Isolation and detection of *Pseudomonas syringae pv. actinidiae* (Psa-V) in kiwifruit green pollen and an evaluation of the ability of Psa-V to survive commercial pollen milling and extractions

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Executive summary

The key purpose of this project was to determine whether Psa-V could survive throughout the different stages of the commercial pollen milling process and remain viable in green kiwifruit pollen. While it was known that Psa-V would be present on flowers from infected orchards, there was less certainty around recovery of Psa-V from flowers collected from orchards with little or no disease. It was also unknown whether viable Psa-V colonies could be recovered from pollen after commercial milling and extraction, thus potentially providing a source of infection for the disease.

The study focussed on kiwifruit green pollen and consisted of five objectives:

1. Analysis of Psa-V detections from pollen produced in 2011 using a new method;
2. Isolation and detection of Psa-V from frozen anthers harvested in 2011;
3. Detection of Psa-V in commercially milled pollen extracted from frozen anther;
4. Isolation of viable Psa-V from pollen collected at all stages of commercial pollen milling;
5. Infection of kiwifruit plants with Psa-V by Psa-V infected pollen.

Key findings

This study confirms that Psa-V is able to survive the commercial milling process.

Re-testing of more than 300 lines of pollen produced in 2011 using a more sensitive real time PCR assay resulted in detection of Psa-V in some samples that were previously negative.

Flowers harvested from 30 orchards in the Bay of Plenty processed through a commercial mill resulted in isolation and detection of Psa-V in the majority of flower, anther and pollen samples at each stage of the milling process using real-time PCR.

Real-time PCR results for milled pollen were strongly positive for all orchards (Cq values from 19-33).

Although viable Psa-V colonies were isolated from samples at each stage throughout the pollen milling and extraction process, results for the 30 orchards were inconsistent and viable colonies were not isolated in every pollen sample.

Viable colonies of Psa-V were isolated from 73 % of the pollen produced from the 30 lines of flower processed through the mill. A range of 10 – 10^4 colony forming units (cfu) per 0.2 g of Psa-V was isolated from these samples.

Detection of Psa-V in pollen harvested in November 2012 returned stronger positive results than in previous years. This could indicate that inoculum levels present in pollen are higher than previous years and explain why we were able to isolate Psa-V V colonies directly from commercially milled pollen samples for the first time.

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1. Introduction

Psa-V was first discovered in New Zealand in an orchard near Te Puke in November 2010 and it has now been detected in all major North Island kiwifruit growing regions. Psa-V can effectively colonise kiwifruit plants throughout the year. The bacteria are thought to be primarily spread by rain and wind, while frost can pre-dispose plants to infection. Transfer of plant material is a major source of infection to new areas and agronomic techniques such as pruning and girdling can contribute to further spread of disease within orchards (Scortichini et al., 2012). While it is also possible that pollen from infected plants could transfer Psa-V bacteria to uninfected plants, there is no evidence to support this (Vanneste et al., 2011).

Male plants are more prone to Psa-V infection, either because of a varietal weakness, or because of the different management that is undertaken on male vines. This has a double effect of reducing the amount of pollen available for pollination in the orchard, and reducing the amount of pollen available for harvest and storage for later use.

A project (Braggins and Saunders, 2012) was undertaken in November 2011 with the aim of detecting Psa-V from pollen material collected from Hayward green kiwifruit vines in the Bay of Plenty (BOP). The key finding of this work found that pollen produced from 24 flower samples collected from different orchards in the BOP were found to be "not detected" for Psa-V by PCR. Results from a project conducted by the Ministry for Primary Industries (MPI) in 2012 isolated viable Psa-V from freshly harvested pollen extracted from closed male kiwifruit gold (cv. CK2, CK3 and Bruce) flowers (MPI, 2011). However, it remains unknown whether viable Psa-V is associated with male kiwifruit green pollen in the same way and whether Psa-V remains viable after commercial milling and storage. A further study by Gallelli et al. (2011) provided new information that viable Psa-V populations can be isolated from kiwifruit pollen extracted commercially from male gold kiwifruit flowers in Italian orchards. However, these findings related to pollen field-vacuumed from open male gold kiwifruit flowers, thereby it was difficult to pinpoint the source of contamination i.e. Psa-V could be present as an external contaminant on flowers or the vine and not actually present in pollen. In New Zealand male gold kiwifruit flowers and the Italian method of vacuuming pollen from plants are not used commercially to extract pollen. In the same Italian study, three commercial lines of pollen sourced from New Zealand showed no evidence of Psa-V contamination. However, the details of the age and source of the New Zealand pollen were not reported in this study and we cannot rule out that Psa-V can be isolated and cultured from New Zealand commercial pollen sources.

Results from a small scale, preliminary trial in Italy suggests that Psa-V can be spread from pollen and colonise kiwifruit (Stefani and Giovanardi, 2011). However, these preliminary experiments were not detailed enough to enable us to make any conclusion with regards of the ability of Psa-V to survive on pollen and spread to kiwifruit under field conditions.

One possible remedy to reduce the potential of spreading Psa-V via pollen is to treat the pollen in a manner that kills any Psa-V bacteria present without affecting pollen viability. Everett, et al. (2012) investigated the effect of using temperature, humidity and time and identified a possible combination that may kill certain levels of Psa-V on pollen in the laboratory. However, the temperature/time combination identified is unlikely to be suitable at this stage as a commercially practical treatment.

2. Objectives

The main objective of this work was to determine whether Psa-V V could survive throughout the commercial milling process on green kiwifruit pollen. While it was known that Psa-V would be present on flowers from infected orchards, it was uncertain if Psa-V was associated with green kiwifruit pollen and if so, would remain viable throughout the different drying and milling processes.

The specific objectives were:

1. Analysis of Psa-V detections from 2011 produced pollen by PCR.

2. To determine if viable or dead Psa-V can be isolated or detected from frozen anther from the 2011 season;

3. To determine if viable or dead Psa-V can be isolated or detected in the pollen extracted from anther frozen in 2011;

4. If it is possible to isolate viable Psa-V populations in commercially harvested and milled pollen, and if so:

5. Can uninfected kiwifruit plants be infected with Psa-V by either dry or wet application of Psa-V infected pollen?

   Once we have analysed the results we will be able to outline the potential risk of using supplementary applied pollen harvested, milled and extracted by commercial means.

3. Methodology

a. Pollen milling process

The aim of the pollen milling process is to extract pollen from male kiwifruit flowers. This pollen can then be stored indefinitely by freezing and can then be applied to orchards to maximise fruit set, size and dry matter.

Male flower is handpicked either from dedicated male kiwifruit blocks, or from male plants in Hayward fruiting orchards. Flower is poured into a mill delivery chute, where it is taken by conveyor belt up into a mill head that crushes the flower to help release the pollen-containing anthers from the flower (Figure 1). This material is then passed through a drum screen that allows the anther material to fall through the screen and be collected in trays (Figure 2). The anther material is then spread onto drying trays (Figure 3). These are then transferred to the drying cabinets. The drying cabinets run at 32^{o}C for 4 hours, and then have the temperature reduced to 29^{o}C until the anther is determined to be “dry”. At this stage, the anther material is vacuumed into a cyclone extractor that releases the pollen from the material, and this pollen is captured in a large jar (Figure 4). After extraction, the pollen is sieved through a fine stainless steel mesh (50 micron) and placed in 250 gram jars.

The sample size was determined as a proportion of the original flower sample, based on the percentage recovery achieved through the commercial mill at each stage.

Figure 1 Flower milling head

Figure 2 Screen drum milled flower passes through

Figure 3 Anther material after passing through screen drum

Figure 4 Cyclone extraction of pollen from dry anther material

We identified the following stages in the flower milling and pollen extraction process that we obtained samples from in order to test for Psa-V.

1. Picked flower from bags
2. Milled flower
3. Extracted anther
4. Wet anther after 4 hours drying
5. Dry anther
6. Anther after cyclone extraction
7. Pollen after cyclone extraction
8. Pollen after sieving

These stages are shown in Table 1 below. Samples were collected by hand and stored in sealed zip-lock bags under refrigeration until delivered to the Ministry of Primary Industries for analysis.

Table 1 Process points for collecting samples for Psa-V testing

Sample number	Process	Sample	Hours after picking	Percentage weight of flower	Amount sampled (grams)
	Pick flower	flower from vine	0		
	Receive flower at mill	flower from bag	7	100%	
1	Mill flower	Crushed flower (including anther)	9	100%	100
2	Screen anther	Wet anther	9	28%	28
3	Begin drying (4 hrs @ 32°C)	Anther after 4 hours	13	17%	17
4	After drying	Anther after drying	29	7%	7
5	Cycloning	Anther after cycloning	29	6%	7
6	Cycloning	Pollen after cycloning	29	1%	1
7	Sieving	Pollen after sieving	31	1%	1

b. Flower Collection

Male kiwifruit flower was collected from 30 orchards in the Bay of Plenty from 13 November to 3 December 2012. The majority of the flower samples were collected during periods where the risk of Psa-V infection was very low (no rainfall recorded) except for flower collected on the 13th (1 sample), 15th November (2 samples), and 16th November (3 samples) where moderate Psa-V risk events were recorded (Appendix 1). The locations of these orchards are shown in Figure 5. Contracted pickers, who were unaware of this trial, picked the flower. Therefore, we obtained a representative field example of flower. The pickers were instructed to pick the male flower at "popcorn" stage, when it is soft to the touch, and not after the flower has opened past bell shape.

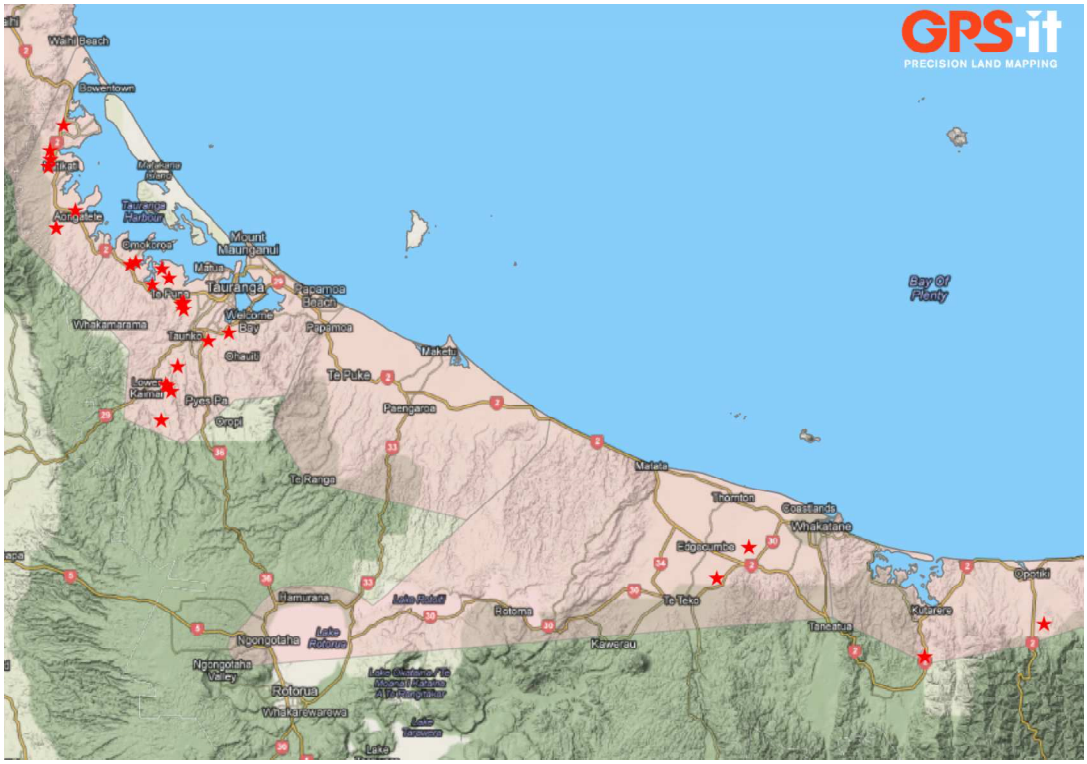

Figure 5 Map showing location of sampled orchards

No special hygiene practices were undertaken while collecting the samples. The onion sacks that the flower is placed in before delivery to the mill were washed in a weak bleach solution (0.3 % sodium hypochlorite) between orchards, and air-dried before use.

One hundred flowers were taken from each sample and these were inspected to identify potential signs of bacterial disease in the flower bud. An example of a sample is shown in Figure 6. This figure also shows the variation in stages of flower material harvested and supplied for pollen extraction.

No special hygiene practices were undertaken in the flower mill and pollen extraction process. It was decided not to undertake any special hygiene protocols while collecting these samples as the project aimed to determine the risk of commercially produced pollen, produced through a commercial pollen mill.

Figure 6 Flower sample from male flower supplied to PollenPlus Te Puna mill

c. Testing approach

Samples were taken as described above, from the seven different milling stages and tested by a real-time PCR assay to detect Psa-V DNA and isolation onto semi-selective media to determine viability. Samples from each of the seven milling stages were tested for the first seven orchards (table 2) and milled pollen samples (step 7) from all 30 orchards were tested (Figure 10). Sample weights tested were proportion to the percentage of sample recovered throughout the commercial mill at each stage (Table 1).

d. Isolation onto semi-selective media

Samples were re-suspended in bacteriological saline (0.85% NaCl w/v) and shaken at 250 rpm at room temperature on an orbital shaker for 30 minutes. The aim of this step was to wash bacteria from the plant suspensions (flower mash, anthers) and pollen grains for plating onto semi-selective media to assess viability and if possible quantify the number of viable colony forming units present in each sample. Aliquots of 100 µl from each suspension were streaked onto KBC, a semi-selective medium developed for *Pseudomonas syringae* pathovars by Mohan and Schaad, 1987. These were incubated overnight at 26^{o}C. Plate counts were undertaken to obtain quantitative data.

e. Polymerase chain reaction (PCR) testing

PCR testing of the plant tissue suspensions (flower mash, anthers, pollen) and bacterial colonies were tested as described by MPI report to KVH (2011). Samples that gave a positive Psa-V test result were then retested using other Psa-V diagnostics to confirm the finding.

4. Results

Observations of harvested flowers for Psa-V symptoms

Figure 7 shows a flower bud with a discoloured petal, and Figure 8 shows a flower bud with discoloured sepals. A number of flowers were found with discoloured petals and or sepals. However, these flowers were not tested for the presence of Psa-V, so we are unable to determine the cause of this symptom. Later in the season, a number of Hayward fruitlets have been observed with deformed parts of fruit that may relate to diseased petals or sepals at pollination. There appeared to be no correlation between the percentages of brown flowers identified from the flower sample to the amount of Psa-V detected in the field. A number of the sample sites that had no brown flowers identified had high levels of Psa-V infection present in the vines.

Figure 7 Flower showing discoloured petal

Figure 8 Flower showing discoloured sepals

Appendix 2 gives a summary of sampling points in the flower milling process. Note that some of the samples from the wet anther that should have been collected 4 hours after drying began were not collected. This was due to this sampling period occurring in the middle of the night, while large volumes of commercial flower material were being processed.

5. Analysis of Psa-V test results from 2011 produced pollen lines (Objective 1)

Over 300 commercially produced pollen lines (2kg of pollen per line) were produced in the 2011 pollen harvest season. These lines were tested for Psa-V using the real-time PCR assay referred to as the 83/84/85 SYBR green assay. This assay was developed at Plant and Food Research (PFR), with further validation being completed at Hill Laboratories. This assay enables the distinction between Psa-V and Psa-V-LV haplotypes based on the sequence variation of the hopA1 gene. Of the 300 pollen lines tested from harvest 2011, none were found to be positive for Psa-V. However, two of the lines had "un-determined" results, indicating a weak positive result.

In mid 2012, Hill Laboratories began using the TaqMan duplex, the new real-time PCR assay as supplied by DNature Limited, Gisborne. This assay was validated by PFR. Internal validation, carried out by Hill Laboratories, which compared the TaqMan assay and the SYBR green-based 83/84/85 assay indicated the TaqMan assay is superior, with a lower detection limit, across a range of sample matrices, including pollen. When some of the lines produced and tested in 2011 were retested in 2012 using the new TaqMan assay, Psa-V was detected. The difference in results between 2011 and 2012 is due to the more sensitive nature of the TaqMan assay.

6. Isolation and detection of Psa-V from frozen anthers and pollen harvested in 2011 (Objective 2)

It was not possible to isolate viable or detect dead Psa-V from the frozen anthers harvested in 2011.

The frozen anther material had significant tissue degradation caused by the freezing and thawing process and it was not possible to extract pollen from these samples.

7. Isolation and detection of Psa-V during commercial pollen milling and extraction (Objective 3)

Real-time PCR can provide semi-quantitative results and average Cq values detected at each step of the pollen milling and extraction process are presented in Table 2. Psa-V was detected from all milled pollen samples from each of the 30 orchards with a range of Cq values from 19 to 33 (Table 3). Cq values in this range are indicative of those for strong positive test results. There was no significant change in the Psa-V Cq values detected at each step of the process. This could indicate that Psa-V inoculum levels did not decline throughout the milling process, or that there was contamination occurring throughout the milling process. However, we were unable to pinpoint precisely where this was occurring because there was no obvious increase in inoculum levels at a particular stage.

While real time PCR can provide a semi-quantitative result, the average Cq values do not distinguish between dead and live cells.

Viable Psa-V colonies were isolated from every step of the milling and extraction process for different lines of flower processed in the pollen mill and tested (Table 2). Pollen produced from all 30 orchards was found to be Psa-V positive by real-time PCR, but viable Psa-V colonies were not isolated from all of these samples. There was an issue with the modified King's medium B (KBC) not solely selecting for Psa-V. This resulted in a significant background of micro-flora being co-isolated thereby interfering in the detection and

quantification of the organism. This created a significant amount of additional work where individual Psa-V-like colonies were further tested by real-time PCR to confirm identity. Despite extensive efforts in screening and isolation of Psa-V colonies on KBC, accurate determination of Psa-V inoculum levels present in pollen was not possible due to Psa-V being swamped out by faster growing background micro-flora. This was contrary to previous work conducted (MPI report 2011) on isolating Psa-V from pollen extracted from gold kiwifruit flowers where the KBC media did effectively select for Psa-V. The reason for the increase in background micro-flora on green kiwifruit pollen is unknown. It is possible that at least some of the orchards are using biological control agents, or applying supplements that encourage the survival of microbes on plant surfaces. Viable Psa-V colonies were isolated using KBC media from pollen collected at the end of the milling process from 18 of the 30 lines of flower processed through the mill. The confirmation that these colonies were Psa-V was checked by using two different Psa-V diagnostic real-time PCR tests. Due to background micro-flora swamping the KBC plates, it is possible that there were viable Psa-V colonies present in the milled pollen where colonies were not isolated onto KBC. Alternatively, it is possible that in samples where viable Psa-V colonies were not detected inoculum levels had fallen to undetectable or non-viable levels.

Pollen samples sent for further testing by Verified Laboratory Services (VLS) using their selective media resulted in the isolation of viable Psa-V colonies from an additional four lines of pollen. Viable colonies of Psa-V were therefore isolated from pollen produced from 22 of the 30 lines of flower processed through the mill. A range of 10 – 10^4 Psa-V colony forming units (cfu) per 0.2 g was isolated from these samples using the VLS media.

Table 2 Real-time PCR detection of plant tissue sampled throughout the pollen milling process

Sampling stages	1. Milled flower	2. Wet anthers	3.Anthers mid-dry	4. Dried anthers	5. Anther waste	6. Pollen after cyclone	7.Pollen after sieving
Average Cq values	26.8	26.9	27.9	27.3	29.2	28.3	27.6
Cq value range	24.2 -33.7	19.8-31.8	25.4–30.9	23.3–32.3	25.9–28.7	25.3–33.6	19.8-33.8

Key for Cq values <37 = positive; 37-42 = undetermined; >42 negative

Table 3 Summary of results from PCR detection of plant tissue (flower, anther, pollen) and isolation of Psa-V onto semi-selective media

Orchards	Disease incidence	1. Milled flower		2. Wet anthers		3. Anthers mid-drying		4. Dried anthers		5. Anther waste after cyclone		6. Pollen after cyclone		7. Pollen after sieving	
		PCR (Cq value)	Viable	PCR (Cq value)	Viable	PCR (Cq value)	Viable	PCR (Cq value)	Viable	PCR (Cq value)	Viable	PCR (Cq value)	Viable	PCR (Cq value)	Viable
1	Low	21.95	+	19.82	-	25.43	-	23.34	-	25.96	-	28.79	-	26.39	+
2	Low	0	-	30.33	-	29.89	-	32.37	-	31.92	-	31.15	-	32.55	+
3	Low	33.73	-	28.57	-	30.29	-	32.57	-	33.65	-	31.31	-	33.80	+
4	Medium	30.01	+	31.87	-	NS	NS	27.21	-	28.73	+	25.18	+	26.99	+
5	Low	29.01	-	28.10	+	NS	NS	24.76	+	30.02	+	30.61	-	31.67	+
6	Low	24.22	+	22.99	-	NS	NS	26.16	+	26.41	+	26.54	+	27.69	-
7	Low	25.33	+	27.15	-	26.38	+	25.27	+	28.71	+	25.33	-	23.59	+
8	Low													27.55	+
9	Low													23.17	+
10	Low													23.40	-
11	Low													22.59	-
12	Low													24.15	+
13	Low													22.39	+
14	Low													22.20	+
15	Medium													25.34	-
16	Low													25.64	-
17	Low													25.49	+
18	High													24.15	+
19	High													22.85	+
20	Medium													23.43	-
21	Medium													24.15	+
22	Low													23.54	+
23	Low													25.01	+
24	Low													25.13	+
25	Low													23.43	-
26	Low													26.97	-
27	Low													19.86	+
28	Low													22.67	+
29	Low													21.71	+
30	High													20.96	+

Key – NS=Not sampled; PCR Cq value <37 = positive; 37-42 = undetermined; >42 = negative; + = viable Psa-V colonies isolated; - no Psa-V colonies isolated

8. Discussion

Viable Psa-V populations were detected throughout each stage of the milling process. All of the orchards we collected flower from were in an area with high disease pressure and were from orchards that were confirmed as having, or were suspected as having, Psa-V infection. The findings of this work do not necessarily apply to regions that have no Psa-V or low levels of Psa-V infection, and/or isolated orchards with Psa-V infection.

This study confirms that Psa-V is able to survive the commercial milling process, and supports the earlier findings in Italy (Gallelli et al., 2011; and Vanneste et al., 2011) that viable Psa-V can be associated with pollen. In this study attempts at using KBC to quantify Psa-V levels in pollen was not successful. However, retesting by VLS using their selective media estimated that there was a range of 10 – 10^4 Psa-V cfu per 0.2 g of pollen. Previous studies (Stefani and Giovanaardii, 2011; Vanneste et al. 2011) that tested a small number of samples have found that Italian pollen was contaminated with a range of $7 \times 10^3 – 1 \times 10^6$ cfu per g. Italian pollen is harvested by vacuum and it is possible the levels of Psa-V in these samples could have been over-estimated due to also collecting extraneous material contaminated with Psa-V (Vanneste et al. (2011). The levels of Psa-V detected in this study at the higher range of 10^4 cfu per 0.2 g are lower than what was detected in the bulk of Italian pollen samples tested (10^6 cfu per g). Previous studies on other phytopathogenic bacteria including *Pseudomonas syringae* have suggested that there is a relationship between population levels and resulting infection levels (for example, Lindemann et al. 1984; Taylor et al. 2003; Upper et al. 2003). Small populations (c. < 10^3 cfu) have a low probability of causing disease, as population levels influence the time required to cause infection and disease symptoms. So as pathogen levels increase, so does the probability of disease development. When pathogen populations remain small to moderate, it is possible for host and pathogen to co-exist without disease especially if conducive conditions are not present, for example, climate, host not at susceptible stage. This has been recently observed for Psa-V where the bacterium has been detected on pollen and leaf surfaces in kiwifruit blocks where no disease was observed (Vanneste et al. 2011; MPI, 2011). Interestingly, in preliminary experiments (Stefani and Giovanardi 2011) where kiwifruit plants were dusted with Psa-V infected pollen (10^6 cfu per gram) and sprayed with a wet suspension of artificially contaminated pollen (10^6cfu per ml), Psa-V was shown to colonise the plant without causing severe disease. They also showed that through artificial pollination colonisation was more effective on flowers by wet suspensions than by dry dusting of pollen, suggesting that dry pollination was less suitable for establishing epiphytic populations. The low to moderate inoculum levels present in pollen may also explain why initial epidemiological work during the early response to the disease could not find a link between pollen application and subsequent Psa-V infection (Richardson et al. 2012).

In comparing PCR test results (Cq values) of pollen samples from 2010 and 2011 it was observed that detections in pollen harvested this year returned stronger positive results than previous years (MPI report 2012; data not shown). This could indicate that inoculum levels present in pollen are higher than previous years and explain why we were able to isolate Psa-V V colonies directly from commercially milled pollen samples for the first time.

It is important to note that these results do not confirm if the disease can be transmitted in the field through pollen application. We still do not know how readily Psa-V is transmitted from infected pollen to a susceptible host and then initiate disease (Objective 5). The possibility of infection of kiwifruit vines via contaminated pollen has yet to be addressed in this work. An initial

pre-trial to set up such experiments with only three plants was not conclusive. No disease was observed on plants sprayed with naturally infested pollen and a positive Psa-V control despite conducive humid and temperate conditions for infection. It is suspected that infection did not occur because the inoculated leaves were too mature. Some pathogenicity test studies have observed that the age of leaf tissue is an important factor in obtaining infection. To conduct this experiment we need to source pathogen-free plants for infection. However, obtaining suitable uninfected mature kiwifruit plants will be a challenge. We did initially have a source of plants set up in Gisborne but these become contaminated in the recent outbreak. We now intend to complete this aspect of the project on kiwifruit seedlings raised from seed sourced from the South Island. We expect this will take another 2-3 months by the time seedlings are propagated from seed and we intend to include the results of this work in an addendum to this report. We note that experiments on kiwifruit seedlings in a laboratory environment are likely to create the "worst case scenario" and may not be reflective of what occurs in an orchard e.g. seedlings are more likely to be susceptible to disease and do not grow true to type.

Kiwifruit Vine Health (KVH) consider the most important factors to minimise orchard-to orchard transmission are orchard visitor, staff and contractor hygiene, tool hygiene, machinery and vehicle hygiene, harvest hygiene, and fruit bin and bee hive hygiene.

One of the objectives of this work was to "Provide pollen clients with a degree of certainty around the potential risk of using commercially produced pollen". In this work, 100% of pollen samples tested positive for Psa-V by PCR. This is further supported by test results where all samples of commercially produced pollen from both the Waikato and Bay of Plenty region in the 2012 season (sent to Hill Laboratories) tested positive for Psa-V. These findings justify the approach to collecting male flower for pollen and applying as outlined by Kiwifruit Vine Health, 2012.

9. Scope for further work

We were unable to determine if any of the Psa-V detections found in samples taken from the pollen mill were a result of cross-contamination from the mill. If we were to obtain flower material from a region known to have no incidence of Psa-V, we could run this through the mill, and take samples from the different pollen milling and extraction stages. It would then be possible to track any introduction or increase in the level of Psa-V at the various milling stages, thereby identifying if there is any source of cross-contamination in the mill.

We did not test the discoloured brown petal material for Psa-V incidence. Quite possibly a complex of bacteria, including other pseudomonads, colonise flower tissues and cause discoloration. It would be useful to identify these bacteria and assess their contribution to disease expression especially for new varieties such as Green14 where a high incidence of flower drop was recorded in spring 2012.

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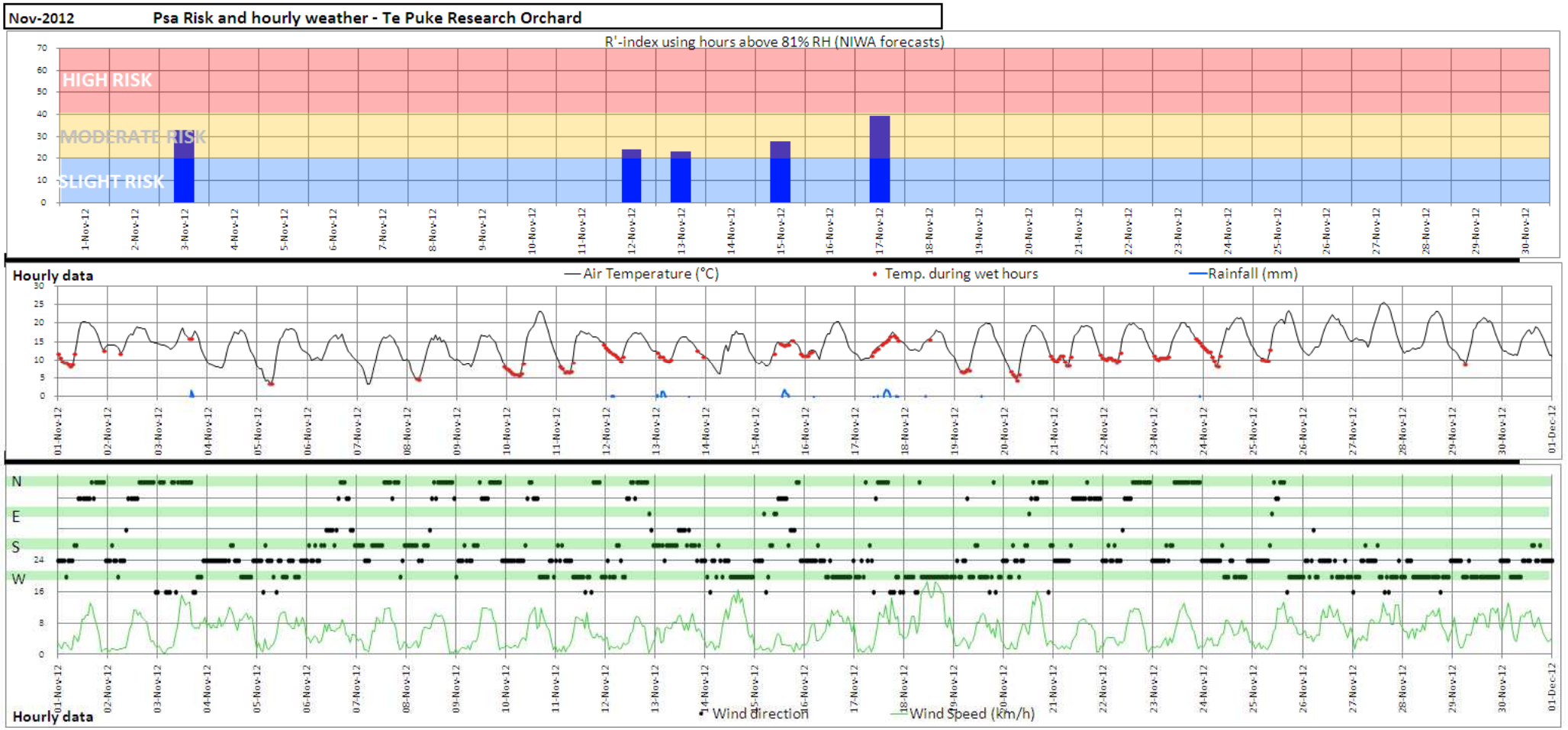
Appendix 1 Kiwifruit Vine Health (KVH) weather data and risk model for Te Puke (November 2012)



Appendix 2 Summary of sampling information

Picking date	ID	Flower Weight (Kg)	% brown buds	Flowers (comments)	Sample weight (grams)						
					milled flowers	Wet anthers	anthers + 4hrs dry	anthers pre-cyclone	anthers post-cyclone	pollen post cyclone	sieved pollen
13/11/2012	1	8.9	0	Test run	300	43.5	missing	missing	missing	missing	missing
15/11/2012	2	8.8	5		440	123.5	74.02	12.01	15.31	23.73	13.62
15/11/2012	3	9	0		440	126.5	76	31.5	15.29	20.39	43.7
16/11/2012	4	8.9	0		445.5	124.6	missing	31.3	15.96	33.49	44.33
16/11/2012	5	9.1	0		455	127.5	missing	29.5	15.5	21.29	48.03
16/11/2012	6	8.2	0		409.5	114.6	missing	28.71	15.17	28.7	37.17
19/11/2012	7	8.7	0		435	122	74	15.03	15.37	20.15	23.45
19/11/2012	8	10.8	1		521	151	68.44	37.79	17.32	43.92	34.36
19/11/2012	9	7.6	0		380	106.4	64.46	26.64	15.06	26.03	28.23
19/11/2012	10	8.1	0		405	113.4	68.95	28.35	15.21	25.53	37.26
21/11/2012	11	10.2	0	warm, left outside	497	140.5	60.9	36.51	15.71	23.33	48.4
21/11/2012	12	9.8	0		121.5	137	55.2	34.23	15.08	27.49	33.54
21/11/2012	13	8.7	0		435	122	36.27	22.84	15.21	18.86	18.98
21/11/2012	14	9.8	0	lots of open flowers	490	133.5	71.63	32.62	15.74	33.14	26.88
21/11/2012	15	8.5	12		425	119	72.1	20.78	15.24	25.92	43.25

Date	ID	Flower weight (Kg)	% brown buds	Flowers (comments)	Sample weight (g)						
					milled flowers	wet anthers	anthers + 4hrs dry	anthers pre-cyclone	anthers post-cyclone	pollen post cyclone	sieved pollen
21/11/2012	16	10.8	0	lots of open flowers	502	151	90	37.21	15.71	21.96	38.62
21/11/2012	17	8.4	0		420	118.5	71	28.11	15.35	27.57	34.98
21/11/2012	18	8.1	0		405	114.5	69.01	28.76	15.39	10.87	24.33
21/11/2012	19	10.8	0		472.5	151	92.08	38.39	15.44	18.14	34.33
21/11/2012	20	8	0		400	112	68.01	28.22	15.21	32.99	32.99
21/11/2012	21	8	0		400	112	68.42	28.95	15.58	21.21	39.99
21/11/2012	22	10.1	0	lots of open flowers	503	141	77.95	35.19	15.01	12.22	30.42
21/11/2012	23	9.15	11		457	129	78	31.2	15.58	23.27	30.61
21/11/2012	24	8.6	10		430	120	73.45	30.64	15.5	15.41	30
21/11/2012	25	12.5	15		488	138.5	missing	41.75	16.53	22.68	35.3
21/11/2012	26	8.8	12		440	123	missing	31.63	15.59	14.18	32.67
29-Nov-12	27	12.5	0		536	176	80	35.86	15.19	18.43	45.2
29-Nov-12	28	10.2	0		511.5	143.5	87	36.13	15.09	31.48	45.88
29-Nov-12	29	9.2	0		460	128	78	32.2	15.1	19.45	28.2
2-Dec-12	30	10.8	0		513	151.5	missing	38.21	15.44	27.74	49.48