Review of the risk factors associated with kiwifruit bacterial canker caused by *Pseudomonas syringae* pv. *actinidiae*

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Abstract Bacterial canker of kiwifruit, commonly referred to as Psa, is caused by *Pseudomonas syringae* pv. *actinidiae,* and the biovar 3 strain has affected kiwifruit vines in New Zealand since 2010. Psa has had an impact on the management and production of kiwifruit in New Zealand. This paper provides a review of the epidemiological risk factors that are associated with Psa disease within orchards. The presence of the pathogen, rain and a susceptible host are sufficient to cause disease in kiwifruit, but there are other risk factors that can increase the amount of disease that develops and the spread of disease, including other environmental factors (e.g. temperature), host factors (e.g. cultivar) and management factors (e.g. pruning practices). The aim of this literature review was to describe the current knowledge of a range of known and postulated risk factors for the development of bacterial canker in kiwifruit.

Keywords *Actinidia chinensis, Actinidia deliciosa,* Psa, Psa-V, risk factors, 'Hayward', 'Hort16A'.

INTRODUCTION

Pseudomonas syringae pv. *actinidiae* (Psa) biovar 3 is the causal agent of bacterial canker of kiwifruit and is causing significant damage to kiwifruit vines in New Zealand, where it has been commonly referred to as Psa-V (Vanneste et al. 2013). There are several strains of Psa found worldwide, with some causing moderate damage (biovars 1,2) and others causing important damage (biovar 3) (McCann et al. 2013). The first countries to report symptoms of bacterial canker in kiwifruit orchards were Japan in 1984 (Takikawa et al. 1989) caused by biovar 1 (Ferrante & Scortichini 2015) and then Korea in the late 1980s (Koh et al. 2010) caused by biovar 2 (Ferrante & Scortichini 2015), followed by Italy in 1992 (Scortichini 1994) (also biovar 1). The results from a 5-year study of Psa in China indicated that kiwifruit bacterial canker was a problem in China as early as 1996 (Li et al. 2001; Li et al. 2004), and another paper stated that bacterial canker had become one of the most serious factors limiting kiwifruit cultivation in the Sichuan area of China (Liu et al. 2012). Recent isolates from China fit within the biovar 3 population (Ferrante & Scortichini 2015).

The strain of Psa (biovar 3) found in New Zealand (McCann et al. 2013; Vanneste et al. 2013) has caused a global pandemic (Scortichini

et al. 2012), and was initially reported from Italy in 2008 (Balestra et al. 2008; 2009b), from Turkey in 2009 (Bastas & Karakaya 2012), followed by New Zealand in 2010 (Everett et al. 2011). In addition, reports of Psa have come from France in 2010 (Vanneste et al. 2011c), Portugal in 2010 (Balestra et al. 2010), Spain in 2011 (Abelleira et al. 2011), Chile in 2011 (ProMed 2011), Slovenia in 2013 (Dreo et al. 2014) and Greece in 2014 (Holeva et al. 2015).

Kiwifruit bacterial canker has been described as a cyclic disease. Canker symptoms express in winter to early spring on infected branches and trunks followed by lesions on green tissues in spring and early summer (Serizawa et al. 1994). Seasonality is strongly linked to temperature and rainfall, which have both been shown to be associated with Psa bacterial population growth (Serizawa & Ichikawa 1993d; Serizawa et al. 1994; Tyson et al. 2012a), along with differing host plant phenology and susceptibility (Serizawa & Ichikawa 1993a; Serizawa et al. 1994). The addition of seasonal management activities of kiwifruit vines and associated risks results in multiple factors contributing to disease expression.

The presence of the pathogen is necessary for the development of bacterial canker of kiwifruit (Everett et al. 2011), but its mere presence is not sufficient to cause a disease outbreak. Water is required for infection and transmission to new hosts and the host material must be susceptible to bacterial entry at the time of exposure. While Psa inoculum, water and a susceptible host are needed to cause disease in kiwifruit, there are many other environmental, host and management factors that can influence the amount of disease that develops.

Pseudomonas syringae pv. *actinidiae* has been reviewed by several authors in recent years, in particular the symptoms (Everett et al. 2011) and the different strains around the world (Vanneste et al. 2011b; Scortichini et al. 2012; McCann et al. 2013; Vanneste 2013; Donati et al. 2014; Ferrante & Scortichini 2015). The aim of this paper is to review the literature to describe the spread and epidemiology of the disease and assess the current knowledge of risk factors for the development of bacterial canker disease in kiwifruit orchards.

PATHOGEN

Distribution of Psa in the host

Psa has been detected on leaves, canes, trunks, leaders, buds, flowers, internal parts of fruit, pollen, bleeding sap and roots (Takikawa et al. 1989; Serizawa & Ichikawa 1993a, d; Balestra et al. 2009b; Everett et al. 2011; Vanneste et al. 2011a; Abelleira et al. 2014). Psa has also been isolated from tissue of apparently healthy flowers, buds, leaves and woody material of vines (Gallelli et al. 2011; Tyson et al. 2014a,c; Taylor et al. 2015). Microscopic examination of Psa in two *Actinidia chinensis* cultivars showed that bacterial cells were present in all regions of the cane down to the cambium layer (Hallett 2012). In some samples they found no bacterial cells in the xylem or woody parts of the cane, even in heavily infected canes (Hallett 2012). In further research Psa was found in leaf hydathodes and in the xylem of canes with and without visible symptoms (P.W. Sutherland, Plant & Food Research, personal communication) and this suggests that entry into the plant has multiple infection pathways. A recent study in Italy reports that Psa was detected from bleeding sap from cut branches in spring (Biondi et al. 2013). Another study of the infection process within inoculated host tissues using green fluorescent protein (GFP)-transformed Psa cells showed that the bacteria can colonise both the outer tissues as well as the xylem and pith, and that migration within host tissue occurs in the xylem (Spinelli et al. 2011). The same study also found Psa on flower anthers, stigmas and the calyx, and noted that small bacterial colonies had been found on flower debris in pollen samples (Spinelli et al. 2011). Spinelli et al. (2011) also showed that Psa could penetrate kiwifruit vines via the stomata, the leaf abscission scar and through damaged trichomes (leaf hairs).

Inoculum

Under high humidity conditions in New Zealand orchards Psa exudate has been observed oozing from leaf spots on the undersides of leaves (Tyson et al. 2012a), which are present from early spring to late autumn. Inoculum has also been

recorded from bleeding sap (Biondi et al. 2013), and as a red exudate or a milky-white exudate from bleeding cankers in early winter (Tyson et al. 2012a). In Spain the white exudate has been observed from both cankers and wounds on trunks and branches (Abelleira et al. 2011).

Symptoms

There are several plant pathogenic bacteria in New Zealand kiwifruit orchards that can cause similar symptoms on leaves and flowers. Severe wind or frost can also cause red bleeding from wounds. The most specific symptoms of Psa in New Zealand kiwifruit orchards are shoot-wilt and dieback, and the presence of white exudate (Vanneste et al. 2011b).

In early spring and summer, Psa symptoms in leaves are typically dark angular necrotic spots, often accompanied by a yellow chlorotic halo around the outer edge of the spot (Everett et al. 2011; Donati et al. 2014). Leaf wilting is also often observed when the bacterium is systemic within the vine and is thought to be caused by blocking of the vascular tissue (Vanneste et al. 2011b). Shoots in New Zealand vines show wilting and dieback, and occasionally appear to have a dark blue/black inky colouration on the shoots and appear flattened and ribbon-like (Vanneste et al. 2011b). Vanneste et al. (2011b) noted that the inky discoloration has not been described in association with Psa previously.

Budrot caused by Psa has been widely reported in New Zealand (Everett et al. 2011; Tyson et al. 2014a; Taylor et al. 2015). Buds are discoloured, with brown staining over part or all of the developing bud (Tyson et al. 2014a). Buds on infected canes may also fail to develop or, if they do develop, they may wilt and drop off (Vanneste et al. 2011b). In five orchards of the cultivars *A. deliciosa* 'Hayward' and *A. chinensis* × *A. deliciosa* 'Zesh004' (commonly known in New Zealand as Green14), Tyson et al. (2014a) detected Psa in 96% of buds with browning symptoms, whereas the kiwifruit blossom blight pathogen (*Pseudomonas* sp.) was detected in 30% of the buds, indicating that Psa is a causal agent of browning and bud drop in addition to the existing blossom blight pathogen.

In woody tissue, Psa symptoms are most obvious in late winter to early spring. Cankers form in trunks and leaders, where they exude reddish or milky white ooze, and in severe cases the whole leader or vine will die (Everett et al. 2011; Vanneste et al. 2011b).

Dispersal of the pathogen

Natural spread

Natural dispersal of Psa in Japan occurs via rain-splash and movement of rainwater by wind (Serizawa & Ichikawa 1993b). Tyson & Manning (2013) provide a comprehensive review of the literature around rain-splash and aerosol spread of pseudomonads.

Tyson et al. (2014c) showed that trap plants placed in infected orchards were able to be infected year-round, particularly in spring, and that infection events were strongly associated with rainfall. They concluded that rain-splash and wind-blown rain were the main mechanisms of localised natural spread between and within vines in New Zealand.

It has also been postulated (Vanneste et al. 2011b) that epiphytic colonies of Psa on kiwifruit leaves could be spread by wind during hot dry conditions in the middle of the day. This has been observed with *Pseudomonas syringae* in green beans by Lindemann & Upper (1985), who found that upward movement of bacteria in aerosols was greatest on days immediately following rain. They considered that rain may either allow bacteria to be more easily removed from the leaves, or that it may promote bacterial growth, allowing more to be available for dispersal. The promotion of *P. syringae* growth by rain was observed by Hirano & Upper (2000). They suggested that the momentum of the raindrops may play an important role in triggering bacterial growth on bean leaves, because growth was lower when screens were used to reduce the velocity of the rain. If Psa behaves in a similar manner to *P. syringae*, the movement of Psa in wind-blown aerosols immediately following rain is likely to be important in the natural spread of the disease.

Human-mediated spread

Another mechanism that has allowed Psa to move between regions post incursion is through

human-mediated spread on infected plant material (grafting material, nursery material and pollen) or via vectors that may be contaminated with Psa. This includes items such as pruning tools, vehicles and machinery, animals and insects, soil and people (Everett et al. 2012a). Human-mediated spread can result in both localised and long-distance movement.

In the 1992 detection of Psa in Italy, Scortichini (1994) suspected that the pathogen had entered the orchard with 2-year-old vines as propagation material before spreading to the older vines, because the 2-year-old 'Hayward' vines were affected by disease, whereas older vines in the same orchards had only minor symptoms. Italian research into the 2008 Psa biovar 3 outbreak suggests that it began from a unique initial focus in the province of Latina (Vanneste et al. 2011b) and then spread between countries (Italy, France and Portugal) through movement of infected plant material. Psa was detected in Spain in 2011 and is suspected to have arrived on infected *A. chinensis* nursery stock in 2010 (Abelleira et al. 2011, 2014).

The spatial dynamics of the New Zealand outbreak were described using spatio-temporal analysis investigating 2066 kiwifruit orchards, of which 1354 were Psa positive (65.5%) (Rosanowski et al. 2013). The study showed that during the first 2 years of the outbreak (November 2010 to February 2013), 98% of the spread was within 10 km of an infected orchard (Rosanowski et al. 2013) and was considered to be localised spread. In addition, Rosanowski et al. (2013) identified 12 unique clusters of infected orchards that were >20 km from infected orchards and were most likely to be due to human-mediated long-distance spread. A further 13 clusters of Psa positive orchards, which were 10–20 km from other infected orchards, could have become infected by either human-mediated long-distance spread or localised spread during extreme wet and windy weather. The arrival of Psa into New Zealand is also likely to have been due to the movement of infected plant material and appeared to have a single point of introduction at or close to the area where it was first detected (Ministry for Primary Industries 2011). The spatial research of Rosanowski et al. (2013) showed that the first orchards to have reported Psa were situated centrally within the area of the highest density of infected orchards, also suggesting a single point source for the New Zealand outbreak.

Vector associated spread

Associated with human-mediated spread is spread via vectors that may be contaminated with Psa. This includes items such as pruning tools, vehicles and machinery, animals and insects, soil and people (Everett et al. 2012a). Potential vectors of Psa were studied in New Zealand on samples collected in light rain conditions from five people (clothing, boots, arms and heads), inside and outside six orchard vehicles and a trailer, 11 orchard tools that had been cleaned using the industry recommendations, and from the feet of two rabbits (Everett et al. 2012a). The researchers isolated bacteria from the swabs and then tested for Psa using PCR. The only personal item found to be positive for Psa was a wet raincoat. The vehicles were Psa-free except for the tyres of four vehicles and the upright section of a trailer that was covered in soil from the tyres. Soil from the feet of both the rabbits also tested positive. All the positive samples were associated with moist soil except the raincoat sample. A key finding was the absence of Psa from people (1/32 samples was weakly positive) despite favourable weather conditions. This implies that the risk of direct transfer of bacteria by clothing is low. It was also reassuring that the tools were not harbouring Psa. The presence of Psa from tyres was concerning and reinforces the need to clean and disinfect vehicles that have been on infected orchards thoroughly prior to entering uninfected orchards. This could be a mechanism for human-mediated long-distance spread of Psa (Everett et al. 2012a).

In general it appears that localised spread of Psa (e.g. <10 km) is likely to be predominantly due to rain and wind with some vectored spread occurring, whereas long distance spread (>10-20 km) is likely to be predominantly due to human mediated

spread via infected plant material. The role of contaminated vectors in long distance spread is uncertain.

HOST PLANT

Spread within the host plant

Serizawa & Ichikawa (1993a) found that the bacteria spread from inoculated leaf lesions to the leaf midrib and down to the petiole where bacterial exudate was observed. They also found that Psa could be isolated from the midrib and petioles of leaves when the tender shoot was inoculated with Psa.

Bacterial exudate from canes was observed to cease in summer at the same time that rapid callus formation occurred, although 20% of diseased canes resumed oozing bacterial exudate the following spring (Serizawa & Ichikawa 1993b). Serizawa et al. (1994) observed that growth of wound-healing tissue (callus) was related to temperature. They found tissue growth increased rapidly above 22°C and declined gradually below 20°C, until it ceased entirely below 15°C. This is important as Serizawa et al. (1994) went on to observe that the bacteria were inhibited by the growth of wound-healing tissue and the bacterial population declined rapidly when this callus tissue was formed. Serizawa et al. (1994) concluded that branch infection observed in winter and early spring was via wounds that were exposed to bacteria in autumn and early winter and these bacteria came from leaf lesions that were formed in the preceding spring.

In New Zealand, a study in the 2011–12 season showed that new leaf spots appeared throughout the summer period, indicating that inoculum was available via rain and rain-splash, and the only period with no new infection was an extended dry period of 4 weeks in summer (Horner & Manning 2012). The observation of new infections year-round in New Zealand is in contrast to Italy (Kay 2011) and Japan (Serizawa & Ichikawa 1993c), where new infections cease in summer. It is thought that this is due to higher summer temperatures in Italy and Japan compared to New Zealand.

Research in New Zealand soon after Psa was first detected showed that when the trunks of small vines were inoculated, the bacteria could readily move, both above and below the point of inoculation, and could transverse the graft union from the scion down to the rootstock (Tyson et al. 2014b). This research also showed that movement of bacteria occurred during autumn, winter and spring, with a maximum movement of 95 cm over 151 days (Tyson et al. 2014b).

Spinelli et al. (2015) used transgenic Psa strains to observe flower colonisation by bacteria *in vivo* and found that Psa first colonises the stigma, then undergoes rapid multiplication before migrating within the style to the ovary or calyx. They also observed systemic invasion from the flower pedicel into the vines, and recorded leaf spots 2 months post-inoculation. This study provides insight into the mechanism of Psa movement within flowers, although it should be noted that the flowers were inoculated with Psa and the disease transmission may not be the same in naturally infected flowers.

Leaf tissue age

Kiwifruit vines are deciduous and lose their leaves in late autumn and early winter (May to July in New Zealand) and begin to produce new leaves in spring (September). Studies on developing leaves in Japan found that the susceptibility to Psa was highest when the leaf blade reached 2 cm in length, which is approximately 1 week old, and decreased as the leaves matured (Serizawa & Ichikawa 1993b). However, season played a large part in leaf susceptibility, as new leaves in spring had much higher disease severity scores than new leaves developing in summer on established vines (Serizawa & Ichikawa 1993b).

In New Zealand, Tyson et al. (2015) found that both detached leaves and leaves on potted plants that were 1 to 3 weeks old when inoculated with Psa (the period of rapid expansion) had a higher percentage of leaves with leaf spots than leaves that were 4 or more weeks old. The difference between results for the summer leaves in Japan and New Zealand is probably due to a higher field temperature and a lack of inoculum for natural infection in the Japanese trial in summer. These studies show that the period of rapid expansion

of leaves is also the period of highest risk of infection. This also coincides with the period of least efficacy of protective sprays (Gaskin 2012). Gaskin (2012) showed that spray coverage was reduced because of rapid leaf expansion which, depending on the mode of action of the protective spray, could reduce the efficacy on newly developing susceptible leaves.

It is possible that kiwifruit tissues show ontogenic resistance, whereby tissues become increasingly resistant to pathogens with age, as has been shown in other deciduous hosts such as grapevines (Ficke et al. 2002). If this is the case in kiwifruit then tissue other than leaves (e.g. shoots and inflorescences) may also show this pattern.

Vine age

Recent reports of the biovar 3 strain in Italy indicate that younger, newly-grafted plants were more susceptible than older plants in the same orchard (Vanneste et al. 2011b). This is in contrast to results from Chinese studies where they found that the older vines showed a higher prevalence of disease (Li et al. 2001; Zhang et al. 2013).

Host susceptibility

There is considerable variation in the susceptibility of different commercial kiwifruit cultivars to bacterial canker. 'Hort16A' and other *A. chinensis* cultivars consistently show higher disease incidence and severity than *A. deliciosa* cultivars (Balestra et al. 2009a,b). Froud et al. (2014) quantified the effect of Psa on the productivity of 'Hort16A' over time compared with 'Hayward' and found that there was a much greater and more rapid impact on 'Hort16A'. A study in New Zealand looking at grower-reported symptoms found that male kiwifruit vines (various *A. deliciosa* cultivars) had a higher prevalence (46%) of shoot wilting and cane dieback than the female cultivar 'Hayward' (31%) (Froud et al. 2015). To date there has been no published information on the differences in susceptibility of new kiwifruit cultivars or other kiwifruit species, although *A. arguta* seems to be less affected by bacterial canker in New Zealand (Vanneste et al. 2014).

Spinelli et al. (2011) showed that Psa could penetrate the leaf surface through damaged leaf hairs and also postulated that these trichomes could provide a very favourable environment for bacterial growth. They also noted that the *A. chinensis* cultivars had very dense trichomes in comparison to those of *A. deliciosa* in Italy and suspected that the presence of dense trichomes may contribute to the susceptibility of *A. chinensis* kiwifruit cultivars.

Host phenology is also very different between cultivars in New Zealand, with the *A. chinensis* cultivars coming into both budburst and flowering 4–6 weeks earlier than 'Hayward'. This adds two additional risk factors to these cultivars: the first being that they are at greater risk from frost, which is strongly associated with Psa infection (Ferrante & Scortichini 2014) (discussed further later), and the second being that a large number of susceptible leaves (1–2 weeks old) are present in vine canopies during the cooler and wetter weather conditions that predominate during the early part of a typical New Zealand spring. Ferrante & Scortichini (2014) found that *A. deliciosa* was more frost tolerant than *A. chinensis*.

In addition to these factors there has been interest in determining the genetic basis for host plant resistance (Scortichini et al. 2012; Cotrut et al. 2013; McCann et al. 2013; Reglinski et al. 2013).

ENVIRONMENTAL RISK FACTORS

Climatic factors

Serizawa & Ichikawa (1993d) found that bacterial populations in leaf lesions were highest in late spring in Japan (10^6 to 10^7 cfu/ml) and dropped rapidly over summer to 10^2 to 10^3 cfu/ml when the mean temperature over the 10 days prior to isolation was between 20°C and 24°C. When the temperature was higher (25–26°C) in late summer, Psa was not detected in some lesions and was low in those where it was present (10– 10^1 cfu/ml). In autumn, the bacterial populations increased again and remained high until early winter (10^4 to 10^7 cfu/ml). A similar pattern was seen for bacterial exudate from leaf lesions, which was high in spring, autumn and

early winter, and low to not present over summer (Serizawa & Ichikawa 1993d).

Field studies on Psa in Japan on 'Hayward' vines indicated that the range of temperature for growth of Psa was 10°C to 20°C, with an optimum temperature of 15°C (± 3°C) (Serizawa & Ichikawa 1993b). They also noted that formation of wound healing tissue was highest in mid-summer, with a mean temperature of 25°C, and this coincided with the cessation of bacterial exudate oozing from parts of affected vines (Serizawa & Ichikawa 1993b). Further studies on inoculated vines in growth chambers at a range of variable and constant day:night temperatures (Serizawa & Ichikawa 1993c) suggested an optimal temperature range for Psa growth of 10–18°C, which was consistent with their field-based observations.

Casonato & Bent (2014) observed that symptoms of disease caused by Psa increased with greater exposure to rainfall compared with kiwifruit vines that were protected from rain by breathable plastic covers.

R.M. Beresford and colleagues (Plant & Food Research) developed a risk model in 2011 to predict Psa infection events in New Zealand based on rain and temperature exposures, and this was described by McKay et al. (2012). The model used daily rainfall and temperature to simulate the bacterial multiplication rate to predict the relative risk of infection each day. This was shown to be highly accurate in predicting days when infection occurred in susceptible trap plants (potted 'Hort16A') during spring, but produced a proportion of false positive predictions during autumn and winter (Beresford & Tyson 2014). It was concluded that some of the false positives arose because, although weather conditions were suitable for infection in autumn and winter, inoculum was less available than in spring.

Studies in Italy observed that frost events during winter (in 2007–2008) were associated with outbreaks of disease the following spring and autumn on gold-fleshed (*A. chinensis)* (Ferrante et al. 2012) and green-fleshed (*A. deliciosa*) kiwifruit (Ferrante & Scortichini 2014). Frost damage allows direct entry of the pathogen into the vine through the damaged tissue (Ferrante & Scortichini 2014), although the exact mechanism of why frost promotes bacterial canker is not yet determined. It is important to note that Psa is not an ice nucleation bacterium like *Pseudomonas syringae* (Rees-George et al. 2010). In Psa-infected orchards in Bay of Plenty, New Zealand, the authors have observed that vines in frost-prone places, e.g. hollows or gullies within a block, and vines adjacent to breaks in shelter, show more disease symptoms than other vines in the same block.

More severe symptoms of Psa bacterial canker in areas where strong winds occurred were observed during a weather risk study (Serizawa & Ichikawa 1993b). They postulated that this could be an important risk factor for infection.

Geographical factors

Regional differences in the prevalence and severity of bacterial canker in New Zealand can in part be explained by the period of time the pathogen has been present in a region and differences in climatic conditions between regions. Cogger & Froud (2015) found differences in time to infection between different regions during the New Zealand outbreak and showed that, while the Te Puke region was severely affected with 10% of orchards infected after 6 months, orchards in the Whakatane region had a much faster rate of symptom appearance following the first detection in the region, with 41% of orchards infected in the first 6 months. This was noteworthy as the density of orchards was lower, the distances between orchards was greater, and there was less planted area of the susceptible *A. chinensis* in Whakatane than in Te Puke. The most obvious difference between the two regions was a higher risk of frost in Whakatane. Li et al. (2001) also found that the prevalence of kiwifruit bacterial canker disease was greater above 750 m elevation in China, and suggested that colder temperatures at the higher elevations may favour the disease.

Shelter

Deciduous shelters may allow greater access for Psa inoculum into the blocks during winter and early spring. In addition, there may be more wind damage to vines during winter providing wound

sites for the entry of Psa. Field assessments have shown a higher prevalence of leaf spotting immediately adjacent to breaks in shelter, indicating access points for the aerially dispersed bacteria (I.J. Horner, Plant & Food Research, personal communication; Serizawa et al. 1989). This was also noted by Casonato & Bent (2014), who observed that Psa symptoms were worse in 'Hort16A' vines immediately adjacent to a gap in artificial shelter in their study block. In another study, researchers postulated that cryptomeria (*Cryptomeria japonica*) may be beneficial by slowing the movement of Psa inoculum transfer between blocks (Vanneste et al. 2012).

ORCHARD MANAGEMENT RISK FACTORS

Pollen

In the initial New Zealand Psa outbreak, imported pollen from Chile and China and locally sourced pollen from New Zealand tested positive for Psa (Ministry for Primary Industries 2011), but there were concerns that the results were false positives (Vanneste et al. 2011a). It was also unknown if the Psa in the pollen samples was alive, or if live Psa on pollen could transmit disease to kiwifruit vines. It is now well understood that pollen can harbour Psa (Gallelli et al. 2011; Vanneste et al. 2011a; Everett et al. 2012d). Psa has been isolated from Italian pollen (Vanneste et al. 2011a) and Everett et al. (2012b) have recovered live Psa from stored New Zealand pollen. Vanneste et al. (2011a) found Psa in pollen from two orchards that were asymptomatic at the time of collection. These had symptoms the following season, and the authors postulated that one of the first signs of orchard infection may be the presence of the pathogen in pollen. This hypothesis is supported by the detection of Psa in commercially collected and stored pollen from New Zealand that was harvested during the 2009 spring flowering, approximately 11 months before the detection of leaf spots and severe systemic infection of Psa in New Zealand (Everett et al. 2012b).

In addition there is evidence that pollen samples collected in infected regions from asymptomatic vines have Psa present (Gallelli et al. 2011; Heuer & Taylor 2015; Taylor et al. 2015). Another factor in the risk of Psa contaminated pollen is that, while the tests used to detect Psa have been optimised, they are still imperfect and have poor sensitivity (false negatives) for pollen (sensitivity = true positives/(true positives + false negatives)) based on studies on *A. chinensis* (Heuer & Taylor 2015; Taylor et al. 2015). Therefore the prevalence of Psa in pollen may be underestimated by testing.

The evidence for pollen transmission of Psa has been strengthened by a study in Italy where it was found that Psa isolates could be recovered from flowers and leaves following application of Psa inoculated pollen and (for 48 hours) after application of naturally infected pollen (Stefani & Giovanardi 2011).

A recent study by Italian researchers investigated the transmission of bacterial canker by naturally contaminated kiwifruit pollen to kiwifruit vines planted 100 km from any known infected orchards (Tontou et al. 2014). They observed leaf spots the following spring and found that application of pollen resulted in transmission of Psa to kiwifruit vines in low numbers. Although infection rates were low, there was sufficient evidence that pollen has the potential to transmit Psa and to establish new disease foci (Tontou et al. 2014). They concluded that, as they had not detected systemic infection or cankers in the first year, the transmission was probably the result of epiphytic Psa overwintering in buds and that transmission via pollen may not present as an outbreak for up to 2 years. There have also been several studies in New Zealand that aim to reduce the risk of inadvertently transmitting Psa with pollen by investigating methods to reduce the amount of viable bacteria present on pollen while still maintaining pollen viability (Everett et al. 2012d).

A recent development of a real-time viable PCR tool using propidium monoazide to determine if oral bacterial biofilms in humans are dead or alive may be applicable to plant pathogenic bacteria in the future, to eliminate the issues that were faced in the Psa outbreak regarding whether viable bacteria were present on pollen (Alvarez et al. 2013).

There is sufficient evidence that live Psa can be present in pollen collected from infected regions. There is also evidence that it can be present in pollen collected from asymptomatic flowers and vines. There is now evidence that transmission of bacterial canker from naturally contaminated pollen is possible and therefore the importance of pollen as a biosecurity risk has been clarified. However the relative importance of contaminated pollen used for artificial pollination has not been established in relation to disease management on orchards.

Transmission via insects

It has been suggested that insects may be associated with both localised spread and human-assisted spread of Psa. The movement of *P. syringae* from infected plants onto bacterial plates via insects was observed in bean crops, but only if there was dew on leaves when the insects traversed the leaf (Hirano & Upper 2000).

Given that Psa is present in kiwifruit flowers and on pollen, and that beehives are used in most New Zealand kiwifruit orchards during flowering to assist pollination, bees have been of particular interest as potential means of Psa transmission. In New Zealand, Pattemore et al. (2014) found that inoculated bees within a containment facility could bring Psa-contaminated pollen back to the hive and Psa contamination was found on the outer frame of the hive after 2 days, but was not found in the centre of the hive. Psa continued to be detected on bees but not in hives after 2 days, and the cfu/bee reduced rapidly to be undetectable by day nine. Because of the artificiality of this experiment, where the bees were not able to forage in the outside environment, the experiment was repeated using a streptomycin-resistant strain of *P. syringae* pv. *syringae* with free-foraging beehives and Pattemore et al. (2014) found very similar results to those obtained with the contained bees. The authors concluded that bees could become contaminated with Psa and potentially contaminate other members of the hive over a short period, and they therefore recommended that hives be rested between orchards for more than 9 days. They also pointed out that contamination does not necessarily prove the ability of bees to transmit disease but it is possible (Pattemore et al. 2014), and this has been shown for fire blight (*Erwinia amylovora*) in apples (de Wael & de Greef 1990; Johnson et al. 1993; Pattemore et al. 2014).

Other common insects in New Zealand kiwifruit orchards that were suspected to be capable of transmitting Psa were cicadas, blowflies and passion-vine hoppers. Everett et al. (2012c) examined these and showed that Psa was present on the bodies of cicadas, blowflies and passion-vine hoppers and from the mouthparts of the latter two. Tyson et al. (2012b) also showed that cicada egg batch wounds had a significantly higher isolation rate of Psa than non-wounded canes and this was more likely to be the result of susceptible wounds than an effective vector. Further studies are required to determine whether disease spread is actually possible via contaminated insects.

Vine management

Kiwifruit vines are extremely vigorous, requiring winter and summer pruning and vine management to control growth and ensure that fruiting canes are available each season. Winter pruning requires removal of old or dead wood and canes with poor buds or poor spacing (Torr 2010). After pruning, the retained canes are tied down to the trellis structure. Winter pruning results in wounds to canes and leaders, and tying down can cause cracking in canes.

Spring and summer pruning prevents excessive extension of shoots and involves cutting off or ripping out blind shoots (shoots with no flower buds) and terminating the vegetative growing tips of fruiting shoots (Torr 2011). Extension growth can be managed by three methods: (1) crushing or squeezing the shoot tip to promote self-termination of the shoot, (2) using 'zero leaf pruning', where the shoot is cut distally to the final flower or fruit stalk so that the presence of the fruit inhibits vegetative shoots from forming and (3) 'gel tipping', which is less common, where the cut shoot is treated with a growth-inhibiting gel to prevent further vegetative growth. During the

growing season bud thinning and fruit thinning are also carried out to maximise fruit quality, and at the end of the growing season fruit are picked.

Girdling, a process of cutting into the bark and cambium of the vine using a handheld chainsaw blade, is used to increase fruit yield and dry matter content. Male vine management involves winter pruning to remove excessive growth and to leave short spurs with flower buds. Rigorous pruning occurs in spring after flowering, followed by tip squeezing, cutting and shoot ripping (KVH 2013). Pruning, thinning or girdling activities all result in wounds that may be sites for Psa infection. Italian field observations showed that Psa lesions could be found on the outer margins of pruning wounds and they concluded that these wounds provide direct entry to the pathogen (Ferrante et al. 2012). In New Zealand, the effects of pruning and girdling on disease development and the potential for pruned material to contribute to infection have been studied. Disease progression in New Zealand orchards was recorded for three seasons after the start of the 2010 outbreak (Horner & Manning 2011, 2012). Psa disease symptoms continued to appear throughout the growing season, indicating that inoculum was available in the orchard whenever vine management activities were undertaken, although Tyson et al. (2014c) showed that rain events are necessary for movement of the inoculum. Miller & Horner (2012) induced bacterial canker symptoms on summer pruning wounds on inoculated canes up to 64 days post pruning. They also observed the development of dieback symptoms within 5 weeks of inoculation onto 24-h-old wounds and the spread of the pathogen systemically into un-inoculated shoots on the same canes. An exploratory study to investigate the risk of spring pruning techniques could not differentiate between each pruning type and the unpruned controls and further work is required to identify which, if any, of these techniques increases the risk of Psa entry and disease developing (Thorp et al. 2012).

Tyson et al. (2012a) showed that leaves from natural leaf fall and pruning waste left on the orchard floor yielded viable Psa throughout the winter period and well into the budbreak period the following spring. These could be an important source of inoculum during the spring infection period, in addition to cankers on living vines.

Callus formation was observed in monitored orchards in New Zealand on pruning cuts made to remove Psa-infected vine material. More rapid and complete healing occurred on pruning cuts made in late spring and summer than on early spring cuts (Horner et al. 2013). This study also showed that Psa lesions were halted where full callus formation was able to occur, but it was unclear whether failure to form callus was related to the presence of Psa or to other factors (Horner et al. 2013). It is possible that this is due to low temperatures in early spring inhibiting callus formation.

Another study (Snelgar et al. 2012) investigated girdling wounds and showed that inoculated vines became infected, and that unprotected girdling wounds remained susceptible for at least 15 days. It was also observed that callus formation was slower on inoculated vines than on un-inoculated vines (Snelgar et al. 2012).

It is unquantified whether pathogen entry via vine management wounds is of greater or lesser importance than pathogen entry via natural plant entry points (i.e. stomata, lenticels, hydathodes) and naturally occurring wounds.

CONCLUSION

This review summarises the key epidemiological risk factors that have been investigated to date. There is sufficient evidence that Psa is spread locally by the means of wind and rain and that long-distance spread via kiwifruit plant material is a risk. In New Zealand, researchers have shown that Psa inoculum is present year-round, with spring being a key infection period, and there is a strong relationship between the disease severity and climatic factors such as rainfall, temperature and frost.

There are still many gaps in the understanding of kiwifruit bacterial canker epidemiology, particularly a full understanding of the life cycle and infection process of the disease in New Zealand, but research completed to date has built a solid platform for understanding the disease. There has been a large amount of research

completed in a very short time on Psa both in New Zealand and internationally, and much of this work is still in commercial reports and waiting to be published and reviewed.

ACKNOWLEDGEMENTS

Thanks to the scientists who have made their work available for this review in the form of published papers and scientific reports.

REFERENCES

Abelleira A, Ares A, Aguín O, Picoaga A, López MM, Mansilla P 2014. Current situation and characterization of *Pseudomonas syringae* pv. *actinidiae* on kiwifruit in Galicia (northwest Spain). Plant Pathology 63: 691-699.

Abelleira A, Lopez MM, Penalver J, Aguin O, Mansilla JP, Picoaga A, Garcia MJ 2011. First report of bacterial canker of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae* in Spain. Plant Disease 95: 1583-1583.

Alvarez G, Gonzalez M, Isabal S, Blanc V, Leon R 2013. Method to quantify live and dead cells in multi-species oral biofilm by real-time PCR with propidium monoazide. AMB Express 3: 1-1.

Balestra GM, Renzi M, Mazzaglia A 2010. First report of bacterial canker of *Actinidia deliciosa* caused by *Pseudomonas syringae* pv. *actinidiae* in Portugal. New Disease Reports 22: 10.

Balestra GM, Mazzaglia A, Quattrucci A, Renzi M, Rossetti A 2009a. Occurrence of *Pseudomonas syringae* pv. *actinidiae* in Jin Tao kiwi plants in Italy. Phytopathologia Mediterranea 48: 299-301.

Balestra GM, Mazzaglia A, Quattrucci A, Renzi M, Rossetti A 2009b. Current status of bacterial canker spread on kiwifruit in Italy. Australasian Plant Disease Notes 4: 34-36.

Balestra GM, Mazzaglia A, Quattrucci A, Spinelli R, Graziani S, Rossetti A 2008. Bacterial canker on *Actinidia chinensis*. Informatore Agrario 64: 75-76.

Bastas KK, Karakaya A 2012. First report of bacterial canker of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae* in Turkey. Plant Disease 96: 452.

Beresford R, Tyson JL 2014. Seasonal accuracy of the Psa risk model. New Zealand Kiwifruit Journal 228: 18-19.

Biondi E, Galeone A, Kuzmanovic N, Ardizzi S, Lucchese C, Bertaccini A 2013. *Pseudomonas syringae* pv. *actinidiae* detection in kiwifruit plant tissue and bleeding sap. Annals of Applied Biology 162: 60-70.

Casonato SG, Bent S 2014. Impact of covered structures on the progression of Psa-V: Phase Two. A confidential report prepared for Zespri Group Limited, SPTS No. 10790. Plant & Food Research, Te Puke, New Zealand. 34 pp.

Cogger N, Froud K 2015. Application of survival analysis to plant protection research. In: Beresford RM, Froud KJ, Kean JM, Worner SP ed. The plant protection data tool box. New Zealand Plant Protection Society, Christchurch, New Zealand. In Press.

Cotrut R, Renzi M, Taratufolo MC, Mazzaglia A, Balestra G, Stanica F 2013. *Actinidia arguta* ploidy level variation in relation to *Pseudomonas syringae* pv. *actinidiae* susceptibility. Lucrari Stiintifice, Universitatea de Stiinte Agricole Si Medicina Veterinara "Ion Ionescu de la Brad" Iasi, Seria Agronomie 56: 29-38.

de Wael L, de Greef M 1990. Influence of the honeybee on the transmission of fireblight. Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent 55: 1107-1111.

Donati I, Buriani G, Cellini A, Mauri S, Costa G, Spinelli F 2014. New insights on the bacterial canker of kiwifruit (*Pseudomonas syringae* pv. *actinidiae*). Journal of Berry Research 4: 53-67.

Dreo T, Pirc M, Ravnikar M, Zezlina I, Poliakoff E, Rivoal C, Nice F, Cunty A 2014. First report of *Pseudomonas syringae* pv. *actinidiae*, the causal agent of bacterial canker of kiwifruit in Slovenia. Plant Disease 98: 1578-1578.

Everett KR, Pushparajah IPS, Vergara MJ 2012a. *Pseudomonas syringae* pv. *actinidiae* on surfaces in the orchard. New Zealand Plant Protection 65: 19-24.

Everett KR, Vergara MJ, Pushparajah IPS 2012b. Testing stored pollen as a source of *Pseudomonas syringae* pv. *actinidiae* (Psa) inoculum. A confidential report prepared for ZESPRI Group Limited. SPTS No. 7235. Plant & Food Research, Auckland, New Zealand. 18 pp.

Everett KR, Larsen NJ, Logan DP, Pushparajah IPS, Rowe C, Vergara MJ 2012c. Potential for insect vectoring. A report prepared for ZESPRI Group Limited. SPTS No. 7213. Plant & Food Research, Auckland, New Zealand. 13 pp.

Everett KR, Taylor RK, Romberg MK, Rees-George J, Fullerton RA, Vanneste JL, Manning MA 2011. First report of *Pseudomonas syringae* pv. *actinidiae* causing kiwifruit bacterial canker in New Zealand. Australasian Plant Disease Notes 6: 67-71.

Everett KR, Cohen D, Pushparajah IPS, Vergara MJ, Curtis CL, Larsen NJ, Jia Y 2012d. Heat treatments to kill *Pseudomonas syringae* pv. *actinidiae* on contaminated pollen. New Zealand Plant Protection 65: 8-18.

Ferrante P, Scortichini M 2014. Frost promotes the pathogenicity of *Pseudomonas syringae* pv. *actinidiae* in *Actinidia chinensis* and *A. deliciosa* plants. Plant Pathology 63: 12-19.

Ferrante P, Scortichini M 2015. Redefining the global populations of *Pseudomonas syringae* pv. *actinidiae* based on pathogenic, molecular and phenotypic characteristics. Plant Pathology 64: 51-62.

Ferrante P, Fiorillo E, Marcelletti S, Marocchi F, Mastroleo M, Simeoni S, Scortichini M 2012. The importance of the main colonization and penetration sites of *Pseudomonas syringae* pv. *actinidiae* and prevailing weather conditions in the development of epidemics in yellow kiwifruit, recently observed in central Italy. Journal of Plant Pathology 94: 455-461.

Ficke A, Gadoury DM, Seem RC 2002. Ontogenic resistance and plant disease management: A case study of grape powdery mildew. Phytopathology 92: 671-675.

Froud K, Cogger N, Beresford R 2014. The relationship between kiwifruit bacterial canker disease (Psa-V (*Pseudomonas syringae* pv. *actinidiae*)) and kiwifruit productivity. New Zealand Plant Protection 67: 34-40.

Froud K, Cogger N, Beresford R, Clark G 2015. Orchardist-observed prevalence of symptoms of kiwifruit bacterial canker disease in 'Hayward' kiwifruit blocks in New Zealand. Acta Horticultrae: 1st International Symposium on Bacterial Canker of Kiwifruit (Psa). In Press.

Gallelli A, Talocci S, L'Aurora A, Loreti S 2011. Detection of *Pseudomonas syringae* pv. *actinidiae*, causal agent of bacterial canker of kiwifruit, from symptomless fruits and twigs, and from pollen. Phytopathologia Mediterranea 50: 462-472.

Gaskin RE 2012. Visualising spray coverage on expanding kiwifruit leaves. A report prepared for Zespri Ltd. Plant Protection Chemistry New Zealand Ltd, Rotorua, New Zealand. 5 pp.

Hallett I 2012. Microscopic examination of the progression of Psa-V in Gold 3. Plant & Food Research (Psa) Research Note, Plant & Food Research, Auckland, New Zealand. 11 pp.

Heuer C, Taylor R 2015. Surveillance strategies for determining presence or absence of disease. In: Beresford R, Froud K, Worner SP, Kean J ed. The plant protection data toolbox. New Zealand Plant Protection Society, Christchurch, New Zealand. In Press.

Hirano SS, Upper CD 2000. Bacteria in the Leaf Ecosystem with Emphasis on *Pseudomonas syringae*, a Pathogen, Ice Nucleus, and Epiphyte. Microbiology and Molecular Biology Reviews 64: 624-653.

Holeva MC, Glynos PE, Karafla CD 2015. First report of bacterial canker of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae* in Greece. Plant Disease 99(5): 723.

Horner I, Manning M 2011. Psa progression within orchards. A confidential report prepared for ZESPRI Group Limited. SPTS No. 5997. Plant & Food Research, Havelock North, New Zealand. 33 pp.

Horner I, Manning M, Casonato SG 2013. Psa progression and intervention. A confidential report prepared for ZESPRI Group Limited. SPTS No. 8596. Plant & Food Research, Havelock North, New Zealand. 13 pp.

Horner IJ, Manning MA 2012. Progression of Psa within kiwifruit orchards. Proceedings

of the New Zealand Plant Protection Society Symposium - A snapshot of Psa. Hamilton, New Zealand. p. 9 (abstract only).

Johnson K, Stockwell V, Burgett D, Sugar D, Loper J 1993. Dispersal of *Erwinia amylovora* and *Pseudomonas fluorescens* by honey bees from hives to apple and pear blossoms. Phytopathology 83: 478-484.

Kay C 2011. Psa and Italian kiwifruit orchards—an observation. Kiwifruit Vine Health Case Study. http://www.kvh.org.nz/vdb/document/164 (15 June 2015). 8 pp.

Koh YJ, Kim GH, Jung JS, Lee YS, Hur JS 2010. Outbreak of bacterial canker on Hort16A (*Actinidia chinensis* Planchon) caused by *Pseudomonas syringae* pv. *actinidiae* in Korea. New Zealand Journal of Crop and Horticultural Science 38: 275-282.

KVH 2013. KVH Best Practice: Protecting Male Plants in a Psa-V Environment. KVH Bulletin, http://www.kvh.org.nz, (accessed 6 May 2015). 5 pp.

Li M, Tan G, Li Y, Cheng H, Han X, Xue L, Li L 2004. Resistance of different Chinese gooseberry cultivars to Chinese gooseberry bacterial canker caused by *Pseudomonas syringae* pv. *actinidiae* and their cluster analysis. Plant Protection 30: 51-54.

Li Y, Cheng H, Fang S, Qian Z 2001. Ecological factors affecting prevalence of kiwifruit bacterial canker and bacteriostatic action of bacteriocides on *Pseudomonas syringae* pv. *actinidiae*. Yingyong Shengtai Xuebao 12: 359-362.

Lindemann J, Upper C 1985. Aerial dispersal of epiphytic bacteria over bean plants. Applied and Environmental Microbiology 50: 1229-1232.

Liu Y, Li S, Zhu T, Shao B 2012. Specific DNA markers for detection of bacterial canker of kiwifruit in Sichuan, China. African Journal of Microbiology Research 6: 7512-7519.

McCann HC, Rikkerink EHA, Bertels F, Fiers M, Lu A, Rees-George J, Andersen MT, Gleave AP, Haubold B, Wohlers MW, Guttman DS, Wang PW, Straub C, Vanneste J, Rainey PB, Templeton MD 2013. Genomic analysis of the kiwifruit pathogen *Pseudomonas syringae* pv. *actinidiae* provides insight into the origins of an emergent plant disease. Plos Pathogens 9: e1003503.

McKay A, Beresford R, McKenna C, Dobson S 2012. Field testing Plant & Food Research's Psa risk prediction model. New Zealand Kiwifruit Journal May/Jun: 14-16.

Miller SA, Horner IJ 2012. Towards better understanding of the risk of summer pruning and Psa infection. Proceedings of the New Zealand Plant Protection Society Symposium: A snapshot of Psa. p. 13. (abstract only).

Ministry for Primary Industries 2011. Psa – Pathway tracing report. Psa Kiwifruit 2010-348. Ministry of Agriculture and Forestry, Wellington, New Zealand. 32 pp.

Pattemore DE, Goodwin RM, McBrydie HM, Hoyte SM, Vanneste JL 2014. Evidence of the role of honey bees (*Apis mellifera*) as vectors of the bacterial plant pathogen *Pseudomonas syringae*. Australasian Plant Pathology 43: 571-575.

ProMed 2011. Bacterial canker, kiwifruit – Chile: first report: (O'Higgins, Maule). ProMed posting (no. 20110325.0940) of 2011-03-25: http://www.promedmail.org (accessed 14 October 2014).

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

Reglinski T, Vanneste JL, Wurms K, Gould E, Spinelli F, Rikkerink E 2013. Using fundamental knowledge of induced resistance to develop control strategies for bacterial canker of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae*. Frontiers in Plant Science 4: Article 24. DOI: 10.3389/fpls.2013.00024.

Rosanowski SM, Carpenter T, Stevenson M, Froud K 2013. Quantification of the spatial distribution and natural rate of Psa spread in New Zealand. Report prepared for Zespri International Ltd and Kiwifruit Vine Health, Massey University, Palmerston North, New Zealand. 34 pp.

Scortichini M 1994. Occurence of *Pseudomonas syringae* pv. *actinidiae* on kiwifruit in Italy. Plant Pathology 43: 1035-1038.

Scortichini M, Marcelletti S, Ferrante P, Petriccione M, Firrao G 2012. *Pseudomonas syringae* pv. *actinidiae*: a re-emerging, multi-faceted, pandemic pathogen. Molecular Plant Pathology 13: 631-640.

Serizawa S, Ichikawa T 1993a. Epidemiology of bacterial canker of kiwifruit: 1. Infection and bacterial movement in tissue of new canes. Annals of the Phytopathological Society of Japan 59: 452-459.

Serizawa S, Ichikawa T 1993b. Epidemiology of bacterial canker of kiwifruit: 2. The most suitable times and environments for infection on new canes. Annals of the Phytopathological Society of Japan 59: 460-468.

Serizawa S, Ichikawa T 1993c. Epidemiology of bacterial canker of Kiwifruit 4. Optimum temperature for disease development of new canes. Annals of the Phytopathological Society of Japan 59: 694-701.

Serizawa S, Ichikawa T 1993d. Epidemiology of bacterial canker of kiwifruit: 3. The seasonal changes of bacterial population in lesions and of its exudation from lesion. Annals of the Phytopathological Society of Japan 59: 469-476.

Serizawa S, Ichikawa T, Suzuki H 1994. Epidemiology of bacterial canker of kiwifruit: 5. Effect of infection in fall to early winter on the disease development in branches and trunk after winter. Annals of the Phytopathological Society of Japan 60: 237-244.

Serizawa S, Ichikawa T, Takikawa Y, Tsuyumu S, Goto M 1989. Occurrence of bacterial canker of kiwifruit in Japan: description of symptoms, isolation of the pathogen and screening of bactericides. Annals of the Phytopathological Society of Japan 55: 427-436.

Snelgar B, Blattmann P, Tyson JL, Manning MA, Curtis C 2012. On-orchard management of *Pseudomonas syringae* pv. *actinidiae* infection and symptom expression: part C: Girdling - possible positive and negative effects on Psa. A report prepared for ZESPRI Group Limited. SPTS No. 6935. Plant & Food Research, Te Puke, New Zealand. 39 pp.

Spinelli F, Donati I, Vanneste J, Costa M, Costa G 2011. Real time monitoring of the interactions between *Pseudomonas syringae* pv. *actinidiae* and *Actinidia* species. Acta Horticulturae 913: 461-466.

Spinelli F, Donati I, Cellini A, Buriani G, Vanneste J, Yu J, Cornish D, Fiorentini L, Rocchi L, Felman CM, Mauri S, Kay C, Giacomuzzi V, Costa G 2015. Colonization of kiwifruit flowers by *Pseudomonas syringae* pv. *actinidiae* and methods to prevent infection. Acta Horticultrae: 1st International Symposium on Bacterial Canker of Kiwifruit (Psa): In Press.

Stefani E, Giovanardi D 2011. Dissemination of *Pseudomonas syringae* pv. *actinidiae* through pollen and its epiphytic life on leaves and fruits. Phytopathologia Mediterranea 50: 489-496.

Takikawa Y, Serizawa S, Ichikawa T, Tsuyumu S, Goto M 1989. *Pseudomonas-syringae* pathovar *actinidiae* new pathovar the causal bacterium of canker of kiwifruit in Japan. Annals of the Phytopathological Society of Japan 55: 437-444.

Taylor R, Surrey M, Alexander B 2015. Evaluation of DNA extraction and enrichment procedures for qPCR detection of *Pseudomonas syringae* pv. *actinidiae* in kiwifruit plants and pollen. Acta Horticultrae: 1st International Symposium on Bacterial Canker of Kiwifruit (Psa). In Press.

Thorp G, Barnett A, Blattmann M 2012. Short-term risk assessment of spring pruning techniques. A confidential report prepared for ZESPRI Group Limited. SPTS No. 7775. Plant & Food Research, Auckland, New Zealand. 7 pp.

Tontou R, Giovanardi D, Stefani E 2014. Pollen as a possible pathway for the dissemination of *Pseudomonas syringae* pv. *actinidiae* and bacterial canker of kiwifruit. Phytopathologia Mediterranea 53: 333-339.

Torr T 2010. Winter Pruning 2010. Technical Tips. EastPac Ltd, Bay of Plenty, New Zealand, Bulletin 81. 8 pp.

Torr T 2011. Managing Canopy Establishment in Hayward. Technical Tips. EastPac Ltd, Bay of Plenty, New Zealand, Bulletin 79. 6 pp.

Tyson JL, Manning M 2013. Review of splash dispersal of pseudomonads. Confidential Report for Plant and Food Research Ltd. Plant & Food Research, Auckland, New Zealand. 20 pp.

Tyson JL, Curtis CL, Manning MA 2014a. Budrot in 'green' kiwifruit (*Actinidia* sp.) varieties – Spring 2014 A confidential report prepared for Zespri Group Limited, SPTS No. 11140, Plant & Food Research, Auckland, New Zealand. 9 pp.

Tyson JL, Rees-George J, Curtis CL, Manning MA, Fullerton RA 2012a. Survival of *Pseudomonas syringae* pv. *actinidiae* on the orchard floor over winter. New Zealand Plant Protection 65: 25-28.

Tyson JL, Horner IJ, Curtis CL, Blackmore A, Manning MA 2015. Influence of leaf age on infection of *Actinidia* species by *Pseudomonas syringae* pv. *actinidiae*. New Zealand Plant Protection 68: 328-331.

Tyson JL, Curtis C, Dobson S, Logan DP, Manning M, Rowe C 2012b. *Pseudomonas syringae* pv. *actinidiae* wound entry sites - cicada egg nest field trial. A report prepared for ZESPRI Group Limited. SPTS No. 7217. Plant & Food Research, Auckland, New Zealand. 17 pp.

Tyson JL, Curtis CL, Manning MA, Rees-George J, Snelgar WP, Blattmann P 2014b. Systemic movement of *Pseudomonas syringae* pv. *actinidiae* in kiwifruit vines in New Zealand. New Zealand Plant Protection 67: 41-47.

Tyson JL, Manning MA, Curtis CL, Dobson SJ, McKenna CE, Vergara MJ 2014c. Inoculum production and infection of kiwifruit plants by *Pseudomonas syringae* pv. *actinidiae* in New Zealand. VIII International Symposium on Kiwifruit. Dujiangyan City, Chengdu, China. p.105 (abstract only).

Vanneste J, Cornish D, Yu J, Stokes C 2014. First Report of *Pseudomonas syringae* pv. *actinidiae* the Causal Agent of Bacterial Canker of Kiwifruit on *Actinidia arguta* Vines in New Zealand. Plant Disease 98: 418-418.

Vanneste J, Yu J, Cornish D, Tanner D, Windner R, Chapman J, Taylor R, Mackay J, Dowlut S 2013. Identification, virulence, and distribution of two biovars of *Pseudomonas syringae* pv. *actinidiae* in New Zealand. Plant Disease 97: 708-719.

Vanneste JL 2013. Recent progress on detecting, understanding and controlling *Pseudomonas syringae* pv. *actinidiae*: a short review. New Zealand Plant Protection 66: 170-177.

Vanneste JL, Moffat BJ, Oldham JM 2012. Survival of *Pseudomonas syringae* pv. *actinidiae* on *Cryptomeria japonica*, a non-host plant used as shelter belts in kiwifruit orchards. New Zealand Plant Protection 65: 1-7.

Vanneste JL, Giovanardi D, Yu J, Cornish DA, Kay C, Spinelli F, Stefani E 2011a. Detection of *Pseudomonas syringae* pv. *actinidiae* in kiwifruit pollen samples. New Zealand Plant Protection 64: 246-251.

Vanneste JL, Kay C, Onorato R, Yu J, Cornish DA, Spinelli F, Max S 2011b. Recent advances in the characterisation and control of *Pseudomonas syringae* pv. *actinidiae*, the causal agent of bacterial canker on kiwifruit. In: Costa G, Ferguson AR ed. Acta Horticulturae. Pp. 443-455.

Vanneste JL, Poliakoff F, Audusseau C, Cornish DA, Paillard S, Rivoal C, Yu J 2011c. First report of *Pseudomonas syringae* pv. *actinidiae*, the causal agent of bacterial canker of kiwifruit in France. Plant Disease 95: 1311-1312.

Zhang H, Li H, Feng J, Xiao J, Song G, Xie M 2013. Investigation and analysis of infection caused by *Pseudomonas syringae* pv. *actinidiae* and its affecting factors in Zhejiang province. Acta Agriculturae Zhejiangensis 25: 832-835.