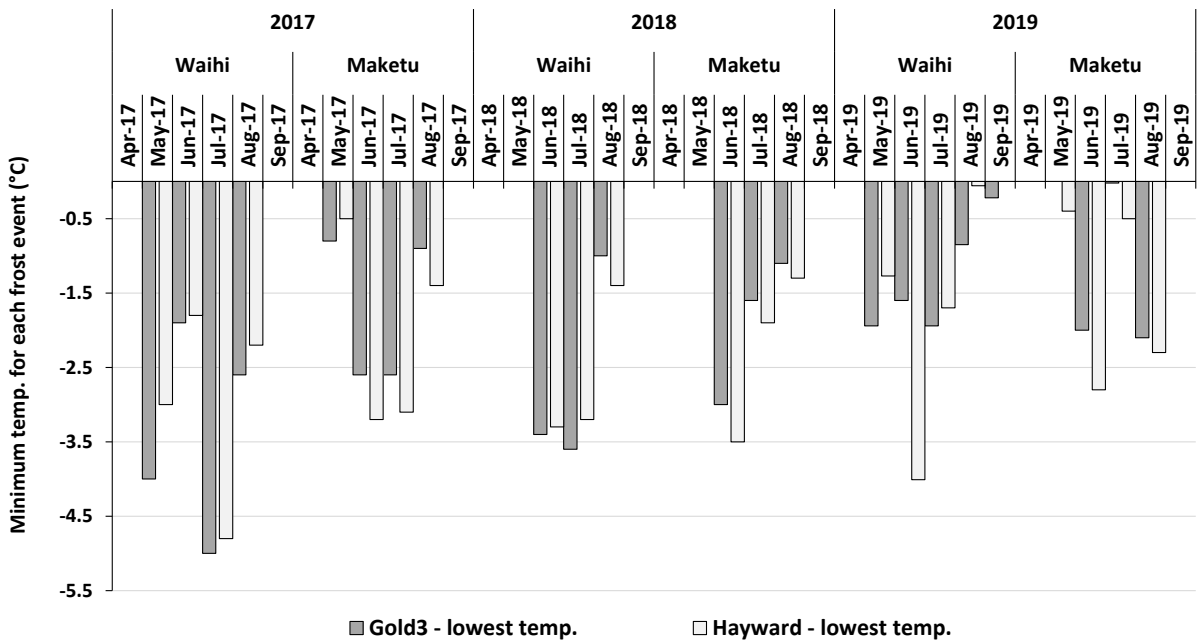


Figure 2. Lowest temperature recorded during the frost events each month (April to September) in 2017, 2018 and 2019 in the *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) and *A. chinensis* var. *deliciosa* 'Hayward' study blocks in the Waihi and Maketu kiwifruit orchards.

2.3 Discussion

The wide range in number of frost events and minimum temperatures among the three years reflects the highly variable nature of frost occurrence at these sites, and, indeed, in kiwifruit orchards generally. There were generally fewer frosts and they were less severe in 2019 than in 2017 or 2018. The three most severe frosts recorded during the whole study were:

- Two in the Waihi Gold3 block on 22 and 23 May 2017, both with durations of 12 hours and minimum temperatures of -4.0 and -4.5°C (42 frost $^{\circ}\text{C}$ hours and 47 frost $^{\circ}\text{C}$ hours)
- One in the Waihi Gold3 block on 31 July 2017, with a duration of 13 hours and a minimum temperature of -5.0°C (55 frost $^{\circ}\text{C}$ hours).

The Psa canker monitoring in the four study blocks during 2017 and 2018 did not find any visible evidence of frost-induced damage in the kiwifruit vines (Beresford et al. 2018 and Beresford et al. 2019). Also, the occurrences of the Psa cankers observed in the blocks during the winter periods were not able to be correlated with frost occurrence because winter pruning, which was necessary for orchard management, removed the cankers.

3 PRE-FROST VS POST-FROST INOCULATION, 2019 (DETACHED CANE ASSAY)

3.1 Background – summary of progress in 2017 and 2018

In 2017, the detached cane assay was used to study Psa lesion development in relation to frost treatments in dormant canes sampled from the Maketu and Waihi orchards in five months (May, June, July, August and October). Lesion length was measured three weeks after inoculation and various 4-hour artificial 'frost' treatments were applied using freezers, refrigerators and ambient laboratory temperatures to give -6°C , -2°C , $+2^{\circ}\text{C}$ and $+20^{\circ}\text{C}$. Key results from the 2017 study (Beresford et al. 2018) were:

- A frost temperature of -6°C for 4 h caused a 23% increase in lesion length on Psa-inoculated canes, whereas a frost temperature of -2°C did not significantly increase lesion length.
- Temperatures of -3°C and -4°C , which had been used in a previous detached cane study (Casonato et al. 2015), did not significantly increase lesion length compared with temperatures above 0°C . This suggested that -6°C (or perhaps -5°C) represents a temperature threshold for frost tissue damage to enhance Psa development in dormant canes.
- A -6°C frost produced a greater increase in lesion length when infection occurred before the frost event (pre-frost inoculation) than when it occurred after the frost event (post-frost inoculation). Although this suggested that frost has a greater effect in making existing infection worse than in inciting new infection in frost-damaged tissue, it was suspected that this might be an artefact produced by the detached cane methodology, e.g., the amount of time after excision from the vine before cane pieces were inoculated may have been important in determining lesion length.
- Psa lesion lengths in Gold3 were 8% greater following inoculation than in 'Hayward' over all the experimental factors for 'frost' treatments other than the -6°C frost for 4 hours (24 frost $^{\circ}\text{C}$ hours) treatment. However, for the -6°C frost, which caused tissue damage in the dormant canes, the increase in lesion length was not significantly different for both cultivars (23% increase). This suggests that Gold3 is more susceptible to Psa than 'Hayward', but when frost-induced tissue damage occurs, both cultivars are similarly susceptible. The detached cane assay, with the temperature treatments used, was not able to confirm the suggestion that Gold3 would be more frost susceptible than 'Hayward', as suggested by Ferrante & Scorichini (2014), who observed that *Actinidia chinensis* cultivars are less frost tolerant than *A. deliciosa* cultivars.
- Lesion length in inoculated canes varied significantly from month to month, and was greater in canes collected in June, July and August (winter) than in May (autumn) and October (spring). This may suggest that the canes are more susceptible to Psa when they are dormant than when they are more physiologically active in autumn and spring. This appears to be the opposite to what would be expected from the frost tolerance results of Pyke et al. (1986) for 'Hayward', where re-analysis and modelling of their results showed there was greater susceptibility to frost damage in May than in June or July (Appendix 2).

In 2018, a second year with the detached cane assay investigated the utility of “frost degree hours” (mean frost temperature multiplied by hours below zero) as a possible way of measuring frost severity for modelling purposes (Beresford et al. 2019). This approach combined both frost intensity (how cold) and frost duration (how long) as a way to explain the effect of frost events on Psa development.

- Canes were collected from Gold3 and ‘Hayward’ at the Maketu and Waihi study orchards in June and July 2018. As in 2017, multiple experimental factors were studied, including collection month, site, cultivar, inoculation (Psa or water), inoculation timing (pre- or post-frost) and frost severity, expressed in frost degree hours.
- The 2018 results were similar to 2017 in terms of the response of lesion length to the various experimental factors. Relationships between frost degree hours and lesion length were weak and did not produce a simple quantitative regression relationship between frost degree hours and Psa development. However, there was a trend for greater significance for the regressions when the milder “frost” events were excluded from the analysis. This suggested that there is a threshold of frost severity below which damage to dormant canes does not occur. Evidence was obtained that at least 16°C hours of frost are required before a frost event exacerbates Psa development (e.g. 4 hours at or below -4°C or 2.6 hours at or below -6°C). However, the threshold of 16°C hours of frost for damage to dormant canes was subsequently reviewed (see below).

In 2019, as reported below, a further detached cane experiment was conducted to verify the finding from the 2017 and 2018 detached cane assays that inoculation pre-frost causes greater Psa lesion development than inoculation post-frost (i.e. frost causes more Psa development in canes that are already infected than in canes that become infected after a frost event). Detached dormant canes were exposed to frost at different intervals before or after inoculation and Psa lesion development was measured.

3.2 Methods

The detached cane assay, as described in Beresford et al. (2018), was used with Gold3 canes collected from the Maketu frost study orchard. The experimental factors and factor levels are shown in Table 2. These factors were analysed using GenStat 17th Edition General Linear Model ANOVA and main effects and selected 2-way interactions are presented. Detached cane and potted plant analyses were done with cane diameter as a covariate, although the covariate was never significant ($p > 0.05$) and is not shown in the presentation of results.

Table 2. Treatment factors and factor levels making up the experimental units (file wounds) used in the pre-frost vs post-frost kiwifruit inoculation experiment using the detached cane assay. Psa = *Pseudomonas syringae* pv. *actinidiae*.

Treatments	Levels
Frost	2 (Frost, no frost)
Inoculation	2 (Psa, no Psa - bacterial saline solution only)
Inoculation timing	6 (pre-5d, pre-2d, pre-1d, post-1d, post-2d, post-5d)
Replicates	8
Total	192
For each of the six inoculation timings	
Frost	2 (frost, no frost)
Inoculation	2 (Psa, no Psa - bacterial saline solution only)
Replicates	8
Total	32

Canes were collected on a series of dates to give intervals of 5, 2 and 1 day from inoculation to frost treatment (pre-frost inoculation) and -1, -2 and -5 days from frost treatment to inoculation (post-frost inoculation) (Table 3). Canes were collected five days prior to each frost treatment and frost treatment was done in freezers with mean temperatures as indicated in Table 3. A single inoculation date was used to ensure that the inoculum and all details of the inoculation procedure were the same for every frost timing treatment. This meant the interval from collection to inoculation varied from 0 to 10 days (Table 3). The sets of four replicate control canes inoculated with bacterial saline (no-Psa) were maintained at 20°C.

Table 3. Timing of kiwifruit cane collection, inoculation, frost treatment and assessment of lesion length in the pre-frost vs post-frost inoculation experiment using the detached cane assay.

Inoculation timing	Collection date	Inoculation date	Days from collection to inoculation	Frost date	Days from inoculation to frost treatment	Frost duration (h)	Mean frost temp. (°C)	Assess. date	Days from inoculation to assess
Pre-frost+5	24-Jun-19	24-Jun-19	0	29-Jun-19	5	4	-4.69	15-Jul-19	21
Pre-frost+2	21-Jun-19	24-Jun-19	3	26-Jun-19	2	4	-5.49	15-Jul-19	21
Pre-frost+1	20-Jun-19	24-Jun-19	4	25-Jun-19	1	4	-5.31	15-Jul-19	21
Post-frost-1	18-Jun-19	24-Jun-19	6	23-Jun-19	-1	4	-5.78	15-Jul-19	21
Post-frost-2	17-Jun-19	24-Jun-19	7	22-Jun-19	-2	4	-5.34	15-Jul-19	21
Post-frost-5	14-Jun-19	24-Jun-19	10	19-Jun-19	-5	4	-5.49	15-Jul-19	21

All cane pieces were surface sterilised by placing them in 70% ethanol for 1 min, 1% sodium hypochlorite for 3 min, 70% ethanol for 1 min, and then triple rinsing with sterile water. Canes were then dried in a laminar flow cabinet before being labelled.

Each cane piece was wounded in two places (3 cm from either end) with a triangular file by making a lateral cut that was 2 mm long. Wounds on each cane piece were then inoculated with either one drop (10 μ L) of a 1×10^9 cells/mL suspension of Psa biovar 3 (10627) or with 0.85% bacterial saline (control). Canes were maintained at 20°C for 21 days after inoculation before measuring lesion length.

For the pre-frost inoculations, cane pieces were inoculated and then incubated in trays with moist paper towels and lids on for five days to establish infection before the 'frost' temperature treatments. For the post-frost inoculations, cane pieces were frosted the day after field collection, incubated in trays (as above) and then wounded and inoculated the appropriate number of days after frosting.

All trays, except for the 20°C controls, were pre-conditioned in a refrigerator with the temperature at 2°C for 1-2 h. The frost treatment trays were then placed into freezers with a nominal temperature between -5 and -6°C. Freezer temperatures were controlled using a microcomputer temperature controller STC-1000. Data loggers were placed in each refrigerator/freezer to record temperatures.

After the inoculation date, for the post-frost inoculation treatments, and after the frosting date for the pre-frost inoculation treatments, trays were incubated at room temperature for 21 days until they were assessed for lesion length. At assessment, the top layer of bark was removed by a blade 2 cm either side of each wound site, and the longitudinal lesion extension either side of the wound (lesion length) was measured with digital callipers. If no lesion was visible, the width of the wound itself was measured (about 2 mm).

3.3 Results and Discussion

The 2019 detached cane assay showed a significant ($p = 0.005$) effect for frost treatment, but, unexpectedly, greater lesion length occurred in the no-frost than the frost treatment (Figure 3). This means that, although inoculation with Psa increased lesion length compared with inoculation with saline, the frost treatments (4 hours at between -4.7 and -5.8°C for the different pre- and post-frost inoculation timings) actually resulted in smaller Psa lesions than those at laboratory temperature (c. 20°C).

Greater lesion length at warmer temperatures, even at some sub-zero temperatures, was also seen in the 2017 detached cane assay and this appeared to occur when the frost temperature was not sufficiently cold to induce tissue damage. In 2017, the Psa lesions became slightly longer as 'frost period' temperature increased up to 20°C and only when the 'frost period' temperature was -6°C, as compared with -2°C, was there sufficient frost damage to cause significantly longer Psa lesions. The frost conditions in the 2019 experiment (4 hours at between -4.7 and -5.8°C) appeared not to have been cold enough to damage tissues in the dormant canes and therefore did not cause an increase in Psa lesion length.

The Frost x Inoculation interaction in Figure 3 was non-significant ($p = 0.272$) because the relative increase in lesion length as a result of Psa inoculation, compared with saline inoculation, was similar with or without frost treatment. This confirms that the temperatures used in the 2019 study were not sufficient to induce frost damage to the cane tissues.

Main effects
 Inoculation (Psa vs saline) $P < 0.001$ ***
 Frost (frost vs no frost) $P = 0.005$ **

2-way interaction
 Frost x Inoculation $P = 0.272$ ns

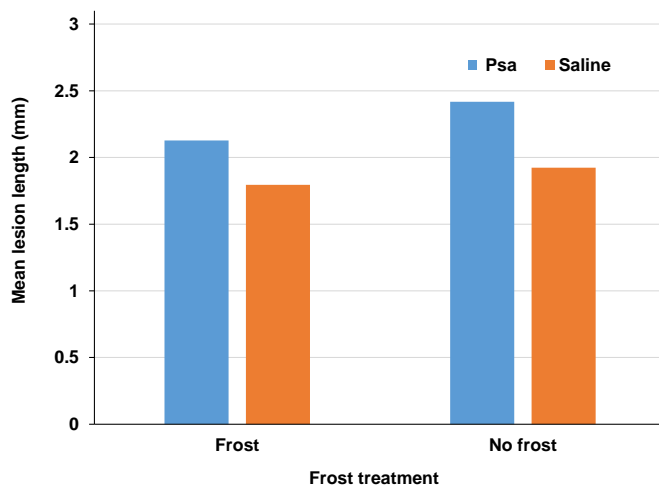


Figure 3. Main effects on mean lesion length in the 2019 detached kiwifruit cane assay for inoculation with either *Pseudomonas syringae* pv. *actinidiae* (Psa) or saline (control) and frost or no-frost treatment, over all pre-frost and post-frost inoculation timings. The Frost x Inoculation interaction was not significant.

Figure 4 shows that there were significant differences in Psa lesion length between the different pre-frost and post-frost inoculation timings; however, these were haphazard in relation to the time sequence of the six pre-frost and post-frost treatments, as shown in Table 3.

Main effect
 Inoculation timing $P < 0.001$ ***

2-way interaction
 Frost x inoc timing $P = 0.104$ ns

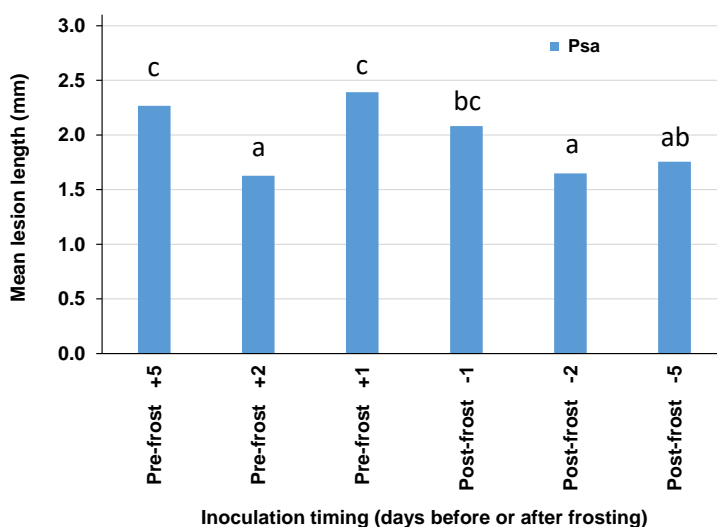


Figure 4. Mean *Pseudomonas syringae* pv. *actinidiae* (Psa) lesion length for frost-treated kiwifruit canes (excluding the 20°C controls) in the pre-frost vs post-frost inoculation experiment using detached canes. This shows analysis of variance main effects for inoculation and inoculation timing and significance of the 2-way interaction between frost and inoculation timing (detail not shown in the graph). Bars accompanied by same letters are not significantly different (Bonferroni test $\alpha = 0.05$).

There are two other considerations in relation to the design of the 2019 detached cane experiment that may have contributed to the haphazard mean lesion lengths for the six inoculation/frost timings:

1. Lesion length tended to increase at warmer frost temperatures, although this trend was not statistically significant ($p > 0.4$) (Figure 5). Frost temperature control in the different inoculation timing treatments was not precise enough to eliminate these temperature differences between the inoculation timing treatments.
2. Lesion length tended to decrease with increasing number of days from time of cane collection to time of inoculation, and to increase with increasing number of days from inoculation to frosting (Figure 6). These were a consequence of the chosen design, which emphasised a constant time (5 days) from cane collection to frost treatment (see Table 3). It would be impossible to design an experiment with all the factors that could contribute towards variation in lesion length held constant.

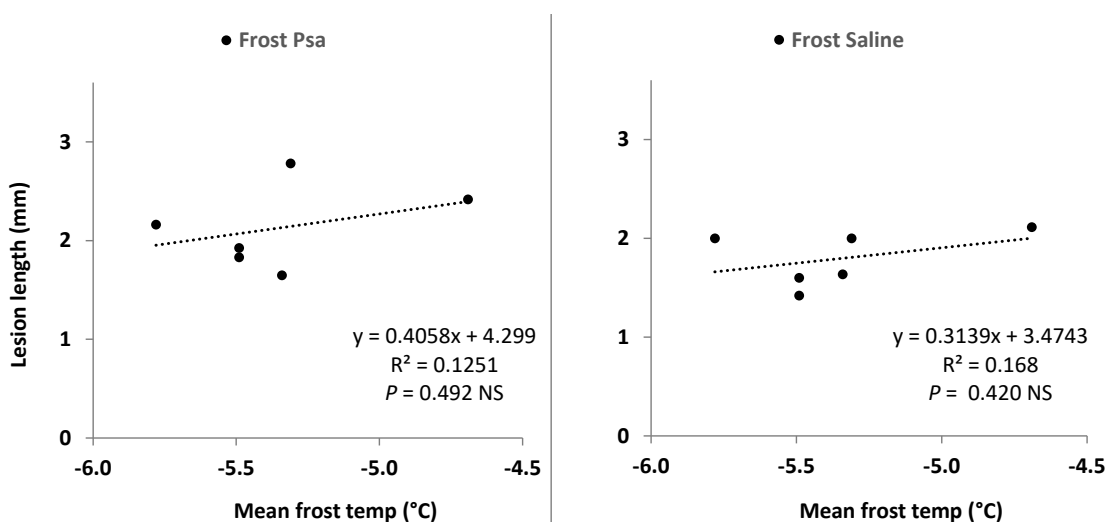


Figure 5. Mean *Pseudomonas syringae* pv. *actinidiae* (Psa) lesion length versus mean temperature during the 4-hour frost events used in the six pre-frost and post-frost inoculation timings in the 2019 detached kiwifruit cane assay, showing the non-significant trend for longer lesions at warmer frost temperatures, for both Psa-inoculated and saline-inoculated kiwifruit canes.

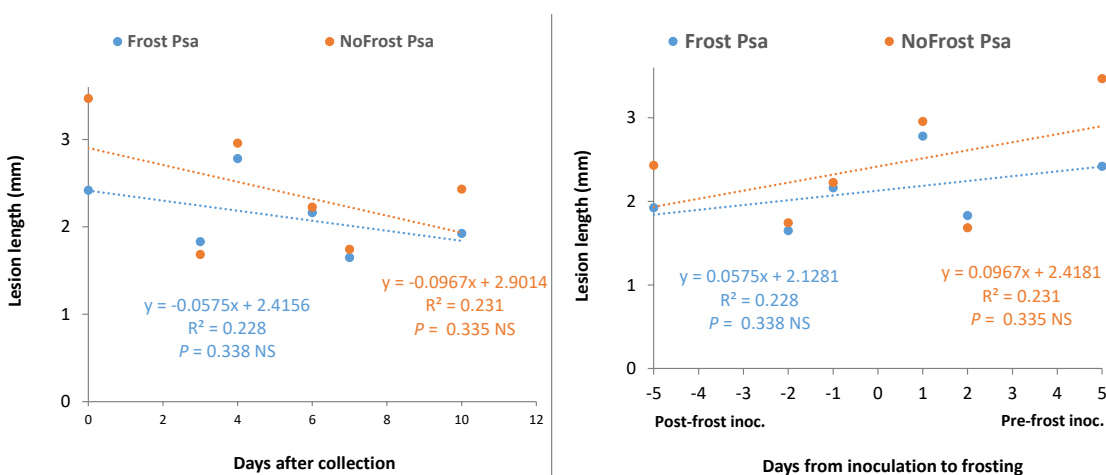


Figure 6. Non-significant trends in *Pseudomonas syringae* pv. *actinidiae* (Psa)-inoculated kiwifruit canes, where lesion length decreased with increasing number of days from time of cane collection to time of inoculation, and also lesion length increased with increasing number of days from inoculation to frost treatment.

3.4 Detached cane assay conclusions

The conclusions from the detached cane experiments over the three years are as follows:

- In 2018, unlike 2017, there was a significant difference in lesion length between the sites, with Waihi having longer lesions than Maketu. There was also a significant difference in lesion length between the two months in 2018 (June and July), whereas in 2017, although lesion length did vary significantly between the five months examined, June and July were not significantly different. The trends in lesion length between months in 2017 suggested greater lesion development when the canes were in full winter dormancy (June to August) than in late autumn (May) or spring (October). The reason for this somewhat unexpected finding was not been explained, but is discussed further under the modelling section (Section 5.2) below.
- It appears that out of all the frost treatments used in the 3 years, only a temperature of -6°C for 4 hours was sufficient to induce tissue damage leading to a significant increase in Psa lesion growth. Pilot experiments in 2016 had shown that -10°C for 4 hours (40 frost $^{\circ}\text{C}$ hours) caused longer lesions than -5°C for 4 hours (20 frost $^{\circ}\text{C}$ hours) and that -10°C for 16 hours (160 frost $^{\circ}\text{C}$ hours) killed canes outright, so that no Psa lesion growth could be measured after three weeks of incubation.
- The 2019 experiment showed similar trends in the response of lesion length to the various experimental factors to those found during 2017 and 2018. These included significantly longer lesions in Gold3 than in 'Hayward', and significantly longer lesions when inoculation occurred pre-frost than post-frost.
- Most of the trends in lesion length in wounded and Psa-inoculated canes, which resulted from the various 'frost' temperature treatments, also occurred in water or saline-inoculated canes. However, the responses were generally significantly greater with Psa, rather than water/saline, inoculation, and it therefore appears that Psa infection exacerbates the effect of wounding. However, when the frost treatment was sufficient to induce tissue damage (-6°C in 2017), the increase in lesion length from Psa inoculation was significantly greater.
- Both frost temperature and duration are important in frost damage, so the use of frost degree hours is helpful for building concepts around frost effects on Psa risk. It appears from the 2017 and 2019 data that a frost event of 24 frost $^{\circ}\text{C}$ hours (e.g. -6°C x 4 hours) is sufficient to cause frost damage, whereas 20 frost $^{\circ}\text{C}$ hours (e.g. -5°C x 4 hours) is not. In the report on the 2018 results (Beresford et al. 2019), it was suggested that 16 $^{\circ}\text{C}$ hours (e.g. -4°C x 4 hours) was sufficient to induce frost damage in dormant tissue, but the new interpretation based on the 2017 and 2019 data suggests a threshold of 24 frost $^{\circ}\text{C}$ hours is more appropriate.
- The 2019 experiment suggested that the detached cane assay may produce some spurious Psa lesion length results in relation to imposed treatments because of time-dependent effects on cane physiology resulting from detachment of the cane pieces from the vine. These effects are not fully understood, but results obtained using this assay need to be interpreted with caution, particularly the apparently greater lesion length in pre-frost inoculated canes compared with those inoculated post-frost.

4 POTTED PLANT ICE PACK EXPERIMENT, 2019

4.1 Background

In 2018, Kabir and Aracely had inoculated canes on potted Gold3 and 'Hayward' plants with Psa and exposed them to a natural frost event in the field. This provided an alternative approach to the detached cane assay to help understand how frost influences Psa development. Unfortunately, the very mild winter conditions in 2018 meant that only one short frost event occurred (-0.5°C for 40 minutes; 0.33 frost °C hours) at the study site (PFR's Te Puke Research Orchard). The main findings from that experiment were:

- The Psa-inoculated canes developed longer lesions when field-exposed to frost than when kept under a shelter, although the difference was only statistically significant for Gold3 and not for 'Hayward'. Lesion lengths were generally significantly greater in Gold3 than 'Hayward', reflecting Gold3's greater susceptibility to Psa.
- A comparison of pre-frost versus post-frost Psa inoculation timing showed that Psa lesions developed to the same degree whether the canes are already infected at the time of frost exposure or whether they became infected immediately after frost exposure. This was in contrast to the detached cane assay which had consistently shown longer lesions with pre-frost inoculation when canes were already infected at the time of the frost event.

The differences between the detached cane and potted plant assays needed to be further investigated. Peter Scott joined the research team in 2019 and suggested another approach using ice packs to generate controlled frost conditions in inoculated potted plants. This experiment replaced one that had been planned to look at the effects of field frost in spring on new shoot growth. This would not have been possible because there were no spring frosts in 2019.

4.2 Methods

Potted vines of Gold3 and 'Hayward' (2 years old) were maintained at the Te Puke Research Orchard (TPRO) under a shelter, comprising a semi-clear roof, one closed side and drip irrigation. This shelter protected the potted plants from rain splash that could disperse Psa inoculum, and it therefore prevented Psa infection.

The experimental approach involved treating the canes (main stems) of the potted plants with combinations of wounding and Psa-inoculation (similar to the detached cane assay) and inducing frost conditions by wrapping gel ice packs around the treated canes. Inoculations before (pre-) and after (post-) frost exposure were also included, as were saline-inoculated controls, as described under the detached cane assay methods section. Table 4 shows the experimental design.

Table 4. A, Treatments used in the potted kiwifruit plant experiment showing factors and factor levels and B, Number of replicate plants (two wounds each) assigned to the *Pseudomonas syringae* pv. *actinidiae* (Psa) inoculation and exposure factors. Gold3 = *Actinidia chinensis* var. *chinensis* ‘Zesy002’; ‘Hayward’ = *A. chinensis* var. *deliciosa* ‘Hayward’.

A	
Factor	Factor levels
Cultivar	2, Gold3 vs ‘Hayward’
Inoculation	2, (Psa vs no Psa - bacterial saline solution only)
Inoculation timing	2, Pre-frost vs Post-frost
Exposure	2, frost (ice pack) vs no frost (no ice pack)
Replicate plants	7-8 replicates (see B, below)
Wounds	2 replicate wounds on the main stem of each plant

B		Inoculation	
		Psa	Water
Exposure	Frost (ice pack)	8	8
	No-frost (no ice pack)	7	7

On 3 July 2019, the pre-frost treatment plants were wounded and inoculated with either Psa (10 µL of a 3×10⁸ cfu/mL cell suspension) or water, as controls (Table 4B). Each cane was wounded at two points with a triangular file by making a 2-mm wide lateral cut. Each wound was located between two dormant buds and well separated. After inoculation canes were placed under plastic tents overnight under the shelter to promote infection.

On 5 July 2019, frost treatment was applied to all the frost-treated plants (pre- and post-) using gel ice packs (Esky Ice Gel Pack – Large; 430 mm high; 250 mm long; 12 mm thick; mass 0.4 kg; SKU 03240466). Ice packs were frozen in a -20°C freezer for more than 24 hours before application. Each inoculation point was marked, wrapped in bubble wrap, to prevent direct contact between the ice pack and cane, wrapped with one ice pack, then wrapped with two layers of bubble wrap for insulation and secured with cable ties. Ice packs were applied between 1600 and 1700 h and left on the canes overnight (more than 12 hours). Preliminary trials indicated that the frost pack system reliably maintained a cane surface temperature between -5 and -10°C for about 4 hours after application. During frost treatment, air temperature and temperature adjacent to the cane surface within the ice packs were recorded with a portable data logger (EasyLog; EL-USB-2-LCD, RH/Temp data logger; www.lascarelectronics.co). Temperatures are shown in Figure 7. On 7 July, the post-frost plants were wounded and inoculated, as described above.

Lesion length for all wounds on all canes was measured on 27 and 28 August 2019. Various statistical comparisons were made, but principally Psa lesion length on inoculated plants treated with the ice packs was compared with that on inoculated plants without ice packs, along with comparisons between pre-frost and post-frost inoculation and cultivar.

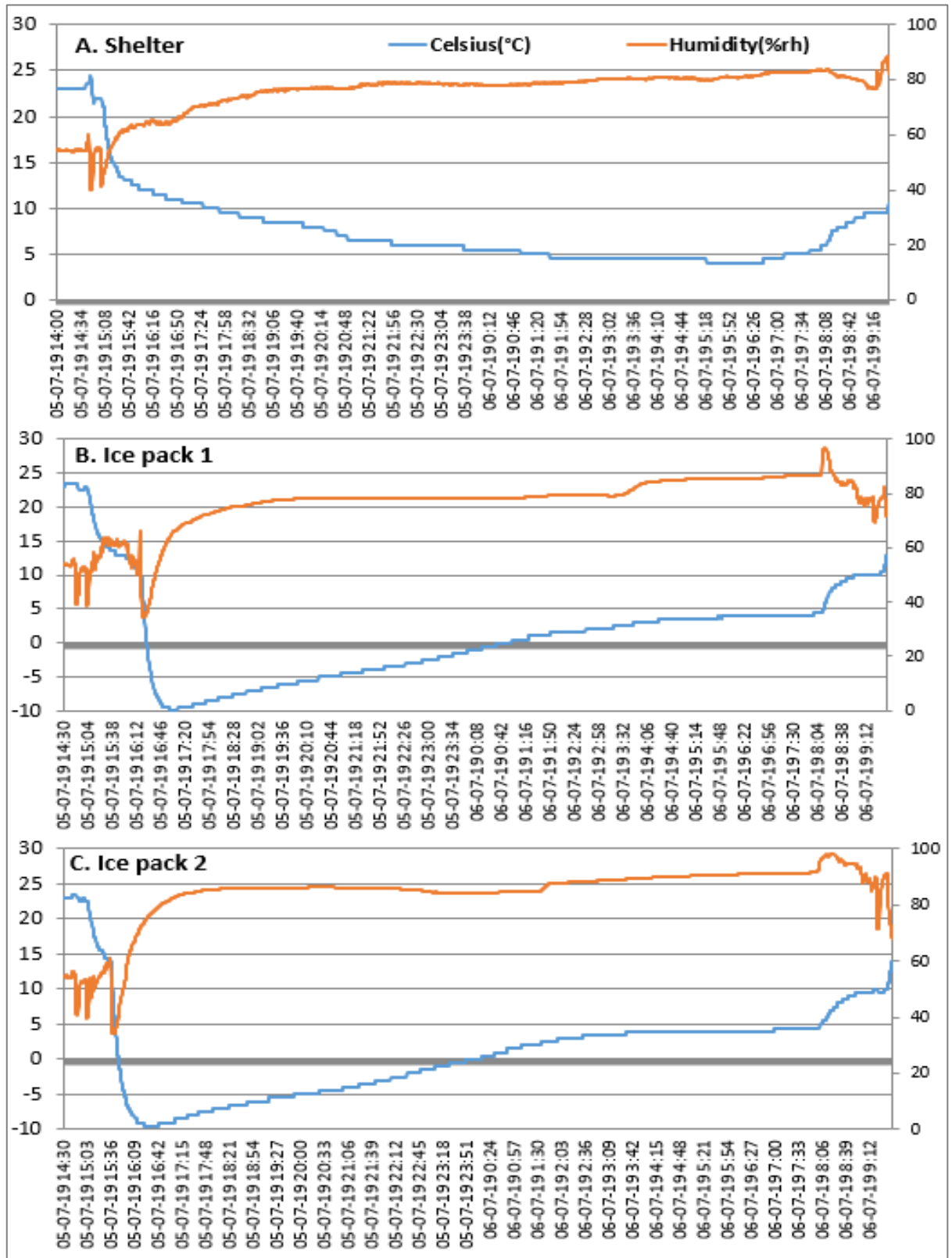


Figure 7. Air temperature and relative humidity during the potted kiwifruit plant ice pack experiment, measured within the inoculation shed (A) and adjacent to two canes within the ice pack wraps used to generate frost simulated conditions (B and C) on 5 July 2019.

4.3 Results

The ice pack system effectively maintained a cane surface temperature below 0°C for around 8 hours, with the minimum temperature briefly at -10°C (Figure 7). This produced a below-zero temperature curve that was a reasonable representation of field-frost conditions. Ice pack 1 had an average temperature of -5.2°C for 8.1 hours (42 frost °C hours; Figure 7B) and ice pack 2 had an average temperature of -5.1°C for 8.0 hours (41 frost °C hours; Figure 7C). The average of the two monitored ice packs was -5.1°C for 8.0 hours (41 frost °C hours).

The frost temperatures induced by ice packs caused significantly ($p < 0.001$) longer lesions than the no-frost conditions in the shelter (main effect for frost in Figure 8). Although the main effect for cultivar was not quite significant ($p = 0.057$), the trend was for a greater mean lesion length in Gold3 than 'Hayward', which is consistent with both the detached cane assays in 2017, 2018 and 2019 and the field frost experiment in 2018, indicating greater Psa susceptibility of Gold3 than 'Hayward'.

For the Psa inoculated plants, there was a significant ($p < 0.01$) three-way treatment interaction between cultivar (Gold3 versus 'Hayward'), frosting (frost versus no frost) and inoculation timing (pre-frost versus post-frost) (Figure 8). Lesions were significantly longer in frosted plants than no-frost plants ($p < 0.001$). As in the 2018 potted plant experiment, there was no significant difference in lesion length between pre- and post-frost inoculation for Gold3. However, 'Hayward' plants inoculated post-frost had significantly longer lesions than those inoculated pre-frost.

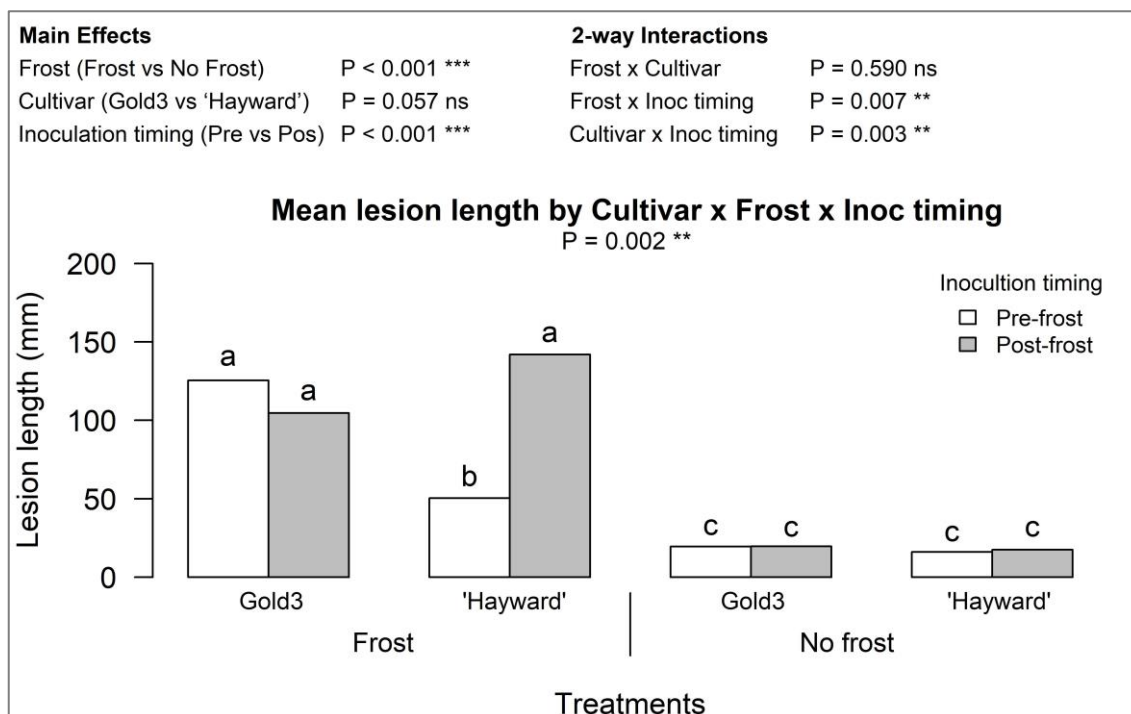


Figure 8. Mean lesion length for *Pseudomonas syringae* pv. *actinidiae* (Psa)-inoculated potted *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) and *A. chinensis* var. *deliciosa* 'Hayward' kiwifruit plants used in the potted plant experiment, showing main effects, 2-way and 3-way interactions from analysis of variance for log-transformed data. Bars with the same letter are not significantly different (Tukey test $\alpha = 0.05$).

4.4 Discussion

The ice pack method used in the 2019 potted plant experiment added a different and valuable perspective on the effects of frost on Psa development. The frost conditions achieved with ice pack 1 (Figure 7B) resulted in 42 frost °C hours ($-5.2^{\circ}\text{C} \times 8.1$ hours, Figure 7B) and with ice pack 2 (Figure 7C) resulted in 41 frost °C hours ($-5.1^{\circ}\text{C} \times 8.0$ hours). The frost conditions achieved with the ice packs were sufficient to increase Psa lesion development as a result of frosting and this is consistent with the detached cane assay results over the three years, where 24 frost °C hours appeared to be a threshold for frost damage leading to greater Psa development.

The overall pre-frost versus post-frost differences across the three experimental approaches are somewhat difficult to interpret. The detached cane assay in 2017 and 2018 suggested that, for both cultivars, there was greater Psa development when canes were already infected prior to occurrence of frost. The natural field frost experiment in 2018 suggested no difference between pre-frost and post-frost inoculation timing for both cultivars. However, the ice pack-induced frost experiment in 2019 suggested that, for Gold3, there was no significant difference between pre-frost and post-frost timing, whereas for 'Hayward', greater lesion development occurred with post-frost inoculation. Given the artificial nature of the detached cane assay and the possibility of artefacts from the methodology, it appears that overall, there may be no real difference between pre-frost and post-frost timing of infection.

5 MODELLING FROST EFFECTS ON PSA RISK

5.1 Psa risk model background

The Psa risk model was originally developed in 2011/12 and implemented as a risk warning service on the KVH website by the National Institute of Water and Atmospheric Research Ltd (NIWA) in April 2012 using weather risk forecast maps.

The service provider was changed in 2016 to HortPlus Ltd and the user interface format was changed to time series graphs instead of maps. The graphs provide more useful information for users by allowing easy interpretation of changes in risk over time and, in addition, the HortPlus platform allows retrospective analysis of risk data, including easy comparisons between seasons and sites. The model algorithm was also updated at that time with a revised temperature function following a further assessment of published literature, which showed that Psa canker development is greatly reduced at temperatures above about 20°C. A diagrammatic representation of the current model's algorithm for calculating daily infection risk is shown in Figure 9. A full scientific description of the model's development has been published (Beresford et al. 2017).

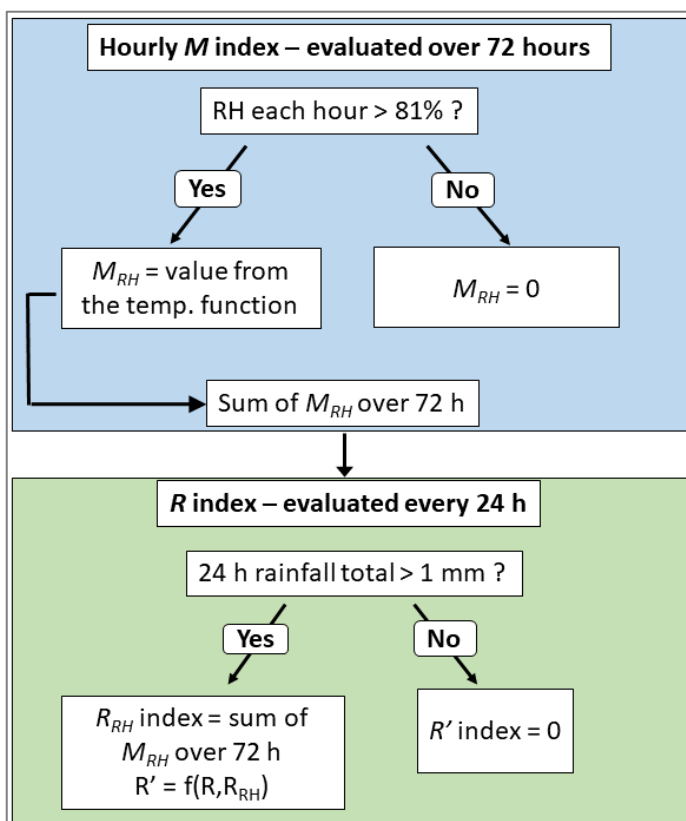


Figure 9. The (*Pseudomonas syringae* pv. *actinidiae*) Psa risk model algorithm, as currently implemented. The variables referred to are as follows:

- M_{RH} = Hourly bacterial multiplication index summed for hours > 81% relative humidity (RH)
- R' = Daily infection risk index using RH (sum of MRH over 72 h)
- R = Daily infection risk index using leaf wetness (used to calibrate R_{RH}).

The possibility of including frost as a risk factor in the Psa risk model was first discussed with KVH in 2015 and after lengthy discussions with the Psa Steering Group, the current project began in 2017.

5.2 Current understanding about how frost affects Psa

5.2.1 Temperatures associated with increased Psa development

Low temperature damage in dormant cane tissues causing an increase in Psa development requires frost temperatures $< 0^{\circ}\text{C}$. It had been suggested by some in the kiwifruit industry that low temperatures above zero might also cause an abnormal increase in Psa development, but evidence from the detached cane assay over three years suggests this is not the case. There was a gradual increase in Psa lesion growth with increasing 'frost' temperature from about -5°C up to 20°C . This gradual response was quite different from the significant increase in Psa lesion length that occurred as a result of freezing damage observed at -6°C .

A major focus of this study was to define the frost temperature threshold at which freezing damage in Gold3 and 'Hayward', leading to greater Psa development, occurs. In the frost experiments we performed, that temperature was defined as the below-zero temperature producing markedly greater Psa lesion growth than seen with higher temperatures. The evidence suggested this occurred at -6°C and that both cultivars were similarly affected, even though Gold3 showed greater susceptibility to Psa than 'Hayward' when there was no freezing damage.

Although the two cultivars did not appear to differ in relation to Psa development following freezing damage, it is possible that the range of frost temperatures we used was not fine enough to detect differences. Ferrante & Scortichini (2014) considered that *Actinida chinensis* cultivars (like Gold3) are less frost tolerant than *A. deliciosa* cultivars (like 'Hayward'), although no quantitative data are available on this.

5.2.2 Frost occurrence in New Zealand kiwifruit orchards

The frosts monitored in this study were typical for Bay of Plenty and Waihi kiwifruit orchards and the lowest recorded frost temperature during the vine dormancy period over the three years was -5°C . The frost temperatures we monitored were mild compared with the -10 to -15°C reported to be associated with increased Psa damage to kiwifruit vines in Italy in 1985 (Testolin & Messina 1987). The regional climatology comparison in the 2017 report suggested that at Riwaka (Tasman region), frosts of -10 to -15°C might occur very occasionally between June and August, but would be unlikely to occur in coastal areas of the Bay of Plenty or Waihi. Hawke's Bay frosts were much less severe and less frequent than those at Riwaka and minimum temperatures of -10 to -15°C would be very unusual. Waikato frosts were not studied, and although some may be more severe than in the Bay of Plenty, they would seldom be as severe as those at Riwaka. It is therefore concluded that frosts that could increase Psa development in dormant kiwifruit vines would be unusual in most New Zealand orchards.

The frost risk that requires vines to be protected by wind machine, water sprinkling or heat input are spring frosts that damage new emerging shoot tissues. None of these occurred in the orchards in this three-year study, with the latest frosts being recorded in August, before budburst. Spring frosts can cause severe tissue damage, even at temperatures as high as -0.5°C (Pyke et al. 1986). Damage associated with such frosts would increase Psa infection, but only in the presence of water splash and runoff from rainfall following frost damage or water sprinkling for (ineffective) frost protection.

5.2.3 Cold hardening of vines and seasonal risk of frost damage

Tissue damage at sub-zero temperatures depends on the state of cold hardening of vines (Pyke et al. 1986; Appendix 2). Cold hardening is physiologically complex, but an important component is the accumulation of sugars and other solutes within the plant that allows super-cooling of tissues and prevents formation of damaging ice crystals. Hardening develops in response to cooling temperatures and shortening days in autumn, and de-hardening occurs in response to increasing temperatures in spring. Hardening is greatest in June or July (Figure A2.3). Frost damage is least likely when vines are fully cold hardened and therefore the 2017 detached cane assay results, where Psa lesion length in field-collected cane pieces was greatest in June and July, may be an anomaly associated with the detached cane assay methodology.

We conclude from our experiments and from field frost monitoring that, under New Zealand conditions, mid-winter frosts that would damage dormant kiwifruit vine tissues sufficiently to exacerbate Psa development are unusual in most kiwifruit-growing regions and may become even less likely in the future with global warming. However, autumn and late winter frosts, when vines are less well cold hardened, could cause tissue damage that increases Psa damage. This was not able to be confirmed in this study. Spring frosts in September, October and occasionally November are problematic for new shoot growth because any temperature below zero can cause damage in those months, as noted above.

5.2.4 Use of frost degree hours versus minimum temperature

It has been suggested that the only important parameter in frost damage is minimum temperature and that longer frost duration does increase the amount of tissue damage. However, preliminary experiments comparing frost temperatures -5 and -10°C for 4 h and 16 h showed that the lower temperature for the longer duration (-10°C for 16 h) caused more tissue damage. The cane tissues were completely killed in this treatment and no Psa lesions developed. Also, the field temperature curves observed in this study showed that, during frosts in Waihi and the Bay of Plenty, the minimum temperature usually only occurs for a short period, e.g. 0.5 to 2 hours. This suggests that using minimum temperature as a predictor of frost damage would often overestimate the effect of a frost.

We therefore investigated the frost degree hour concept to describe frost events for incorporation into the Psa risk model. We did not find a strong correlation between frost degree hours and Psa lesion length; however, it appears that most of our frost treatments were not sufficiently cold to induce tissue damage. We concluded that the degree hour threshold below which tissue damage does not occur is 24 frost $^{\circ}\text{C}$ hours.

5.3 Conclusions from the three-year study

This study has provided important insights into how frost conditions affect kiwifruit cane tissues and how this interacts with Psa development. However, we have not produced a perfect set of quantitative data that can be used to model Psa risk in relation to frost conditions. The reasons for this have been the complexity of the interactions between factors that potentially contribute to both cold-induced tissue damage and Psa development, and questions about how well the detached cane assay represents the physiological effects of frost and Psa on kiwifruit vines in orchards.

The detached cane assay was initially the only technique available for doing controlled Psa inoculations at controlled temperatures, as controlled environment facilities were not available. Our attempts to use field frost conditions for experiments were hampered by mild winter conditions combined with biosecurity restrictions on where field Psa inoculations could be done.

Despite these challenges, the study has produced the following key conclusions:

- A frost temperature lower than -6°C is required to induce tissue damage leading to increased Psa development. Very severe frost conditions can cause such severe tissue damage that Psa infection cannot occur. This was shown during pilot experiments in 2017 using -10°C for 16 h.
- Frost damage depends on both frost temperature and frost duration. The frost degree hours concept combines temperature and duration and is useful for modelling the effects of frost on Psa. The frost degree hour threshold for tissue damage leading to increased Psa development is 24 frost $^{\circ}\text{C}$ hours.
- It is still uncertain whether the effect of freezing temperatures on Psa development is the same whether canes are already infected by Psa prior to the frost event or not.
- Gold3 is more susceptible to Psa infection than 'Hayward', but when the frost temperature is low enough to damage dormant canes (e.g. $< -6^{\circ}\text{C}$), the increase in Psa development is similar irrespective of cultivar susceptibility. Gold3 and 'Hayward' may each have a different degree of frost tolerance, but this was not identified in this study.
- Spring frost affecting new growth is a risk at any temperature $< 0^{\circ}\text{C}$ and this damages young shoots whether Psa is present or not, but it could exacerbate Psa in an orchard.
- The detached cane assay, using wounding and Psa inoculation showed that months differed in the degree of Psa development, with longer lesions in June, July and August, when vines were fully dormant, than in May and October. These results appear counter-intuitive in relation to what is known about cold hardening in kiwifruit, where less frost damage occurs when vines are fully cold hardened in June and July.

5.4 Adding frost functionality to the Psa risk model

To incorporate frost risk functionality into the Psa risk model requires modification of the algorithm, such as that in the scheme shown in Figure 10.

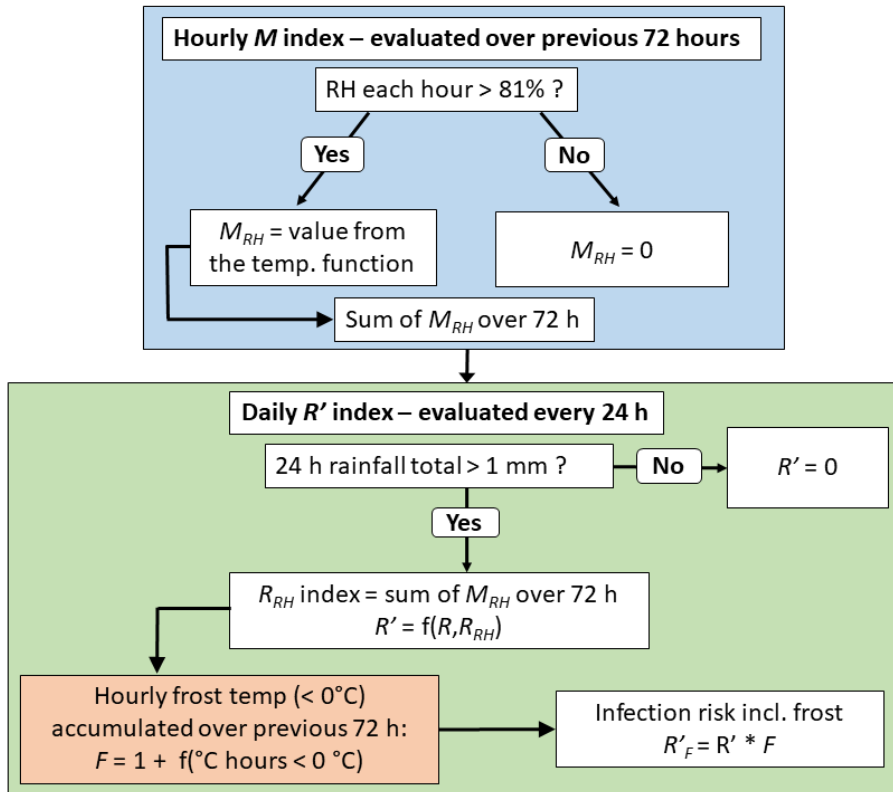


Figure 10. Proposed (*Pseudomonas syringae* pv. *actinidiae*) Psa risk model algorithm incorporating the effect of frost on Psa risk. The variable descriptions are as follows:

- M_{RH} = Hourly bacterial multiplication index summed for hours > 81% relative humidity (RH)
- R' = Daily infection risk index using RH (sum of MRH over 72 h)
- R = Daily infection risk index using leaf wetness (used to calibrate R_{RH})
- F = Increased risk due to frost. This is a function of the 72-hour sum of hourly frost temperature (< 0°C) expressed as frost °C hours/day).

A possible function to describe the increase in Psa infection risk due to frost (F), based on the results of this study and using frost degree hours, is shown in Figure 11. This relationship takes into account the 24 frost °C hours required for tissue damage to start occurring and hypothetically proposes that Psa infection risk is doubled once 80 to 100 frost °C hours is reached. This function is accumulated hourly over the previous 72 hours then divided by 3 to give the average frost degree hours per day. The F function value is then multiplied by the risk model's daily R' index to give the R'_F index, which is the same as R' when there is no frost and, as with R' index, is zero on days with < 1 mm of rainfall.

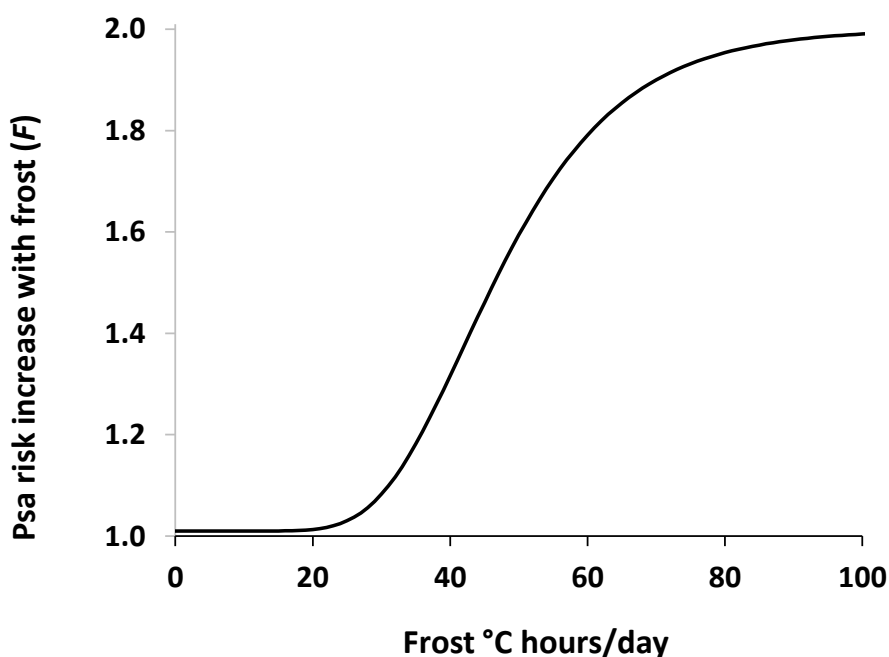


Figure 11. The F function, a hypothetical relationship describing the increase in risk of *Pseudomonas syringae* pv. *actinidiae* (Psa) development in kiwifruit canes with increasing frost severity, as indicated by frost degree hours.

A possible way of displaying frost risk information by the Psa risk model was developed under a Strategic Science Investment Fund allocation to kiwifruit risk modelling and is shown in Figure 12. This hypothetical output shows the occurrence of frost temperatures on 8, 9, 10 and 12 August 2016. The increased Psa infection risk associated with these frosts is displayed for 12 and 13 August, which were days with > 1 mm of rainfall that allowed new infection. This analysis assumed that the frost degree day threshold was 0 and not the 24 frost °C days subsequently determined in this project. This type of representation for frost risk could be adapted for the HortPlus™ display format on the KVH website.

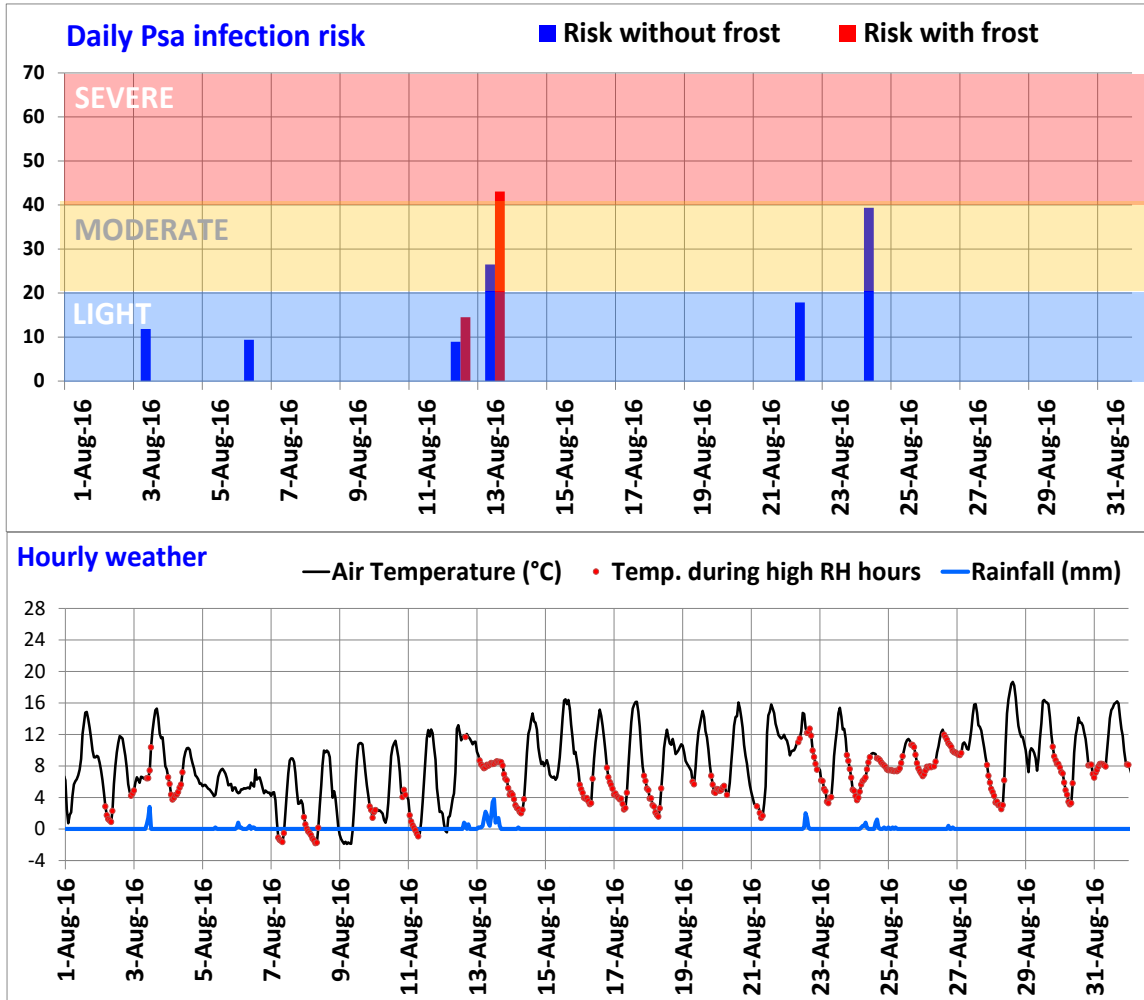


Figure 12. Mock-up of (*Pseudomonas syringae* pv. *actinidiae*) Psa risk model output showing how frost risk could be displayed. Upper graph: Blue bars indicate Psa infection risk on days with rain and no frost in the previous 3 days and red bars indicate increased infection risk when frost (temp. < 0°C) occurs within the previous 3 days. Lower graph: Hourly temperature, including hours with high relative humidity (red dots), and rainfall.

5.5 Next steps and unanswered questions

Good progress has been made towards understanding and modelling the effects of frost on Psa risk, despite the challenges in interpreting the results from some of the experiments. To finish incorporating frost risk into the Psa risk model, the following further information needs to be obtained:

1. Quantitative data to verify the assumptions about the frost degree hour thresholds in the preliminary F function and how the thresholds differ for different cultivars. The potted plant/ice pack experimental approach is probably the best way to achieve this.
2. Further experiments to finally resolve the pre-frost versus post-frost inoculation question. This is crucially important because it determines how risk model predictions about frost risk should be interpreted. Should growers expect orchards with existing Psa infection to be at greater risk from frost events than orchards with low Psa infection? The answer also helps to identify what management actions should be taken in response to frost events. The potted plant/ice pack experimental approach is again probably the best way to answer this question.
3. There are some aspects of the data from the detached cane assays in previous years that should be re-analysed to help to answer specific questions about factor interactions that have arisen since that work was done.

Once frost risk has been incorporated into the Psa risk model to the satisfaction of kiwifruit industry personnel and researchers, then HortPlus Ltd needs to be contracted to implement the upgraded model on the KVH website.

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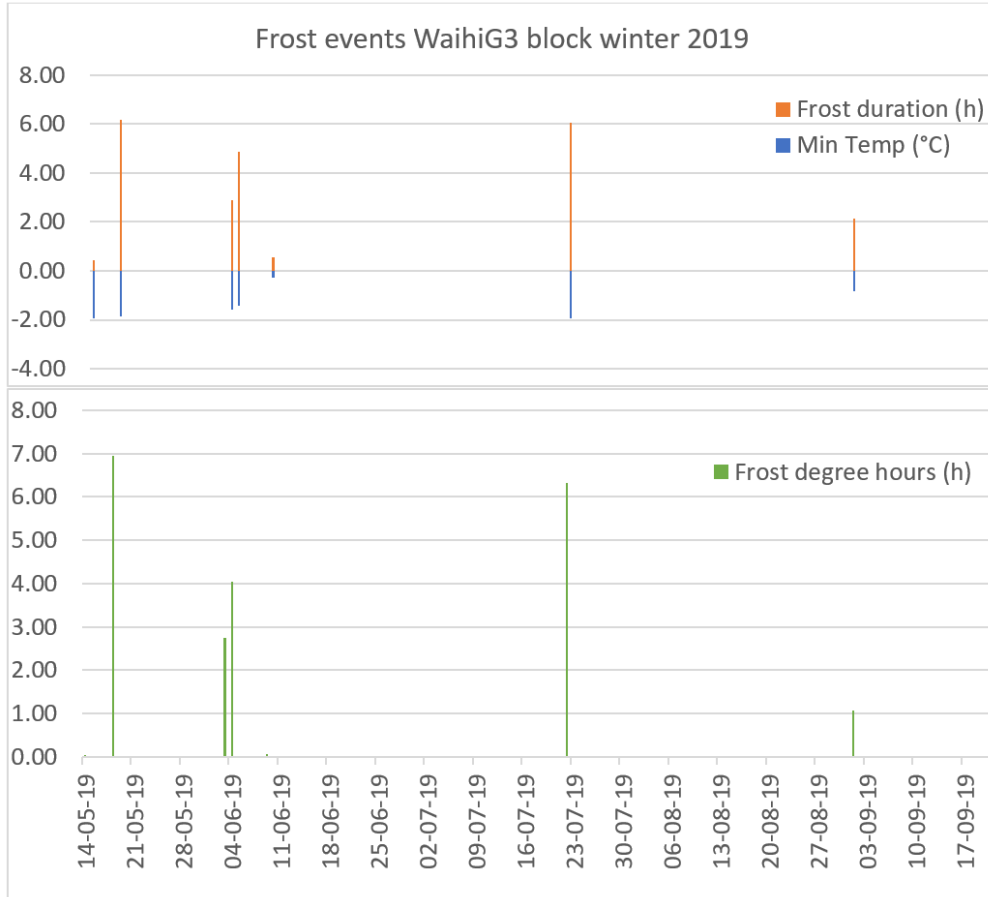
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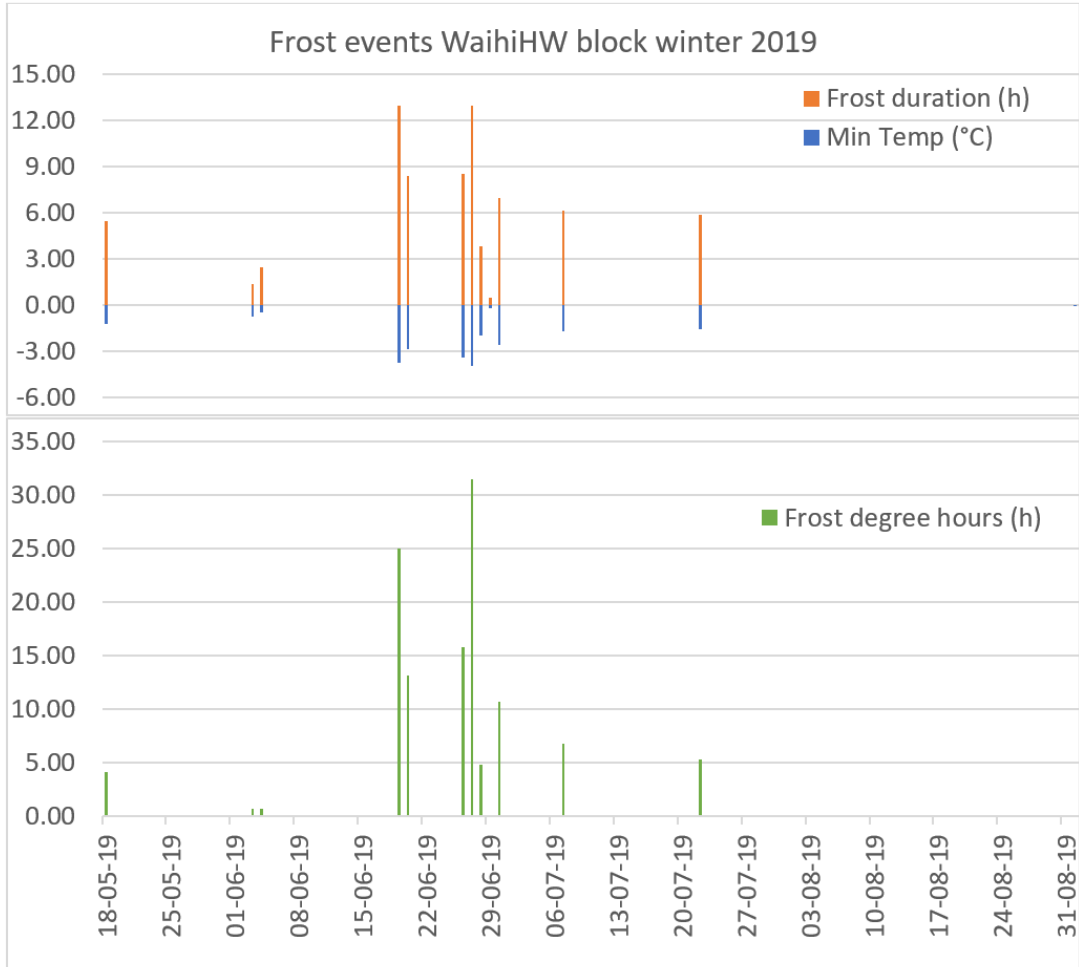
Testolin R, Messina R 1987. Winter Cold Tolerance of Kiwifruit - a Survey after Winter Frost Injury in Northern Italy. *New Zealand Journal of Experimental Agriculture* 15(4): 501-504.

APPENDIX 1

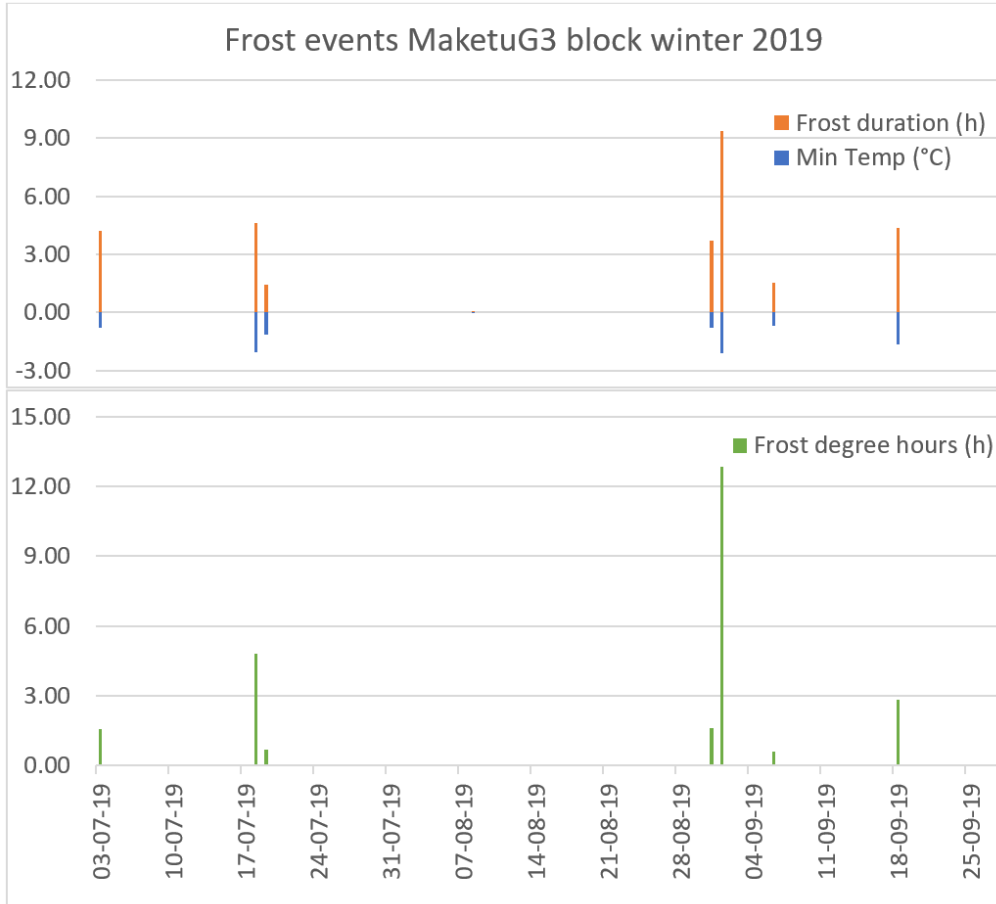
Frost events (overnight periods when temperature was $\leq 0^{\circ}\text{C}$ for > 5 minutes) recorded in four kiwifruit orchard study blocks from May to September 2019, showing duration (number of hours $\leq 0^{\circ}\text{C}$), minimum temperature and frost degree hours (product of hours $\leq 0^{\circ}\text{C}$ and average temperature while $\leq 0^{\circ}\text{C}$).



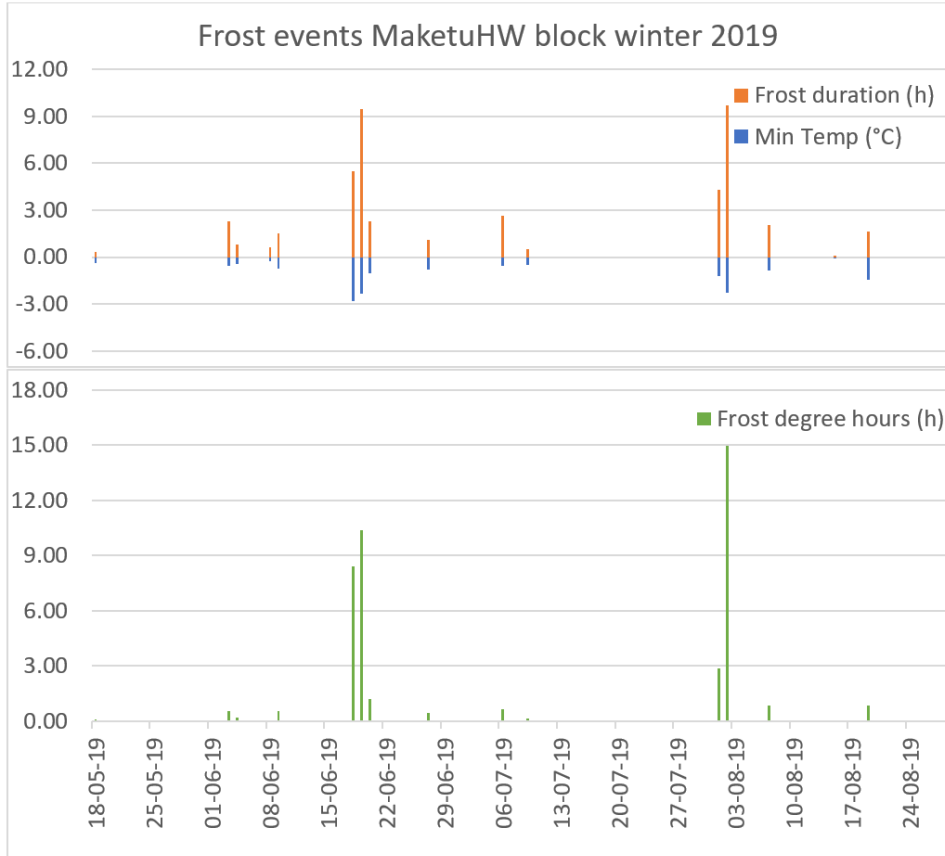
Appendix 1 cont.



Appendix 1 cont.



Appendix 1 cont.



APPENDIX 2

Modelling seasonal frost tolerance of *Actinidia chinensis* var. *deliciosa* 'Hayward' from Pyke et al. (1986)

R. M. Beresford, September 2016 (presented in the original frost project description)

Summary

This study modelled the data of Pyke et al. (1986) who subjected potted 'Hayward' vines that were naturally hardened in autumn to various freezing temperatures in a controlled environment facility in autumn, winter and spring. Frost effects were assessed in spring in terms of damage to buds and shoots. The main findings were:

- Vines showed increasing frost tolerance as they hardened during autumn
- Vines reached maximum frost tolerance in July, when they could tolerate temperatures of -6°C without damage
- De-hardening occurred in late winter and vines were damaged by a -0.5°C frost in early September, just before budburst.

Methods

Published data on 'Hayward' frost tolerance (Pyke et al. 1986) were modelled to summarise the effects of different frost temperatures during different months on plant damage. The data were from a controlled temperature study in the DSIR National Climate Laboratory in Palmerston North. Potted 'Hayward' plants were held at different constant temperatures below 0°C for 6 h in different months following natural "hardening off". Plant damage was assessed the following spring. The Boltzman equation was used to model the relationship between temperature and damage for each month studied and cubic polynomials were used to define the Boltzman equation parameters for different months.

The two-parameter Boltzman equation is a symmetrical growth function with a lower asymptote of 0 and an upper asymptote of 1 (Equation 1)

$$Y = -1 / (1 + \text{EXP}((X-M) / R)) \quad (1),$$

where Y is a measure of frost damage, X is the temperature (below 0°C), M is the mid-point parameter describing the temperature at which 50% damage occurs and R is the rate parameter describing how fast the response occurs.

Results

Figure A2.1 shows the Boltzman curves fitted to the presented data. In September > 50% plant damage occurred at -0.5°C , whereas there was much greater frost tolerance in May, with 50% plant damage occurring at -7°C . In June and July frost tolerance was at its greatest, with 50% plant damage occurring at about -8°C .

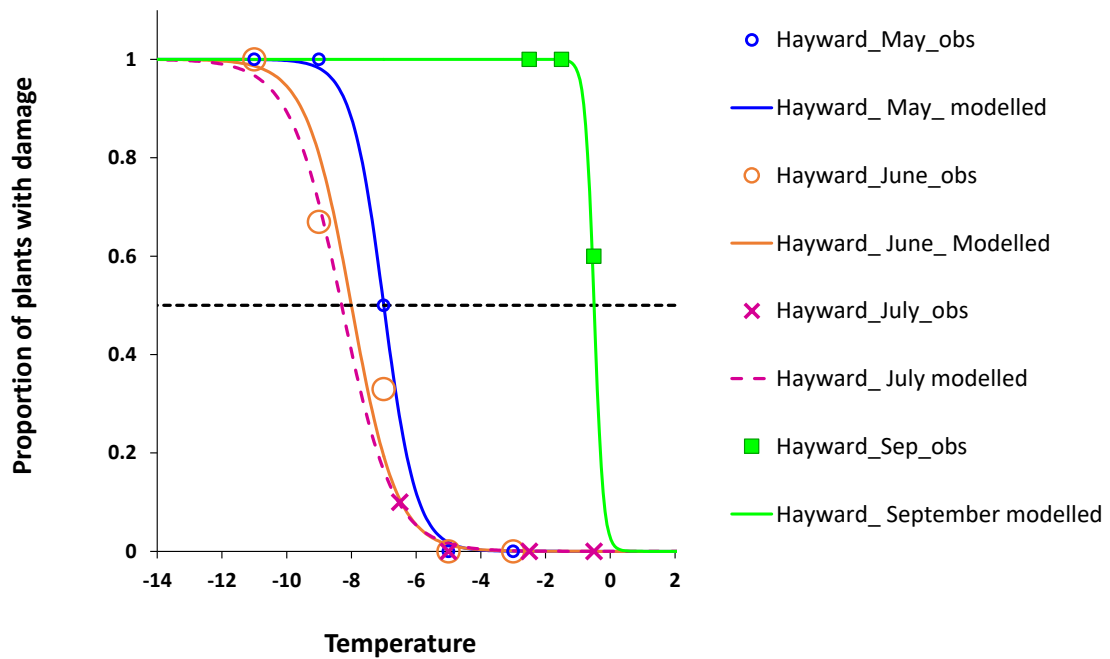


Figure A2.1. Boltzman equations fitted to observed data on plant damage in relation to temperature from Pyke et al. (1986).

Figure A2.2 shows Boltzman parameters in relation to the month in which potted plants were subjected to freezing temperatures. To describe the entire relationship between autumn and spring, an additional data point was added for the rate and mid-point parameters that assumed frost sensitivity in April would be the same as in September.

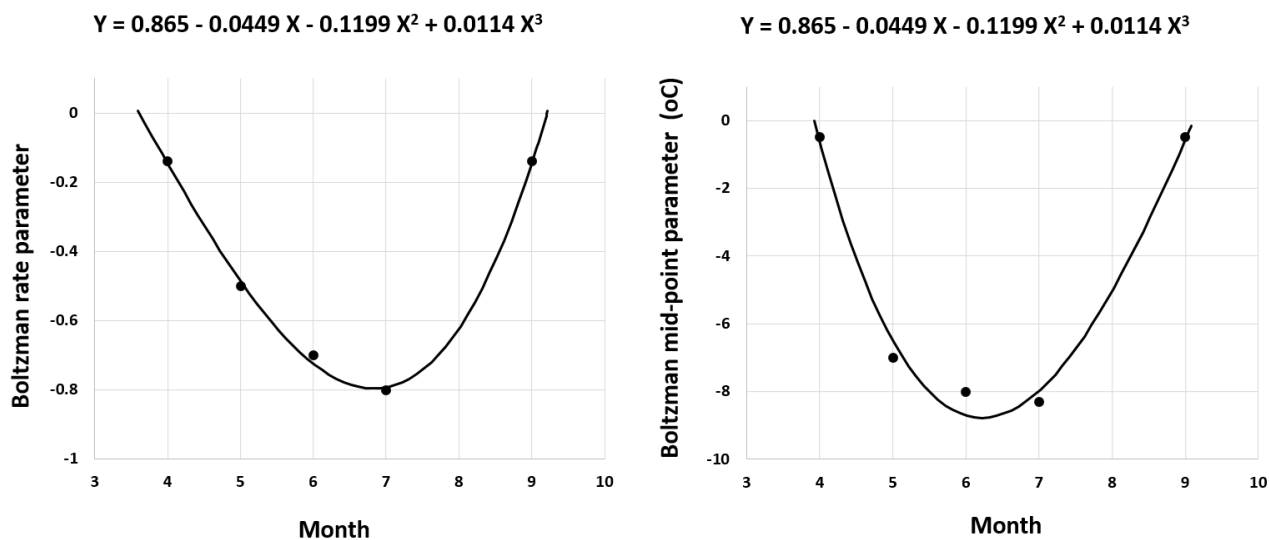


Figure A2.2. Cubic polynomials describing relationships between month and the rate and midpoint parameters of the Boltzman function.

The overall model showed that plants with active shoot growth in April and September were very susceptible to frost injury, for example, -0.5°C for 6 h caused $> 50\%$ damage (Figure A2.3). Frost tolerance increased gradually as dormancy developed during May and was maximum in late June and early July. At that time a temperature of -6°C could be tolerated with very little plant damage expressed the following spring. Frost tolerance decreased again during August and by mid-September plants were again very frost sensitive.

**Frost damage to 'Hayward' shoots in relation to temperature and month.
From Pyke et al. (1986)**

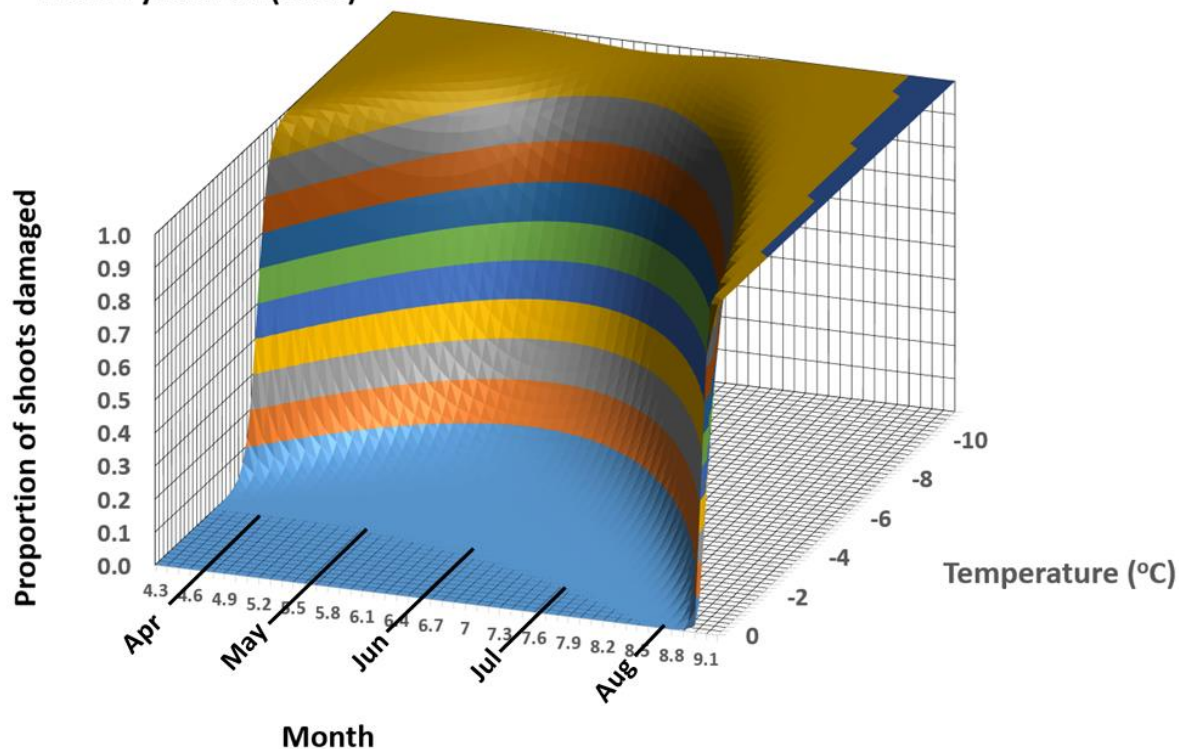


Figure A2.3. Response surface for the relationship between proportion of 'Hayward' kiwifruit plants with shoot damage, freezing temperature and the mid-point of each month in which a 6 h frost occurs.

Further refinement of his model is required and the small amount of 'Bruno' data provided by Pyke et al. (1986) should be compared with the 'Hayward' data examined here.

Reference

Pyke NB, Stanley CJ, Warrington IJ 1986. Kiwifruit: frost tolerance of plants in controlled frost conditions. *New Zealand Journal of Experimental Agriculture* 14: 443-447.



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