PSEUDOMONAS SYRINGAE pv. ACTINIDIAE
IN NEW ZEALAND

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SUMMARY

There are several populations of Pseudomonas syringae pv. actinidiae (Psa) that differ in virulence, in genetic characteristics, and in phenotypic characters. Primary concern is with highly virulent strains, here called Psa – V. They cause most damage in gold fruit cultivars of Actinidia chinensis. So far, attempts to manage the disease by pruning, by sprays and by other measures have been unsuccessful.

INTRODUCTION

Much information has been gathered concerning bacterial epidemiology in the last 50 years, particularly of the pathovars of P. syringae. Much of this literature has been forgotten and does not inform the present problem. The virulent strains of P. s. actinidae (Psa-V) have been studied for 3-4 years in Italy (Ferrante and Scortichini, 2010) and for two seasons in NZ. Although this is a small research and observational base, there is enough information in the literature, from past general experience, and in local observations, to draw preliminary conclusions. At present, virulent strains of P. s. actinidae (Psa-V) are found in New Zealand in the Bay of Plenty, the main growing region, and cannot be eradicated from the country. All outbreaks are identified in ‘priority zones’ by MAF Biosecurity as part of an effort to contain the pathogen.

P. s. actinidiae strains. At least three populations of P. s. actinidiae have been reported. The first was recorded in Japan (Takikawa et al., 1989), South Korea (Koh et al., 1996, 2003, 2010) and in Italy in 1992 (Scortichini, 1994) about 20 years ago. This pathogen formed the basis of the pathovar and first epidemiological observations. Subsequently, a second population that causes a more serious form of the disease has been recorded in gold kiwifruit (H16A) in Italy since 2008 and in New Zealand in November 2010. These two populations appear to be genetically very similar and are considered to be members of the same haplotype (Fig. 1). The virulent and weakly virulent populations are now referred to as Psa-V and Psa-LV, respectively. The third population has been identified only in New Zealand so far (Chapman et al., 2011). It is a distinct genetic haplotype of the pathogen that, as inferred from its widespread distribution, has been present in New Zealand for many years without being noticed. It appears to be weakly virulent. Actinidia appears to be a diverse Chinese genus. It seems probable that Psa is also diverse and that the three identified haplotypes only reflect a small proportion of the diversity of the pathovar as a whole. Differentiating these populations by name is cumbersome, but the most accurate terminology, until further studies clarify matters, is to recognise the pathovar, P. syringae pv. actinidae haplotype 1, which includes Psa – V and Psa-LV. Haplotype 2 is here designated Psa-LV (NZ) (Fig. 1).

Distribution of Psa. Psa has now been recorded in all countries where Actinidia chinensis and A. deliciosa have been distributed; in Japan, Korea, Italy, France, Portugal, Brazil, Chile, China and New Zealand (Everett et al., 2011, Scortichini et al., 2011).

Since it was first observed in New Zealand in November, 2010, Psa-V is now (December 2011) widely distributed in the Bay of Plenty, the main growing region (Table 1). Its very rapid spread in the 2010-11 season suggests that the pathogen was introduced very few seasons ago. In March, 2011, Psa-V was identified on orchards comprising fewer than 500 ha. Since then the affected area has risen exponentially to (November 2011) almost 5000 ha. Until recently, all outbreaks were to be found in the Bay of Plenty. A single outbreak in the Manukau area has occurred that seems to have no obvious connection with previous records.

Kiwifruit cultivars. Favoured cultivars of gold kiwifruit (Actinidia chinensis) are Hort16A, Jin Tao and Soreli. Other cultivars are now under investigation for fruit quality and tolerance to Psa. Psa-V is more virulent in these gold kiwifruit cultivars (A. chinensis) than in green kiwifruit cv. Hayward (A. deliciosa). Nevertheless,
the pathogen moves progressively in ‘Hayward’ and its impact on this cultivar has not yet been adequately determined.

**Identification.** *Psa* is a member of LOPAT group I of *Pseudomonas syringae* (Lelliott *et al.*, 1966). With isolates so identified, several DNA based methods have been reported to differentiate *Psa* from other pathovars (Rees-George *et al.*, 2010). Mazzaaglia *et al.* (2011) and Ferrante and Scortichini (2010) report methods that differentiate *Psa-V* and *Psa-LV*. Based on MLSA studies, *Psa* has been allocated to a genomospecies that comprises two of those created by Gardan *et al.* (1999), viz. 3 and 8 using DNA-DNA hybridisation (Fig. 1). It can be allocated reliably to this genomospecies by any one of a number of comparisons of housekeeping genes, such as *acnB*, *dnaX*, *cts*, *gapI*, *gltA*, *gyrB*, *pfk*, *pgi* and *rpoD* (unpublished information). Primers for Real-time PCR detection/identification have been developed that differentiate the pathogenic populations and are now being tested.

**Aetiology.** The three major environmental factors affecting primary infection are moisture, temperature and light. When leaves transpire, the stomata are so numerous and open so widely that the lower leaf surface offers practically no barrier to gas exchange or bacterial infection. Light is probably the most critical factor involved in stomatal opening.

Quorum sensing (QS) is a well-established phenomenon in which bacteria are stimulated to specific metabolic action only when physically closely associated cells act in concert. It has been shown that QS influences many metabolic processes including several disease steps in *P. syringae* (von Bodman *et al.*, 2003; Quiniones *et al.*, 2005). The involvement of QS is being investigated as a possible means of inhibiting the main steps in infection. However, the available data indicate that the multiplication of individual pathogenic cells begins within a few hour of inoculation while the enlarged populations capable of QS develop after one or more. This indicates that QS is not essential for multiplication. Evolutionary considerations suggest that it would be fatal for a single-celled pathogen to require the multiple invasions of infection sites for development.

**Epidemiology.** The occurrence and severity of epidemics is primarily determined by seasonal weather. The micro-climate in orchards is critical in determining the local severity and extent of disease. This means that, while it may be possible to determine the extent to which an area may be at risk, more detailed predictions are impossible.

Weather conditions that favour bacterial infection are usually sporadic, being confined to particular seasons and are associated with rainy, wet humid, conditions. Once infected, disease development is largely determined by temperature.

Practically all literature on *Psa* concerns the behaviour of *Psa-LV* in Japan and *Psa-V* in Italy. Detailed epidemiological studies have been made only in Japan (Serizawa and Ichikawa, 1993a, 1993b, 1993c; Serizawa *et al.*, 1989, 1994) Like *P. syringae* (Young, 1991), *Psa* causes disease at relatively low temperatures, being most active at ca.18°C and progressively reducing in severity above 20°C and below 15°C. Cankers in trunks and leaders, developing mostly in late winter and spring, are the most serious symptom because they girdle whole limbs and kill entire vines. In Italy, infection of vegetative and fruiting canes occurs in spring. Bacteria in cankers usually die out in spring-summer when temperatures rise above 20°C (M. Scortichini, personal communication). This is not the case in New Zealand, where disease in canes continues through summer.
Table 1. Distribution of *Psa-V* in New Zealand at 30 November 2011 (http://www.kvh.org.nz/statistics)

<table>
<thead>
<tr>
<th>Number of infected orchards</th>
<th>889</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affected orchards (%)</td>
<td>24</td>
</tr>
<tr>
<td>Hectares of affected orchards</td>
<td>4356</td>
</tr>
<tr>
<td>Infected hectares (%)</td>
<td>32</td>
</tr>
</tbody>
</table>

| 79% of New Zealand’s kiwifruit hectares are now in a Priority Zone. |
| 35% of New Zealand’s kiwifruit hectares are in orchards with a *Psa-V* positive result. |

**Symptoms.** Symptoms in canes may primarily be the result of leaf scar infections that occur in autumn. The pathogen in leaf scars may rest during winter, becoming active in spring, spreading in first and second year tissue. Infected canes lead to girdling and wilting of flowering shoots. In Italy, infection and disease cease in canes in summer, being carried over in infected leaves. However, in New Zealand’s growing regions, which have a mild maritime, practically frost-free, climate, disease spreading in canes throughout the summer. It has yet to be determined if cane cankers carry over to develop in winter. Severe cankers appear in late winter and spring. These produce gumming cankers from which the pathogen can initially be isolated. Subsequently, cankers are invaded by bactericidal plant phenolics, resulting in red-brown gummosis from which the pathogen is increasingly difficult to isolate.

Leaf-spots in summer are usually trivial but they are the likely source of next season’s inoculum; the pathogen being carried over autumn in and on leaves to leaf scars. Leaves remain infected into autumn. Infections of leaf scars appear to remain dormant until early spring when the pathogen becomes active and infects new season’s growth.

There is little evidence of significant movement of individual bacterial cells either in the parenchyma or vasculature. Cells multiply at lesion margins, pushing young cells into the adjacent tissue, thus advancing the lesion.

In trunks and leaders spread of the pathogen appears to be through several tissue elements, including outer parenchyma (Fig. 2C,D) and internal woody sclerenchyma (Fig. 2E). It has been suggested that infection is systemic, moving from leaf spots down into canes through the vascular cortex. However, invasion of leaf veins from leaf spots (Fig. 2B) appears to be rare. Shoots emerging in late spring can be severely infected (Fig. 2A). As yet, sites of infection, at the abaxial or adaxial ends of cankers, have not been established. The role of systemic

**Fig. 2.** A. Infected emerging shoot (courtesy of R. Baker). B. Leaf lesions invariably delimited by veins: absence of systemic spread (courtesy of J. P. Wilkie). C. Infection of outer parenchyma (courtesy of G.M. Balestra). D. Incipient girdling of trunk (courtesy of M. Scortichini). E. *Pseudomonas syringae pv. actinidiae* oozing from woody sclerenchyma (courtesy of R. Baker).
movement in vines is unclear (Y. Takikawa, personal communication).

**Dispersal.** It has been known for decades that bacterial pathogens are dispersed for hundreds of metres, perhaps kilometres in clouds of inoculum generated as aerosols in wind-driven rain, which spread between blocks and orchards. Where a localized infection site is observed, then it follows that the pathogen is already present in all surrounding vines and blocks. Spread between orchards on implements can occur, but it will be secondary in importance to natural spread. Courtesy requires that orchardists observe sensible hygienic measures moving equipment between orchards.

Localized expression, sometimes only in one part of a single vine, is due to the highly specific environmental conditions required for disease development. Fundamental to an understanding of bacterial epidemiology is that epidemics usually occur sporadically, sometimes after years of bacterial association with the host at sub-clinical levels (e.g., *P. syringae pv. pisi*). Given the virulence of *Psa*-V, epidemics can be expected to be more regular than those occurring in weaker pathogens, but absence of symptoms of the pathogen is not evidence of its absence.

**Containment.** Only significant geographical breaks between infected and uninfected orchards will delay further spread of the pathogen, with rigorous attention to preventing the movement of infected material between regions. The recent confirmations of a single isolated outbreak of *Psa*-V spread outside the initial infection zone is most probably on inadequately disinfected vehicles or equipment. For some vehicles, decontamination is practically impossible. Attempts at active containment in other crops in the past have never been effective (the eradication of citrus canker in Florida in the 1930s using draconian measures being the exception). Recent attempts at eradication of citrus canker and *Candidatus Liberibacter spp.* (Gottwald, 2010) were ineffective and this confirms that efforts to contain bacterial pathogens, including *Psa*, by local eradication in identified containment zones are unrealistic.

**Climate.** Epidemics occur under precisely prescribed conditions, usually occurring regionally or sub-regionally. Southern growing regions are likely to be at greater risk. Several important questions about the behaviour of *Psa* could be framed in terms of NZ’s seasonal conditions compared with those in Italian regions. A study of historical daily temperatures and rainfall may indicate to what extent conditions mimic those of Italy and Japan and hence likely regional severity. Only when the pathogen is distributed more widely, in a wider climatic range, will it be possible to establish possible areas for future planting and those to avoid.

**Management and Control.** Any proposed control protocol must be cost-effective; the return in crop yield from the treatment must exceed labour and material costs. It is only by crop surveys that the severity of a disease can be described accurately.

**Pruning.** Pruning was introduced to control fungal diseases in the 19th century when it was realized that spore production occurred mainly after plant tissue was killed. Spore production and dispersal in this way was a main source of further fungal infection. This practice has been recommended for bacterial control since then:

(i) To remove prunings to reduce future infection in subsequent years. Compared with inoculum still present in living plants, infected prunings are only likely to be a secondary source. Stringent removal of prunings is unlikely to lead to significant reduction in disease in the long term. Numbers of pruning wounds is small compared with natural infection sites.

(ii) To prevent further spread of disease in a block, orchard or region (‘aggressive containment’; the removal of all leaders, canes and leaves, shortening the vine to trunk stumps). Aggressive pruning appears to arise from a confusion between eradication and control. Disease will certainly reappear with any new growth, arising from the block itself or from adjacent orchards. Recovery of vines that have been aggressively pruned may be doubly difficult because the gross imbalance between roots and shoots means that sprouts and grafted scions are the most susceptible of all young tissue and now are confronted with a virulent pathogen.

Following the initial outbreak, a compensation scheme was introduced that indemnified all growers who agreed to the removal of blocks and orchards as part of aggressive containment. This scheme was retracted when it was realized that it was ineffective.

*Psa*-V does infect through pruning cuts, but recent trials in Italy have shown that the cuts are, for practical purposes, impossible to sterilize, even in a rigorous trial involving cauterizing wounds with a blow-lamp and sealing them immediately after pruning (C. Kay, Zespri, personal communication). Commercial pruning will not be effective as a disease preventative.

Numerically, there are many more natural infection sites than pruning wounds and, although the latter are more susceptible, they make a lesser contribution to infection. There have been many attempts to control pathogens by pruning. Only in conditions where the pathogen induces relatively mild symptoms have there been claims of success. Pruning is usually for general hygiene, not for bacterial control.

**Spray control.** Attempts at bacterial control with in-season biocidal sprays have been made for more than a century. Many compounds have been tested with little or no success and have been abandoned. In testing the
efficacy of sprays, the most common practice has been to assay leaf spots and leaf damage. Past observations suggest that this does not give a measure of crop yield.

Copper sprays are most commonly recommended but it has been shown that many plants, including kiwifruit (unpublished information), suffer from severe phytotoxicity. Once the pathogen has penetrated leaves, whether or not symptoms are expressed, it is impossible for control sprays, coppers or antibiotics, to affect them. Sprays in spring therefore have no efficacy. However, in desperation, kiwifruit growers continue to apply exotic compounds including organic extracts (garlic extract, citrus seed extract, propolis), hypochlorite, quaternary ammonium compounds, terpenes, chitin-based products, and other unproved ‘snake-oils’.

A spray programme, proved to be effective in other tree crops and pathogens, entails copper sprays applied during leaf-fall to cover leaf scars as infection sites in autumn (Young, 1982 and other references).

Streptomycin was applied to many crops but is now banned in most jurisdictions. In trials in many crops in the past, the antibiotic gave unreliable results. Serizawa et al. (1989) report the reduction of *Psa* on kiwifruit leaves using streptomycin in spring. Past studies of injection of streptomycin into kiwifruit trunks resulted in the direct transmission of the antibiotic to the leaves where it caused chlorosis with severe stunting of the leaf margins (unpublished information).

The genes for streptomycin and copper resistance have been reported in *Psa* (Marcelletti et al., 2011; Nakajima et al., 2002) and in other pathogens. However, it is rare to find a report in which resistant strains are proved to be the cause of epidemics (Young, 1977).

**Biocontrol.** There appears to be only one approach to characterizing biocontrol agents. Saprobes are screened in culture for antagonism to the target pathogen. In glass-house trials, antagonistic strains are screened in culture for antagonism to the target pathogen. Saprobes are dependent on the host cell wall, releasing the cellular contents as nutrients for their multiplication and secondarily for saprobes. Although pathogens grow more slowly than saprobes in culture, the latter are dependent on the prior activity of the pathogenic cells for growth in the plant. Saprobes survive in or on leaves for some hours or days. If the populations are examined over extended periods, it will be found that the saprobes decline except under unusual circumstances, but the pathogen multiplies exponentially after a resting phase of a few hours. It follows that the saprobe can have little influence on the pathogenic population. Spore-forming bacteria as biocontrol agents are also inactive in their plant association but they instantly form resting spores in adverse conditions, which is why high populations can be recorded after copper sprays.

Bacteriophages were tested exhaustively in the 1970s-80s without success. The reason is probably that phage particles are immobile and unable to reach bacterial populations in plants.

**Breeding for resistance.** As noted, some cultivar selections of gold kiwifruit offer tolerance to *Psa* V and may serve as substitutes for the present favoured cultivars. The only long-term solution is to introduce resistance genes into existing cultivars that have favourable marketing characteristics. Studies in China indicate that there are several possible sources of resistance (Li et al., 2004). The selection of crosses of resistant and commercially favourable cultivars will be slow and painstaking.

**Epilogue.** Canker of kiwifruit is so severe in Italy and New Zealand that producing organizations are considering abandoning cv. Hort16A in these countries.

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**REFERENCES**


