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VI20098: Gold3 bud abortion

Kabir MS, Barton C, Parry BE, Tyson JL, Scott P, Beresford RM

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Contents

Executive summary

VI20098: Gold3 bud abortion

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Bud rot can be a serious problem for the kiwifruit industry. Since 2016, a number of growers have reported flower abortion in *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) kiwifruit. Initially it was not clear if all growers were describing the same symptoms, and the details of symptoms and timing of bud abortion were not identified. Recently *Pseudomonas syringae* pv. *actinidiae* (Psa), was found to be the causal agent of one flower abortion syndrome, 'Gold3 bud rot'. The symptoms were described and 'Gold3 bud rot' (previously 'Gold3 flower abortion') was distinguished from 'Gold3 physiological bud abortion'. Further research is required to determine the risk factors associated with bud rot and to minimise the losses it causes.

To address the knowledge gaps around Gold3 bud rot, this study aimed to:

- Determine the flower bud growth stage at which Psa is present on/in the buds and open flowers, and how the symptoms develop
- Assess Gold3 bud rot incidence and progression
- Survey Gold3 bud rot on 'Bounty71' rootstocks compared with 'Bruno' rootstocks to better understand the cause of Psa-related bud rot
- Determine the effect that timing of flower thinning has on bud rot.

This is the final report of the two-year project. Laboratory and field experiments were conducted to satisfy these aims. The presence of Psa in symptomless flower buds was checked at four different growth stages BBCH (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie growth stage) 51 (closed buds, greenish sepals), BBCH 53 (closed buds, elongating reddish peduncles), BBCH 55 (sepals begin to separate, a white-greenish corolla is visible) and BBCH 56 (sepals continue to separate, peduncles continue to elongate and thicken, corolla visible and white). There were three different collections based on flower bud symptoms: 'symptomless', 'symptoms developing' and 'advanced symptomatic'; these were dissected to identify how Psa moves through Gold3 flower buds.

For field experiments, three sites (two commercial and one research) in the Bay of Plenty were chosen where growers carried out their usual management practice. At each site, 15 vines were monitored for this study and from each vine; 100 flower buds were assessed for bud rot.

In laboratory experimental results, Psa was first detected on flower buds when buds were at BBCH 53 (developing closed buds) at Site 1 and Site 2. These results suggest that protection measures are required before this growth stage. It was also observed, based on results from Psa detection from dissected flowers, that Psa is moving from external parts (sepals) into internal parts (petals, anthers and ovary); this has previously been identified in green-fleshed cultivars.

In field experiments, monitoring of bud rot incidence and progression was carried out at three sites on Gold3–'Bounty71' vines. At all three sites, symptoms of bud rot were seen during the last week of October. Incidence increased in the following weeks with the final percentage of symptomatic buds ranging between 2–35%. Rainfall events before this week, along with Psa inoculum, might have initiated bud rot.

Bud rot was compared between Gold3–'Bruno' and Gold3–'Bounty71' vines at one commercial and one research site. Significantly higher bud rot was observed on Gold3–'Bounty71' (2–35%) vines compared with Gold3–'Bruno' (less than 1%). Although 'Bounty71' rootstock has some advantages over 'Bruno' (such as the production of more floral shoots, king flower buds and lateral flowers resulting in more total trays/ha with early maturity), growers may need to pay more attention to Psa management when Gold3 is on 'Bounty71' rootstock.

Timing of flower thinning was modified to assess whether late thinning could minimise the rate of bud rot. Late thinning was conducted 10 days after industry thinning. Although late thinning resulted in a ~6% reduction in bud rot in 2019, this was not statistically significant. Late thinning alone might not be sufficient to reduce bud rot.

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1 Introduction

Since 2016, a number of growers have reported flower abortion in *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) kiwifruit. Initially it was not clear if all growers were describing the same symptoms. The details of symptoms and timing of bud abortion were not identified.

In a recent study, two types of bud abortion symptoms were described (Kabir et al. 2019). In type 1, flower buds emerged in the leaf axils of the young shoots following bud break, but the pedicel and flower remained small and undeveloped. These undeveloped flower buds sometimes turned brown, falling off the vine after a few days or weeks. Although growth usually ceased after bud emergence, sometimes the symptomatic flower buds continued to develop very slowly, but did not turn into well-developed flowers. The syndrome observed was tentatively termed 'Gold3 physiological bud abortion', since bacterial pathogens were not associated with it and it occurred at an early developmental stage.

In type 2, symptoms started to appear in late October when the flowers were about to open. Flower pedicels developed brown necrosis from the cane ends, the bud end, or from a lateral bud scar that was caused by bud thinning. For a time, the rest of the pedicel remained green, then the whole pedicel gradually became brown, necrotic, shrivelled and very dry, as did the sepals and petals. Flower development stopped when these symptoms appeared and the developing flowers eventually dropped from the vine. *Pseudomonas syringae* pv. *actinidiae* (Psa) was found to be associated with 'Gold3 flower abortion' (Kabir et al. 2019).

Based on industry preference, type 1 and 2 were termed 'Gold3 bud abortion' and 'Gold3 bud rot', respectively (Whiteman et al. 2019).

Gold3 bud rot can be a serious problem for the kiwifruit industry. Almost 29% of flowers were aborted in one monitored orchard in 2018 (Kabir, personal communication). Further research is required to determine the risk factors associated with bud rot and to minimise the losses it causes.

To address the knowledge gaps around Gold3 bud rot, this study aimed to:

- Determine the growth stage at which Psa is present on/in the buds and dissecting flower buds, and how the symptoms develop
- Assess Gold3 bud rot incidence and progression of symptoms
- Survey Gold3 bud rot on 'Bounty71' rootstocks compared with 'Bruno' rootstocks across the industry to better understand the cause of Psa-related bud rot
- Determine the effect the timing of flower thinning has on bud rot.

2 Materials and methods

2.1 Experimental sites

The research was conducted on three Gold3 sites within the Bay of Plenty in 2019 and 2020; two were commercial orchards and one was a research block as shown in Table 1.

Table 1. Orchard locations of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) kiwifruit in 2019 and 2020, showing the relevant project aims and rootstock information.

Site	Location	Orchard	Aims	Rootstock
1	Paengaroa	Commercial	a) Psa[1] detection	'Bounty71'
			b) Bud rot incidence and progression	'Bounty71'
			c) Rootstock comparison	'Bounty71' & 'Bruno'
2	Te Puke	Research	a) Psa detection	'Bounty71'
			b) Bud rot incidence and progression	'Bounty71'
			c) Rootstock comparison	'Bounty71' & 'Bruno'
			d) Timing of flower thinning	'Bounty71'
3	Paengaroa	Commercial	b) Bud rot incidence and progression	'Bounty71'

[1]Psa = Psa = *Pseudomonas syringae* pv. *actinidiae*

At Site 1 and Site 2 Gold3 bud rot was monitored on both 'Bounty71' and 'Bruno' rootstock. At Site 3 Gold3 bud rot was monitored on 'Bounty71' rootstock only.

At each site, 15 vines were selected at random for monitoring. This was carried out for both rootstocks where applicable; all vines were within the same block at each site. At Site 1, the Gold3–'Bounty71' vines were on the west side of the block and Gold3–'Bruno' on the east. At Site 2, the Gold3–'Bounty71' and Gold3–'Bruno' vines were planted side by side in the same bays. Orchard managers carried out their standard management practices at all three sites, except Site 2 where the timing of flower thinning on bud rot was investigated, requiring some canes on the monitored vines to not be thinned (Table 2).

Table 2. Spray diary (relevant to *Pseudomonas syringae* pv. *actinidiae* (Psa) management), rootstock and flower thinning of two commercial and one research *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) kiwifruit orchards in 2019 and 2020.

Year	Site	Spray programme		Rootstock	Flower thinning date
		Products and rates/100L	Application date		
2019	Site 1	Nordox™ 75 WG (60g)	25 June 2019	'Bounty71' and 'Bruno'	25 Oct. 2019
		Nordox 75 WG (60g)	20 July 2019		
		Nordox 75 WG (60g)	8 Aug. 2019		
		Nordox 75 WG (55 g)	3 Sept. 2019		
		Nordox 75 WG (37 g)	12 Sept. 2019		
		Kasumin® (500mL)	27 Sept. 2019		
		Kocide® Opti™ (70 g)	24 Oct. 2019		
	Site 2	Copper sulphate (600g)	13 June 2019	'Bounty71' and 'Bruno'	26 Oct. 2019 Late: 5 Nov. 2019
		Kocide Opti (70 g)	18 Aug. 2019		

Year	Site	Spray programme		Rootstock	Flower thinning date
		Products and rates/100L	Application date		
		Kocide Opti (70 g)	11 Sep. 2019		
		Kocide Opti (70 g)	18 Oct. 2019		
	Site 3	Nordox 75 WG (70 g)	4 June 2019	'Bounty71'	21 Oct. 2019
		Nordox 75 WG (70 g)	19 June 2019		
		Nordox 75 WG (70 g)	28 July 2019		
		Nordox 75 WG (55 g)	29 Aug. 2019		
		Kocide Opti (70 g)	20 Sept. 2019		
		Kocide Opti (70 g)	7 Oct. 2019		
		KeyStrepto™ (70 g)	18 Oct. 2019		
		Kocide Opti (70 g)	28 Oct. 2019		
2020	Site 1	Kocide Opti (70 g)	1 May. 2020	'Bounty71' and 'Bruno'	21 Oct. 2020
		Kocide Opti (70 g)	19 May. 2020		
		Kocide Opti (70 g)	3 Jun. 2020		
		Nordox 75 WG (70 g)	2 Jul. 2020		
		Nordox 75 WG (70 g)	23 Jul. 2020		
		Kocide Opti (70 g)	8 Sept. 2020		
		Kocide Opti (70 g)	19. Sept. 2020		
		Actigard™ (20 g)	28 Sept. 2020		
		Kocide Opti (70 g)	28 Sept. 2020		
		KeyStrepto (60 g)	8 Oct. 2020		
		Kocide Opti (70 g)	22 Oct. 2020		
		Kocide Opti (70 g)	21 Nov. 2020		
	Site 2	Copper sulphate (600 g)	13 Jun. 2020	'Bounty71' and 'Bruno'	21 Oct. 2020 Late 27 Oct. 2020
		Kocide Opti (70 g)	18 Aug. 2020		
		Kocide Opti (70 g)	21 Sept. 2020		
		Aureo Gold (50 g)	9 Oct. 2020		
		Aureo Gold (50 g)	22 Oct. 2020		
	Site 3	Kocide Opti (70 g)	9 April. 2020	'Bounty71'	19 Oct. 2020
		Kocide Opti (20 g)	24 April. 2020		
		Actigard (20 g)	24 April. 2020		
		Kocide Opti (70 g)	16 May. 2020		
		Kocide Opti (70 g)	19 Sept. 2020		
		Kasumin (400 mL)	3 Oct. 2020		
		Actigard (20 g)	3 Oct. 2020		
		Kocide Opti (70 g)	24 Oct. 2020		
		Kocide Opti (70 g)	5 Dec. 2020		

2.2 Aim 1: Psa detection and symptom development

Detection of Psa from the whole and dissected Gold3 flower buds were performed using bacterial isolation and quantitative polymerase chain reaction (qPCR). This was carried out from the samples of Site 1 and Site 2 on flower buds from Gold3–'Bounty71'. Each site had 15 vines randomly selected within one block before bud break. Flower bud samples were taken from these vines or adjacent ones when suitable buds were not able to be located on the monitored vines.

2.2.1 Field sampling

Flower buds were collected from the field over September–November 2019 and 2020. There were three different groups of samples collected (Table 3).

Table 3. Flower bud collection, symptoms, bud growth stages, collection dates, bud processing and sample number from one commercial and one research *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) kiwifruit orchards in 2019 and 2020.

Year	Site	Group	Bud symptom	Bud growth stage	Date collected	Processed	Number of buds
2019	Site 1	1	Symptomless	BBCH 51	25 Sept.	Whole	40
				BBCH 53	2 Oct.	Whole	40
				BBCH 55	20 Oct.	Whole	40
				BBCH 56	5 Nov.	Whole	40
		2	Symptomless	BBCH 53 & 55	8 Oct.	Dissected	5
				BBCH 53 & 55	16 Oct.	Dissected	10
			Developing	BBCH 55 & 56	29 Oct.	Dissected	15
			Advanced	BBCH 56	5 Nov.	Dissected	15
		3	Advanced	BBCH 55, 56, 57	5 Nov.	Whole	75
	Site 2	1	Symptomless	BBCH 51	25 Sept.	Whole	40
				BBCH 53	2 Oct.	Whole	40
				BBCH 55	24 Oct.	Whole	40
				BBCH 56	7 Nov.	Whole	40
		2	Symptomless	BBCH 53 & 55	15 Oct.	Dissected	10
				BBCH 55	17 Oct.	Dissected	5
			Developing	BBCH 55 & 56	31 Oct.	Dissected	15
			Advanced	BBCH 55 & 56	7 Nov.	Dissected	15
		3	Advanced	BBCH 56	7 Nov.	Whole	75
2020	Site 1	1	Symptomless	BBCH 51	23 Sept.	Whole	40
				BBCH 53	29 Sept.	Whole	40
				BBCH 55	12 Oct.	Whole	40
				BBCH 56	27 Oct.	Whole	40
		2	Symptomless	BBCH 53 & 55	05 Oct.	Dissected	15
			Developing	BBCH 55	19 Oct.	Dissected	15
			Advanced	BBCH 56	02 Nov.	Dissected	15
		3	Advanced	BBCH 56	02 Nov.	Whole	60
	Site 2	1	Symptomless	BBCH 51	23 Sept.	Whole	40

Year	Site	Group	Bud symptom	Bud growth stage	Date collected	Processed	Number of buds
				BBCH 53	29 Sept.	Whole	40
				BBCH 55	12 Oct.	Whole	40
				BBCH 56	30 Oct.	Whole	40
			Symptomless	BBCH 53 & 55	05 Oct.	Dissected	15
		2	Developing	BBCH 55	20 Oct.	Dissected	15
			Advanced	BBCH 56	30 Oct.	Dissected	15
		3	Advanced	BBCH 56	02 Nov.	Whole	60

BBCH = Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie growth stage scale (Salinero et al. 2009).

Group 1 comprised symptomless flower buds at four developmental stages, categorised by the BBCH (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie growth stage) scale (Salinero et al. 2009); these were 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) and BBCH 56 (sepals continue to separate, peduncles continue to elongate and thicken, corolla visible and white) (Figure 1). Forty buds were collected from each site at each developmental stage. Flower buds were processed whole to check for the presence of Psa. This sampling aimed to see which flower bud growth stage Psa is first able to be detected. No Psa symptoms were seen within the orchards (e.g. leaf spot or bud browning) at BBCH 51, so these samples were picked at random from the selected vines. As male vines and female vines began to display Psa symptoms, the later bud collections of BBCH 53, 55 and 56 targeted female vines from areas near to the Psa disease.

Group 2 focussed on flower buds that were at growth stage BBCH 53 or above (Figure 1) so they could be dissected into five distinct flower parts: stalk, sepals, petals, anthers and ovary (Figure 2). Each flower part was then checked for Psa. There were three different collections based on the flower bud symptoms — symptomless, developing and advanced symptoms. Symptomless buds had no visible bud browning, ‘developing symptomatic’ targeted buds that had browning on either the stalk and/or 1–3 sepals, and ‘advanced symptomatic’ buds had browning over most of the sepals and stalk. At each site, 15 flower buds were collected per symptom category.

Group 3 flower bud collection targeted buds with advanced symptoms and was carried out just once before flowering in early November 2019 at both sites. A total of 75 flower buds were collected from each site and processed for the presence of Psa.

Figure 1. 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 (courtesy of Kabir et al. 2018).

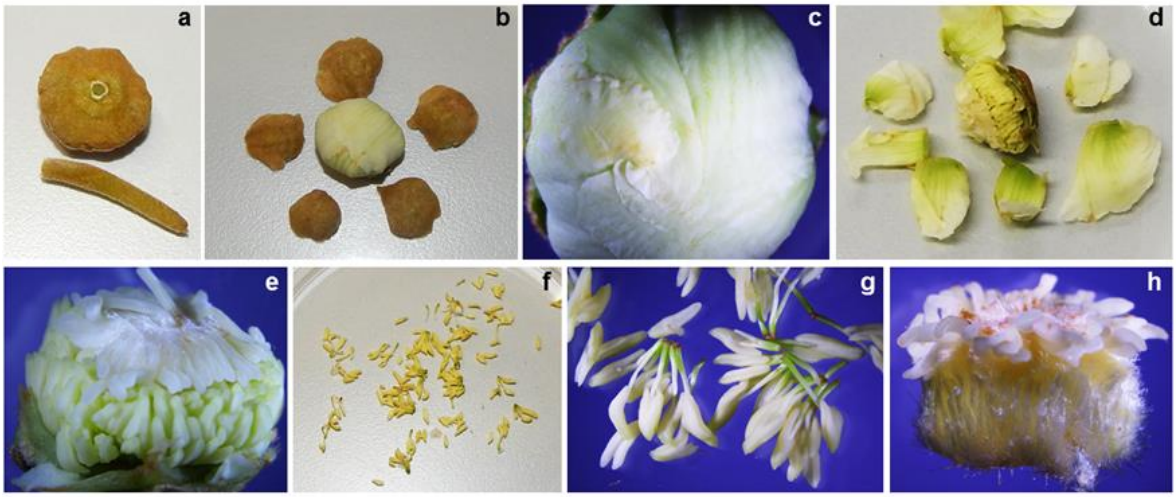

Figure 2. Dissected kiwifruit flower buds of *Actinidia chinensis* var. *deliciosa* 'Hayward': a) stalk separated from a bud, b) sepals separated from the inner parts of a bud, c) bud after sepal removal, d) petals separated from inner parts of a bud, e) bud after petal removal, ovary surrounded by anthers, f) anthers, g) anthers after removal from a bud, and h) ovary (courtesy of Kabir et al. 2018).

2.2.2 Laboratory assessment of flower buds

The flower buds collected at each sampling time were photographed and their description was recorded.

2.2.3 Bacterial isolation from flower buds

All flower buds were surface sterilised by immersing in 70% ethanol for 1 min, 1% sodium hypochlorite for 3 min, 70% ethanol for 30 s, then double rinsed in sterile reverse osmosis water and left to air dry in the laminar flow before further processing.

Each flower bud (sample) was macerated in 1 mL bacterial saline (BS, 0.85% sodium chloride NaCl). Once macerated, each sample was left for 5 min, after which 100 µL of the resultant suspension was spread onto King's BC medium (KBC), a semi-selective medium for *Pseudomonas syringae* (Mohan and Schaad 1987). All isolation plates were incubated at room temperature for 72–120 h, then assessed for bacterial growth.

2.2.4 DNA extractions

Isolation plates with a low number of bacterial colonies (<20 colony forming units; cfu) were streaked across the plate to increase biomass; those with colony numbers <50 were looped directly into 900 µL BS. Plates that had mixed bacterial cultures of >50 colonies had 1 mL BS added to each plate and an aliquot (100 µL) of each of the resultant suspensions was added to 900 µL BS.

All tubes of suspensions were then centrifuged at 8500 rpm for 5 min. The supernatant was discarded and the pellet was re-suspended in 1 mL BS and centrifuged at 8500 rpm for 5 min. The supernatant was discarded and the pellet was re-suspended in 1 mL EDTA (1 mM). An aliquot (200 µL) of the final suspension was then placed in a heat block at 100°C for 5 min, then immediately placed on ice for 10–15 min. The tubes were again centrifuged for 5 min at 13,000 rpm and the resultant DNA sample was diluted 2.5-fold, then stored at -20°C until being used for qPCR.

2.2.5 Detection of Psa using qPCR

qPCR was performed using a Rotor-Gene Q (Qiagen) to detect Psa. The Psa-specific primers PsaF3 and PsaR4, developed by Rees-George et al. (2010), were used to detect Psa. Each 10-µL reaction consisted of 2.5 µL of diluted DNA, 5 µL Rotor-Gene® SYBR® Green 2x, 1.5 µL RNase-free water and 0.5 µL of 5 µM forward and reverse primers. The qPCR ran under the following conditions: 95°C for 10 min; 40 cycles of 95°C for 5 s; 65°C for 7 s; 72°C for 7 s; followed by melting-curve analysis, with a temperature profile slope from 65°C to 97°C.

The Psa detection assay included a positive control (*P. syringae* pv. *actinidiae* isolate cc691 from New Zealand *Actinidia* sp.) and negative control (*P. syringae* pv. *syringae* isolate cc726, from New Zealand *Actinidia* sp.).

A sample with a Ct (crossing point or threshold value) value below 30 and a melting point within 0.5°C of the positive control was interpreted as Psa positive.

2.3 Aim 2: Bud rot incidence and progression

Bud rot incidence was assessed on Gold3–'Bounty71' rootstock at all three sites, from just after bud break to just before flowering (September – November in 2019 and 2020) (Table 1). There were seven assessments carried out at Site 1 and Site 2, with six assessments at Site 3 (Table 4). At each site, the 15 monitored vines had two canes randomly selected, one on each side of the bay, to check for bud rot. One-hundred flower buds were assessed from each vine, giving a total of 1500 flower buds at each assessment, at each site.

Table 4. Flower bud assessment dates from two commercial and one research *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) kiwifruit orchards in 2019 and 2020.

Year	Assessment number	Site 1	Site 2	Site 3
2019	1	1 Oct. 2019	3 Oct. 2019	30 Sep. 2019
	2	7 Oct. 2019	9 Oct.2019	7 Oct. 2019
	3	14 Oct. 2019	18 Oct. 2019	14 Oct. 2019
	4	21 Oct. 2019	24 Oct. 2019	21 Oct. 2019
	5	29 Oct. 2019	1 Nov. 2019	29 Oct. 2019
	6	4 Nov. 2019	7 Nov. 2019	5 Nov. 2019
	7	13 Nov. 2019	15 Nov. 2019	Not conducted
2020	1	29 Sept. 2020	29 Sept. 2020	29 Sep. 2020
	2	05 Oct. 2020	05 Oct. 2020	05 Oct. 2020
	3	12 Oct. 2020	12 Oct. 2020	12 Oct. 2020
	4	19 Oct. 2020	19 Oct. 2020	19 Oct. 2020
	5	27 Oct. 2020	27 Oct. 2020	27 Oct. 2020
	6	02 Nov. 2020	02 Nov. 2020	02 Nov. 2020
	7	09 Nov. 2020	09 Nov. 2020	Not conducted

2.4 Aim 3: Rootstock effect on bud rot

Bud rot incidence was also assessed on Gold3–'Bruno' rootstock at Site 1 and Site 2, from just after bud break to just before flowering (October – November, 2019 and 2020) (Table 1). There were seven assessments carried out at Site 1 and Site 2 (Table 4). At each site the 15 monitored vines had two canes randomly selected, one on each side of the bay, to check for bud rot. One-hundred flower buds were assessed from each vine, giving a total of 1500 flower buds at each assessment, at each site. This information was then compared against the Gold3–'Bounty71' data (see Section 2.3) to assess whether there is a difference in Gold3 bud rot incidence on the two different rootstocks.

In addition to the bud development stage (BBCH), any symptoms of Psa such as cane dieback, oozing, cankers and leaf spot were also recorded.

2.5 Aim 4: Timing of flower thinning and bud rot

At Site 2, on the 15 monitored Gold3–'Bounty71' vines, flower thinning was carried out at two different times (early and late) to see if either gave a difference on bud rot incidence. Early thinning relates to orchard practice (OP control) that was carried out on 26 October 2019 and late thinning was carried out on 5 November 2019 (10 days apart). The following year (2020), early and late thinning were carried out on 21 October and 27 October 2020, respectively (6 days apart). During thinning only triplets were removed from the king flowers. Controls consisted of canes on which the flowers were not thinned. Each vine had six canes randomly selected to be monitored, two for each thinning treatment and the control. Three assessments were carried out (1, 7 and 15 November in 2019 and 26 October, 2 and 9 November in 2020); 100 buds were assessed for each thinning treatment on each vine, at each assessment.

2.6 Analysis

Binomial generalised linear models were used to analyse the proportion of symptomatic buds at each assessment time for the three orchards (six assessment dates for Site 1 and 3, seven assessment dates for Site 2 (Section 3.2).

Binomial generalised linear models were used to analyse the proportion of symptomatic buds for the two orchards (Site 1 and Site 2) and for each of the assessment dates (Section 3.3) to compare the two rootstocks ('Bruno' and 'Bounty71'). The same model was used to analyse the proportion of symptomatic buds to compare the three thinning treatments (OP, late and none) for the three assessment dates (1, 7 and 15 November) in 2019 and four assessment dates (19 October, 27 October, 2 November and 9 November) in 2020.

Fitted means and 95% confidence intervals were estimated to identify significant differences. Analyses were carried out using Genstat 20th Edition (2019, VSN International), and R version 3.5.1 (R core team, 2018).

3 Results

3.1 Psa detection and symptom development

Group 1, symptomless flower buds, were collected from Gold3–'Bounty71' at four bud development stages (BBCH 51, BBCH 53, BBCH 55 and BBCH 56) from two sites (Site 1 and Site 2) to identify at which developmental stage Psa is present.

In 2019, Psa was not detected from any of the flower buds at BBCH 51 and BBCH 53 (Figure 3). Psa began to be detected from BBCH 55 (when sepals begin to separate) at both sites. In 2020, Psa was detected in a small percentage of flower buds (5–8%) when buds were at BBCH 53 (developing buds, closed).

Figure 4 shows the growth stages at which Psa was detected (Gold3–'Bounty71' flower buds). The percentage of flower buds with Psa was higher at Site 2 than at Site 1.

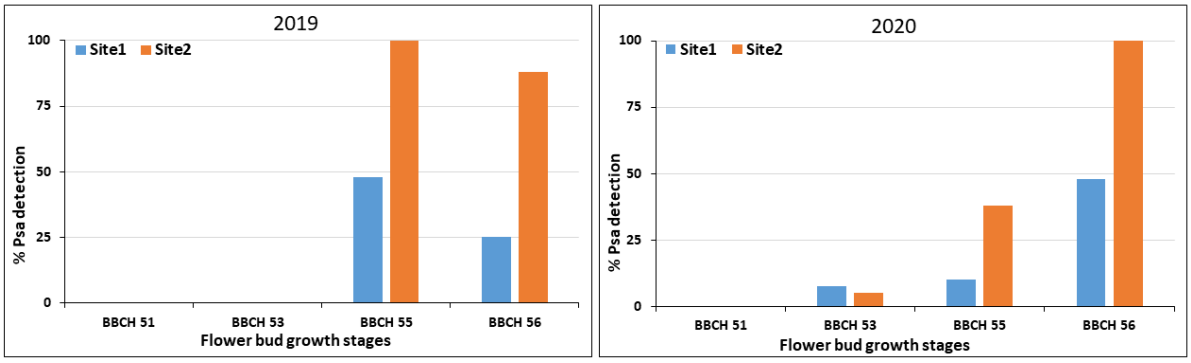

Figure 3. Percentage of flower buds showing detection of *Pseudomonas syringae* pv. *actinidiae* (Psa) from Site 1 and Site 2 at four development stages BBCH 51, BBCH 53, BBCH 55 and BBCH 56 (Salinero et al 2009) of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3)–'Bounty71' flower buds in 2019 and 2020. Data presented by mean; n=40 site/BBCH. 'BBCH'=Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie growth stage.

Figure 4. Representative images of symptomless *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3)–'Bounty71' flower buds that were checked for *Pseudomonas syringae* pv. *actinidiae* (Psa) using quantitative polymerase chain reaction (qPCR). No Psa was detected from buds at BBCH 51 and BBCH 53; Psa started to appear at BBCH 55 and continued at BBCH 56. 'BBCH'=Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie growth stage.

Group 2, flower dissection (stalk, sepals, petals, anthers and ovary) was carried out on three flower bud symptoms (symptomless, developing and advanced symptomatic buds) to determine when and where Psa is present within the flower tissue.

In 2019 at Site 1, Psa was detected in all flower parts of symptomless flower buds, although at low rates from the internal tissues. In the symptomless flower buds the highest detection rate was from sepals (67%); for the other four flower parts (stalk, petals, anthers and ovary) Psa detection ranged from 13–27% (Figure 5). When the flowers were developing symptoms, almost all of the flower parts were colonised by Psa (100% pedicel and sepals; 93% sepals, anthers and ovary). When flowers had advanced symptoms, Psa was present in all flower parts (100%). At Site 2 there was a similar pattern to Site 1. Psa was detected in all flower parts of the symptomless buds, although at low rates from the internal tissues, and with a higher rate of detection from sepals (Figure 5). Psa was present in all flower parts of developing and advanced symptomatic buds from this site (100%).

A similar trend of tissue colonisation was observed at both sites in 2020. Psa was only detectable from sepals (7%) of symptomless flowers at Site 1 (Figure 5). In developing symptoms, all flower parts were showing some symptoms (13–53%) with the highest detection rate in sepals. In flowers with advanced symptomatic buds, higher detection was observed from all flower parts (53–73%). At Site 2, Psa was detected in all flower parts at the symptomless stage, at very low detection rates (all 7%). When flowers were at the developing and advanced stages, almost all flower parts were colonised by Psa.

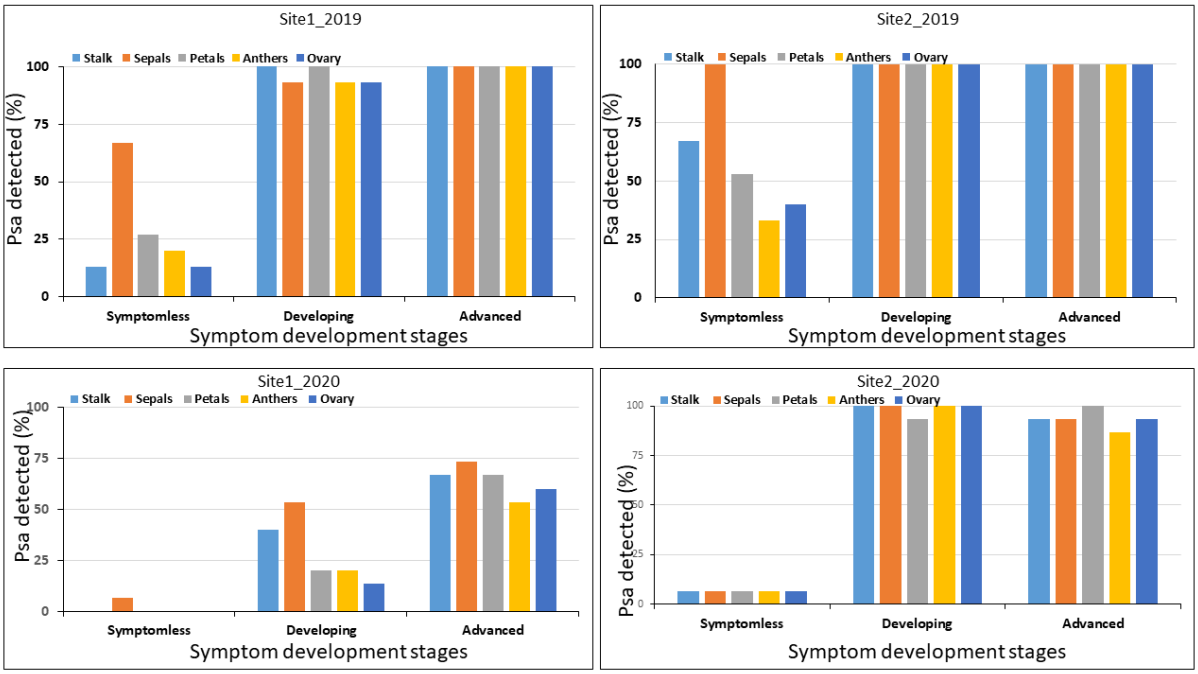

Figure 5. Dissected flower parts (stalk, sepals, petals, anthers and ovary) showing percent detection of *Pseudomonas syringae* pv. *actinidiae* (Psa) from symptomless, developing and advanced symptomatic flower buds collected from Site 1 (left) and Site 2 (right) in 2019 and 2020. Data presented by mean, n=15.

Images were taken of symptomless, developing and advanced symptomatic buds before dissection (Figure 6) and in the field (Figure 7). Symptomless buds had no visible bud browning. Developing symptomatic buds showed browning on the stalk and/or 1–3 sepals. Advanced symptomatic buds had browning over most of the sepals and stalk.

Figure 6. Representative images of *Actinidia chinensis* var. *chinensis* ‘Zesy002’ (Gold3)–‘Bounty71’ bud rot development. No pedicel or sepal browning on symptomless flowers (top row); developing flowers are showing slightly browned sepals (middle row) and in advanced stage pedicel, sepals, and petals are showing necrosis (bottom row).

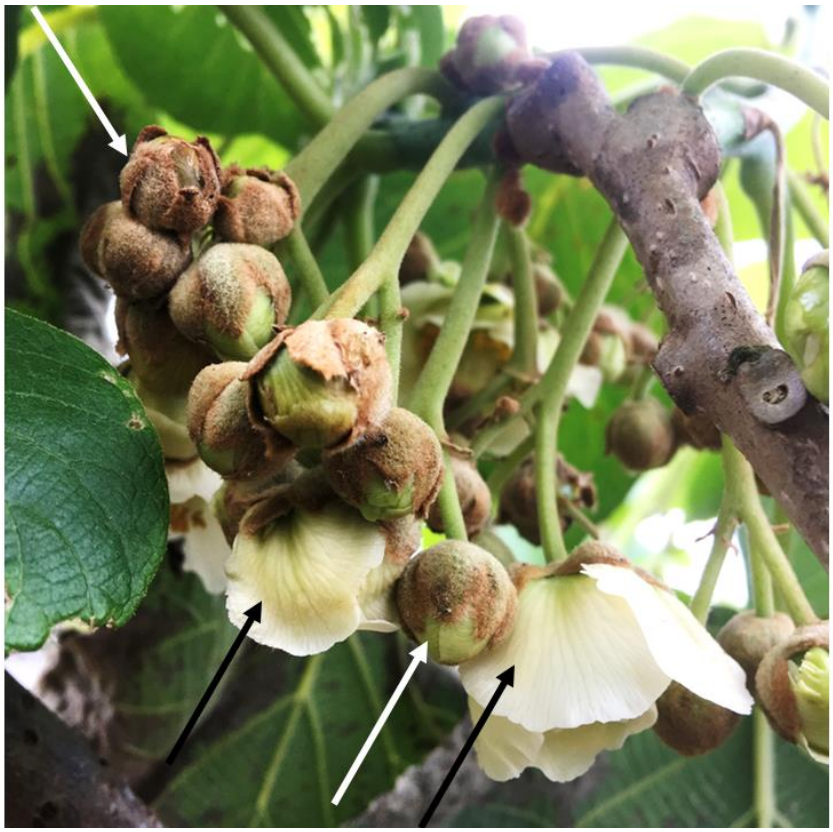

Figure 7. Field images of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) flower buds showing symptomless (black arrow), developing (white arrow) and advanced (red arrow) symptoms of bud rot.

Group 3, advanced symptomatic flower buds, were collected from Gold3–'Bounty71' at Site 1 and Site 2 at the end of the experiment for confirmation of Psa association. Psa was detected from all symptomatic bud samples (collected from Site 1 and Site 2; 75 flower buds/site) in 2019. Psa was also detected from all flower buds from Site 2 in 2020 but 97% from Site 1.

3.2 Bud rot incidence and progression

Bud rot incidence and progression was monitored from all three Gold3–'Bounty71' sites. In 2019, bud rot significantly increased at all three sites during the week starting 28 October compared with the previous assessment dates (Figure 8). Flower bud growth stage from 21–28 October was BBCH 55 and in the following week (28 October – 4 November) it was BBCH 56. Bud rot increased significantly over the following week (4–11 November) with a final percentage of symptomatic buds of 9.1%, 34.8% and 7.9% at Site 1, Site 2 and Site 3, respectively. Although bud rot incidence was very low in 2020 compared to 2019, bud rot progression started to increase significantly from the 2 November assessment.

Weather data were recorded from all three sites (Figure 9), which showed there were several rainfall events between 11 and16 October 2019. Total rainfall was less in 2020 and mainly occurred from 12–15 October and during the first week of November.

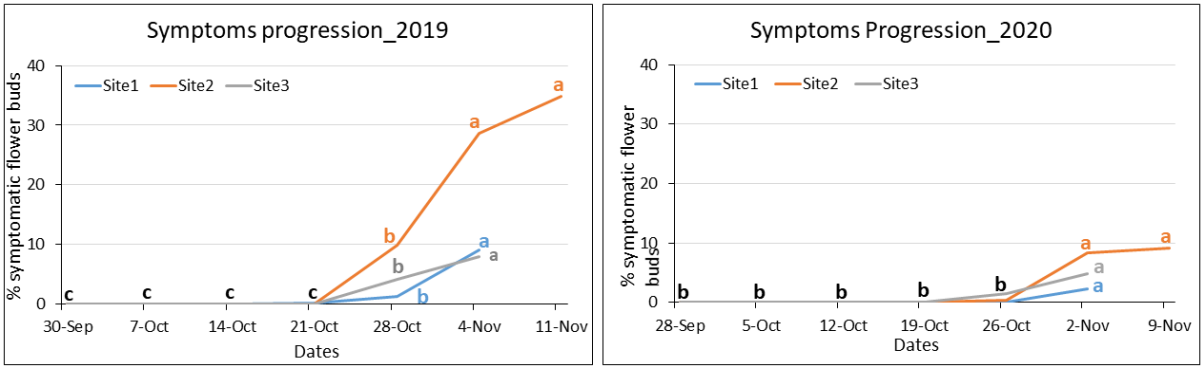

Figure 8. Progression of bud rot symptoms of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) at three commercial sites on seven dates in 2019 and 2020. Different letters for each site (within each line) show significant differences (p< 0.001). Data displayed are back-transformed means, n=15.

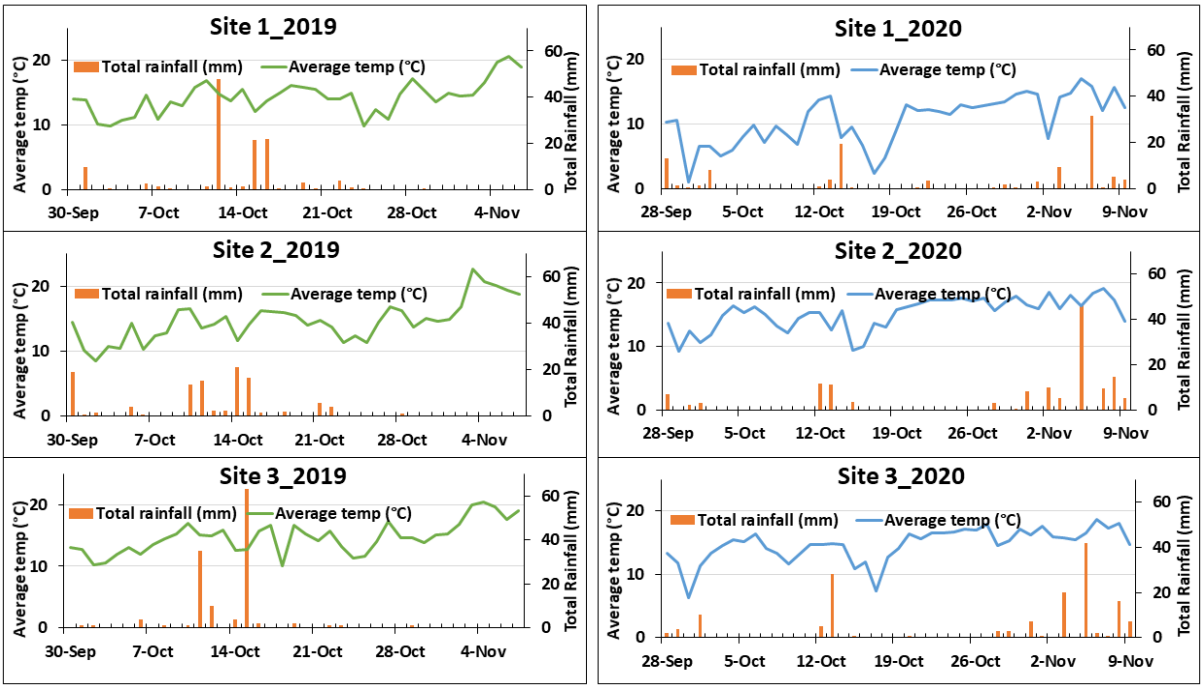

Figure 9. Total rainfall and the average daily temperature at Site 1, Site 2 and Site 3 located in the Bay of Plenty, in 2019 and 2020.

Monitoring of symptom progression continued after flowering at Site 2. Pedicel necrosis was recorded at the fruitlet stage which terminated fruitlet growth (Figure 10).

Figure 10. Necrosis on stalk (white arrows) which terminated the growth of kiwifruit fruitlet.

3.3 Rootstock effect on bud rot

The timing of bud break through to flower opening differed by approximately 1 week between the two rootstocks in 2019. At the first assessment, during the first week of October, the three Gold3–'Bounty71' sites were mostly at BBCH 51, while the two Gold3–'Bruno' sites had leaves that had not yet expanded — classed as BBCH 11 (Table 5). This difference in timing carried on through to the final assessments in early November when the Gold3–'Bounty71' were at BBCH 65 (at least 50% of the flowers had opened) and the Gold3'-Bruno' were closer to BBCH 56 (flower bud). The one-week slower growth was also observed in 2020 (Table 6).

Table 5. Flower bud assessment dates and average growth stages (categorised by the BBCH scale; Salinero et al. 2009) across the site from *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) kiwifruit grown on two different rootstocks in 2019.

Gold3 rootstock	Date of assessment (2019)					
	1/10/2019	7/10/2019	14/10/2019	21/10/2019	29/10/2019	4/11/2019
'Bounty71'	BBCH 51	BBCH 53	BBCH 53	BBCH 55	BBCH 56	BBCH 65
'Bruno'	BBCH 11	BBCH 51	BBCH 53	BBCH 53	BBCH 55	BBCH 56

'BBCH'=Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie growth stage.

Table 6. Flower bud assessment dates and average growth stages (categorised by the BBCH scale; Salinero et al. 2009) across the site from *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) kiwifruit grown on two different rootstocks in 2020.

Gold3 rootstock	Date of assessment (2020)					
	29/09/2020	05/10/2020	12/10/2020	19/10/2020	27/10/2020	02/11/2020
'Bounty71'	BBCH 51	BBCH 53	BBCH 53	BBCH 55	BBCH 56	BBCH 65
'Bruno'	BBCH 11	BBCH 19	BBCH 51 & 53	BBCH 53	BBCH 55	BBCH 56

Even taking into account the difference in growth stages (by comparing bud rot rates at similar growth stages rather than on the same date), the same rates of bud rot were not seen on the two rootstock (data not shown). In 2019, Site 1 had a significantly higher percentage of symptomatic flower buds recorded on Gold3–'Bounty71' vines on 28 October and 4 November compared with Gold3–'Bruno'

vines. At site 2, the percentage of symptomatic flower buds was significantly higher on Gold3–'Bounty71' rootstock compared with Gold3–'Bruno' when symptoms started to appear on 28 October (Figure 11). This difference continued to be observed over the following 2 weeks (4 and 11 November) with 34.8% symptomatic buds in Gold3–'Bounty71' and 0.4% in Gold3–'Bruno (Figure 12).

In 2020, significantly higher bud rot incidence was also observed on Gold3–'Bounty71' rootstock compared with Gold3–'Bruno' on 2 November at site 1. Significantly higher bud rot incidence was also found at on Gold3–'Bounty71' rootstock on 2 and 4 November compared with Gold3–'Bruno' at site 2.

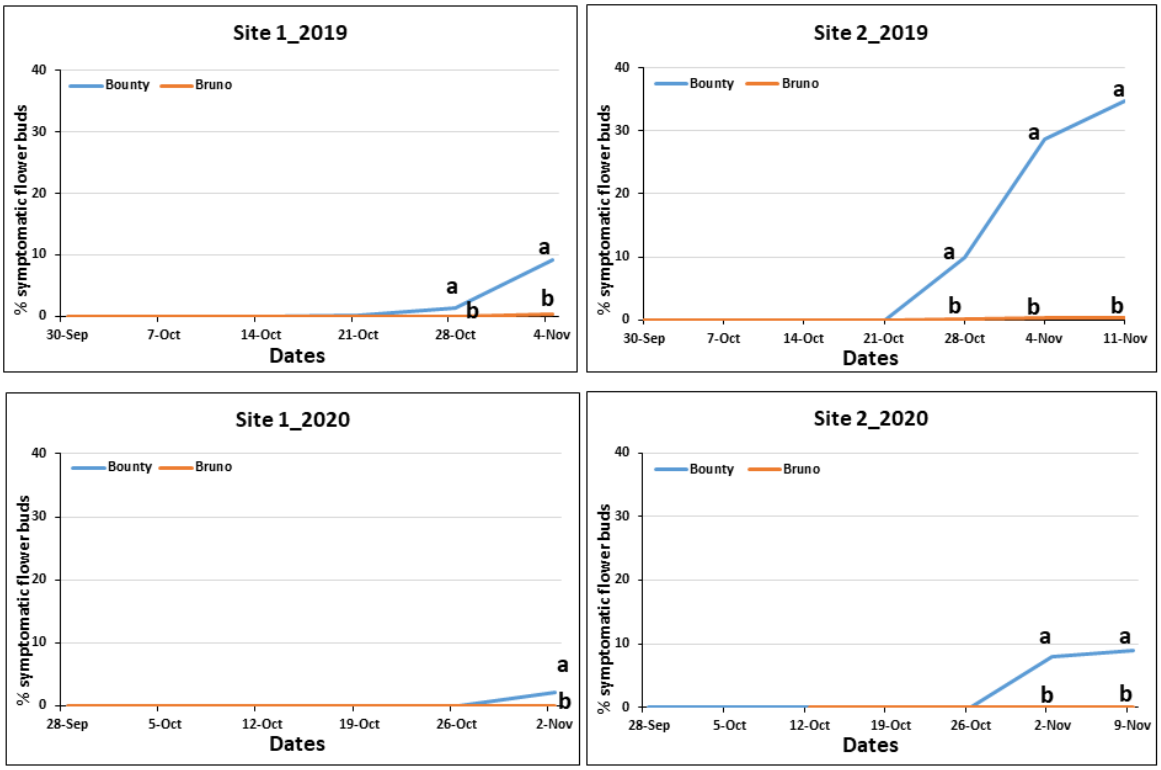

Figure 11. Comparison of the percentage of symptomatic flower buds between *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3)–'Bounty71' and Gold3–'Bruno' on seven dates in 2019 and 2020 at two sites (Site 1 and Site 2). Different letters on each date show significant differences between the rootstocks ($p<0.001$). Data presented as mean, n=15.


a


b

Figure 12. Comparison of bud rot between *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3)–'Bruno' and Gold3–'Bounty71' vines. An overview on a) the right two canes from Gold3–'Bounty71' vines show symptomatic flower buds (orange coloured), whereas the left cane from Gold3–'Bruno' vines show healthy flower buds, b) a close view of a cane from a Gold3–'Bounty71' vine showing mostly aborted flowers and few healthy fruitlets, c) a close view of cane from a Gold3–'Bruno' vine showing mostly flower buds. Images are taken from Site 2 where both vines were co-located in one bay.

3.4 Timing of flower thinning and bud rot

The timing of flower thinning was modified to assess if late thinning would reduce the number of symptomatic flower buds. In 2019, two assessments carried out after late thinning (8 and 15 November 2019) showed a lower percentage of symptomatic buds (about 6% in both assessments) from late thinning (29% on 15 November 2019) compared with OP timing (35% on 15 November), however, it was not significantly different (Figure 13).

In 2020, bud rot incidence on late thinning and OP appeared to be almost similar in both observations (2 and 9 November). However, bud rot incidence on no thinning treatment had significantly less bud rot incidence (4% and 6% less bud rot infection compared with the late thinning treatment on 2 and 9 November, respectively).

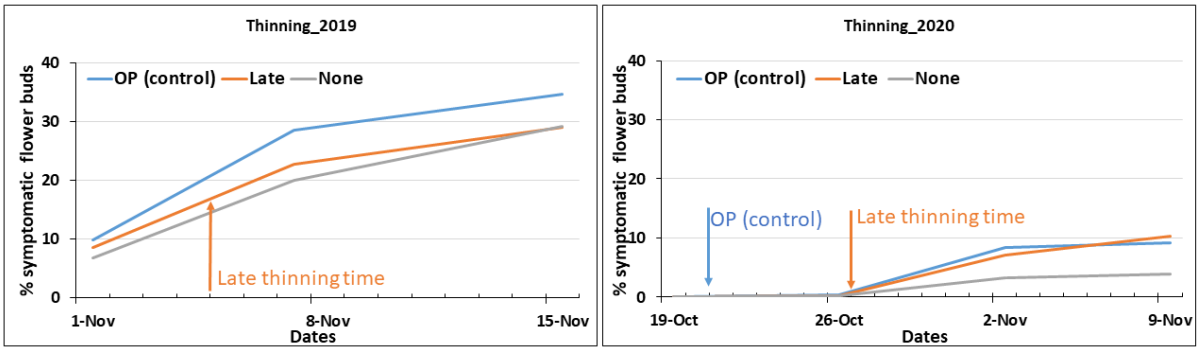

Figure 13. Effect of timing of flower thinning on the percentage of symptomatic flower buds on Gold3-'Bounty71'. Orchard practice (OP) and late thinning were carried out on 26 October and 5 November respectively in 2019; OP and late thinning were carried out on 21 and 27 October respectively in 2020. 'None' represents no thinning at all during the experiment. No significant difference was observed at the 8 and 15 November time points in 2019; late thinning and OP were significantly higher than no thinning on November 2 and 9. Data presented as mean, n=15.

4 Discussion

This two-year epidemiological study was carried out on Gold3 orchards experiencing bud rot associated with Psa (previously called flower abortion, as in Kabir et al. 2019) and aimed to identify when Psa enters the flower buds and how it moves. Field experiments were also conducted to monitor incidence and progression of the disease, the effect of rootstock and timing of flower bud thinning.

Detection and symptoms development

Determining when Psa is present on flower buds can indicate the most beneficial time to apply protection measures to control Psa. In this study, Psa was found on flower buds between developing closed buds (BBCH 53) and the buds when sepals began to separate (BBCH 55). As isolations were made from surface-sterilised buds, the detection of Psa at this stage is predominantly from infected flower tissues. If the source of Psa inoculum is external and Psa moves from external (i.e. sepals) to internal parts of the flower buds, as with *Actinidia chinensis* var. *deliciosa* 'Hayward' and *Actinidia chinensis* var. *chinensis* 'Zesh004' (Green14) (Kabir et al. 2019a), Psa protection measures need to be taken before developing bud stage (BBCH 53). Developing bud stage (BBCH 53) is approximately 4 weeks after bud break for Gold3. Here bud break refers to 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), and when canopy are at 50% of this stage.

Psa was detected in flower buds of Gold3 at a comparatively later growth stage than in the green-fleshed cultivars. Although the rates were low, Psa was detected at early growth stages (BBCH 51 and BBCH 53) in green-fleshed cultivars (Kabir et al. 2019a). On Gold3, bud rot appeared in 7% of buds at BBCH 53 in 2020 but not in buds at BBCH 51 at all. It could be the case with bud break occurring later in green-fleshed cultivars (late September) than Gold3 (mid-September) that the spring weather had increased Psa inoculum in the orchard allowing it to infect green-fleshed cultivars earlier, and/or the green-fleshed cultivars are susceptible at an earlier growth stage.

The percentage of buds from which Psa was detected varied by location. In this study, rates of Psa were mostly higher at Site 2 than at Site 1. One possible reason might be that more bud samples from Site 2 were collected from Psa leaf spotted areas. Furthermore, Site 2 is a research block and does not receive as much Psa management as a commercial site. This suggests that external inoculum sources impact on Psa within the buds.

Psa detection from dissected flower buds indicated that even when a flower bud is symptomless, Psa may be present. When 'developing symptomatic' flower buds were dissected (stalk browning and/or few sepals browning), Psa was present in most of the flower tissues. This result is similar to the movement of Psa in green-fleshed cultivars 'external to internal' (Kabir et al. 2019a). Psa detection was more often in sepals compared to other flower tissues which was also found in green-fleshed cultivar (Kabir et al. 2019a).

Gold3 bud rot incidence and progression

Bud rot incidence was monitored on Gold3–'Bounty71' vines, as vines on this rootstock had significant flower loss in a previous study (Kabir et al. 2019). Bud rot symptoms started to increase significantly from the last week of October when buds were at growth stage BBCH 55–56 (between sepals beginning to separate and white petals becoming visible).

It is possible that bud rot appearance time may be affected by rain events. In 2019, bud rot incidence significantly increased in the week prior to the assessment date of 28 October; a significant amount of rain had been recorded 2 weeks earlier. However, in 2020, bud rot incidence increased significantly in the week prior to the 2 November assessment; there was a moderate rainfall event 3 weeks previously. These results suggest that growers need to consider applying Psa protectants when there is a chance of rain before flowering.

Bud rot symptoms varied from site to site in each season. In 2019 and 2020, bud rot incidence was greater at Site 2 compared with the other two sites. Inoculum availability might be one of the factors responsible for the greater amount of bud rot at this site. There were rows of *Actinidia chinensis* var. *chinensis* 'Zes008' (Red19) vines located near the experimental rows (within the same block) in the 2019 season; bud rot was visible on these about 2 weeks before the monitored Gold3 vines. This result suggests that cultivars susceptible to Psa may need to be kept at a distance from Gold3 to reduce bud rot.

Psa management practice may be another reason for the higher bud rot incidence at Site 2. This was a research site and Psa was not managed in the same way as industry practice at commercial sites. Although protectants were applied, no antibiotic was applied in either year. Antibiotics were applied in both commercial sites.

Bud rot also varied from year to year on the same site. At Site 1, in the 2018 flowering season, there was 22% flower loss (Kabir et al. 2019), whereas in 2019 and 2020, incidence fell to 9% and 2% respectively. There are likely to be a few reasons behind this, including rain during flower bud thinning, application of protectants at an appropriate time and sources of Psa inoculum around the site. At Site 1, there was significant rainfall during thinning in 2018. Over the next 2 years there was little or no rain during thinning and good Psa management was carried out through the season.

Bud rot variation was also observed on Site 2 between the 2019 and 2020 seasons. At this site, bud rot incidence was 35% and 9% in 2019 and 2020 respectively. Two reasons may have accounted for this. Firstly, the removal of an inoculum source. In winter 2019, a nearby bud rot that affected cultivar Red19 was removed. This cultivar flowers 2 weeks earlier than Gold3 which suggesting that there was a significant reduction of usual inoculum production prior to the Gold3 flowering. Secondly, this site was managed differently in each of the 2 years. In addition to other management practices, three protectants were applied from bud break until flowering in 2020 instead of only one protectant application in 2019. This result suggests that careful protection of the canopy from bud break until flowering can minimise bud rot incidence

Other than at our experimental sites, the information on bud rot incidence is anecdotal. Growers have found that bud rot largely affects vines grafted to 'Bounty71' rootstock and that it tends to be patchy throughout orchard blocks. One grower noted during a conversation that he had 50% bud rot in the 2019 flowering season. Amongst all the growers, the highest percentage of bud rot (80%) was observed in a site where the grower picked 9000 trays/ha. This represents a significant crop loss from bud rot.

Although bud rot can be a severe problem on 'Bounty71' grafted Gold3 vines (see next section), it can be managed effectively. Data from the two commercial sites provides a good example of this. Site 1 had 29% flower loss in 2018, with sporadic incidence all over the orchard; this was reduced to 2% by 2020. Site 3, which had 50% flower loss in 2019, was reduced to 5% by 2020. However, antibiotics were applied to these two commercial orchards, which organic growers are not allowed to use.

Organic growers are also not allowed to apply Hi-cane, thus their bud break can be very irregular. Suitable bud rot management practices for organic orchards need to be investigated further.

Psa associated flower loss can impact on fruit loss. At the time of last assessment (on 8 November 2019, at Site 2), the stalk necrosis continued into fruit set, causing fruitlets to terminate.

Rootstock effect

Significantly higher bud rot was recorded on Gold3–'Bounty71' vines compared with Gold3–'Bruno' in the both sites in 2019 and 2020. This result confirms the findings of Perie et al. (2019) who compared this at various locations. During visual observations, it was noted that although there were fewer flower buds on Gold3–'Bounty71' vines, there were comparatively larger fruit on these vines than on Gold3–'Bruno' vines. Fruit weight, dry matter and crop load were not studied in this project. Although flower loss is an important factor in Gold3–'Bounty71', it may be that up to a certain rate flower loss is still workable for the industry. This requires further investigation.

Flower thinning timing

The timing of flower thinning did not show a significant difference in flower loss in either of the years, although late thinning resulted in lower incidence of bud rot compared with industry practice in 2019. The idea for later flower thinning was investigated after observing a progression in necrotic lesions on the stalk (Kabir et al. 2019) from the lateral bud scar (after removing lateral buds). Even though the result was not statistically significant this year, perhaps due to OP (Orchard Practice/industry practice) thinning and late thinning both taking place during dry weather, project results still suggest that thinning wounds are an infection point for Psa in this disease (bud rot). Therefore, it is very important to thin flower buds in 'low risk periods' which means thinning in a dry weather conditions and ensuring vines are well protected (e.g. by Psa spray program) to avoid infection entering wounds created when side flowers are removed.

5 Acknowledgements

We would like to thank the growers, orchard managers and other staff for their co-operation throughout the trial. We also acknowledge Zespri Group Limited/Kiwifruit Vine Health (KVH) for funding.

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Appendix

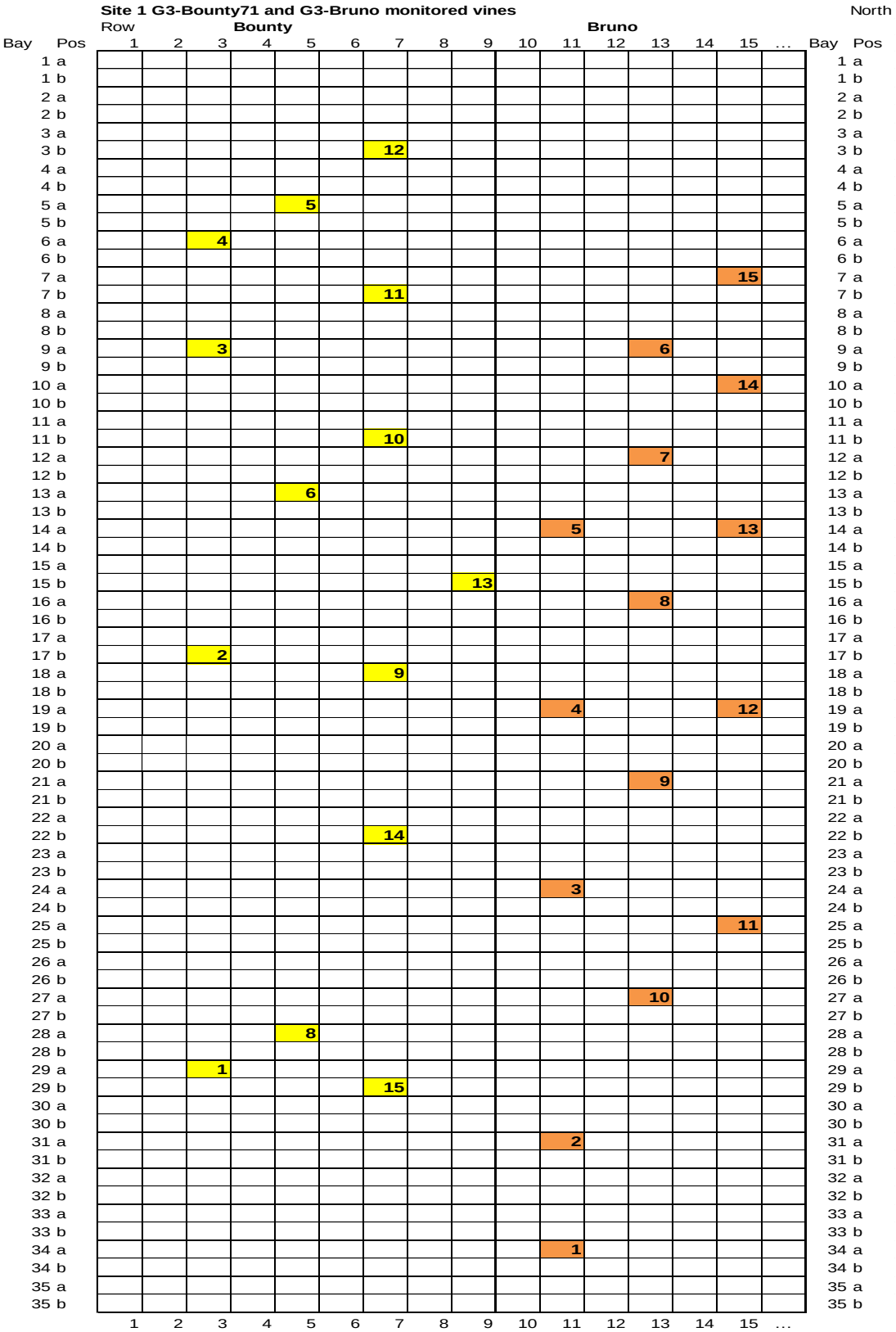

Figure 1A. The layout of Site 1 located at Paengaroa, Bay of Plenty. Fifteen vines of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) each from 'Bounty71' and 'Bruno' rootstock were marked for this study.

Site 2 G3-Bounty71 and G3-Bruno monitored vines North

Bay	Pos	Row ...	12	13	14	15	16	17	18	...	Bay	Pos
2	a										2	a
2	b										2	b
3	a										3	a
3	b										3	b
4	a		7						8		4	a
4	b		7						8		4	b
5	a										5	a
5	b										5	b
6	a								9		6	a
6	b								9		6	b
7	a		6								7	a
7	b		6								7	b
8	a								10		8	a
8	b								10		8	b
9	a		5								9	a
9	b		5								9	b
10	a										10	a
10	b										10	b
11	a		4								11	a
11	b		4								11	b
12	a								11		12	a
12	b								11		12	b
13	a										13	a
13	b										13	b
14	a								12		14	a
14	b								12		14	b
15	a		3								15	a
15	b		3								15	b
16	a								13		16	a
16	b								13		16	b
17	a										17	a
17	b										17	b
18	a		2								18	a
18	b		2								18	b
19	a								14		19	a
19	b								14		19	b
20	a										20	a
20	b										20	b
21	a								15		21	a
21	b								15		21	b
22	a		1								22	a
22	b		1								22	b
23	a										23	a
23	b										23	b
24	a										24	a
24	b										24	b
25	a										25	a
25	b										25	b
		...	12	13	14	15	16	17	18	...		

Figure 2A. The layout of Site 2 located at Te Puke, Bay of Plenty. Fifteen vines of *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) each from 'Bounty71' and 'Bruno' rootstock were marked for this study. Vines were co-located in a bay.

Site 3 G3-Bounty71 monitored vines North

Bay	Row 30	31	32	33	34	35	36	37	38	39	40	Bay
…												…
9												9
10			**11**				**10**					10
11												11
12												12
13									**1**			13
14			**12**									14
15							**9**					15
16												16
17			**13**									17
18												18
19									**2**			19
20												20
21												21
22			**14**									22
23									**3**			23
24												24
25							**8**					25
26									**4**			26
27												27
28			**15**				**7**					28
29												29
30									**5**			30
31												31
32												32
33							**6**					33
34												34
…												…

Figure 3A. The layout of Site 3 located at Paengaroa, Bay of Plenty. Fifteen vines of *Actinidia chinensis* var. *chinensis* ‘Zesy002’ (Gold3)–‘Bounty71’ were marked for this study.

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