A Review of Research and Development Undertaken on Psa

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Contents

1 EXECUTIVE SUMMARY

***Pseudomonas syringae* pv. *actinidiae* (Psa) is the causal agent of bacterial canker in kiwifruit, a significant threat with wide reaching economic consequences. Psa has been estimated to have cost over $1 billion to the New Zealand kiwifruit industry since 2010. Although productivity has recovered, Psa still remains a prevalent threat as the majority of orchards harbour the bacteria and only one kiwifruit growing region in New Zealand has yet to exhibit infection. Control methods are currently in place and being used to manage the incidence and progression of Psa infection. This has relied upon chemical products such as copper, antibiotics and elicitors in addition to orchard management practises such as the removal of infected material. More recently the use of other biological organisms to control Psa have been trialled and included in a comprehensive control programme. All these products are effective to various extents, but it has been found that some samples of Psa have acquired low level resistance to some of the chemical controls, which could potentially render them ineffective. Subsequently a continuing KVH / Zespri funded research programme is essential as an effective response to ensure the ongoing ability to control Psa, addressing the occurrence of resistance, optimising control methods, and identifying new ways of combating bacterial canker.**

This document aims to review past and ongoing research addressing the threat of Psa to New Zealand kiwifruit, identifying areas where research needs to be strengthened and highlighting new and novel areas which could have potential in combatting Psa. This review covers the epidemiology of Psa, biological and chemical control of the bacteria, on-orchard management and the breeding of Psa tolerance varieties of kiwifruit.

2 INTRODUCTION

2.1 GENERAL INTRODUCTION

The bacterium *Pseudomonas syringae* is one of the most prevalent plant pathogens, existing as more than 50 varieties, and is able to infect over 180 plant species (González et al., 2000). *P. syringae* pv. *actinidiae* (Psa) is one such variety and is the cause of bacterial canker in kiwifruit (*Actinidia* spp.). The disease presents itself through leaf spotting, bud browning, bud drop, cane dieback and red or white exudate from canes or trunks, and extreme cases even vine death (Balestra et al. 2009). Psa can be classified into several types of biovars. Biovars 1 and 2 were involved in outbreaks causing moderate damage in kiwifruit industries in Japan, China, Korea and Italy. Recent outbreaks causing serious damage to kiwifruit, including that of New Zealand, have been caused by Psa biovar 3, otherwise known as Psa-V. Psa was historically typed into a forth biovar, known as Psa-LV, however this bacterium is no longer considered a type of Psa (Froud *et al*. 2015).

Although not much is known about how this bacterium causes disease, it is thought to be spread primarily by water splash, and thrives in humid and wet conditions. It is thought to initially survive on the surface of the plant like many of its relatives (Arnold et al. 2011), and when the opportunity arises, it will invade the plant through natural openings or wounds to spread through the plant and either survive asymptomatically or to cause disease in the leaves, cane and trunks of kiwifruit (Vanneste et al. 2011a). It is noted that rain is a key component of infection, in the absence of rainfall, bacterial populations of *P. syringae* remain static (Everett 2011b).

Although it is relatively easy to kill on a lab bench or a surface, there is no cure for Psa once infection takes hold (Donati et al. 2014), meaning the control of Psa relies on prevention and control. Currently disease management is limited to physical orchard practises such as the removal of infected plant material and trying to slow the spread of Psa. Chemical compounds such as copper, elicitors and antibiotics are used to prevent the disease from occurring, however these practises may become less and less effective unless bacterial resistance is managed carefully.

2.2 TIMELINE

Psa was first described in China and Japan during the 1980s which soon spread to neighbouring provinces, countries and even as far as Italy (Takikawa et al. 1989, Mazzaglia et al. 2012). Although quietly active, the disease did not rear its head significantly until an Italian outbreak in 2008 (Balestra et al. 2009), later spreading to France and Portugal which affected both *A. deliciosa* and *A. chinensis* varieties of kiwifruit, of particular significance to Zespri and KVH; Hort16A and Hayward (Vanneste et al. 2011b, Balestra et al. 2010). In November 2010, the disease had reached New Zealand (Everett et al. 2011a). Psa has since spread to Spain (Balestra et al. 2011), Switzerland (EPPO 2011a), Australia (EPPO 2011b), Germany (EPPO 2013), Chile (Anonymous 2011), Slovenia (Dreo et al. 2014) and Greece (Holeva et al. 2015).

Thought to originate from China (Butler et al. 2013), Psa biovar 3 (Psa-V), henceforth referred to as Psa unless otherwise stated, was first discovered in the Te Puke region of the Bay of Plenty, where it spread to surrounding areas before becoming widespread, as of August 2016 it was estimated that at least 84% of orchards were infected with Psa (Kiwifruit Vine Health, 2016). Symptoms were first found on the Hort16A species, and then soon after on the Hayward variety (Everett et al. 2011b).

Hort16A was found to be especially susceptible to Psa (Balestra et al. 2009), exhibiting more severe symptoms, such as whole vine collapse, leading to the removal of large volumes of infected canopy and vines which affected downstream productivity. Hayward was found to be susceptible to Psa, but to a lesser extent, expressing later development of symptoms and less effect on productivity (Froud et al. 2014). As a result, the success of the industry primarily driven by Hort16A seemed unlikely. Fortunately, Plant and Food Research (PFR) and Zespri breeding programmes had produced new cultivars which were originally designed to access the early market. The cultivar Gold3 (G3) was released to growers in 2010 to rapidly replace cultivars of Hort16A. G3 has shown to more tolerant than Hort16A in regards to Psa infection (Hoyte et al. 2011). It was hoped that by the predominant use of the tolerant cultivars Hayward and G3 Psa could be effectively managed.

The incidence of Psa has increased rapidly since 2010; with the rate slowing as the number of Psa positive orchards becomes saturated (figure 1). Nelson remains the only kiwifruit growing region to date, which has no Psa-positive orchards.

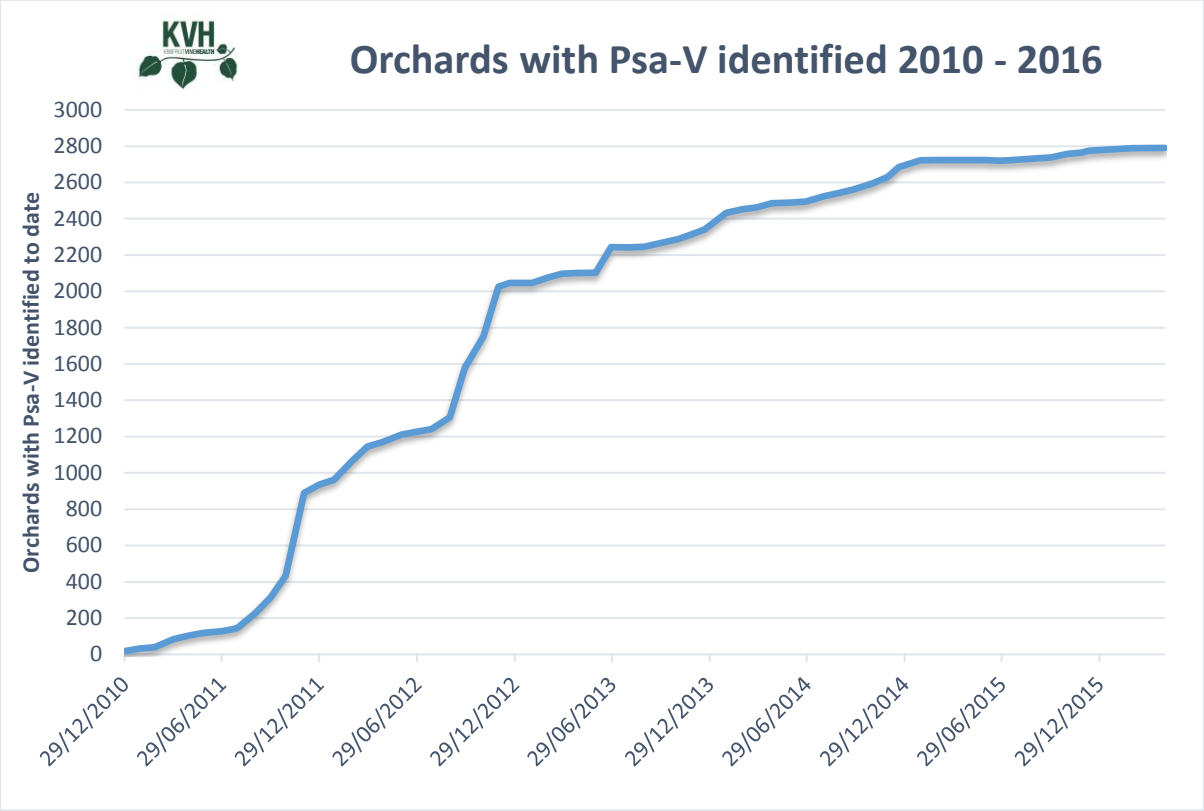

Figure 1: The incidence of Psa in New Zealand orchards from 2010 to 2016. Courtesy of Kiwifruit Vine Health 2016.

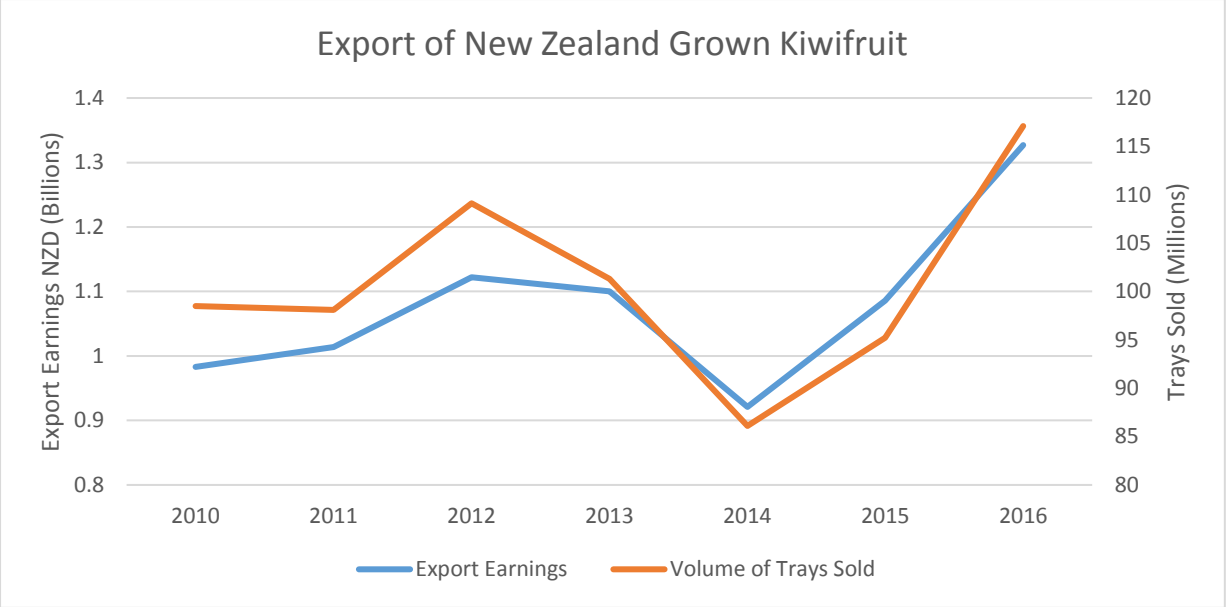

Figure 2: The export of New Zealand grown Kiwifruit since the incidence of Psa (Zespri Annual Reports 2009/2010 – 2015/2016).

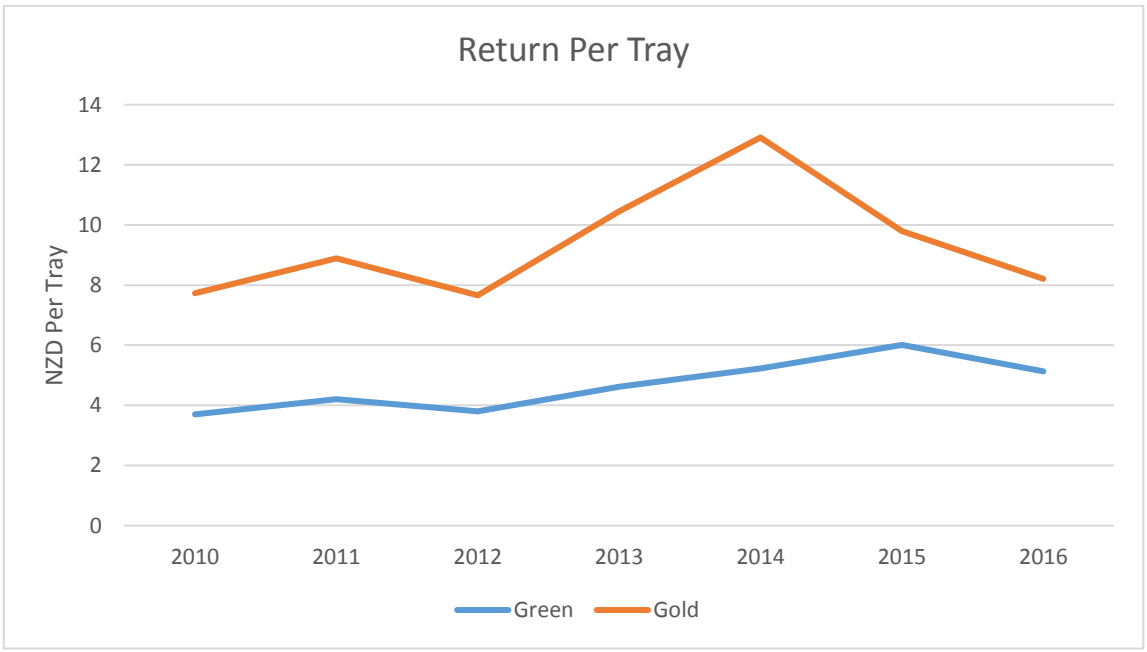

Figure 3: Return price per Tray for New Zealand Grown Green and Gold Varieties of Kiwifruit (Zespri Annual Review 2015/2016)

Kiwifruit is one of New Zealand's most valuable and biggest export industries, producing over 100 million trays of fruit from over 11,000 hectares of orchard, with around 80% of production coming from the Bay of Plenty region. In 2010 the industry exports from New Zealand were valued at over $1 billion. However, after the onset of Psa, the industry production declined by over 10%. In 2012, it was originally predicted that Psa would cost the New Zealand kiwifruit industry up to $410 million by 2017 (Greer and Saunders 2012). However, in 2014 it was thought that the industry had lost close to $900 million since the onset of Psa (Birnie and Livesey 2014).

Zespri annual report statistics (figure 2) suggest that the overall cost of Psa was not truly felt until 2014 when both exported kiwifruit volume and export earnings dropped to pre-2010 levels. Arguably the cost of Psa was felt much earlier at an individual or orchard level. This trend is inversely correlated with the return for a tray of each variety of kiwifruit (figure 3).

Despite the high incidence of Psa, the kiwifruit industry is growing at an unprecedented rate. Since 2015 the revenues generated from exporting New Zealand grown kiwifruit has surpassed 2014 annual predictions (Birnie and Livesey 2014) in addition to exceeding revenue generated prior to Psa. This growth in productivity to support industry growth relied heavily on a successful response to combat Psa and bacterial canker. Although economically the industry is improving, maintaining research and active management of Psa is essential to prevent further widespread outbreaks and productivity loss.

2.3 New Zealand Industry Response to Psa

Kiwifruit Vine Health Inc (KVH) was established in December 2010 to lead the New Zealand kiwifruit industry response to the Psa epidemic. KVH addresses areas where the knowledge of Psa and how it causes infection is poor, and commissions research to address this.

Zespri, is contracted by KVH, to coordinate and facilitate a comprehensive research programme. This will allow the better understanding of the bacteria and determine effective techniques to prevent infection and spread.

This document will review the research and accumulated knowledge of the epidemiology, control and prevention of Psa in New Zealand – with the aim to identify areas of research that may require more attention. The five main research areas are:

- Epidemiology
- Detection and Psa Genetics
- Chemical and Biological Control
- Orchard Management
- Psa Tolerance Breeding Programme

3 Research

The scientific and horticultural communities have been aware of Psa for decades (Takikawa et al. 1989). When Zespri variety Hort16A became infected in Italy, New Zealand became involved and contributed to scientific research aimed at understanding this bacterium. When the disease arrived in New Zealand very little was known about how it survives and causes disease in this climate, this translated into uncertainties with regard to best practices in controlling this disease. The understanding of the conditions in which Psa thrives and investigating the process of how infection is caused and developed will allow us to effectively prevent, control and manage the disease.

3.1 EPIDEMIOLOGY

The understanding of the biology and physiology of Psa in 2011 was limited, which was evident by commissioned reviews screening the available scientific literature (Everett 2011b). But still, in 2016, our knowledge of the epidemiology is far from comprehensive.

3.1.1 Psa Infection

Psa can be found associated with a wide variety of kiwifruit plant tissues. Interestingly, Psa can be found in healthy tissue, and not always detected in diseased tissue. To date the bacterium has been found on leaves, buds, flowers, roots, pollen and the woody tissues of vines (Froud et al. 2015; Sutherland 2013; Horner *et al*. 2011).

The most paragidmatic symptom of Psa is the formation of cankers in the woody tissue. Generally found on the trunk or the leaders within the vine, they are most readily observed during early spring. Cankers are associated with the production of red or cloudy exudate (Figure 4d). This exudate is a mixture of plant sap and may contain Psa (Vanneste *et al*. 2011d).

During late spring to summer, leaves start to emerge and are one of the primary infection sites for Psa. They tend to exhibit dark brown spots at the site of infection surrounded by a yellow halo around the circumference (Figures 4a and 4b). While younger leaves appear to be more susceptible to Psa infection there are cultivar differences with regards to symptom expression with Hayward leaves tending to exhibit an increased amount of leaf spot as compared to the G3 cultivar. Leaf spotting can lead to the wilting or necrosis of the leaf tissue when infection progresses (Figure 4c), this then can extend to the shoots and canes as Psa progresses through the vine. Cane die-back is where the cane takes a blackened, dehydrated appearance and fails to produce any viable buds or flowers, this is likely to be due to the blockage of vasculature. Die-back starts at the point where Psa enters the cane, presumably from the infected leaf and stem, and can move bilaterially through the cane and eventually infect the leader (Vanneste *et al*. 2011d).

Another important secondary symptom is budrot, which is the partial or full browning of buds indicating tissue death. Infected buds can fail to develop and may wilt and drop off, affecting yield and productivity (Vanneste *et* al. 2011d). This symptom is not unique to Psa, but can be caused by other *Pseudomonas spp*. Including the casual agent of blossum blight. Psa is now however recognised as the main contribution *Pseudomonas* species to budrot in green kiwifruit varieties (Tyson *et al*. 2015b). Budrot is less prevelant in the G3 variety of Kiwifruit.

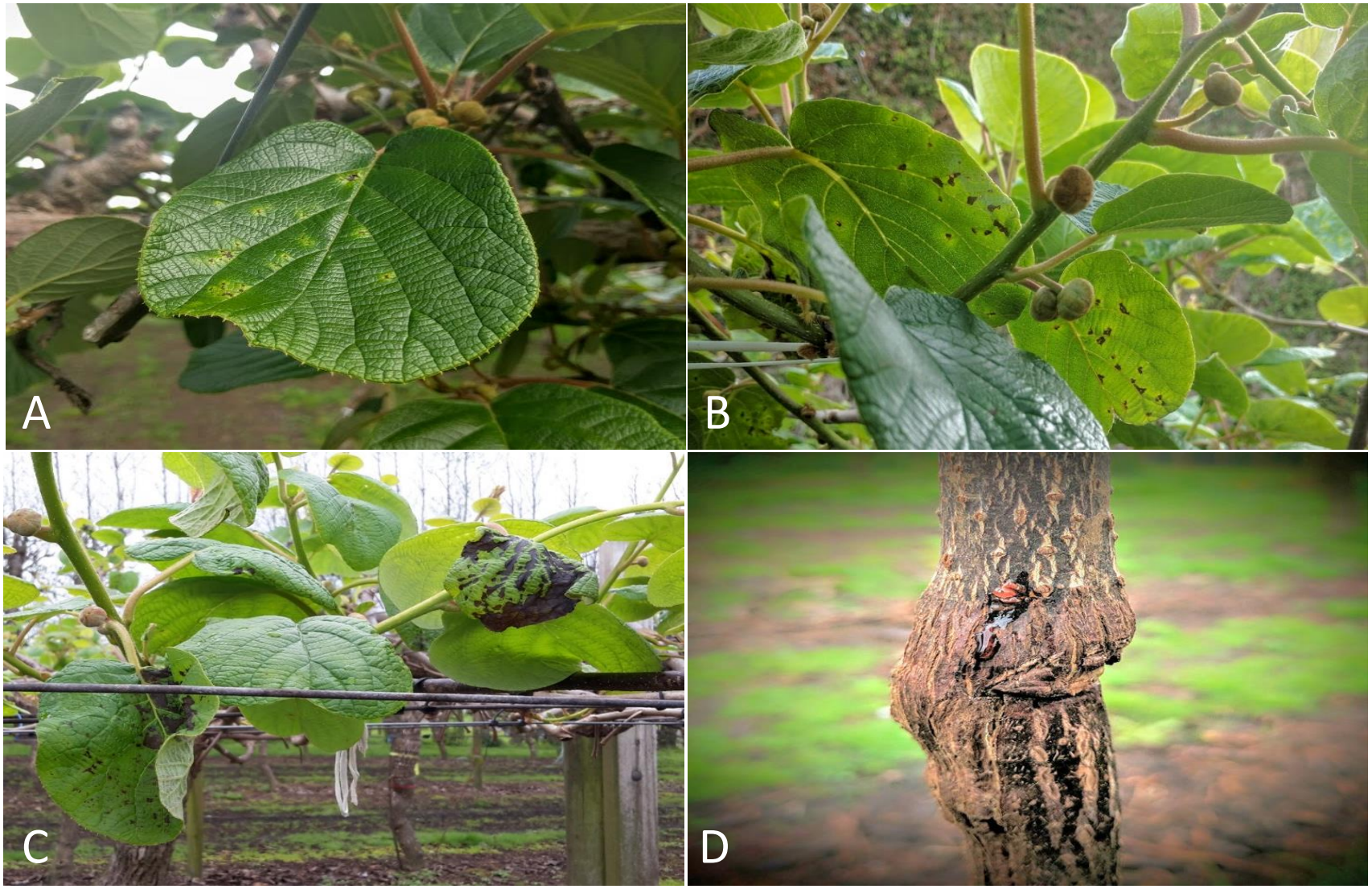

Figure 4: Examples of the common symptoms of Psa; Leaf spot (A & B); Budrot (B); Leaf Wilt and Necrosis (C) and production of red exudate from an infected lenticel (D).

3.1.2 Life Cycle and Pathogenesis of Psa

In New Zealand, Psa has the ability to infect kiwifruit and produce viable inoculum all year around, but is more prominent in spring and autumn (Tyson *et al*. 2014c). As rainfall or water is essential to the infection process, it was thought that Psa may be naturally spread through a combination of rain and wind mediated splash. Bacterial spread through aerosols were found to increase after rainfall. The water is likely to either be essential to the replication of the bacteria facilitating infection, or for the movement of Psa from one location to another where it is better located to cause infection. Wind and hail can also damage the vine and provide additional entry points for Psa, this is most evident as an increase in the severity of symptoms was observed associated with gaps in shelter belts or unprotected areas (Casonato and Bent 2014).

Temperature is another key factor, as the optimal temperature for Psa proliferation and spread of infection is around 10 °C to 20 °C, where callus formation in the vine was found to occur at temperatures around 25 °C, which correlates with the decline of Psa populations and symptoms (Froud *et al*. 2015). Conversely temperatures which promote frost can also promote Psa infection. Extreme cold events or frosts can damage the vine and act as an additional entry point for Psa, thereby increasing the incidence of Psa. Frost also promotes the proliferation and progression of Psa throughout the vine, although the reason is unknown (Ferrante and Scortichini 2014).

Once Psa reaches the surface of the vine, it will migrate and invade the plant either through natural openings in the leaf, or through wounds or scars. Methods which allowed the visualisation of bacteria showed that Psa can enter through stomata, and through leaf scars and broken trichomes (surface hair-like projection) (Spinelli *et al*. 2011). Other potential infection sites have also been identified.

Figure 5: Lenticels are gas exchange nodules found on the surface of woody material within the kiwifruit vine. This image shows the presence of lenticels on a Psa-infected cane.

Lenticels are gas exchange nodules found on the stem of woody plant material. It was found that Psa could colonise these pores, and subsequently invade the cells beneath. Young lenticels were found to be more susceptible and it has been concluded that Psa is likely to be more successful at colonising lenticels during spring (figure 5) (Everett *et al*. 2012a). Cicadas are insects which cause damage to the kiwifruit vine by laying eggs into canes. Research has shown that Psa can potentially use these wound sites to infect the vine. However, this is not always statistically significant (Tyson *et al*. 2012). Other work has shown that the root system of kiwifruit vines is a potential point of entry for Psa infection (Vanneste 2013), although growers have reported no increase in infection susceptibility when wounding the roots in a practise called root pruning.

It has been reported that once infected, Psa can migrate through the kiwifruit vine to become a systemic infection though tissues may remain asymptomatic. When Psa was artificially wound inoculated within the trunks of Hort16A cultivars the bacterium was found to have travelled up to a meter either direction from the point of entry (Tyson *et al*. 2014b). Although an important technique to remove a substantial amount of inoculum, removing infected material is unlikely to remove all Psa from the vine and symptom expression may reoccur at a later date. Psa has also been found in the root stocks meaning re-grafting might not necessarily prevent infection of the new scion. This impacts potential management techniques (Horner *et al*. 2011). More research needs to be conducted to find the consequence of the presence of bacteria in asymptomatic tissue, and the extent of contribution to re-infection and disease.

3.1.2.1 Seasonality and Vine Age

Kiwifruit vines are deciduous and lose their leaves in the autumn/winter period and will grow new leaves in spring. This cyclic characteristic is also observed in Psa infection, with symptoms beginning to express in spring/autumn and becoming absent in winter. This is thought to correlate with weather and temperature, with spring and autumn harbouring more rainfall and milder temperatures (Froud *et al*. 2015). This incidence of infection could also be correlated with the age of the plant tissue, as it has been shown that young leaves (up to four weeks old) are significantly more susceptible to Psa than their older counterparts (Tyson *et al*. 2015a). This correlates with reports from New Zealand growers that young growth, or new grafts are more susceptible than older plants in the same orchard, however the idea that young plants are more susceptible is disputed (Vanneste *et al*. 2011d; Zhang *et al*. 2013).

3.1.2.2 Inoculum Sources

Once infection has taken hold it can present in a variety of symptoms. Important symptoms regarding the transmission of Psa are leaf spotting and the production of exudate. Psa leaf spotting is a common symptom within kiwifruit orchards, occurring from early spring to late autumn, with up to 50% of Hayward leaves exhibiting these lesions. Although infected woody material is often removed, leaves exhibiting infection remain within the vine. Research has shown that these leaf spots are viable sources of live Psa, which under high humidity can provide inoculum to infect neighbouring plants, or re-infect the original vine (Casonato *et al*. 2014a). Infected plant material will produce exudate or 'bleeding sap' and can be milky white or red in colour. Using previously established genetic tests, live Psa has been identified in both types of exudate and are therefore a viable source of inoculum (Biondi *et al*. 2013).

3.1.2.3 Environmental Inoculum Sources of Psa

Psa is an epiphytic bacterium and can survive outside the host plant. For this reason, it is critical to examine and identify the environments which have the potential to support and harbour Psa. This knowledge can help prevent and limit the spread of infection. In addition to plant material, soil has been shown to be an important vector of Psa, with surfaces or equipment becoming contaminated after contact with Psa containing soil (Everett *et al*. 2012c). This subchapter aims to highlight other possible environmental sources of Psa.

3.1.2.3.1 Compost

Compost may be used to add organic matter and increase nutrient content to soils on kiwifruit orchards. If Psa can survive in compost, this could increase the risk of Psa within and to surrounding orchards.

To investigate whether Psa could survive in compost, and whether this implicated compost as a potential source of infection, compost made from non-kiwifruit green waste, was tested for the presence of Psa. No level of Psa could be detected. This could be due to the composting process, or due to the compost not containing kiwifruit and therefore no Psa contaminated material.

When Psa was artificially added to the compost, it was determined that Psa could not multiply and few bacteria survived for more than a few days. Other bacteria were identified within the compost which could be inhibiting the growth of Psa, but due to the nature of these organisms they have not been pursued as viable biocontrol agents (Vanneste *et al*. 2013b). Compost does not support the growth of Psa and is unlikely to be a viable source of Psa.

3.1.2.3.2 Honeybees and Hives

Honeybees collect pollen from flowers and will distribute this to different vines. There is a concern that these bees can either become contaminated with Psa from infected vines, or transfer infected material such as pollen from one vine to another, facilitating the spread of Psa infection.

In experiments in which honeybees were exposed to pollen contaminated with *P. syringae* bacteria, and exposed to honeybees. It was found that the bacteria could be recovered from the bees for up to 2 weeks after the initial contact. Bacteria could also be recovered from non-exposed bees within the same hive, showing that not only could *P. syringae* survive within the hive, but it could rapidly spread between honeybees (Pattemore *et al*. 2011). This work implicates hives and honeybees as a viable source of infection and can facilitate the spread of Psa.

3.1.2.3.3 Pollen

To produce fruit a female kiwifruit flower must become pollinated by pollen from male plants. This is primarily facilitated by wind and insect driven pollination. Psa may be present in the flowers of infected plants, so it is possible that the associated pollen could also contain and be a source of Psa. Pollen may therefore transmit the infection to other plants through the process of natural or artificial pollination. Italian researchers have showed through genetic and culturing techniques that they were able to detect and isolate viable Psa from harvested pollen from Hayward flowers. This contaminated pollen was then used to pollinate several kiwifruit plots. The use of contaminated pollen resulted in the transfer of Psa and infection of previously clean plants (Stefani and Giovanardi 2011; Vanneste *et al*. 2011c). This work implicates pollen and the process of pollination from infected males as a possible source of Psa.

3.1.2.3.4 Leaf litter and Pruned Plant Debris

During the life cycle of an orchard, leaves and vine material will become deposited on the ground. As this material is capable of harbouring Psa, the potential of leaf litter and vine debris to act as sources of Psa was investigated (Horner *et al*. 2011). Infected leaves and cane material was incubated for a set amount of time in a variety of conditions, to assess the recoverability and survival of Psa. It was found that Psa can survive in the infected leaves and leaf litter for up to 3 months. Psa was also found to survive in winter cane pruning's for a similar amount of time. This work shows that pruning's and leaf litter can be a source of Psa which has the potential to infect nearby plants. This has provided valuable information to growers to help shape their management practices to minimise their risk of Psa infection. It is recommended that digester and copper products are used on mulched pruning's and leaf material to promote tissue break down and prevent this material acting as a inoculum source of Psa.

3.1.2.3.5 Shelter and Weeds

Research has established that Psa and its relatives can survive on the surface of a plant without causing disease (Vanneste *et al*. 2011a). Work has investigated whether Psa could survive on nearby non-kiwifruit plants such as weeds or shelter (Horner *et al*. 2011). This could provide an additional source of Psa infection. Weeds found on orchard floors, and plant species from shelter belts were tested for their ability to harbour Psa. It was found that in all cases, Psa may survive for a short period of time, however, it could not replicated and hence shelterbelts and weeds are not likely to be a source of Psa innoculum.

3.1.2.3.6 Water

P. syringae has been found in water in New Zealand and in other countries (Vanneste *et al*. 2008; Morris *et al*. 2010). Essential to the infection process, water has been thought to allow the bacteria to survive and multiply allowing the bacteria to become widespread. Work has been performed to assess whether Psa, like its close relatives, can also survive in water and if infected orchard water could be a source of Psa infection (Horner *et al*. 2011). Psa was artificially added to several water sources including tap and rainwater to assess the ability of the bacteria to survive. Psa could not survive in tap water, this may be due to the chemical treating process. Psa could survive indefinitely in sterilised rain water, however struggled to survive when the rainwater was un-sterilised. This was attributed to the presence of other microorganisms in the water which out-competed or inhibited the growth of Psa.

When water from orchards was tested for the presence of Psa, none could be found either from culturing or by genetic testing. High concentrations of other microorganism were found, which may be responsible for preventing Psa survival and replication. This work reports that water is unlikely to harbour Psa and subsequently presents a low infection risk.

3.1.3 Modelling Risk

During the Italian outbreak of Psa it was noted that spring and autumn were important times for the progression of infection. This was likely to be because spring and autumn harbour the optimal temperatures for the bacterium's replication, whereas summer would lead to a decrease in bacterial populations and winter would prevent the progression of the disease. Temperatures over 20 °C correlated with the absence of new symptoms (Vanneste 2013a).

However, in New Zealand summer and winter temperatures generally never get hot or cold enough to reduce bacterial numbers or prevent the spreading of infection respectively. This means that infection can occur all year around theoretically. Previous predictive modelling software for other bacterial disease had been updated to include data on the incidence of Psa during different weather conditions, including wind, humidity and rain-fall (Beresford 2011). This allowed growers to predict the risk of Psa depending on the weather and apply appropriate protection (http://www.kvh.org.nz/kiwi_psa).

3.1.3.1 Validation and Optimisation

The Psa risk model was subsequently validated using young hort16A seedlings so called 'trap plants' (McKay 2012). These trap plants were placed within an orchard block in several locations and exposed to both the weather and environmental Psa inoculum for a week. The severity of leaf spotting was compared to the calculated risk of Psa infection and it was found that the two highly correlated, suggesting the model was accurate and that growers could use this to accurately assess their risk of Psa infection.

To improve the risk modelling, Tyson et al. (2014a) sought to provide data that included seasonal variation. They found that rainfall was still an extremely important factor with no Psa symptoms occurring without prior rainfall conditions. The results from this project suggested that the Psa risk model had greater predictive power during spring and winter, whereas performed poorer over autumn and winter. It was speculated that this could be due to differences in the plant maturity, levels of environmental Psa, in addition to environmental factors (Tyson *et al*. 2014a). There are further proposals to incorporate the risk posed by frost events into the current model (Beresford, R. Personal Communication 2016).

3.2 DETECTION

New Zealand kiwifruit orchards can contain several strains of *Pseudomonas* bacteria and subtypes of Psa. It is important to be able to distinguish between them to accurately assess the disease state of an orchard and to effectively manage the disease.

Originally there were four biovars of Psa that had been identified world-wide. New Zealand found itself host to two subtypes of Psa, one which is the highly virulent strain which is causing the disease found in orchards; referred to as Psa-V, the other is a low virulent strain which causes leaf spotting; historically known as Psa-LV; now newly designated *P. syringae* pv. *actinidifoliorum* pv. nov. (Pfm) (Cunty *et al*. 2015). This meant that the presence of leaf spotting, would not necessarily reflect the disease status of the orchard. Therefore, a test to distinguish between the two organisms was critical.

Historically, candidate Psa bacteria would be cultured and isolated from infected material in a process which would span at least a week. The DNA of the bacteria would then be extracted and subsequently tested to screen for a unique region of the genome which would identify the subtype of Psa (Vanneste 2013). This method is both time consuming and labour intensive. Therefore, unique methods of identifying Psa and the subsequent subtype which did not rely on time consuming culturing and isolation were investigated.

3.2.1 Genetic Based Detection

The possibility of a fast, sensitive test that did not rely on culturing bacteria was investigated. Rikkerink *et al*. (2011) Identified key genes which were likely to contain differences between both Psa and other bacteria and between Psa-V and Pfm. These genes included those responsible for the differences observed in virulence between the subtypes. Once the genomes for these bacteria had been published, the search included universal genes shared by these related bacteria but focused on finding those with subtle differences, such as the presence or absence of a particular stretch of DNA. Upon identifying regions of DNA which differed between Psa-V and Pfm, quantitative-PCR assays were developed providing the ability to distinguish between Psa-V or Pfm and other bacterial species. These assays were then validated independently (figure 6).

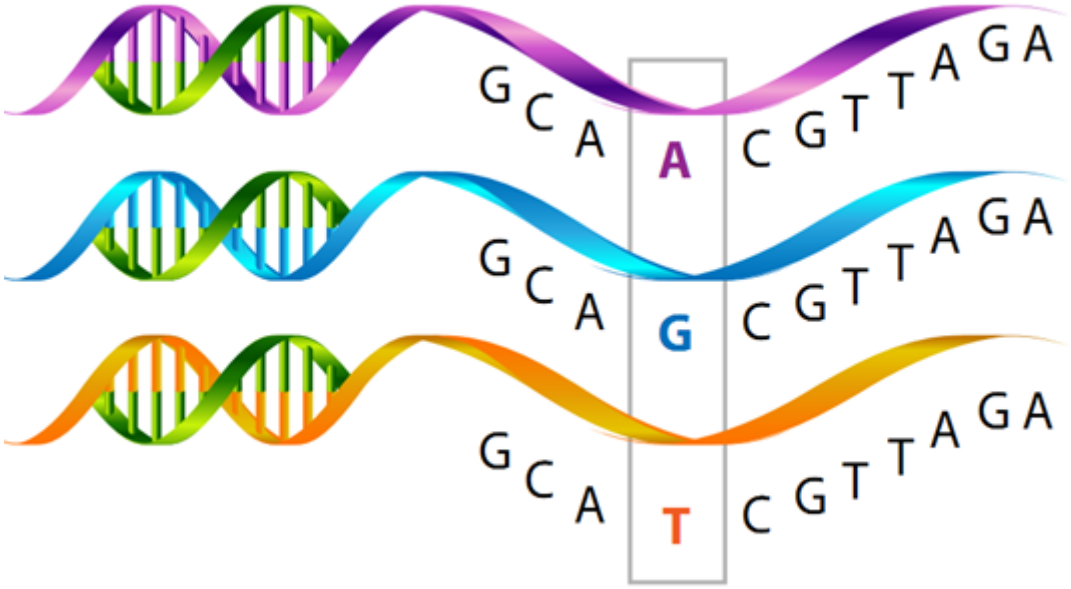

Figure 6: Differences in the bacterial DNA sequence allows the identification of Psa and subtypes. (http://www.biomedheads.com/restriction-digests.html)

This work provided a rapid, and sensitive assay which could detect and type Psa bacteria extracted from leaf and cane tissue, allowing the disease status of the orchard to be determined rapidly.

More recently PFR have worked to utilise novel genetic technologies to detect and type Psa (Notomi *et al*. 2000, Bühlmann et al. 2013). Similar to qPCR, loop-mediated isothermal amplification (LAMP) was used to detect the presence of Psa-V using unique DNA signatures (Ruinelli et al. 2016). Unlike other genetic techniques, LAMP does not require expensive machinery to manipulate temperature and can be used in the field. Results can be produced quickly, but may require experienced analysis. There are validated products on the market which can be used to perform LAMP assays to detect Psa-V which produce easy to interpret results in around 15 minutes which can determine if plant material is infected prior to the onset of symptoms. However, this research still requires more work to validate its reliability and assess suitable demand in the industry.

3.2.2 Immunohistochemical Based Detection

Alternative approaches to detecting Psa within plant tissue have also been investigated. Proteins called antibodies have been created which recognise and bind specifically to Psa. This provides a potential method of determining the presence and location of Psa within the kiwifruit plant (Sutherland *et al*. 2013).

The process involves taking infected plant material, treating it and rendering it biological inert. The samples are then exposed to the antibodies which bind to a secreted sugar on the bacterial surface called lipopolysaccaride (LPS). These antibodies were shown to be very sensitive, those designed to bind to Psa-V would only bind to Psa-V LPS and not to Pfm or any other Pseudomonas bacteria, and visa-versa. These antibodies were also able to detect Psa-V in a wide range of plant tissue, including leaves, flowers and canes (figure 7).

This is a reliable method which allows for further scientific study especially in investigating the process of infection and how it spreads. However, these antibodies are expensive to produce and are maunfactured via the blood serum of laboratory animals.

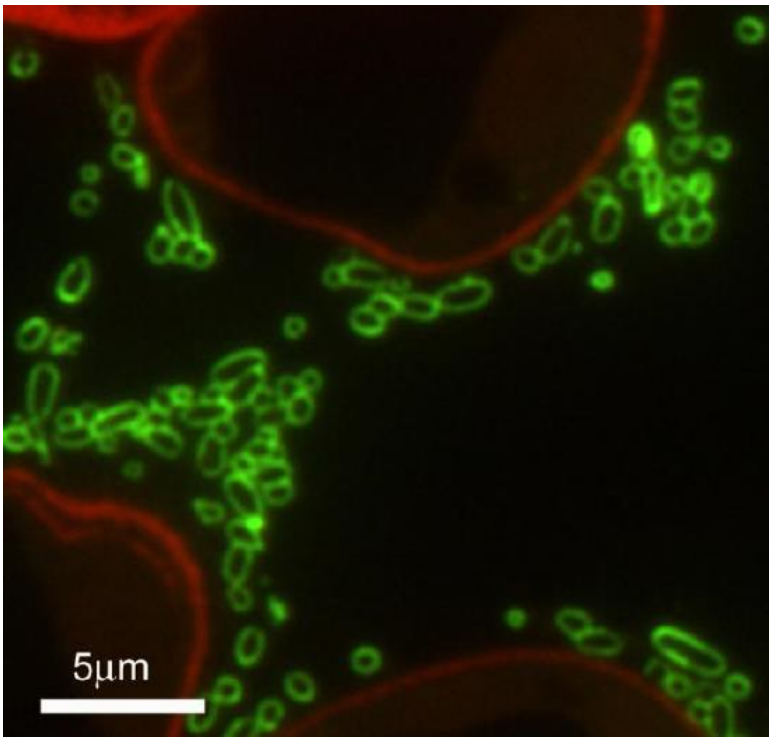

Figure 7: The fluorescent staining of Psa-V (green) in between kiwifruit leaf cell walls. (Sutherland *et al*. 2013).

3.2.3 Satellite and Aerial Imagery Detection

Remote detection of plant diseases through satellite images have been previously used in combating rice sheath blight in the USA (Qin and Zhang 2005). It was proposed that the same techniques could be used to monitor orchards to detect Psa. During early season development of the vine canopy, low vigour could be visually identified and subsequent Psa infection was confirmed. However, later in the season, using canopy visuals to identify disease status becomes difficult (Taylor and Whelan 2012). This work was preliminary and would require a significantly increased amount data collection

and optimisation before this technique of disease detection could be applied to season-wide Psa detection in kiwifruit orchards.

3.2.4 Future Direction of Psa Detection

Important steps have been taken to identify Psa within infected orchards. This allows the effective management and the placement of strategies to prevent spreading of the infection. However, research has yet to develop an effective, reliable technique to identify Psa on-orchard rapidly. LAMP technology is interesting and has the potential to meet this gap in the market if proved to be reliable and cost-effective.

Although the presence of Psa can be confirmed, the quantification of Psa, or measure of bacterial load is not currently practised in New Zealand. By knowing the bacterial load of a sample, the extent of infection can be determined and therefore the risk of potential infection or spread of Psa could also be established and intercalated into pre-existing models. The technology currently used to detect Psa; qPCR, can also been used to accurately assess the relative number of bacteria. However, this type of detection and quantification does not discriminate between alive and dead bacteria.

Work has however been undertaken to investigate techniques to distinguish live and dead Psa populations. Currently DNA intercalators have been shown to bind free environmental DNA or the DNA of membrane compromised dead bacteria and prevent amplification, thus removing their signals from the qPCR analysis such that only live cells are detected (Goh and Gin 2015; Yasunaga *et al*. 2013).

The use of these or similar methods if appropriately scaled up for industrial use could provide useful information for the assessment of Psa infection risk and to accurately assess the amount of live Psa and the level of inoculum present within sampled tissue. This knowledge could provide valuable information for modelling risk and to accurately determine the likelihood of infection when compared to weather conditions.

3.3 Chemical and Biological Control of Psa

The most effective method of controlling an unwanted pathogenic organism is to prevent it entering the country in the first instance. However, once invasion into New Zealand occurred, like all plant pathogens, control of Psa was extremely difficult. Although relatively easy to kill on an inert surface, once Psa becomes associated with the vine, either as an asymptomatic or pathogenic state there is no cure. A significant screening programme was started in New Zealand in 2011 to identify products that may help control the disease. Candidate control products that had shown to have efficacy against Psa (stage 0) were taken on for further tests using potted seedlings (stage 1) and field trials (stage 2) (figure 8). These trials consisted of measuring the severity of Psa related symptoms when control products were absent or present. Successful products were categorised based on their mode of action. These currently include protectants, elicitors, sanitisers and biological control. Protectants are chemicals such as copper and antibiotics, which kill the bacterium before it causes infection, and therefore prevents the disease. Elicitors stimulate a plant immune response which increases the tolerance to Psa. Biological controls are the use of other microorganisms to combat or compete with Psa (Jeyakumar *et al*. 2014).

Adjuvants or detergents are often added to chemicals to increase the coverage of control products. Adjuvants themselves do not normally possess any activity against Psa, instead they directly modify the properties of other control products, such as improved spreading or prevention of chemical drift.

To date over 400 products have been screened for activity against Psa. Only a small percentage have shown efficacy against Psa in both in vitro and greenhouse potted plant trials (www.kvh.org.nz/product_testing). However due to a large number of compounds and time constraints only significantly effective products that exhibited a decrease in the severity of leaf spot were taken forward. This could have excluded other efficacious products which reduced secondary symptoms. Initial work showed that copper and streptomycin based products had the greatest potential to combat Psa in the field (Everett 2011c).

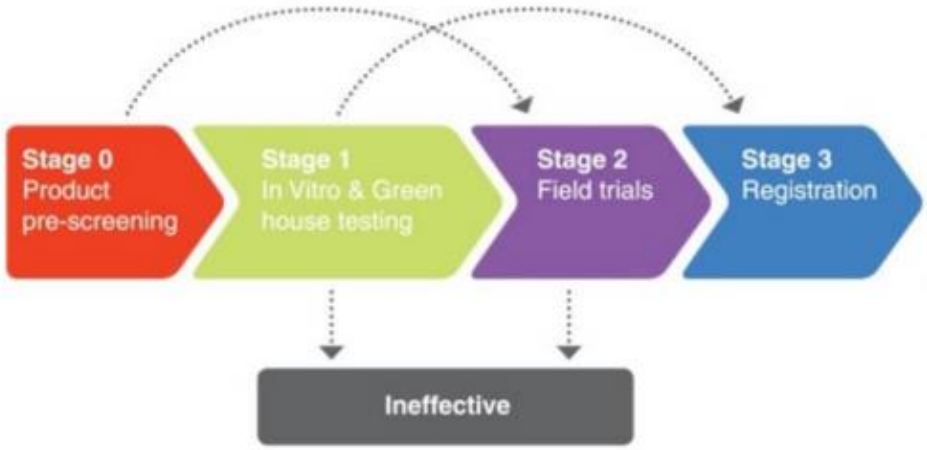

Figure 8: The rationale of control product testing.

3.3.1 Protectants

3.3.1.1 Copper Based Chemical Sprays

Copper was first used to control Psa in Japan (Serizawa *et al*. 1989) where it was found that using copper significantly reduced Psa symptoms on affected leaves. Copper sprays are still considered to be the best form of protection against Psa in agriculture (Everett 2011c). Copper ions disrupt the cellular processes within the bacterium leading to its death, preventing the ability to cause infection.

Copper efficacy is determined by a number of factors including particle size, retention and solubility.

Particle size governs both the efficacy of the copper product but also its retention time, the duration in which the particles remain on the vine surface. Smaller particle size results in a greater surface area, which increases the presence of copper particles and subsequently copper ions in a given space. This increase in density increases the efficacy of the antibacterial activity. An increase in surface area also allows the copper particles to remain on the surface of the vine for longer. Larger copper particles are prone to weathering and becoming dislodged from the plant.

Solubility governs the duration of protection copper can provide. More soluble forms of copper erode quicker and will need to be applied more often. Whereas more insoluble forms release copper ions for longer and require less frequent applications.

Plant growth is another important consideration, if new leaves and shoots develop after an application of copper, gaps will appear between adjacent particles increasing the amount of plant material which is unprotected and thus susceptible to Psa.

3.3.1.1.1 Copper Based Agrichemicals

Table 1: KVH Recommended Copper Based Agrichemicals for use in the Kiwifruit growing industry, including the recommended timeframe of application, copper formulation and label claim against Psa.

Product	*Copper Formulation*	*Label Claim*	*Timing*
Tri-base Blue	Copper sulphate	Limited	Pre-Flowering
AG Copp 75	Cuprous Oxide	Limited	Full Season (Apply alone to open flowers)
HORTCARE® Copper Hydroxide 300	Copper hydroxide	Limited	Full Season (Do not apply to open flowers)
Coptyzin	Chelated	Limited	Full Season (Do not apply to open flowers)
Champ® DP	Copper hydroxide	Limited	Recommended Pre-Flowering and Post-Harvest Only
Kocide® Opti™	Copper hydroxide	Full	Full Season (Do not apply to open flowers)
Nordox 75 WG™	Cuprous Oxide	Full	Full Season (Do not apply to open flowers)

3.3.1.1.2 Efficacy

There has been a substantial amount of research investigating the efficacy or effectiveness of copper products in reducing the symptoms of Psa. The more positive reports find that the usage of specific copper products perform similarly to antibiotics, significantly reducing the severity of leaf spot and improving the fruitset of Hayward cultivars (Fruitfed Supplies 2014). Other research shows that Hort16A canes are equally susceptible to Psa regardless of the levels of copper (Mauchline and Stannard 2012a). Although lab-based research contains discrepancies and contradictions, field observations consistently suggest copper is an effective preventative, reducing the incidence of Psa symptoms.

3.3.1.1.3 Phytotoxicity

An effective control product must be concentrated enough to ensure the killing of Psa, however it should not be concentrated enough to start damaging the vine and cause phytotoxicity. Copper can

cause discolouration and damage of the leaf surface, and fruit marking as well as a decrease in vine vigour. This can be caused by a variety of conditions including but not limited to the concentration of copper, the pH of the solution, wetness of the vine, temperature of the product, humidity of the local environment and the plant growth stage (Kiwifruit Vine Health 2016a). Concerns about the application of copper on New Zealand kiwifruit led to the investigation of commonly used copper products and the symptoms they caused. Kocide (90 g /100 L), Champ (75 g/100 L) and Nordox (38 and 75 g/100 L) were used 3 – 5 times after flowering. These products were found to cause light phytotoxic symptoms in Hort16A and Hayward, but symptoms did not correlate with the concentration of copper used (Hawes 2012). Other research investigating the phytotoxic effects of various copper products during different periods of the growth cycle using Hayward and G3 have also shown that copper usage is unlikely to cause phytotoxic effects (Lupton and Owen 2013a; 2013b; Hawes 2014).

3.3.1.1.3.1 Rainfastness and usage of Adjuvants

To ensure an optimal dose of copper is delivered and present within the vine, the effect of rainfall on copper residue was investigated (Gaskin *et al*. 2011a; Gaskin *et al*. 2011b). Heavy rain was simulated upon kiwifruit plants whilst using three commonly used copper products. It was found that four sequential copper sprays upon kiwifruit did not exceed maximum residue limits, even when rainfall was not simulated. Upon testing the levels of copper residues after varying levels of rainfall, it was found that these products were highly resistant to washing off when applied to kiwifruit and canes, and that the products were moderately resistant to washing off from the leaves and foliage.

Adjuvants are sometimes added to chemicals to improve coverage. The effect of this addition on the properties of this copper products was also tested. When added, it was found that the adjuvant could increase copper coverage upon leaves, but had no effect on the rainfastness or levels of copper residue measured on the kiwifruit, canes and foliage.

3.3.1.1.4 Bacterial Resistance to Copper

Copper is one of the most effective control product to combat Psa. However, it could become less effective if high levels of resistance develop. Bacteria require small amounts of copper, but in excess it is toxic and it exhibits bactericidal effects. It has been reported that there are various naturally occurring methods of bacteria resisting high levels of copper. This includes the expression of the *cop* genes, which encode proteins which allow Psa to tolerate increased concentrations of copper. The presence of proteins which bind and chelate copper and proteins which pump copper outside of the bacterial cell both confer copper resistance (Masami *et al*. 2004). The presence or acquisition of these or similar genes have allowed samples of Psa in New Zealand to exhibit low levels of resistance to copper in the lab.

One explanation for the occurrence of copper-resistance is the abundance of mobile genetic elements within the environment, which contain genes that confer resistance to copper. These resistance genes can be obtained by Psa from other bacteria within the environment allowing them to become copper resistant (Colombi *et al.* 2016).

The occurrence of copper resistance can be exacerbated by the use of sub-optimal levels of copper control. Even though an optimal concentration of copper for the control of Psa within an orchard has not been established, the use of copper products at a sub-recommended label level, especially in 2013 and 2014, may have accelerated the acquisition of these resistance genes (figure 9).

This emphasises the need to identify and optimise the use of copper to effectively control Psa and keep the occurrence of resistance to a minimum.

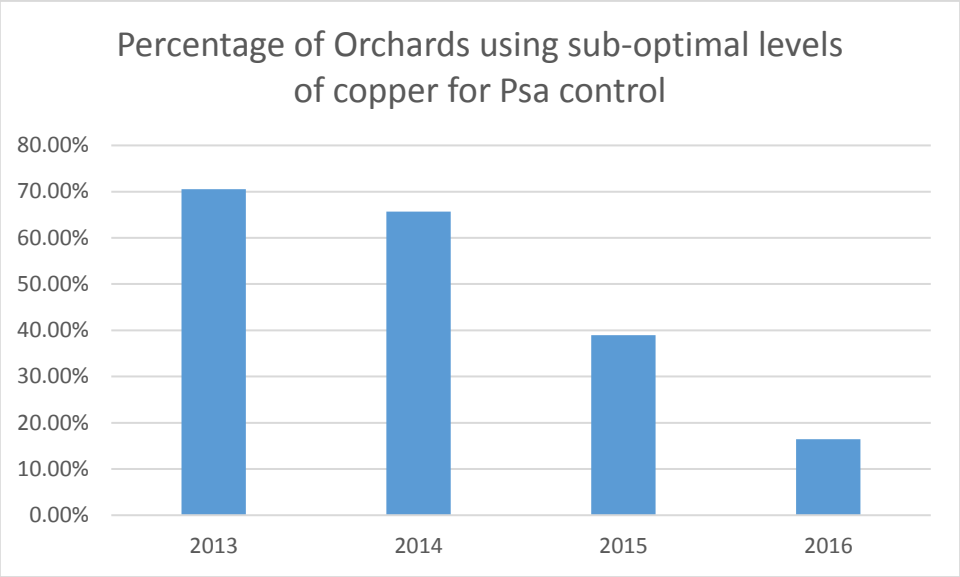

Figure 9: The use of sub-optimal levels of copper in the control of Psa. Data courtesy of Zespri Spray Programme.

3.3.1.1.5 Detection of Psa Copper Resistance

Due to the plethora of genetic elements that may contribute to copper resistance, the development of a genetic test to confirm the presence of resistant Psa has not yet been realised. Instead bacteria are exposed to increasing amounts of copper in solid media in order to determine the level of tolerance. To date there have been no physiological levels of resistance or product failure found within New Zealand orchards.

3.3.1.1.6 Consequences of Long Term Copper Usage

The use of copper to control agricultural pests leads to the accumulation of copper within the soil. In 2012 it was found that orchards in the Bay of Plenty region of New Zealand contained up to 35 mg/kg of copper within the topsoil (Jeyakumar *et al*. 2014). Although no phytotoxic effects have been reported as a product of this copper accumulation, the long-term effects in New Zealand orchards are currently being studied.

One possible consequence of copper accumulation in the soil is the changing of the phylosphere or the community profile of soil dwelling microorganisms. Work investigating the microbial dynamics of China's copper contaminated soil showed that when copper contamination increased not only the level of bacteria dropped but also the level of bacterial diversity (Li *et al*. 2015). The long-term effects of this potential change on the agricultural crop is unknown. One possible consequence could be the decrease in beneficial microorganisms which promote growth or protect against disease, leaving kiwifruit vulnerable to stunted growth or further disease (Hayat *et al*. 2010).

The use of copper and subsequent accumulation within agricultural soils has also resulted in the detection of an increased amount of copper resistance genes within the local soil microbial population (Altimira *et al*. 2012). Although this may not be an immediate problem allowing aforementioned beneficial microorganisms to survive, this also provides a local source of resistance genes which can be easily transferred or acquired by pathogenic microbes such as Psa. This could allow Psa to acquire resistance quicker and decrease the efficacy of copper treatments.

Recently the presence of copper in agricultural soils has also been linked to the accumulation of antibiotic resistance (Seiler and Berendonk 2012; Hu *et al*. 2016). In environments where specific antibiotics are not present and there is no selection pressure to acquire resistance, bacteria are still acquiring these resistance genes. It has been found the use of heavy metals, including copper, can not only select for genes encoding respective metal resistance, but also the acquisition of antibiotic resistance. This can include Psa, which can directly gain these antibiotic genes, or acquire them through the local microbial population. On average orchards that harbour copper-resistant and/or streptomycin resistant Psa are less likely to use copper at the correct rate as compared to other orchards nationwide (Zespri Spray Diaries 2016). This sub-optimal concentration of copper instead of killing Psa and the local population of microorganisms could instead lead to various resistance becoming acquired. This has far-reaching consequences, including limiting other methods of agricultural control such as streptomycin or kasumin, but also in spreading antibiotic resistance in human-related diseases.

3.3.1.1.7 The Future of Copper in New Zealand Agriculture

Copper is one of the most efficient products in the control of Psa. Work is being undertaken to understand the effects of copper usage in more detail.

Currently the bioavailability of copper in the orchard is not known. Copper is currently used at around a concentration of 4 mM. Higher concentrations result in an increase in phytotoxicity in the kiwifruit vine. Copper ions are responsible for the bactericidal effect on Psa, however it is not known how much of the copper product is in this state and what governs the rate of copper ion release. Work underpinned by the Sustainable Farming Fund is looking at researching the bioavailability of these copper ions within orchards and the quantitative effect on Psa populations. This includes the influence of weather events and the effects of other commonly used control products on the release of copper ions. Work is also underway to address the long-term effects of copper usage on the plant and soil microbiomes, including looking at changes in biodiversity and the abundance of various heavy metal and antibiotic resistance markers.

3.3.1.2 Bactericidal Antibiotics

Antibiotics have shown one of the highest efficacies against Psa in the lab. However, most countries prohibit the use of these chemicals in agriculture due to the risk to human health. Countries like New Zealand, the USA and China, allow the use of antibiotics in extreme circumstances, for example, when there are very limited options to control a disease. There are two approved antibiotic products that can be used against Psa in New Zealand; streptomycin and kasugamycin. The Agricultural Compounds and Veterinary Medicines Group (ACVM) have approved them for use.

3.3.1.2.1 Streptomycin

Streptomycin is a type of antibiotic called an aminoglycoside, historically used in human health to treat bacterial infection. They work by targeting the ribosome – protein making machinery within

the bacteria, meaning they cannot undergo cellular processes and die. Streptomycin was found to be effective against Psa not only in the lab (Everett 2011c), but also in the field (Fruitfed Supplies 2014; Kiwifruit Vine Health 2014). In 2011 limited approval was granted for use of the streptomycin product KeyStrepto from bud break until a week before flowering. This product must not be used during flowering due to the risk of residues on kiwifruit and the threat and risk of residues to bees and the bee industry. From 2016, KeyStrepto could only be used under justified approval from Zespri (Kiwifruit Vine Health 2016b). Although effective, concerns over the usage of antibiotics, especially those involved in human health, has limited the potential of this control product.

3.3.1.2.1.1 Psa Streptomycin Resistance

As with copper resistance, bacteria will also acquire resistance to antibiotics when inappropriately used or over long periods of exposure. It can also be transferred naturally through horizontal gene transfer, even in the absence of streptomycin. Bacterial resistance to streptomycin was discovered only a few years after the introduction of streptomycin to agricultural pest management. This would suggest that after the approval of the streptomycin, resistance would shortly follow.

Streptomycin resistance can occur in one of two ways. The first mechanism of streptomycin resistance is a change in the DNA or mutation of the *rpsL* gene mutation which confers resistance to streptomycin by preventing the antibiotic from working (Springer *et al*. 2001). The second mechanism of streptomycin resistance is the acquisition of the genes encoding StrA and StrB, obtained from other bacteria. These proteins are streptomycin modifying enzymes which render the antibiotic inert (Chiou and Jones 1995).

Since the use of KeyStrepto in New Zealand orchards, resistance has been identified, possibly reducing the effectiveness of streptomycin as a control option. The first streptomycin resistant samples were identified in 2015 by a KVH / Zespri and Verified Lab Services monitoring, four years after the commercial usage. Samples were first identified as being streptomycin resistant by culturing on increasing concentrations of streptomycin. However, this method was slow, as culturing could take weeks. In 2016, rapid qPCR genetic tests for both types of streptomycin resistance was developed. These tests could determine both the presence of *strA* and *strB* genes and the sensitive or resistant status of the *rpsL* gene (Mackay and Waters 2016). Although useful in identifying orchards that harbour streptomycin resistant Psa, this test has yet to be independently verified.

3.3.1.2.1.2 Withdrawal of Streptomycin

Although ACVM has approved the use of streptomycin to combat Psa, Zespri has removed KeyStrepto from the crop protection standard as part of an integrated pest and disease management strategy, effective from August 2016. This means KeyStrepto must only be used under exceptional circumstances under Justified Approval. This is in part due to the fact that KeyStrepto was only intended to be used as a temporary control product. Other driving factors included the international pressure to eliminate the use of antibiotics from agriculture and the emergence of streptomycin resistant Psa.

3.3.1.2.2 Kasumin

Kasugamycin or Kasumin is another ACVM registered aminoglycoside antibiotic which is effective against Psa in the lab (Everett and Vergara 2012a) and in the field (Kiwifruit Vine Health 2014; Fruitfed Supplies 2014). This antibiotic has never been used in human or animal health. It functions in a similar way to streptomycin by disrupting protein synthesis. However, it uses a different

molecular target so streptomycin resistant Psa would still be sensitive to kasumin, there is no cross-resistance. Kasumin has also been shown to be non-phytotoxic when used at the recommended label rate and no kasumin residues were found on kiwifruit when applied before flowering (Eurofins Agroscience Services 2015).

3.3.1.2.2.1 Psa Kasumin Resistance

Although a relatively new product in the management of Psa in New Zealand, there are concerns about the development of Kasumin resistance. Work has already shown that resistance can spontaneously occur in the causal agent of fire blight (*Erwinia amylovora*) or possibly be acquired from neighbouring bacteria. However, kasumin resistant *E. amylovora* are shown to have a growth defect and grow slower as compared to their kasumin sensitive counterparts (McGhee and Sundin 2011). Although harbouring growth defects, kasumin resistant bacteria have the potential to thrive and spread when kasumin is constantly used. This emphasises the importance of using kasumin as part of a management programme with other control products and at the appropriate concentration to prevent and effectively manage the incidence of resistance.

Kasumin resistance is currently being monitored using culturing methods. Although kasumin resistant Psa have not been identified to date, it would be advantageous to be prepared by developing the ability to perform high-throughput genetic qPCR assays to rapidly identify resistance markers, as with streptomycin.

3.3.1.3 The Usage of Protectant Control Products in New Zealand Kiwifruit Agriculture

Preventative control products are the best option available for the control of Psa. However, we do not fully understand the long-term effects of constant use. Resistance to copper and streptomycin has already been observed, limited options for effective control. Resistance combined with pressure to remove antibiotics from the management strategies emphasises the need for new and novel control products, where resistance is unlikely to develop as well as the continued research into the long-term effects of continued use of such strategies.

3.3.2 Elicitors

Most of the control methods of preventing Psa infection rely on using heavy metals (copper) and antibiotics, which can become toxic if used at high enough concentrations or at the wrong time of the growing season. This led to the screening of alternative control products that could show efficacy against Psa infections independently and in combination with protective control products (Gilbertson Associates 2013; Reglinkski *et al*. 2011). There is only one commonly commercial elicitor used in New Zealand, with more being tested for potential activity against Psa.

3.3.2.1 Actigard

Elicitors are compounds which induce plant resistance to bacteria, meaning they are less likely to succumb to infection. Acibenzolar-S-methyl (ASM) or Actigard is a commonplace elicitor used in various fruit industries to control bacterial disease. Actigard mimics the plant hormone salicyclic acid which is produced during Psa infection and co-ordinates the plant response to the bacteria (Reglinski *et al*. 2013; Reglinksi *et al*. 2011). The application of Actigard to kiwifruit leaves, primes the tissue to expect Psa infection and can deal with it effectively. Application is recommended before Psa infection and usage is recommended in the weeks prior to flowering and after harvest. Actigard cannot be used post-flowering due to the likelihood of the detection of ASM residues on fruit.

Actigard has been shown to be significantly effective at preventing Psa infection, as compared with other hormone mimic products (Reglinski *et al*. 2013; Fruitfed Supplies 2014; Kiwifruit Vine Health 2015). Alone Actigard reduced the incidence of Psa infection, however, it was found that Actigard in combination with other products such as copper and KeyStrepto, resulted in a higher rate of effectiveness. To date, Actigard is the most effective ACVM registered elicitor product for controlling Psa in the orchard.

3.3.2.1.1 Phytotoxicity

There were several concerns from growers and commercial users that applying Actigard resulted in reduced canopy growth and orchard yield, due to the induction of a plant hormone stress response. Trials commissioned by Zespri and Actigard manufacturers Syngenta investigated the effects the product has on the kiwifruit vine. These trials have found that no negative effects on canopy growth or yield can be attributed to the use of Actigard, full trial results will be published later this year. Trials in France have also shown there are no negative effects on the canopy even when two applications of Actigard are used before flowering (Cazy and Willaert 2016). Nevertheless, the use of Actigard on stressed vines is not recommended.

3.3.2.1.2 Mode of Action

Both Actigard and Psa induce the expression of a certain set of genes. When leaves treated with Actigard are inoculated with Psa, the response is greater, suggesting Actigard primes the plant to react quicker or stronger to Psa, reducing the incidence of symptoms (Reglinkski *et al*. 2013). However, this work is preliminary and the full extent of the kiwifruit vine response to Psa/ASM has not been determined, and should be investigated to understand the full mode of action of Actigard and its efficacy when applied to various varieties of kiwifruit.

3.3.2.2 Novel Elicitor Products

Although Actigard is the most effective elicitor product at controlling Psa in the orchard, it cannot be used on fruiting vines due to the presence of residues on fruit. As most control products are best used in combination with others, novel or alternative plant elicitors are being researched to identify those that can be used throughout the entirety of the growing season (Hoyte *et al*. 2013).

One of the most promising elicitor products currently being trialled is an elicitor mix named 'EMix'. It is a PFR product which has been shown to have significant activity against Psa in potted plant trials similar to Actigard. EMix possesses the potential to be used all season, as it is thought to leave little or no detectable residues. The commercial development of EMix is recommended as an effective Psa control product, but also as an important component in combination with other novel control products (Elmer *et al*. 2015; Elmer *et al*. 2014a).

3.3.3 Biological Organisms

The use of biological organisms to control agricultural pests was first demonstrated in 1929 (Mandadi and Scholthof 2013). This practise is the use of microorganisms or their products to control the population of an agricultural pest, in this case Psa. As part of the next generation biopesticides research programme, biocontrol agents (BCAs) are essential for kiwifruit growers who want non-chemical control options for the management of Psa. In 2011 a review was conducted to investigate the suitability of existing agricultural BCAs. It was determined that although recommended organisms could show activity against Psa, they were most likely to be successful as part of an integrated control strategy including both chemical and biological controls (Stewart *et al*.

2011). Several types of biological organisms or products have been subsequently researched following these recommendations. This includes; endophytic or antagonistic strains of yeast and trichoderma, bacteriophages, and marine natural products.

3.3.3.1 BOTRY-Zen

BOTRY-Zen is the only KVH recommended biological control product currently boasting a limited label claim for activity against Psa. This product contains the fungus *Ulocladium oudemansii*. It colonises dead and decaying plant material but does not damage live plant tissue. Generally used for the control of fungal infections in a range of crops, BOTRY-Zen was found to significantly reduce the amount of leaf spotting in Hayward kiwifruit during potted plant field trials (Kiwifruit Vine Health 2013).

3.3.3.2 Yeast

Yeast microorganisms are effective biocontrol agents as they can rapidly colonise leaf surfaces and survive for long periods of time under different environments and can tolerate commercial fungicides (Rosa-Magri *et al*. 2011). PFR have collected various yeast samples which have been isolated from the surface of several crops including kiwifruit. These have been used previously to combat Botrytis blight in apples. Based on activity against *Botrytis spp*. yeast isolates were tested for activity against Psa, both individually and as combinations (Hoyte *et al*. 2013).

Activity against Psa was investigated when yeast samples were applied to the leaves of Hayward and Hort16A seedlings with and without Actigard. It was found that yeast cultures coded YM1 and YM2, exhibited some of the highest activity against Psa. Although YM2 was chosen to progress to field trials and further validation, contingency studies were conducted to identify other mixes of yeast isolates effective against Psa, if YM2 was not effective in the field (Elmer *et al*. 2014a).

Potted plant trials also showed the potential of YM2 as a biocontrol product of Psa (Elmer *et al*. 2014b), however, it was shown that the individual components; YCom1 and YCom2 exhibited moderate and high susceptibility to Kocide opti respectively. When YM2 was tested in field trials, it was shown not to be commercially acceptable despite being fast tracked and issued with provisional registration.

Following the cessation of research using YM2, focus shifted to second generation yeast BCA products from PFR; YBCA4 and YBCA5. Although they possessed similar efficacy against Psa in both potted plant and field trials, YBCA5 was cheaper, exhibited increased copper tolerance and overall survival on the leaf surface as compared to YBCA4 (Elmer *et al*. 2015). To register YBCA5 as a Psa control product more research must be conducted. This includes investigating the effect of YBCA5 on pollination, bee health, and efficacy against Psa in combination with other commonly used control products.

3.3.3.2.1 Mode of Action

The molecular basis of yeast activity against Psa is unknown. It is likely to be related to direct competition for nutrients, physical prevention of colonisation of plant material or the production of antibacterial products (Hatoum *et al*. 2012). This presents a new line of research enquiry, to investigate the Psa-Yeast interface and how yeast interacts with Psa. This could also be exploited to identify commercially viable antimicrobial products with activity against Psa.

3.3.3.3 Trichoderma

Trichoderma spp. are fungi which colonise root surfaces and penetrate roots. They have been successfully used as BCAs since 1930, to combat a variety of agricultural disease. These genera possess the ability to antagonise plant pathogens through a variety of methods, including competition for nutrients and space, production of antimicrobial products, and inducing plant resistance (Hoyte *et al*. 2013). These actions can also indirectly promote plant growth and increase productivity (Hermosa *et al*. 2012).

The Bio-Protection Research Centre in Lincoln University isolated root endophytic Trichoderma from healthy kiwifruit vines where there was a high incidence of Psa. The potential of pre-existing, new isolates and mixtures of *Trichoderma spp*. against Psa was investigated using potted plant assays. Various combinations of these BCAs applied to the roots with and without chemical controls were used and the most effective combinations were recommended for further trials. TMix1 and TMix2 showed little efficacy, and were only effective when combined with other control products. Nonetheless they were validated further in field trials (Elmer *et al*. 2014a; Elmer *et al*. 2014b; Hoyte *et al.* 2013). TMix1 and TMix2 showed poor efficacy when applied alone to roots in field trials, however, a second-generation Trichoderma mix; TMix3, now named KiwiVax, is being further validated as it exhibited moderate efficacy against Psa in the field.

3.3.3.3.1 Mode of Action

Not unlike yeast, the molecular basis of Trichoderma activity against Psa has not been researched. If TMix3 or another similar product shows efficacy in field trials and is commercially viable, it would be valuable to investigate how these fungi antagonise Psa.

3.3.3.4 Bacteriophages

Bacteriophages are virus-like entities that exclusively infect bacteria. They infect a bacterial cell and take over the cellular machinery for its own viral replication. This ultimately leads to the lysis (bursting) of the cell and the progeny viruses can go on to infect other bacteria (figure 10). Bacteriophages have been used to control similar bacterial diseases in a range of plants (Obradovic *et al*. 2004; Balogh *et al*. 2008).

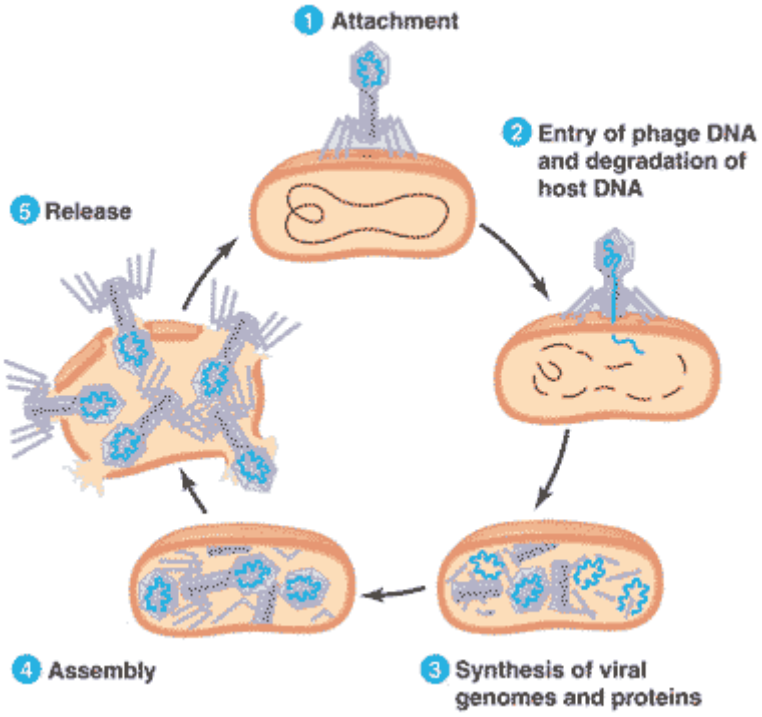

Figure 10: The Bactericidal action of Bacteriophages. https://smithlhhsb122.wikispaces.com/Kyle+R.

Bacteriophages were first found associated with plant bacteria in 1924 and were proposed as an option to control agricultural disease (Moore 1926; Jones *et al*. 2012). However, the efficacy of using bacteriophage in the prevention or treatment of agricultural disease is limited. This is partly because they are extremely sensitive to environmental conditions. For example, populations of bacteriophage rapidly decline when exposed to UV rays (Iriarte *et al*. 2007). The use of delivery vectors such as non-virulent bacteria, could increase the longevity of bacteriophages. However, as bacteriophage are limited to a specific host range, the challenge is finding a suitable bacteriophage which will infect both the vector and pathogenic bacteria, or a related non-pathogenic bacterium that could host the efficacious bacteriophage (Tanaka *et al*. 1990). For the combat of Psa, the naturally occurring non-pathogenic *Pseudomonas fluorescens* is being researched as a potential vector. As non-pathogenic strains are desirable, one could argue an avirulent strain of Psa or Pfm could be a better suited vector due to a high level of genetic similarity.

Bacteriophages are useful as they would not discriminate between copper and antibiotic resistant Psa as compared to susceptible Psa. However, there are reports of naturally occurring bacteriophage resistance, and this must be considered when evaluating viable bacteriophage BCAs. Resistance is generally due to the mutation or change of the site at which the bacteriophage initiates infection. The usage of a mixture of bacteriophages with varying methods of infection could negate the risk of bacteriophage resistance.

3.3.3.5 Predatory bacteria

Bacterial predators enter prey bacteria, reproduce and burst the bacterium from the inside. It is currently thought that predatory bacteria could be an alternative approach to combatting antibiotic resistant bacterial infections in humans (Kadouri *et al*. 2013).

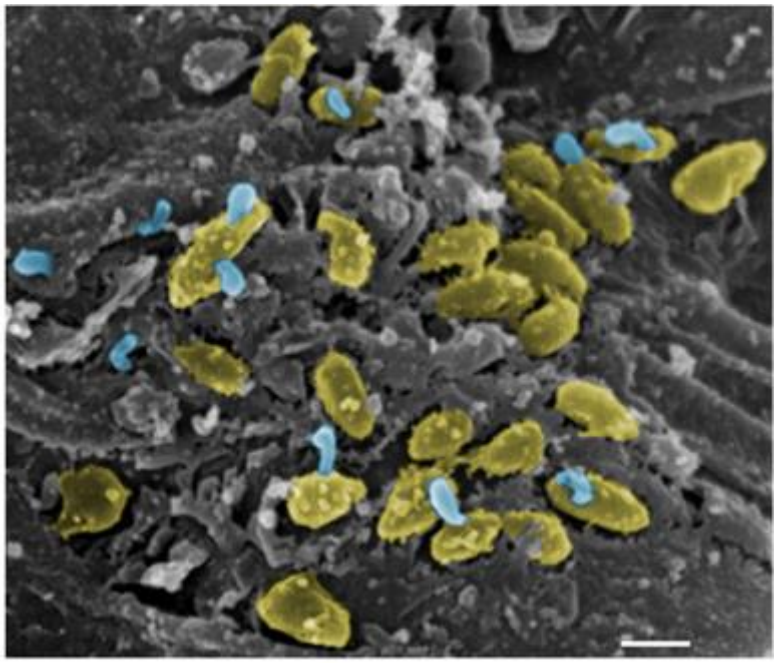

Figure 11: Scanning Electron Microscope Image of *Bdellovibrio bacteriovorus* (blue) infecting *Pseudomonas tolaasii* (yellow). Saxon *et al*. 2014.

Predatory bacteria have also been used in agriculture, protecting against brown-blotch in super market mushrooms. Brown-blotch is caused by *Pseudomonas tolaasii*, which is closely related to Psa. When the predatory bacteria *Bdellovibrio bacteriovorus* HD100 was applied to the mushrooms, numbers of *P. tolaasii* and severity of symptoms reduced significantly (figure 11) (Saxon *et al.* 2014). This treatment has potential for use against Psa in kiwifruit. Although it has already been shown that *B. bacteriovorus* has activity against other *P. syringae* pathovars (Barel *et al*. 2005; Scherff 1973), work would need to establish if Psa was a viable target for available strains of predatory bacteria. If viable, this could provide a specific treatment for Psa which would not target commensal, growth promoting bacteria.

3.3.4 Miscellaneous Control Products

3.3.4.1 Ambitious

Forchlorfenuron or CPPU is commonly used world-wide as a potent growth regulator of non-NZ kiwifruit, blueberries, apples and grapes. CPPU is used to increase fruit size (Kim *et al*. 2006; Curry and Greene 1993; Reynolds *et al*. 1992; NeSmith 2002). Although there are many other CPPU products, this report will focus on Ambitious as the only CPPU product to be used within the kiwifruit industry against Psa.

Under the Zespri quality system, CPPU cannot be used as a growth regulator of kiwifruit. However, since 2014, Ambitious was the only CPPU granted a full label ACVM claim to control Psa on Hayward kiwifruit vines and can be used as an additional control product since 2014. It was shown that Ambitious reduced the severity of leaf spot, when used between bud break and flowering (Hawes 2015). It was determined that ambitious showed similar efficacy to Actigard, however did not protect against bud rot or secondary symptoms. Ambitious also resulted in increased canopy growth, and healthier leaves consistent with its role as a growth promoter (Fruitfed Supplies 2015). Ambitious use on G3 varieties is not permitted as it is likely to cause flowering and fruit defects.

It is not known how this growth promotion product protects against Psa, and more research is needed to fully understand how the use of CPPU prevents the incidence of leaf spot.

3.4 Orchard Management

Standard orchard management requires the maintenance of vines to ensure a high quality crop is produced annually. This is important to ensure economic viability of the orchard. Since the incidence of Psa, management techniques have had to adapt to minimise the risk of Psa infection occurring and subsequent spreading through the orchard. Orchard management of Psa is a delicate interplay between ensuring the economic status of an orchard and minimising the risk, presence and spread of Psa.

The ultimate goal of orchard management is to prevent Psa from entering the orchard and initiating infection. Once it does, inoculum builds up and the risk of Psa spreading through the orchard increases. Once infection has established focus must shift to preventing spread, by managing the risk of conditions that promote Psa infection. This includes the use of protective measures such as copper, antibiotics and other products, as well as the physical removal of inoculum. This chapter will discuss the risks that govern the incidence and spread of Psa on an orchard and options to prevent or control it.

3.4.1 Risk factors for the introduction of Psa and spread of Infection

There are several practises which are common within normal orchard management that can either introduce Psa from contaminated orchards to clean orchards or spread Psa from one infected vine to others within an orchard. This subchapter discusses the factors which promote the spread and progression through the kiwifruit vine.

3.4.1.1 Cultivar Tolerance

One of the most important factors governing Psa infection and the subsequent spread is the susceptibility of the cultivar to the bacterium. Cultivars of *A. chinesis* such as Hort16A consistently showed higher incidence of disease than *A. deliciosa*. It was thought that differences in the physiology and phenology could be partially responsible for this. Hort16A possess more trichomes which translates as more potential Psa infection entry points (Spinelli *et al*. 2011). They also develop and flower earlier in the season, which means delicate young tissues is susceptible to damage when Psa is most prevalent. There are also reports that *A. delicosa* males exhibit a higher incidence of symptoms than *A. delicosa* female vines, suggesting they are more susceptible (Froud *et al.* 2015), although this may be due to different management requirments. Although the replacement of susceptible varieties may reduce the impact of Psa, the effect on the level of Psa is not known.

3.4.1.2 Plant Material and Bud-Wood

Root stocks and grafted plants from nurseries or bud-wood from suppliers are used to propagate kiwifruit varieties in new orchards and have the potential to spread Psa. This has led to schemes such as the Kiwifruit Plant Certification Scheme (KPCS) which govern the movement of plant material to prevent the spread of Psa from region to region. This prevents Psa-positive material from leaving the region, and prevents material from a Psa-positive region from being exported to a clean region or a region with a lower risk of Psa. Although these schemes are in place, as a grower it is important to recognise the potential risk of importing Psa from contaminated plant material and make appropriate decisions to prevent or limit this accordingly.

Research is ongoing to explore the possibility of sterilising bud-wood material to remove the risk of spreading Psa. This would be beneficial as it would allow the distribution and propagation of new varieties of kiwifruit which have been grown in Psa-positive regions to all regions. Initial work used heat-treatments and were able to demonstrate that no viable Psa remained when bud-wood was heated to 50 degrees for five minutes (Everett 2011d). However, it is thought that after this heat treatment, the bud-wood was no longer viable. Research investigating methods of removing Psa from propagation material is ongoing.

3.4.1.3 Hygiene

Figure 12: Example of the introduction of on-orchard hygiene practises.

Another method of introducing Psa onto orchard or the spread of infection is through the contaminated equipment, clothing, shoes and vehicles. Upon swabbing several surfaces in contact with the orchard environment, Psa was detected on the surfaces which had come into contact with the soil, including the sides and tyres of vehicles and even on the feet of rabbits (Everett *et al*. 2012c). Further testing showed that Psa could be detected on the soles of footwear and pruning equipment (Everett *et al*. 2012d). It should be noted that this work used genetic identification which is likely to have gave false positive results, however, this should not detract from this likely method of spreading Psa.

With the knowledge that Psa can potentially spread through the introduction of contaminated items, work began on identifying disinfectant products to decontaminate various surfaces (Dowlut and Judd 2012). Many disinfectants showed total killing after as little as 10 seconds of contact with the contaminated surface. This presents an effective strategy to prevent the spread of Psa between orchards and from vine to vine. However, there are many reports that the use of disinfectant products in the orchard needs optimising. It is recommended all tools, vehicles and persons that enter an orchard are decontaminated, and that tools are decontaminated between each pruning event (figure 12) (Kiwifruit Vine Health 2016d).

3.4.1.4 Pruning

Kiwifruit vines are regularly pruned in spring and winter as part of the normal management programme. This is to ensure the presence of fruiting cane and control excessive growth. However, this creates wounds and provides entry sites for Psa into the vine (Ferrante *et al*. 2012; Miller 2012). This problem is exacerbated in areas with a high inoculum of Psa.

To try and prevent infection and protect wound sites, protectants are often applied after every pruning event. Greenseal is arguably the industry standard for wound protectant, showing significant activity against Psa in the lab. Commonly used wound protectants such as Greenseal are used at high concentrations in the orchard, however, when tested in the lab, concentrations of 5% - 20% of each product would result in the complete killing of high levels of Psa in under 2 hours (Dowlut *et al*. 2013). However, Greenseal failed to significantly protect against Psa in Bruno field trials (Everett *et al*. 2014).

Other field trials showed that the application of the wound protectants; InocBloc and copper pastes significantly reduced the presence of Psa in the cane after wounding as compared to plants which were not protected. It was also demonstrated that wound protection is paramount as there was a dramatic increase in the incidence of Psa during winter and spring in unprotected plants (Everett *et al*. 2016). Unfortunately, these trials used Hort16A, a pre-commercial variety and G14 kiwifruit plants, which do not represent the majority of the industry. Research is currently ongoing to trial these products in G3 and Hayward and to include protection of wounds caused by girdling and grafting. This work seeks to optimise the use of wound protectants to recommend the most effective products and methods of protecting the vine against infection via wounds. It should also be noted that the use of tools or brushes to apply protective pastes or wound protectants also have the potential to aid the progression of Psa. In order to prevent this, protectants in the form of sprays should be sought.

3.4.1.5 Girdling

Girdling is the process of ring barking the trunk of the vine, removing the vasculature which transports sugars from the foliage to the roots. If the girdle is applied too deep water transport could be affected and this could lead to the death of the vine. Applying a trunk girdle in spring can improve the fruit size and applying girdles in summer can increase fruit dry matter. There are financial incentives for a grower to increase both size and dry matter of one's harvest. The act of girdling, however creates a wound within the vine and it has been shown to be an entry point for Psa in Hayward and Hort16A. (Snelgar *et al*. 2012). Further work has also shown that Psa is more likely to infect vines through the girdle when applied too deep healing is impaired (Casonato *et al*. 2014b).

3.4.1.6 Pollination

As discussed earlier, pollen and honeybees can potentially spread Psa from vine to vine or from nearby orchards. The same can apply to the practise of artificial pollination.

Artificial pollination is the process of manually applying pollen to female flowers. This is generally adopted when local pollen or means of pollen transmission is limiting. Male kiwifruit vines may have produced less than desirable flower numbers for various reasons, including Psa challenge. This means that there is not enough pollen to effectively pollinate the female flowers, and external pollen must be brought in. In Italy, there are constraints on the availability of honeybees and natural pollination cannot be relied on (Goodwin *et al*. 2011). In the event of lack of natural transmission of pollen, artificial pollination is adopted.

Flowers are picked from the male vine, and then undergo the milling process. The flower is crushed and the pollen containing anthers are released. Another process then separates the anthers from the rest of the plant material. Once dried the anthers are vacuumed and the pollen is extracted. Through this process the pollen is exposed to the external part of the flower and may become contaminated with Psa if not already. Psa survives this milling process and is subsequently present on contaminated pollen used for artificial pollination (Holmes *et al*. 2013).

This combined with the knowledge that honeybees can also carry Psa means that pollination; natural or artificial poses a risk of spreading Psa.

3.4.1.7 Climatic Factors

As discussed earlier, Psa requires rain and cold temperatures in order to cause and progress infection. Therefore, an orchard which is located in a region which is subject to colder temperatures and more rainfall would expect a higher incidence of Psa, as compared to better situated orchards. This also includes orchards or regions which are exposed to an increased amount of wind or other damaging weather events (Casonato and Bent 2014; Froud *et al*. 2015).

3.4.2 Management Options for the Prevention of Psa and Spread of Infection

3.4.2.1 Psa Related Pruning

Psa related pruning is a controversial method of physically removing infected plant material from the vine. This is thought to reduce local Psa inoculum, especially in material with visible exudate or leaf spotting. In Hayward orchards, there was minimal Psa progression when infected material was cut out up to 40 cm from the most proximal symptom site. When the same practise was applied to Gold3 vines, progression of Psa was not halted, however the rate of spread was reduced. This may have been due to the ineffective callusing of wounds. If this is the case, it is recommended to cut further closer to the leader, as Psa may have progressed beyond the 40-cm mark. Callus formation can be improved by pruning in late spring (Horner *et al*. 2013). Other methods such as cauterisation showed little effect on preventing Psa progression through the vine.

This process of removing infected material is controversial as the removal of parts of the vine also reduces overall yield, especially when leaders are infected with Psa. Some growers may opt to not prune infected material if it will lead to a significant loss of productivity. Some vines managed in this manner have reportedly dealt with the infection and have been productive in the following seasons, however it is unknown how this may impact the health and productivity of the vine long term.

3.4.2.2 Vine and Canopy Management

Growing kiwifruit in a Psa environment is a challenge and may necessitate a significant amount of material being removed from the orchard due to infection impacting on orchard productivity. To minimise the loss of productive fruiting canopy, there has been work investigating vine and canopy management methods to minimise the effect Psa and subsequent pruning has on overall yield.

When grafting multiple budwood of either G3 or Hayward into established Bruno stumps, it was found that four leaders could be successfully grown. The existence of multiple leaders reduces the impact of cankers on the canopy and fruit yields if a single leader is required to be removed (Currie *et al*. 2014).

It is also prudent to thin the canopy, as this may result in reduced secondary symptoms. Reducing the amount of overlapping canes from both female and male green kiwifruit canopies may reduce budrot. This is thought to be a product of the reduction of damage due to cane rubbing against each other, but may also be the result of reduced humidity and less conducive conditions for disease establishment (Scarrow and Underwood 2013).

3.4.2.3 Psa Control Programme

Although the removal of infected material is effective, sometimes this is not practical, especially with infected leaders or leaf spotting, these constant sources of Psa must be managed appropriately. The level of inoculum and the potential of further infection to occur can be minimised by the application of the appropriate control products.

An effective control programme includes the application of protectant products which minimise the levels of inoculum and protect vine surfaces, preventing further infection. The use of elicitors or other biological products can induce defence responses within the vine and promote the effective resolution of new infection, or try to hinder systemic infection.

3.4.2.4 Managing the Environment

In additional to using chemical and biological controls of Psa, growers may choose to reduce the environmental risk of infection. This involves modifying the local environment to protect against extreme weather conditions. This can include covers to protect against wind and rain, wind shelter belts and frost protection.

3.4.2.4.1 Artificial Covers

As Psa needs rainfall for infection to occur and progress, plastic breathable covers have been used to prevent the vine being exposed to excessive moisture. These covers prevent rainfall from entering the orchard and in theory minimise the risk of Psa infection. Initial work suggested that diseased plants showed improvement when placed under cover. However, when plants which have not been exposed to Psa are covered, infection is slow to establish if at all (Casonato *et al*. 2013). Further trials showed that even in diseased plants the progression of Psa is slowed when covered. However, the feasibility of plastic covers is variable as high wind can destroy the protective canopies (Casonato *et al*. 2014c). The Chilean kiwifruit industry have reported great success in a comprehensive trial concerning the use of plastic covers, correlating the reduction in local humidity and the reduction of Psa infection (C. Abud & CIA Personal Communication 2016).

Shelter Belts and other artificial covers also protect against wind and other weather damage. They act as a physical barrier and prevent weather-mediated damage to the vine and the creation of additional Psa entry points. Shelterbelts comprised of deciduous trees or that contain gaps expose

proximal vines to the wind and these may show a greater incidence of Psa symptoms. For example, during winter, deciduous shelterbelts lose foliage and therefore fail to shelter the vines from the wind (Froud *et al*. 2015).

3.4.2.4.2 Frost Protection

When the temperature drops below 0 °C frosts are likely, this leads to damage and promotes Psa infection. Growers can prevent this by using frost protection methods. Most popular methods include overhead irrigation and the use of frost fans.

Frost damages the plant tissue by forming ice crystals and dehydrates the plant cells. Overhead irrigation instead promotes the formation of ice outside of the plant preventing injury. However, this method spreads significant amounts of water over the surface of the vine which potentially could aid the progression of Psa. In areas where there is poor drainage, soils can become waterlogged through irrigation and cause additional stress to the vine.

Frost fans can also prevent frost. This is achieved by blowing air horizontally to mix warmer air with the colder ground level air, this raises the overall temperature of the orchard and prevents frost. However, fans are not always suitable for all orchard landscapes (Kiwifruit Vine Health 2016c).

3.4.2.5 Girdling to Control Psa

Bud and flower rot in green kiwifruit is a significant secondary symptom that causes a reduction in the productivity and yield of an orchard. Although bud and flower rot is not specific to Psa, it was associated with the majority of bud rot in green kiwifruit in New Zealand (Tyson et al. 2015b). The use of several products was evaluated for the control of budrot. Although many products reduced the levels of leaf spotting, they did not significantly affect the incidence of bud rot. Only the application of a girdle seemed to reduce the levels of budrot, although leaf spotting was unaffected (Fruitfed Supplies 2014). The success of the girdle depends on its application relative to flowering, as when applied closer to flowering, the incidence of budrot increased. Optimal results were obtained when a girdle was applied 30 days prior to flowering (Casonato *et al*. 2015).

It is unknown how girdling affects Psa and therefore the expression of symptoms, however there are three theories.

3.4.2.5.1 Girdling Effects the Microbial Population on and in Flower Buds.

The application of the pre-flower girdle is thought to change the microbial communities within the flower bud. This then could go on to affect the levels of Psa and therefore budrot. Girdling seemed to result in lower populations of bacteria including Psa. However, this needs to be further investigated with larger studies (Richardson *et al*. 2016).

3.4.2.5.2 Girdling Effects on Carbohydrate Status

Girdling disrupts the vasculature, governing the transport of sugar and carbohydrates from the foliage to the roots. This leads to a build-up of sugars within the vine. Indeed, an increase in total sugar concentration was found in the leaves, flower buds and ovaries on girdled vines. This change in sugar concentration could potentially lead to a decrease in budrot or Psa populations although the mechanism is unknown (Richardson *et al*. 2016).

3.4.2.5.3 Girdling Effects on Water Dynamics

Figure 13: The expulsion of guttation from kiwifruit flower buds (Richardson *et al*. 2016).

Figure 14: Guttation staining on brown sepals of infected buds

The final hypothesis is that girdling effects water pressure within the vine, which in turn prevents Psa entry into the buds. Buds expel fluid due to root pressure in a process known as guttation (figure 13). The production of this fluid could be a viable entry point of Psa (figure 14). Girdled vines exhibit reduced root pressure and reduced bud guttation. Therefore, reduced guttation fluid is thought to result in less Psa. However, a link between guttation fluid and Psa infection has not been satisfactorily shown to date (Richardson *et al*. 2016).

3.4.2.6 Root Pruning

Root pruning is an orchard management technique which can be used to increase the dry matter of kiwifruit. When root pruning is practised it induces a systemic response within the plant similar to girdling. It is also thought that root pruning has similar effects on the root pressure within the plant and would also reduce guttation. The possibility of root pruning, timing and the dynamics of the subsequent responses relevant to Psa needs to be explored.

3.4.2.7 Nutrition and Plant Health

There is evidence to suggest that the nutritional status or the health of plants can affect susceptibility to pathogens and disease. Therefore, the nutritional status of kiwifruit was investigated to determine if there was a link between specific nutrients and the relative tolerance of Psa infection. Work showed that the nutrient composition of soil and therefore the nutrition of the vine is important not only in the health and productivity of the vine but also in the severity of Psa symptoms. However, maybe less important in more mature plants, due to the size of root systems and the increased storage capacity. The presence of the various forms of nitrogen had the most evident effect on the severity of symptoms and suggests nitrogen and other soil nutrition should be a consideration in Psa control (Miller and Dean 2015).

3.5 Psa Tolerance Breeding Programme

Plant and Food Research boasts the largest kiwifruit breeding programme in the world. It focuses on improving taste and quality in new cultivars to command a market premium. Following the onset of Psa, new cultivars such as G3 and Green14 were released which were coincidentally less susceptible to the bacterial infection. Psa tolerance is now an important selection trait for new cultivars of kiwifruit. Seedlings and older kiwifruit cultivars are screened for their tolerance of Psa and score for the severity of symptoms. This provides information of the Psa tolerance of a cultivar relative to the susceptibility of Hort16A. Interestingly, there is no formal research quantifying the Psa tolerance of recently commercial varieties. There are other assays available which claim to be able to detect differences in tolerance to Psa between cultivars, however, there is little evidence to back the reliability of these assays (Mauchline *et al*. 2012b).

To truly manipulate the breeding of a Psa tolerant cultivar, genetic and molecular understanding of resistance is paramount. For example, the genetic difference between Hort16A and G3 has never been understood, this knowledge could not only aid the breeding programme but provide a fundamental comprehension of how Psa causes disease and potentially highlight further control options. For example, a difference in cell physiology could hinder the initiation of Psa infection, or a differential host response could allow more effective clearance or prevent the progression of infection.

Other avenues of exploration could include the investigation of differential microorganism communities harboured by different cultivars. For example, Hayward could promote the growth of certain beneficial microbes, which are lacking in Hort16A, which could antagonise Psa.

4 THE FUTURE, SCOPE AND OUTREACH

Pseudomonas syringae pv. *actinidiae* (Psa) is the etiological agent of bacterial canker in kiwifruit, which is a significant threat to a number of kiwifruit producing countries. Psa has been estimated to have cost over $1 billion to the New Zealand kiwifruit industry since 2010. In response to the New Zealand outbreak, KVH contracted Zespri to coordinate and facilitate a comprehensive research programme. The aim of this programme is to increase the understanding of the bacteria and the characteristics of infection in New Zealand and identify methods of controlling the incidence and spread of Psa.

This programme governed the identification and optimisation of control products used within the industry, as well as identifying the most effective orchard management practises to minimise risk and manage infection. As the demands of the industry changed the scope of Psa research changed with it. For example, due to the need for organic certified products and the looming threat of Psa-resistance to some chemical controls, attention shifted towards researching the use of biological organisms such as yeast and fungi to control Psa, with varying success.

Although the scope of Psa is expansive, there is a distinct lack of knowledge concerning the molecular mechanisms of Psa interaction and infection of the kiwifruit vine. There is a vast amount to be gained by understanding the factors governing infection allowing the potential for exploitation and other control strategies. This is most evident when comparing endophytic, epiphytic and actively infectious Psa. This direction of research could also explore the changes that govern changes in bacterial behaviour, and the initiation of infection.

The molecular detection of Psa is one of the most developed areas of research conducted. There is scope to apply molecular techniques on the orchard to provide fast and cheap identification of Psa, however it is uncertain if there is a demand for this. Ongoing optimisation of this technology could include the quantification of levels of Psa, which would aid epidemiology knowledge greatly. For example, the ability to determine levels of Psa could be valuable in the management of infection on orchard, by accurately assessing the inoculum risk and the control approach that should be utilised.

Since the initial introduction of Psa to New Zealand, there has been a magnitude of research conducted into understanding and preventing the spread of the bacteria using control products and orchard management, however Psa has become widespread throughout New Zealand making it very likely that it will be a persistent ongoing problem which needs to be managed effectively and sustainably. The scope of commissioned research is now including an in-depth understanding into the mechanism of action for specific control products and the long-term effects upon the local environment. For example, work relating to the Sustainable Farming Fund involves the further progression of understanding investigating the characteristics of copper, allowing the sustainable use of copper and management of copper resistant Psa in the future.

Psa resistance to KeyStrepto and Copper products is also a significant concern, which is likely to be addressed by the removal of KeyStrepto from the crop protection standard. However, copper is a one of the most useful chemical controls used on orchard and therefore the ongoing threat of resistance must be managed effectively. This includes the ongoing engagement with growers highlighting the importance of correct application and rates. Another way this research is addressing

the occurrence of resistance is through thorough understanding of how resistance is acquired and how this could affect the use of current and future control products.

The use of biological organisms and new chemical products such as Kasumin, address the issue of resistance in the short term, however Psa can also potentially acquire resistance to each of these in turn. This emphasises the need for a control programme incorporating several control products each with varying mechanisms of action to prolong the life and on-orchard effectiveness. Ongoing research is also suggested in identifying novel ways of using biological organisms to control Psa, such as bacteriophages and predatory bacteria.

Orchard management techniques and practises are arguably one of the most effective ways of controlling Psa infection. Management responses to Psa have resulted in the increase in productivity and yield, however much more can be learned. Although practises have been widely adopted, there are plenty of opportunities to further understand how specific techniques control Psa. There is a lack of innovation in this space, and New Zealand must look to other kiwifruit growing countries to incorporate novel practises and invest in renewing efforts in those seen as impractical.

In theory, the production of more Psa-tolerant cultivars of kiwifruit is desired. This would relieve the Psa-burden on growers and could potential decrease the costs of orchard management and control programmes. However, in reality there are a variety of factors which supersede tolerance to Psa when breeding kiwifruit, including cultivation, storage characteristics and fruit quality. There seems to be a lack of published data comparing the Psa-tolerance of new cultivars, and since Psa arrived in New Zealand, there has been no significant advances towards Psa-tolerance. As this is a long-term project, transparency is desirable.

As a whole the kiwifruit industry in New Zealand has made scientific advances in the understanding of Psa. However, one major drawback is the establishment of a culture where data is often unpublished, through choice or otherwise, or subject to limited review in journals such as the New Zealand Plant Protection Journal. Peer-reviewed collaborative science will greatly aid future progress and increase confidence within this area.

Psa is a global problem and as such the response is equally widespread. There are a multitude of research institutes from a variety of countries in both the northern and southern hemisphere contributing to the research initiative to counter the outbreak of Psa, with biennial symposiums to present, discuss and disseminate forefront Psa research.

International Symposium on Bacterial Canker of Kiwifruit (Psa):

- I – Mount Maunganui, New Zealand (2013)
- II – Bologna, Italy (2015)
- III – Santiago, Chile (2017)

As Psa evolves and changes, the industry must also change and respond. This means applying calculated and measured approaches to ongoing research, consolidating the industry with analytical and quantitative science, challenging a culture of unpublished or unsatisfactorily reviewed data and ultimately plan for the future of kiwifruit with sustainable solutions.

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