Psa – Pathway tracing report

Response title: Psa Kiwifruit 2010-348

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Ministry of Agriculture and Forestry
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BIOSECURITY NEW ZEALAND

NEW ZEALAND FOOD SAFETY AUTHORITY

INTRODUCTION

The first validated report of *Pseudomonas syringae actinidiae* (Psa) in New Zealand (subsequently confirmed as the more virulent form Psa V) was made in an orchard in Te Puke, on 5th November 2010. Since that time the disease has spread rapidly throughout the Te Puke region (to an extent that currently 773 orchards or 75% of all orchards in Te Puke are now infected)

The disease has also seen progressive radial spread outwards around the Bay of Plenty to other growing regions in Tauranga (first identified on 9th August), Waihi (12th September),Katikati (27th Sept), Whakatane (29th Sept), Opotoki (20th October) and across to Auckland (being identified in the Franklin growing region on 18th November). Currently the number of orchards in New Zealand confirmed as being infected with Psa V stands at 927.

Pathway Tracing Report

A pathway tracing report is prepared by MAF when there is a need to better understand how and when a new animal or plant pest reached New Zealand. The report is primarily a technical document that analyses information about the arrival, spread, biology and impacts of a new pest.

The report identifies potential pathways by which the pest reached New Zealand and assesses the risks of the different pathways

Sometimes it will be obvious how and when a pest or disease arrived in New Zealand, unfortunately in the case of Psa this is not the case. More often, however, there will be a range of possible pathways identified, information about the pest and its movements will be incomplete, and the presence or absence of the pest in other countries may be uncertain. In these cases the report can only assess the most probable pathways and give an assessment of the likelihood of each of these being the actual pathway

The pathway tracing report assesses three aspects of the possible pathways:

- Location – was the pathway or event linked to the location of the areas where the pest was initially found?
- Timeline – does the date of the finding indicate an association with particular pathways or events?
- Plausibility – is there scientific evidence that the pest or disease can be transmitted by the pathway? Alternatively, can similar or related organisms be spread by the pathway or event?

Analysis of these three can rule some pathways in and others out. It can also provide the information that enables the biosecurity risks around different pathways to be assessed and compared.

SUMMARY OF THE PSA PATHWAY TRACING REPORT

When did Psa V arrive in New Zealand and where did it come from?

The analysis undertaken by MAF indicates that it is most likely that Psa V arrived in New Zealand no more than 18 months before the first symptoms were observed on kiwifruit in October 2010. The initial infection probably arose from a single point of introduction at or close to the area where the first infected vines were identified. Psa V could have arrived in New Zealand from any of the European countries where it is found, or alternatively, from another country where it is present but this presence has yet to be confirmed.

1. Interviews with kiwifruit orchard staff indicated that the Psa V symptoms of heavy leaf spotting had been observed in two orchards around 10 and 23 October 2010. Under the mild and humid weather conditions that are found round Te Puke, Psa-V has spread rapidly between vines and orchards and in some orchards the disease has progressed quickly from initial leaf spotting to leaf and shoot collapse and death. In some cases the progression from primary to secondary disease symptoms has happened in only three to four weeks, though the average time is15 weeks.

2. The symptoms of Psa V are obvious, and growers and orchard staff working in and around orchards would notice them relatively easily. It is unlikely that Psa-V had been in gold orchards in the affected area for an extended period prior to its detection in October 2010.

3. The pattern and timing of spread from the sites where Psa V was initially found also suggest that the disease arose from a single point of introduction. It spread from this site by natural means (wind and rain) to adjacent orchards, and by the actions of people more widely. Human induced spread could include movement of kiwifruit cuttings or plant material, equipment movements or bacterial contamination on clothing or footwear.

4. The virulent form of Psa, known as Psa V, is known to be in Italy and believed to have infected orchards across other countries in Europe (Spain, France, Switzerland, Portugal). Gold kiwifruit orchards in Italy have been seriously affected by Psa-V. The haplotype, or form, found in New Zealand is genetically similar to the European haplotype. The form of Psa that has caused some damage in Japan and Korea is genetically distinct from the New Zealand Psa V. A lower virulence haplotype, Psa-LV, has also been found in New Zealand and Australia. Chile and China are reported to have Psa but the haplotype has not yet been confirmed. China is the native origin of kiwifruit and it has a diverse range of kiwifruit species and varieties growing in cultivation and in the wild.

5. Psa V could have arrived in New Zealand from any of the countries where it is confirmed, or alternatively, from another source where it is present but not yet confirmed. Genetic studies of Psa V in different countries might, in time, confirm the sources of the infection and help confirm the likely pathway or mechanism by which Psa V reached New Zealand.

Assessment of different entry pathways

6. The following potential entry pathways have been assessed or investigated:
    - Imported kiwifruit pollen and pollen trials;
    - Imported kiwifruit plant material – budwood and tissue culture;
    - Imported kiwifruit plant material – seed;
    - Imported kiwifruit plant material – fruit
    - Illegal importation of plant material;
    - Importation of orchard equipment (including pollen related);
    - People movements;
    - Research.

The findings from the Psa pathway tracing report on each of these pathways is summarised below.

Imported kiwifruit pollen and pollen trials

> *Whilst MAF has assessed the overall risk from imported kiwifruit pollen and from pollen trials as uncertain but probably low based on current information, we can not rule it out. Further information about the viability of Psa V associated with pollen and about the presence or absence of Psa V in other countries would assist any future reassessment of risk from these pathways.*

7. The pollination of kiwifruit in commercial orchards is undertaken in two primary ways. In some orchards beehives are placed in the orchards during the spring flowering of male and female vines. The hives are removed after flowering and returned to honey production by the apiarists.

8. In other orchards pollen is commercially sourced and blown over the open female flowers using machine blowers. This pollen is sourced from male flowers and is milled or processed to separate the pollen from the flowers and then stored for use. This pollen has been sourced from New Zealand orchards and has also been imported.

9. While the DNA from Psa has been detected by PCR (polymerase chain reaction) in pollen samples, these tests do not provide information on the viability of the bacteria or its potential for transmission.

10. Extensive investigation was undertaken of the potential pollen pathway including interviews with stakeholders and pollen importers, consideration of pollen import and export records and testing of some imported pollen samples. While pollen would initially appear to be a potential pathway for both the initial incursion and subsequent spread, the very short period between the reported use of pollen and the noticing of disease symptoms on vines suggests that this was not the source of the initial incursion.

11. There have been some inconsistencies and uncertainties in information received by MAF about imported pollen and its use in New Zealand. Uncertainty also remains over whether viable Psa V could survive on milled and stored pollen in

sufficient quantity or virulence, to initiate disease in vines after pollination and thus confirm pollen as a viable pathway for Psa V.

12. However, the overall risk from commercial pollen imports has been assessed as uncertain but probably low based on current information.

13. Trials of artificial pollination techniques were undertaken on three orchards in 2006 and 2009. The tests were of pollination techniques and pollination equipment. These trials were reported as involving only New Zealand sourced pollen and equipment only used in New Zealand. None of the 2009 trial orchards has reported infected vines at August 2011 and the 2006 trials were undertaken before pollen was imported for commercial pollination. They were also well outside the likely window of entry of Psa V to New Zealand. The risk from these trials has also been assessed as uncertain but probably low based on current information.

Imported kiwifruit plant material – budwood and tissue culture

MAF assesses the risk from imported plant material as negligible, noting that it is a possible pathway but unlikely due to inspection, testing and other controls on this pathway.

14. Host plant material is generally considered to be the highest risk for the long distance spread and establishment of new pests and diseases. Consequently there are strict provisions required by the import health standard for the import of dormant cuttings and tissue culture samples of kiwifruit species. Both require importation and growth in Level 3 Post Entry Quarantine (PEQ) facilities.

15. The plants are now grown for a minimum of six months in quarantine and inspected and tested for virus diseases and Psa. A 2001 import pre-dated these requirements, but was tested for viruses (but not Psa) while held in PEQ for 7 years. It was also imported considerably before Psa V was first detected overseas. Some of the plants released from quarantine have been traced and inspected. No Psa V symptoms were observed on any of these plants and Psa V was not detected in leaf tissues that were sampled. Some of the plants that have been released from quarantine are being grown in areas which are still free of Psa V.

Imported kiwifruit plant material – seed

MAF assesses the risk from imported kiwifruit seed as negligible as seed is not a known vector, seed is quarantined and grown before release, and the origins of imported seed do not match known Psa sources.

16. As with plant material, there are strict provisions required by the import health standard for the import of kiwifruit seeds. Seeds have to have a phytosanitary certificate and are imported into a Level 3 PEQ facility where they are germinated and grown for a minimum of six months. The plants are tested for viruses and inspected for pest and disease symptoms. There is no specific requirement to test for Psa. Only healthy plants can be released from quarantine. The imported seeds themselves cannot be released from quarantine

17. Although seeds are not a known vector of Psa, they cannot be released from quarantine without being grown into plants and tested. Although this testing is for viruses and not Psa, if any were infected with Psa, the symptoms would be seen on seedlings as they grew in PEQ.

Imported kiwifruit plant material – fruit

MAF assesses the pathway risk from fruit to be negligible as fruit is not a known pathway for Psa

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18. Green kiwifruit can be imported from Italy and the USA under an import health standard. The IHS, was first approved in 1999 and was reviewed in 2008 and 2010. No changes were made as there was no new scientific evidence about the risk from mature fruit as a pathway. Since 2000, kiwifruit imports to New Zealand have totalled 6,700 tonnes from Italy and 300 tonnes from the United States.

19. International consensus is that there is no scientific evidence that mature fruit is a pathway for the entry, establishment and spread of the disease. A visiting scientist recently stated that DNA from Psa can be detected in otherwise healthy fruit. This report is yet to be published, is not supported by validated scientific papers and does not yet confirm the presence of viable bacteria or a pathway for infection.

Illegal importation of plant material

MAF assesses the risk presented by the illegal importation of kiwifruit plant material as low, noting the lack of drivers or incentives, and the absence of credible evidence of illegal imports

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20. Samples of kiwifruit vegetative material, seeds or pollen could potentially have been brought to New Zealand illegally by individuals or groups such as backyard breeders, commercial growers, home gardeners and scientists. MAF reviewed the possible motives and import pathways that might have been used by such people or groups.

21. The motives of home gardeners and enthusiasts who illegally import plants or seeds is often to obtain species, colours or varieties that are difficult to obtain in New Zealand. With kiwifruit, New Zealand holds, grows and breeds world leading varieties which are generally available for growing. For those who want new material for breeding or pollination there are legal and controlled pathways for these imports. In addition there are wild vines established in the Bay of Plenty and seeds from fruit available in New Zealand could be used.

22. The commercial industry is also tightly regulated and there is little incentive to access and grow varieties which would not be packed and exported through the existing marketing structures. There is also little evidence of previous attempted illegal imports of kiwifruit material into New Zealand. No kiwifruit material has been intercepted at the International Mail Centre since 2000.

Importation of orchard equipment (including pollen related)

MAF assesses the risk presented by the import of orchard equipment including machinery, pollen spreading devices and hand equipment as

negligible for large machinery and uncertain but probably low for pollen equipment.

23. While Psa can survive and remain viable on inanimate objects such as equipment for several weeks, the viability of the whole pathway including shipping to New Zealand, border inspection, and infection of vines is less well understood. Imports have been made from Psa infected countries such as Italy, along with imports from countries free of Psa. Some of this is new equipment and some is second hand.

24. The risks presented by new tractors or other heavy equipment are low and second hand accessories are inspected at the border for soil and plant and other contamination. There has also been some movement of mechanised and hand operated pollen application and dusting equipment between New Zealand and Italy. Some of this activity took place before Psa V was reported in Italy, and the timing of later imports does not align well with the first detections of Psa V in New Zealand.

People movements

MAF assesses the risk presented by people travelling to and from New Zealand for a range of reasons as unknown and difficult to define but consider the risk probably low, but possible.

25. The groups of people potentially presenting a risk of accidentally importing Psa V include tourists, particularly tour groups from overseas, kiwifruit orchardists returning to New Zealand, and scientists and industry representatives travelling between New Zealand and other kiwifruit growing locations. They could potentially transfer Psa in soil on footwear or clothing, or on personal effects such as camera bags.

26. Several times a year groups of Korean and Japanese kiwifruit growers visit New Zealand kiwifruit packhouses and orchards. Similar groups of Chilean and Italian growers also visit from time to time. While they are visiting orchards the groups are with growers, supervisors and industry representatives, but it is unknown if any specific hygiene measures are implemented for the groups.

27. A number of Te Puke growers and related businesses have personal or business links with Italian kiwifruit growers and the wider industry there. It is likely that some have visited infected orchards and returned to work in or visit orchards here. Again, the extent of disease hygiene measures that are implemented by these people is unknown.

28. A significant number of scientists, industry representatives and agronomists visit or receive visitors from Italy to exchange information, attend workshops or conferences, and collaborate on research programmes. Although these groups may have a better understanding of disease transmission risks it is again uncertain what measures individuals implement to reduce this risk.

29. The risk from people movements depends to a large degree on the persistence of viable Psa V bacteria on shoes, clothing and other personal effects that are taken into orchards here. In ideal conditions some Psa bacteria may be able to survive on these objects for several weeks. However, the next steps through to infection

of vines – mechanism for transfer to vines and infection – are not well understood.

30. The risk from people movements comprises a range of different activities but overall MAF assesses the risk from this pathway to be unknown and difficult to define, but probably low.

Research

MAF assesses the risk presented by research activities to be low to negligible as personnel involved in plant pathology research are generally aware of the risks involved when handling new pathogens.

31. The New Zealand scientific community is relatively small and is well networked nationally and internationally. Scientists from Plant & Food Research, in particular, have research collaborations with French and Italian research groups. These have increased since the discovery of Psa in New Zealand resulted in additional resources being been focussed on understanding how to prevent and manage the disease.

32. Researchers have imported kiwifruit plant materials into New Zealand for research and breeding purposes. More recently diagnostic work for Psa from Italian vines was undertaken in Hamilton under MAF permit and containment conditions. Other Psa V cultures used for diagnostic purposes have been held in Auckland – a considerable distance from the centre of infection in Te Puke. One incident occurred when Psa infected Italian kiwifruit vine samples were being brought to New Zealand under permit for diagnostic purposes. The samples were in personal luggage and when inspected on arrival, the outer packaging was found to be potentially compromised. The samples were destroyed at the border.

33. Researchers, particularly those involved in plant pathology, generally have a good understanding of the needs for and mechanisms to ensure containment of plant diseases during research activity.

34. The more general risks from scientists' travel and visits to kiwifruit orchards overseas is addressed in the section on people movements above.

PSA - PATHWAY TRACING TECHNICAL REPORT

INTRODUCTION

During the *Pseudomonas syringae* pv. *actinidiae* (Psa) response MAF undertook a range of investigative activities to identify possible entry pathways for Psa into New Zealand. This work was initially undertaken to help identify the extent of incursion, and evolved into a range of work with the following goals:

Primary Objectives

- To consider potential entry pathways for Psa V
- To assess the likelihood of these pathways being the entry point for Psa V

Secondary Objectives

- To identify high risk sites for surveillance or monitoring based on tracing activity
- To identify any areas of improvement to minimise the risk of similar entries in the future (not considered in this document)

This work was constrained by available resources as MAF was primarily focused on responding to the outbreak. Additionally, independent and reliable information was not readily available to assess all pathways. The level of information presented does not correlate with the likely risk of those pathways.

It is important to note that it is unlikely that a definitive entry pathway will ever be conclusively demonstrated.

This report is a summary of all work undertaken to date and the assessments represent current understanding as at 5 December 2011. As new information becomes available this assessment may require review.

The following potential pathways have been assessed or investigated.

1. Imported kiwifruit pollen and pollen trials
2. Imported kiwifruit plant material - budwood / tissue culture
3. Imported kiwifruit plant material – seed
4. Imported kiwifruit plant material – fruit
5. Illegal importation of plant material
6. Importation of orchard equipment (including pollen related)
7. People movements
8. Research

This list does not include all possible pathways, just those where MAF has undertaken some investigation or analysis.

GENERAL BACKGROUND AND ASSUMPTIONS

This document represents the best understanding at the date of preparation and may require review as new information becomes available.

Likely period of Psa V introduction into New Zealand: Less than 18 months prior to detection in November 2010

Significant Psa like symptoms (heavy spotting) were first observed on Restricted Place number 1 (RP1) in New Zealand around 23 October 2010, and reported to MAF on 5 November 2010. In addition, early Psa like symptoms were noticed in Restricted Place 2 around 10 October 2010.

Based on the observed rate of Psa V spread in New Zealand, favourable weather for Psa bacterial disease expression and spread in 2010 and the rapid onset of secondary symptoms observed in the initial reported gold orchards in Te Puke and also observed for both green and gold orchards in the surrounding area, (in some cases from primary to secondary symptoms in three to four weeks though on average industry observed 15 weeks (standard deviation 9)) it is unlikely that Psa V was present in gold orchards in the affected area for an extended period prior to detection. Te Puke has a relatively mild humid climate with few severe frosts that damage plant defences, (mild frosts are protected against by using a range of frost protection techniques) and has a temperature range which is likely to support all year round viability of bacteria (Everett & Henshall1994).

In Italy high summer temperatures and low humidity appear to lower Psa pathogen pressure, however there was no lull in bacterial disease expression and progression to secondary symptoms over summer 2010-11 in Te Puke, indicating temperatures in Te Puke are favourable for Psa development and dispersal for longer periods.

This conclusion assumes a competent level of observation and reporting by industry of the disease as to date there have been no reports of secondary-like symptoms in orchards preceding the 2010/2011 growing season. This conclusion also assumes that climatic conditions were likely to be conducive for infection in previous years. Preliminary data indicates that weather conditions in Spring 2009 were similar to those in Spring 2010.

Psa V has been isolated from both gold and green orchards, however there appears to be a much higher percentage of secondary symptoms reported in gold vines. As at the end of July 2011 less than 12% of reported secondary symptoms were in green varieties.

As of the end of September 2011, Psa V has not been detected in other regions outside the Bay of Plenty in New Zealand despite testing of suspect plant material (based on leaf spot symptoms). However, on 28 November 2011 Psa V was confirmed on a property near Pukekohe. The Psa LV haplotype has been identified on at least 21 properties outside of the Bay of Plenty area.

Investigations into potential risk factors and pattern of disease spread to date indicate a point source of vine infection, rather than multiple introduction sites (e.g., pathway with multiple incursions). RPs 1 and 2 are likely to be close to the index case based on the timing of symptom progression.

Based on these observations it is considered most likely that the Psa V outbreak is due to a recent introduction likely to be less than 18 months between introduction and disease expression.

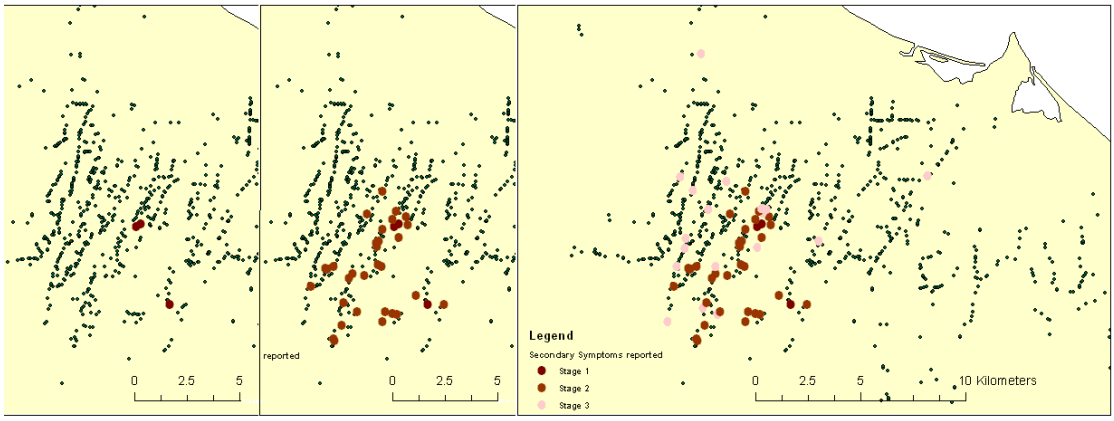

Figure 1 – Spatial distribution of secondary symptoms broken into three stages from week 1 to week 38 of the outbreak (Stage 1 = week 1-11; Stage 2 = weeks 12 - 24; Stage 3 = weeks 25 – 38).

There is a second possible scenario where Psa V was established for some time in a location on a host (possibly green) where the disease expressed no significant symptoms in New Zealand, and either a natural or human assisted event spread it to a susceptible host or new, more favourable location. It is unlikely that Psa V would have been detected in this scenario given that there were no specific field surveys for the detection of Psa V. No research on kiwifruit bacterial leaf spotting diseases has been conducted in the last 10 years and no symptoms were reported to inform a passive surveillance notification to the scientific community or MAF.

There have been management changes noted in gold crops in the last two years, such as increased use of artificial pollination and girdling. Both of these practices may have changed the disease triangle dynamics of host, pathogen and environment.

General bacterial spread information

Bacteria can be dispersed in a range of ways. The most common method of bacterial spread is through wind and rain. Rain mediates bacterial spread in several ways, with rain drop hitting a leaf causing splash which distributes the bacteria, rain may also create a medium for bacteria to be carried in and wet leaves become more turgid, opening leaf stomata and allowing bacteria to enter.the leaf. Wind may also spread the water droplets containing the bacteria, potentially increasing the distance the bacteria may spread. However, natural spread is by nature relatively local, and although it may explain the distribution within Te Puke, it doesn't explain the entry of the disease into Te Puke.

Human activity, movement of plant material and machinery movements have been proposed as modes of transmission for the distribution of Psa. Based on recent unpublished work undertaken at Plant and Food Research (Vanneste pers com) Psa may survive for up to several weeks on inanimate objects and some non-host material. This has been demonstrated for other species, for example, in field

experiments *Erwinia amylovora* has been reported to survive as an epiphyte on landscape plants (non host) (Johnson et al. 2006). The implications of this are investigated in this report.

Based on the speed of spread and development of secondary symptoms it is unlikely that large inoculum levels are required for establishment on the surface of the plant. Inoculum is just one of three principle factors required for the successful spread and establishment of a pathogen. The other two factors required are a conducive host and environment to support the pathogens life cycle. It is the interaction of these three factors that govern how fast a pathogen multiplies.

The transfer of low numbers of bacteria to the plant may occur with splashing rain, but it is the growth of the pathogen on the surface of the plant and the transfer to suitable infection sites in sufficiently high numbers on the plant that results in infection. It is unclear what the minimum population size is for a successful infection (for example, one bacterium landing in the perfect spot under optimal environmental conditions and a very susceptible host MAY be sufficient, but this is a very unlikely scenario, and it is likely that a larger population than one bacterium is required to cause disease). For example, in the fire blight model, it was found that a small population of *E. amylovora* has a low probability of causing disease as populations levels influence the time required to cause infection and disease symptoms and have generally been considered of little epidemiological significance (Taylor et al. 2003; Thomson 2000).

Potential sources: Europe (specifically Italy, France, or others where Psa V status is confirmed) and possibly China or Chile (where Psa V status is unreported)

Whilst Psa is relatively widely spread, significant impacts have only been reported from Spain (Balestra et al. 2011), Italy, Switzerland (EPPO), Portugal, France, Korea and Japan. In New Zealand, two haplotypes of Psa have been identified, where one appears highly virulent and acts as a strong host pathogen (Psa V), and the second does not appear so strongly pathogenic (Psa LV), and so is often referred to as the less virulent strain. The Italian (and therefore possibly French, Swiss, Portuguese and Spanish) populations appear genetically consistent with Psa V. This is consistent with the hypothesis of the new variety ‘Summerkiwi’ plant material from Italy distributing the pathogen to France, and presumably Spain and Portugal (Limmer, 2011). The Korean and Japanese populations are genetically distinct from other Psa populations, as is the New Zealand Psa LV haplotype.

Australia has recently announced detection of Psa, but this is genetically similar to Psa LV.

Chile has recently announced detection of Psa, but it is unknown which haplotype exists in Chile. The new kiwifruit variety ‘Summerkiwi’ was exported from Italy to both Chile and Argentina in either 2005 or 2006 (Fruit Today, 2009). This occurred prior to the Psa V outbreak in Italy.

Psa is reported as present in China; however information on the level of impact and haplotype information has not been reported. It is worth noting that China is the native origin of kiwifruit and has a diverse range of *Actinidia* (kiwifruit) species and varieties.

ENTRY PATHWAY ASSESSMENT METHODS

The potential pathways were qualitatively assessed as either 'yes' (✓), 'no' (✗) or unknown or unreliable (?) on three categories:

- Location: Was the pathway or event located in the main Te Puke priority zone as described on the 31 May 2011? Pathways or events with direct links to orchards which demonstrated secondary symptoms in November and December 2010 were assessed as direct matches;

- Timeline: Did the pathway or event occur prior to symptoms being observed, with sufficient time allowed for pathogen multiplication? As discussed above, the risk period is defined as between May 2009 and November 2010 and more credence given to pathway or events occurring between May and November 2010; and

- Scientific plausibility: Is there scientific evidence that this pathway can transmit Psa V?

The pathway or event was then assigned a further qualitative, epidemiology based assessment of Negligible, Low, Medium or High.

1. Imported commercial pollen and pollen trials

Risk summary - Imported Commercial Pollen – Uncertain but probably low based on current information *– known application periods do not match infection and pollen source does not match known Psa V infections. Cross contamination and unreported use of imported pollen in previous years are possible but unlikely pathways*

Risk summary – Imported Pollen used in Trials – Uncertain but probably low based on current information *– there is no evidence that imported pollen was used; however this can not be confirmed by independent sources. Cross contamination, unreported trials and unreported use of imported pollen in these trials are possible but unlikely pathways*

Pathway assessment

Event	Location	Timeline	Scientific Plausibility	Probability
Imported Commercial Pollen	✓	×?	?	Uncertain but probably low
Pollen Trials	✓	?	?	Uncertain but probably low

Background and assumptions

Presence and viability of Psa in pollen

The consistent detection of Psa by polymerase chain reactions (PCR) tests on a large number of New Zealand pollen samples indicate that Psa is likely to be associated with pollen in New Zealand. Pollen harvested in New Zealand, Italy, Chile and China has tested positive by PCR tests for presence of Psa (Vanneste et al. 2011, MAF unpublished data). The primers (Psa F1/R2 and Psa F3/R4) used in the PCR pollen tests are currently the best validated and most specific published primers to distinguish Psa from other bacteria known to be present on kiwifruit (Rees-George et al. 2010, Vanneste et al. 2010, MAF unpublished data). Sequencing of amplified products from PCR tests on pollen has confirmed these positive results (MAF unpublished data). PCR methods detect the presence of Psa DNA, but do not provide information on the viability of the bacteria or the bacteria's potential for transmission.

Pollen as a pathway

Bacterial diseases have been associated with pollen and there has been scientific speculation that pollen may transmit such diseases. However, there is no scientific testing that demonstrates the efficient transmission of phytopathogenic bacteria from a pollen grain to a susceptible host. A recent review reported that there are no pollen-transmitted bacteria (Card et al 2007). However, it should be noted that Psa is an emerging pathogen and further research is required to fully understand its transmission.

There was an obvious coincidence that the property directly adjacent to the initial notification site has strong business ties with a major kiwifruit pollen producer.

That producer was also the only pollen company to import pollen into New Zealand, was a long term and significant user of artificial pollination in its organic gold orchard and was severely affected by Psa infection (later determined to be Psa V). This led to a need to investigate the potential for pollen as the pathway for stimulation of a Psa outbreak in New Zealand.

Laboratory trials indicate that if a high inoculum of Psa is directly applied to a plant or flower then Psa can be detected in pollen of the plant (Spinelli, 2011), however these trials were undertaken in very artificial conditions and there is only preliminary evidence that the application of infected pollen will result in infection in a plant.

Furthermore, the method of collection, processing, storage, transport and application may all impact viability and bacterial longevity. These activities may or may not reduce survival of bacteria in the long term.

Pollen pathway tracing investigation activities

Prior to haplotype information being available, imported pollen was not thought to be a viable introduction pathway of Psa to New Zealand as the distribution records of this product could not account for the widespread incidence of Psa found throughout New Zealand. However, since this original analysis, two differing Psa haplotypes have been identified (Psa V and Psa LV) where Psa V appeared to have very limited distribution at the time of first detection.

A range of investigative activities have been undertaken including;

- Multiple interviews with key industry stakeholders and other connected parties
- Consideration of import and export records
- Testing of imported material

Six commercial consignments of kiwifruit pollen have been imported from Chile (4) and China (2) beginning in 2008 (Table 1).

Only one commercial company has imported kiwifruit pollen into New Zealand. That company has been importing clean milled pollen from Chile since 2008 with four consignments received between 15/12/2008 and 06/06/2010.

The consignments were given biosecurity clearance by MAF on arrival in New Zealand as all the import requirements were met at the border under MAF import health standard 155.02.06: Importation of Nursery Stock (i.e. a phytosanitary certificate endorsed with additional declaration confirming that the pollen had been milled from hand collected unopened male flower buds).

There was industry concern that the Chilean pollen may have come from Italy via Chile (there is known to be a strong market for kiwifruit pollen in Chile imported both from New Zealand and Italy). However, based on available import documentation with supporting assurances from the Servicio Agricola y Ganadero (SAG) in Chile, documented export of milling equipment from New Zealand to Chile in 2007 and 2008 and interviews with the pollen importer, there is significant evidence that the pollen imported from Chile was sourced from Chile. MAF has control of all known remaining Chilean pollen and it has tested positive for Psa by

PCR, however viable cultures of Psa could not be isolated and the haplotype can not be determined.

Two consignments of imported pollen have also been received from China: 24/06/2009 and 06/06/2010. These shipments of pollen imported from China were small and were reported as either discarded (approximately 15 grams of anthers) or were tested and unused due to low pollen viability (the second shipment was damaged during border inspection).

The information provided on Chinese origin pollen appears reliable. MAF has control of all remaining Chinese pollen and it has tested strongly positive for Psa by PCR, however viable cultures of Psa could not be isolated and the haplotype can not be determined.

Based on procedures and timing there is potential for small amounts of cross contamination between New Zealand sourced pollen and Chilean pollen.

Table 1 – Summary of all pollen imports into New Zealand and issues associated with consignment details.

Date of import	Date released	Consignment #	Quantity	Country of origin	Reported fate	Any issues
15/12/08	20/01/09	c2008/352699	12.965 kg	Chile	Re-exported to the Northern Hemisphere	Based on a paperwork reconciliation 0.75 kg may be unaccounted for (i.e. not re-exported, or seized by MAF) . This may be due to errors in records.
28/03/09	28/03/09	c2009/67312	4 units	Chile	Reported as re-exported to the Northern Hemisphere. However an order to France was cancelled	
28/11/09	1/12/09	c2009/296408	11 units	Chile	Reported as used in the 2010 season	
30/04/10	3/05/10	c2010/113285	3 units	Chile	Reported as used in New Zealand Gold orchards (2010 season)	Unlikely to be the source of the 2010 outbreak as initial symptoms observed before pollen application
24/06/09	30/06/09	c2009/140782	1 unit	China	Imported as anthers, Reported as discarded	MAF informed that following viability testing all material was discarded. Disposal process

						was described but not able to be independently verified. Only a very small amount of material was imported approx 15gm of pollen.
6/06/10	16/06/10	c2010/161762	1 kg	China	Damaged during border inspection, low viability	Retained & tested +ve to Psa by PCR. Unlikely to be the source of the 2010 outbreak as initial symptoms observed before pollen application. No material was missing from the consignment when handed over to MAF.

Based on interviews, imported pollen was first used in New Zealand in the 2010 season. This information appears to be accurate. However, earlier use of previous consignments either through cross-contamination, mixing with New Zealand collected pollen or by experimental use can not be ruled out through independent sources. Assuming imported pollen was first used in 2010, imported pollen is unlikely to be the source of direct infection as significant symptoms were observed on RP1 within 10 days of artificial pollen application and reported on RP2 around or just before pollen application.

Imported pollen was repackaged in the same facility as New Zealand pollen giving rise to the potential for cross contamination of New Zealand sourced pollen with contaminants from imported pollen. Cross contamination of New Zealand origin pollen with imported pollen is chronologically feasible but likely to be very limited and in most cases was out of synchronisation with packaging of NZ sourced pollen. The unreported use of imported pollen in 2008 or 2009 is also a chronologically feasible pathway and can not be ruled out by independent sources based on investigations.

Based on interviews, RP1 first used artificial pollination in 2010 on 13 and 16 October 2010. RP1 reported that this occurred before Psa symptoms were initially observed. Significant symptoms (heavy spotting on leaves and flowers) were noted on 23rd October, secondary symptoms including wilting of shoots and curling of leaves was observed by MAF by the second week of November.

It was initially understood that only New Zealand pollen was used on RP1. However, subsequent information received makes this less certain. We have been unable to find any independent evidence as to whether imported pollen was or was not used on RP1. Additionally the applicator used on RP1 was reported as using imported pollen in 2010 over the same period on other orchards.

RP2 is a certified organic property, requiring use of certified organic pollen. Based on this, and interviews with RP2, it is highly unlikely imported pollen was used on this property. Based on interviews with orchard staff at RP2, possible

symptoms (spotting) in RP2 were reportedly noticed just prior to or during pollen application around 10 October 2010.

Preliminary work analysing factors which may influence the relative risk of Psa V presence on an orchard, showed no significant difference in reported Psa V infection rates between properties that used artificial pollination and those that did not. This analysis does not preclude pollen as an entry pathway.

Pollen Trials

Questions were also raised regarding pollen trials.

Based on interviews, application trials were undertaken on three orchards in October or November 2006 and one in November 2009. These trials were for testing pollen application techniques and equipment. The November 2009 trial is the only likely introduction pathway time-wise and was undertaken in Pongakawa. Associated orchards are classified as "not detected" as of August 2011. In all cases pollen was reported as of New Zealand origin and equipment only ever used in New Zealand. Cross contamination, contaminated equipment, unreported trials or unreported use of imported pollen are the only viable explanations for a successful introduction through these trials. No evidence to support these hypotheses has been provided to date.

Risks remaining

There have been some inconsistencies in information obtained by MAF on pollen related pathways and information on this pathway has been unable to be verified through independent sources.

Direct connections between RP2 and the pollen company may also have led to introduction to RP2 through people movements. This is difficult to assess.

Uncertainty also remains relating to commercially prepared pollen as a viable pathway for the entry of Psa, and the required contamination level or load sufficient to achieve infection from pollen. This creates difficulty in estimating the volume of pollen required to initiate the disease outbreak. Potential cross contamination between imported and New Zealand pollen during packaging and/or application is also a concern.

Neither China nor Chile has reported the presence of Psa V or virulent Psa infections. Should either of these countries be confirmed with Psa V, this assessment will require review.

2. Imported plant material – budwood / tissue culture

Summary: Negligible - unlikely due to inspection, testing and other controls on this pathway. *This is supported by tracing and further testing for Psa activity.*

Pathway assessment

Event	Location	Timeline	Scientific Plausibility	Probability
Budwood or tissue culture	×	×	✓	Negligible

Background

Host plant material is generally considered the highest risk material for the entry and establishment of new pests and diseases. To reduce the risk only dormant cuttings and tissue cultures of *Actinidia* spp. nursery stock are eligible for import into New Zealand, and are required to conform to MAF's import health standard 155.02.06: Importation of Nursery Stock (http://www.biosecurity.govt.nz/files/ihs/155-02-06.pdf).

Dormant cuttings are imported into a Level 3 post entry quarantine (PEQ) facility and are grafted on to New Zealand origin root stocks. Tissue cultures are imported into a Level 3 PEQ tissue culture laboratory and must be de-flasked into a Level 3 PEQ greenhouse before the quarantine period begins. Greenhouse plants undergo a minimum 6 months quarantine period where they require mandatory testing for viruses and Psa, and growing season inspection for pest and disease symptoms. The plants have at least four inspections MAF inspectors during the growing season, as well as regular inspections by the operator of the PEQ facility.

The specific testing requirements for Psa were first added to the import health standard in 2004. Note: Detection of Psa V and significant symptoms was not reported in Italy until 2007-2008 (Ferrante and Scortichini, 2009).

In addition to the import requirements for budwood, and tissue cultures described above, due to the sterile nature of the growing medium, plant tissue is sanitised prior to tissue culturing. Any fungal and bacterial contaminants are usually easy to detect as vigorous, spreading growths, overwhelming plant material within the container due to the absence of microbial competition. Any tissue culture plantlets contaminated in this way are discarded due to the risk of contaminating further cultures. It is theoretically possible but unlikely that tissue culture plantlets may be contaminated with undetected pathogenic bacteria which are present in an epiphytic manner (not causing disease). This is considered particularly unlikely, where the pathogen is considered aggressive or virulent in nature.

Nursery stock pathway tracing investigation activities

Fourteen consignments of *Actinidia* sp. budwood or plants have been imported into New Zealand since 2000, six of which were destroyed either at the border or while in post entry quarantine (PEQ). Details of the remaining eight consignments are presented in Table 2. Six consignments were given biosecurity clearance prior to the detection of Psa in New Zealand. Of these six consignments, five tested negative for Psa prior to release from PEQ as part of the normal testing protocol required by the import health standard. One consignment given biosecurity clearance which was not tested by PCR (imported under permit issued in 2001), was imported from China and held in PEQ for a total of 7 years due to virus testing requirements. During which time it was subjected to inspection and testing for general bacterial symptoms. It should also be noted that this consignment was imported prior to Psa V causing epidemics overseas.

All plant material from the six consignments released prior to the Psa outbreak were traced and underwent inspection and if required PCR testing. No Psa symptoms have been observed on these plants and Psa was not detected in leaf

tissue from the tested consignments. Furthermore, some of these imported plants are in areas that still remain free of any form of Psa for example, Kerikeri. The inspection and (some) re-testing of the imported nursery stock (or daughter material) was conducted in June 2011. Two consignments were still held in PEQ at the time of the Psa outbreak. These two consignments have since been given biosecurity clearance with further testing for Psa additional to the requirements of the import health standard, including growing the plants under conditions optimal for symptom expression of Psa and testing at two time points using multiple PCR tests.

Table 2 – Summary of budwood imports and any issues

Date given biosecurity clearance	Consignment #	Country of origin	Species	# of plants released	Tested for Psa while in PEQ	Outcome of tracing	Issues / comments
21/12/2007	C2006/214481	Italy	*A. deliciosa*	8 greenhouse plants	Yes, neg	PCR Neg	Field tested material has been through tissue culture since arrival in New Zealand Imported prior to Italian outbreak.
24/07/2008	C2004/58742	China	*A. chinensis*	11 greenhouse plants	No	PCR Neg	China is not known to have Psa V
8/07/2008	C2007/285730	NSW, Australia	*A. chinensis*	8 greenhouse plants	Yes, neg	Healthy leaves, not tested	Australia is not known to have Psa V
18/06/2009	C2008/88846	Italy	*A. deliciosa*	8 greenhouse plants + 644 tissue culture derived from the 8 greenhouse plants	Yes, neg	No leaves suitable for testing	Derived from tissue culture imports
13/02/2009	C2008/128599	Greece	*A. deliciosa*	11 greenhouse plants	Yes, neg	PCR Neg	Greece is not known to have Psa V
6/08/2009	C2008/252900	China	*A. chinensis*	4 greenhouse plants	Yes, neg	PCR Neg	China is not known to have Psa V
4/05/2011	C2010/20873	Italy	*A. chinensis*	18 greenhouse plants	Yes, neg	PCR Neg	Given biosecurity clearance after Psa outbreak
11/02/2011	C2010/42670	Italy	*A. deliciosa; A. valvata*	1 greenhouse plant of each	Yes, neg	PCR Neg	Given biosecurity clearance after

				spp			Psa outbreak

Remaining risk

Various combinations of factors such as the extended length of time that imported plant material was held in post entry quarantine, the country of origin, the use of tissue culture material, the timing of importation or release from post entry quarantine, one or more negative PCR test results for all consignments and the imported material now being located in an area which is not known to have Psa V are inconsistent with a virulent Psa haplotype capable of rapid dispersal being introduced via these consignments. This gives a very reasonable level of confidence that budwood or tissue culture imports via MAF-regulated channels were not the pathway of Psa V introduction. I

3. Imported plant material – seed

Summary: Negligible *as seed is not a known vector, seed was grown into plants in PEQ and the origins of the imported seed do not match known Psa sources*

Pathway assessment

Event	Location	Timeline	Scientific Plausibility	Probability
Seed	✓	✓	×	Negligible

There is no evidence that Psa is seed transmitted in *Actinidia* spp. seed. Hu et al. (1998) concluded that due to its small size and extraction method kiwifruit seed would be unlikely to be a vector for different *Pseudomonas* spp. Storage of seed is also unlikely to be consistent with bacterial survival in the longer term.

Actinidia seed is eligible for import under the import health standard 155.02.05: Importation of Seed for Sowing (http://www.biosecurity.govt.nz/files/ihs/155-02-05.pdf). Seed must be accompanied by a phytosanitary certificate and imported into a Level 3 post entry quarantine facility where the seed is germinated. Resulting seedlings undergo a minimum 6 months quarantine period where they require specific testing for viruses and growing season inspection for pest and disease symptoms (including bacteria). There is no specific testing requirement for Psa.

Only plants that have been grown in the greenhouse, undergone growing season inspections and testing for viruses are eligible for biosecurity clearance. Seed that has not been germinated is not eligible for biosecurity clearance.

As young plants are anecdotally more susceptible to Psa and these plants are inspected at least four times by the MAF Inspector during their six month growing period, as well as regular inspection by the operator of PEQ facility, it is likely that a Psa V infection would have been detected.

In all cases post entry quarantine was undertaken at the Plant and Food Facility, Mt Albert, Auckland.

Since 2000, ten consignments have been imported from China and one consignment from Nepal. However, the seeds have only been germinated in PEQ

in recent years, and the resulting plants were only given biosecurity clearance after August 2009, as identified in the table below.

The Psa status of Nepal is unknown. There is no published information on the haplotype(s) present in China.

Date released	Consignment number	Country of origin	Species	# of plants released
October 2010, April 2011	C2009/240270	China	*A. chinensis*	30 & 10 plants respectively
No plants given biosecurity clearance from these consignments	C2005/253226	China	*A. chinensis*	
	C2004/185944	China	*A. chinensis*	
	C2004/185931	China	*A. chinensis*	
	C2004/185941	China	*A. chinensis*	
	C2002/49320	China	*A. callosa*	
	C2002/49321	China	*A. chinensis*	
June 2009	C2001/42182	China	*A. arguta*	44 plants
June 2010	C2006/207140	Nepal	*A. callosa*	6 plants
August 2009, February 2010, June 2010, August 2010, April 2011, April 2011	C2001/40510	China	*A. deliciosa*	132, 36, 15, 24, 27 & 14 plants respectively.
August 2009	C2006/207115	China	*A. chinensis*	37 plants

4. Imported plant material – fruit

Summary: Negligible *as fruit is not a known pathway for Psa.*

Pathway assessment

Event	Location	Timeline	Scientific Plausibility	Probability
Fruit	?	✓	×	Negligible

Green kiwifruit (*Actinidia deliciosa*) can be imported under the import health standard (IHS) 152.02 from two countries - the USA and Italy (http://www.biosecurity.govt.nz/files/ihs/152-02.pdf). Importation volumes since 2000 are presented below.

Country	Weight (kg)	# consignments
Italy	6,700,000	285
USA	300,000	23

In 1999 when MAF issued the IHS for kiwifruit from Italy, the risk assessment which underpinned the development of the IHS considered Psa as it was already reported from Italy. At that time and currently, the international consensus is that there is no scientific evidence that mature fruit is a pathway for the entry, establishment and spread of the disease.

Consequently the 1999 IHS made no recommendations for measures to manage this organism. The assessment was reviewed in 2008 and July 2010 and as there was no new scientific evidence that the mature fruit is a pathway no changes were made to the IHS.

In a recent presentation by a visiting Italian scientist (Stefani 2011), it was stated that Psa was detected by PCR from internal fruit structures of kiwifruit however the Psa bacteria was not cultured. The detection of Psa in fruit not supported by validated scientific papers.

Even if this finding is validated, this work represents a detection of genetic material associated with Psa, not proof of viability or proof of a pathway for infection.

5. Illegal importation of plant material

Summary: Low, *unable to determine likely driver or incentive. No credible evidence presented*.

Pathway assessment

Event	Location	Timeline	Scientific Plausibility	Probability
Back yard enthusiast	?	?	✓	Low
Scientists	?	?	✓	Low
Pollen	?	?	✓	Low

Background

Kiwifruit (*A. deliciosa*) was first introduced to New Zealand in 1906 via seeds from China and the first fruit was produced in 1910. In 1987 the leading cultivars were Abbott, Allison, Bruno, Greensill, Hayward and Monty, with the two male varieties Matua and Tomuri. Abbott, Allison, Bruno and Hayward all originated from chance seedlings discovered in the 1920's. Greensill and Monty originated from later selections. In 1977, DSIR obtained further kiwifruit seeds from China, which were later designated as *Actinidia chinensis*. This triggered the Plant and Food Research's kiwifruit breeding program, from which Plant Variety Rights for the gold kiwifruit Hort16A were granted in 1996.

New varieties, including male varieties such as Bruce, continue to be developed under a contractual arrangement between Plant and Food Research and Zespri. In this breeding programme, varieties are rigorously assessed on key production attributes such as fruit size, colour, storage and shelf life, and consumer qualities such as taste, appearance and texture over a number of years and at a number of sites in New Zealand. Funding for kiwifruit variety breeding is not a limiting factor as $35.7M was made available for this program through both FRST funding ($15.2M) and a large contribution from Zespri ($20.5M) in November 2009

(Foundation for Research, Science and Technology, 2009). Access to new genetic material has improved considerably since late 2007, through the legal importation of budwood, tissue culture and seeds (as discussed above).

The New Zealand kiwifruit industry is tightly regulated by virtue of Zespri's export marketing structure and holds little incentive for the commercial release of a new variety outside of this arrangement. A key component of the industry is the control of plant variety right licences for the fruit production of Gold (Hort16A) and any newly released varieties.

Additionally, growers wishing to export fruit of any variety must meet the quality control requirements of Zespri's Kiwigreen, GLOBALGAP and British Retail Consortium programs and this includes using true to type (quality) nursery stock. The emergence of a new kiwifruit variety outside of existing historical varieties, the Plant and Food Research breeding programme or through MAF importation channels would be immediately suspicious.

However, backyard enthusiasts not wishing to develop kiwifruit varieties for commercial gain would not be limited by industry regulations and controls. Border statistics show that air passengers do not always declare risk goods as required on the arrival declaration. In 2005-06, undeclared risk goods were seized from four out of every one thousand arriving passengers (Waite 2006). Furthermore, although tools such as searching, X-rays and dogs are used to detect these undeclared risk goods, there are low levels of undeclared and undetected risk goods (slippage) through the system (Taulau & Rowsell 2010).

The illegal importation of pollen is also considered in this section. This can be split into two sections, pollen for plant breeding purposes and pollen for commercial application.

Pollen for breeding purposes

The availability of funding and genetic resources and the meticulous, detailed genealogy records required when registering kiwifruit varieties for patents or Plant Variety Rights is likely to limit the appeal of pollen smuggled for plant breeding purposes.

Pollen for commercial purposes

During flowering, growers may apply supplementary pollen at rates between 250 gm – 750 gms per hectare. Artificial pollen application can help produce larger fruit size and fruit set and has developed into a profitable industry in its own right. In 2009, due to the increase in demand for artificial pollen for both domestic and international markets, a shortage of pollen occurred. However, this situation is unlikely to have prompted the smuggling of pollen from overseas as a regulated importation pathway already existed, and the volumes of pollen required for commercial operations (kgs) are unlikely to enter the country undetected in mail or personal luggage.

Investigation findings

Backyard enthusiast

No information has been received by MAF to indicate that activity of this nature has occurred in the Te Puke area (or others). An abundance of non-commercial kiwifruit propagative material was potentially available to a back yard enthusiast

due to vines becoming naturalised (wild) in the Bay of Plenty region. Wild kiwifruit vines have been present in the area since the 1970's and were the target of a control programme for 8 years prior to the Psa outbreak. No kiwifruit nursery material (without MAF permit) has been intercepted at the International Mail Centre since 2000 (Christopher Waite, MAF, *pers comm*.)

Scientists

No information has been received which indicates a secondary process for obtaining new kiwifruit genetic material. Since the discovery of Psa, Plant and Food Research (and other organisations) have followed appropriate processes for movement of *Actinidia* sp. plant material (infected or otherwise) and for the use of Psa material for research purposes.

Pollen

No information has been received which indicates pollen was smuggled into New Zealand for breeding or commercial purposes.

6. Importation of orchard equipment (including pollen related)

***Summary: Negligible for large machinery, uncertain but probably low for pollen equipment*.**

Pathway assessment

Event	Location	Timeline	Scientific Plausibility	Probability
Heavy equipment	✓	×	✓	Negligible
Pollen equipment	✓	?	✓	Low

Background

Although it is understood that Psa can remain viable for several weeks on inanimate objects, the parameters required for survival and successful inoculation, for example, amount/volume of inoculum, surface type, temperature and humidity are not well understood. In relative terms, a wooden object heavily contaminated with or any equipment harbouring infected host plant material is assumed to be a higher risk compared with a smooth metal object which has been cleaned of all plant residue (eg. secateurs).

Investigation actions

Heavy equipment

Tracing of equipment from Italy was conducted for a number of equipment types, heavy machinery, parts and pollen equipment.

Interviews conducted with the local tractor distributor identified that only new machinery is imported from Italy. However the machinery is likely to be test driven prior to shipping and it is not known what is involved during this test driving process. Parts (gear boxes, drive shafts, pumps etc), sprayers and wheels are

also imported from Italy. Second-hand trailers are imported from the United Kingdom. This tracing is not considered exhaustive as second-hand kiwifruit vineyard machinery may have been imported by other specialty machinery dealers located elsewhere in New Zealand. However, all second-hand machinery is inspected for soil, plant and other contamination by MAF staff. New, containerised machinery is inspected by an Approved Person at the point of de-vanning. R&R Tractors in Te Puke reported that all containerised new machinery was imported as clean equipment.

Pollen equipment

In 2005 or 2006 a tractor-mounted pollen application unit was shipped to Italy for trials before being returned to New Zealand. A number of pollen dusters (handheld) have been imported from Italy, some of which have been used by New Zealand orchardists in November 2008 on a trial basis. It was originally understood that none of the pollen dusters had been used in Italy (imported new); subsequent information indicated that that they had been tested in Italy using New Zealand-sourced pollen. This information is unable to be independently verified.

7. People movements

Summary: Unknown and difficult to define but probably low. *Tour groups considered low due to the level of supervision. Level of expertise or awareness of risk may vary with returning orchardists or scientists.*

Pathway assessment

Event	Location	Timeline	Scientific Plausibility	Probability
Tour groups	?	✓	✓	Probably low
Returning New Zealand orchardists	✓	?	✓	Probably low
Scientists	✓	?	✓	Probably low

Background

In a study of soil contaminants on international aircraft passengers' footwear, McNeill et al (2011) recorded high incidences, counts and diversities of viable bacteria, fungi, nematodes and seeds as well as several live arthropods. Bacteria from the *Pseudomonas* genera were cultured and identified from a subsample in the study. Limited investigations have been conducted examining fungal pathogens transferred directly by human movements and is considered a significant risk factor when managing spread of *Phytophthora* species in recreational environments. Sheridan (1989) found overseas passengers originating from a farm carried significantly greater number of (fungal) spore types than those of urban or transit origin and more rust urediniospores than all the other groups combined. No specific data is available to calculate the probability of bacterial establishment following exposure from similar inoculum sources, therefore accidental transmission of Psa V via international visitors or returning New Zealand residents cannot be defined. However, it is a scientifically plausible

introduction pathway. Risk awareness and assessment at international airport arrival is likely to be triggered by the requirement to declare whether passengers have visited an orchard or farm in the previous 30 days prior to arrival in New Zealand although it is unlikely that orchardists or scientists undertook biosecurity hygiene measures beyond cleaning their footwear prior to the discovery of Psa.

Investigation findings

Tour groups

MAF has received reported estimates that since 2000 to the present day, at least 20 groups of approximately 25 Korean or Japanese kiwifruit growers have toured New Zealand kiwifruit orchards and pack houses over a 5-6 day trip. These tour groups have become more common in the last 5 years and occur at any time of the year except November and December. Similar groups of Chilean and Italian growers are also known to have visited. Whilst in New Zealand, the tour groups are under the supervision of organisers, growers, Zespri and/or a consultant; however it is not known if special hygiene procedures were implemented. The specific orchards visited and dates of tour group visits which occurred in the Te Puke region prior to the discovery of Psa have not been obtained.

Returning New Zealand orchardists

A number of Te Puke orchardists have personal and business connections with Italy and Italian kiwifruit growers. Zespri and various pack houses have significant investments in Italian kiwifruit orchards. Key staff members regularly (annually or more frequent) travel to Italy to monitor orchards, conduct trials and conduct business. One of the initial infected properties connected with a pollen exporting/importing business has a number of business connections with Italy. MAF understands that the pollen producers' director visits Italy every year in late February and/or March to conduct trials and sales however this person is a rare visitor to their home orchard blocks. We do not know whether this timeframe would be consistent with first symptoms being observed in October 2010. Limited information has been obtained for other New Zealand orchardists or their personal effects. However given the volume of this exchange and travel several orchards within the Te Puke priority zone could have had an interaction of this type.

Scientist and agronomist exchange

A large number of Plant and Food Scientists, Zespri staff and other agronomists regularly (annually, biennially) visit or receive visitors from Italy or other countries to exchange kiwifruit agronomy practices and other information. Detailed visitation or travel information has not been obtained. Again given the volume of this exchange and travel, several orchards within the Te Puke priority zone could have had an interaction of this type.

8. Research

Risk summary: Low to negligible. *Personnel involved in plant pathology research are generally aware of dangers involved when handling new pathogens.*

Pathway assessment

Event	Location	Timeline	Scientific Plausibility	Probability
Plant and Food Research	✓	?	✓	Low
Other	×	?	✓	Negligible

Background

The New Zealand scientific community is a small interconnected network of highly professional scientists. By reputation, this environment has enabled strong collaborations to develop between New Zealand and many international scientific communities. Plant and Food Research scientists have a number of collaborative links to Italian and French research groups. These collaborations have increased since the discovery of Psa in New Zealand and the resulting funding becoming available to research control options, resistant varieties and best cultural practices for Psa control. This section considers experimental work, diagnostic testing, transport and storage of Psa risk materials in containment facilities within New Zealand.

Containment facilities by and large enjoy excellent safety records. Sadly there are some unfortunate examples of containment breaches worldwide such as the effluent processing facility at the Pirbright laboratory in the United Kingdom implicated in a localised 2007 outbreak of Foot and Mouth Disease (Spratt, 2007).

Investigation findings

Breeding

International sources of kiwifruit genetic material for variety development are discussed in budwood/tissue culture, seed (above) and illegal plant material (below).

Plant and Food Research

Through the New Zealand-owned orchards in Italy and the strong collaborative links between Zespri and Plant and Food Research, it is known that diagnostic work for Psa from Italian vines was being conducted in a Ruakura (Hamilton) laboratory under MAF permit and containment conditions. The transportation of these samples for diagnostics was via personal courier (luggage) as specimen submission was on an ad hoc basis. An event where the outer packaging of Italian vine specimens infected with Psa V was potentially compromised has been documented. These samples were destroyed at the border.

It is understood that a Plant and Food Research scientist had imported pollen samples from Italy under MAF permit into containment for experimental purposes. A condition of the permit was the destruction of all pollen on completion of the trial. It is understood that this work was conducted under containment at Ruakura and it is unlikely that transmission outside of the laboratory would occur in this scenario.

Pollen application research conducted by commercial entities has been discussed with pollen pathways or equipment (above).

Other than the transportation incident above, no containment issues have been notified to MAF (a requirement of the containment standard).

Other

For purposes of reliable positive control and test development, MAF scientists obtained cultures of Psa. These cultures were imported into and utilised in containment at a location in Auckland remote from the Te Puke region.

CONCLUSION

- Due to the controls in place and information available, legally imported plant material, heavy machinery, research and seed pathways are considered to represent a low likelihood of being the entry pathway.
- Due to the lack of driver or incentives illegal importation is also considered to be a low likelihood of being the entry pathway.
- There is no scientific evidence for fruit as a pathway for Psa, consequently imported fruit is considered to be a low likelihood of being the entry pathway.
- Pollen and pollen equipment is the most difficult to accurately assess due to the level of uncertainty in information obtained and inability to independently verify much of this information. Should Chile be confirmed as Psa V positive there may be potential to trace New Zealand sales of imported pollen and some surveillance in outlier regions could be initiated based on use of Chilean pollen, however there is little point in initiating this surveillance in the absence of any information that Chile has Psa V or that pollen is an effective transmission agent. In the event that pollen transmission of Psa is scientifically demonstrated, further emphasis may be assigned to this pathway. At this stage and without further scientific evidence we conclude that pollen is a possible entry pathway.
- Due to the high level of apparent connectivity, reported significant survival on non organic material and mixed levels of biosecurity awareness, people movements, whilst generally low risk can clearly not be excluded and are a possible entry pathway.

Despite significant work undertaken to identify, assess and investigate possible introduction pathways, significant uncertainty remains as to a specific entry point or a specific pathway. This is frustrating for industry and MAF as it reduces our ability to target specific activities to prevent further spread or further introduction through similar pathways. This is not unusual however and it is rare to be able to confidently identify the introduction pathway of a new pest or disease particularly when its biology is not well known.

Key information that could assist in further refining our understanding of the entry pathway and would trigger a reassessment are;

- New information on the biology of the bacteria, particularly survival and transmission ecology through all vectors (natural dispersal and long distance vectored spread).
- Obtaining information on the haplotypes present in other parts of the world (origin of infection), in particular Chile and China.
- New information on the timeline and location of first infection within New Zealand through more detailed tracing interviews of all the orchards within the centre of the hot zone, as initial focus was mostly on the report properties.

Further pathway of entry investigation is unlikely to add to the current knowledge of how best to manage the disease in New Zealand and reduction of spread to new areas via scientifically credible pathways is where research should be focused.

GLOSSARY

Psa	*Pseudomonas syringae* p.v. *actinidiae* (kiwifruit bacterial canker)
Psa V	Virulent haplotype
Psa LV	Less virulent haplotype

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