Mitigating the Risk of *Pseudomonas syringae* pv. *actinidiae* Introduction by Pollen

ZESPRI Innovation Project V11285 Report

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1 Executive Summary

1.1 Project Purpose

The key purpose of this project is to ensure growers have access to pollen with a known minimal risk of contamination of live *Pseudomonas syringae pv. actinidiae* of the virulent strain *(Psa*-V*).*

This project focused on identifying the hurdles needed to ensure pollen with less than 10^6 colony-forming units (cfu) per gram is available for pollen production and decontamination.

The study consisted of four objectives:

1. To determine the pollen contamination risk associated with sourcing pollen from orchards located at varying distances from known *Psa*-V infected orchard sites (Geographic Distance Risk).
2. Establish the sampling protocols and frequency required for confirming *Psa*-V contamination within single commercial batches of pollen.
3. To determine the amount of reduction in *Psa*-V bacteria achieved by spraying flowers in-situ with a current best-practice bactericide.
4. To assess the quantum and variability in *Psa*-V contamination of pollen obtained from orchards assessed as low risk, based on the KVH pollen production best-practice pollen source guidelines.

1.2 Key Findings and Recommendations

1.2.1 Geographic Distance Risk

- *Psa*-V was found on flower buds from all 15 orchards sampled along an 11 km easterly path that included the orchard that first tested positive to *Psa* in Te Puke in October 2010.
- *Psa*-V levels were similar along the 11 km path suggesting a similar risk of selecting *Psa*-V infected flowers from the Te Puke region tested.
- One third of anther samples taken from a subsample of the sampled flower buds were positive of *Psa*-V. These samples were from the 5 eastern-most orchards.
- Four flower bud samples and 7 anther samples from 14 orchards sampled along a 22 km north-south path in Edgcumbe (a more-recently infected region) were positive for *Psa*-V.
- There was an equal risk of selecting *Psa*-V positive flowers along the 22 km path.

1.2.2 Sampling Protocols and Testing Frequency of Commercial Batches of Pollen

- A 0.25g sub-sample taken from a well-mixed sample of commercially harvested pollen gives acceptable test variability (n = 3, mean Cq = 28.06, sd = 0.58, % CV = 2.05) and a single test is representative of a sample batch of pollen.
- The quantitative polymerase chain reaction (qPC*R*) *Psa*-V test method used by Hill Laboratories based on PCR primers recently develop by Plant & Food Research (PFR), could detect no less than 2.6 x 10^3 cfu per 0.1 mL when calibrated against viable bacterial inoculum. The *Psa*-V test was about 15 times less sensitive that the qPCR *Psa* test using F1/R2 primers. A recent MAF report has also noted the qPCR *Psa*-V test is less sensitive and suggested further work by PFR to enhance the method's sensitivity.
- Until such time that a more sensitive Psa-V test is developed, is recommended that future *Psa* testing of pollen should first use the qPCR *Psa* test using F1/R2 primers (detects *Psa*-V and *Psa*-LV), followed by a confirmation of Psa-V presence using the qPCR *Psa*-V test using the hop-1 primer set.

1.2.3 Reduction of Psa-V on flowers and anthers by spraying

- All 30 unsprayed and 30 sprayed flower bud samples taken from a highly infected orchard were highly positive for *Psa*-V by qPCR (mean Cq values of 22.0 and 24.5, respectively), with apparent *Psa*-V bacterial load of 2 x 10^8 and 1 x 10^8 cfu per g of flower buds, respectively.
- Twenty-seven out of thirty (90%) anther samples from both the unsprayed and sprayed vines were also positive for *Psa*-V by qPCR.
- Attempts to enumerate live *Psa*-V bacteria were unsuccessful so differentiation between live and dead bacteria was not possible. Therefore, the effectiveness of using a bactericide spray could not be determined.
- It is recommended that a better viable *Psa* bacteria recovery method be developed before the next pollen season if this work is to be repeated.

1.2.4 Risk of Psa-V contamination of pollen harvested according to the KVH Pollen Production Best-practice Pollen Source Guidelines

- Flower buds harvested, according to best-practice guidelines, from 24 separate orchards were reported as 'not detected' for *Psa*-V by qPCR.
- Representative samples of pollen commercially processed on the same days as the flower buds were harvested, were reported as 'not detected' for *Psa*-V by qPCR.
- These results give a degree of assurance to growers that they have access to pollen with a known minimal risk of contamination of live *Pseudomonas syringae pv. actinidiae (Psa*-V*).*

2 Introduction

Pseudomonas syringae pv. *actinidiae*-virulent strain (*Psa*-V) infection of Kiwifruit vines is well established in Te Puke region and is also present in other parts of the Bay of Plenty.

In addition to climatic factors such as wind and rain, insects, animals, people, irrigation water and equipment[1] can facilitate *Psa*-V dispersal between plants and orchards. Loss of male vines on orchards, due to *Psa*-V infection, can necessitate greater reliance on artificial pollination to ensure high yields of fruit with market preferred quality and size. Transfer of pollen between kiwifruit orchards could, in some situations, pose a risk of dispersing *Psa*-V[2].

Currently, the most effective heat treatment methods of decontaminating pollen are reliant on pollen having a contamination level below 10^6 colony-forming units (cfu) per gram to be effective.

This project focused on identifying the hurdles needed to ensure pollen with less than 10^6 cfu per gram is available for pollen production and decontamination.

The study consisted of four objectives:

1. To determine the pollen contamination risk associated with sourcing pollen from orchards located at varying distances from known *Psa*-V infected orchard sites.
2. Establish the sampling protocols and frequency required for confirming *Psa*-V contamination within single commercial batches of pollen.
3. To determine the amount of reduction in *Psa*-V inoculum achieved by spraying flowers in-situ with a current best-practice bactericide.
4. To assess the quantum and variability in *Psa*-V contamination of pollen obtained from orchards assessed as low risk, based on the KVH pollen production best-practice pollen source guidelines.

This report describes the orchard sampling protocols, sample preparation and laboratory analysis procedures, reports the experimental results and key findings, and makes recommendations to the industry for future work.

[1] Bashan, (1985), Field dispersal of Pseudomonas syringae pv. tomato, Xanthomonas campestris pv. vesicatoria, and Alternaria macrospora by animals, people, birds, insects, mites, agricultural tools, aircraft, soil particles and water sources. Canadian Journal of Botany 64: 276 – 281.

[2] Vanneste et al., (2011). Detection of Pseudomonas syringae pv. actinidiae in kiwifruit pollen samples. New Zealand Plant Protection 64: 246-251.

3 Methods

3.1 Sampling Procedures

3.1.1 Geographic Distance Risk

3.1.1.1 Flower Sampling

Fifteen Kiwifruit orchards planted in Hayward vines, with varying degree of *Psa* infection, were sampled along a line transecting the epicentre of the original *Psa* infection in Te Puke. One of two optional orchards was selected for sampling at 750 metre intervals along the 11 kilometre transect using a map of infected orchards in the region provided by Kiwifruit Vine Health (KVH).

A second series of samples was taken from 14 Hayward orchards along a 22 km transect of a more recent *Psa* infection in Edgecombe.

At each sampling point, GPS coordinates, the variety of Kiwifruit vine (Hayward per hectare), visual *Psa*-V rating (scale of 0 to 10, where 10 is severe), and previous orchard *Psa* test results were recorded.

Hygiene best practice protocols, including the use of hairnets, gloves, and sanitizers, were followed on each orchard.

One kilogram of flower buds (approximately 1,000 buds) were sampled from each orchard by combining buds selected along the transect line that cut across each orchard. The flower buds were at the "Pop Corn" stage of maturity, which is the same stage of flower maturity used by commercial pollen producers for pollen production.

Each kilogram batch of flower buds was placed in a large labelled wax coated paper bag, sealed, and stored overnight in a refrigerator. Samples were then transferred into a clean plastic zip-lock bag and mixed thoroughly. Two x 250g sub-samples were taken and placed into separate clean zip-lock bags for sample preparation.

3.1.1.2 Sample Preparation

1 Bacteriological Saline Wash

To one 250g sub-sample of flower buds was added 500mL of 0.85% bacteriological saline (8.5 g NaCl per litre of non-chlorinated water). The zip-lock bag was sealed and shaken to wash the surface of the flower buds. The bags were agitated two more times over 10 minutes before a bottom corner of the bag was cut and 80mL of saline wash decanted into bar code labelled 80mL flip-lid plastic containers. Samples were stored in a refrigerator before being dispatched in a cooler-bin to the laboratory for analysis.

2 Anther Collection

Flower anthers were exposed by carefully plucking flower petals from individual flower buds contained in the second 250g sub-sample zip-lock bag. Special care

was taken not to contaminate the anthers with flower petals. Filaments with attached anthers were excised using surface-sterilised (70% ethanol) nail scissors on clean collection paper. The filaments, with attached anthers, collected from each bag were then placed into separate bar coded 80mL flip-lid plastic containers and stored refrigerated until being dispatched in a cooler-bin to the laboratory for analysis. All surfaces and equipment were sterilised with 70% ethanol between each sample preparation.

3.1.2 Sampling Protocols and Testing Frequency of Commercial Batches of Pollen

As at the time of this experiment, no known cases of *Psa*-V infected *Actindia deliciosa* pollen were found in commercially produced pollen in New Zealand, the following protocol was used to produce *Psa*-V infected pollen.

Four kilograms of male kiwifruit flowers was harvested from a heavy infected Hayward orchard in Te Puke. A 250g sub-sample was taken for a bacterial saline wash to confirm *Psa*-V presence on flowers.

The flower petals were removed using unsterilised equipment and technicians rubbed their hands through both petals and anthers during the pollen harvesting process. Filaments with attached anthers were removed using unsterilised nail scissors, placed onto plastic Petri dishes and dried in a food dehydrator at 28^{o}C for 24 hours to release pollen from the anthers. Contents from the Petri dish were place into a 200 mesh per square inch stainless steel filter and shaken onto aluminium foil. The filter allowed the filament with attached anthers to mix with pollen as the pollen sifted through the mesh onto the collection surface.

Collected pollen was the placed into a bar coded 80mL flip-lid plastic containers and stored in a refrigerator until ready for dispatch in a cooler-bin to the laboratory for analysis.

3.1.3 Reduction of Psa-V on flowers and anthers by spraying

Thirty male *Actinidia deliciosa* kiwifruit vines from an orchard with *Psa V* symptoms in Te Puke were treated with an approved kiwifruit leaf surface sterilant (Spotless) by a commercial spray contractor two days before collection of flowers. Thirty vines from the same orchard that had not been sprayed were used to collect untreated flowers.

As many flowers as possible at the “Pop Corn” stage were collected from each vine from both treated and untreated vines into large labelled wax prove paper bags, sealed and placed in a refrigerator overnight.

Bacteriological saline washings and anthers were prepared according to the protocol described in Section 1 (Geographic Distance Risk).

3.1.4 Risk of Psa-V contamination of pollen harvested according to the KVH Pollen Production Best-practice Pollen Source Guidelines

The KVH orchard screening guidelines were used to identify 23 Hayward orchards suitable for pollen collection. One kilogram of flowers from each orchard was collected from two commercial KVH-certified pollen mills' daily flower receipts. Samples were taken from each bag receipted from each orchard line.

Flowers were placed in a large labelled wax lined paper bag, sealed, refrigerated overnight, then bacteriological saline washings prepared according to the protocol in Section 1 (Geographic Distance Risk).

To obtain a representative sampling of commercially produced pollen, pollen samples were taken from each 2 kg batch of commercially prepared pollen produced from flowers receipted on the same days as the flowers were sampled from 23 Hayward orchards.

From each of eight 250g plastic jars that make up a 2 kg batch of pollen, was taken 5 x ~45 mg subsamples (40 all told) and transferred into one 1.8mL bar coded plastic screw-capped vial. Samples were stored in a refrigerator until ready for dispatch in a cooler-bin to the laboratory for analysis.

During sample processing, all surfaces and equipment were sterilised with 70% ethanol between each sample preparation.

3.2 Laboratory Procedures

3.2.1 Saline wash samples

Plastic containers filled with bacteriological saline (0.85%) washings of kiwifruit flowers were gently shaken to re-suspend particulates. A 15-mL aliquot of sample was transferred into a 15 mL conical plastic tube and centrifuged at 5,000 rpm for 10 minutes to precipitate the particulates and bacteria. All but 1.5 mL of the supernatant was removed. The remaining solution and precipitate was vortexed and transferred to 2-mL tubes that were centrifuged at 10,000 rpm for 5 minutes.

The supernatant was removed and discarded. The precipitate was washed by re-suspending in 1.0-mL of bacteriological saline (0.85%), vortexing and re-centrifuging at 10, 000 rpm for 5 minutes. The supernatant was discard.

DNA was extracted by adding 1.0 mL of CTAB buffer and 20 µL proteinase K. The tubes were incubated at 65^{o}C for 15 minutes with shaking, then centrifuged for 2 min at 12,000 rpm. 420 µl of the supernatant was then extracted using the InviMag\ Plant DNA extraction kit and a MagMax automated DNA extraction instrument according to the manufacture's protocol. Purified DNA was eluted into 100 µL of elution buffer and stored at -20 ^{o}C until required for PCR analysis.

3.2.2 Anthers

Each container of anthers was inverted several times to mix the contents thoroughly. A 10-g subsample was transferred to a 50 mL container and homogenized (Ultraturrax) with 20-mL of bacteriological saline (0.85%). A 100 µL aliquot of the homogenized suspension was transfer into a 2mL vial. 1.0mL of CTAB buffer and 20 µL proteinase K was added and the tubes were incubated at 65^{o}C for 15 minutes with shaking, then centrifuged for 2 min at 12,000 rpm. 420 µl of the supernatant was then extracted using the InviMag\ Plant DNA extraction kit and a MagMax automated DNA extraction instrument according to the manufacture's protocol. Purified DNA was eluted into 100 µL of elution buffer and stored at -20 ^{o}C until required for qPCR analysis.

3.2.3 Pollen

Dry pollen samples were mixed thoroughly by inverting the container several times. A 0.25g aliquot was transferred into a 2 mL tubes and 1.0mL of CTAB buffer and 20 µL proteinase K was added and the tubes were incubated at 65^{o}C for 15 minutes with shaking, then centrifuged for 5 min at 13,000 rpm. 420 µl of the supernatant was then extracted using the InviMag\ Plant DNA extraction kit and a MagMax automated DNA extraction instrument according to the manufacture's protocol. Purified DNA was eluted into 100 µL of elution buffer and stored at -20 ^{o}C until required for qPCR analysis.

3.2.4 Sampling Protocols and Testing Frequency of Commercial Batches of Pollen

To help establish the sampling protocols and sampling frequency of pollen required for confirming *Psa* contamination within commercial batches of pollen, two experiments were undertaken. For the first experiment, *Psa-V* contaminated pollen was increasingly diluted with uncontaminated pollen to determine how sensitive the qPCR *Psa-V* assay is to detect Psa in naturally infected pollen. As at the time of this experiment's design, the PCR assay had only been validated with pollen spiked with isolated *Psa-V* bacteria.

The second experiment, bulk samples of 'contaminated' and 'uncontaminated' pollen were repeatedly sampled and tested by qPCR for Psa-V content to determined the variability of the qPCR Cq value and give an indication of the uncertainty of measurement of the test.

3.2.4.1 Serial Dilution of Infected Pollen

Pollen known to be contaminated with *Psa* was serially diluted 2-fold, to give a dilution range of 2 to 1024 times, with pollen known to be clear (uncontaminated) of *Psa* infection (tested by qPCR). Five replicate 0.25g subsamples from each of the 2-fold dilutions were tested for Psa by qPCR.

3.2.4.2 Variability of the PCR Psa-V test

Thirty replicate 0.25g sub-samples of the "contaminated" and the "uncontaminated' pollen were tested according to the protocol described in section 3.2.3 to assess variability of the laboratory sub-sampling and testing protocol.

3.2.5 Determination of apparent Psa colony forming units (cfu) in saline wash samples

Since it is difficult to reliably isolate viable *Psa* bacterial from pollen, a calibration curve of known levels of bacteria vs. qPCR *Psa*-V Cq values was created. This calibration curve allows estimations of the bacterial load, expressed as apparent colony forming units (cfu), present on flowers buds, anthers and pollen.

A fresh culture of *Psa*-V bacteria was used to prepare a bacteriological saline of known cfu concentration. An array of x10 serial dilutions in bacteriological saline was prepared to give a range of 10^1 to 10^{10} cfu per mL. Aliquots of each of these dilutions of viable bacteria were tested by qPCR and used to calibrate a standard curve to estimate apparent *Psa* cfu concentrations in samples from their Cq values when tested by qPCR. Two calibration curves were prepared. One by doing a direct DNA extraction and qPCR on an aliquot of the saline dilution, the other by emulating the extraction procedure used to prepare the bacteriological saline washings. The latter curve takes into account any procedural losses that might occur during the preparation of bacteriological saline washings.

3.2.5.1 Direct determination of Psa-V DNA

Duplicate 100 µL aliquots of each dilution of *Psa-V* bacteria were immediately transferred to 2 mL tubes and 1.0mL of CTAB buffer and 20 µL proteinase K was added and the tubes incubated at 65^{o}C for 15 minutes with shaking, then centrifuged for 5 min at 13,000 rpm. 420 µl of the supernatant was then extracted using the InviMag⟨ Plant DNA extraction kit and a MagMax automated DNA extraction instrument according to the manufacture's protocol. Purified DNA was eluted into 100 µL of elution buffer and stored at -20 ^{o}C until required for PCR analysis.

3.2.5.2 Emulation of Flower Saline Wash Preparation

A 100 µL aliquot of each serial dilution was added to 14.9 mL of bacteriological saline in conical centrifuge tubes, mixed thoroughly and contents centrifuged at 5,000 rpm for 10 minutes to precipitate the bacteria. All but 1.5 mL of the supernatant was removed. The remaining solution and precipitate was vortexed and transferred to 2-mL tubes that were centrifuged at 10,000 rpm for 5 minutes.

The supernatant was removed and discarded. The precipitate was washed by re-suspending in 1.0-mL of bacteriological saline (0.85%), vortexing and re-centrifuging at 10, 000 rpm for 5 minutes. The supernatant was discard.

DNA was extracted by adding 1.0 mL of CTAB buffer and 20 µL proteinase K. The tubes were incubated at 65^{o}C for 15 minutes with shaking, then centrifuged for 2 min at 12,000 rpm. 420 µl of the supernatant was then extracted using the InviMag⟨ Plant DNA extraction kit and a MagMax automated DNA extraction instrument according to the manufacture's protocol. Purified DNA was eluted into 100 µL of elution buffer and stored at -20 ^{o}C until required for PCR analysis.

3.2.6 Reduction of Psa-V on flowers and anthers by spraying

Six samples of flower saline washes, that gave the highest level of Psa contamination by qPCR, from each of the pre- and post-spray groups were selected to determine if the positive qPCR results originated from live or dead *Psa* bacteria. qPCR alone cannot distinguish between DNA from live bacteria or DNA from dead bacteria.

A 100 µL aliquot of the same saline wash initially use to detect *Psa* by qPCR was plated onto King's medium B agar and incubated at 28 ^{o}C for 16 hours. All bacterial colonies were harvested by washing the surface of the agar with 1.0 mL of bacteriological saline and transferred to a 2 mL storage tube. After thorough mixing, DNA from 100 µL of the suspension was extracted using the InviMag\ Plant DNA extraction kit and a MagMax automated DNA extraction instrument according to the manufacture's protocol. Purified DNA was eluted into 100 µL of elution buffer and stored at -20 ^{o}C until required for PCR analysis.

At a later date, this experiment was repeated using a more selective media, KBC. This media contains boric acid (1.5 mg/mL), cephalexin (80µg/mL), and cycloheximide (200µg/mL). Extended incubation time, greater than 16 hours was also investigated.

3.2.7 Psa Determination by PCR

All samples, except those from the serial dilution of infected pollen experiment, were tested by qPCR using primer sets (83/84/85 –hop1) that could differentiate between *Psa*-V and *Psa*-LV based on different DNA melt curves (Rikkerink et al, 2011[3]). For the serial dilution experiment, only the *Psa*-V specific 83/84 primer pair was used. DNA extracts were diluted either 10 or 100 fold before qPCR to remove any influence of inhibitory substances in the extracts. Preliminary experiments with DNA extracts were done to determine the most appropriate dilution. Hill Laboratories, Hamilton, did all laboratory sample preparations, *Psa*-V bacterial growth experiments and qPCR tests.

[3] Rikkerink E, Andersen M, Rees-George J, Cui W, Vanneste J, Templeton M. December 2011. Development of a rapid tool for the molecular characterisation of Psa haplotypes. A confidential report prepared for Zespri Group Limited, VI1256. Plant & Food Research Client Report No. 46010.

4 Results and Discussion

4.1 Geographic Distance Risk

All saline washings of the exterior of 'Popcorn' Kiwifruit flower buds taken from 15 orchards along the Te Puke transect (Figure 1) tested either positive or weakly positive for *Psa*-V by qPCR analysis. The Te Puke area has a well-established *Psa-V* infection with sample orchards having relatively high *Psa* Infection Visual Ratings (median = 4, mean = 4.2, sd = 2.2, range 2 – 9).

Figure 1. Te Puke Sampling Transect (the 5th flag from the left represents the approximate location where *Psa* was first detected). Flags on the map indicated the latitude and longitude coordinates of the sampling points.

There was no significant change in the *Psa*-V Cq values of bacteriological saline washing of flowers collected from orchards along the length of the Te Puke sampling transect, suggesting a similar risk of *Psa* infection independent of geographical location (Figure 2 and Appendix 1). Five of the fifteen anther samples taken from the orchards were positive or weakly positive for *Psa-V*. Interestingly, these samples came from the orchards furthermost from the first infected orchard (Figure 2). Possible contamination during sample preparation cannot be discounted.

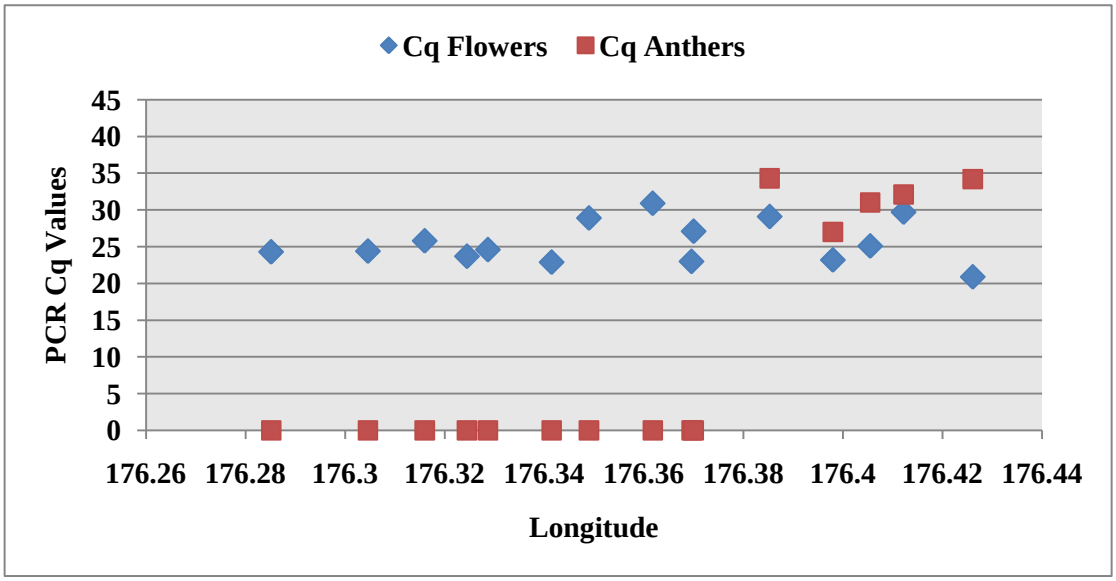

Figure 2. qPCR Cq values for bacteriological saline washings (Cq Flowers) and flower anthers (Cq Anthers) plotted according to the longitude location of the sampling point along the Te Puke Transect. The left-most point represents the orchard where *Psa* was first detected [Orchard 1], and the right-most point represents the orchard [Orchard 15] further most from the first infected orchard. Longitude was used because the transect ran east-west, better representing geographical distance between orchards.

The Edgecumbe transect (Figure 3) represented an area of more recent *Psa* infection with lower *Psa*-V Infection Visual Ratings (median = 0.5, mean = 1.4, sd = 2.5, range = 0 – 9).

Figure 3. Edgecumbe Sampling Transect. Flags on the map indicated the latitude and longitude coordinates of the sampling points.

Four out of fourteen saline washings of flower buds from orchards sampled in the Edgecumbe area tested positive/weakly positive for *Psa*-V by qPCR. Seven anther samples were also positive/weakly positive for *Psa*-V (Figure 4 and Appendix 1). Six of the anther samples that tested positive/weakly positive did not test positive for their corresponding saline washings of flower exteriors. This suggests infection of anthers by *Psa*-V has occurred through internal movement of *Psa* bacteria. Three saline wash samples were positive/weak positive for Psa but their corresponding anther samples were negative for *Psa*-V by qPCR, suggesting that these samples were contaminated through movement of bacteria in the environment. One sample was positive for *Psa*-V in the saline wash and the anthers.

The appeared to be no significant geographical pattern to Psa-V infection along the Edgecumbe Transect, and the results suggest an equal risk harvesting contaminated flowers from any point along the transect (Figure 4).

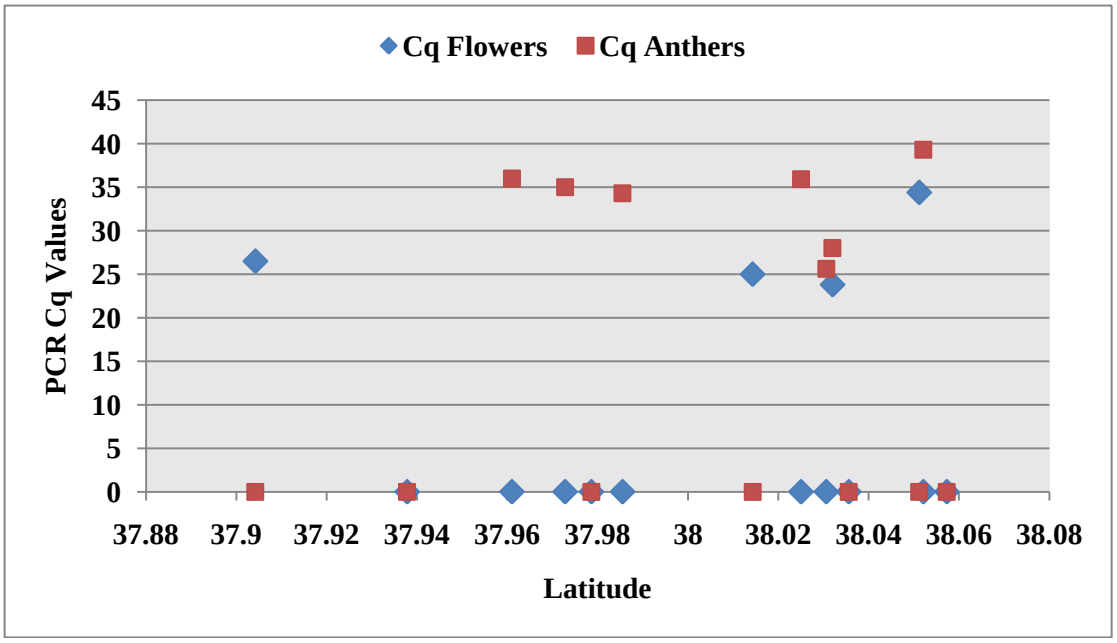

Figure 4. qPCR Cq values for bacteriological saline washings (Cq Flowers) and flower anthers (Cq Anthers) plotted according to the latitude location of the sampling point along the Edgecumbe Transect. Latitude was used because the transect ran north-south, better representing geographical distance between orchards.

4.2 Sampling Protocols and Testing Frequency of Commercial Batches of Pollen

4.2.1 Serial Dilution of Infected Pollen

Pollen contaminated with *Psa*-V was serially diluted with pollen harvested according to the KVH protocol and tested as not containing *Psa*-V to determine sensitivity and repeatability of the qPCR *Psa*-V test offered by Hill Laboratories.

Although every effort was made during the pollen harvesting process to contaminate the pollen with *Psa*-V, the resultant 'contaminated' pollen gave a relatively high Cq value by q PCR (low level of *Psa*-V contamination). Thus, few serial dilutions were needed before the limit of detection of the method was reached (Table 1 and Appendix 2). In addition, the laboratory found a significant dilution (x10 for the undiluted sample and x100 for serially diluted samples) of the extracted DNA was required before the qPCR assay to prevent inhibitors in the extracts affecting the PCR assay. Consequently this reduces the sensitivity of the test. Nevertheless, acceptable sampling repeatability of replicates gives confidence that 0.25g of a sample will give a confident representation of the total sample.

Table 1. Serial dilutions of 'contaminated' pollen with 'uncontaminated' pollen

Dilution	Replicates Tested	Replicates Psa positive	Mean	1 x sd	% CV
Undiluted	3	3	27.3	0.3	1.1
2 x	5	5	29.1	0.5	1.7
4 x	5	5	30.2	0.9	3.1
8 x	5	5	31.5	0.9	3.0
16 x	5	5	31.6	0.4	1.3
32 x	5	5	33.0	1.5	4.6
64 x	5	4	34.1	1.1	3.4
128 x	5	4	33.3	0.3	0.9
256 x	5	2	32.5	1.6	4.8
512 x	5	0	-	-	-
1024 x	5	1	35.3	-	-

4.2.2 Variability of the qPCR Psa-V test

Thirty 0.25g replicates of the 'uncontaminated' and the 'contaminated' pollen batches were tested for *Psa-V* content by qPCR. All 'uncontaminated' sample replicates did not give PCR melt curves indicative of *Psa*-V and were determined not to contain *Psa*-V. The thirty replicates of 'contaminated' pollen gave a mean *Psa*-V Cq value of 28.06, and a standard deviation of 0.58 (% coefficient of variation = 2.05).

These results give confidence that the protocol used to collect and process the pollen samples and the repeatability of the laboratory test are appropriate to be able to reliably detect *Psa* contamination of a batch of pollen consisting of flowers originating from multiple orchards.

4.2.3 Determination of apparent Psa colony forming units (cfu) in saline wash samples

Since it is difficult to reliably isolate viable *Psa* bacterial from pollen, a calibration curve of known levels of bacteria vs. qPCR *Psa*-V Cq values was created. This calibration curve allows estimations of the bacterial load, expressed as apparent colony forming units (cfu), present on flowers buds, anthers and pollen.

Two calibration curves were prepared, one using 0.1mL of *Psa* bacteria inoculated saline solution (Direct), and the other processing 0.1mL of *Psa* bacteria inoculated saline solution through the protocol used to prepare the bacteriological saline washes of the flower buds (Saline Wash).

An acceptable correlation for the Direct (R^2 = 0.994) and Saline Wash (R^2 = 0.982) calibration curves were obtained (Figure 5, Appendix 3). The qPCR could detect about 2.6 x10^3 cfu per 0.1mL of either untreated inoculated saline or inoculated saline processed by the protocol used to prepare the bacteriological saline washes of the flower buds. However, there is evidence of procedural loss of bacteria, particularly at lower cfu levels, when preparing the saline washes.

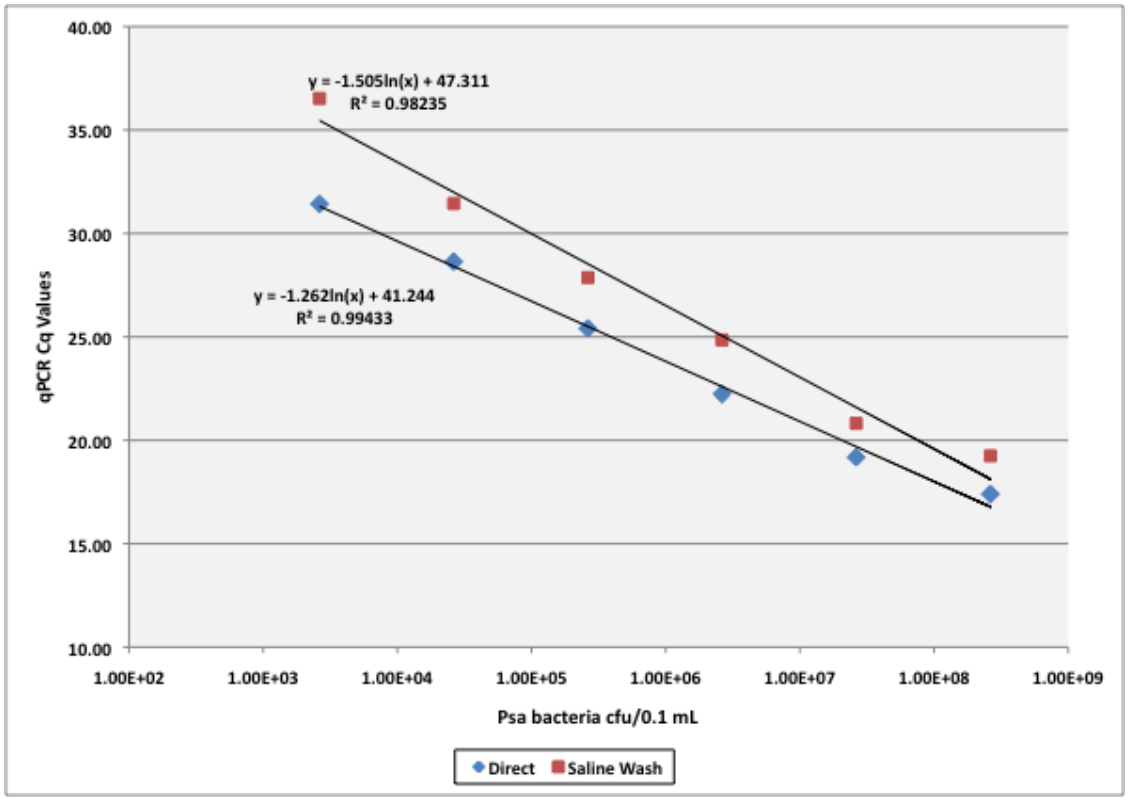

Figure 5. Calibration curves of qPCR Cq values and *Psa*-V bacteria cfu for (a) 0.1mL of *Psa* bacteria inoculated saline solution (Direct), and (b) 0.1mL of *Psa-V* bacteria inoculated saline solution processed through the protocol used to prepare the bacteriological saline washes of the flower buds (Saline Wash).

The sensitivity of the qPCR assay using the F1/R2 primers, that are not specific for *Psa*-V, is reported to be as low as 10 cfu (Rees-George, et al. 2010[4]). A more recent report[5] suggests that the qPCR assay using primers (83/84/85 – hop1), that are specific to *Psa*-V, is less sensitive that the assay using the F1/R2 primers. The magnitude of the difference in sensitivity was not reported.

To quantify the difference in sensitivity of the two assays, the DNA extracts of serial dilutions of *Psa* bacteria inoculated saline solution use for the aforementioned calibration curve (Direct) were tested using the F1/R2 and the hop1 primer sets.

The qPCR assay using the F1/R2 primer set was found to be about 15 times more sensitive than the qPCR assay using the hop-1 primer set when comparing calibration curves (Figure 6). The F1/R2 primer set could detect about 100 cfu in 0.1mL.

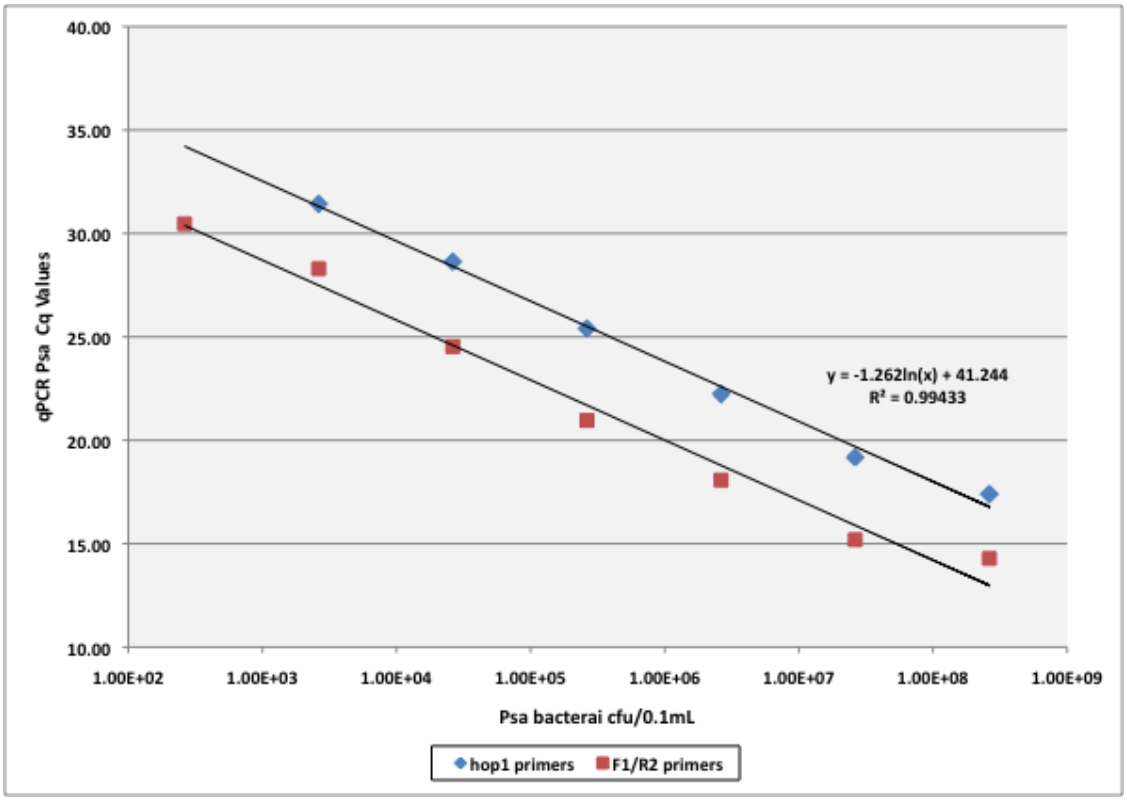

Figure 6. Calibration curves of qPCR Cq values and *Psa-V* bacteria cfu for 0.1mL of *Psa* bacteria inoculated saline solution (Direct) derived from qPCR tests using (a) hop-1 and (b) F1/R2 primer sets.

[4] Rees-George J, Vanneste JL, Cornish DA, Pushparajah IPS, Yu J, Templeton MD, Everett KR, 2010. Detection of *Pseudomonas syringae* pv. *actinidiae* using polymerase chain reaction (PCR) primers based on the 16S–23S rDNA intertranscribed spacer region and comparison with PCR primers based on other gene regions. *Plant Pathology* **59**, 453–464.

[5] Detection of Pseudomonas syringae pv. actinidiae from leaves and pollen collected from symptomatic and asymptomatic Actinidia chinensis in Te Puke, Bay of Plenty. IDC and Response, Ministry of Agriculture and Forestry, PO Box 2095, Auckland 1140, New Zealand. December 2011

4.2.4 Reduction of Psa-V on flowers and anthers by spraying

Thirty male *Actinidia deliciosa* Kiwifruit vines from an orchard with Psa-*V* symptoms in Te Puke were treated with an approved kiwifruit leaf surface sterilant (Spotless) by a commercial spray contractor two days before collection of flowers. Thirty vines from the same orchard that had not been sprayed were used to collect a similar number of unsprayed flower samples. Saline washes of flower bud exteriors and dissected anthers were tested by qPCR for *Psa-V* content.

All unsprayed and sprayed flower bud saline wash samples were highly infected with *Psa-V* (mean Cq values of 22.0 and 24.5, respectively), with apparent Psa-V bacterial load of 2 x 10^8 and 1 x 10^8 cfu per g of flower buds, respectively (Table 2, Appendix 4). Twenty-seven out of thirty (90%) Anther samples for both the unsprayed and sprayed vines were also positive for *Psa-V* by qPCR (Table 2, Appendix 4).

Since the qPCR test cannot distinguish the difference between DNA from live or DNA from dead bacteria, a representative selection of six samples of flower bud washing from the unsprayed and sprayed groups were plated onto King's Medium B agar and incubated overnight at 25^{o}C. All bacterial growth was harvested and the extracted DNA was tested for *Psa*-V content by qPCR. It was expected that if viable *Psa*-V bacteria were present in a sample, there would be growth of *Psa*-V bacteria and an increase in *Psa*-V Cq values in the qPCR test. If the spray was effective, then samples from the spray treatment should show no increase in Cq values.

Bacterial growth was observed on all agar plates after overnight incubation. However, the observed colonies did not appear to be characteristic of *Psa* colonies. Subsequent qPCR of harvested colonies could not detect any *Psa*-V.

This experiment was repeated using a more selective King's media and plates were incubated for up to 48 hours. Again microbial growth was not indicative of *Psa*-V. Failure to grow *Psa*-V in the laboratory might well have been due to the considerable time (3 weeks) the sample extracts were stored refrigerated before testing. Therefore, it cannot be determined if the application of Spotless killed or did not kill *Psa*-V bacteria on the flower buds or on the anthers.

What can be deduced from this experiment is that flowers from heavily infected Kiwifruit vines contain anthers with high levels of *Psa*-V infection.

Table 2. Frequency of *Psa*-V positive samples of saline washing of flower buds and anthers, mean qPCR Cq values of positive samples and estimated Psa bacteria (cfu/g) load of flower samples taken from a heavily infected orchard before and after spraying with a bactericide (Spotless).

	Unsprayed Vines		**Sprayed Vines**	
	Flower bud	**Anthers**	**Flower bud**	**Anthers**
Positive (n)	30	27	30	27
Not Detected (n)	0	3	0	3
Mean Cq of Positives	22.9	28.4	24.5	28.9
SD of Positives	2.1	2.9	1.3	1.9
% CV of Positives	9.4	10.1	5.3	6.6
~ cfu load (per g)	2.0e 08	1.8e 07	1.0e 08	6.0e 06

4.2.5 Risk of Psa-V contamination of pollen harvested according to the KVH Pollen Production Best-practice Pollen Source Guidelines

Bacteriological saline washes of flower buds collected, according to the KVH Pollen Production Best-practice Pollen Source Guidelines; from 23 Hayward orchards (over a number of days) as part of a commercial pollen harvesting process were tested for Psa-V contamination by qPCR. On the same day as these samples arrived at the pollen processing plant, 40 replicate pollen sub-samples from each 2kg batch of commercial pollen were combined and sent to Hill Laboratories for qPCR analysis. A total of X commercial batches of pollen were tested.

Psa-V was not detected in any of the saline washes or the pollen samples submitted to the laboratory, suggesting that pollen harvested from orchards according to the KVH Pollen Production Best-practice Pollen Source Guidelines have low risk of *Psa*-V contamination (Appendix 5).

5 Appendices

5.1 Appendix 1: Geographic Distance Risk Data

	Laboratory Job Number:	956729	Objective 1																
	Date Registered:	26/11/11 13:28	Te Puke Transect																
	File Creation Date:	8/12/11 9:56																	
	Quote Number:	47161																	
							KPIN and PSA Status												
Sample Number	Sample ID	Sample Type	Dilution Factor	Psa Cq Value	Psa Result		KPIN	Psa Test Result	Isolate Type	Orchard Address	Town	Region	Priority Zone	Variety (HW / Ha)	GPS coordinates Long	Lat	Visual Psa V Rating		
1	2100000022182	Bacteriological saline washings	10	24.3	Positive		4596	Positive	Psa-V	17 Bayly Rd	Te Puke	TE PUKE	Te Puke	2	176.28515	-37.8334^	4		
2	2100000022199	Bacteriological saline washings	10	24.4	Positive		6979	Positive	Psa-V	349 No 2 Rd	Te Puke	TE PUKE	Te Puke	4.57	176.3046	-37.8302	4		
3	2100000022205	Bacteriological saline washings	10	25.8	Positive *		5417	Positive	Psa-V	11 Cheetham Ave	Te Puke	TE PUKE	Te Puke	2.75	176.316	-37.8262	7		
4	2100000022212	Bacteriological saline washings	10	23.7	Positive		8738	Positive	TND	Te Matai Rd	Te Puke	TE PUKE	Te Puke	3.8	176.3245	-37.8293	9		
5	2100000022229	Bacteriological saline washings	10	24.6	Positive		5123	Positive	Psa-V	487 Te Matai Rd	Te Puke	TE PUKE	Te Puke	6.35	176.3287	-37.8302	4		
6	2100000022236	Bacteriological saline washings	10	22.9	Positive		8577	Positive	Psa-V	157 Mark Road	Te Puke	TE PUKE	Te Puke	12.4	176.3415	-37.8326	5		
7	2100000022243	Bacteriological saline washings	10	28.9	Positive		5185	Positive	Psa-V visual	337 Brown Rd	Te Puke	TE PUKE	Te Puke	5.04	176.349	-37.8215	4		
8	2100000022250	Bacteriological saline washings	10	30.9	Weak positive		5181	Positive	Psa-V	Gridley Road	Te Puke	TE PUKE	Te Puke	22.7	176.3618	-37.8268	2		
9	2100000022267	Bacteriological saline washings	10	23	Positive		6739	Positive	Psa-V	412 Rangiuru Rd	Te Puke	TE PUKE	Te Puke	2.54	176.3696	-37.8242	6		
10	2100000022274	Bacteriological saline washings	10	27.1	Positive		3616	Positive	Psa-V	130 Casuarina Dr	Te Puke	TE PUKE	Te Puke	8.96	176.37003	-37.8403^	3		
11	2100000022281	Bacteriological saline washings	10	29.1	Positive		8921	Not Detected	-	Karner Dr	Te Puke	TE PUKE	Te Puke	6	176.3853	-37.8223	3		
12	2100000022298	Bacteriological saline washings	10	23.2	Positive		1879	Positive	Psa-V	316 State Highway 33	Paengaroa	TE PUKE	Te Puke	9.31	176.398	-37.8301	9		
13	2100000022304	Bacteriological saline washings	10	25.1	Positive		8008	Positive	Psa-V	329 State Highway 33	Paengaroa	TE PUKE	Te Puke	15.06	176.4055	-37.8372	3		
14	2100000022311	Bacteriological saline washings	10	29.7	Positive		4225	Positive	Psa-V	Old Coach Rd	Te Puke	TE PUKE	Te Puke	4.83	176.4122	-37.8286	3		
15	2100000022328	Bacteriological saline washings	10	20.9	Positive		8143	Positive	Psa-V	Milford Park Dr	Paengaroa	TE PUKE	Te Puke	5.38	176.4261	-37.8357	3		
16	2100000022359	Kiwifruit Flowers / Buds	10	42.6	Not Detected		4596	Positive	Psa-V	17 Bayly Rd	Te Puke	TE PUKE	Te Puke	2	176.28515	-37.8334^	4		
17	2100000022380	Kiwifruit Flowers / Buds	10	ND	Not Detected		6979	Positive	Psa-V	349 No 2 Rd	Te Puke	TE PUKE	Te Puke	4.57	176.3046	-37.8302	4		
18	2100000022410	Kiwifruit Flowers / Buds	10	42.1	Not Detected		5417	Positive	Psa-V	11 Cheetham Ave	Te Puke	TE PUKE	Te Puke	2.75	176.316	-37.8262	7		
19	2100000022441	Kiwifruit Flowers / Buds	10	ND	Not Detected		8738	Positive	TND	Te Matai Rd	Te Puke	TE PUKE	Te Puke	3.8	176.3245	-37.8293	9		
20	2100000022472	Kiwifruit Flowers / Buds	10	ND	Not Detected		5123	Positive	Psa-V	487 Te Matai Rd	Te Puke	TE PUKE	Te Puke	6.35	176.3287	-37.8302	4		
21	2100000022502	Kiwifruit Flowers / Buds	10	44.8	Not Detected		8577	Positive	Psa-V	157 Mark Road	Te Puke	TE PUKE	Te Puke	12.4	176.3415	-37.8326	5		
22	2100000022533	Kiwifruit Flowers / Buds	10	ND	Not Detected		5185	Positive	Psa-V visual	337 Brown Rd	Te Puke	TE PUKE	Te Puke	5.04	176.349	-37.8215	4		
23	2100000022564	Kiwifruit Flowers / Buds	10	ND	Not Detected		5181	Positive	Psa-V	Gridley Road	Te Puke	TE PUKE	Te Puke	22.7	176.3618	-37.8268	2		
24	2100000022595	Kiwifruit Flowers / Buds	10	ND	Not Detected		6739	Positive	Psa-V	412 Rangiuru Rd	Te Puke	TE PUKE	Te Puke	2.54	176.3696	-37.8242	6		
25	2100000022625	Kiwifruit Flowers / Buds	10	40.6	Not Detected		3616	Positive	Psa-V	130 Casuarina Dr	Te Puke	TE PUKE	Te Puke	8.96	176.37003	-37.8403^	3		
26	2100000022656	Kiwifruit Flowers / Buds	10	34.3	Weak positive		8921	Not Detected	-	Karner Dr	Te Puke	TE PUKE	Te Puke	6	176.3853	-37.8223	3		
27	2100000022687	Kiwifruit Flowers / Buds	10	27	Positive		1879	Positive	Psa-V	316 State Highway 33	Paengaroa	TE PUKE	Te Puke	9.31	176.398	-37.8301	9		
28	2100000022717	Kiwifruit Flowers / Buds	10	31	Weak positive		8008	Positive	Psa-V	329 State Highway 33	Paengaroa	TE PUKE	Te Puke	15.06	176.4055	-37.8372	3		
29	2100000022748	Kiwifruit Flowers / Buds	10	32.1	Weak positive		4225	Positive	Psa-V	Old Coach Rd	Te Puke	TE PUKE	Te Puke	4.83	176.4122	-37.8286	3		
30	2100000022779	Kiwifruit Flowers / Buds	10	34.2	Weak positive		8143	Positive	Psa-V	Milford Park Dr	Paengaroa	TE PUKE	Te Puke	5.38	176.4261	-37.8357	3		
	* = Also minor LV Positive		^ Lat, Ln coordinates derived from street address																
	Laboratory Job Number:	958643	Objective 1																
	Date Registered:	2/12/11 10:13	Edgecumbe / Whakatane Transect																
	File Creation Date:	8/12/11 18:23																	
	Quote Number:	47161																	
							KPIN and PSA Status												
Sample Number	Sample ID	Sample Type	Dilution Factor	Psa Cq Value	Psa Result		KPIN	Psa Test Result	Isolate Type	Orchard Address	Town	Region	Priority Zone	Variety (HW / Ha)	GPS coordinates Long	Lat	Visual Psa V Rating		
1	2100000030729	Bacteriological saline washings	10	33	Not Detected		2831	Positive	Psa-V	147 Otakiri Road	Edgecumbe	Whakatane	Whakatane	0.2	176.811	-37.9855	1		
2	2100000030750	Bacteriological saline washings	10	32.1	Not Detected		5150	Positive	Psa-V	1927 State Highway 30	Te Teko	Whakatane	Whakatane	21.3	176.8198	-38.0356	1		
3	2100000030781	Bacteriological saline washings	10	29.6	Not Detected		4048	Not tested	-	158 Galatea Road	Whakatane	Whakatane	Whakatane	2.1	176.808	-38.0521	0		
4	2100000030811	Bacteriological saline washings	10	37.1	Not Detected		7977	Positive	Psa-V	389 Mcdonald Road	Whakatane	Whakatane	Whakatane	1.3	176.8307	-38.0573	4		
5	2100000030842	Bacteriological saline washings	10	34.4	Weak positive		3397	Not Detected	-	326 McDonald Road	Te Teko	Whakatane	Whakatane	27.2	176.8233	-38.0512	0		
6	2100000030873	Bacteriological saline washings	10	23.8	Positive		5356	Positive	Psa-V	118 B MacDonald Road	Whakatane	Whakatane	Whakatane	1.7	176.8311	-38.032	2		
7	2100000030903	Bacteriological saline washings	10	38.4	Not Detected		5544	Positive	Psa-V	88 MacDonald Road	Te Teko	Whakatane	Whakatane	3.12	176.8324	-38.0306	9		
8	2100000030934	Bacteriological saline washings	10	33.7	Not Detected		1947	No data	-	2 Paul Road		Whakatane	Whakatane		176.83799	-38.0250^	0		
9	2100000030965	Bacteriological saline washings	10	25	Positive		1907	Not Detected	-	46 Maunder Rd	Whakatane	Whakatane	Whakatane	1.6	176.8461	-38.0143	1		
10	2100000030996	Bacteriological saline washings	10	30.5	Not Detected		7278	Not tested	-	121 Western Drain Rd	Whakatane	Whakatane	Whakatane		176.871493	-37.9727^	0		
11	2100000031023	Bacteriological saline washings	10	29.2	Not Detected		3276	Not tested	-	67 Luke Rd	Whakatane	Whakatane	Whakatane	4.18	176.8887	-37.9786	0		
12	2100000031054	Bacteriological saline washings	10	31.8	Not Detected		2435	Not tested	-	162 College Rd	Edgecumbe	Whakatane	Whakatane	1.9	176.8332	-37.961	0		
13	2100000031085	Bacteriological saline washings	10	39.3	Not Detected		8870	Not tested	-	275 Gow Road	Whakatane	Whakatane	Whakatane	15	176.8134	-37.9378	0		
14	2100000031115	Bacteriological saline washings	10	26.5	Positive		9288	Not Detected	-	33 Burt Road	Whakatane	Whakatane	Whakatane	7.9	176.766	-37.9042	1		
15	2100000031146	Kiwifruit Flowers / Buds	10	34.3	Weak positive		2831	Positive	Psa-V	147 Otakiri Road	Edgecumbe	Whakatane	Whakatane	0.2	176.811	-37.9855	1		
16	2100000031177	Kiwifruit Flowers / Buds	10	42.3	Not Detected		5150	Positive	Psa-V	1927 State Highway 30	Te Teko	Whakatane	Whakatane	21.3	176.8198	-38.0356	1		
17	2100000031207	Kiwifruit Flowers / Buds	10	39.3	Weak positive		4048	Not tested	-	158 Galatea Road	Whakatane	Whakatane	Whakatane	2.1	176.808	-38.0521	0		
18	2100000031238	Kiwifruit Flowers / Buds	10	0	Not Detected		7977	Positive	Psa-V	389 Mcdonald Road	Whakatane	Whakatane	Whakatane	1.3	176.8307	-38.0573	4		
19	2100000031269	Kiwifruit Flowers / Buds	10	40.9	Not Detected		3397	Not Detected	-	326 McDonald Road	Te Teko	Whakatane	Whakatane	27.2	176.8233	-38.0512	0		
20	2100000031290	Kiwifruit Flowers / Buds	10	28	Positive		5356	Positive	Psa-V	118 B MacDonald Road	Whakatane	Whakatane	Whakatane	1.7	176.8311	-38.032	2		
21	2100000031320	Kiwifruit Flowers / Buds	10	25.6	Positive		5544	Positive	Psa-V	88 MacDonald Road	Te Teko	Whakatane	Whakatane	3.12	176.8324	-38.0306	9		
22	2100000031351	Kiwifruit Flowers / Buds	10	35.9	Weak positive		1947	No data	-	2 Paul Road		Whakatane	Whakatane		176.83799	-38.0250^	0		
23	2100000031382	Kiwifruit Flowers / Buds	10	44.1	Not Detected		1907	Not Detected	-	46 Maunder Rd	Whakatane	Whakatane	Whakatane	1.6	176.8461	-38.0143	1		
24	2100000031412	Kiwifruit Flowers / Buds	10	35	Weak positive		7278	Not tested	-	121 Western Drain Rd	Whakatane	Whakatane	Whakatane		176.871493	-37.9727^	0		
25	2100000031443	Kiwifruit Flowers / Buds	10	42.1	Not Detected		3276	Not tested	-	67 Luke Rd	Whakatane	Whakatane	Whakatane	4.18	176.8887	-37.9786	0		
26	2100000031474	Kiwifruit Flowers / Buds	10	36	Weak positive		2435	Not tested	-	162 College Rd	Edgecumbe	Whakatane	Whakatane	1.9	176.8332	-37.961	0		
27	2100000031504	Kiwifruit Flowers / Buds	10	40.4	Not Detected		8870	Not tested	-	275 Gow Road	Whakatane	Whakatane	Whakatane	15	176.8134	-37.9378	0		
28	2100000031535	Kiwifruit Flowers / Buds	10	42.2	Not Detected		9288	Not Detected	-	33 Burt Road	Whakatane	Whakatane	Whakatane	7.9	176.766	-37.9042	1		
			^ Lat, Ln coordinates derived from street address																

5.2 Appendix 2a: Sampling Protocols and Testing Frequency of Commercial Batches of Pollen Data

Laboratory Job Number:	958621														
Date Registered:	3/12/15 9:58														
File Creation Date:	21/12/15 10:23														
Quote Number:	47284														
Sample Number	**Sample Name**	**Sample Type**	**PCR dilution**	**Cq Value**	**Psa Result**	**Cq value**									
1	2100000032624 (Infected)	Kiwifruit pollen	10	27.2	Positive	27.24									
2	2100000032594 (Clean)	Kiwifruit pollen	10	35.4	Not Detected	35.36									
3	Sample 1 (2x) Rep 1	Kiwifruit pollen	100	29.6	Positive	29.57									
4	Sample 1 (2x) Rep 2	Kiwifruit pollen	100	28.9	Positive	28.92									
5	Sample 1 (2x) Rep 3	Kiwifruit pollen	100	29.5	Positive	29.45									
6	Sample 1 (2x) Rep 4	Kiwifruit pollen	100	28.3	Positive	28.33	Mean	1 x sd	%cv			Replicates	Replicates			
7	Sample 1 (2x) Rep 5	Kiwifruit pollen	100	29.1	Positive	29.12	29.08	0.49	1.69	**Dilution**		**tested**	**positive**	**mean**	**1 x sd**	**% CV**
8	Sample 2 (4x) Rep 1	Kiwifruit pollen	100	29.9	Positive	29.92				Undiluted		3	3	27.3	0.3	1.1
9	Sample 2 (4x) Rep 2	Kiwifruit pollen	100	28.8	Positive	28.78				2 x		5	5	29.1	0.5	1.7
10	Sample 2 (4x) Rep 3	Kiwifruit pollen	100	30.9	Weak positive	30.86				4 x		5	5	30.2	0.9	3.1
11	Sample 2 (4x) Rep 4	Kiwifruit pollen	100	31.1	Weak positive	31.13	Mean	1 x sd	%cv	8 x		5	5	31.5	0.9	3.0
12	Sample 2 (4x) Rep 5	Kiwifruit pollen	100	30.3	Weak positive	30.28	30.20	0.92	3.05	16 x		5	5	31.6	0.4	1.3
13	Sample 3 (8x) Rep 1	Kiwifruit pollen	100	31.2	Weak positive	31.23				32 x		5	5	33.0	1.5	4.6
14	Sample 3 (8x) Rep 2	Kiwifruit pollen	100	31	Weak positive	31.03				64 x		5	4	34.1	1.1	3.4
15	Sample 3 (8x) Rep 3	Kiwifruit pollen	100	33	Weak positive	32.99				128 x		5	4	33.3	0.3	0.9
16	Sample 3 (8x) Rep 4	Kiwifruit pollen	100	31.7	Weak positive	31.65	Mean	1 x sd	%cv	256 x		5	2	32.5	1.6	4.8
17	Sample 3 (8x) Rep 5	Kiwifruit pollen	100	30.5	Weak positive	30.47	31.47	0.95	3.02	512 x		5	0	-	-	-
18	Sample 4 (16x) Rep 1	Kiwifruit pollen	100	31.6	Weak positive	31.63				1024 x		5	0	-	-	-
19	Sample 4 (16x) Rep 2	Kiwifruit pollen	100	32.1	Weak positive	32.14										
20	Sample 4 (16x) Rep 3	Kiwifruit pollen	100	31	Weak positive	30.97										
21	Sample 4 (16x) Rep 4	Kiwifruit pollen	100	31.7	Weak positive	31.69	Mean	1 x sd	%cv							
22	Sample 4 (16x) Rep 5	Kiwifruit pollen	100	31.5	Weak positive	31.48	31.58	0.42	1.33							
23	Sample 5 (32x) Rep 1	Kiwifruit pollen	100	33.7	Weak positive	33.70										
24	Sample 5 (32x) Rep 2	Kiwifruit pollen	100	34.4	Weak positive	34.38										
25	Sample 5 (32x) Rep 3	Kiwifruit pollen	100	33.2	Weak positive	33.22										
26	Sample 5 (32x) Rep 4	Kiwifruit pollen	100	33.3	Weak positive	33.32	Mean	1 x sd	%cv							
27	Sample 5 (32x) Rep 5	Kiwifruit pollen	100	30.4	Weak positive	30.41	33.01	1.52	4.61							
28	Sample 6 (64x) Rep 1	Kiwifruit pollen	100	33.2	Weak positive	33.17										
29	Sample 6 (64x) Rep 2	Kiwifruit pollen	100	35.3	Weak positive	35.28										
30	Sample 6 (64x) Rep 3	Kiwifruit pollen	100	34.9	Weak positive	34.92										
31	Sample 6 (64x) Rep 4	Kiwifruit pollen	100	33.1	Weak positive	33.10	Mean	1 x sd	%cv							
32	Sample 6 (64x) Rep 5	Kiwifruit pollen	100	35.7	Not Detected	*	34.11	1.14	3.35							
33	Sample 7 (128x) Rep 1	Kiwifruit pollen	100	33.6	Weak positive	33.55										
34	Sample 7 (128x) Rep 2	Kiwifruit pollen	100	32.9	Weak positive	32.94										
35	Sample 7 (128x) Rep 3	Kiwifruit pollen	100	35	Not Detected	*										
36	Sample 7 (128x) Rep 4	Kiwifruit pollen	100	33.3	Weak positive	33.26	Mean	1 x sd	%cv							
37	Sample 7 (128x) Rep 5	Kiwifruit pollen	100	33.5	Weak positive	33.53	33.32	0.29	0.86							
38	Sample 8 (256x) Rep 1	Kiwifruit pollen	100	33.3	Not Detected	*										
39	Sample 8 (256x) Rep 2	Kiwifruit pollen	100	33.6	Weak positive	33.59										
40	Sample 8 (256x) Rep 3	Kiwifruit pollen	100	34.6	Not Detected	*										
41	Sample 8 (256x) Rep 4	Kiwifruit pollen	100	31.4	Weak positive	31.38	Mean	1 x sd	%cv							
42	Sample 8 (256x) Rep 5	Kiwifruit pollen	100	34.2	Not Detected	*	32.48	1.56	4.81							
43	Sample 9 (512x) Rep 1	Kiwifruit pollen	100	34.2	Not Detected	*										
44	Sample 9 (512x) Rep 2	Kiwifruit pollen	100	33.8	Not Detected	*										
45	Sample 9 (512x) Rep 3	Kiwifruit pollen	100	33.9	Not Detected	*										
46	Sample 9 (512x) Rep 4	Kiwifruit pollen	100	33	Not Detected	*										
47	Sample 9 (512x) Rep 5	Kiwifruit pollen	100	33.5	Not Detected	*										
48	Sample 10 (1024x) Rep 1	Kiwifruit pollen	100	40.9	Not Detected	*										
49	Sample 10 (1024x) Rep 2	Kiwifruit pollen	100	0	Not Detected	*										
50	Sample 10 (1024x) Rep 3	Kiwifruit pollen	100	35.4	Weak positive	35.374542										
51	Sample 10 (1024x) Rep 4	Kiwifruit pollen	100	43	Not Detected	*										
52	Sample 10 (1024x) Rep 5	Kiwifruit pollen	1000	37.2	Not Detected	*										

5.3 Appendix 2b Sampling Protocols and Testing Frequency of Commercial Batches of Pollen Data

Laboratory Job Number:	958621								
Date Registered:	3/12/15 9:58	Thrity Replicates of 'contaminated' and 'uncontaminated' pollen							
File Creation Date:	21/12/15 10:23								
Quote Number:	47284								
Sample Number	**Sample Name**	**Sample Type**	**PCR dilution**	**Cq Value**	**Psa Result**	**Cq value**			
53	2100000032624 Rep 1 (958621.1)	Kiwifruit pollen	100	28.2	Positive	28.183082			
54	2100000032624 Rep 2 (958621.1)	Kiwifruit pollen	100	27.3	Positive	27.334353			
55	2100000032624 Rep 3 (958621.1)	Kiwifruit pollen	100	28.3	Positive	28.2852742			
56	2100000032624 Rep 4 (958621.1)	Kiwifruit pollen	100	27.9	Positive	27.929382			
57	2100000032624 Rep 5 (958621.1)	Kiwifruit pollen	100	27.7	Positive	27.7066767			
58	2100000032624 Rep 6 (958621.1)	Kiwifruit pollen	100	28.2	Positive	28.1727549			
59	2100000032624 Rep 7 (958621.1)	Kiwifruit pollen	100	27.7	Positive	27.7443593			
60	2100000032624 Rep 8 (958621.1)	Kiwifruit pollen	100	27.7	Positive	27.6809293			
61	2100000032624 Rep 9 (958621.1)	Kiwifruit pollen	100	27.9	Positive	27.8955824			
62	2100000032624 Rep 10 (958621.1)	Kiwifruit pollen	100	27.9	Positive	27.9283042			
63	2100000032624 Rep 11 (958621.1)	Kiwifruit pollen	100	28	Positive	28.0445746			
64	2100000032624 Rep 12 (958621.1)	Kiwifruit pollen	100	27.7	Positive	27.7485453			
65	2100000032624 Rep 13 (958621.1)	Kiwifruit pollen	100	27.7	Positive	27.7285732			
66	2100000032624 Rep 14 (958621.1)	Kiwifruit pollen	100	28.4	Positive	28.3844564			
67	2100000032624 Rep 15 (958621.1)	Kiwifruit pollen	100	26.3	Positive	26.3318481			
68	2100000032624 Rep 16 (958621.1)	Kiwifruit pollen	100	28	Positive	28.0010879			
69	2100000032624 Rep 17 (958621.1)	Kiwifruit pollen	100	27.9	Positive	27.8741384			
70	2100000032624 Rep 18 (958621.1)	Kiwifruit pollen	100	27.6	Positive	27.5556029			
71	2100000032624 Rep 19 (958621.1)	Kiwifruit pollen	100	27.7	Positive	27.6766631			
72	2100000032624 Rep 20 (958621.1)	Kiwifruit pollen	100	28.6	Positive	28.603266			
73	2100000032624 Rep 21 (958621.1)	Kiwifruit pollen	100	28.4	Positive	28.3896857			
74	2100000032624 Rep 22 (958621.1)	Kiwifruit pollen	100	29.5	Positive	29.462256			
75	2100000032624 Rep 23 (958621.1)	Kiwifruit pollen	100	28.2	Positive	28.1596441			
76	2100000032624 Rep 24 (958621.1)	Kiwifruit pollen	100	28.5	Positive	28.4875949			
77	2100000032624 Rep 25 (958621.1)	Kiwifruit pollen	100	28.4	Positive	28.4269829			
78	2100000032624 Rep 26 (958621.1)	Kiwifruit pollen	100	29.2	Positive	29.1817951			
79	2100000032624 Rep 27 (958621.1)	Kiwifruit pollen	100	28.2	Positive	28.1525648			
80	2100000032624 Rep 28 (958621.1)	Kiwifruit pollen	100	28.8	Positive	28.7729595			
81	2100000032624 Rep 29 (958621.1)	Kiwifruit pollen	100	27.5	Positive	27.537556	Mean	1 x sd	%cv
82	2100000032624 Rep 30 (958621.1)	Kiwifruit pollen	100	28.4	Positive	28.3679826	28.06	0.58	2.05
83	2100000032594 Rep 1 (958621.2)	Kiwifruit pollen	100	0		*			
84	2100000032594 Rep 2 (958621.2)	Kiwifruit pollen	100	38.4	Not Detected	38.4265963			
85	2100000032594 Rep 3 (958621.2)	Kiwifruit pollen	100	39.3	Not Detected	39.2530744			
86	2100000032594 Rep 4 (958621.2)	Kiwifruit pollen	100	40.7	Not Detected	40.6860675			
87	2100000032594 Rep 5 (958621.2)	Kiwifruit pollen	100	40.5	Not Detected	40.5431086			
88	2100000032594 Rep 6 (958621.2)	Kiwifruit pollen	100	42	Not Detected	42.0016609			
89	2100000032594 Rep 7 (958621.2)	Kiwifruit pollen	100	41.9	Not Detected	41.9495383			
90	2100000032594 Rep 8 (958621.2)	Kiwifruit pollen	100	42.2	Not Detected	42.2466826			
91	2100000032594 Rep 9 (958621.2)	Kiwifruit pollen	100	0	Not Detected	*			
92	2100000032594 Rep 10 (958621.2)	Kiwifruit pollen	100	44.4	Not Detected	44.3819811			
93	2100000032594 Rep 11 (958621.2)	Kiwifruit pollen	100	0	Not Detected	*			
94	2100000032594 Rep 12 (958621.2)	Kiwifruit pollen	100	42.2	Not Detected	42.2463121			
95	2100000032594 Rep 13 (958621.2)	Kiwifruit pollen	100	42.6	Not Detected	42.5987837			
96	2100000032594 Rep 14 (958621.2)	Kiwifruit pollen	100	43.2	Not Detected	43.2348427			
97	2100000032594 Rep 15 (958621.2)	Kiwifruit pollen	100	39	Not Detected	38.9910001			
98	2100000032594 Rep 16 (958621.2)	Kiwifruit pollen	100	38.4	Not Detected	38.3521746			
99	2100000032594 Rep 17 (958621.2)	Kiwifruit pollen	100	43	Not Detected	42.9606412			
100	2100000032594 Rep 18 (958621.2)	Kiwifruit pollen	100	0	Not Detected	*			
101	2100000032594 Rep 19 (958621.2)	Kiwifruit pollen	100	0	Not Detected	*			
102	2100000032594 Rep 20 (958621.2)	Kiwifruit pollen	100	43	Not Detected	43.0006211			
103	2100000032594 Rep 21 (958621.2)	Kiwifruit pollen	100	34.7	Not Detected	34.7482785			
104	2100000032594 Rep 22 (958621.2)	Kiwifruit pollen	100	39.7	Not Detected	39.7367931			
105	2100000032594 Rep 23 (958621.2)	Kiwifruit pollen	100	36.5	Not Detected	36.5155182			
106	2100000032594 Rep 24 (958621.2)	Kiwifruit pollen	100	42.4	Not Detected	42.3696801			
107	2100000032594 Rep 25 (958621.2)	Kiwifruit pollen	100	40.3	Not Detected	40.3267911			
108	2100000032594 Rep 26 (958621.2)	Kiwifruit pollen	100	37.8	Not Detected	37.84135			
109	2100000032594 Rep 27 (958621.2)	Kiwifruit pollen	100	37.3	Not Detected	37.288227			
110	2100000032594 Rep 28 (958621.2)	Kiwifruit pollen	100	39	Not Detected	39.0058752			
111	2100000032594 Rep 29 (958621.2)	Kiwifruit pollen	100	37.3	Not Detected	37.3048125	Mean	1 x sd	%cv
112	2100000032594 Rep 30 (958621.2)	Kiwifruit pollen	100	38	Not Detected	38.0198105	40.16	2.49	6.20

5.4 Appendix 3a Determination of apparent Psa colony forming units (cfu) in saline wash samples data

Cq Values							
		Direct PCR			Saline Wash PCR Results		
Number of cfu/mL	Number of cfu/0.1 mL tested	Sample Cq	Duplicate Sample Cq	Direct	Sample Cq	Duplicate Sample Cq	Saline Wash
2.63E+00	2.63E-01	35.16	35.35	35.25*	42.95	44.37	43.66*
2.63E+01	2.63E-01	37.25	36.66	36.95*	39.22	39.04	39.13*
2.63E+02	2.63E+01	36.26	36.17	36.21*	42.03	43.41	42.71*
2.63E+03	2.63E+02	37.42	36.80	37.10*	-	44.82	44.81*
2.63E+04	2.63E+03	31.55	31.31	31.43	36.53	-	36.53
2.63E+05	2.63E+04	28.41	28.86	28.63	31.52	31.37	31.44
2.63E+06	2.63E+05	25.23	25.58	25.41	28.13	27.60	27.87
2.63E+07	2.63E+06	22.13	22.34	22.24	24.77	24.94	24.85
2.63E+08	2.63E+07	19.09	19.26	19.18	20.95	20.67	20.81
2.63E+09	2.63E+08	18.18	16.62	17.40	18.96	19.54	19.25
* Samples did not give the characteristic melt curve for Psa-V so were omitted from the calibration curve							

5.5 Appendix 3b Comparison of Primers set testing serial dilutions of 2.63E+09 cfu, then DNA extraction

83/84/85 Primer Set Cq Values

			Direct PCR Results			Saline Wash PCR Results		
Dilution	Number of cfu/mL	Number of cfu/0.1 mL tested	Sample Cq	Duplicate Sample Cq	Average	Sample Cq	Duplicate Sample Cq	Average
1.00E-09	2.63E+00	2.63E-01						
1.00E-08	2.63E+01	2.63E+00						
1.00E-07	2.63E+02	2.63E+01						
1.00E-06	2.63E+03	2.63E+02						
1.00E-05	2.63E+04	2.63E+03	31.55	31.31	31.43	36.53		36.53
1.00E-04	2.63E+05	2.63E+04	28.41	28.86	28.63	31.52	31.37	31.44
1.00E-03	2.63E+06	2.63E+05	25.23	25.58	25.41	28.13	27.60	27.87
1.00E-02	2.63E+07	2.63E+06	22.13	22.34	22.24	24.77	24.94	24.85
1.00E-01	2.63E+08	2.63E+07	19.09	19.26	19.18	20.95	20.67	20.81
1.00E+00	2.63E+09	2.63E+08	18.18	16.62	17.40	18.96	19.54	19.25

F1/R2 Cq Values

			Direct PCR Results			Saline Wash PCR Results		
Dilution	Number of cfu/mL	Number of cfu/0.1 mL tested	Sample Cq	Duplicate Sample Cq	Average	Sample Cq	Duplicate Sample Cq	Average
1.00E-09	2.63E+00	2.63E-01	-	-		-	-	
1.00E-08	2.63E+01	2.63E+00	-	-		-	-	
1.00E-07	2.63E+02	2.63E+01	-	-		-	-	
1.00E-06	2.63E+03	2.63E+02	30.08	30.85	30.47	-	-	
1.00E-05	2.63E+04	2.63E+03	28.03	28.56	28.30	30.15	29.31	29.73
1.00E-04	2.63E+05	2.63E+04	24.58	24.49	24.53	26.32	25.84	26.08
1.00E-03	2.63E+06	2.63E+05	21.11	20.81	20.96	23.26	21.83	22.55
1.00E-02	2.63E+07	2.63E+06	18.19	17.96	18.08	21.27	20.33	20.80
1.00E-01	2.63E+08	2.63E+07	15.20	15.19	15.19	15.52	15.98	15.75
1.00E+00	2.63E+09	2.63E+08	14.03	14.54	14.29	13.22	13.69	13.45

5.6 Appendix 4a Reduction of Psa-V on flowers and anthers by spraying data

Laboratory Job Number	958664	Objective 3 Sprayed	KPIN and PSA Status			Orchard location						
			KPIN	Psa Test result	Isolate Type	Orchard Address	Town	Region	Priority Zone or HRA	Variety HW / Ha	Long	Lat
Date Registered:	2/12/11 10:26											
File Creation Date:	12/12/11 12:43		5111	Positive	Psa-V	849 No1 Rd	Te Puke	TE PUKE	Te Puke Priority Zone	1.61	176.2935	-37.8546
Quote Number:	47283											

Sample Number	Sample Name	Sample Type	Dilution Factor	Cq Value	Result	Test Comments	Flower Weight	Saline (mL)
1	2100000027125	Bacterological saline washings	10	23.6	Positive		175	350
2	2100000027156	Bacterological saline washings	10	23.3	Positive		142	284
3	2100000027187	Bacterological saline washings	10	23.4	Positive		71	142
4	2100000027217	Bacterological saline washings	10	25.2	Positive		193	386
5	2100000027248	Bacterological saline washings	10	27	Positive		98	196
6	2100000027279	Bacterological saline washings	10	23.4	Positive		160	320
7	2100000027309	Bacterological saline washings	10	25.4	Positive		66	132
8	2100000027330	Bacterological saline washings	10	22.2	Positive		108	216
9	2100000027361	Bacterological saline washings	10	24.9	Positive		245	490
10	2100000027392	Bacterological saline washings	10	26.3	Positive		73	146
11	2100000027422	Bacterological saline washings	10	25.1	Positive		148	296
12	2100000027453	Bacterological saline washings	10	22.5	Positive		192	384
13	2100000027484	Bacterological saline washings	10	23.1	Positive		95	190
14	2100000027514	Bacterological saline washings	10	25.2	Positive		157	314
15	2100000027545	Bacterological saline washings	10	23.1	Positive		98	196
16	2100000027576	Bacterological saline washings	10	23.6	Positive		123	246
17	2100000027606	Bacterological saline washings	10	24.1	Positive		118	236
18	2100000027637	Bacterological saline washings	10	25.2	Positive		65	130
19	2100000027668	Bacterological saline washings	10	26.2	Positive		92	184
20	2100000027699	Bacterological saline washings	10	25.2	Positive		25	50
21	2100000027729	Bacterological saline washings	10	24.5	Positive		195	390
22	2100000027750	Bacterological saline washings	10	24.2	Positive		179	358
23	2100000027781	Bacterological saline washings	10	25.2	Positive		154	308
24	2100000027811	Bacterological saline washings	10	22.1	Positive		153	306
25	2100000027842	Bacterological saline washings	10	25.2	Positive		29	58
26	2100000027873	Bacterological saline washings	10	24	Positive		196	392
27	2100000027903	Bacterological saline washings	10	26.8	Positive		147	294
28	2100000027934	Bacterological saline washings	10	25.3	Positive		142	284
29	2100000027965	Bacterological saline washings	10	25.1	Positive		205	410
30	2100000027996	Bacterological saline washings	10	24.2	Positive		212	424
31	2100000028924	Kiwifruit Flowers / Buds	10	31.7	Weak positive		175	
32	2100000028955	Kiwifruit Flowers / Buds	10	30.4	Weak positive		142	
33	2100000028986	Kiwifruit Flowers / Buds	10	28.3	Positive		71	
34	2100000029013	Kiwifruit Flowers / Buds	10	27.9	Positive		193	
35	2100000029044	Kiwifruit Flowers / Buds	10	28.8	Positive		98	
36	2100000029075	Kiwifruit Flowers / Buds	10	31	Weak positive		160	
37	2100000029105	Kiwifruit Flowers / Buds	10	29	Positive		66	
38	2100000029136	Kiwifruit Flowers / Buds	10	31	Weak positive		108	
39	2100000029167	Kiwifruit Flowers / Buds	10	*	Not Detected		245	
40	2100000029198	Kiwifruit Flowers / Buds	10	28.4	Positive		73	
41	2100000029228	Kiwifruit Flowers / Buds	10	*	Not Detected		148	
42	2100000029259	Kiwifruit Flowers / Buds	10	31.3	Weak positive		192	
43	2100000029280	Kiwifruit Flowers / Buds	10	26.3	Positive		95	
44	2100000029310	Kiwifruit Flowers / Buds	10	28.8	Positive		157	
45	2100000029341	Kiwifruit Flowers / Buds	10	30.4	Weak positive		98	
46	2100000029822	Kiwifruit Flowers / Buds	10	27.6	Positive		123	
47	2100000029853	Kiwifruit Flowers / Buds	10	27.1	Positive		118	
48	2100000029884	Kiwifruit Flowers / Buds	10	29.9	Positive		65	
49	2100000029914	Kiwifruit Flowers / Buds	10	28.7	Positive		92	
50	2100000029945	Kiwifruit Flowers / Buds	10	26.2	Positive	Only 3 g of sample	25	
51	2100000029976	Kiwifruit Flowers / Buds	10	28.3	Positive		195	
52	2100000030002	Kiwifruit Flowers / Buds	10	29.2	Positive		179	
53	2100000030033	Kiwifruit Flowers / Buds	10	24.1	Positive		154	
54	2100000030095	Kiwifruit Flowers / Buds	10	26.7	Positive	Only 3 g of sample	153	
55	2100000030125	Kiwifruit Flowers / Buds	10	27.4	Positive		29	
56	2100000030064	Kiwifruit Flowers / Buds	10	29.8	Positive		196	
57	2100000030156	Kiwifruit Flowers / Buds	10	31.4	Weak positive		147	
58	2100000030187	Kiwifruit Flowers / Buds	10	31.6	Weak positive		142	
59	2100000030217	Kiwifruit Flowers / Buds	10	28	Positive		205	
60	2100000030248	Kiwifruit Flowers / Buds	10	*	Not Detected		212	

Buds	Positives/Weak Positive	ND	
	30	0	
	Mean	sd	%cv
	24.5	1.3	5.3

Anthers	Positives/Weak Positive	ND	
	27	3	
	Mean	sd	%cv
	28.9	1.9	6.6

5.7 Appendix 4b Reduction of Psa-V on flowers and anthers by spraying data

			KPIN and PSA Status									
			KPIN		Psa Test result	Isolate Type	Orchard Address	Region	Priority Zone or HRA	Variety HW / Hg	Long	Lat
Laboratory Job Number	958716	Objective 3. Non-sprayed										
Quote Number:	47283		5111		Positive	Psa-V	849 No1 Rd	TE PUKE	Te Puke Priority Zone	1.61	176.2935	-37.8546
Sample Number	Sample Name	Sample Type	Dilution Factor	Result Text			Flower Weight	Saline (mL)				
1	2100000028016	Bacteriological saline washings	10	23.3	Positive		165	330				
2	2100000028047	Bacteriological saline washings	10	25.2	Positive		109	218				
3	2100000028078	Bacteriological saline washings	10	25.3	Positive		129	258				
4	2100000028108	Bacteriological saline washings	10	23	Positive		189	378				
5	2100000028139	Bacteriological saline washings	10	23.2	Positive		231	462				
6	2100000028177	Bacteriological saline washings	10	19.81	Positive		169	338				
7	2100000028207	Bacteriological saline washings	10	20.7	Positive		224	448				
8	2100000028238	Bacteriological saline washings	10	20.1	Positive		245	490				
9	2100000028269	Bacteriological saline washings	10	20.6	Positive		241	482				
10	2100000028290	Bacteriological saline washings	10	24.1	Positive		114	228				
11	2100000028320	Bacteriological saline washings	10	26	Positive		76	152				
12	2100000028351	Bacteriological saline washings	10	23.7	Positive		192	384				
13	2100000028382	Bacteriological saline washings	10	25.1	Positive		168	336				
14	2100000028412	Bacteriological saline washings	10	24.5	Positive		118	236				
15	2100000028443	Bacteriological saline washings	10	21.6	Positive		212	424				
16	2100000028474	Bacteriological saline washings	10	22.5	Positive		104	208				
17	2100000028504	Bacteriological saline washings	10	22.4	Positive		167	334				
18	2100000028535	Bacteriological saline washings	10	20.2	Positive		181	362				
19	2100000028566	Bacteriological saline washings	10	17.66	Positive		264	528				
20	2100000028597	Bacteriological saline washings	10	23.3	Positive		176	352				
21	2100000028627	Bacteriological saline washings	10	20.8	Positive		168	336				
22	2100000028658	Bacteriological saline washings	10	25.8	Positive		218	436				
23	2100000028689	Bacteriological saline washings	10	24.3	Positive		185	370				
24	2100000028719	Bacteriological saline washings	10	21	Positive		104	208				
25	2100000028740	Bacteriological saline washings	10	20.4	Positive		163	326				
26	2100000028771	Bacteriological saline washings	10	25.1	Positive		244	488				
27	2100000028801	Bacteriological saline washings	10	25.3	Positive		220	440				
28	2100000028832	Bacteriological saline washings	10	22.9	Positive		214	428				
29	2100000028863	Bacteriological saline washings	10	24.2	Positive		171	342				
30	2100000028894	Bacteriological saline washings	10	24.1	Positive		220	440				
31	2100000029372	Kiwifruit Flowers / Buds	10	24.1	Positive		165					
32	2100000029402	Kiwifruit Flowers / Buds	10	24.5	Positive		109					
33	2100000029433	Kiwifruit Flowers / Buds	10	28.4	Positive		129					
34	2100000029464	Kiwifruit Flowers / Buds	10	29.7	Positive		189					
35	2100000029495	Kiwifruit Flowers / Buds	10	30.5	Weak positive		231					
36	2100000029525	Kiwifruit Flowers / Buds	10	32.2	Weak positive		169					
37	2100000029556	Kiwifruit Flowers / Buds	10	28.6	Positive		224					
38	2100000029587	Kiwifruit Flowers / Buds	10	36.7	Weak positive		245					
39	2100000029617	Kiwifruit Flowers / Buds	10	30.4	Weak positive		241					
40	2100000029648	Kiwifruit Flowers / Buds	10	26.8	Positive		114					
41	2100000029679	Kiwifruit Flowers / Buds	10	25.1	Positive		76					
42	2100000029709	Kiwifruit Flowers / Buds	10	28.9	Positive		192					
43	2100000029730	Kiwifruit Flowers / Buds	10	28.8	Positive		168					
44	2100000029761	Kiwifruit Flowers / Buds	10	25	Positive		118					
45	2100000029792	Kiwifruit Flowers / Buds	10	30.3	Weak positive		212					
46	2100000030279	Kiwifruit Flowers / Buds	10	25.2	Positive		104					
47	2100000030309	Kiwifruit Flowers / Buds	10	25.2	Positive		167					
48	2100000030330	Kiwifruit Flowers / Buds	10	29.6	Positive		181					
49	2100000030361	Kiwifruit Flowers / Buds	10	24	Positive		264					
50	2100000030392	Kiwifruit Flowers / Buds	10	29.6	Positive		176					
51	2100000030422	Kiwifruit Flowers / Buds	10	28.3	Positive		168					
52	2100000030453	Kiwifruit Flowers / Buds	10	*	Not Detected		218					
53	2100000030484	Kiwifruit Flowers / Buds	10	*	Not Detected		185					
54	2100000030514	Kiwifruit Flowers / Buds	10	*	Not Detected		104					
55	2100000030545	Kiwifruit Flowers / Buds	10	27.4	Positive		163					
56	2100000030576	Kiwifruit Flowers / Buds	10	31.4	Weak positive		244					
57	2100000030606	Kiwifruit Flowers / Buds	10	27.6	Positive		220					
58	2100000030637	Kiwifruit Flowers / Buds	10	29.2	Positive		214					
59	2100000030668	Kiwifruit Flowers / Buds	10	30	Positive		171					
60	2100000030699	Kiwifruit Flowers / Buds	10	28.8	Positive		220					

Buds	Mean	sd	%cv
	22.9	2.1	9.4
Anthers	Positives/Weak Positive	ND	
	27	3	
	Mean	sd	%cv
	28.4	2.9	10.1

Summary Statistics

	Unsprayed Vines		Sprayed Vines	
	Flower bud	Anthers	Flower bud	Anthers
Positive (n)	30	27	30	27
Not Detected (n)	0	3	0	3
Mean Cq of Positives	22.9	28.4	24.5	28.9
SD of Positives	2.1	2.9	1.3	1.9
% CV of Positives	9.4	10.1	5.3	6.6
~ cfu load (per g)	2.0e 08	1.8e 07	1.0e 08	6.0e 06

5.8 Appendix 5 Risk of Psa-V contamination of pollen harvested according to the KVH Pollen Production Best-practice Pollen Source Guideline

956728	Objective 4				
26-Nov-11					
47168		qPCR		Psa	
Sample Name	**Sample Type**	**Dilution Factor**	**Cq Value**	**Result**	**Test Comments**
2100000023479	Bacterological saline washings	10	34.8	Not Detected	Weak positive for LV
2100000023509	Bacterological saline washings	10	35.6	Not Detected	
2100000023530	Bacterological saline washings	10	34.2	Not Detected	
2100000023561	Bacterological saline washings	10	37.1	Not Detected	
2100000023592	Bacterological saline washings	10	32.8	Not Detected	
2100000023622	Bacterological saline washings	10	33.3	Not Detected	
2100000023653	Bacterological saline washings	10	33.5	Not Detected	
2100000023684	Bacterological saline washings	10	33.7	Not Detected	
2100000023714	Bacterological saline washings	10	33.1	Not Detected	
2100000023745	Bacterological saline washings	10	35.5	Not Detected	
2100000023776	Bacterological saline washings	10	34.3	Not Detected	
2100000023806	Bacterological saline washings	10	34	Not Detected	
2100000023837	Bacterological saline washings	10	36.7	Not Detected	
2100000023868	Bacterological saline washings	10	35.3	Not Detected	
2100000023899	Bacterological saline washings	10	38	Not Detected	
2100000023929	Bacterological saline washings	10	36.8	Not Detected	
2100000023950	Bacterological saline washings	10	31.9	Not Detected	
2100000023981	Bacterological saline washings	100	35	Not Detected	Note different dilution
2100000024018	Bacterological saline washings	10	30.1	Not Detected	
958618					
2100000032471	Bacterological saline washings	10	33.3	Not Detected	Weak Positive for LV
2100000032501	Bacterological saline washings	10	32	Not Detected	
2100000032532	Bacterological saline washings	10	33.6	Not Detected	
2100000032563	Bacterological saline washings	10	35.1	Not Detected	