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Pest risk analysis report for *Pseudomonas syringae* pv. *actinidiae* associated with *Actinidia* (kiwifruit) propagative material

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Cover image: Leaf spots surrounded by chlorotic halos on leaves and bacterial ooze formed on a young shoot of *Actinidia* by *Pseudomonas syringae* pv. *actinidiae*.

www.biosecurity.govt.nz/files/regs/.../high.../peq-actinidi-testing.pdf

Contents

Summary

Australia initiated this pathogen-based pest risk analysis (PRA) following the expansion of the aggressive strain of *Pseudomonas syringae* pv. *actinidiae* (*Psa*) into countries that export kiwifruit propagative material to Australia. This PRA evaluates the likelihood and consequences of *Psa* strains being introduced into Australia on *Actinidia* propagative material from all sources and reviews import conditions for propagative material. The review of existing policy indicates that the current requirements for propagative material are inadequate for preventing the introduction of *Psa* strains not present in Australia. Stronger mitigation measures are proposed to minimise the risk of *Psa* strains not present in Australia entering on *Actinidia* propagative material.

Australia's established policy for the importation of *Actinidia* (kiwifruit) nursery stock and pollen from New Zealand was suspended in November 2010 due to the detection of *Psa*. The suspension was subsequently extended to all other countries. Following the suspension of import conditions, a less aggressive strain of *Psa* was detected in Victoria and from a collection in Western Australia. This PRA examines all strains of *Psa* which are not present in Australia.

Prior to suspension, all imported consignments of kiwifruit propagative material (dormant cuttings only) were subjected to mandatory on-arrival inspection, fumigation and growth in a closed government post-entry quarantine (PEQ) facility with visual pathogen screening for three months. Separate conditions existed for *Actinidia* propagative material from New Zealand.

This PRA has identified that *Psa* strains not present in Australia could enter Australia with kiwifruit propagative material (dormant cuttings, tissue cultures and pollen) and proposes quarantine measures to manage the risks. The PRA proposes strengthening of the existing policy for all countries and withdrawing New Zealand specific conditions. The proposed risk management measures for the different propagative materials are:

Dormant cuttings

- Mandatory on-arrival methyl-bromide fumigation, hot water treatment (50 °C for 30 minutes), surface sterilisation (1% NaOCl for 10 minutes), then newly established plants are grown at 15±3 °C in closed government PEQ facilities for a minimum period of 12 months for visual observation; and
- Molecular testing techniques including polymerase chain reaction (PCR) test.

Pollen

- Pollen must be sourced from countries or areas demonstrated to be free of *Psa*.

Tissue culture

- Growth in closed government PEQ facilities at 15±3 °C for a minimum period of six months for visual observation; and
- Molecular testing techniques including polymerase chain reaction (PCR) test.

Interested parties are encouraged to provide comments and submissions to Plant Biosecurity within the consultation period. Plant Biosecurity will consider any comments received before finalising the pest risk analysis and quarantine policy recommendations.

1 Introduction

1.1 Australia's biosecurity policy framework

Australia's biosecurity policies aim to protect Australia against the risks that may arise from exotic pests[1] entering, establishing and spreading in Australia, thereby threatening Australia's unique flora and fauna, as well as those agricultural industries that are relatively free from serious pests.

The pest risk analysis (PRA) process is an important part of Australia's biosecurity policies. It enables the Australian Government to formally consider the risks that could be associated with proposals to import products into Australia. If the risks are found to exceed Australia's appropriate level of protection (ALOP), risk management measures are proposed to reduce the risk to an acceptable level. If it is not possible to reduce the risks to an acceptable level, then no trade will be allowed.

Successive Australian governments have maintained a conservative, but not a zero-risk, approach to the management of biosecurity risks. This approach is expressed in terms of Australia's ALOP, which reflects community expectations through government policy and is currently described as providing a high level of protection aimed at reducing risk to a very low level, but not to zero.

Australia's PRAs are undertaken by Plant Biosecurity using teams of technical and scientific experts in relevant fields, and involves consultation with stakeholders at various stages during the process. Plant Biosecurity provides recommendations for animal and plant quarantine policy to Australia's Director of Animal and Plant Quarantine (the Secretary of the Australian Department of Agriculture, Fisheries and Forestry). The Director or delegate is responsible for determining whether or not an importation can be permitted under the *Quarantine Act 1908*, and if so, under what conditions. The Australian Quarantine and Inspection Service (AQIS) is responsible for implementing appropriate risk management measures.

More information about Australia's biosecurity framework is provided in the *Import Risk Analysis Handbook 2007* (update 2009) located on the Biosecurity Australia website www.daff.gov.au/ba.

1.2 This pest risk analysis

This pest risk analysis (PRA) evaluates the quarantine risks posed by *Psa* strains (that are not present in Australia) entering Australia on *Actinidia* (kiwifruit) propagative material from all sources. This PRA also reviews the existing import conditions for *Actinidia* (kiwifruit) propagative material from all sources and proposes additional measures where required.

Propagative material represents one of the highest plant quarantine risks, as it can harbour various forms of pathogens. The introduction of plant pathogens, especially pathogens with latent infection, is of particular concern in propagative material. Due to the latency period between infection and the expression of symptoms, propagative material may appear healthy at harvest but later manifest the disease, especially when harvested in the early stages of an outbreak or where fungicides are being used to reduce the prevalence of the disease. A range

[1] A pest is any species, strain or biotype of plant, animal, or pathogenic agent injurious to plants or plant products (FAO 2009).

of exotic arthropod pests and pathogens can be introduced and established via propagative material when imported in a viable state for ongoing propagation purposes.

1.2.1 Background

Previously, Australia had an established policy for the importation of *Actinidia* propagative material from all sources. Prior to suspension, all imported consignments of kiwifruit propagative material (dormant cuttings only) were subjected to mandatory on-arrival inspection, fumigation and growth in a closed government or AQIS approved private post-entry quarantine (PEQ) facility with visual pathogen screening for three months. Separate conditions existed for *Actinidia* propagative material from New Zealand, with a Phytosanitary Certificate. In this circumstance propagative material was released after on-arrival inspection following fumigation only. However, the import conditions for *Actinidia* propagative material from all sources including New Zealand were suspended after the detection of *Psa* in several countries. Following the suspension of import conditions, the less aggressive strain of *Psa* was detected in Victoria and from a collection in Western Australia. This PRA examines all strains of *Psa* which are not present in Australia.

1.2.2 Scope

The scope of this analysis is limited to all strains of *Psa* currently absent from Australia—as a less aggressive strain has been recorded in Australia. References to *Psa* strains not present in Australia are hereafter abbreviated to *Psa*.

In this PRA, Plant Biosecurity has assessed the likelihood of entry, establishment and spread and associated potential consequences of *Psa* for Australia. This PRA process forms the basis for the development of import policy to manage the risks of *Psa* entering Australia.

1.2.3 Import policy for *Actinidia* propagative material

Dormant cuttings from all sources other than New Zealand

Prior to the suspension of imports, importation of *Actinidia* species propagative material occurred through a closed government or AQIS-approved PEQ facility. All consignments of *Actinidia* species propagative material imported prior to the suspension were subject to quarantine/biosecurity measures set out in the import conditions for *Actinidia* nursery stock and Condition C7300 ‘General Import requirements, nursery stock for all species’. The general requirements included:

- an AQIS import permit;
- freedom from regulated articles including soil, disease symptoms and other extraneous contamination of quarantine concern;
- on-arrival inspection;
- mandatory methyl-bromide fumigation; and
- growth under closed quarantine, at either a government or AQIS-approved PEQ facility for three months with pathogen screening.

Dormant cuttings from New Zealand

Actinidia species budwood was allowed entry from New Zealand if accompanied by a Phytosanitary Certificate with the following declaration:

> "The Scion wood is free from pests and diseases. The consignment was dipped pre-shipment in a solution of mancozeb or chlorpyrifos".

Following on-arrival fumigation, *Actinidia* species budwood consignments from New Zealand were released from quarantine without any further quarantine concerns. However, consignments of *Actinidia* species budwood from New Zealand without phytosanitary certification, with the above mentioned additional declaration, were subject to the same conditions for *Actinidia* species budwood from other countries.

Tissue culture from all sources other than New Zealand

Actinidia species tissue cultures from all sources other than New Zealand were subject to visual inspection and grown in closed quarantine either at a government or AQIS-approved private PEQ facility for a minimum of three months for visual disease screening.

Tissue culture from New Zealand

Actinidia species tissue cultures from New Zealand were subject to the AQIS general tissue culture import conditions. If the general conditions were met, the consignments were released from quarantine without any further quarantine measures, that is, no growth in PEQ required.

Pollen from New Zealand

Actinidia species pollen for breeding purposes was only allowed entry from New Zealand and subject to an import permit. The pollen had to be collected from unopened flowers and accompanied by a Phytosanitary Certificate with the following declaration:

> "The pollen in the consignment is of New Zealand origin only and has been tested by the New Zealand Plant Protection Centre for the presence of *Pseudomonas syringae* pv. *actinidiae*. It is free from this bacterial pathogen".

2 Method for pest risk analysis

Plant Biosecurity has conducted this pest risk analysis (PRA) in accordance with the International Standards for Phytosanitary Measures (ISPMs), including ISPM 2: *Framework for pest risk analysis* (FAO 2007) and ISPM 11: *Pest risk analysis for quarantine pests, including analysis of environmental risks and living modified organisms* (FAO 2004). The standards provide a broad rationale for the analysis of the scientific evidence to be taken into consideration when identifying and assessing the risk posed by quarantine pests.

Following ISPM 11, this pest risk analysis process comprises three discrete stages:

- Stage 1: Initiation
- Stage 2: Pest Risk Assessment
- Stage 3: Pest Risk Management

Phytosanitary terms used in this PRA are defined in ISPM 5 (FAO 2009).

2.1 Stage 1: Initiation

The *initiation* of a risk analysis involves identifying the reason for the PRA and the identification of the pest(s) and pathway(s) that should be considered for risk analysis in relation to the identified PRA area.

This qualitative pathogen-based pathway risk assessment was initiated due to the expansion in range of *Psa* and the identification of new pathways for its potential entry into Australia.

In the context of this assessment, kiwifruit propagative material (dormant cuttings, tissue culture and pollen) is a potential import 'pathway' by which *Psa* can enter Australia.

For this PRA, the 'PRA area' is defined as Australia for pests that are absent from Australia or of limited distribution and under official control in Australia.

2.2 Stage 2: Pest Risk Assessment

A Pest Risk Assessment (for quarantine pests) is: 'the evaluation of the probability of the introduction and spread of a pest and of the likelihood of associated potential economic consequences' (FAO 2009).

This is a qualitative, pathogen-based pathway risk analysis and expresses risk in terms such as high, moderate or low. In a qualitative assessment, risk is estimated through a standard set of factors that contribute to introduction, establishment success, spread or economic impact potential. Risk assessment evaluates the unrestricted pest risk to determine if the risk is sufficient to warrant mitigation.

In this PRA, the assessment was divided into the following interrelated processes:

2.2.1 Pest categorisation

Pest categorisation is a process to examine, for each pest identified in Stage 1 (*Initiation of the PRA process*), whether the criteria for a quarantine pest are satisfied. The process of pest categorisation is summarised by ISPM 11 (FAO 2004) as a screening procedure based on the following criteria:

- identity of the pest;
- presence or absence in the endangered area;
- regulatory status;
- potential for establishment and spread in the PRA area; and
- potential for economic consequences (including environmental consequences) in the PRA area.

2.2.2 Assessment of the probability of entry, establishment and spread

Details for assessing the 'probability of entry', 'probability of establishment' and 'probability of spread' of a pest are given in ISPM 11 (FAO 2004).

ISPM 11 states that in the case of propagative material imports, the concepts of entry, establishment and spread have to be considered differently. Plant material intended for ongoing propagation purposes is deliberately introduced, distributed and aided to establish and spread. That is, this material will enter and then be maintained in an intended habitat, potentially in substantial numbers and for an indeterminate period. Significant resources are utilised to ensure the continued welfare of imported propagative material. Therefore, the introduction and establishment of plants from imported propagative material, in essence, establishes the pests and pathogens associated with the propagative material. Pathogens, in particular, may not need to leave the host to complete their life cycles, further enabling them to establish in the PRA area. Furthermore, propagative material is expected to be shipped at moderate temperatures and humidity which is unlikely to adversely affect any pest that is present during shipment.

For the purposes of this PRA, *Actinidia* propagative material is assumed to come from areas where *Psa* specifically occurs and no phytosanitary measures have been applied. Therefore, *Psa* will enter into the PRA area. Plants imported into the PRA area for planting will be very widely distributed through production nurseries. Movement of *Psa* associated with imported propagative material in the nursery trade is considered the primary means for long-distance dispersal of this bacterium. *Psa* could cause loss or damage to hosts plants in the PRA area.

In its qualitative PRAs, Plant Biosecurity uses the term 'likelihood' for the descriptors it uses for its estimates of probability of entry, establishment and spread. Qualitative likelihoods are assigned to the probability of entry (comprising of an importation step and a distribution step), the probability of establishment and the probability of spread. Six descriptors are used: high; moderate; low; very low; extremely low; and negligible. Definitions for these descriptors and their indicative probability ranges are given in Table 2.1.

Table 2.1: Nomenclature for qualitative likelihoods

Likelihood	Descriptive definition	Indicative probability (P) range
High	The event would be very likely to occur	$0.7 < P \leq 1$
Moderate	The event would occur with an even probability	$0.3 < P \leq 0.7$
Low	The event would be unlikely to occur	$0.05 < P \leq 0.3$
Very low	The event would be very unlikely to occur	$0.001 < P \leq 0.05$
Extremely low	The event would be extremely unlikely to occur	$0.000001 < P \leq 0.001$
Negligible	The event would almost certainly not occur	$0 \leq P \leq 0.000001$

The likelihood of entry is determined by combining the likelihood that the pest will be imported into the PRA area and the likelihood that the pest will be distributed within the PRA

area, using a matrix of rules (Table 2.2). This matrix is then used to combine the likelihood of entry and the likelihood of establishment. The likelihood of entry and establishment is then combined with the likelihood of spread to determine the overall likelihood of entry, establishment and spread.

Table 2.2: Matrix of rules for combining descriptive likelihoods

	High	Moderate	Low	Very low	Extremely low	Negligible
High	High	Moderate	Low	Very low	Extremely low	Negligible
Moderate	Low	Low	Very low	Very low	Extremely low	Negligible
Low	Very low	Very low	Very low	Extremely low	Extremely low	Negligible
Very low	Extremely low	Extremely low	Extremely low	Extremely low	Negligible	Negligible
Extremely low	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible
Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible

2.2.3 Assessment of potential consequences

The objective of the consequence assessment is to provide a structured and transparent analysis of the likely consequences if the pests were to enter, establish and spread in Australia. The assessment considers direct and indirect pest effects and their economic and environmental consequences. Considered together, these assessments and evaluations constitute a 'risk assessment' for each relevant quarantine pest.

The basic requirements for the assessment of consequences are described in the Sanitary and Phytosanitary (SPS) Agreement, in particular Article 5.3 and Annex A. Further details on assessing consequences is given in the 'potential economic consequences' section of ISPM 11 (FAO 2004). This ISPM separates the consequences into 'direct' and 'indirect' and provides examples of factors to consider within each. In this PRA, the term 'consequence' is used to reflect the 'relevant economic factors'/ 'associated potential biological and economic consequences', and 'potential economic consequences' terms as used in the SPS Agreement and ISPM 11 (FAO 2004), respectively.

The direct and indirect consequences were estimated over four geographic levels, defined as:

- **Local**: an aggregate of households or enterprises (a rural community, a town or a local government area).
- **District**: a geographically or geopolitically associated collection of aggregates (generally a recognised section of a state or territory, such as 'Far North Queensland').
- **Regional**: a geographically or geopolitically associated collection of districts in a geographic area (generally a state or territory, although there may be exceptions with larger states such as Western Australia).
- **National**: Australia wide (Australian mainland states and territories and Tasmania).

The magnitude of the potential consequence at each of these levels was described using four categories, defined as:

- **Indiscernible**: pest impact unlikely to be noticeable.
- **Minor significance**: expected to lead to a minor increase in mortality/morbidity of hosts or a minor decrease in production but not expected to threaten the economic viability of production. Expected to decrease the value of non-commercial criteria but not threaten the criterion's intrinsic 'value'. Effects would generally be reversible.
- **Significant**: expected to threaten the economic viability of production through a moderate increase in mortality/morbidity of hosts, or a moderate decrease in production. Expected

to significantly diminish or threaten the intrinsic 'value' of non-commercial criteria. Effects may not be reversible.

- **Major significance**: expected to threaten the economic viability through a large increase in mortality/morbidity of hosts, or a large decrease in production. Expected to severely or irreversibly damage the intrinsic 'value' of non-commercial criteria.

The estimates of the magnitude of the potential consequences over the four geographic levels were translated into a qualitative impact score (A–G)[2] using Table 2.3[3]. For example, a consequence with a magnitude of 'significant' at the 'district' level will have a consequence impact score of D.

Table 2.3: Decision rules for determining the consequence impact score based on the magnitude of consequences at four geographic scales

		Geographic scale			
		Local	District	Region	Nation
Magnitude	Indiscernible	A	A	A	A
	Minor significance	B	C	D	E
	Significant	C	D	E	F
	Major significance	D	E	F	G

The overall consequence for each pest is achieved by combining the qualitative impact scores (A–G) for each direct and indirect consequence using a series of decision rules (Table 2.4). These rules are mutually exclusive, and are assessed in numerical order until one applies.

Table 2.4: Decision rules for determining the overall consequence rating for each pest

Rule	The impact scores for consequences of direct and indirect criteria	Overall consequence rating
1	Any criterion has an impact of '**G**'; or more than one criterion has an impact of '**F**'; or a single criterion has an impact of '**F**' and each remaining criterion an '**E**'.	Extreme
2	A single criterion has an impact of '**F**'; or all criteria have an impact of '**E**'.	High
3	One or more criteria have an impact of '**E**'; or all criteria have an impact of '**D**'.	Moderate
4	One or more criteria have an impact of '**D**'; or all criteria have an impact of '**C**'.	Low
5	One or more criteria have an impact of '**C**'; or all criteria have an impact of '**B**'.	Very Low
6	One or more but not all criteria have an impact of '**B**', and all remaining criteria have an impact of '**A**'.	Negligible

[2] In earlier qualitative IRAs, the scale for the impact scores went from A to F and did not explicitly allow for the rating 'indiscernible' at all four levels. This combination might be applicable for some criteria. In this report, the impact scale of A-F has changed to become B-G and a new lowest category A ('indiscernible' at all four levels) was added. The rules for combining impacts in Table 2.4 were adjusted accordingly.

[3] The decision rules for determining the consequence impact score are presented in a simpler form in Table 2.3 from earlier IRAs, to make the table easier to use. The outcome of the decision rules is the same as the previous table and makes no difference to the final impact score.

2.2.4 Estimation of the unrestricted risk

The unrestricted risk estimate for each pest is determined by combining the likelihood estimates of entry, of establishment and of spread with the overall potential consequences. This is done using the risk estimation matrix shown in Table 2.5. The cells of this matrix describe the product of likelihood of entry, establishment or spread and consequences of entry, establishment or spread.

Table 2.5: Risk estimation matrix

Likelihood of pest entry, establishment and spread	Negligible	Very low	Low	Moderate	High	Extreme
High	Negligible risk	**Very low risk**	Low risk	Moderate risk	High risk	Extreme risk
Moderate	Negligible risk	**Very low risk**	Low risk	Moderate risk	High risk	Extreme risk
Low	Negligible risk	Negligible risk	**Very low risk**	Low risk	Moderate risk	High risk
Very low	Negligible risk	Negligible risk	Negligible risk	**Very low risk**	Low risk	Moderate risk
Extremely low	Negligible risk	Negligible risk	Negligible risk	Negligible risk	**Very low risk**	Low risk
Negligible	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Negligible risk	**Very low risk**

Consequences of pest entry, establishment and spread

2.2.5 Australia's appropriate level of protection (ALOP)

The SPS Agreement defines the concept of an 'appropriate level of sanitary or phytosanitary protection' (ALOP) as the level of protection deemed appropriate by the WTO Member establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory.

Australia expresses its ALOP in qualitative terms. Australia's ALOP, which reflects community expectations through government policy, is currently expressed as providing a high level of sanitary or phytosanitary protection aimed at reducing risk to a very low level, but not to zero. The band of cells in Table 2.5 marked 'very low risk' represents Australia's ALOP.

2.3 Stage 3: Pest Risk Management

Pest risk management describes the process of identifying and implementing phytosanitary measures to manage risks to achieve Australia's ALOP, while ensuring that any negative effects on trade are minimised.

The conclusions from pest risk assessment are used to decide whether risk management is required and if so, the appropriate measures to be used. Where the unrestricted risk estimate exceeds Australia's ALOP, risk management measures are required to reduce this risk to a very low level. The guiding principle for risk management is to manage risk to achieve Australia's ALOP. The effectiveness of any proposed phytosanitary measure (or combination of measures) is evaluated, using the same approach as used to evaluate the unrestricted risk, to ensure it reduces the restricted risk for the relevant pest or pests to meet Australia's ALOP.

ISPM 11 (FAO 2004) provides details on the identification and selection of appropriate risk management options and notes that the choice of measures should be based on their effectiveness in reducing the probability of entry of the pest.

2.3.1 Identification and selection of appropriate risk management options

Phytosanitary measures to prevent the introduction and spread of quarantine pests may include any combination of measures including pre- or post-harvest treatments, inspection at various points, surveillance, official control, or certification. A measure or combination of measures may be applied at any one or more points along the continuum between the point of origin and the final destination. Pest risk management explores options that can be implemented (i) in the exporting country, (ii) at the point of entry or (iii) within the importing country. The ultimate goal is to prevent the introduction of identified quarantine pests in the PRA area.

Examples of phytosanitary measures which may be applied to propagative material consignments include:

- Importation from pest free areas only (ISPM 4, 10)—the establishment and use of a pest free area by a NPPO provides for the export of plants from an exporting country to an importing country without the need for application of additional phytosanitary measures when certain requirements are met.
- Inspections or testing for freedom from regulated pests—this is a practical measure for visible pests or for pests which produce visible symptoms on plants.
- Inspection and certification (ISPM 23, 7, 12) —the exporting country may be asked to inspect the shipment and certify that the shipment is free from regulated pests before export.
- Specified conditions for preparation of the consignment—the importing country may specify steps which must be followed in order to prepare the consignment for shipment. These conditions can include plants required to have been produced from appropriately tested parent material.
- Pre-entry or post-entry quarantine—the importing country may define certain control conditions, inspection and possible treatment of shipments upon their entry into the country.
- Removal of the pest from the consignment by treatment or other methods—the importing country may specify chemical or physical treatments which must be applied to the consignment before it may be imported.

Measures can range from total prohibition to permitting importation subject to visual inspection. In some cases more than one phytosanitary measure may be required in order to reduce the pest risk to an acceptable level.

Phytosanitary measures implemented in the exporting country

Sourcing propagative material from pest free areas (country freedom)

Area freedom is a measure that might be applied to manage the risk posed by the identified pests in propagative material. The requirements for establishing pest free areas are set out in ISPM 4: *Establishment of pest free areas* (FAO 1995). ISPM 4 (FAO 1995, p. 37) identifies a pest free area as being ‘an area in which a specific pest does not occur as demonstrated by

scientific evidence and in which, where appropriate, this condition is being officially maintained'.

The establishment and use of a pest free area (PFA) by NPPO provides for the export of plants and other regulated articles from the exporting country to the importing country without the need for application of additional phytosanitary measures when certain requirements are met. Thus, the pest free status of an area may be used as the basis for the phytosanitary certification of plants and other regulated articles with respect to the stated pest(s). The exporting country may also inspect the crop to confirm freedom from the pest and provide that certification. The requirements for the establishment, and subsequent maintenance, of a PFA include:

- systems to establish freedom (general surveillance, specific survey);
- phytosanitary measures to maintain freedom (regulatory actions, routine monitoring, extension advice to producers); and
- checks to verify freedom has been maintained.

Sourcing propagative material under systems approach

ISPM 14: *The use of integrated measures in a systems approach for pest risk management* provides guidelines on the use of systems approaches to manage pest risk. According to ISPM 14 (FAO 2002, p. 165), 'a systems approach requires the integration of different measures, at least two of which act independently, with a cumulative effect.'

Systems approaches, which integrate measures for pest risk management in a defined manner, could provide an alternative to single measures to meet the appropriate level of phytosanitary protection of an importing country. They can also be developed to provide phytosanitary protection in situations where no single measure is available. A systems approach requires the integration of different measures, at least two of which act independently, with a cumulative effect. Systems approaches range in complexity. Exporting and importing countries may consult and cooperate in the development and implementation of a systems approach. The decision regarding the acceptability of a systems approach lies with the importing country, subject to consideration of technical justification, minimal impact, transparency, non-discrimination, equivalence, and operational feasibility.

Sourcing propagative material from pest free place of production

Pest free place of production is a measure that might be applied to manage the risk posed by the identified pests in propagative material. The requirements for establishing pest free places of production are set out in ISPM 10: *Requirements for the establishment of pest free places of production and pest free production sites* (FAO 1999).

This standard uses the concept of "pest freedom" to allow exporting countries to provide assurance to importing countries that plants, plant products and other regulated articles are free from a specific pest or pests and meet the phytosanitary requirements of the importing country when imported from a pest free place of production. In circumstances where a defined portion of a place of production is managed as a separate unit and can be maintained pest free, it may be regarded as a pest free production site.

Requirements for the establishment and maintenance of a pest free place of production or a pest free production site as a phytosanitary measure by the NPPO include:

- systems to establish pest freedom
- systems to maintain pest freedom

- verification that pest freedom has been attained or maintained
- product identity, consignment integrity and phytosanitary security.

Where necessary, a pest free place of production or a pest free production site also includes the establishment and maintenance of an appropriate buffer zone.

Administrative activities required to support a pest free place of production or pest free production site involve documentation of the system and the maintenance of adequate records concerning the measures taken. Review and audit procedures undertaken by the NPPO are essential to support assurance of pest freedom and for system appraisal. Bilateral agreements or arrangements may also be needed.

Testing: Freedom based on field inspection and testing—the importing country may request testing to verify freedom from pests of quarantine concern. For example, visual inspections during growing season and Polymerase chain reaction (PCR) or an ELISA-based test for latent or low level of infection of propagative material can be used to verify pest freedom.

Treatment: Removal of the pest from the consignment by treatment or other methods—the importing country may specify chemical or physical treatments which must be applied to the consignment before it may be imported.

Certification: The importing country may specify that production of the commodity be undertaken under an officially monitored certification scheme to ensure stock is free from pests.

Phytosanitary measures implemented in the importing country

On-arrival inspection

On-arrival inspection is conducted by the NPPO for freedom from regulated articles and compliance with the import and certification requirements. The purpose of the inspection is to ensure that import requirements for freedom from the pest in question have been met and to detect new pests which may not have been categorised for their pest risk.

Post entry quarantine

In cases where plant material is imported without any certification, the NPPO may allow imports of the propagative material through growth in post entry quarantine facilities for visual and active disease screening.

Phytosanitary certification

Pest risk management includes the consideration of appropriate compliance procedures. The most important of these is export certification (refer to ISPM 7: *Export certification system*). The issuance of Phytosanitary Certificates (refer to ISPM 12: *Guidelines for phytosanitary certificates*) provides official assurance that a consignment meets specified import requirements and confirms that pest risk management options have been followed.

ISPM 12 states that importing countries should only require Phytosanitary Certificates for regulated articles including plants, bulbs and tubers, or seeds for propagation.

3 Pest Information

3.1 *Pseudomonas syringae* pv. *actinidiae* (*Psa*)

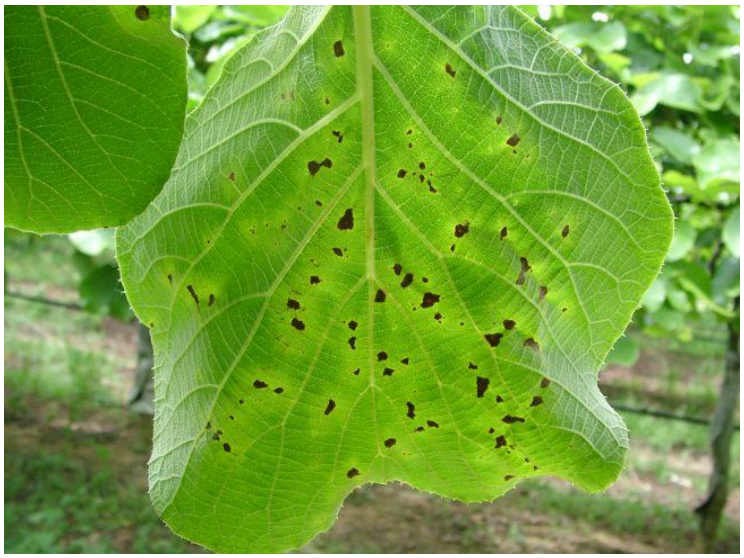
Psa was first described as a new pathovar of *Pseudomonas syringae* in Japan in 1984 (Takikawa *et al*. 1989; Serizawa *et al*. 1989). The bacterium is a gram-negative, obligate aerobic, non-sporing rod bacterium (Takikawa *et al*. 1989). It has been reported that the bacterium originated from wild *Actinidia* species distributed in northern areas of Japan (Ushiyama *et al*. 1992a, b) and was introduced in the 1970s to the Shizuoka Prefecture, Japan (Serizawa *et al*. 1989).

Psa causes bacterial canker disease which is characterized by a red-rusty exudation, blight on young canes and plants, and dark brown spots with a yellowish halo on leaves (Takikawa *et al*. 1989). *Pseudomonas syringae* pathovars produce a wide spectrum of phytotoxins (Bender *et al*. 1999) including syringomycin, syringopeptin, coronatine, phaseolotoxin and tabtoxin (Han *et al*. 2003c). The phytotoxins produced are a characteristic trait of *P. syringae* pathovars (Han *et al*. 2003c). *Psa* and *P. syringae* pv. *phaseolicola* produce phaseolotoxin (Tamura *et al*. 2002). Phaseolotoxin produced by *Psa* contributes to the formation of chlorotic halo lesions in *Actinidia* species (Tamura *et al*. 2002).

3.1.1 Symptoms caused by *Psa*

Psa symptoms are visible on trunks, leaders, leaves, canes and flowers (Serizawa *et al*. 1989; Takikawa *et al*. 1989). *Psa* causes small water-soaked spots to appear on expanded leaves. Spots become brown to dark brown, angular in shape and surrounded by yellow halos (Figure 3.1) (Serizawa *et al*. 1989). The chlorotic halos around lesions on the foliage are caused by phaseolotoxin produced by *Psa* (Tamura *et al*. 2002).

Figure 3.1: Foliage symptoms: Brown spots surrounded by yellow halos



Source: http://photos.eppo.org/index.php/image/3845-pdsmak-01

Further development of the spots is dependent on climatic conditions. For example, in high humidity and cool conditions, the spots remain water-soaked and expand and coalesce to form larger lesions without halos, resulting in a blighted and shrivelled

leaf (Serizawa *et al*. 1989). Bacterial ooze can be observed on the lower surface of the leaf.

The bacterium also infects canes, turning them dark green and water-soaked in appearance. Bacterial ooze is released from infected cracks in the tissue and from lenticels on apparently healthy parts of canes, adjacent to lesions. Lesions become elongated as they increase in size, causing wilting and shoot blight. When canes are infected late in the season, trunk lesions become surrounded by calluses; stem cankers usually ooze red exudates (Serizawa *et al*. 1989) (Figure 3.2).

Figure 3.2: Trunk symptoms: Bacterial canker exudates production from trunks

Source: Balestra *et al*. 2009b

Wilting of foliage occurs when the bacterium colonize the vascular tissues (Figure 3.3). Most infected flowers turn brown and wither without opening, or open prematurely before petals are fully developed, then necrotic lesions develop on sepals (Serizawa *et al*. 1989).

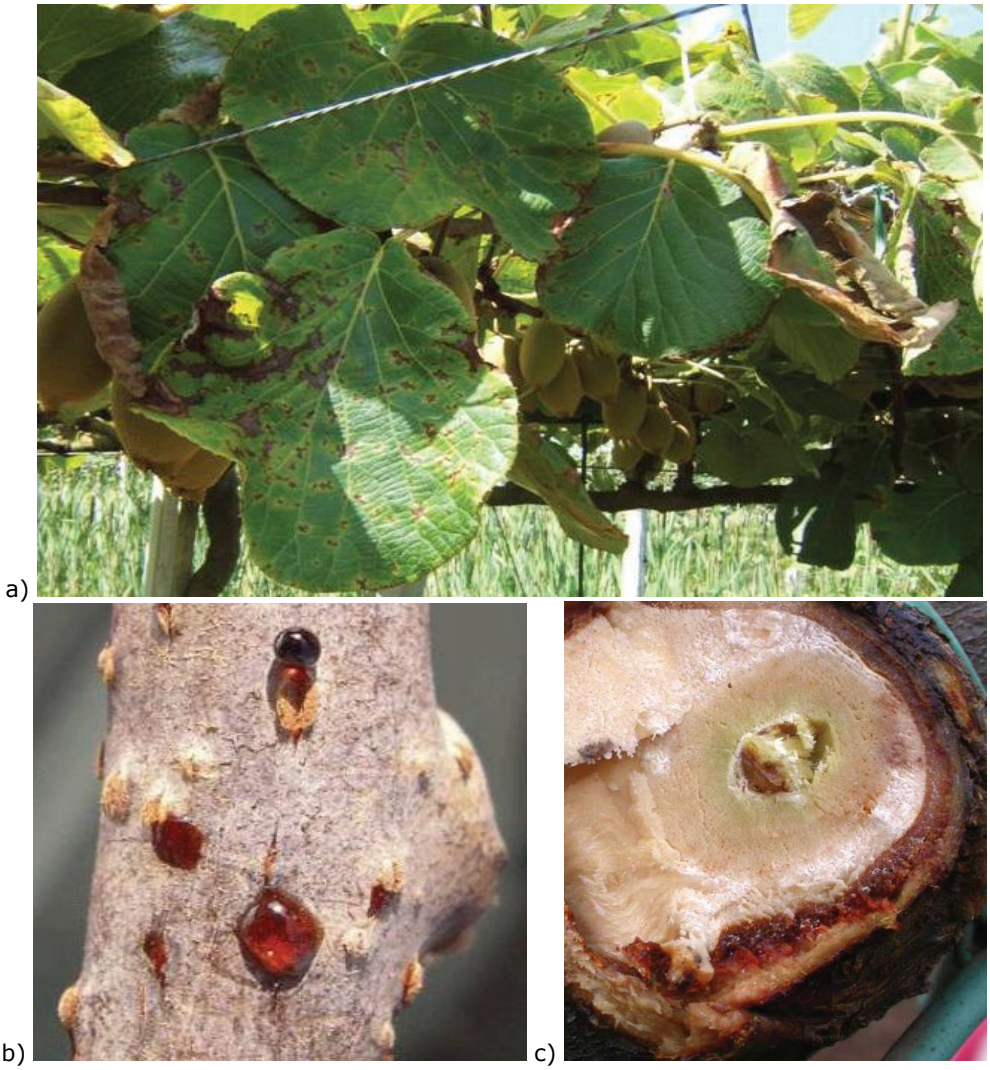
Figure 3.3: Foliage and flower symptoms

Source: http://photos.eppo.org/index.php/image/3843-psdmak-03

Psa symptoms on *Actinidia deliciosa* in Italy were described as a "rusty-brown exudation on bark of twigs and trunks, blight of young canes and plants and angular leaf spots surrounded by chlorotic haloes and tiny cankers on the twigs"(Scortichini 1994). During 2007 and 2008; *Psa* was found on *Actinidia chinensis* in Italy (Ferrante and Scortichini 2009). The symptoms on *A. chinensis* include browning of the buds

and flowers, brown angular spots surrounded by yellow halos on the leaves, and cankers with reddish exudates on the twigs, leaders and trunks (Balestra *et al*. 2009a) (Figure 3.4). In addition, fruits may collapse before fully forming (Balestra *et al*. 2009b).

Figure 3.4: Foliage symptoms (a) canker and (b) red exude on twig (c) and section of leader



Source: Balestra *et al.* 2009a

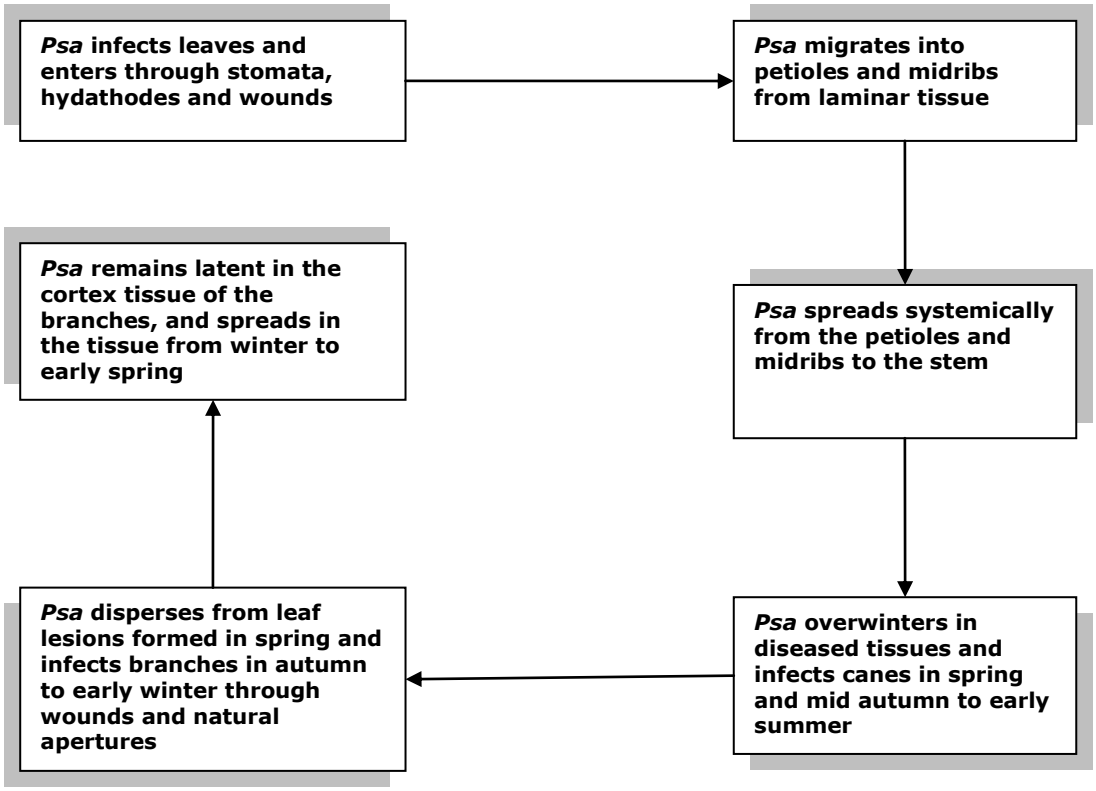
3.1.2 Biology

Psa causes a cyclical disease that damages the kiwifruit vine in winter and spring. The bacterium overwinters in the stem, damaging the main vine structure. This phase has direct effects on yield by reducing the size of the productive vine (Serizawa *et al*. 1989). In spring the bacterium damages all the new season growth (including leaves, flowers, and canes) and this phase plays an important role in bacterial dispersal (Serizawa *et al*. 1989).

Psa infection occurs through stomata, hydathodes or wounds (Serizawa and Ichikawa 1993a) caused by pruning, frost and wind and leaf scars (Serizawa and Ichikawa 1993c; Serizawa *et al*. 1989). The bacterial infection is promoted by cold weather, and frosts that cause injuries and strong winds or heavy rain (Serizawa and Ichikawa

1993c; Serizawa *et al*. 1989). The bacteria can multiply in the infected plant without expressing disease symptoms (Vanneste *et al*. 2010). Young plants (less than five years old) are more susceptible to the bacterium (Vanneste *et al*. 2010). The *Psa* infection process (Serizawa and Ichikawa 1993a; Serizawa *et* al. 1994) is provided in Figure 3.5.

Figure 3.5: Infection process of *Psa*



It has been noted that the cells of *Psa* can survive on leaf surfaces for 20 days before infecting the plant (Serizawa and Ichikawa 1993c). Rainfall and temperature are important factors in symptom development (Hirano and Upper 1990). For example in Japan, disease development during spring increases rapidly when temperature is 10–20 °C; and optimal temperature for disease development is 15±3 °C (Serizawa and Ichikawa 1993c). However, during summer at higher temperatures (>20 °C but < 25 °C) the disease occurred only under atypical cool, rainy conditions (Serizawa and Ichikawa 1993c). At temperatures above 25 °C, no new symptoms were observed. Later studies indicate that the optimum range for disease development is 10–18 °C (Serizawa and Ichikawa 1993b).

Temperature is an important factor affecting the host plants ability to fight *Psa* infection (Serizawa *et al*. 1994). For example, at temperature above 22 °C, the host plant develops calluses around the infected area. This results in a rapid decline in the bacterial population. However, when the temperature drops to 20 °C callus formation decreases. At temperatures below 15 °C callus formation ceases completely (Serizawa *et al*. 1994) and the bacterial population is unaffected.

3.1.3 Strains of *Psa*

The genetic variability in *Psa* populations from various countries has been demonstrated and several strains are recognised. *Psa* strains can be distinguished by the detection of genes coding for phaseolotoxin, coronatine and effector proteins (Ferrante and Scortichini 2010) and their ability to infect different *Actinidia* species (Takikawa *et al*. 1989; Han *et al*. 2003a; Scortichini 1994; Ferrante and Scortichini 2010).

Isolates of *Psa* from Japan are phaseolotoxin producers, whereas isolates of *Psa* from South Korea are coronatine producers (Han *et al*. 2003c). Genomic and phenotypic characteristics of strains of *Psa* from South Korea and Japan indicate that they may have different phylogenic origins (Lee *et al*. 2005). South Korean strains of *Psa* are sensitive to streptomycin whereas most of the Japanese strains of *Psa* are highly resistant to streptomycin. Japanese strains are relatively more resistant to oxytetracycline than South Korean strains (Lee *et al*. 2005).

Isolates of *Psa* from the recent epidemic in Italy are different from strains previously recorded in Japan, South Korea and Italy (Ferrante and Scortichini 2010; Takikawa *et al*. 1989; Koh *et* al. 1994; Scortichini 1994; Han *et al* 2003a). Isolates of the recent epidemic in Italy on *Actinidia chinensis* (yellow kiwifruit) did not possess gene coding for phaseolotoxin or coronatine but had an effector protein (*hopA1*) (Ferrante and Scortichini 2010). However, this effector protein (*hopA1*) is absent from strains causing past outbreaks in Japan and Italy (Ferrante and Scortichini 2010). Additionally, this new strain can cross-infect either *A. chinensis* or *A. deliciosa* (Ferrante and Scortichini 2010), whereas other strains (Korean, Italian) infect only *A. deliciosa* (Han *et al*. 2003a; Scortichini 1994). The *Psa* strain reported on *A. deliciosa* in Italy in 1994 did not cause significant losses (Scortichini 1994). However, the *Psa* strain from the recent epidemic in Italy did cause considerable losses (Ferrante and Scortichini 2009) indicating the involvement of a more virulent strain (Ferrante and Scortichini 2009).

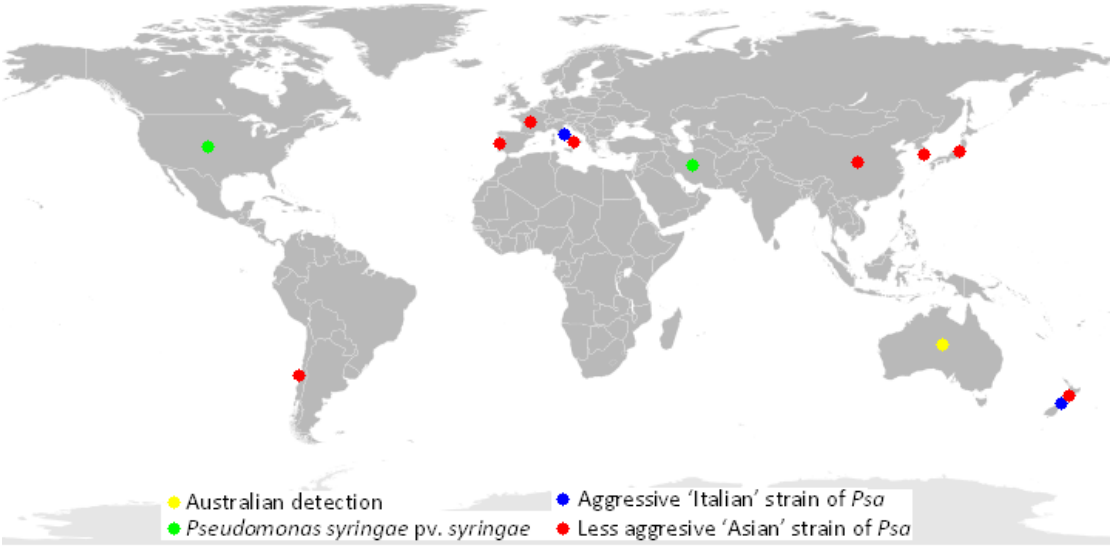
Copper based bactericides and antibiotic compounds can be effective in inhibiting the growth of all *Psa* strains, including the new more aggressive 'Italian' strain (Ferrante and Scortichini 2010). However, the bacterium is capable of developing a resistance to both copper and streptomycin, as previously reported from Japan and South Korea (Goto *et al*. 1994; Han *et al*. 2003a; 2003b).

3.1.4 Global occurrence

Psa was first recorded in Japan on *Actinidia deliciosa* in 1984 (Serizawa *et al*. 1989). Subsequently, *Psa* was found in South Korea (Koh and Lee 1992), Italy (Scortichini 1994), China (Li *et al*. 2004), Portugal (Balestra *et al*. 2010), France (EPPO 2010), New Zealand (Biosecurity New Zealand 2010) and Chile (SAG 2011) (Figure 3.6).

The more aggressive strain was first detected in Italy (Ferrante and Scortichini 2010) and subsequently in New Zealand. Both of these countries also have the less aggressive strains of the pathogen. The more aggressive strains are currently absent from Australia. A less aggressive strain has been recently recorded in Australia in Victoria. A single record of *Psa* in Western Australia has also been shown to be from a less aggressive strain.

Figure 3.6: Distribution of *Pseudomonas syringae* pathovars infecting kiwifruit



There are also records of ''*Pseudomonas* canker'' of kiwifruit plants from California (Opgenorth *et al*. 1983) and ''bacterial canker'' from Iran (Mazarei and Mostofipour, 1994). In both cases, the causal agent was identified as *P. syringae* pv. *syringae* (Mazarei and Mostofipour 1994; Koh and Nou 2002) and not *Psa*.

3.1.5 Spread of *Psa*

Propagative material is considered the main pathway for spread of *Pseudomonas syringae* (Hirano and Upper 1990). *Psa* infects plants systemically and may spread through a variety of means:

- Kiwifruit was introduced to Shizuoka Prefecture, Japan in the 1970s and *Psa* may have been introduced with the host plant; however, it was not noticed until ideal environmental conditions occurred to initiate the epidemic of 1984 (Serizawa *et al*. 1989).
- The bacterium may have been introduced with imported kiwifruit seedlings from Japan into Korea in the mid 1980s soon after the bacterial canker epidemic had occurred in Japan (Lee *et al*. 2005).
- The bacterium can be dispersed in aerosols and can be carried between trees and adjacent orchards in wind-driven rain (Serizawa *et al*. 1989). As a wound-infecting pathogen, it can also be mechanically transmitted by orchard equipment such as pruning implements (CABI 2007).
- *Psa* is able to infect flowers (Hu *et al*. 1998; Serizawa *et al*. 1989). Most infected flowers turn brown and wither without opening or open prematurely before petals are fully developed (Serizawa *et al*. 1989). There are no known pollen-transmitted bacteria (Card *et al*. 2007), but this does not exclude pollen contamination. *Psa* has been detected in pollen imported from Italy into New Zealand and in pollen collected in New Zealand (Biosecurity New Zealand 2010). Therefore, *Psa* may spread with contaminated pollen.
- Pollinators visiting infected flowers may carry the contaminated pollen; therefore, pollinators may have role in *Psa* spread.

The more aggressive strain involved in the recent epidemic in Italy has spread from Latina in the Lazio region of central Italy to the other major kiwifruit growing regions

in northern Italy within two years (Balestra *et al*. 2009b; Balestra *et al*. 2009c; EPPO 2010; Ferrante and Scortichini 2009; Spadaro *et al*. 2010). Propagative material is considered responsible for spread between regions and establishment of a 'high-health' certification scheme was proposed as the most effective measure to prevent further spread (Balestra *et al*. 2009b).

3.1.6 Hosts of *Psa*

The main hosts of this bacterium are *Actinidia* species including *A. arguta*, *A. chinensis, A. deliciosa*, and *A. kolomikta* (EPPO 2010). Field observations suggest that damage caused by the more aggressive strain is more severe on *A. chinensis* cultivars (yellow fleshed kiwifruit) than on *A. deliciosa* cultivars (green fleshed) (Balestra *et al*. 2009b).

4 Risk assessments for *Psa*

This qualitative pathogen-based pathway risk assessment was initiated due to the recent detection of aggressive strains of *Psa* and expansion in range of these strains to other countries. The strains currently not recorded in Australia were identified as quarantine pathogens for Australia because:

- Several strains including the more aggressive strain of *Psa* (hereafter *Psa*) are not present in Australia;
- *Psa* is regulated on propagative material entering into Australia;
- *Psa* is established in areas with a wide range of climatic conditions (EPPO 2010) and the pathogen can spread independently or by human activities (Serizawa *et al.* 1989; CABI 2007). Therefore, *Psa* has the potential for establishment and spread in Australia; and
- *Psa* is considered the most destructive pathogen of kiwifruit (Takikawa *et al*. 1989) as it can destroy an orchard within a short period of time (Koh and Nou 2002). Therefore, *Psa* has a potential for economic consequences in Australia.

In this PRA, dormant cuttings and pollen were assessed as potential pathways for the importation of *Psa* into Australia. The illegal introduction of budwood has a very high risk of introducing *Psa* as the usual quality and quarantine checks are bypassed. However, the assessment of risk posed by potential illegal introductions is outside the scope of this PRA.

The risk assessments in this section focus on the major pathways (dormant cuttings and contaminated pollen) identified for the potential introduction of *Psa*. The risks of establishment and spread of *Psa* depend on the pathways on which *Psa* has entered Australia. The risks of establishment and spread have therefore been assessed separately for each of the two pathways. However, the assessment of potential consequences have been assessed as one for all the pathways considered here. Biosecurity Australia recognises that there are several different strains of the *Psa* bacterium, but has used the highly aggressive strain as a baseline to assess potential risk.

4.1 Pathway 1—Dormant cuttings

4.1.1 Probability of entry

Probability of importation

The likelihood that *Psa* will arrive in Australia with trade in dormant cuttings from countries where the pathogen is present is **HIGH**.

- *Psa* has been reported in association with *Actinidia* species (Serizawa and Ichikawa 1993a, b) and overwinters in infected plants (Serizawa *et al*. 1989). Therefore, propagative material can provide a pathway for the importation of *Psa* into Australia.
- *Psa* can remain latent for 2–3 years before displaying symptoms (Koh and Nou 2002). This may lead to the propagation and distribution of infected propagative material. Importation of infected propagative material led to the introduction of *Psa* into South Korea (Lee *et al.* 2005) and France (EPPO 2010). Therefore,

infected propagative material can provide a pathway for the importation of *Psa* into Australia.

- The primary conditions for survival of *Psa* are fulfilled by the presence of the live propagative material and the associated environmental conditions. Therefore, association with propagative material can provide long term survival for this bacterium.
- The introduction of *Psa* into South Korea (Lee *et al*. 2005) and France (EPPO 2010) through the importation of infected propagative material indicates that *Psa* is able to survive transport and storage.
- The detection of the aggressive strain in Italy is suggested to be the result of an introduction or independent evolution event in central Italy (Ferrante and Scortichini 2009).

Probability of distribution

The likelihood that *Psa* will be distributed in Australia in a viable state as a result of imported dormant cuttings from countries where the pathogen is present, is: **HIGH**.

- *Psa* arriving in Australia with dormant cuttings will not need to move from the import pathway to a suitable host as the bacterium is already within a suitable host.
- Dormant cuttings would be distributed to multiple destinations throughout Australia for propagation. The distribution of infected dormant cuttings commercially will facilitate the distribution of *Psa*.

Overall probability of entry (importation x distribution)

The overall probability of entry of *Psa* is determined by combining the probability of importation with the probability of distribution using the matrix of rules for combining descriptive likelihoods (Table 2.2).

- The likelihood that *Psa* will enter Australia with imported dormant cuttings from countries where the pathogen is present and transferred to a suitable host is: **HIGH**

4.1.2 Probability of establishment

The likelihood that *Psa*, having entered on imported dormant cuttings, will establish within Australia, based on a comparison of factors in the source and destination areas considered pertinent to its survival and reproduction, is: **HIGH**.

- Propagative material intended for ongoing propagation or horticultural purposes is deliberately introduced, distributed and aided to establish. This material will enter and then be maintained in a suitable habitat, potentially in substantial numbers and for an indeterminate period. Therefore, the introduction and establishment of plants from imported dormant cuttings in essence establishes the pathogens associated with the propagative material.
- Association of *Psa* with infected dormant cuttings provides a distinct epidemiological advantage to the bacterium as infected cuttings will result in infected shoots (Serizawa *et al*. 1989). This will result in the establishment of this bacterium in new areas. Additionally, dormant cuttings will be planted directly into regions suitable for kiwifruit production within Australia; environmental conditions are likely to be conducive to disease development and establishment.

- In spring and early summer, the pathogen develops in expanding shoots and leaves. Small cankers develop on extending vines, and leaves develop angular leaf spots. In winter and early spring, extending cankers form on trunks and branches (Serizawa *et al*. 1994). *Psa* is most invasive at relatively low temperatures (10–20 °C; optimum 15±3 °C), being almost completely inhibited above 25 °C (Serizawa and Ichikawa 1993a, b). These optimum temperatures for *Psa* occur during the kiwifruit growing season in Australia and would facilitate the establishment of *Psa* in Australia.
- The latent period of infection before visible symptoms appear may result in non-detection of these pathogens. *Psa* can remain latent for 2–3 years before displaying symptoms (Koh and Nou 2002) therefore, *Psa* will have ample time to establish into new areas.
- Various strains of *Psa* have established successfully in the kiwifruit growing regions of China, Italy, Japan, Portugal, South Korea (Lee *et al*. 2005; Balestra *et al*. 2010; Rees-George *et al*. 2010), France (EPPO 2010), New Zealand (Biosecurity New Zealand 2010) and Chile (SAG 2011). The current reported distribution of *Psa* suggests that there are similar environments in parts of Australia that would be suitable for the establishment of this bacterium.

4.1.3 Probability of spread

The likelihood that *Psa*, having entered on imported dormant cuttings, will spread within Australia, based on a comparison of those factors in the source and destination areas considered pertinent to the expansion of the geographic distribution of the pathogen, is: **HIGH**.

- If established through this pathway, the spread of *Psa* within Australia would rely on the ability of this bacterium to spread independently and in association with infected propagative material (Serizawa *et al*. 1989; Lee *et al*. 2005), contaminated pollen or pollinators (Biosecurity New Zealand pers. comm. 2010).
- *Psa* can spread both independently and in association with infected propagative material (Serizawa *et al*. 1989; Lee *et al*. 2005). Independent spread is facilitated by the production of bacterial ooze exudation on infected tissues (Serizawa *et al*. 1989) which become air-borne during rain and could spread through air currents (Serizawa *et al*. 1989). However, spread would be limited to the local area.
- *Psa* can also spread in association with infected propagative material (Serizawa *et al*. 1989; Lee *et al*. 2005) and as such, long distance spread is facilitated by the commercial distribution of infected planting material. The long latent period of infection (2–3 years) before visible symptoms appear (Koh and Nou 2002) may contribute to the inadvertent propagation and distribution of infected material that will help spread *Psa* within Australia.
- Infected dormant cuttings are unlikely to be grown in isolation, providing greater opportunity for the spread of *Psa* to other plants. Production of bacterial ooze exudation on infected tissues (Serizawa *et al*. 1989) serves as the primary inoculum, spreading the pathogen to healthy leaves and shoots under appropriate environmental conditions (Serizawa *et al*. 1989).
- Pollinators may also spread pollen contaminated with *Psa*. For example, pollen carried by bees has tested positive for *Psa* (Biosecurity New Zealand pers. comm. 2010), indicating a potential spread of the bacterium by pollinators.

- As a wound-infecting pathogen, it can also be transmitted on orchard equipment such as pruning implements (CABI 2007).
- The managed environment in Australian nurseries, garden centres and private gardens are all favourable for the natural spread of *Psa*. In the absence of statutory control *Psa* could spread quickly in Australia by trade of host propagative material.

Overall probability of entry, establishment and spread

The probability of entry, establishment and spread is determined by combining the probability of entry, of establishment and of spread using the matrix of rules for combining descriptive likelihood (Table 2.2).

- The likelihood that *Psa*, having entered on imported dormant cuttings, be distributed in a viable state to suitable hosts, establish in the PRA area and subsequently spread throughout Australia, is: **HIGH**.

4.2 Pathway 2—contaminated pollen

4.2.1 Probability of entry

Probability of importation

The likelihood that *Psa* will arrive in Australia with trade in contaminated pollen from countries where the pathogen is present, is: **HIGH**.

- Pollen has relatively few pests associated with it, compared with those that affect plants and seeds. There are no reports of arthropods, nematodes or insects being transmitted by pollen at any stage of their lifecycles. There are no pollen-transmitted bacteria (Card *et al*. 2007) but this does not exclude pollen contamination. *Psa* has been detected in pollen imported from Italy into New Zealand and in pollen collected in New Zealand (NZ Biosecurity 2010).
- Pollen is generally collected by vacuum extraction from open flowers or closed flowers, therefore it is likely that pollen may be contaminated with *Psa* (NZ Biosecurity 2010).
- *Psa* has been detected in pollen imported from Italy into New Zealand and pollen collected in New Zealand (Biosecurity New Zealand pers. comm. 2010). Therefore, contaminated pollen can provide a pathway for the importation of *Psa* into Australia.
- In 2010, *Psa* was detected from pollen collected in 2007 (Biosecurity New Zealand pers. comm. 2010), indicating the ability of the bacterium to survive over a long period of time. Therefore, association with pollen can provide long term survival for *Psa*.
- There is a risk that pollen imported for use in artificial pollination may be contaminated by live bacteria. Supplementing natural pollination with artificial pollination annually will increase the risk of introduction of *Psa*.

Probability of distribution

The likelihood that *Psa* will be distributed in Australia in a viable state as a result of imported contaminated pollen from countries where the pathogen is present, is: **LOW**.

- *Psa* arriving in Australia with contaminated pollen would be distributed to multiple destinations throughout Australia for use in artificial pollination. The

distribution of contaminated pollen commercially will facilitate the distribution of *Psa* in Australia.

- Considering artificial pollination practices, even a consignment of pollen with low levels of contamination is a risk; for pollen can be applied in aqueous suspension or spread dry mixed with talc (Hopping and Hacking 1983), both of which would facilitate dispersal and coverage by *Psa.*
- *Psa* infects the plant through natural apertures (stomata, lenticels) and wounds (EPPO 2010). Artificial pollination, in its application, is indiscriminant in coverage. It is not only directed at flowers, but is likely to contact all vine surfaces. Therefore, artificial pollination using *Psa* contaminated pollen would distribute the bacteria in the environment.
- There is no published scientific evidence for transmission of *Psa* through pollination or on the artificial pollination pathway despite the bacteria being detected in pollen consignments.

Overall probability of entry (importation x distribution)

The overall probability of entry of *Psa* is determined by combining the probability of importation with the probability of distribution using the matrix of rules for combining descriptive likelihoods (Table 2.2).

- The likelihood that *Psa* will enter Australia with imported contaminated pollen from countries where the pathogen is present and transferred to a suitable host is: **LOW**.

4.2.2 Probability of establishment

The likelihood that *Psa*, having entered on imported pollen, will establish within Australia, based on a comparison of factors in the source and destination areas considered pertinent to its survival and reproduction, is: **MODERATE**.

- The establishment of *Psa* entering Australia through contaminated pollen will depend upon the ability of this bacterium to infect host plants through natural apertures (stomata, lenticels) and wounds (EPPO 2010).
- Association of *Psa* with contaminated pollen provides a distinct epidemiological advantage to the bacterium as contaminated pollen will be used for artificial pollination. Even a slightly contaminated imported pollen, can be applied in aqueous suspension or spread dry after being cut with a separator like talc (Hopping and Hacking 1983), both of which facilitate increased dispersal and coverage by the contaminating bacteria on its preferred host.
- *Psa* is most invasive at relatively low temperatures (10–20 °C; optimum 15±3 °C), being almost completely inhibited above 25 °C (Serizawa and Ichikawa 1993a, b). These optimum temperatures for *Psa* occur during the kiwifruit growing season in Australia thus will facilitate the establishment of *Psa* in Australia.
- Artificial pollination practices may facilitate the entry of the bacterium in the host tissues. It is well documented that *Psa* infects the plant through stomata, hydathodes and wounds (Serizawa *et al*. 1989; EPPO 2010). Artificial pollination in its application is indiscriminant in coverage, not just directed at flowers but likely to contact all vine surfaces. Therefore *Psa* would have ample opportunity to infect and establish through natural openings of its preferred host.

- There is no published scientific evidence for transmission of *Psa* through the pollination or artificial pollination pathway. However, *Psa* has been detected in pollen (NZ Biosecurity 2010) thus supplementing artificial pollination annually will increase the risk of introduction and establishment.

4.2.3 Probability of spread

The likelihood that *Psa*, having entered on contaminated pollen, will spread within Australia, based on a comparison of those factors in the source and destination areas considered pertinent to the expansion of the geographic distribution of the pathogen, is: **HIGH**.

- If established through this pathway, the spread of *Psa* within Australia would rely on the ability of this bacterium to spread independently and in association with infected propagative material (Serizawa *et al*. 1989; Lee *et al*. 2005), contaminated pollen or pollinators (Biosecurity New Zealand pers. comm. 2010).
- Independent spread of *Psa* is facilitated by the production of bacterial ooze exudation on infected tissues (Serizawa *et al*. 1989) which become air-borne during rain and could spread through air currents (Serizawa *et al*. 1989; Koh and Nou 2002). However, spread would be limited to the local area.
- *Psa* can also spread in association with infected propagative material (Serizawa *et al*. 1989; Lee *et al*. 2005) and as such, long distance spread is facilitated by the commercial distribution of infected planting material. The long latent period of infection (2–3 years) before visible symptoms appear (Koh and Nou 2002) may contribute to the inadvertent propagation and distribution of infected material that will help spread *Psa* within Australia.
- Pollinators may also spread pollen contaminated with *Psa*. For example, pollen carried by bees has tested positive for *Psa* (Biosecurity New Zealand pers. comm. 2010), indicating a potential spread of the bacterium by pollinators.
- As a wound-infecting pathogen, *Psa* can also be transmitted on orchard equipment such as pruning implements (CABI 2007).
- *Psa* may have originated in Japan (Serizawa *et al*. 1989) and subsequently spread to South Korea (Koh and Lee 1992), Italy (Scortichini 1994), China (Li *et al*. 2004), Portugal (Balestra *et al*. 2010), France (EPPO 2010), New Zealand (BNZ2010) and Chile (SAG 2011). There are similarities in the natural and urban environments of these areas with those in Australia, which suggests that *Psa* could be capable of spread within Australia.
- The managed environment in Australian nurseries, garden centres and private gardens are all favourable for the natural spread of *Psa*. In the absence of statutory control *Psa* could spread quickly in Australia by trade of host propagative material.

Overall probability of entry, establishment and spread

The probability of entry, establishment and spread is determined by combining the probability of entry, of establishment and of spread using the matrix of rules for combining descriptive likelihood (Table 2.2).

- The likelihood that *Psa*, having entered on imported contaminated pollen, be distributed in a viable state to suitable hosts, establish in Australia and subsequently spread throughout Australia, is: **LOW**.

4.3 Consequences

The consequences of the entry, establishment and spread of *Psa* in Australia have been estimated according to the methods described in Tables 2.3.

The assessment of potential consequences is provided below.

Criterion	Estimate and rationale
Direct Impact	
Plant life or health	**Impact score: E** – Significant at the regional level. Strains of *Psa* can be quite destructive in kiwifruit and have become a major production constraint on kiwifruit production in Japan (Serizawa *et al.* 1989), South Korea (Koh and Lee 1992) and Italy (Scortichini 1994). • The bacterium overwinters in the stem, damaging the main vine structure. This phase has direct effects on yield by reducing the size of the productive vine (Serizawa *et al.* 1989). In spring the bacterium damages all the new season growth and this phase plays an important role in bacterial dispersal (Serizawa *et al.* 1989). • Typical symptoms on infected vines include the reddish exudation along the main trunk and branches (Ferrante and Scortichini 2010, Balestra *et al.* 2009a); browning of the buds and flowers, angular leaf spots, blight of young canes, and cankers on the twigs, leaders and trunks (Scortichini 1994; Balestra *et al.* 2009a); leaf wilting, twig die-back and plant wilting (Ferrante and Scortichini 2010). • Most Infected flowers turn brown and wither without opening. Infected flowers can open prematurely before petals have fully developed, then necrotic lesions develop on sepals (Serizawa *et al.* 1989), and fruits collapse before fully forming (Balestra *et al.* 2009b). Infected vine leaves shrivel and plants may die, or dying vines may produce vigorous suckers at the base of the trunk (Scortichini 1994). In the recent epidemic in Italy, *Psa* caused the death of branches on 3–5% of the plants present in the orchard (Ferrante and Scortichini 2009).
Other aspects of the environment	**Impact score: A** – Indiscernible at the local level. • There are no known direct consequences of *Psa* on the natural or built environment as the bacterium is limited to *Actinidia* species only.
Indirect Impact	
Eradication, control etc.	**Impact score: D** – significant at the district level If *Psa* was introduced to kiwifruit growing regions of Australia, variable costs of kiwifruit production would increase due to the need for changes in management strategies. The pathogen can destroy an orchard within a very short period of time; therefore, early detection is critical to control the pathogen (Koh and Nou 2002). • Programs to minimise the impact of *Psa* on kiwifruit are likely to be costly and include regular application of antibiotics, copper compounds or trunk injection of antibiotics to partially control the pathogen (Koh and Nou 2002). • Chemical control is considered to be unsuccessful after the symptoms appear (Koh and Nou 2002). Some strains of *Psa* have developed a resistance against antibiotics in some regions (Lee *et al.* 2005); antibiotics are not allowed to be used in agriculture in Australia. • An eradication campaign for *Psa*, should it be detected early, is likely to be expensive as it would require eradication of many infected plants. As a result of the latency period, removal of only symptomatic plants may allow nearby infected plants to remain in the kiwifruit orchard. Therefore, plants adjacent to symptomatic plants would also need to be removed.

Criterion	Estimate and rationale
Domestic trade	**Impact score: D** – Significant at district level • The presence of *Psa* in kiwifruit production areas is likely to result in some domestic movement restriction for host plants. Interstate restrictions on nursery stock and pollen for artificial pollination may lead to a loss of markets, which in turn would be likely to require industry adjustment.
International trade	**Impact score: D** – Significant at district level • Although *Psa* is present in most kiwifruit producing countries, it is absent from parts of the Americas. Restrictions on Australian exports of nursery stock to the Americas would be anticipated if *Psa* was to become established in Australia.
Environmental and non-commercial	**Impact score: B** – minor significance at the local level • Additional control activities may be required to control and/or eradicate this pathogen. However, this is not considered to have significant consequences for the environment.

Based on the decision rules described in Table 2.4, that is, where the consequences of a pest with respect to one or more criteria are '**E**', the overall consequences are estimated to be **MODERATE**

4.4 Unrestricted risk estimate

Unrestricted risk is the result of combining the probability of entry, establishment and spread with the outcome of overall consequences. Probabilities and consequences are combined using the risk estimation matrix shown in Table 2.5. The unrestricted risk estimation for *Psa* is summarised in Table 4.1.

Table 4.1: Unrestricted risk estimates of *Psa* for different pathways

Pathway	Overall probability of entry, establishment and spread	Consequences	Unrestricted risk
Nursery stock (dormant cuttings)	High	Moderate	Moderate
Contaminated pollen	Low		Low

The unrestricted risk for *Psa* has been assessed as 'moderate–low' which exceeds Australia's ALOP. Therefore, specific risk management measures are required for *Psa.*

5 Pest risk management

Pest risk management evaluates and selects risk management options to reduce the risk of entry, establishment or spread of quarantine pests identified with an unrestricted risk exceeding Australia's ALOP.

The detailed risk assessment identified *Actinidia* propagative material as a direct pathway for *Psa*. To effectively prevent the introduction of *Psa* associated with an identified pathway a series of important safeguards, conditions or phytosanitary measures must be in place. The proposed pest risk management measures for *Psa* on various pathways are summarised in Table 5.1.

Table 5.1: Proposed phytosanitary measures for *Psa* for different pathways

Pathway	Risk mitigation measure
Dormant cuttings	On-arrival inspection, fumigation, hot water treatment, sodium hypochlorite treatment and growth in PEQ
Tissue culture	On-arrival inspection and growth in PEQ
Pollen	Country or area freedom

Plant Biosecurity considers that the risk management measures proposed in this pest risk analysis will achieve Australia's ALOP. While the following measures are proposed by Plant Biosecurity, any other measure that provides an equivalent level of protection could be considered.

5.1 Existing risk management measures for propagative material

All imported nursery stock consignments are subject to the quarantine/biosecurity measures set out in Condition C7300 'General import requirements, nursery stock for all species'.

5.1.1 Existing policy to import *Actinidia* propagative material

Currently, there are no import conditions for *Actinidia* propagative material on the AQIS Import Conditions (ICON) Database. However, prior to the recent suspension of *Actinidia* propagative material, Australia's import policy conditions only allowed the entry of dormant cuttings. All consignments of *Actinidia* nursery stock imported (except from New Zealand) prior to 2010 were subjected to mandatory on-arrival inspection, fumigation and growth under a closed quarantine facility, at either a government or AQIS-approved PEQ facility with visual pathogen screening for three months.

After on-arrival inspection and fumigation, *Actinidia* budwood consignments from New Zealand with a Phytosanitary Certificate were released from quarantine without any further quarantine measures. However, consignments of *Actinidia* budwood from New Zealand without phytosanitary certification were subject to the same conditions for *Actinidia* species budwood from other countries.

Specific disease expression requirements and the long latency period (2–3 years) between infection and the expression of symptoms indicate that current requirements for propagative material, that is, growth in PEQ for a minimum of three months and visual inspection, are inadequate for *Psa*. Stronger mitigation measures are proposed to minimise the risk of *Psa* entering Australia in *Actinidia* propagative material from all sources.

5.2 Proposed risk management measures for *Actinidia* propagative material

The review of existing policy indicates that current requirements for propagative material are inadequate for the virulent strain of *Psa*. The PRA proposes strengthening of the existing policy for all countries and withdrawing New Zealand specific conditions. The proposed import conditions for *Actinidia* propagative material (dormant cuttings, tissue culture and pollen) are based on tiered safeguards. This process ensures that if one mitigating measure fails, other safeguards exist to ensure that the risk is progressively reduced and managed.

5.2.1 Proposed policy to import dormant cuttings

The proposed policy on *Actinidia* dormant cuttings comprises of:

- mandatory on-arrival inspection and fumigation
- mandatory sodium hypochlorite treatment by dipping; and
- mandatory hot water treatment;
- mandatory growth of newly established plants in closed government PEQ facilities with pathogen screening

Mandatory on-arrival inspection

All imported dormant cuttings require mandatory on-arrival visual inspection to verify freedom from disease symptoms, live insects, soil and other extraneous contaminants of quarantine concern. If diseased material is detected during on-arrival inspection, the pathogen should be identified.

On-arrival visual inspection may not detect latent infection caused by *Psa*. Reliance on on-arrival visual inspection only to detect pathogens is inefficient in the case of nursery stock, including *Actinidia* dormant cuttings. For this reason, visual inspection is not considered an appropriate measure to mitigate the risk posed by *Psa*. Therefore, additional risk management measures are required for *Psa*.

Mandatory on-arrival inspection is a standard measure applied to all imported nursery stock to manage the risk posed by disease symptoms, live insects, soil and extraneous contaminants. While mandatory on-arrival inspection will not specifically manage the risk posed by *Psa*, it is recommended to be implemented to manage these other quarantine risks.

Mandatory on-arrival fumigation

Mandatory on-arrival fumigation of dormant cuttings from all sources is proposed. Treatments for kiwifruit dormant cuttings other than methyl-bromide fumigation will be considered on a case by case basis by Plant Biosecurity if proposed by an exporting country. Prior to the acceptance of an alternative treatment for cuttings

Plant Biosecurity would need to assess the efficacy of that fumigant to ensure it gives an equal level of protection to methyl-bromide for all pests likely to be associated with the commodity.

Mandatory on-arrival fumigation may not be effective against pathogens, including *Psa*. Therefore, additional risk management measures are required for this pathogen.

Mandatory on-arrival fumigation is a standard measure applied to all imported nursery stock to manage the risk posed by arthropod pests. While mandatory on-arrival fumigation will not specifically manage the risk posed by *Psa*, it is recommended to be implemented to manage these other quarantine risks.

Mandatory sodium hypochlorite treatment

Imported *Actinidia* dormant cuttings must undergo sodium hypochlorite treatment (1% NaOCl for 10 minutes) for surface sterilisation. This risk management measure will be effective against superficial contaminating bacterial propagules.

Treatment with sodium hypochlorite alone may not be effective against an endophytic bacterial inoculum such as *Psa*. Therefore, additional mitigation measures are required for *Psa*.

Mandatory hot water treatment

Hot water treatment (HWT) is applied to minimise the risk of accidental introduction of pathogens. This risk management option is currently employed in Australia to reduce the risk of entry, establishment and spread of pathogens associated with *Citrus* and *Vitis* species budwood.

Psa is heat sensitive and displays symptoms at relatively low temperatures (10–20 °C); however, the optimum temperature for disease expression is 15±3 °C while temperatures above 25 °C completely inhibit the bacterium (Serizawa and Ichikawa 1993a, b).

It is recommended that imported dormant cuttings be subject to hot water treatment at 50 °C for 30 minutes (core temperature). However, hot water treatment alone may not be effective in eliminating *Psa* from the budwood. Therefore, additional mitigation measures are required for *Psa*.

Mandatory growth in PEQ facilities with pathogen screening

It is recommended that imported *Actinidia* dormant cuttings must be grown in closed government PEQ facilities under conditions that are conducive to symptom expression of *Psa* until the required pathogen screening/testing is complete. This increases the likelihood that *Psa* will be detected.

It is proposed that resultant plants from imported *Actinidia* propagative material must be grown at 15±3 °C for a minimum period of twelve months for visual observation of disease symptoms and until the required pathogen screening/testing is completed.

Pseudomonas syringae pv. *actinidiae* screening

Although visual assessment is an important method for screening pathogens, *Actinidia* plants may be infected and not display any obvious disease symptoms due to cultivar susceptibility, environmental conditions or other plant related factors. Therefore, in

addition to the observation for symptoms, Plant Biosecurity recommends molecular testing using polymerase chain reaction (PCR) to screen for *Psa.*

Polymerase chain reaction protocols have been developed to identify *Psa* (Koh and Nou 2002; Han *et al*. 2003c; Ferrante and Scortichini 2009; Ferrante and Scortichini 2010). It is proposed that testing for *Psa* using PCR should not be by direct extraction. The bacterium should be cultured first and then the PCR undertaken on the cultured medium (to ensure inoculum levels are high enough to confidently determine the identity of the bacterium).

5.2.2 Proposed policy to import *Actinidia* pollen

Pollen, unlike plants and seeds, has relatively few pests associated with it. There are no reports of arthropods or nematodes being transmitted by pollen at any stage of their lifecycles. There are no known pollen-transmitted bacteria (Card *et al*. 2007); however, *Psa* has been detected, potentially as a contaminant, in pollen imported from Italy into New Zealand and in pollen collected in New Zealand (Biosecurity New Zealand pers. comm. 2010). Therefore, *Psa* could enter Australia through pollen imported from known infected countries.

The proposed policy on *Actinidia* pollen comprises of:

- sourcing from countries free of *Psa*; or
- sourcing from pest free areas

Sourcing pollen from countries free of *Psa*

It is recommended that kiwifruit pollen be sourced from countries which are currently free of *Psa.* This requirement will apply to all countries regardless of the strain of *Psa* known to occur. This is due to the continued research being undertaken to identify strains and the need to ensure no new strains enter Australia.

Sourcing pollen from pest free areas

Area freedom is a measure that might be applied to manage the risk posed by *Psa* in pollen imported into Australia. The requirements for establishing pest free areas or pest free places of production are set out in ISPM No. 4: *Establishment of pest free areas* (FAO 1996) and ISPM No. 10: *Requirements for the establishment of pest free places of production and pest free production sites* (FAO 1999).

Before area freedom could be adopted as a phytosanitary measure it would be necessary for the exporting party to scientifically demonstrate the establishment, maintenance and verification of area freedom. Australia's evaluation and acceptance of this claim will be based on ISPM 4 or 10 guidelines (as appropriate) and must be consistent with Australia's ALOP. Failure to adequately establish, maintain or verify area freedom is likely to result in the risk of *Psa* in pollen.

Surveys and molecular testing required to establish a pest free area may be hampered by the time-lag between infection and *Psa* symptom expression (Koh and Nou 2002). Plants infected with *Psa* bacteria may be asymptomatic, in the initial stages of infection and could be easily overlooked. For this reason, any proposal for area freedom status will need to be assessed by Plant Biosecurity on a case by case basis.

Molecular testing for *Psa* in pollen is not definitive. False negatives are possible and could lead to the importation of *Psa* contaminated pollen for artificial pollination into Australia.

5.2.3 Proposed policy to import *Actinidia* tissue culture

The safest and preferred method for inter-country *Actinidia* germplasm movement is *in vitro* tissue cultures. *In vitro* techniques are effective in eliminating most fungal and bacterial pathogens. However, currently there are no import conditions for tissue cultures. To minimize the entry and establishment of *Psa* in Australia, effective testing procedures are required to ensure that imported tissue culture is free of *Psa*.

Tissues cultures represent an inherently lower risk than most other forms of nursery stock (e.g. cuttings) and, as such, require fewer phytosanitary measures. However, tissue cultures still require some form of quarantine measures as many pathogens are capable of surviving the tissue culturing process. The proposed policy is based on tiered safeguards, which ensures that if one mitigating measure fails, other safeguards exist to ensure that the risk is progressively reduced and managed.

The proposed policy for *Actinidia* species tissue cultures comprises:

- mandatory on-arrival inspection; and
- mandatory growth in closed government PEQ facilities with pathogen screening.

Mandatory on-arrival inspection

It is recommended that imported tissue cultures be subject to on-arrival AQIS inspection to verify freedom from fungal and bacterial contamination. The agar culture media must be clear and not contain antibiotics. If diseased material is detected during on-arrival inspection, sections of diseased material must be plated out for isolation and identification of the pathogen.

Mandatory growth in PEQ facilities with pathogen screening

It is recommended that imported cultures must be de-flasked and grown for a minimum of six months in a government PEQ station for pathogen screening. The tissue culture must be maintained in conditions suitable for disease expression and must undergo general disease screening and PCR testing.

6 Conclusion

The findings of this draft qualitative, pest-initiated pathway risk analysis report are based on a comprehensive analysis of relevant scientific and other appropriate literature on *Pseudomonas syringae* pv. *actinidiae* (*Psa*).

The pest risk analysis identified dormant cuttings, tissue cultures and pollen as potential pathways for the introduction of *Psa* strains currently not present in Australia. These potential pathways have an unrestricted risk that exceeds Australia's ALOP; therefore, risk management measures are required. The PRA proposes strengthening of the existing policy for all countries and withdrawing New Zealand specific conditions.

Plant Biosecurity considers that the risk management measures proposed in this draft report are adequate to mitigate the risks posed by *Psa*. The proposed risk management measures for the different propagative materials are:

Dormant cuttings

- Mandatory methyl-bromide fumigation on-arrival, hot water treatment (50 °C for 30 minutes), surface sterilisation (1% NaOCl for 10 minutes), then newly established plants are grown at 15±3 °C in closed government PEQ facilities for a minimum period of 12 months for visual observation; and
- Molecular testing techniques including polymerase chain reaction (PCR) test.

Pollen

- Pollen must be sourced from countries or areas demonstrated to be free of *Psa*.

Tissue culture

- Growth in closed government PEQ facilities at 15±3 °C for a minimum period of six months for visual observation; and
- Molecular testing techniques including polymerase chain reaction (PCR) test.

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