Nursery levy at work: **Building Resilience & Biosecurity Capacity**

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1 Purpose and background of this contingency plan

This contingency plan is designed to enhance Australia's capacity to respond to and manage an incursion of exotic Pseudomonas syringae pathovars and type species with special emphasis on production nurseries.

As this contingency plan was developed specifically for the Nursery and Garden Industry Australia (NGIA), it is focused on production nurseries covered by this association. In the event of an incursion, operations not covered by the NGIA (e.g. retail outlets) will not be eligible for Owner Reimbursement Costs, as defined in the Emergency Plant Pest Response Deed, if affected by actions carried out under an approved Response Plan.

2 Australian nursery industry

The Australian production nursery industry supports a number of horticultural sectors including urban horticulture, food supply via fruit and vegetable cropping, fibre production through forestry and the environment under land care and revegetation. Any industry reliant on planting healthy seedlings to produce a crop is underpinned by the production nursery industry. Production nurseries produce about 1-2 billion plants each year and have a farm gate value of about $2.4 billion (Nursery industry statistics – NY17008).

Endemic and exotic pests and diseases represent a major threat to the health, productivity and profitability of Australian production nursery businesses, as well as the industries they support. The nursery industry is particularly vulnerable compared to other horticultural industries, mainly due to the great diversity of plant species involved (>10 000 plant lines), and the multitude of pathogens and pests associated with these hosts. The extensive domestic and international movement of nursery stock through commercial trade also provides an ideal pathway for the spread of pests and diseases.

3 Impact of exotic *Pseudomonas syringae* pathovars and strains

The bacterial plant pathogen *Pseudomonas syringae* has impacted many crop and orchard industries with its various pathovars and type species. As an example, the kiwifruit industry in New Zealand has suffered huge losses since the first known outbreak of *P. syringae* pv. *actinidiae* back in 2007. At the time, New Zealand was second to Italy in the total volume of kiwifruit exports making an annual revenue of $NZ 1 billion, making it the most economically valuable export in the country. In 2014 this pathogen caused loss of exports as high as NZ$930 million. In addition to this, growers had to pay for treatments, and removal of infected vines, as well as suffering the loss of capital value in their orchards. There were reports of orchard values dropping from NZ$450,000/ha to $70,000/ha after the outbreak, which was the price of bare land. The total loss of equity for the country of New Zealand was as high as NZ$2 billion.

4 Eradication decision support matrix

Production nurseries are important as pathways for the potential entry and spread of exotic *P. syringae* pathovars, type species and strains. Following an outbreak of an exotic *P. syringae* disease, the response needs to be clearly explained, decisive, coordinated and rapidly implemented. Initially it will be assumed that eradication of the exotic pathovar or type species of *P. syringae* disease is possible; containment will be the second option. Containment measures will be based on the biology of the pathogen and the institutional and commercial structures in place for the management of plant disease outbreaks.

The decision matrix to aid in the decision between eradication and containment is shown in Figure 1 and Table 2.

Figure 1. Decision outline for the response to an exotic pest incursion and a summary of the basis on which each decision could be made.

Basis for technical feasibility: ○ Early detection ○ Confined space/restricted area of dispersal ○ Known distribution of host plants ○ Effective, reliable, quick detection method ○ Support from industries, businesses and communities involved.
Basis for economic feasibility: ○ Value of crop destroyed by uncontrolled pest is more than cost of controlling the pest ○ Value of environmental amenity (native species lost) vs cost or loss of other amenity (loss of native insects due to spraying in native forests etc)
Basis for quarantine containment: ○ Legislation to create a pest quarantine area (PQA) ○ Resources to maintain the PQA, inspection points, staffing, detection equipment, diagnostics ○ Support of industry and community to make the PQA work
Basis for destruction/control strategies required: ○ How much destruction and or control measures are industry and individuals prepared to undertake? ○ What level of destruction is technically feasible? ○ Do the benefits of destruction outweigh the problems created?
What would containment or ongoing management look like? ○ Is containment feasible? ○ What would ongoing management really mean? ○ Many similar features to eradication, but at less intense / restrictive levels.

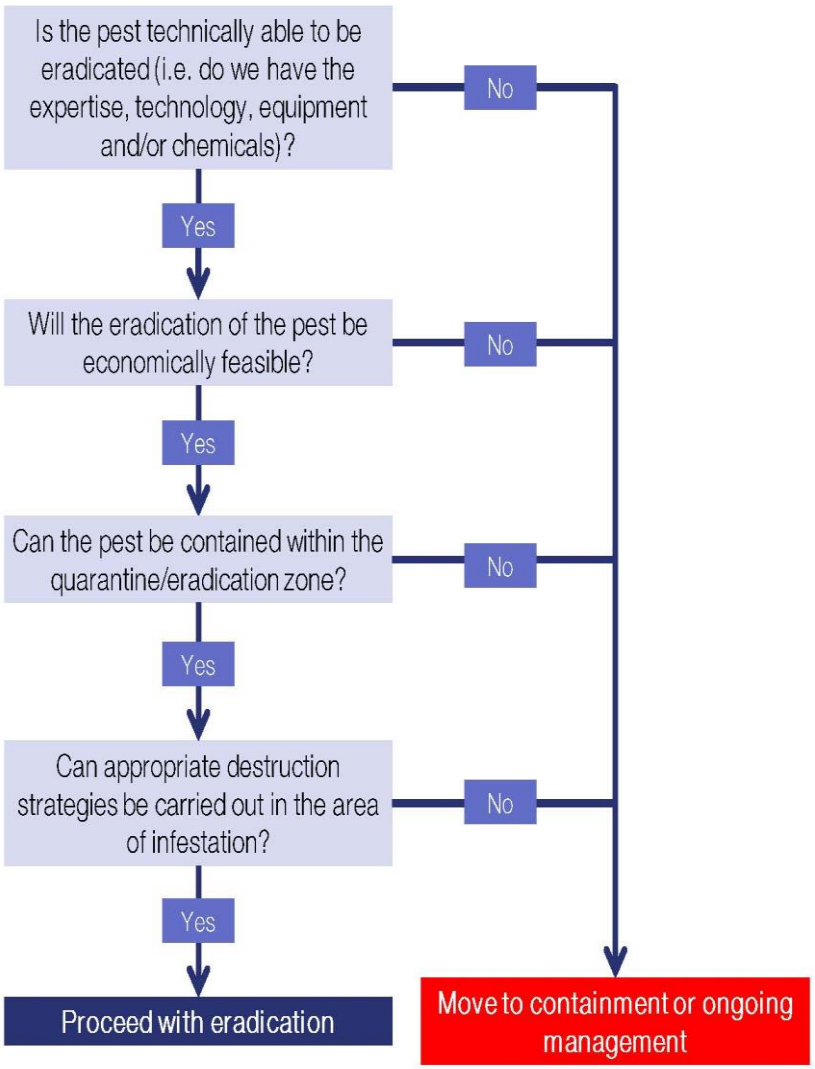


Table 2. Factors considered in determining whether eradication or alternative action will be taken for an Emergency Plant Pest (EPP) incident from PLANTPLAN (Plant Health Australia, 2016 Table 2).

a)	the capability to accurately diagnose or identify the EPP.
b)	the effectiveness of recommended control technique options, which are likely to be the most cost-effective in eradicating the EPP.
c)	the ability to remove or destroy all EPPs present by the recommended control techniques.
d)	the ability to remove the EPP at a faster rate than it can propagate until proof of freedom can be achieved.
e)	the recommended control techniques are publicly acceptable (taking into consideration cultural and social values, humaneness, public health impacts, non-target impacts and environmental impacts)
f)	whether Emergency Containment measures have been put in place by the Lead Agency(s).
g)	whether there are controls methods, commonly employed for endemic pests and diseases, that may limit or prevent the establishment or impact of the EPP.
h)	any legislative impediments to undertaking an emergency response.
i)	the resources e.g. chemicals, personnel etc. required to undertake an emergency response are accessible or available.
j)	the ability to delimit the known area of infestation.
k)	the ability to identify the pathway for entry into, and trace the spread of the EPP, within Australia.
l)	the ability to determine whether the likelihood of further introductions is sufficiently low.
m)	the dispersal ability of the EPP (that is, whether the EPP is capable of rapid spread over large distances).
n)	the capability to detect the EPP at very low densities for the purpose of declaring freedom, and that all sites affected by the EPP have or can be found.
o)	the ability to put in place surveillance activities to confirm Proof of Freedom for sites possibly infested by the EPP.
p)	whether community consultation activities have or will be undertaken.

5 Pest information/status

5.1 Pest details

Scientific name: *Pseudomonas syringae* complex including various pathovars and type species.

Domain	Bacteria
phylum	Proteobacteria
class	gamma subdivision
order	Pseudomonadales
family	Pseudomonadaceae
genus	*Pseudomonas*
species	*syringae*

The taxonomy of *Pseudomonas syringae* sensu lato has changed dramatically over the years and today this diverse group is considered a 'species complex' consisting of closely related identities that are not easy to distinguish due to recombination between them being sufficiently high to blur taxonomic boundaries. The species complex currently consists of type species and pathovars. Type species are isolates to which the scientific genus and species name of that organism is formally attached to a different *Pseudomonas* species under the rules of prokaryote nomenclature, even though it phylogenetically falls within the *P. syringae* species complex. Pathotype strains are similar to type strains but recognised as the species *P. syringae* with the addition of specific pathogen/host information.

Thakur et al., (2016) and Baltrus et al., (2017) split the *P. syringae* complex into approximately 64 type species and pathovars based on host range and pathogenic characteristics which form seven to 13 phylogoups, based on multilocus sequence and 16s rRNA analysis (Berg *et al.,* 2014; Hwang *et al.,* 2005; Sarkar and Guttman 2004; Young, 2010) (Tables 3 and 4).

Around the same time Gomila et al (2017) proposed the *P. syringae* complex consists of 19 putative species within 6 genomic groups (Table 5). Phylogenetic trees produced from whole genome analysis show placement of pathovars and type species within these (Figure 2).

More recently, Dillon et al., (2019) analysed the genomes of a diverse collection of 391 *P. syringae* complex strains representing 11 of the 13 *P. syringae* proposed phylogroups. Their phylogenetic trees show phylogroups were supported but that host families were not consistent with this, sometimes being spread over more than one phylogroup (Figure 3). Plant pathogens were distributed between phylogroups 1, 2, 3, 4, 5, 6, 7 and 11, with the remaining phylogroups (8, 9, 10, 12 ad 13) made up of strains isolated from environmental substrates (Tables 3, 4 and 5).

Formally, the *P. syringae* species complex currently includes the following 15 closely related plant pathogenic 'type species'; *P. amygdali, P. austuriensis, P. avellanae, P. cannabina, P. caricapapayae, P. caspiana, P. cerasi, P. cichorii, P. congelans, P. fiscuserectae, P. meliae, P. savastanoi, P. syringae, P. tremae and P. viridiflava*. However, some of these species are quite similar at the genetic

level and are consequently proposed to be synonyms (e.g. *P. amydagdali, P. meliae, P. savastanoi* and *P. fiscuserectae*), some are not monophyletic (e.g. *P.syringae* and *P. viridiflava*) and distinct names have not historically been assigned based on uniform criteria.

Although the type species, pathovars and phylogeny of the *Pseudomonas syringae* species complex are still under review, for the purposes of this document, the pathovars and type species named in Tables 3 and 4 will be considered to belong to the *P. syringae* complex as this represents the most comprehensive list of isolates for the species complex.

Table 3: Worldwide *Pseudomonas syringae* pathovars and type species from Thakur (2016) and Baltrus (2017)

Pseudomonas species	Pathovar	Phylogroup	States reported to be present in Australia[1]	Original host common name	Original host scientific name
P. syringae	*antirrhini*	1	NSW	Snapdragon	*Antirrhinum majus*
P. syringae	*apii*	1	WA	Celery	*Apium graveolens* var. dulce (mill.) Pers.
P. syringae	*berberidis*	1		Barberry	*Berberis* sp.
P. syringae	*delphinii*	1		Delphinium	*Delphinium* sp.
P. syringae	*maculicola*	1	NSW, QLD, VIC, WA	Cauliflower	*Brassica oleraceae* var. *Botrytis*
P. syringae	*philadelphi*	1		Mock orange cv aureus	*Philadelphus coronarius*
P. syringae	*spinaceae*	1		Spinach	*Spinacia oleracea*
P. syringae	*theae*	1		Tea	*Camellia sinensis*
P. syringae	*tomato*	1	NSW, QLD, VIC	Tomato	*Lycopersicon esculentum*
P. syringae	*viburni*	1		Viburnum	*Viburnum* sp.
P. syringae	*aceris*	2		Maple	*Acer* sp.
P. syringae	*aptata*	2	NSW QLD	Sugar beet	*Beta vulgaris*
P. syringae	*atrofaciens*	2		Wheat	*Triticum aestivum*
P. syringae	*coryli*	2		European hazelnut	*Corylus avellana*
P. syringae	*lapsa*	2		Corn hybrid	*Zea* sp.
P. syringae	*papulans*	2		Apple	*Malus* x *domestica* Borkh.
P. syringae	*solidagae*	2		Tall golden rod	*Solidago altissima*
P. syringae	*syringae*	2	NSW, SA, QLD, VIC, TAS	Lilac	*Syringa vulgaris*
P. congelans		2		Grass phyllosphere	NA
P. syringae	*aesculi*	3		Horse chestnut	*Aesculus indica* Colebr.
P. syringae	*broussonetiae*	3		Paper mulberry	*Broussonetia kazinoki* Sieb. X *B. papyrifa* Vent.
P. syringae	*castaneae*	3		Japanese chestnut	*Castanea crenata* Sieb. & Zucc.
P. syringae	*cerasicola*	3		Cherry	*Prunus* x *yedoensis*
P. syringae	*ciccaronei*	3		Carob	*Creatonia siliqua*
P. syringae	*cunninghamiae*	3		Chinese fir	*Cunninghamia lanceolata*

Pseudomonas species	Pathovar	Phylogroup	States reported to be present in Australia[1]	Original host common name	Original host scientific name
P. syringae	*daphniphylli*	3		Himeyuzuriha	*Daphniphyllum teijsmanni* Zoll.
P. syringae	*dendropanacis*	3		Kakuremino	*Dendropanax trifidus*
P. syringae	*eriobotryae*	3	NSW	Laquot	*Eriobotrya japonica*
P. savastanoi	*fraxini*	3		European ash	*Fraxinus excelsior*
P. savastanoi	*glycinea*	3	NSW, QLD	Soybean cv. Disoy	*Glycine max*
P. syringae	*hibisci*	3		Chinese hibiscus	*Hibiscus rosa-sinensis*
P. syringae	*lachrymans*	3	NSW, QLD	Cucumber	*Cucumis sativus*
P. syringae	*mellea*	3		Tobacco	*Nicotiana tabacum*
P. syringae	*mori*	3	NSW, QLD	White mulberry	*Morus alba*
P. syringae	*myricae*	3		Chinese bayberry	*Myrica rubra*
P. savastanoi	*nerii*	3		Oleander	*Nerium oleander*
P. savastanoi	*phaseolicola*	3	NSW, QLD, VIC, WA	Bean	*Phaseolus vulgaris*
P. syringae	*photiniae*	3		Japanese photina	*Photina glabra*
P. savastanoi	*retacarpa*	3		Broom bus	*Retama sphaerocarpa*
P. syringae	*rhaphiolepidis*	3		Sharinbai	*Rhaphiolepis umbellata*
P. savastanoi	*savastanoi*	3	NSW, SA	Olive	*Lolea europaea*
P. syringae	*sesame*	3		Sesame	*Sesamum indicum*
P. syringae	*tabaci*	3	NSW, QLD	Tobacco	*Nicotiana tabacum*
P. syringae	*ulmi*	3		Elm	*Ulmus* sp.
P. amygdali		3		Almond	*Prunus dulcis*
P. ficuserectae		3		Inubiwa	*Ficus erecta*
P. meliae		3		Chinaberry	*Melia azedarach*
P. syringae	*atropurpurea*	4		Italian ryegrass	*Lolium multiflorum* Lam.
P. syringae	*garcae*	4		Coffee	*Coffea arabica*
P. syringae	*oryzae*	4		Rice cv. Akihikari	*Oryza sativa*
P. syringae	*porri*	4	QLD, SA, VIC, WA	Leek	*Allium porrum*

Pseudomonas species	Pathovar	Phylogroup	States reported to be present in Australia[1]	Original host common name	Original host scientific name
P. tremae	*tremae*	4		Urajiroenoki	*Trema orientalis* Blume
P. syringae	*zizaniae*	4		Wild rice	*Zizania aquatica*
P. syringae	*alisalensis*	5		Brococoli raab	*Brassica rapa* subsp. *rapa*
P. syringae	*coriandricola*	5	NSW, VIC	Cilantro	*Coriandrum sativum*
P. cannabina	*cannabina*	5		Indian hemp	*Cannabis sativa*
P. syringae	*helianthi*	6	WA	Sunflower	*Helianthus annuus*
P. syringae	*tagetis*	6	NSW, QLD	African marigold	*Tagetes erecta*
P. caricapapayae		6		Pawpaw, papaya	*Carica papaya*
P. syringae	*primulae*	7		Polyanthus	*Primula* sp.
P. syringae	*ribicola*	7		Golden currant	*Ribes aureum*
P. viridiflava		7	NSW, QLD, TAS, VIC, WA	Bean	*Phaseolus vulgaris*

[1]Pathovars and type species belonging to the *Pseudomonas syringae* complex recorded as being in Australia according to APPD and EPPO records. Note there is an additional 330 plus records of *Pseudomonas syringae* not identified to pathovar in APPD. A blank cell indicates that it has not recorded as being in Australia according to APPD and EPPO.

Table 4. Additional worldwide *Pseudomonas syringae* complex type species and pathovars not listed in Thakur (2016) and Baltrus (2017)

Pseudomonas species	Pathovar	Phylogroup	State reported to be present in Australia[1]	Common Host name	Scientific Host name
P. syringae	*actinidiae*	1	VIC	Kiwifruit	*Actinidia delicosa*
P. syringae	*actinidifoliorum*	1	VIC, WA	kiwifruit	*Actinidia delicosa*
P. syringae	*avii*	1		Cherries	*Prunus avium*
P. syringae	*morsprunorum*	1 & 3	NSW	Plum	*Prunus* sp.
P. syringae	*passiflorae*	1	Australia	Passionfruit	*Passiflora edulis*
P. syringae	*persicae*	1		nectarine, peach	*Prunus persica, P. salicina*
P. avellanae		1 & 2		Hazelnut	Corylus avellana
P. syringae	*dysoxylii*	2		Kohekohe	*Dysoxylum spectabile*
P. syringae	*pisi*	2	NSW, TAS, VIC, WA	Pea	*Pisum sativum*
P. cerasi		2		Cherry	*Prunus sp.*
P. syringae	*averrhoi*	3		Star fruit	*Averrhoa carambola*
P. syringae	*loropetali*	3		Fringe flower	*Loropetalum chinensis*
P. syringae	*allii*	4		Onion	*Allium* spp.
P. syringae	*alliifistulosi*	4		Onion	*Allium* spp.
P. coronafaciens		4		Oat, Corn	*Avena sativa, Zea mays*
P. asturiensis		7		Soybean	*Glycine max*
P. cichorii		11	NSW, NT, QLD, VIC, WA	Chicory	*Cichorium intybos*
P. caspiana		UD		Citrus	*Citrus* spp.

UD = undetermined

[1]Pathovars and type species belonging to the *Pseudomonas syringae* complex recorded as being in Australia according to APPD and EPPO records. Note there is an additional 330 plus records of *Pseudomonas syringae* not yet identified to pathovar level in APPD. A blank cell indicates that it has not recorded as being in Australia according to APPD and EPPO.

Pseudomonas syringae valid pathovar names are listed on the following website:
https://www.isppweb.org/names_bacterial_pant2005.asp

Table 5. Gomila et al (2017) Proposed the *Pseudomonas syringae* complex consists of 19 putative species within 6 genomic groups

Genomic Branch	Phylogenomic group	Phylogenomic species and representative strains	Number of strains assessed
I	2	*P. congelans*	3
	2	*P. syringae*	9
	2	*P. cerasi*	1
	2	Species A (strain B728a)	2
II	1	*P. avellanae*	13
	1	*P. tomato'* (DC3000)	7
	5	*P. cannabina*	3
	5	*P. coriandricola'* (ICMP 12471)	1
	5	Species B (strain CC1583)	2
	4	*P. coronafaciens'* (LMG5060)	11
IV	3	**P.amygdali, (= P. melliae, = P. savastonoi, = P. ficuserectae)*	57
	6	*P. caricapapayae*	3
V	7	*P. asturiensis*	1
	7	Species C (strain CC1417)	2
	7	*P. viridiflava*	7
VI	11	*P. cichorii*	1
	UD	Species D (strain UB246)	1
	UD	*P. caspiana*	1
	UD	Species E (strain S25)	1

*Study confirmed *P. melliae*, *P. savastonoi* and *P. ficuserectae* are later synonyms of *P. amygdali*, and that '*P. coronafaciens*' should be revived as a nomenspecies.

Red phylogroup assigned by assessing position in Gomila (2017) compared to those known from Baltrus (2017). Gomila (2017) phylogenomic branch VI is additional to those of Thakur (2016) and Baltrus (2017).

UD = undetermined

Figure 2. Phylogeny of Gomila et al (2017) 19 putative species within 6 genomic groups

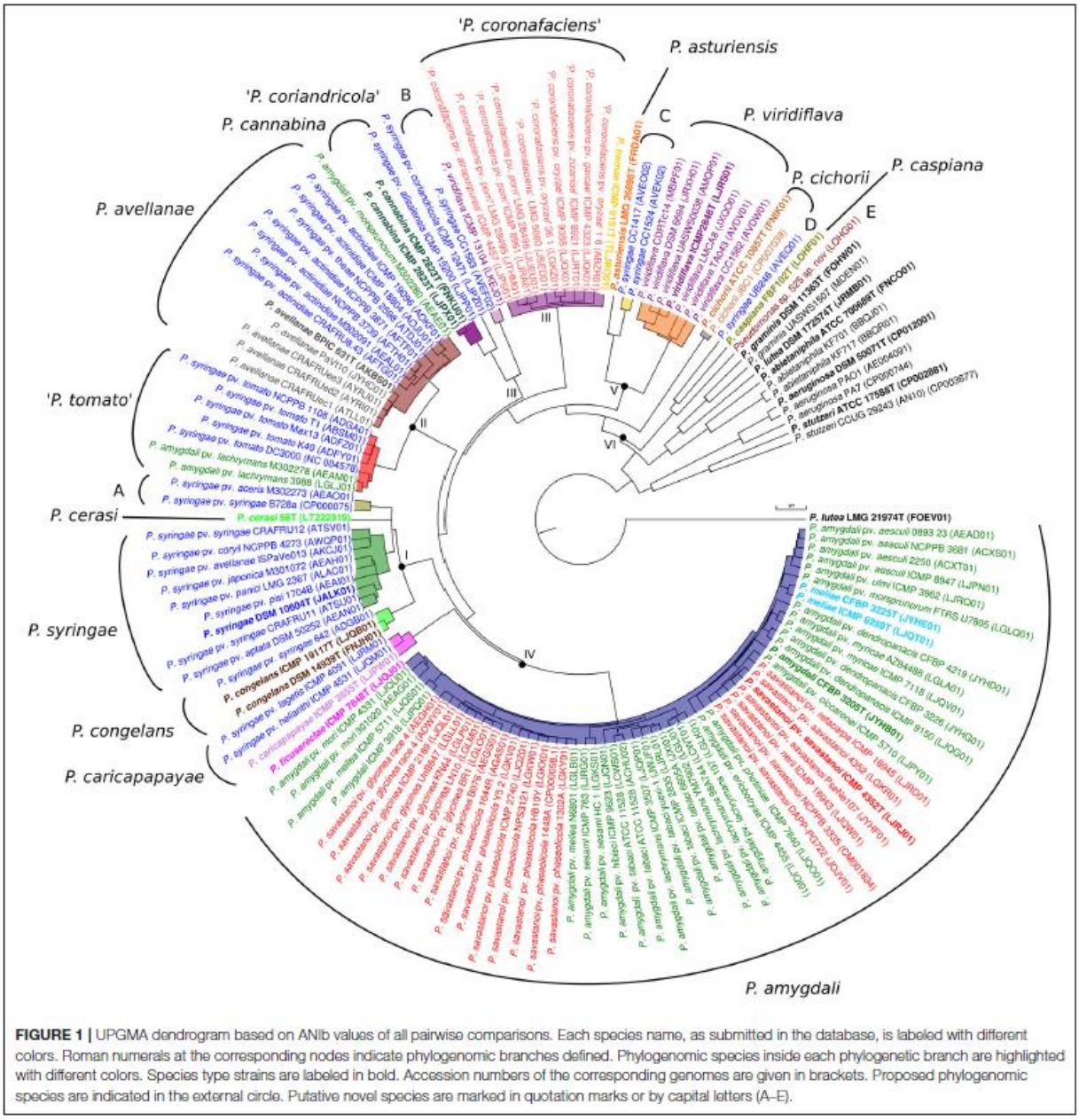


FIGURE 1 | UPGMA dendrogram based on ANIb values of all pairwise comparisons. Each species name, as submitted in the database, is labeled with different colors. Roman numerals at the corresponding nodes indicate phylogenomic branches defined. Phylogenomic species inside each phylogenetic branch are highlighted with different colors. Species type strains are labeled in bold. Accession numbers of the corresponding genomes are given in brackets. Proposed phylogenomic species are indicated in the external circle. Putative novel species are marked in quotation marks or by capital letters (A–E).

Figure 3. From Dillon et al., (2019), Phylogeny of *Pseudomonas syringae* complex depicting 11 of 13 phylogroups and host range.

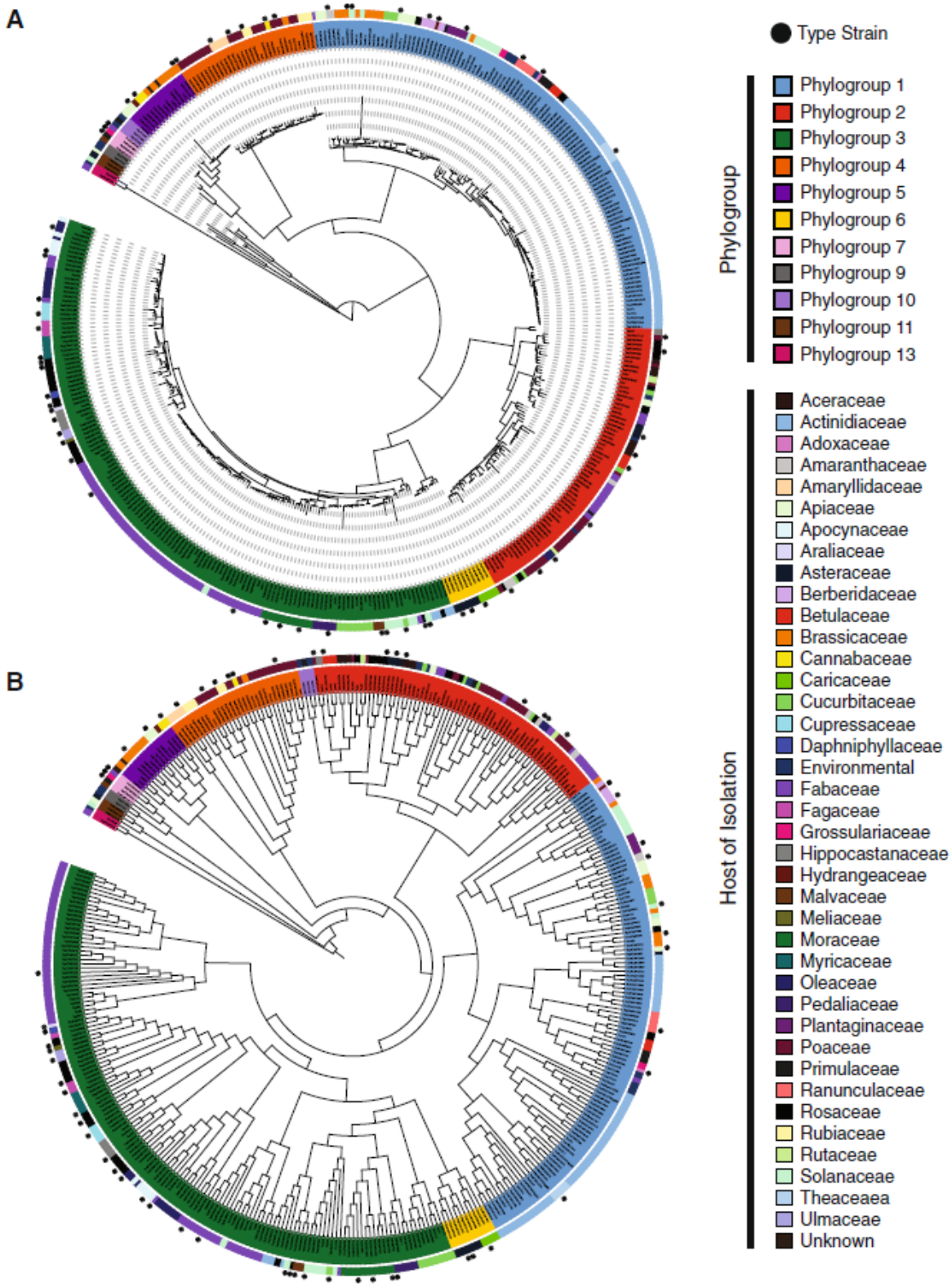


Fig. 2 Core (**a**) and pan (**b**) genome phylogenies of *Pseudomonas syringae* strains. The core genome, maximum-likelihood tree was generated from a core genome alignment of the 2410 core genes present in at least 95% of the *P. syringae* strains analyzed in this study. The pan-genome tree was generated by hierarchical clustering of the gene content in each strain using the Jaccard coefficient method for calculating the distance between strains and the Ward hierarchical clustering method for clustering. Strain phylogroups, hosts of isolation, and whether the strain is a type or pathotype strain are shown outside the tree

5.2 Biology of pathogenic species types and pathovars

For annual hosts, *P. syringae* overwinters on seed and infected crop debris. Groundcover and weeds within and outside a field represent another potential source of inoculum of *P. syringae*. In particular, when annual crops are harvested and no leaf surface of the crop is available, the pathogen can shift to weeds and groundcover where it multiplies, thereby ensuring a constant source of inoculum. The bacterium is transferred onto healthy plants via water splash from irrigation or rain. Once on a host it can enter the plant via natural openings or wounds. Bacteria are highly dependent on natural openings and wounds to access internal parts of plant tissue. Overall, stomata, hydathodes and trichomes are the most important entry ports of annual plants (Agrios 2005). In addition, the wounds caused by biotic (humans, insects, animals) and abiotic factors (hailstones, frost) are another important port of entry. Consequently, infection usually occurs following heavy rain, frost or physical damage.

After entering the host tissue, the bacterium spreads through the intercellular spaces and enters parenchyma cells of the cortex and pith. Bacteria-filled cavities are formed, and vascular tissues are also attacked from where the bacteria may spread to stipules, leaflets and the insides of pods and, via the funicle and micropyle, into the seed. Seeds may also become infected at harvest by contact with diseased material. Contaminated seed represents the most important source of primary inoculum for a large number of plant species. Both external and internal contamination can lead to disease development although internal contamination might result in greater disease severity. Seed-borne pathogens are introduced into new regions on contaminated seeds but may also be introduced through edible infested leafy vegetables such as spinach that are harvested and traded worldwide. Seed infestation is the main cause of long-distance pathogen dissemination and is the most common pathway for a nursery infestation. Although, introduction of infected plants and inadequate disinfestation after an infestation can also provide sources of inoculum.

Foliar disease symptoms are very common on annual plants although P. syringae can also cause symptoms on fruits of bean, cantaloupe, cucumber, okra and tomato. Bacterial cells ooze from these symptomatic tissues and spread to healthy plants via water splash (rain or irrigation), wind, insects, tools and machinery (Lamichhane et al., 2015).

The phylloplane of almost all annual plants are colonized by this pathogen. Hence, epiphytic populations on asymptomatic plants are the immediate source of inoculum for disease development.

For perennials, *P. syringae* overwinters on infected plant tissues such as regions of necrosis or gummosis (sap oozing from wounds on the tree) but can also overwinter in healthy looking plant tissues. In the spring, water from rain or other sources will wash the bacteria onto leaves and blossoms where it will grow and survive throughout the summer. This is the epiphyte phase of the *P. syringae* life cycle where it will multiply and spread but will not cause a disease. Once it enters the plant through a wound, leaf's stomata or necrotic spots on either leaves or woody tissue, then the disease will start. The pathogen will then exploit and grow in intercellular spaces causing the leaf spots and canker symptoms typical of *P. syringae*.

This bacterium can survive in temperatures slightly below freezing. These below freezing temperatures increase the severity of infection within trees like sour cherry, apricot, and peach.

Disease by *P. syringae* tends to be favoured by wet, cool conditions—optimum temperatures for disease tend to be around 12–25 °C, although this can vary according to the pathovar or type species involved. The bacteria tend to be seed-borne and are dispersed locally between plants by rain splash.

Although it is a plant pathogen, it can also live as a saprotroph in the phyllosphere when conditions are not favourable for disease. Some saprotrophic strains of *P. syringae* have been used as biocontrol agents against pathogenic strains causing postharvest rots (Figure 4).

Mechanisms of pathogenicity

- The mechanisms of *P. syringae* pathogenicity can be separated into several categories: ability to invade a plant, ability to overcome host resistance, biofilm formation, and production of proteins with ice-nucleating properties.

Ability to invade plants

- Planktonic *P. syringae* is able to enter plants using its flagella and pili to swim towards a target host. It must enter the plant via wounds or natural opening sites, as it is not able to breach the plant cell wall. An example of this is the partnership with the leaf-mining fly *Scaptomyza flava*, which creates holes in leaves during oviposition that the pathogen can use to enter the plant. The role of taxis in *P. syringae* has not been well-studied, but the bacteria are thought to use chemical signals released by the plant to find their host and cause infection.

Overcoming host resistance

- *Pseudomonas syringae* isolates carry a range of virulence factors called type III secretion system (T3SS) effector proteins. These proteins primarily function to cause disease symptoms and manipulate the host's immune response to facilitate infection. The major family of T3SS effectors in *P. syringae* is the *hrp* gene cluster, coding for the Hrp secretion apparatus.
- The pathogens also produce phytotoxins which injure the plant and can suppress the host immune system. One such phytotoxin is coronatine, found in pathovars *P. syringae* pv. *tomato* and *P. syringae* pv. *glycinea*.

Biofilm formation

- *Pseudomonas syringae* produces polysaccharides which allow it to adhere to the surface of plant cells. It also releases quorum sensing molecules, which allows it to sense the presence of other bacterial cells nearby. If these molecules pass a threshold level, the bacteria change their pattern of gene expression to form a biofilm and begin expression of virulence-related genes. The bacteria secrete highly viscous compounds such as polysaccharides and DNA to create a protective environment in which to grow.

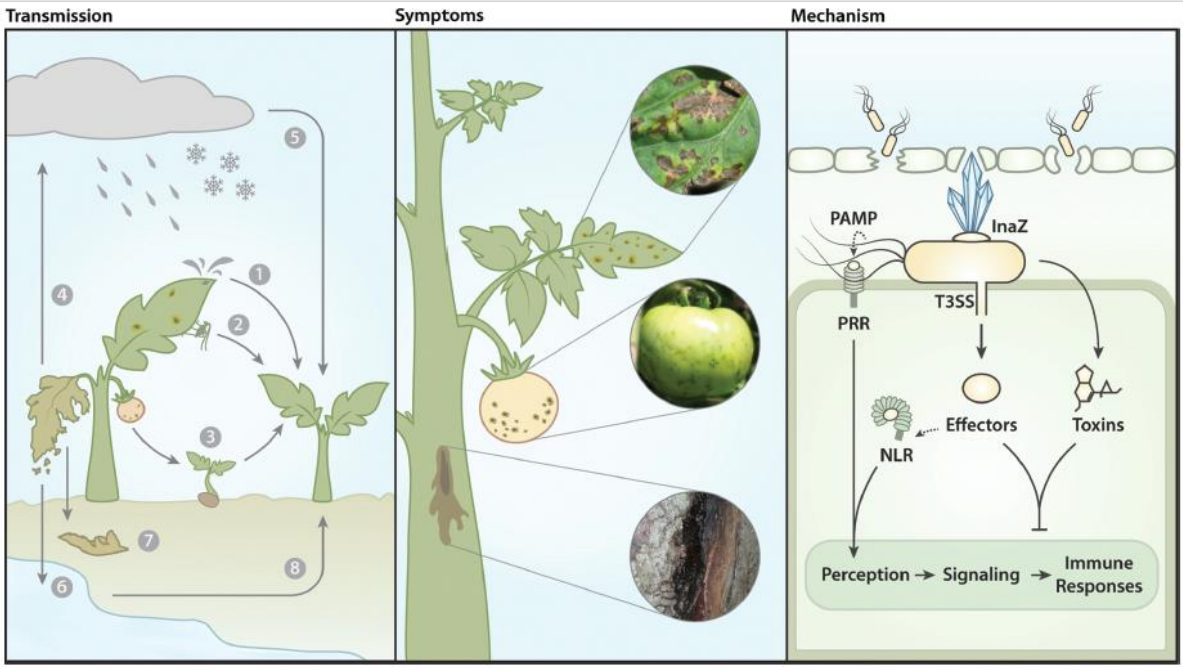
Ice-nucleating properties

- *Pseudomonas syringae*—more than any mineral or other organism—is responsible for the surface frost damage in plants exposed to the environment. For plants without antifreeze proteins, frost damage usually occurs between -4 and -12 °C as the water in plant tissue can remain in a supercooled liquid state. *P. syringae* can cause water to freeze at temperatures as high as −1.8 °C, but strains causing ice nucleation at lower temperatures (down to −8 °C) are more common. The freezing causes injuries in the epithelia cell layer and makes the nutrients in the underlying plant tissues available to the bacteria.
- *Pseudomonas syringae* has INA (ice nucleation-active) genes that make INA proteins which translocate to the outer bacterial membrane on the surface of the bacteria, where the proteins

act as nuclei for ice formation. Artificial strains of *P. syringae* known as ice-minus bacteria have been created to reduce frost damage.

- *Pseudomonas syringae* has been found in the centre of hailstones, suggesting the bacterium may play a role in Earth's hydrological cycle.

Figure 4: Disease transmission, symptoms and mechanism for pathogenicity of *Pseudomonas syringae* from Arnold and Preston (2019)



Graphical abstract

The lifecycle and pathogenicity mechanisms of *Pseudomonas syringae*. **Transmission**: *P. syringae* can be disseminated by rainsplash, aerosols and airborne plant particles (1), insect vectors (2) or as a seed-borne pathogen (3). If carried into the atmosphere (4), ice-nucleating *P. syringae* strains can contribute to ice nucleation in clouds and be disseminated in snow or rainfall (5). *P. syringae* can also be disseminated through terrestrial water systems (6, 8) and through plant debris in soil (7, 8). **Symptoms**: *P. syringae* infections are commonly characterized by chlorosis and necrosis of leaves, stem tips, buds and flowers (top); by necrotic lesions and delayed ripening or altered development of fruit (middle); and by cankers and galls of woody tissues (bottom). **Mechanism**: *P. syringae* enters plant tissues through wounds and natural openings such as stomata. Some strains of *P. syringae* can increase frost damage to plant tissues through ice nucleation promoted by proteins such as InaZ. Pathogen-associated molecular patterns (PAMPs) produced by *P. syringae*, such as flagellin, are recognized by plant pattern recognition receptors (PRRs), triggering the induction of plant immune responses. *P. syringae* counters plant immune responses through the production of toxins, and the secretion of effector proteins via a type III secretion system (T3SS). Effector proteins and toxins disable or subvert plant immune responses and alter plant metabolism and physiology to promote *P. syringae* infection. Effectors can also be directly or indirectly recognized by plant immune receptors, notably nucleotide-binding domain and leucine-rich repeat region (NLR)-containing proteins, thereby triggering plant immune responses. Picture: Nattapong Sanguankiattichai.

5.3 Dispersal

Pseudomonas syringae can be transmitted and dispersed locally via water splash (e.g. rain and irrigation), aerosols, airborne plant particles, terrestrial water systems and plant debris in the soil. It can be dispersed longer distances via infected plant material, insect vectors, pollen and infected seed (i.e. seed borne).

5.4 Affected hosts

5.4.1 Host range

Each *P. syringae* type and pathovar mostly has its own unique and narrow host range, with the exception of *P. syringae* pv. *syringae* which can infect over 180 different monocot and dicot plant species. The complex has been split into 13 distinct phylogenetic groups (phylogroups), based on multi-locus sequence typing, although the exact breadth of the species complex (and hence the number of phylogroups) is not universally agreed upon. Host range families within these phylogenetic groups is diverse. The ability of type/pathovars to produce various groups of effector proteins is better aligned with their phylogeny, although not always consistent within phylogenetic groups.

A combination of the pathogen's effector genes and the plant's resistance genes is thought to determine which host species a particular type/pathovar can infect. Plants can develop resistance to a type/pathovar by recognising pathogen-associated molecular patterns (PAMPs) and launching an immune response. These PAMPs are necessary for the microbe to function, so cannot be lost, but the pathogen may find ways to suppress this immune response, leading to an evolutionary arms race between the pathogen and the host.

Table 6. *Pseudomonas syringae* pathovars and types NOT reported in Australia and industry they could impact based on known host range. Note that almost all of these are relevant to the production nursery industry (excluding grains), though some are very minor crops.

Pseudomonas species	Pathovar	Geographic distribution	Host common name	Host value in Australia
P. syringae	*aceris*	USA	Maple	Ornamental tree
P. syringae	*aesculi*	India	Horse chestnut	Ornamental tree with edible nuts
P. syringae	*allii*	South Africa	Onion	4th largest vegetable crop. In 2017–18, onion production was valued at $192 million (LVP) with fresh exports valued at $21.7 million.
P. syringae	*alliifistulosi*	Japan	Onion	as above
P. syringae	*alisalensis*	USA	Crucifers (in particular broccoli, broccoli Raab), grasses, tomato	10th largest vegetables crop. gross value of $101.2 million in 2008/09
P. asturiensis		Spain		
P. syringae	*atrofaciens*	NZ	Wheat	Major crop with gross annual value of $6 billion p.a.
P. syringae	*atropurpurea*	Japan	Italian ryegrass	Ryegrass seed 0.1 Million USD p.a.
P. avellanae				
P. syringae	*averrhoi*	Taiwan		
P. syringae	*avii*	France		
P. syringae	*berberidis*	NZ, England	Barberry	Weed and berries
P. syringae	*broussonetiae*	Japan	Paper mulberry	Ornamental tree
P. caspiana				
P. syringae	*castaneae*	Japan	Japanese chestnut	The farm gate value of production in 2016 was valued at A$12.5 million. This is expected to grow to more than A$16 million by 2025
P. cerasi		Poland		
P. syringae	*cerasicola*	Japan	Cherry	$164m of Australian cherry sales (for year ending June 2016)
P. syringae	*ciccaronei*	Italy	Carob, olive	Food additive, stock feed, pet food thickener. Over 10 million p.a. 1998. Olive oil industry.
P. syringae	*coryli*	Italy	European hazelnut	Ornamental tree with edible nuts
P. syringae	*cunninghamiae*	China	Chinese fir	Ornamental tree
P. syringae	*daphniphylli*	Japan	Himeyuzuriha	Ornamental tree
P. syringae	*delphinii*	NZ	Delphinium	Ornamental flowers
P. syringae	*dendropanacis*	Japan	Kakuremino	Ornamental tree
P. syringae	*dysoxylii*	NZ		
P. savastanoi	*fraxini*	Austria, France, Netherlands, Russia, Serbia, Switzerland, UK	European ash	Ornamental tree
P. syringae	*garcae*	Brazil	Coffee	Small export industry in QLD and NSW
P. syringae	*hibisci*	USA	Chinese hibiscus	Ornamental and hedging
P. syringae	*lapsa*	-	Corn hybrid	25-35 million p.a., mostly domestic market.

P. syringae	*loropetali*	USA		
P. syringae	*mellea*	Japan	Tobacco	$27 million farm gate value in Victoria
P. syringae	*myricae*	Japan	Chinese bayberry	Emerging market in Australia for berries
P. savastanoi	*nerii*	Spain	Oleander	Ornamental flowering shrub
P. syringae	*oryzae*	Japan	Rice cv. Akihikari	av 600,000 to 800,000 tonnes p.a. depending on rainfall.
P. syringae	*papulans*	Canada	Apple	300,000 tonne p.a. only 1-2% exported
P. syringae	*persicae*	NZ, Croatia, France	Nectarine, peach	Peach and nectarine industries over 150,000 tonne p.a.
P. syringae	*philadelphi*	UK	Mock orange cv aureus, Philadelphus spp.	Ornamental
P. syringae	*photiniae*	Japan	Japanese photina	Ornamental shrub
P. syringae	*primulae*	USA	Polyanthus	Ornamental flower
P. savastanoi	*retacarpa*	Spain	Broom bus	Environmental weed
P. syringae	*rhaphiolepidis*	Japan	Sharinbai	Ornamental shrub
P. syringae	*ribicola*	USA	Golden currant	Ornamental shrub
P. syringae	*sesame*	Yugoslavia	Sesame	Small local industry
P. syringae	*solidagae*	Japan	Tall golden rod	ornamental shrub, weed
P. syringae	*spinaceae*	Japan	Spinach	Vegetable produce
P. syringae	*theae*	Japan	Tea	Ornamental hedging and green tea production
P. tremae	*tremae*	Japan	Urajiroenoki	Ornamental tree, Medicinal
P. syringae	*ulmi*	Yugoslavia	Elm	Ornamental tree
P. syringae	*viburni*	USA	Viburnum	Ornamental hedging
P. syringae	*zizaniae*	USA	Wild rice	Not present in Australia
P. amygdali		Afghanistan, Greece, Turkey	Almond	Nut, oil and milk production
P. cannabina	*cannabina*	Hungary	Indian hemp	Hemp and medicinal industries
P. caricapapayae		Brazil	Pawpaw, papaya	Fruit
P. ficuserectae		Japan	Inubiwa	Not in Australia
P. meliae		Japan	Chinaberry	Native host of Australia

5.4.2 Current geographic distribution

The geographical distribution within Australia of the 25 *P. syringae* pathovars and type species recorded as being in Australia are listed in Tables 3 and 5. The global distribution of the 55 pathovars and type species currently not recorded as being in Australia are listed in Table 6, although it is recognised that new detections in other countries may not be captured in this summary.

5.4.3 Symptoms

Pseudomonas syringae infections are commonly characterized by chlorosis and necrosis of leaves, stem tips, buds and flowers; by necrotic lesions and delayed ripening or altered development of fruit and by cankers and galls of woody tissues (Figure 4). However, there can be variations for individual pathovars on specific hosts. Some examples:

5.4.3.1 Typical symptoms of Pseudomonas syringae pv. persicae symptoms

Symptoms of this pathogen can vary slightly between hosts but in general, symptoms may include:

Fruit: abnormal shape, gummosis, black or brown lesions: scab or pitting.

Leaves: abnormal colours, necrotic areas, wilting.

Roots: necrotic streaks or lesions.

Stems: canker on woody stem, dieback, internal discoloration.

Whole plant: unusual odour, dieback, plant dead.

In nectarine and peach, symptoms include shoot dieback, limb and root injury, leaf spots, fruit lesions and tree death.

On Japanese plum, symptoms are mainly confined to dieback, occasional limb death, and leaf spots. Dieback of terminal shoots can occur in autumn and in spring following the development of girdling lesions from nodal infections. Small elliptical lesions may develop at internodes. The rootstock can also be infected showing symptoms similar to those on woody shoots. Leaf infection results in small, angular, water-soaked spots, the tissue of which becomes brown. The necrotic tissue subsequently falls out, causing a ‘shot hole’ effect. On fruits, small, round, dark, oily spots occur. These can be spread within the fruit tissue, causing sunken, deforming lesions that ooze gum.

Some symptoms of bacterial dieback due to *P. syringae* pv. *persicae* can be confused with those of bacterial canker of stone fruits (*P. syringae* pv. *syringae*, *P. syringae* pv. *morsprunorum*) and symptoms of leucostoma canker (*Leucostoma* spp.) or frost injury. Distinctive characteristics of dieback are discoloration of wood in branches above the necrosis and the absence of an obvious boundary between the morbid and healthy bark in the lower parts of the tree. Bacterial dieback can be disseminated with infected plants for planting or via contaminated pruning tools.

5.4.3.2 Typical Pseudomonas syringae pv. alisalensis *(bacterial blight of Broccoli and Broccoli Raab)*

Initial symptoms consist of small water-soaked flecks on the lower foliage. These flecks expand and become surrounded by bright yellow borders, which eventually may coalesce and result in large necrotic areas. This disease is economically important because large necrotic areas may form on foliage, rendering the crop unmarketable.

5.5 Diagnostic information

Morphological and biochemical methods can delineate *P. syringae* from other species, but molecular methods and pathogenicity testing is required to delineate pathovars or type species.

5.5.1 Morphological methods

Pseudomonas syringae is a Gram-negative aerobic bacterium with rod shaped cells, which are typically 1.5 µm long and 0.7–1.2 µm in diameter. The cells are motile, using at least one polar flagellum. The optimal temperature for growth ranges from 22–30˚C, depending on the pathovar or type species.

5.5.2 Biochemical methods

Pseudomonas syringae is negative for oxidase and arginine dihydrolase activity. Many *P. syringae* strains produce the polysaccharide levan and elicit the hypersensitive response on tobacco, which are elements of the levan, oxidase, potato rot, arginine dihydrolase, tobacco hypersensitivity (LOPAT) test.

One defining and unifying feature across nearly all strains phenotypically (via LOPAT testing) classified as *P. syringae* is the presence of a type III secretion system (Lindeberg et al., 2012).

5.5.3 Molecular methods

Currently, the *P. syringae* species complex is subdivided into over 62 type and pathotype strains defined by pathogenic characters, nine genomospecies defined by DNA–DNA hybridization and 13 phylogenetic groups (phylogroups) defined by multilocus sequence analysis (Thakur et al., 2016; Berge et al., 2014; Hwang et al., 2005; Sarkar and Guttman, 2004; Young, 2010) and whole genome sequencing (Dillon et.al., 2019; Gomilla et al., 2017).

A number of economically important pathovars have had pathovar specific diagnostic tests developed for them.

Four conserved housekeeping genes have been shown to distinguish pathovars *cts* (encoding citrate synthase also known as *gltA*), *gapA* (glyceraldehyde-3-phosphate dehydrogenase A), *rpoD* (RNA polymerase sigma70 factor) and *gyrB* (gyrase B) using the Morris MLST schema of the Plant Associated and Environmental Microbes Database (Plant Associated and Environmental Microbes Database (PAMDB), **http://genome.ppws.vt.edu/cgi-bin/MLST/home.pl**) in combination with *gapA* and *gyrB* of the Hwang PAMDB schema (Berg et al., 2014). They report that the partial *gapA* and *cts* sequences were the most efficient sequences for phylogroup delimitation.

Currently *cts* is the preferred gene for identifying *Pseudomonas syringae* pathovars and type species (Dr Cherie Gambly, DAF QLD pers. Comm.).

A good resource on MLST data and genome sequence for the *P. syringae* complex can be found at **https://guttman.csb.utoronto.ca/resources-software/**

5.5.4 Pathogenicity tests

The *P. syringae* species complex is subdivided into pathovars depending on the plant species they infect.

6 Pest risk assessment

6.1 Entry of the pathogen

The current import conditions for plant material that could potentially bring with it exotic and unwanted *P. syringae* pathovars and type species will depend on the plant species being imported and the country from which they are being imported. According to the Australian Biosecurity Import conditions website (**https://bicon.agriculture.gov.au/**) there are currently no specific import conditions for *P. syringae* pathovars or type species associated with any host plant. However, prior to export, all plants or plant products are required to be inspected or tested by the National Plant Protection Organisation (NPPO) according to appropriate procedures and be considered free from biosecurity pests.

It is therefore recommended the following guidelines be applied when reviewing an application to import a host plant that could potentially harbour an exotic *P. syringae* pathovar or type species that presents a risk to Australian industries:

- **For dormant cuttings**: introduction of hot water and surface sterilization treatments; have a minimum 3-month post entry quarantine (PEQ) period to allow detection of the *P. syringae* pathovar or type species in question; introduction of specific climatic conditions for growth in PEQ (for disease expression); and disease screening, including the introduction of molecular testing techniques (PCR).
- **For tissue cultures**: have a minimum PEQ period of three months for detecting the *P. syringae* pathovar or type species in question, during which time, implement disease screening, including using molecular testing (PCR and sequencing).
- **For pollen and seed:** pollen and seed must be sourced from countries or areas demonstrated to be free of the *P. syringae* pathovar or type species in question.

6.1.1 Entry potential

Rating: Medium

Given *P. syringae* can exist on plant material in a non-pathogenic state (epiphyte) where it can be present without symptoms, it is considered a medium risk for being introduced to Australia.

6.1.2 Establishment potential

Rating: Medium

Given exotic *P. syringae* pathovars and type species can exist on plant material in a non-pathogenic state (epiphyte) where it can be present without symptoms, it is considered a medium risk for becoming established in Australia should it be introduced and should host plants be present.

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6.1.3 Spread potential

Rating: medium

Pseudomonas syringae can be transmitted and dispersed locally via water splash (e.g. rain and irrigation), aerosols, airborne plant particles, terrestrial water systems and plant debris in the soil. It can be dispersed longer distances via infected plant material, insect vectors, pollen and infected seed (i.e. seed borne).

6.1.4 Economic impact

Rating: low to High

The economic impact would depend on the pathovar, or type species and the host impacted. Host importance ranges from weeds to large economically important industries with smaller crops and ornamentals in between.

6.1.5 Environmental impact

Rating: Medium
A number of pathovars and type species are pathogenic to ornamental and amenity trees, many of which are found in parks and private gardens in Australia.

6.1.6 Overall risk

Rating: Medium
Based on the individual ratings above, the combined overall risk is considered medium.

7 Surveillance and collection of samples

Information provided in the following sections provides a framework for the development of early detection and delimiting surveys for exotic pathovars and type species of the *Pseudomonas syringae* complex.

7.1 Surveillance

Detection and delimiting surveys are required to delimit the extent of the outbreak, ensuring areas free of the pest retain market access and appropriate quarantine zones are established.

Where an exotic pathovar or type species of *P. syringae* is found in a production nursery that is in close proximity to potential host plants (including weeds), periodically inspect nearby hosts for symptoms caused by the exotic pathovar of *P. syringae* by examining plants closely. Infected sources within a production nursery may provide an opportunity for exotic *P. syringae* pathovars and type species to spread outside the production nursery.

Determine the most typical symptom across the range of hosts that show symptoms. Agricultural inspectors and other production nursery visitors should not move infested plant material between production nurseries. Shoes, tools and vehicle tyres should be thoroughly washed of soil and then sanitised with a registered disinfectant to reduce spread of soil-borne diseases that may be present at the survey area.

7.1.1 Technical information for planning surveys

When developing surveys for the presence, and/or distribution, of new *P. syringae* pathovars and type strains, the following characteristics of the pathogen provide the basic biological knowledge that impacts on the survey strategy:

- If other pathovars or type strains share many of the same hosts.
- If host species in Australia are likely to be numerous and widely dispersed.
- If symptoms may look similar to other abiotic or biotic stress symptoms.
- If it can be asymptomatic in many hosts.
- If movement of *P. syringae* can occur by human assistance through the transfer of nursery stock.
- If the risk of pathogen movement on machinery, equipment and personal effects is high.
- If production nursery greenhouses and significant proportions of Australia have favourable climatic conditions for the spread and establishment of the new pathovar or type species of *P. syringae.*

7.1.2 Surveys for early detection of an incursion in a production nursery

The success of an eradication response to an exotic *Pseudomonas syringae* incursion in a production nursery is more likely following early detection of the pest before it has had the opportunity to disperse to a wide area. It is therefore necessary to consider pathways and plan surveys accordingly. Important points to consider when developing early detection surveys for *P. syringae* pathovars and type species in production nurseries are:

- Systematic and careful inspection of crops and propagative plant material is essential to prevent introduction of exotic *P. syringae* pathovars that are pathogenic and limit its spread

within and from contaminated outdoor and greenhouse production areas. Early detection of the pathogen while at low levels, will provide the best chance of eradication.

- An inspector must be trained to recognise disease symptoms of the exotic *P. syringae* pathovar or type species (see Section 5.4.3), and other similar disorders for comparison.
- Awareness information should be targeted at people who are in regular close contact with potential hosts in high-risk areas or movement vectors (e.g. production nursery operators).
- Should the presence of an exotic *P. syringae* pathovar or type species be detected in Australia and movement of potential host material is permitted, any new host material entering nurseries from suspected areas of infection should be quarantined prior to distribution throughout the property to allow for visual inspection or testing for the presence of the pest.

7.1.3 Delimiting surveys in the event of an incursion

- In the event of an incursion, delimiting surveys are essential to inform the decision-making process.
- The size of the survey area will depend on the size of the infested area and the severity of the infection, as well prevailing winds and movement of plant material during the period prior to detection. Other considerations are for example, movement of people or plant material equipment as a result of trace-forward and trace-backs.
- Initial surveys should be carried out in a 2 km radius of the initial detection and expanded from there depending on results. The exact radius to be surveyed will depend on the biology of the pathogen present, the type of environment in the area, extent of host plants present, presence of insects that could be carriers of infective spores, prevailing wind direction etc.
- All potential host species should be surveyed, with particular attention paid to the species in which the pest was initially detected (refer to Section 5.4.1 for current host lists).
- In addition to inspection of possible host plants, material should be collected for diagnostic purposes, including asymptomatic host plants (EPPO 2016). Complete destruction should not occur until sufficient material has been collected for diagnostic purposes.
- If the incursion is in a populated area, publication and distribution of information sheets and appeals for public assistance may be helpful.

7.2 Collection of samples

Protocols for the collection, transport and diagnosis of suspect Emergency Plant Pests (EPPs) must follow PLANTPLAN (Plant Health Australia 2016). Any personnel collecting samples for assessment should notify the diagnostic laboratory prior to submitting samples to ensure expertise is available to undertake the diagnosis.

7.2.1 Sampling procedures for *Pseudomonas syringae*

- In addition to being a plant pathogen causing disease symptoms on host plants, *P. syringae* pathovars and strain species can also exist in water ways, in rain and ice, in soil, in plant debris, on insects, on the surface of healthy plants and inside plants that appear healthy. Sampling for surveillance will therefore need to include plant, insect, and environmental samples.
- Plant infections are commonly characterized by chlorosis and necrosis of leaves, stem tips, buds and flowers; by necrotic lesions and delayed ripening or altered development of fruit and by cankers and galls of woody tissues. However, there can be variations for individual pathovars on specific hosts.
- Direct PCR using primers specific for the pathovar, and type strain of interest would need to be developed for the pathovars and type species exotic to Australia. At present there is a direct PCR available to detect nine of the 13 phylogroups (Borschinger *et. al.,* 2015) including all but 4 of the phylogroups (phylogroups 5, 6, 11 and 12).
- In addition, a pure culture needs to be isolated and stored for later varification. Plant and insect tissue samples can be macerated in water and streaked onto King's B medium, CSGA or PGS (Lamichhane & Varvari 2013) and cultured at 25C for 48 hrs. Similarly, water samples can be added to these media for selective cultivation and bulking. PCR can also be used directly with cells from colonies thereby permitting unbiased sorting of colonies for further characterization. This eliminates the need for expert recognition of *P. syringae* colonies or the bias that can be caused due to unexpected phenotypic variability in traits that have been used in the past to select putative *P. syringae* strains. Typical *P. syringae* colony morphology can also be masked by the mixture of *P. syringae* with other bacteria (Guilbaud *et al.,* 2016).

7.3 Stakeholder engagement

It is recommended that factsheets for all relevant industries be developed and made available to growers and other key stakeholders.

Groups that should be engaged following a detection include:

- Local councils/main road authorities that may have roadside host plants, e.g. oleander.
- Parks and garden organisations, e.g. botanic gardens, national/state parks.
- Relevant community groups, e.g. groups that maintain community gardens.
- Industry groups:
    - Greenlife Industry Australia (GIA), state nursery and garden industry peak bodies; production nurseries and retail outlets.
    - Relevant host industry groups (e.g. AUSVEG, Onions Australia, Australian Nut industry Council, Australian grains, Summerfruit Australia, Almond Board Australia, Australian Olive Association, Australian Subtropical Coffee Association, etc).

7.3.1 Activities for ongoing general surveillance following a detection

Undertake General Surveillance elements for Pseudomonas disease. To establish effective General Surveillance in Australia, several elements require additional support. The following is recommended to address gaps in these elements:

- Awareness material on state DPI websites.
- Inclusion of awareness material for PHA industry members.
- Inclusion of regulations to limit movement of plant material and equipment for jurisdictions with proof of freedom.
- Establish dedicated Australian web resource(s) as a repository of information for the public, affected plant industries and transport industries.

8 Course of action – immediate response to a detection

For a range of specifically designed procedures for the emergency response to a pest incursion and a general communication strategy refer to PLANTPLAN (Plant Health Australia 2016).

8.1 Tracing

Trace backs and trace forwards are essential for delimiting survey activities following an initial detection. Trace backs attempt to determine the source of the infection whereas trace forwards further define potential spread of and dissemination of the infection. There are many potential sources of trace backs/trace forwards. These are summarized to assist in the investigations to locate potential populations of exotic pathovars and type species of *P. syringae*. However, not all of these will be relevant to all scenarios so one must determine the importance of certain lines of investigation on a case-by-case basis. In any case, trace backs and trace forwards will identify movement linked to IPs, CPs and SPs.

8.1.1 Trace backs

Investigate where the infected material may have been purchased or obtained, this may include (not an exhaustive list):

- Retail nursery, weekend or road-side market, or internet sale.
- Production nursery – trace back to mother stock plants.
- Staff, visitors, etc., both domestic & international.
- Legal or illegal importation of plant material.
- Items of equipment, machinery and vehicles which have been shared between properties (e.g. storage and transport bins).

Trace back plant movements should focus on stock that was received within twelve months of the detection, or longer if deemed necessary.

8.1.2 Trace forwards

- Long distance movement of plants via sale of plants:
    - At production nurseries should maintain records of where consignments of plants have been sold. Sales of all host plants should be investigated from the last 6 months, or longer if deemed necessary.
    - At retail outlets, markets etc. – this will cause the scope of residential surveillance to be widened substantially.

For both trace forward and trace back plant movements, the critical period could be longer than the stated time periods, as symptoms may take longer than this to appear. This period of time should, of course, be modified based on the individual circumstances of the detection. However, an initial period of six months for trace forward and twelve months for trace back is suggested as a suitable compromise between scientific rigour and the practicalities of responding to a detection.

8.2 Quarantine and movement controls

Consult PLANTPLAN (Plant Health Australia 2016) for administrative details and procedures.

8.2.1 Quarantine priorities

Plant material and growing media/soil at the site of infestation to be subject to movement restrictions.

Machinery, equipment, vehicles, and disposable equipment in contact with infested plant material or growing media/soil, or present in close proximity to the site of infestation to be subject to movement restrictions.

8.2.2 Movement controls

- Movement controls need to be put in place to minimise the potential for transport of the pathogen, and this will apply to all plant material, growing media and other items within the quarantined area.
- Movement of people, vehicles, equipment, and plant material, from and to affected properties or areas, must be controlled to ensure that the pest is not moved off-property. Movement controls can be achieved through the following, however specific measures must be endorsed in the Response Plan:
- Signage to indicate quarantine area and restricted movement into and within these zones.
- Fenced, barricaded or locked entry to quarantine areas.
- Movement of equipment, machinery, plant material or growing media/soil by permit only. Therefore, all non-essential operations in the area or on the property should cease.
- Where no dwellings are located within these areas, strong movement controls should be enforced.

- Where dwellings and places of business are included within the Restricted and Control Areas movement restrictions are more difficult to enforce, however limitation of contact with infested plants should be enforced.
- If a production nursery is situated within the Restricted Area, all nursery trading in host and non-host material must cease and no material may be removed from the site without permission, due to the high likelihood of pest spread. Movement restrictions would be imposed on both host and non-host material.
- Residents should be advised on measures to minimise the inadvertent transport of vectors, should the pathogen and vector both be present.
- Clothing and footwear worn at the infested site should either be double bagged prior to removal for decontamination or should not leave the site until thoroughly disinfected, washed and cleaned.
- Plant material or plant products must not be removed from the site unless part of an approved disposal procedure.
- All machinery and equipment should be thoroughly cleaned down with a high-pressure cleaner or by scrubbing with a detergent/degreaser, followed by application of an appropriate disinfectant, prior to leaving the affected area. Machinery should be inspected for the presence of insects and if found, treatment with insecticide may be required. The clean down procedure should be carried out on a hard surface, preferably a designated wash-down area, to avoid mud being re-collected from the affected site onto the machine. When using high pressure water, care should be taken to contain all plant material and mud dislodged during the cleaning process.

8.3 Zoning

The size of each quarantine area will be determined by a number of factors, including the location of the incursion, the time of year, climatic conditions and the proximity of the infested property to other infested properties. The size of the zones will be determined by the consultative committee and agreed by the National Management Group during the production of the response plan. Immediately after an initial detection the zones in the following sections should be identified.

For private residences, in the first phases of a suspected incursion, government agencies in each jurisdiction will attempt to work with residents to gain permission to access premises for the purposes of surveillance or eradication. Once confirmation of an incursion has occurred (i.e. validation diagnosis has been made), legislation in most jurisdictions provides greater powers to access premises. For private residences, access may be possible to backyards and surrounds but entry into houses is limited without invitation from the resident.

If denied access, confirmatory diagnosis may be required in most jurisdictions before being able to enter premises or conduct treatments. For these reasons, eradication or management programs requiring establishment of treatment zones or restricted areas must be coupled with communication programs to achieve best outcomes.

8.3.1 Destruction/treatment zone

The size of the destruction zone (i.e. zone in which the pathogen and all host material is destroyed) will depend on the ability of the pathogen to spread, distribution of the pathogen (as determined by

delimiting surveys), time of season (and part of the pathogen life cycle being targeted) and factors which may contribute to the pathogen spreading.

All host plants should be destroyed after the level of infestation has been established. The delimiting survey will determine whether or not neighbouring plants are infested and need to be destroyed. Non-host plant material within this zone may be destroyed, based on recommendations in the Response Plan. The Destruction Zone may be defined as contiguous areas associated with the same management practices as, or in contact with, the infested area (i.e. the entire production nursery, property or area if spread could have occurred prior to the infection being identified).

Particular care needs to be taken to ensure that plant material (including non-hosts) is not moved into surrounding areas.

8.3.2 Restricted area

The Restricted Area is defined as the zone immediately around the infected premises and suspected infected premises. The Restricted Area is established following initial surveys that confirm the presence of the pathogen. The Restricted Area will be subject to intense surveillance and movement control with movement out of the Restricted Area to be prohibited and movement into the Restricted Area to occur by permit only. Multiple Restricted Areas may be required within a Control Area.

8.3.3 Control area

The Control Area is defined as all areas affected within the incursion. The Control Area comprises the Restricted Area, all infected premises and all suspected infected premises and will be defined as the minimum area necessary to prevent spread of the pest from the Quarantine Zone. The Control Area will also be used to regulate movement of all susceptible plant species to allow trace back, trace forward and epidemiological studies to be completed.

8.4 Destruction strategy

8.4.1 Destruction protocols

General protocols:

- No plant material or associated growing media or pots should be removed from the infested area unless part of the disposal procedure.
- Disposable equipment, infested plant material or growing media/soil should be disposed of by autoclaving, high temperature incineration or deep burial.
- Any equipment removed from the site for disposal should be double bagged.
- Machinery used in destruction processes need to be thoroughly washed, preferably using a detergent or farm degreaser, followed by application of an appropriate disinfectant.

8.4.2 Availability of control measures

Preventative measures are of critical importance to the management of diseases caused by exotic races of *P. syringae*, as there are currently no chemical curative treatments available for the pathogen in

Australia. Quarantine and phytosanitary procedures to exclude the pathogen are the first line of defence but must be supported by other strategies such as the use of resistant varieties (if available), cultural and hygiene practices.

Currently there is no effective way to 100% eradicate *P. syringae* from a plant or plant tissue. Eradication will involve destroying symptomatic or otherwise infested plant material. The most common way to manage this pathogen is to spray bactericides with copper compounds or other heavy metals that can be combined with fungicides or other pest control chemicals. Chemical treatments with fixed copper such as bordeaux and copper hydroxide are used to stop the spread of *P. syringae* by killing the bacteria while it is in the epiphyte stage on leaves, or woody parts of trees. Spraying antibiotics such as streptomycin and organic bactericides is another way to control *P. syringae* but is less common than the methods listed above and is not available for that use in Australia.

Strict hygiene practices used in fields on mother stock, along with pruning in early spring and summer, were proven to make the trees more resistant to *P. syringae.* Cauterizing cankers found on orchard trees can save the trees life by stopping the infection from spreading.

Breeding plants for resistance is another somewhat effective way to avoid *P. syringae*. It has been successful in the cherry rootstock with *P. syringae* pv. *syringae*, but so far, no other species are 100% resistant to this pathogen. Resistance breeding is a slow process, especially in trees. Unfortunately, *P. syringae* bacteria can adapt genetically to infect resistant plants, after which the process for resistance breeding has to start over again.

8.4.2.1 General procedures for control

- Keep traffic out of affected areas and minimise movement in adjacent areas.
- Adopt best-practice property hygiene procedures to retard the spread of the pest between glasshouses, fields and adjacent properties.
- After surveys are completed, and permission has been obtained from the Chief Plant Health Manager, destruction of the infested plant material is an effective control.
- On-going surveillance of infected areas to ensure the pest is eradicated.
- Do not use any material from infected plants for propagation.

8.4.2.2 Quarantine exclusion and phytosanitary measures

Quarantine exclusion is the first line of defence against the introduction of exotic pathovars and type species of the *P. syringae* complex into Australia.

8.4.2.3 Chemical control

There is currently no curative chemical treatment available for the control of *P. syringae* pathovars and type species.

8.4.2.4 Cultural control

Practicing good hygiene in the nursery, avoid overhead irrigation, keep relative humidity low by spacing plants appropriately, quarantine of incoming stock, monitoring for the presence of *P. syringae*, identification of the disease through lab-based diagnostics, removal and destruction of infected plants (and neighbouring symptomless plants).

8.4.3 Decontamination protocols

Machinery, vehicles, equipment, structures, clothing and footwear in contact with infested plant material or growing media/soil, or present within the Quarantine Area, should be first washed to remove plant material and growing media/soil using high pressure water or scrubbing with a detergent/degreaser, followed by application of an appropriate disinfectant (e.g. quaternary ammonium compound) in a designated wash down area. When using high pressure water, care should be taken not to spread plant material. High pressure water should be used in wash down areas which meet the following guidelines:

- Located away from crops or sensitive vegetation.
- Readily accessible with clear signage.
- Access to fresh water and power.
- Mud free, including entry and exit points (e.g. gravel, concrete or rubber matting).
- Gently sloped to drain effluent away.
- Effluent must not enter water courses or water bodies.
- Allow adequate space to move larger vehicles.
- Away from hazards such as power lines.
- Wastewater, growing media/soil or plant residues should be contained.
- Disposable overalls and rubber boots should be worn when handling infested plant material or growing media/soil. Boots, clothes and shoes in contact with infested plant material or growing media/soil should be disinfected at the site or double-bagged to remove for cleaning.
- Skin and hair in contact with infested plant material or growing media/soil should be washed.

Procedures for the sterilisation of plant containers and growing media are provided within the BioSecure *HACCP* Guidelines, however, in the event of an exotic pathovar of type species of *P. syringae* incursion, additional or modified procedures may be required for the destruction of the pest. Any sterilisation procedure must be approved for use in the endorsed Response Plan.

8.4.4 Priorities

- Confirm the presence of the pathogen.

- Limit movement or people and prevent movement of vehicles and equipment through affected areas.
- Stop the movement of any plant material that may be infected with the pathogen.
- Determine the strategy for the eradication/decontamination of the pathogen and infested host material.
- Determine the extent of infestation through survey and plant material trace back and trace forward which would be assessed on a case-by-case basis and included within the response plan.

8.4.5 Plants, by-products and waste processing

- Any growing media/soil or infected plant material removed from the infected site should be destroyed by (enclosed) high temperature incineration, autoclaving or deep burial.
- As the pest can be spread with plant material, plant debris from the destruction zone must be carefully handled and transported.
- Infested areas or production nursery yards should remain free of susceptible host plants until the area has been shown to be free from the pathogen.

8.4.6 Disposal issues

- Particular care must be taken to minimise the transfer of infected plant material and growing media from the area.
- Host material including leaf litter and growing media should be collected and incinerated or double bagged and deep buried in an approved site.

9 Recommendations for preparedness activities

9.1 Assessment of current preparedness for Pseudomonas

Diagnostics capacity and capability – Experience from other countries has shown that *Pseudomonas syringae* complex consisting of over 60 pathovars and type species have a wide host range. There is a need to taxonomically sort out the *P. syringae* complex and to be able to detect at the pathovar and type species level and ensure tests are effective for Australian hosts, consider ‘surge’ capacity to test large numbers of samples that may be experienced in the event of an incursion, develop rapid field tests and ensure diagnostic tests are available.

Communication and awareness – Improving awareness of the significance and impact of different pathovars and type species of *P. syringae* amongst plant industries should be undertaken through development of support material such as websites, fact sheets and industry newsletters. Consideration could also be given to improving awareness in other groups such as traveler’s, environmental groups, researchers and government staff. Coordination of material would be useful to ensure consistent messaging is being delivered.

Planning and preparedness – Activities such as development of a cross-industry pest contingency plan, delivery of a simulation exercise, development of a regional containment plan, ensuring that all affected industries are signed to the EPPRD, and ensuring *P. syringae* is included within biosecurity plans for potentially affected industries will assist in Australia's preparedness for an exotic *P. syringae* incursion.

Research, development and extension – Nationally coordinated R&D for the hosts (including Australian native species), asymptomatic hosts, potential economic impact, resistant cultivars, pathovar specific host ranges and pathway analyses. Fast and efficient molecular diagnostics need to be developed for those pathovars and type species that pose a significant threat to Australian industries.

Surveillance – To confirm Australia's status for *P. syringae* pathovars and type species, and to improve our likelihood of early detection of them should one enter Australia, improved surveillance for the pathogens is required. This should include:

- At the border – surveying potential hosts for the presence of symptoms in the vicinity of high risk points of entry, including quarantine approved premises;
- Post-border – specific surveys targeting potential hosts. Surveillance program including specific surveys for the pathogen in high risk hosts. General surveillance programs that increase awareness of *P. syringae* symptoms and reporting mechanisms for industry and communities.

Control and eradication — Preparedness activities that provide information for control of the pathogen are required. These could include improved knowledge of potential eradication strategies and distances of buffer and quarantine zones. Preparedness information is also required on management options in the event that the pathovar of *P. syringae* is not technically feasible to eradicate. This could include strategies that slow the spread or minimise the impact of the pathovar in question, by identifying management priorities and potential movement control requirements.

9.2 Priorities for future exotic pathovars and type species of *Pseudomonas syringae* biosecurity preparedness activities

Hold a workshop to consider a range of preparedness activities based on potential impact and ease of implementation. Key areas to be determined to be of highest priority and would result in the highest impact were as follows:

Awareness

Development of awareness material suitable for multiple audiences would achieve considerable impact. Types of audiences included government staff, R&D providers, industry and growers, the public and biosecurity inspectors.

Information should be provided on the impact of *P. syringae* exotic pathovars, what to look for and how to report suspected samples. Training within industry and government in identification, surveillance and reporting was also seen as part of awareness activities.

Incursion simulation exercise

Given the large number of industries and jurisdictions that could be involved in the event of a detection of an exotic pathovar of P. syringae in Australia, a simulation exercise that assisted with preparedness for a response was seen as a high priority. A simulation exercise should involve industry and government and will assist with improving capacity and capability, planning and coordination and identifying any gaps in preparedness.

Host identification

A review of plant species (including Australian) known to be hosts/infected from affected countries was also identified as important.

Surveillance and diagnostic capacity

There is a need to undertake assessment of current diagnostic capacity & capability as well as surge capacity requirements should large numbers of samples need to be processed. A review of the current diagnostic protocol/s to address any issues with diagnostics in a range of hosts is required.

A nationally coordinated surveillance strategy and protocol for exotic pathovars of *P. syringae* is needed to confirm and support Australia's plant health status, including whether surveillance should focus on early warning or proof of freedom, and to improve Australia's capacity for early detection of these exotic pathovars.

Regional containment

As part of preparedness activities, improved knowledge is needed to gain a better understanding of regional containment requirements should an incursion of an exotic pathovar of *P. syringae* be deemed not technically feasible to eradicate. Planning is needed on measures that may be required such as the size of a host free buffer zone, control options, surveillance requirements, determination of the potential role of asymptomatic hosts and risk pathways for spread within Australia.

These outcomes will be considered by governments and industry in the context of future preparedness investment, with some activities to address these priorities already in progress.

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11 Appendices

11.1 Important nursery contacts

National Nursery governing body ‘Greenlife industry’:

https://www.greenlifeindustry.com.au/

Australia Plant Protection Standards website:

https://nurseryproductionfms.com.au/

11.2 Resources and facilities

Each state and territory have their own government supported plant and pest disease diagnostic laboratory. Refer to the following National Plant Biosecurity Diagnostic Network (NPBDN) website for an up to date and comprehensive list of diagnostic laboratories in Australia:

https://www.plantbiosecuritydiagnostics.net.au/laboratory-directory/