



VI1957 - Bud rot disease cycle (Objective 2)

Cover Note

Background:

Flower bud rot caused by *Pseudomonas syringae* pv. *actinidiae* (Psa) can decrease fruit productivity of 'Hayward' Kiwifruit. Symptoms include discoloured sepals, flowers that fail to open, partially opened flowers and shrivelled flowers. Flower buds with bud rot can lead to shrivelled fruit stalks and small shrivelled fruitlets and in extreme cases buds dropping off. These symptoms develop during spring and can reduce fruit set significantly. Therefore, it is important to understand when flower buds are at most risk so they can be protected from Psa infection.

Aims:

The study aimed to identify the bud growth stage(s) that are most susceptible to Psa in Hayward.

Methodology:

An experiment was conducted in the pre-flowering period during spring of 2018, 2019, and 2020 (October-November) on 'Hayward' kiwifruit vines, in a commercial orchard with a history of flower bud rot in the Bay of Plenty in New Zealand. Vines were exposed to rainfall and natural Psa inoculum during four week-long uncovered "Window" treatments during different flower bud growth stages. In 2018 and 2019 only natural rainfall occurred but in 2020 additional artificial rain was also applied during each uncovered window. Bud rot incidence, Psa leaf spot, severity, and bud growth stages were recorded weekly, and weather data were recorded for the entire experimental period..

Key findings:

- Flower buds were most susceptible to Psa infection early in their development, for only 1-2 weeks after they first emerged.
- In the first two years, high rainfall coincided with the early bud development stage, so the bud rot that developed could have been caused either by the early stage or the high rainfall. However, during the third year, the artificial rain applied equally in each uncovered window treatment proved the buds were only susceptible at the early stage.
- Symptoms can take up to 2 weeks to develop after the infection takes place.

Overall conclusion:

- The crucial role rainfall plays in Psa bud, leaf and cane infection is captured in the Psa risk model but we now know that buds are only susceptible for 1-2 two weeks after they first emerge.
- Early bud protection is key in protecting your flower buds from Psa infection.



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VI1957 var2: Bud rot disease cycle

Kabir MS, Barton C, Parry BE, Tyson JL, Scott P, Mellow KD, Lewis K, Beresford RM

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Report prepared by:

Shahjahan Kabir
Scientist, Epidemiology and Disease Management
May 2021

Report approved by:

Beccy Ganley
Science Group Leader, Plant Pathology
May 2021

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Executive summary

VI1957 var2: Bud rot disease cycle

Kabir MS¹, Barton C¹, Parry BE¹, Tyson JL², Scott P¹, Mellow KD², Lewis K², Beresford RM²
Plant & Food Research: ¹Te Puke, ²Auckland

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Flower bud rot of *Actinidia chinensis* var. *deliciosa* 'Hayward' kiwifruit, caused by *Pseudomonas syringae* pv. *actinidiae* (Psa) can decrease vine productivity. Growers are managing this disease mainly by applying protectants. However, potential high risk orchards are still facing about 40% bud rot infection. Part of the solution to the bud rot problem is likely to be the ability to target application of control products to the susceptible bud growth stages. However, it is not known when buds are most susceptible to the infections that lead to bud rot, or the environmental conditions associated with it. The aim of this research is to identify the bud growth stage(s) that are most susceptible to bud rot infection in the field.

This is a report of an extended two-year project (conducted in spring of 2018 and 2019), which was conducted based on artificial rain on top of natural rain. The experiment was conducted on 'Hayward' kiwifruit vines, in a commercial orchard with a history of bud rot in the Bay of Plenty. Sections of the kiwifruit canopy were covered when the vines were dormant by raising plastic tunnel houses (3 x 2 m) over parts of the bays. Vines were exposed to rainfall and natural Psa inoculum during four week-long uncovered "Window" treatments during different flower bud growth stages. Control treatments included Completely Covered, No Cover (received no Psa management), Grower Control (received grower's standard Psa management programme) and Water Control (received sprinkler water for entire duration of experiment). Bud rot incidence, Psa leaf spot and severity and bud growth stages were recorded weekly, and weather data was recorded for the experimental periods (from bud break to flowering) in 2020 at the experimental site.

Bud rot incidence was greatest in the earliest exposed window, Window 1 (73%) when buds had just emerged in leaf axils and were still closed (approximately 3 weeks after bud break). Incidence was significantly higher than other windows (2–13%) and the Completely Covered Control (8%). Among the positive controls, No Cover (74%) and Water Control (83%) had similar amounts of bud rot to Window 1. However, Grower Control had significantly less incidence (31%) of bud rot compared to the other two positive controls. These results suggest that buds are highly susceptible to Psa if significant rainfall occurs and Psa inoculum is present in the orchard. Psa management in the Grower Control included pre-flower girdling and protectants (e.g. copper, antibiotics) to manage Psa. Results also suggested that these management practices significantly reduced the amount of bud rot in the Grower Control.

Bud rot progression was also monitored in each window. Bud rot incidence significantly increased in Window 1 (32%) by 2 weeks since after the canopy was opened. These results suggest that bud rot symptoms appears in the field at about 2 weeks after infection takes place. Symptom development also took similar time in other windows.

Leaf spot severity was monitored qualitatively (visual estimates of percentage leaf area damaged by Psa). Higher Psa leaf spot was observed in Window 1 compared to other windows. These results also show that unprotected, newly emerged tissues are susceptible to infection if rainfall occurs.

Our conclusion from the experimental results is that the bud growth stage at Window 1 (early flower buds, approximately 3 weeks after bud break) is when flower buds are most susceptible to Psa infection. Growers need to protect early flower buds to manage Psa infection, this includes removal of all sources of inoculum in the canopy and a good protectant programme. If the bud break happens evenly this will be a very short period of time, but may be longer for organic growers if bud breaks unevenly. We recommend the effectiveness of various products to be trialled during this early bud growth stage for management of Psa bud rot, in both conventional and organic orchard. Canopy density could also be investigated as a cultural control to manage Psa.

For further information please contact:

Shahjahan Kabir
Plant & Food Research Te Puke
412 No. 1 Road
RD2
Te Puke 3182
NEW ZEALAND
Tel: +64 7 928 9800
DDI: +64 7 928 9792
Fax: +64 7 928 9801
Email: Shahjahan.Kabir@plantandfood.co.nz

1 Introduction

Bud rot caused by *Pseudomonas syringae* pv. *actinidiae* (Psa) (Tyson et al. 2015) can be a serious problem in green kiwifruit cultivars. The symptoms of Psa bud rot are discoloured sepals, flowers that fail to open, partially opened flowers and shrivelled flowers. Flower buds with bud rot can lead to shrivelled fruit stalks and small shrivelled fruitlets (Hunkin 2013) and buds dropping off. These symptoms develop during spring and can reduce fruit set. In an extreme case, 80% of flower or fruitlet loss was recorded in a Green14 orchard (*Actinidia chinensis* var. *chinensis* x *A. chinensis* var. *deliciosa* 'Zesh004'; Hunkin 2013).

Growers are attempting to manage bud rot in various ways such as applying protectants (e.g. copper-based products, antibiotics) and practicing pre-flower girdling. However, potential high risk orchards are still facing about 40% bud rot infection (Kabir et al. 2019). Part of the solution to the bud rot problem is being able to better target the application of control products to the time when the host tissue is most susceptible to infection. However, it is not currently known when these tissues are most susceptible and if susceptibility changes over time.

Research over the previous two flowering seasons (2018, 2019) focused on determining the most susceptible flower growth stages. Covered (protected) vines were set up in a commercial orchard; these were consecutively uncovered to create "windows" during which the vines were exposed to rainfall and natural inoculum.

In the previous 2 years of research (spring 2018 and 2019), it appeared that bud rot incidence was greatest in the earliest exposed window, Window 1 (47% and 72% in 2018 and 2019 respectively) when buds had just emerged in leaf axils and were still closed (Kabir et al 2020). This window received a significant amount of rain in both years, hence the results suggest that buds at this growth stage are highly susceptible to Psa. However, several of the later windows received rain only in one of the two years or no rain at all. Due to the unequal amounts of rainfall in the different window treatments, robust conclusions could not be drawn about the susceptibility of buds to bud rot during the later windows.

During the 2-year trial, the first assessment of bud rot symptoms was at the end of Window 1, when the covers were re-closed. However, buds were not assessed for browning prior to Window 1 (when buds had just emerged), which may have resulted in very early infections being missed.

The aim of this research (2020 flowering season) is to identify the flower bud growth stages that are most susceptible to bud rot infection in the field, using artificial rain during each window to account for the uneven rainfall experienced in previous years. Infection 'windows' were created by covering vines to protect flower buds from rainfall and associated Psa inoculum and opening the covers for week-long 'windows' during sequential bud growth stages to allow natural infection to occur. Further to this, aim was also extended to assess earlier buds to know the inoculum availability of Psa at the time of earliest window.

2 Materials and methods

2.1 Experimental site and covering structure

This study was conducted in a commercial *Actinidia chinensis* var. *deliciosa* 'Hayward' orchard near Paengaroa, Bay of Plenty, where there was a history of bud rot over the last few years. The 'Hayward' scions were 13–14 years old and the rootstock was 'Bruno'. This experiment was carried out in the flowering season of 2020 (October – November).

Fifteen plastic tunnel houses (3 x 2 m) were raised (on 7 September 2020) over c. 30% of the canopy of individual vines prior to bud break (on 14 September 2020); the sides below the wires had short 'skirts' of plastic sheeting to further protect the flower buds from rain-splashed *Psa* inoculum (Figure 1). Each tunnel house comprised one replicate of a covered treatment.



Figure 1. Overview of tunnel houses above the kiwifruit canopy (top); close view of tunnel house on top of canopy (bottom left); short 'skirt' of plastic sheeting under each tunnel house (bottom right).

2.2 Windows of exposure, bud rot assessment

The 15 tunnel houses comprised five covered treatments, with three replications of each. Four of these treatments were opened/uncovered for 1 week at a time over 4 consecutive weeks. These were known as Windows 1–4. Each window was designed to expose the flower buds to natural Psa inoculum and the environmental conditions during exposure at various bud growth stages, over the 4 week period that buds developed from being newly emerged with small pedicles (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie growth stage 51; BBCH 51) to buds that were close to opening (BBCH 56), with elongated pedicles and separated sepals exposing the white corolla (Figure 2, Table 1). In addition, four controls were used for this experiment, also with three replications each:

1. Completely Covered Control (covered for the entire duration of flowering; BBCH51 to BBCH 56)
2. No Cover Control (received no Psa management and no simulated rainfall)
3. Water control (no cover and received sprinkler water for entire duration of flowering BBCH51 to BBCH 56)
4. Grower Control (no cover and no simulated rainfall, and received grower's standard Psa management).

All windows and the uncovered and covered controls were set in one row with three replicates of each treatment blocked within the row. These vines did not receive any sprays or girdling. The Grower Control vines were set two rows away, and received all the grower's Psa management practices.



Figure 2. Growth stages of flower buds from *Actinidia chinensis* var. *deliciosa* 'Hayward' vines, categorised by the BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie growth stage) scale (Salinero et al. 2009). BBCH 51 = closed buds, greenish sepals; BBCH 53 = closed buds, elongating reddish peduncles; BBCH 55 = sepals begin to separate, a white-greenish corolla is visible; BBCH 56 = sepals continue to separate, peduncles continue to elongate and thicken, corolla visible and white (source: Kabir et al. 2018).

All vines were assessed weekly for bud rot incidence immediately after the first window was opened and up until flowering. Bud rot incidence was assessed by visually inspecting 200 buds in each replicate. These 200 buds were randomly marked from random shoots of several (5 to 7) canes (from base of leader to the end) that lay within the 2m x 3m covered frame. Each bud was given a bud browning (BB) score from 0–5; 0 = no visible bud browning and 1–5 represented the number of sepals with bud browning (Elizabeth Popowski, personal communication, mentioned in Kabir et al. 2018).

Table 1. Timing of window exposures for *Actinidia chinensis* var. *deliciosa* 'Hayward' kiwifruit flower bud growth stage assessment and bud rot assessment in 2020 flowering season.

Windows and controls (treatment)	Cover removed	Cover replaced	Bud growth stage assessment date	Bud rot incidence assessment dates
Window 1	07 Oct	14 Oct	07 Oct	07, 14, 21, 28 Oct & 04 Nov
Window 2	14 Oct	21 Oct	14 Oct	07, 14, 21, 28 Oct & 04 Nov
Window 3	21 Oct	28 Oct	21 Oct	07, 14, 21, 28 Oct & 04 Nov
Window 4	28 Oct	04 Nov	28 Oct	07, 14, 21, 28 Oct & 04 Nov
Completely Covered Control	Covered for entire experimental time		Not assessed	07, 14, 21, 28 Oct & 04 Nov
No Cover Control	Never covered		Not assessed	07, 14, 21, 28 Oct & 04 Nov
Water Control	Never covered		Not assessed	07, 14, 21, 28 Oct & 04 Nov
Grower Control	Never covered		Not assessed	07, 14, 21, 28 Oct & 04 Nov

2.3 Application of sprinkler water to simulate rain

To simulate rain, filtrated grower's town supply water (filtrated by MP Series Maxiplus Filter Housing 10") was applied on the canopy. Two sprinklers (Meteor Stake Adapter 10mm BSPF to 5mm Barb with a S2000 White/Grey RL Rotor 10mm) with all other necessary accessories was installed by Waterite Pumps and Electrical Ltd, Te Puke at the top of the tunnel house structure (3m x 2m) area (Figure 3). Automated overhead sprinkler water was set to run for 4 h/day and for 3 days/weeklong window (Thursday, Saturday and Monday) to receive a substantial amount of water equivalent to 53.8 mm of rain in a weeklong window. This system was run between late afternoon and evening (4–8 pm) to prevent the water droplets from drying out too quickly.



Figure 3. Automated overhead sprinkler system. Two sprinklers set at the top of the canopy (left) and the water filtration system (right) used to purify supplied water from grower's town supply water.

2.4 Analysis

All analysis were conducted using R version 3.5.1 (R core team, 2018). Bud rot incidence data were fitted using binomial model (glm, link =logit). Number of symptomatic buds (Total bud rot, Bud rot 4+) was fitted as binomial success and total number of buds was fitted as binomial total. Dates of assessment were fitted as fixed effect. Leaf spot percentage data were fitted using beta-binomial model (link=logit), treatment was fitted as fixed effect. Back-transformed means and 95% confidence intervals were estimated to identify significant differences.

3 Results

3.1 Bud rot incidence at final assessment

Bud rot incidence is presented in two ways; firstly including all levels of bud browning severity (1–5 brown sepals) and secondly, including only buds with four or more (4+) brown sepals. The 4+ category was used to represent severely browned buds that were unlikely to set fruit.

When the incidence was analysed based on all sepal browning scores, significantly higher bud rot incidence was recorded in Window 1 (73%, Figure 4) than in all other windows ($p < 0.05$). Window 2 had significantly higher bud rot (13%) than Window 3 but not Window 4. There was no significant difference in bud rot incidence between Window 1 and the No Cover and Water controls. The Grower control had significantly less bud rot (31%) than the No Cover Control (74%) and Water Control (83%).

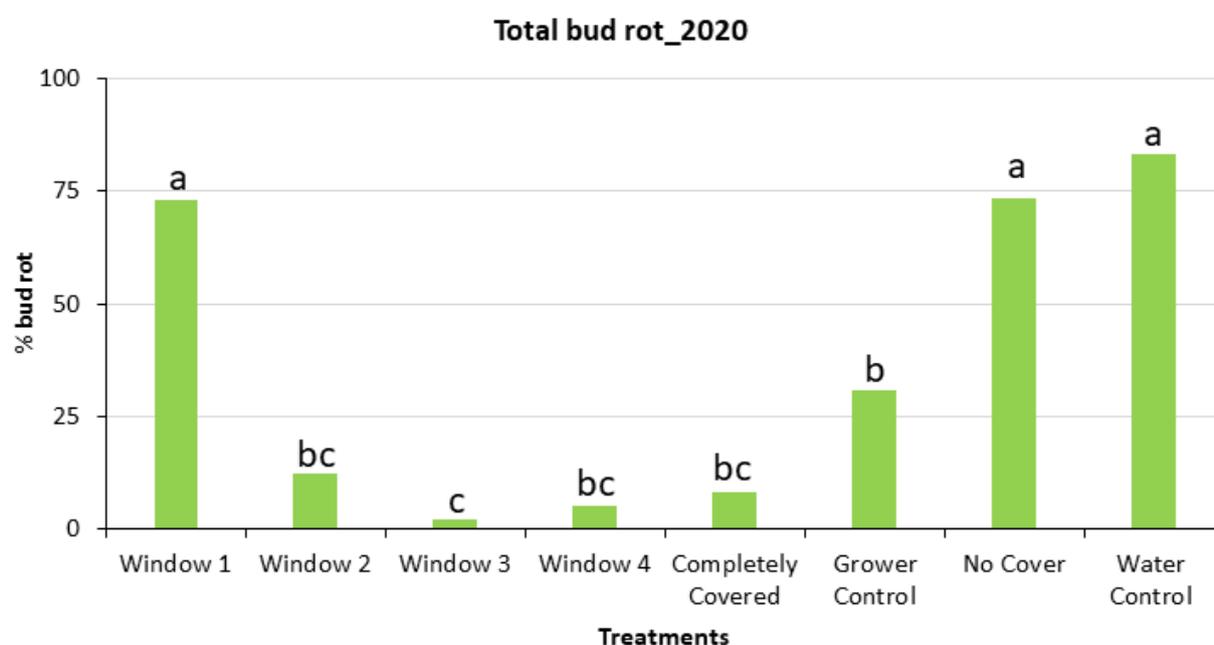


Figure 4. Bud rot incidence based on all types of bud browning scores of 1–5, where 1=one sepal brown and 5=all sepals brown at the final assessment on the 4 November in 2020 on *Actinidia chinensis* var. *deliciosa* 'Hayward'. Data are back-transformed means of 200 buds/ replicate. Common letters on each bar indicate that the means are not significantly different ($p < 0.05$).

When bud rot incidence was counted including only buds with bud browning severity scores greater or equal to 4 (4+ brown sepals), there was significantly higher bud rot incidence in Window 1 (15%, Figure 5) than in all other windows ($p < 0.05$). There was no significant difference between Window 1, the No Cover Control (11%) and the Grower Control (3%). The highest bud rot incidence was seen in the Water Control (34%); this was significantly higher than the incidence in Window 1.

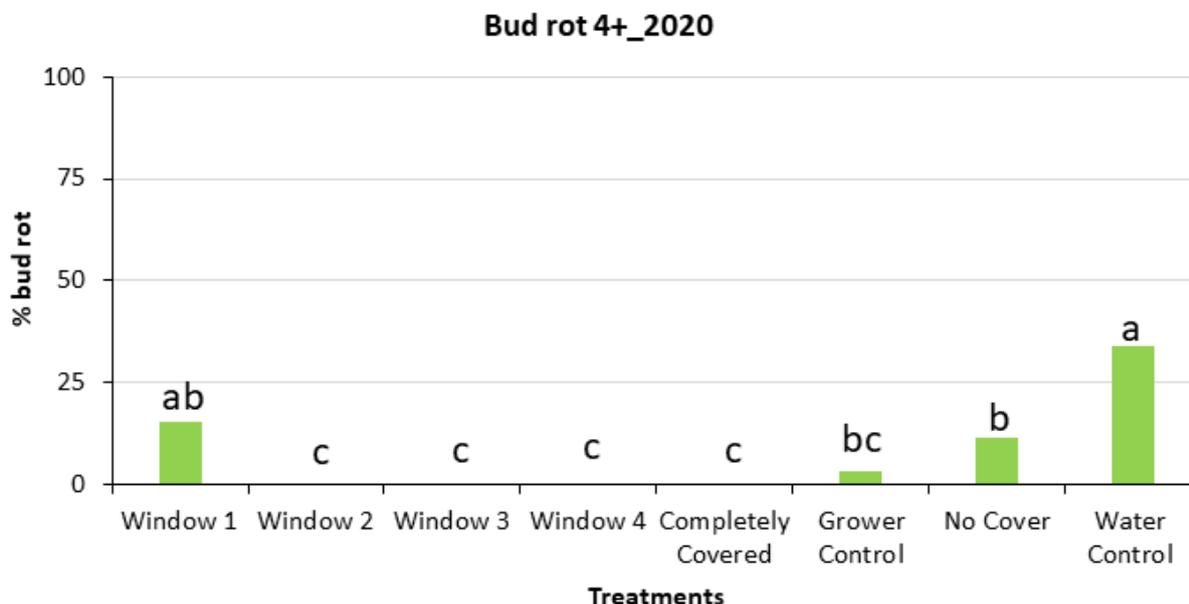


Figure 5. Bud rot incidence based on four and above sepals browning at final assessment on 4 November in 2020 on *Actinidia chinensis* var. *deliciosa* 'Hayward'. Data are back-transformed means of 200 buds/replicate. Common letters on each bar indicate that the means are not significantly different ($p < 0.05$).

3.2 Infection progression considering all types of sepal browning

The growth stage of 'Hayward' kiwifruit flower buds during each 'window' treatment, the time period over which the treatments were open, and rainfall amount (both natural and artificial) and number of rain days during each window are shown in Table 2.

Table 2. Percentage of *Actinidia chinensis* var. *deliciosa* 'Hayward' kiwifruit flower buds at each of four growth stages (at the beginning), and rainfall amount and frequency during the time each window was open in 2020.

Treatment	Exposure dates	BBCH* stage				Comment on growth stage	Total rainfall (mm)		No. days with rain	
		51	53	55	56		Natural	Artificial	Natural	Artificial
Window 1	7 Oct. 2020	76	24	0	0	BBCH51 to early BBCH53	24.5	17.9	3	3
Window 2	14 Oct. 2020	5	95	0	0	Early to mid BBCH53	4.5	17.9	3	3
Window 3	21 Oct. 2020	0	100	0	0	Mid BBCH53	0.5	17.9	1	3
Window 4	28 Oct. 2020	0	40	60	0	Late BBCH53 to BBCH55	15	17.9	4	3

*BBCH= Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie growth stage scale.

Window 1 was exposed to natural Psa inoculum on 7 October 2020 for 7 days; all buds were at the growth stage BBCH51 to early BBCH53 when the covers were removed (Figure 6 and Table 2). Bud rot symptoms were first observed during the assessment on 21 October 2020. In this treatment, bud rot incidence significantly increased (32%, Figure 6) after 14 days (21 October) ($p < 0.05$) and the highest incidence (73%) was observed on 4 November. The bud growth stage and bud rot incidence for this treatment are shown in Figure 10. This window received 24.5 mm and 53.8 mm of natural and artificial rainfall respectively.

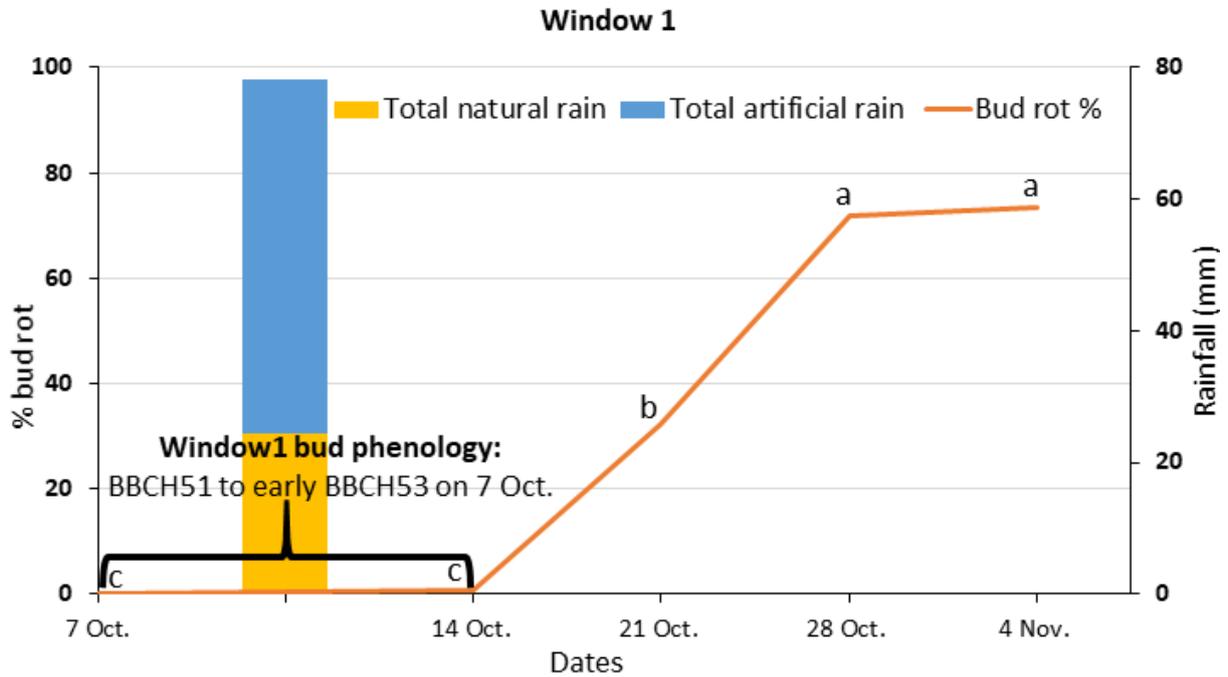


Figure 6. Progression of bud rot incidence in Window 1 in 2020. Percentage of bud rot incidence and total rainfall (during whole window exposed time) indicated on left and right y-axis respectively. Dates are indicated in x-axis and black bracket in each figure indicate an uncovered window. Data are back-transformed means of 200 buds/replicate. Common letters on the line indicate that the means are not significantly different ($p < 0.05$).

Window 2 was opened to natural inoculum on 14 October 2020 (early to mid BBCH53) for 1 week. Incidence increased significantly ($p < 0.05$) one week after the window was re-covered with very low bud rot incidence (less than 7%) (Figure 7). This treatment received 4.5 mm and 53.8 mm of natural and artificial rainfall respectively, the highest bud rot incidence was 13%. The bud growth stage and bud rot incidence for this treatment are shown in Figure 10.

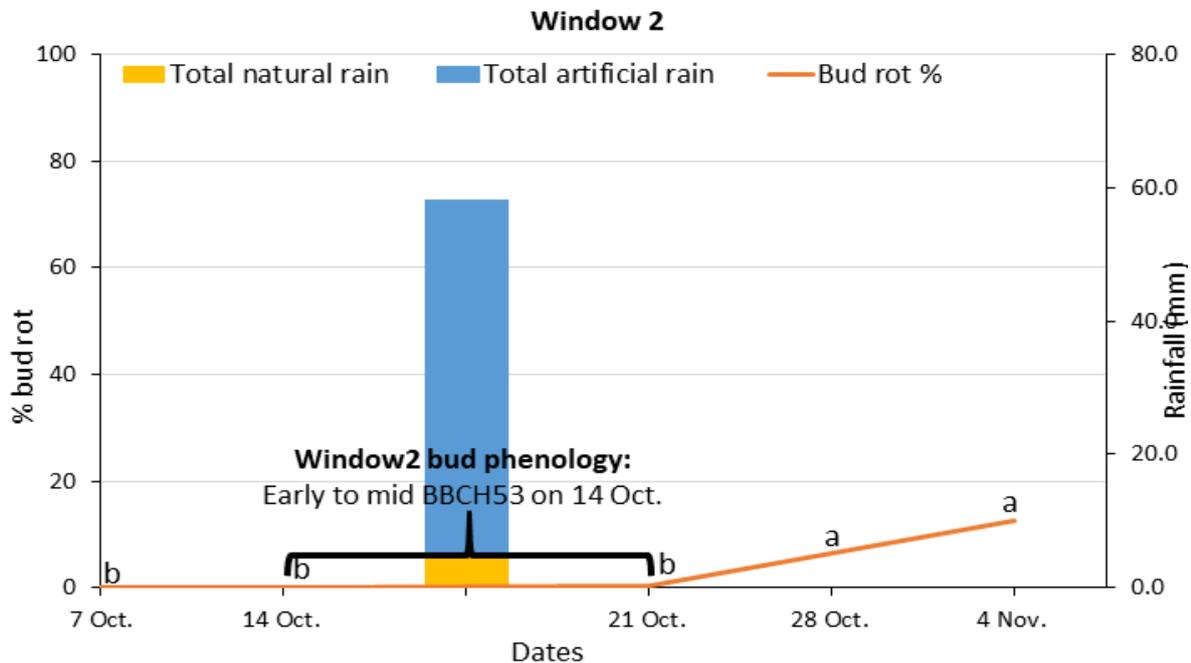


Figure 7. Progression of bud rot incidence in Window 2 in 2020. Percentage of bud rot incidence and total rainfall (during whole window exposed time) indicated on left and right y-axis respectively. Dates are indicated in x-axis and black bracket in each figure indicate an uncovered window. Data are back-transformed means of 200 buds/replicate. Common letters on the line indicate that the means are not significantly different ($p < 0.05$).

In Window 3, (mid BBCH53 growth stage), bud rot incidence increased significantly (Figure 8) at the end of Window 3 (28 October 2020). Despite of receiving artificial rain (53.8 mm) and a small amount of natural rain (0.5 mm), the highest amount of bud rot incidence was 2% on 4 November. The bud growth stage and bud rot incidence for this treatment are shown in Figure 10.

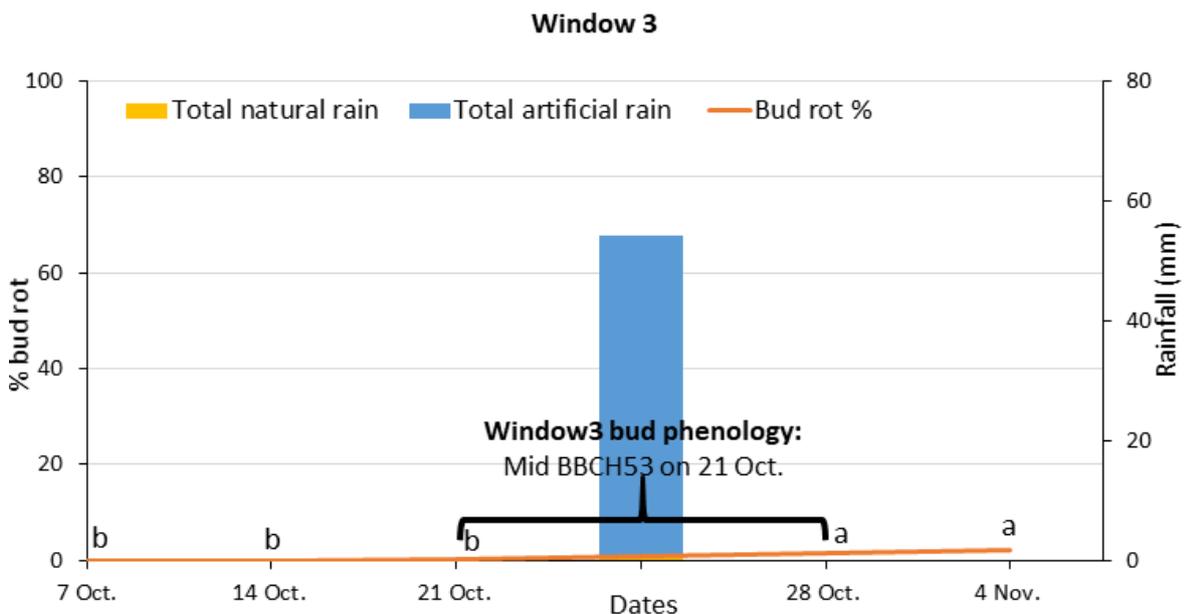


Figure 8. Progression of bud rot incidence in Window 3 in 2020. Percentage of bud rot incidence and total rainfall (during whole window exposed time) indicated on left and right y-axis respectively. Dates are indicated in x-axis and black bracket in each figure indicate an uncovered window. Data are back-transformed means of 200 buds/replicate. Common letters on the line indicate that the means are not significantly different ($p < 0.05$).

No significant increase in bud rot incidence was observed when the canopy was exposed at Window 4 (late BBCH53 to BBCH55) in spite of 15 and 53.8 mm of natural and artificial rainfall respectively (Figure 9). Highest bud rot incidence was noted 5% in this window. The bud growth stage and bud rot incidence for this treatment are shown in Figure 10.

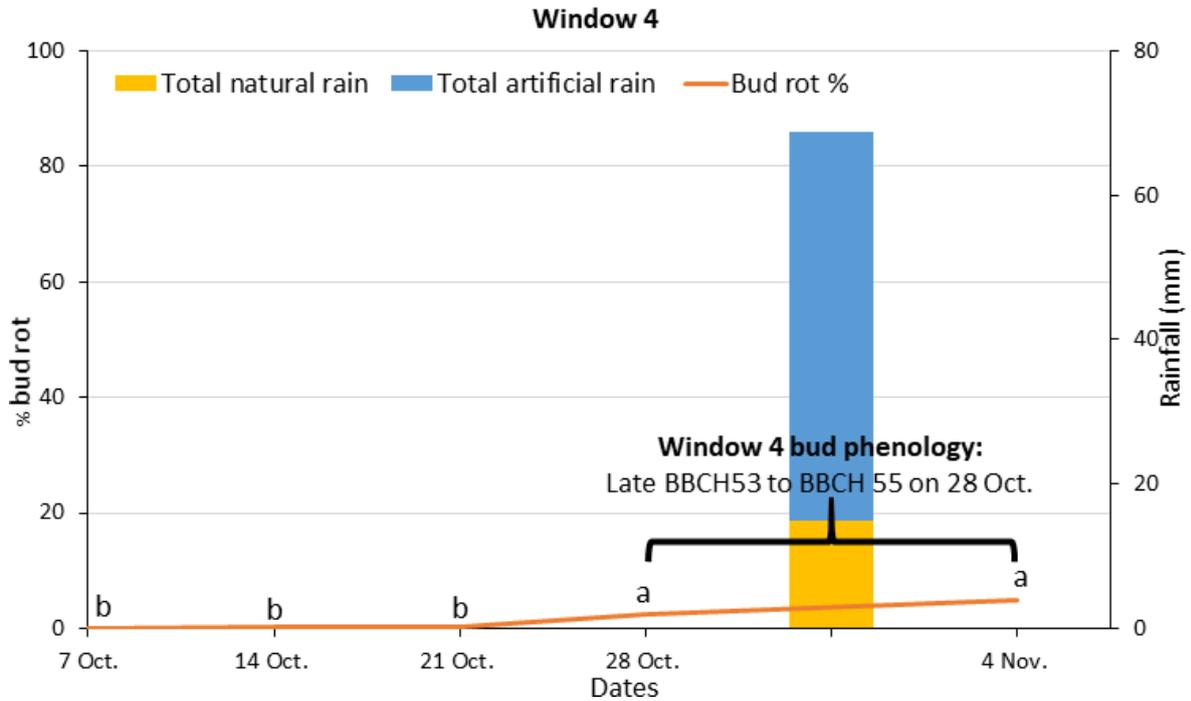


Figure 9. Progression of bud rot incidence in Window 4 in 2020. Percentage of bud rot incidence and total rainfall (during whole window exposed time) indicated on left and right y-axis respectively. Dates are indicated in x-axis and black bracket in each figure indicate an uncovered window. Data are back-transformed means of 200 buds/replicate. Common letters on the line indicate that the means are not significantly different ($p < 0.05$).



Figure 10. Bud growth stage during each 'Window' in 2020 (left column) and symptoms seen at the final assessment (right column). A, B, C and D indicates 'Window 1', 'Window 2', 'Window 3' and 'Window 4' respectively. A. Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie growth stage (BBCH) 51 (left), bud browning and *Pseudomonas syringae* pv. *actinidiae* (Psa) leaf spot (right). B. Bud growth stage early to mid BBCH 53 (left), low incidence of bud rot and Psa leaf spot (right). C. Bud growth stage mid to late BBCH 53 (left), most buds are free from bud rot and Psa leaf spot (right). D. Bud growth stage Late BBCH 53 to BBCH 55 (left), most buds are free from bud rot and Psa leaf spot (right).

In the Completely Covered Control, bud rot incidence increased significantly on 28 October 2020, with a maximum incidence of 8% (Figures 11 and 13).

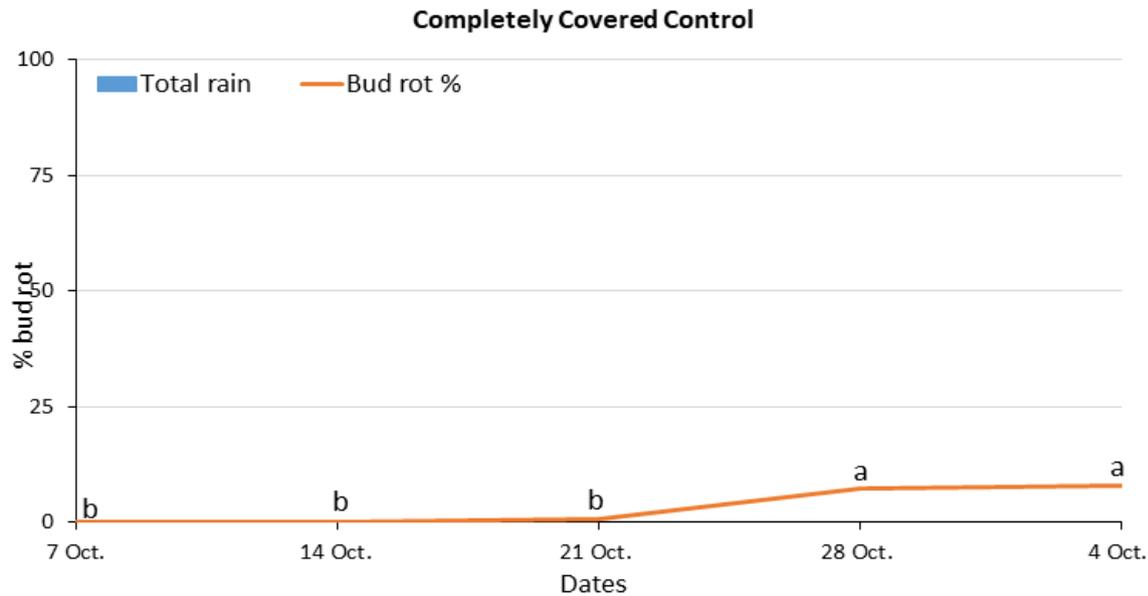


Figure 11. Progression of bud rot incidence in Completely Covered Control in 2020. Percentage of bud rot incidence and dates are indicated on y and x axis respectively. There was no rainfall in this control as it was covered for the experimental period. Data are back-transformed means of 200 buds/replicate. Common letters on the line indicate that the means are not significantly different ($p < 0.05$).

Bud rot incidence was monitored in all positive controls (Figures 12 and 13). Incidence increased significantly on the third assessment (21 October 2020) in Water Control and kept rising sharply till the fourth assessment and then plateaued. The highest point of infection (83%) was observed at the final assessment on 4 November 2020. The same trend was followed for No Cover Control but a lower percentage of infection throughout was observed, with a highest infection of 74%. Although Growers Control showed significant bud rot infection on the second assessment (14 October), it increased slowly over the entire experiment with a highest infection of 31% at the end. This treatment received four protectants (from bud break till flowering) and pre-flower girdling to manage PsA.

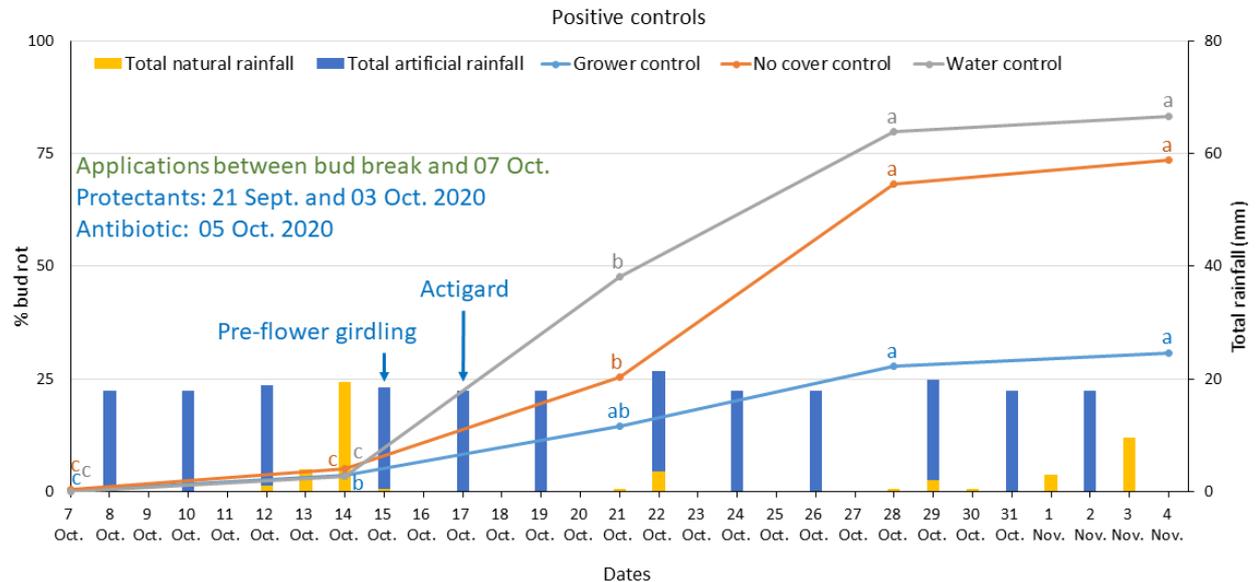


Figure 12. Progression of bud rot incidence (BRI) in positive controls; Grower Control, No Cover Control and Water Control, in 2020. Percentage of BRI and total rainfall indicated on left and right y-axis respectively. BRI of Grower Control and No Cover Control is based on natural rainfall. Grower management are indicated by blue arrows where grower had 3 protectants (including an antibiotic) prior to 7 October. BRI of Water Control is based on both natural and artificial rainfall. Data are back-transformed means of 200 buds/replicate. Common letters on the line indicate that the means are not significantly different ($p < 0.05$).

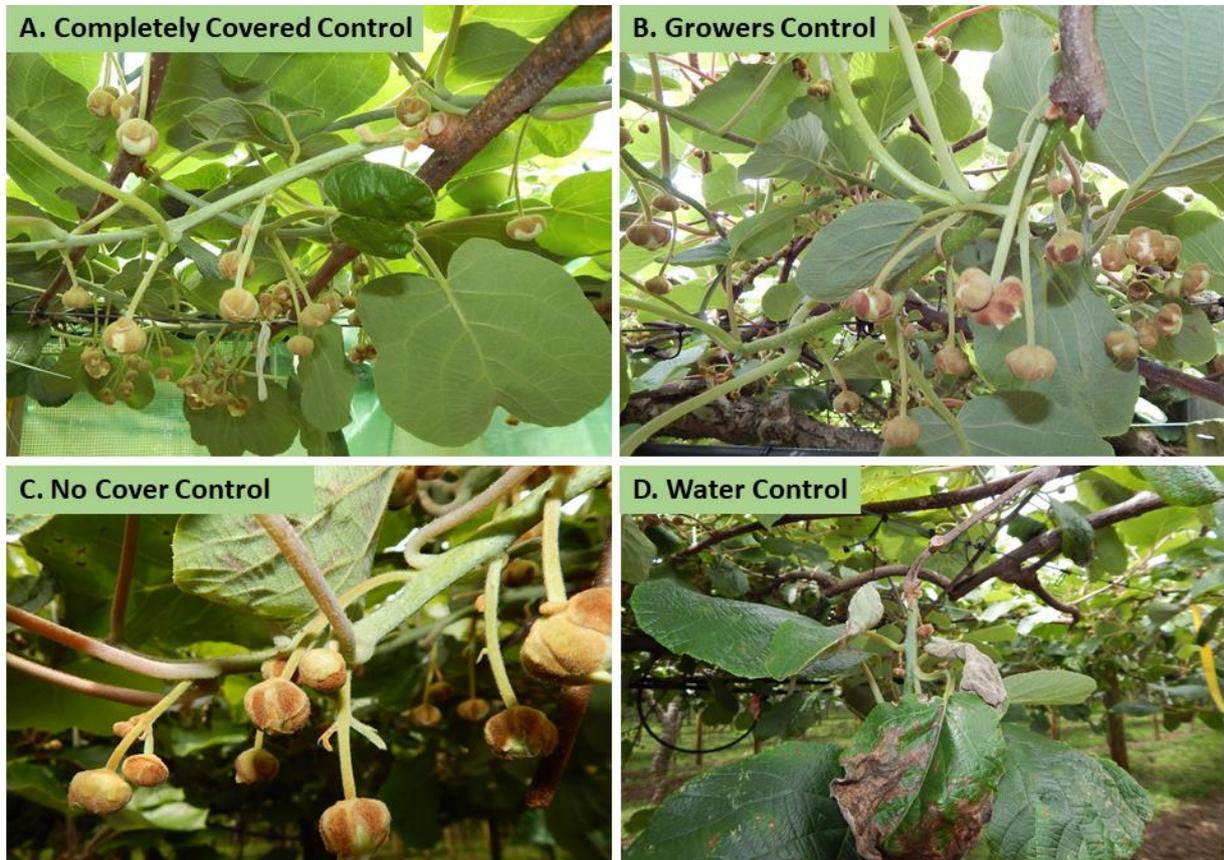


Figure 13. Representative images of buds and leaves of controls at final assessment in 2020 in *Actinidia chinensis* var. *deliciosa* 'Hayward'. A. Completely Covered Control with no bud rot or *Pseudomonas syringae* pv. *actinidiae* (Psa) leaf spot, B and C infected buds with Psa leaf symptoms, D. Severely infected buds and leaves.

3.3 Leaf infection at final assessment

Leaf spot severity was estimated by visual detection of the leaf area damaged by Psa in both years. Window 1 had significantly higher Psa leaf spot ($p < 0.05$) compared with Windows 3 and 4 (Figure 14). The percentage of leaf spot in Window 1 was 5%, which was almost the same as Grower Control and No Cover Control. Among the positive controls, Water Control (11%) had the highest severity, significantly higher than the No Cover Control, Growers Control and Window 1.

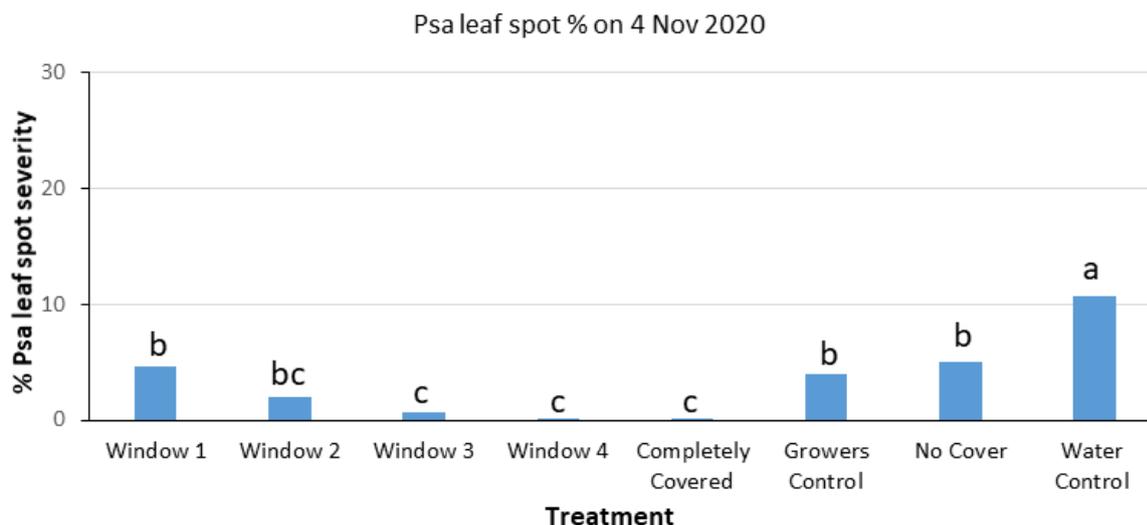


Figure 14. Leaf spot severity at final assessment on 4 November 2020 in all treatments. Data are back-transformed means and common letters on each line indicate that the means are not significantly different ($p < 0.05$). Severity was a qualitative estimation of all canopy leaves that were damaged by leaf spot.

3.4 Extending research into earlier buds

The flower buds of the Completely Covered and No Cover Control were assessed at an early growth stage (Window 1 opening time). No bud rot symptoms were found in the Completely Covered Control. However, a negligible amount of infected buds (0.5%) were seen in at the same time on the No Cover Control treatment. These infected buds were sporadic amongst the replications and were in the category of BB1 (bud browning scale 1).

4 Discussion

Despite Psa management strategies and the importance of adopting Psa best practices between bud break and flowering being well documented, growers are still experiencing Psa bud rot related issues in 'Hayward' kiwifruit. This project aimed to identify bud growth stage(s) that are most susceptible to bud rot infection. A previous 2-year trial was conducted in 2018 and 2019. This trial depended on natural rainfall (Kabir et al. 2020) and as such received an unequal amount of rain in the exposed 'window' treatments (different growth stages of flower buds). Also, several of the windows only received rain in one of the two years. Therefore, in 2020 this experiment included a significant amount of artificial rain in addition to the natural rainfall.

The bud rot incidence in Window 1 was significantly higher than in the other three windows, suggesting that the early buds, newly emerged on the shoot axil approximately 3 weeks after bud break (the developmental state when leaf margins, leaf vasculature and green tissue is first visible from a broken bud, i.e. between BBCH 07 and BBCH 09, Salinero et al. 2009), are highly susceptible to Psa infection. This finding supports the results of 2018 and 2019 conducted under natural rain (Kabir et al. 2020). Coincidentally there were significant natural rain events in 2018 and 2019 during this window, and substantial rain also fell in 2020. From the 3 years of rainfall data, it appears likely that rain is common over this highly susceptible time. Growers will need to find a suitable day to apply a Psa protectant immediately prior to this window.

The flower bud growth stage during Window 2 is less sensitive to Psa infection than Window 1. Although there was some bud rot in 2019 (Kabir et al. 2020), it was only limited to low bud rot severity (less than 4+ in severity scale).

The bud growth stages in Window 3 and 4 (mid BBCH53 to BBCH55) are tolerant to bud rot. Bud rot incidence in Window 3 of 2018 supports this findings (Kabir et al. 2020), where in spite of significant rain there was negligible amount of bud rot. As with bud rot, tissue age is also important for Psa leaf spotting, where younger leaves are most susceptible, becoming progressively less susceptible as they age (Tyson et al. 2015a).

Bud growth stages in these trials are based on conventional growing systems and defined periods of time. As such, Hi-Cane was applied in the trial site and bud break was very regular. However, bud growth stages in organic orchards are likely to be overlapping and longer due to irregular bud break as they are not allowed to apply Hi-Cane.

A small amount of bud rot (less severe, mostly one/two sepal browning) developed in the Completely Covered Control, indicating that the design of the covering structures could be improved. Although the canopy was covered on the top and sides, small rain droplets or splash carrying Psa bacteria could have reached some buds. Covering the entire experimental time, Completely Covered treatment showed substantial amount of shoot growth, which made the canopy very dense. There might be increased humidity due to this overgrowth and this might lead to less bud rot incidence. Alternatively, the possibility that some bud and leaf infection could have arisen internally from within the plots cannot be ruled out from the evidence gathered using this experimental approach.

Three positive controls were used in this study: the Grower Control which received all Psa management practices, the No Cover Control which received no management, and Water Control which received a significant amount of simulated rain each week. The Grower Control had significantly lower bud rot incidence than the No Cover Control, suggesting that the grower's current management approach reduced the bud rot incidence. This year (spring 2020), the grower took Psa protection

measures at the early bud development phase, based on the findings of 2018 and 2019 (Kabir et al. 2020).

The highest bud rot incidence in the Water Control confirmed that the artificial application of water was successful. It had been a concern that significant water may wash Psa from the buds/leaf or might not be suitable for Psa infection, although the bore water was filtered to eliminate iron.

Knowing the source of Psa inoculum is another important piece of information required to manage bud rot infection. Leaves are likely to be one of the main sources of inoculum. Research found flower buds on nearby spotted leaves were positive for Psa inoculum (Kabir et al. 2018) and rain splash disseminates the inoculum (Tyson et al. 2014). This study supports these findings as Window 1 had the highest amount of leaf spot compared with all other windows (Kabir et al. 2020). These results suggest that leaf protection measures are also necessary to restrict the transfer of Psa inoculum from the leaves to the buds early in the season. Research was extended to earlier buds and leaves (Window 1 growth stage) this year in both Window 1 and No Cover Control treatments. Very sporadic and negligible buds showed some initial symptoms but no leaf spot was observed on any of the treatments. This could be the case that leaf infection has occurred but visible symptoms has not been appeared yet.

The windows of infection approach used in this study may have provided more complete information on the availability of Psa inoculum and tissue susceptibility in relation to age if all the exposure windows had been of constant length in relation to each bud developmental stage. In addition to this, due to the size and complexity of raising tunnel houses above the canopy, there were only three replications of the eight treatments. Although more replications would have given more power to the study, due to the size and complexity this was not feasible. However, the current design of the study was still able to detect differences in susceptibility over the bud growth stages and has given fundamental knowledge to move forward with.

Another limiting factor was application of artificial water. We initially had planned to apply the total amount of water equally by matching with the weather forecast. However, as the forecast was variable and rain was patchy, we instead applied the same amount of water on top of natural rain. This automated rain sometimes coincided with natural rain, meaning the total amount of rain applied was not even across the treatments. Artificial rain application was timed between late afternoon and evening time to avoid quick drying of water. The amount of rain under the canopy was measured but leaf wetness was not measured. It would be fair to compare between artificially applied water versus no artificially applied water in each window (or treatment). However, it will bring more complexity as well as make the experiment very laborious.

To conclude, from the experimental results bud growth stage at Window 1 (early flower buds) are most susceptible to Psa infection. Growers need to protect early flower buds to manage Psa infection. This is a very short period of time if the bud break occurs evenly, but may turn into longer for organic growers if bud breaks unevenly. The effectiveness of various products should be trialled during this window to manage Psa bud rot in conventional and organic orchards. Canopy density should also be investigated as a cultural control to manage Psa.

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Appendix. *Actinidia chinensis* var. *deliciosa* 'Hayward' trial site

		Trial site								
Bays		1	2	3	4	5	6	7	8	Rows
1				M	F	M	F	M	F	
2				M	F	M	F	M	F	
3				M	Window 1	M	F	M	F	Replication 1
4				M	F	M	F	M	F	
5				M	Window 2	M	F	M	F	
6				M	F	M	F	M	F	
7				M	Window 3	M	F	M	F	
8				M	Water control	M	F	M	F	
9				M	Window 4	M	F	M	F	
10				M	F	M	F	M	F	
11				M	Completely covered	M	F	M	F	
12				M	F	M	F	M	F	
13				M	No cover	M	F	M	F	Replication 2
14				M	F	M	F	M	F	
15				M	Window 2	M	F	M	F	
16				M	Water control	M	F	M	F	
17				M	Window 4	M	F	M	F	
18				M	F	M	F	M	F	
19				M	Window 1	M	F	M	F	
20				M	F	M	F	M	F	
21				M	Window 3	M	F	M	F	
22				M	F	M	F	M	F	
23				M	Completely covered	M	F	M	F	
24				M	F	M	F	M	F	Replication 3
25				M	No cover	M	F	M	F	
26				M	F	M	F	M	F	
27				M	Window 3	M	F	M	F	
28				M	Water control	M	F	M	F	
29				M	Window 2	M	F	M	F	
30				M	F	M	F	M	F	
31				M	Window 4	M	F	M	F	
32				M	F	M	F	M	F	
33				M	Window 1	M	F	M	F	
34				M	F	M	F	M	F	
35				M	Completely covered	M	F	M	F	
36				M	F	M	F	M	F	
37				M	No cover	M	F	M	F	

Figure 1A. The layout of experimental site located at Bay of Plenty. 'M' and 'F' indicate male and female vines.

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