Kiwifruit bacterial canker in 'Hayward' kiwifruit:

The application of observational study design and epidemiological techniques to the study of disease outbreaks affecting plant health

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Abstract

Bacterial canker of kiwifruit, caused by *Pseudomonas syringae* pv. *actinidiae* (Psa) biovar 3, was first recorded in New Zealand in November 2010 and quickly made production of the gold-fleshed kiwifruit cultivar, 'Hort16A', which is highly susceptible to Psa, no longer viable in the Bay of Plenty region. Production of the green-fleshed cultivar, 'Hayward' has remained viable but there is uncertainty around its long-term productivity. This thesis investigated aspects of Psa in commercial 'Hayward' orchards using observational studies. The aims were to: 1) quantify a change in productivity associated with disease; 2) determine the prevalence of disease in orchards; 3) identify factors that altered the initial development of disease and 4) identify factors that impact on the presence of severe disease. Severe disease was defined as 5% or more female vines in a block showing the systemic symptoms of green shoot wilt and cane dieback. To determine Psa effects on productivity historical data from 2599 'Hayward' orchards were analysed. No reduction in productivity was found until 1 year after initial detection of Psa, after controlling for other orchard inputs that affect productivity. A cross-sectional survey was sent to all Psa confirmed 'Hayward' orchards and 430 growers provided information about one of their 'Hayward' orchard blocks. The survey found 84% of orchard blocks were affected by disease and 57% had green shoot-wilt and/or cane dieback reported. Blocks typically had a low within block prevalence of systemic symptoms (Median = 5% of vines). In 194 orchards that were asymptomatic at the start of the study period the probability of disease developing in a block increased in association with use of Psa protectant sprays immediately post-pruning and using artificial pollination. A lower probability of disease developing was associated with undertaking summer girdling and with the presence of older male vines. The probability of developing severe disease was investigated in 331 orchard blocks that were symptomatic. The probability increased with time after Psa was first detected in a block and was highest when frost damage occurred, when poplar, cypress or pine shelter belts were present and when artificial pollination was used. The probability of severe bacterial canker was lower when spring girdling of female vines was undertaken. The results of this study can be used to prioritise future research. The thesis has also demonstrated the utility of observational studies for plant disease research.

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CHAPTER 1

1 Introduction

Kiwifruit is grown primarily for the export market and is the largest fresh fruit horticultural export from New Zealand (Aitken & Hewett 2012) and was worth $1.143 billion in 2016 (Zespri International Ltd 2016a). *Pseudomonas syringae* pv. *actinidiae* (Psa) biovar 3 the causal agent of bacterial canker of kiwifruit in New Zealand (Vanneste et al. 2013), was first detected in November 2010 and has caused significant damage to kiwifruit vines in New Zealand (Everett et al. 2011). Psa biovar 3 has been described as causing a global outbreak (Scortichini et al. 2012) and was initially reported from Italy in 2008 (Balestra et al. 2008; Balestra et al. 2009b). In 2012 the export value of the kiwifruit industry to New Zealand was $1.0457 billion and in the same year the cost of Psa to the New Zealand kiwifruit industry was estimated to be approximately $126 million (Greer & Saunders 2012) with an estimated on-going cost of $740 to $885 million over the next 15 years. Bacterial canker has been referred to as the most destructive kiwifruit disease worldwide (Vanneste et al. 2013).

Prior to the detection of Psa in 2010 New Zealand kiwifruit production was based on two kiwifruit species and cultivars, namely *Actinidia chinensis* var. *deliciosa* 'Hayward' and *A. chinensis* var. *chinensis* 'Hort16A'. 'Hort16A' vines are highly susceptible to Psa (Ferrante & Scortichini 2009; Everett et al. 2011; Ferrante et al. 2012; Greer & Saunders 2012; Vanneste 2012; Vanneste et al. 2013) and have been replaced almost entirely in infected regions with less susceptible cultivars (Tanner 2015). The result has been that 'Hayward' is now the main cultivar grown in New Zealand (Tanner 2015; Prencipe et al. 2016).

A key assumption in Greer & Saunders's (2012) report is that productivity in 'Hayward' will remain unchanged in Psa infected regions. However, in 2011 there was mounting evidence that challenged that assumption as leaf spotting, which is considered a 'mild' symptom of exposure to Psa inoculum, had become common in 'Hayward' orchards within Psa infected regions and reports of more severe kiwifruit bacterial canker symptoms such as green shoot wilt, cankers and cane dieback were being received by industry in September 2011 (spring). It was not known what, if any, impact Psa was having on 'Hayward' productivity in New Zealand and no other researchers had investigated this relationship. Following the rapid collapse of 'Hort16A' within 12 months of the first detection of Psa, it was essential that productivity of 'Hayward' kiwifruit was maintained to keep the industry afloat.

During the emergence of a new disease epidemic, like Psa, industry representatives make decisions and recommendations based on intuition and expert opinion when scientific evidence is not available. As Tanner (2015) stated, in learning from the New Zealand Psa response:

"A crisis also means that there needs to be information available quickly, readily and when it is not, somebody needs to give clear direction, even in the absence of information."

Observational studies can fill the gap in existing field trial methods to assess disease effects under natural inoculum without the ethical or economic risk of exposing orchards to crop losses. Observational studies can also provide rapid insight into disease prevalence and risk factors that should be prioritised for research to enable decision making in outbreak situations. Observational studies are underutilised in botanical epidemiology to study plant disease (Thebaud et al. 2006). However observational studies are extensively used in medical and veterinary epidemiology, and emerged around the 1950's, when interest in infectious diseases had reduced due to use of antibiotics, vaccination and improved hygiene and with an increase in importance of chronic diseases such as cancer (Thrusfield 2007 Pg 8).

The benefits of observational studies is that they can be used where an experimental study may not be feasible for reasons including: *i*) The investigated factors are not easily manipulated in the field for practical, ethical or economic reasons, e.g. when a very large number of experimental replicates would be required to achieve statistical power; *ii*) The pest or disease cannot be practically manipulated, such as controlled pathogens or pests during a biosecurity incursion; *iii*) Interactions between multiple factors are of interest but are too complex to manipulate experimentally, including complex ecosystems; i*v*) Factors of interest cannot practically be manipulated experimentally, e.g., soil type, frost, size of orchard and elevation; and *v*) A plant or animal health outbreak of unknown cause or origin is to be investigated or the aim is hypothesis generation (Thebaud et al. 2006; Dohoo et al. 2009e; Froud et al. 2014). In addition to these situations an observational study can be used to understand the natural history of a pest or pathogen in the absence of experimental manipulation.

A key difference between observational and experimental studies is that extraneous factors, called confounders, are not able to be managed through randomisation and are typically controlled for at the analysis stage using multivariable statistical models (Dohoo et al. 2009e). If observational study designs can be combined with traditional approaches to botanical epidemiology and plant protection research an exciting new toolbox of approaches to the study of plant diseases or pests will open.

This thesis applied observational studies to investigate the impact of Psa in commercial orchards to 1) quantify a change in productivity associated with disease; 2) determine the

prevalence of disease in orchards; 3) identify factors that altered the initial development of disease and 4) identify factors that impact on presence of severe disease.

Chapter Two of this thesis is a literature review relating to the risk factors and impact of bacterial canker disease in New Zealand kiwifruit caused by Psa.

Chapter Three is a second literature review that investigates the historical separation of the field of epidemiology between plant health and human or animal health in more detail and then describes observational studies and the issues that arise when using population based studies.

Chapter Four determines the effect of Psa on 'Hayward' productivity after Psa had been present in New Zealand orchards for two growing seasons and uses retrospective data obtained from industry databases of productivity, agrichemical use and disease status.

Chapter Five describes the issues associated with the application of a mailed questionnaire to kiwifruit growers. It serves as a discussion around design principles and explores potential for response bias.

Chapter Six briefly describes the percentage of commercial orchard blocks that observed different symptoms commonly associated with Psa.

Chapter Seven provides a description of the population in terms of the frequency with which certain management practises are done and environmental features of the orchard.

Chapters Eight and Nine explore the association between the management and environmental factors and disease in different sub-sets of 'Hayward' orchard blocks. Chapter Eight examines the factors associated with the development of disease symptoms in a block that was symptom free in March 2012. Chapter Nine is limited to orchard blocks that had disease symptoms in February 2013 and considers factors associated with the presence of severe symptoms.

The thesis concludes with a general discussion of the research results in relation to the effect that Psa has had on the productivity of 'Hayward' kiwifruit, a description of the typical commercial orchard and the management practices that are used, the prevalence of disease in commercial orchards and discussion of factors that are associated with disease in 'Hayward' orchards. We also discuss the design, data collection and use of observational studies for investigating plant health, the issues faced in applying these studies and the potential for further use.

NOTE: The usage of recently changed nomenclature for 'Hayward' and 'Hort16A' kiwifruit was adopted during the writing of this thesis. It changed from *Actinidia deliciosa* and *Actinidia chinensis to Actinidia chinensis* var. *deliciosa* and *Actinidia chinensis* var. *chinensis*. In published chapters the correct nomenclature at the time of publication has been retained.

All references cited in the PhD are listed at the end of the relevant chapter.

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CHAPTER 2

2 Literature Review – Kiwifruit bacterial canker

2.1 Introduction

Kiwifruit have been grown in New Zealand since 1904 and is the country's largest fresh fruit horticulture export. In 2010, the New Zealand kiwifruit industry was hit by a devastating disease, kiwifruit bacterial canker, caused by a bacterium called *Pseudomonas syringae* pv. *actinidiae* biovar3 (Psa) (Everett et al. 2011; Vanneste et al. 2013). The cost of Psa to the New Zealand kiwifruit industry has been estimated to be approximately $126 million in 2012 with an on-going cost for the next 15 years of between $740 to $885 (Greer & Saunders 2012). Research to prevent Psa spread or better manage Psa in commercial orchards in New Zealand has predominantly applied descriptive field trials and experimental trials in small orchard plots. This review describes New Zealand's kiwifruit production and is followed by a description of Psa including factors associated with disease spread or severity.

2.2 Kiwifruit production

In New Zealand Kiwifruit is grown primarily for the export market with the industry worth $1.18 billion in 2015 (Aitken & Hewett 2015). New Zealand kiwifruit production prior to the detection of Psa in 2010 was based on two kiwifruit species and cultivars, namely *Actinidia chinensis* var. *deliciosa* 'Hayward' and *A. chinensis* var. *chinensis* 'Hort16A'. The green-fleshed 'Hayward' cultivar was developed in 1928 and is the most widely grown cultivar in New Zealand and worldwide (Campbell & Haggerty 2012). The gold-fleshed 'Hort16A' cultivar was released for commercial production in 2000 (Aitken et al. 2004) and attracted a premium return for growers. Unfortunately, 'Hort16A' vines are highly susceptible to Psa (Ferrante & Scortichini 2009; Everett et al. 2011; Ferrante et al. 2012; Greer & Saunders 2012; Vanneste 2012; Vanneste et al. 2013) and have been replaced almost entirely in infected regions with less susceptible cultivars, particularly *A. chinensis* var. *chinensis* 'Gold3' (Tanner 2015). The result is that 'Hayward' is the main cultivar now grown in New Zealand, and will be until 'Gold3' comes into full production (Tanner 2015; Prencipe et al. 2016).

2.3 Worldwide distribution of Psa

Pseudomonas syringae pv. *actinidiae* (Psa) biovar 3 is the causal agent of bacterial canker of kiwifruit in New Zealand (Vanneste et al. 2013). There are several strains of Psa found worldwide, with some causing moderate damage (biovars 1,2) and others causing important damage (biovar 3) (McCann et al. 2013). The first countries to report symptoms of bacterial canker in kiwifruit orchards were Japan in 1984 (Takikawa et al. 1989) caused by biovar 1 (Ferrante & Scortichini 2015) and then Korea in the late 1980s (Koh et al. 2010) caused by biovar 2 (Ferrante & Scortichini 2015), followed by Italy in 1992 (Scortichini 1994) (also biovar

1). The results from a 5-year study of Psa in China indicated that kiwifruit bacterial canker was a problem in China as early as 1996 (Li et al. 2001; Li et al. 2004), and bacterial canker became one of the most serious factors limiting kiwifruit cultivation in the Sichuan area of China (Liu et al. 2012) although the specific biovar was not known. Recent isolates from China fit within the biovar 3 population (Ferrante & Scortichini 2015).

The strain of Psa (biovar 3) found in New Zealand (McCann et al. 2013; Vanneste et al. 2013) has caused a global outbreak (Scortichini et al. 2012). Psa (biovar 3) was initially reported from Italy in 2008 (Balestra et al. 2008; 2009b), from Turkey in 2009 (Bastas & Karakaya 2012), followed by New Zealand in 2010 (Everett et al. 2011). In addition, records of Psa biovar3 have come from France in 2010 (Vanneste et al. 2011d), Portugal in 2010 (Balestra et al. 2010), Spain in 2011 (Abelleira et al. 2011), Chile in 2011 (ProMed 2011), Slovenia in 2013 (Dreo et al. 2014), Greece in 2014 (Holeva et al. 2015) and Georgia in 2013 (Meparishvili et al. 2016).

2.4 Distribution of Psa in the host

Psa has been detected on leaves, canes, trunks, leaders, buds, flowers, internal parts of fruit, pollen, bleeding sap and roots (Takikawa et al. 1989; Serizawa & Ichikawa 1993d, c; Balestra et al. 2009b; Everett et al. 2011; Vanneste et al. 2011c; Biondi et al. 2013; Abelleira et al. 2014). Psa has also been isolated from tissue of apparently healthy flowers, buds, leaves and the woody material of vines (Gallelli et al. 2011; Tyson et al. 2014a; 2014c; Taylor et al. 2015). Microscopic examination of Psa in two *A. chinensis* var. *chinensis* cultivars showed that bacterial cells were present in all regions of the cane down to the cambium layer (Hallett 2012). In unpublished studies Psa was also found in leaf hydathodes and in the xylem of canes with and without visible symptoms (P.W. Sutherland, Plant and Food Research, personal communication) which suggests that there are multiple entry pathways into the plant. Psa can colonise outer plant tissues as well as the xylem and pith, and migration within host tissue occurs in the xylem (Spinelli et al. 2011). Psa is also found on flower anthers, stigmas and the calyx, and small bacterial colonies have been found on flower debris in pollen samples (Spinelli et al. 2011). Spinelli et al. (2011) showed that Psa could penetrate kiwifruit vines via the stomata, the leaf abscission scar and through damaged trichomes (leaf hairs). Nardozza et al. (2015) have recently shown that Psa growth in xylem sap is associated with the seasonal phenology of vines with higher growth rates from budburst to the popcorn stage of flowering which corresponds to higher levels of hexoses (glucose and fructose) in the xylem which Psa was able to utilise.

Under high humidity conditions in New Zealand orchards Psa exudate has been observed oozing from leaf spots on the undersides of leaves (Tyson et al. 2012b) which are present from early spring to late autumn. Inoculum has also been recorded from bleeding sap (Biondi et al. 2013), and as a red exudate or a milky-white exudate from bleeding cankers in early winter (Tyson et al. 2012b). In Spain the white exudate has been observed from both cankers and wounds on trunks and branches (Abelleira et al. 2011).

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

Bacterial exudate from canes was observed to cease in summer at the same time that rapid callus formation occurred, although 20% of diseased canes resumed oozing bacterial exudate the following spring (Serizawa & Ichikawa 1993b). Serizawa et al. (1994) observed that growth of wound-healing tissue (callus) was related to temperature with tissue growth increasing rapidly when the temperature was above 22°C, declining when temperatures were below 20°C until it ceased entirely when temperatures were below 15°C. The relationship between healing and temperature is important as Serizawa et al. (1994) went on to observe that the bacteria were inhibited by the growth of wound-healing tissue and the bacterial population declined rapidly when this callus tissue was formed.

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

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

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

2.5 Kiwifruit bacterial canker symptoms

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

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

Bud-rot caused by Psa has been widely reported in New Zealand (Everett et al. 2011; Tyson et al. 2014a; Taylor et al. 2015). Buds are discoloured, with brown staining over part or all of the developing bud (Tyson et al. 2014a). Buds on infected canes may also fail to develop or, if they do develop, they may wilt and drop off (Vanneste et al. 2011b).

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

Kiwifruit bacterial canker symptoms are strongly linked to environmental factors such as temperature and rainfall, which have both been shown to be associated with Psa bacterial population growth (Serizawa & Ichikawa 1993c; Serizawa et al. 1994; Tyson et al. 2012b), along with differing host plant phenology and susceptibility (Serizawa & Ichikawa 1993d; Serizawa et al. 1994).

Traditionally we understood that the earlier Psa biovar 1 from Japan was a cyclic disease with canker symptoms expressed in winter to early spring on infected branches and trunks followed by lesions on green tissues in spring and early summer (Serizawa et al. 1994). Serizawa et al. (1994) concluded that branch infection observed in winter and early spring was via wounds that were exposed to bacteria in autumn and early winter and these bacteria came from leaf lesions that were formed in the preceding spring.

While Psa biovar3 follows a similar cyclic lifecycle to biovar1, the mechanisms for the development of severe symptoms associated with the Psa biovar3 type of kiwifruit bacterial canker are not yet fully understood. Leaf wilting and shoot and cane dieback is often observed when the bacterium is systemic within the vine and is thought to be caused by blocking of the vascular tissue (Vanneste et al. 2011b). More recently it has been postulated that shoot and cane dieback and leaf wilt symptoms are caused by a proliferation of bacteria specifically in the xylem vessels which blocks the conductance of water (Nardozza et al. 2015).

2.6 Dispersal of the pathogen

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

Tyson et al. (2014c) showed in New Zealand that trap plants placed in Psa (biovar 3) infected orchards were able to be infected year-round, particularly in spring, and that infection events were strongly associated with rainfall. They concluded that rain-splash and wind-blown rain were the main mechanisms of localised natural spread between and within vines in New Zealand.

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

movement of Psa in wind-blown aerosols immediately following rain is likely to be important in the natural spread of the disease.

2.6.1 Human-mediated spread

Another mechanism that has allowed Psa to move between regions post incursion is through human-mediated spread on infected plant material (grafting material, nursery material and pollen). Alternatively it may be spread by vectors including pruning tools, vehicles and machinery, animals and insects, soil and people (Everett et al. 2012b). Human-mediated spread can result in both localised and long-distance movement.

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

In the 1992 detection of Psa in three orchards in Italy, Scortichini (1994) suspected that the pathogen had entered the orchards with the 2-year-old vines as propagation material before spreading to the older vines as the 2-year-old ‘Hayward’ vines were affected by disease, whereas older vines in the same orchards had only minor symptoms.

Italian research into the 2008 Psa biovar 3 outbreak suggests that it began from a unique initial focus in the province of Latina (Vanneste et al. 2011b) and then spread between countries (Italy, France and Portugal) through movement of infected plant material. Psa was detected in Spain in 2011 and is suspected to have arrived on infected *A. chinensis* var. *chinensis* nursery stock in 2010 (Abelleira et al. 2011; 2014).

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

In addition there is evidence that pollen samples collected in infected regions from asymptomatic vines have Psa present (Gallelli et al. 2011; Heuer & Taylor 2015; Taylor et al. 2015). Another factor in the risk of Psa contaminated pollen is that, while the tests used to detect Psa have been optimised, they are still imperfect. Specifically, the tests have a poor sensitivity, that is, the ability to detect Psa when present, which can result in false negatives (Heuer & Taylor 2015; Taylor et al. 2015). The result of using a test with poor sensitivity is that the prevalence of Psa in pollen may be underestimated.

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

A recent study by Italian researchers investigated the transmission of bacterial canker by naturally contaminated kiwifruit pollen to kiwifruit vines planted 100 km from any known infected orchards (Tontou et al. 2014). They observed leaf spots the following spring and found that application of pollen resulted in transmission of Psa to kiwifruit vines in low numbers. Although infection rates were low, there was sufficient evidence that pollen has the potential to transmit Psa and to establish new disease foci (Tontou et al. 2014). The authors concluded that, as they had not detected systemic infection or cankers in the first year, the transmission was probably the result of epiphytic Psa overwintering in buds and that transmission via pollen

may not present as an outbreak for up to 2 years. There have also been several studies in New Zealand that aim to reduce the risk of inadvertently transmitting Psa with pollen by investigating methods to reduce the amount of viable bacteria present on pollen while still maintaining pollen viability (Everett et al. 2012d).

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

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

2.6.2 Invertebrate associated spread

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

Given that Psa is present in kiwifruit flowers and on pollen, and that beehives are used in most New Zealand kiwifruit orchards during flowering to assist pollination, bees have been of

particular interest as potential means of Psa transmission. In New Zealand, Pattemore et al. (2014) found that inoculated bees within a containment facility could bring Psa-contaminated pollen back to the hive. Furthermore, Psa contamination was found on the outer frame of the hive for up to 2 days, although none was found in the centre of the hive. Psa was detected on bees for up to nine days, and the cfu/bee reduced rapidly to be undetectable by day nine. Because of the artificiality of this experiment, where the bees were not able to forage in the outside environment, the experiment was repeated using a streptomycin-resistant strain of *P. syringae* pv. *syringae* with free-foraging beehives and Pattemore et al. (2014) found very similar results to those obtained with the contained bees. The authors concluded that bees could become contaminated with Psa and potentially contaminate other members of the hive over a short period, and they therefore recommended that hives be rested between orchards for more than 9 days. They also pointed out that contamination does not necessarily prove the ability of bees to transmit disease but it is possible (Pattemore et al. 2014) and this has been shown for fire blight (*Erwinia amylovora*) in apples (de Wael & de Greef 1990; Johnson et al. 1993; Pattemore et al. 2014).

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

In summary, it appears that localised spread of Psa (<10 km) is likely to be predominantly due to rain and wind with some human mediated spread occurring. In contrast the majority of long distance spread (>10-20 km) is most likely due to human mediated spread via infected plant material. The role of contaminated tools, vehicles and animals in long distance spread is uncertain.

2.7 Host susceptibility

2.7.1 Leaf tissue age

Kiwifruit vines are deciduous and lose their leaves in late autumn and early winter (May to July in New Zealand) and begin to produce new leaves in spring (September). Studies on

developing leaves in Japan found that the susceptibility to Psa was highest when the leaf blade reached 2 cm in length, which is approximately 1 week old, and decreased as the leaves matured (Serizawa & Ichikawa 1993b). However, season played a large part in leaf susceptibility, as new leaves in spring had much higher disease severity scores than new leaves developing in summer on established vines (Serizawa & Ichikawa 1993b).

In New Zealand, Tyson et al. (2015) found that both detached leaves and leaves on potted plants that were 1 to 3 weeks old when inoculated with Psa (the period of rapid expansion) had a higher percentage of leaves with leaf spots than leaves that were 4 or more weeks old. The difference between results for the summer leaves in Japan and New Zealand is probably due to a higher field temperature and a lack of inoculum for natural infection in the Japanese trial in summer. The two studies show that the period of rapid expansion of leaves is also the period of highest risk of infection. This also coincides with the period of least efficacy of protective sprays (Gaskin 2012). Gaskin (2012) found that spray coverage was reduced because of rapid leaf expansion which, depending on the mode of action of the protective spray, could reduce the efficacy on newly developing susceptible leaves.

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

2.7.2 Vine age

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

2.7.3 Cultivars

There is considerable variation in the susceptibility of different commercial kiwifruit cultivars to bacterial canker. 'Hort16A' and other *A*. *chinensis* var. *chinensis* cultivars consistently show higher disease incidence and severity than *A. chinensis* var. *deliciosa* cultivars (Balestra et al. 2009b, a). Froud et al. (2014) quantified the effect of Psa on the productivity of 'Hort16A' over time compared with 'Hayward' and found that there was a much greater and more rapid impact on 'Hort16A'. A study in New Zealand looking at grower-reported symptoms found that male kiwifruit vines (various *A. chinensis* var. *deliciosa* cultivars) had a higher prevalence (46%) of shoot wilting and cane dieback than the female cultivar 'Hayward' (31%) (Froud et al. 2015,

Chapter 6). To date there has been no published information on the differences in susceptibility of new kiwifruit cultivars or other kiwifruit species, although *A. arguta* seems to be less affected by bacterial canker in New Zealand (Vanneste et al. 2014). Recent research in Italy has shown that Psa may affect fruit quality in diseased 'Hayward' orchards (Prencipe et al. 2016).

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

Host phenology is also very different between cultivars in New Zealand, with the *A. chinensis* var. *chinensis* cultivars coming into both budburst and flowering 4 to 6 weeks earlier than 'Hayward'. Consequently the *A. chinensis* var. *chinensis* is exposed to two other risk factors for Psa. First budburst and flowering occurs at a time when the risk of frost is greater and this is a factor that has been strongly associated with Psa infection (Ferrante & Scortichini 2014). Secondly, the species has susceptible leaves (1–2 weeks old) present in vine canopies during early spring which in New Zealand is typically cool and wet.

2.8 Environmental risk factors

2.8.1 Climatic factors

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

Field studies on Psa in Japan on 'Hayward' vines indicated that the range of temperature for growth of Psa was 10°C to 20°C, with an optimum temperature of 15°C (± 3°C) (Serizawa & Ichikawa 1993b). They also noted that formation of wound healing tissue was highest in mid-summer, when the mean temperature was 25°C, and this coincided with the cessation of

bacterial exudate oozing from parts of affected vines (Serizawa & Ichikawa 1993b). Further studies on inoculated vines in growth chambers at a range of variable and constant day:night temperatures (Serizawa & Ichikawa 1993a) suggested an optimal temperature range for Psa growth of 10–18°C, which was consistent with their field-based observations.

In 2011 an epidemiological disease risk model was developed to predict Psa infection events in New Zealand (Beresford & Tyson 2014) based on rain and temperature exposures as described by McKay et al. (2012). The model used daily rainfall and temperature to simulate the bacterial multiplication rate to predict the relative risk of infection each day. The model was shown to be highly accurate in predicting days when infection occurred in susceptible trap plants (potted 'Hort16A') during spring, but produced a proportion of false positive predictions during autumn and winter (Beresford & Tyson 2014). It was concluded that some of the false positives arose because, although weather conditions were suitable for infection in autumn and winter, inoculum was less available than in spring. The rainfall component of the risk model is supported by Casonato & Bent (2014) who observed that symptoms of disease caused by Psa increased with greater exposure to rainfall compared with kiwifruit vines that are protected from rain by breathable plastic covers.

Studies in Italy observed that frost events during winter were associated with outbreaks of disease the following spring and autumn on *A. chinensis* var. *chinensis* (Ferrante et al. 2012) and *A. chinensis* var. *deliciosa* kiwifruit (Ferrante & Scortichini 2014). Ferrante and Scortichini (2014) found that *A. chinensis* var. *deliciosa* was more frost tolerant than *A. chinensis.* Frost damage allows direct entry of the pathogen into the vine through the damaged tissue (Ferrante & Scortichini 2014) although the exact mechanism of why frost promotes bacterial canker has not yet been determined. It is important to note that Psa is not an ice nucleation bacterium like *Pseudomonas syringae* (Rees-George et al. 2010). More severe symptoms of Psa bacterial canker in areas where strong winds occurred were observed during a weather risk study (Serizawa & Ichikawa 1993b). They postulated that this could be an important risk factor for infection.

2.8.2 Geographical factors

Regional differences in the prevalence and severity of bacterial canker in New Zealand can in part be explained by the period of time the pathogen has been present in a region and differences in climatic conditions between regions. Cogger & Froud (2015) found differences between regions in time to an orchard became infected once Psa was first identified in the region. While the Te Puke region was severely affected with 10% of orchards infected after 6

months, orchards in the Whakatane region had a much faster rate of symptom appearance following the first detection in the region, with 41% of orchards infected in the first 6 months. This was noteworthy as the density of orchards was lower, the distances between orchards was greater, and there was less planted area of the susceptible *A. chinensis* var. *chinensis* in Whakatane than in Te Puke. The most obvious difference between the two regions was a higher risk of frost in Whakatane. Li et al. (2001) also found that the prevalence of kiwifruit bacterial canker disease was greater above 750 m elevation in China, and suggested that colder temperatures at the higher elevations may favour the disease.

2.8.3 Shelter

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

2.9 Orchard management risk factors

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

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

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

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

Tyson et al. (2012b) showed that leaves from natural leaf fall and pruning waste left on the orchard floor yielded viable Psa throughout the winter period and well into the bud-break period the following spring. They postulated that these could be an important source of inoculum during the spring infection period, in addition to cankers on living vines, however more recent research has shown that there is minimal splash of Psa from this material (Tyson et al. 2016).

Callus formation was observed in monitored orchards in New Zealand on pruning cuts made to remove Psa-infected vine material. More rapid and complete healing occurred on pruning cuts made in late spring and summer than on early spring cuts (Horner et al. 2013). This study also

showed that Psa lesions were halted where full callus formation was able to occur, but it was unclear whether failure to form callus was related to the presence of Psa or to other factors (Horner et al. 2013). It is possible that this is due to low temperatures in early spring inhibiting callus formation.

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

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

2.10 Conclusion

This review summarises the production structure and value of kiwifruit to New Zealand's primary industry export revenue and details the key epidemiological risk factors that have been investigated to date.

There is sufficient evidence that Psa is spread locally by the means of wind and rain and that long-distance spread via kiwifruit plant material is a risk. In New Zealand, researchers have shown that Psa inoculum is present year-round, with spring being a key infection period, and there is a strong relationship between the disease and climatic factors such as rainfall, temperature and frost. There remains uncertainty around how orchard layout and vine or disease management factors contribute to the development of bacterial canker and what the long-term impact of this disease will be on 'Hayward' kiwifruit vines in New Zealand.

However, there are still many gaps in the understanding of kiwifruit bacterial canker epidemiology, particularly a full understanding of the life cycle and infection process of the disease in New Zealand and the impact of orchard management on disease development. As the Psa outbreak continues in New Zealand it is important to rapidly identify the risk factors that have the greatest impacts on infection and severity of disease in commercial orchards so that management changes can be put in place to reduce the impact while more traditional experimental research is undertaken to find better ways to manage these risks.

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CHAPTER 3

3 Literature Review – Observational Studies

3.1 Introduction

Research to prevent the spread of Psa and better manage the effects of Psa in commercial orchards in New Zealand has predominantly used laboratory or experimental trials in small orchard plots. In contrast, this thesis uses descriptive and analytical observational studies in commercial orchards with the aim of prevention and management of kiwifruit bacterial canker. Observational studies are commonly used in animal and human health to investigate disease at a population level for the purposes of managing disease. They have been applied to the study of a wide range of human and animal disease and non-disease health or production outcomes. For simplicity 'disease' is used throughout this literature review to refer to outcomes that are studied using observational methods and includes infectious and non-infectious disease and non-disease health or production outcomes, such as pregnancy or productivity. Observational studies have rarely been used in plant pathology, by botanical epidemiologists, to investigate plant disease (Sanogo & Yang 2004; Thebaud et al. 2006). This review describes the historical separation of the field of epidemiology between plant health and human or animal health, specifically around the use of observational studies to investigate multiple factors affecting disease in populations. Further, the important features of observational study designs for investigating Psa in 'Hayward' kiwifruit are detailed.

3.2 Brief history of observational studies – in search of a common origin

To understand why observational studies are largely absent from botanical epidemiology, the origin of epidemiology within human, animal and plant pathology disciplines is now discussed. The most frequently referenced example of an early medical epidemiological approach was John Snow's work during the 1850's Cholera epidemics in London when he mapped cases and showed that they related to a single contaminated water source (Vandenbroucke 1988).

During the same period the field of phytopathology (the study of plant diseases) developed as a separate scientific discipline following the European potato famine during the 1850's (Zadoks & Koster 1976). Interestingly, the discipline of phytopathology was being practised at that time by medical doctors. The first phytopathology textbook was published by Julius Kühn in 1858 where he noted that epidemics in crops were similar to epidemics in humans and animals (Zadoks & Koster 1976; Wilhelm & Tietz 1978). Phytopathology started to be taught in universities and agricultural colleges in the late 1800's and was increasingly practised by botanists, agronomists and mycologists, rather than medical practitioners (Zadoks & Koster 1976).

Epidemiology for human, plant and animal disease was effectively ignored from the late 1800's to the mid 1900's during the period when specific microorganisms were identified as the causal agents of diseases (Zadoks & Koster 1976; Wilhelm & Tietz 1978; Dohoo et al. 2009 Pg. 3.). A small number of phytopathologists researched disease forecasting and developed disease prediction techniques during the early 20th century, particularly the influence meteorology had on disease epidemics (Zadoks & Koster 1976; Madden et al. 2007 Pg 4-5.).

Medical and veterinary epidemiology was revived around the 1950's, when the strong interest in infectious diseases had reduced due to improved hygiene, nutrition, vaccination and antibiotics, and when chronic diseases non-infectious diseases were increasing in importance (Thrusfield 2007 Pg. 8). There was a corresponding increase in the collection of human and animal health data and the analysis of a wide range of factors that could contribute to disease in both the human and veterinary health sectors (Dohoo et al. 2009 Pg. 3).

Quantitative botanical epidemiology, also emerged in the 1950's with the work of E.C. Large on potato blight (Gregory 1982). Large compared the sigmoid progress curve of potato blight with that of the human population growth curve and then provided methodologies for developing disease progress curves for calibration to crop yield loss (Large 1966). Botanical epidemiology developed as a separate plant pathology discipline in the 1960's (Zadoks & Koster 1976; Madden et al. 2007). There was a botanical epidemiology workshop in 1963, where there was consensus that all of the different topics of epidemiology should be brought together into a common guideline (Zadoks & Koster 1976). The workshop coincided with the development and subsequent publication of van der Plank's book "Plant diseases: epidemics and control" (1963) which was almost entirely focused on predictive modelling of plant disease epidemics and this book became the guiding principle for the discipline. Botanical epidemiology and the methods that are used came directly from within phytopathology with links to meteorology and disease resistance breeding (Zadoks & Koster 1976). The botanical epidemiology methods were extended by Van der Plank (1963) and Zadoks and Schein (1979) in parallel with growing international interest in ecology and environmentalism (Zadoks & Koster 1976). The impact of ecological and environmental awareness was that plant protection scientists needed to research ways to reduce the number of applications of plant protection agrichemicals based on predictive disease models. Research focused on the development of targeted agrichemical chemistry and developing monitoring systems for applying agrichemicals based on a demonstrable need such as meeting an economic threshold for pests or diseases (West et al. 2003). In conjunction with this was the need to conserve agrichemicals that organisms were developing resistance to, by carefully monitoring their use and only applying them when

necessary (Gilligan 2008). Key drivers for the development of botanical epidemiology within plant pathology were related to the high reliance on protectant fungicides which have the best efficacy when applied prior to disease infection periods (Madden 2006). This required plant pathologists to predict infection periods across populations and under certain climatic conditions using predictive modelling (Madden 2006). Predictive modelling in botanical epidemiology is based on combining experimental data about disease development and infection processes and climatic data to model infection risk and potential crop loss (Large 1966; Zadoks 1985; Madden 2006). In comparison medical and veterinary epidemiology tended towards development of new study designs to collect observational data and development of analytical techniques to control for multiple factors (Dohoo et al. 1996). While Madden et al. (2007) refer to multiple factors for plant disease, they only provide tools for exploring these factors experimentally as opposed to using study designs for observational data as used for human or animal health applications. In the review of the history of botanical epidemiology, the use of experimentation to predict disease is seen as a large step forward in the 1950's that medical researchers were not able to benefit from due to ethical reasons (Zadoks & Koster 1976). This provides an insight as to why observational studies were not pursued in botanical epidemiology, that is, the focus was on disease prediction and the use of experimental approaches. The result of this has been that the extensive development of observational study design, methodology and analysis to understanding the causes of disease used in medical and veterinary epidemiology has been underutilised in plant health (Dallot et al. 2004; Thebaud et al. 2006; Vicent et al. 2012; Cogger & Froud 2015).

The definition of epidemiology is slightly different between medical or veterinary epidemiology and botanical epidemiology. Both medical and veterinary epidemiology were defined by Morabia (2004) as:

> *"…the investigation of causes of health-related events in populations."*

This definition shows a wider scientific discipline for medical and veterinary epidemiology than what is encompassed within the current definition of botanical epidemiology which is given by Madden et al. (2007 Pg. 1) as:

> *"The study of plant disease epidemics*"

Madden et al. (2007 Pg. 1) further define an epidemic as:

> *"Change in disease intensity in a host population over time and space."*

Where disease intensity refers to the amount of disease present on plants within a population, and is often measured as the proportion of foliage with lesions. Scortichini (2010) gave a similar but expanded definition for plant bacterial pathogens:

> *"In plant pathology, epidemiology concerns the incidence of the disease in time, its spread in space and survival of the pathogen in the environment" (Scortichini 2010).*

Scortichini et al. (2010) included the life cycle of the pathogen in the description of epidemiology. However, while this is an important feature of understanding the wider system, it does not appear to be part of the core research aim of epidemiologic studies in the human or animal systems.

The origin of modern botanical epidemiology, as discussed by Van der Plank (1963), was largely based on the description of disease progress in time using the logistic transformation of disease severity, which was used as a population growth model to derive a descriptor for the rate of disease increase, called the "apparent infection rate". The effects of reducing initial inoculum, plant resistance and fungicides on the apparent infection rate were considered key in his discussion. Van der Plank (1963 Pg. 118) states:

> *"At any time during the course of an epidemic the amount of disease is determined by how much inoculum there was at the start and how fast disease has developed since"*

There is no reference to the use of observational studies to investigate factors contributing to disease in Van der Plank's book (1963). Zadoks and Schein (1979) reference multiple factors affecting disease, however, they investigate them using experiments and epidemic simulation modelling. Madden et al. (2007) also refer to multiple factors contributing to disease throughout their textbook and provide excellent tools for exploring these factors when using experimental data. Neither reference book provides research methods to investigate risk factors for disease using observational data, although they do reference another agronomy and soil science text (Schabenberger & Pierce 2001) which provides statistical methods for dealing with observational data.

The discipline of botanical epidemiology is confined to plant pathology and is strongly focussed on infectious diseases of plants and on predictive modelling of epidemics for decision support, resistance management and yield loss estimation (Madden et al. 2007). Pests and weeds that influence plant health are not included in the discipline other than as vectors of diseases. While medical and veterinary epidemiology does include predictive modelling of infectious diseases it is one topic within a much broader scope.

Epidemiology in human and animal health involves measuring the frequency of disease and identifying factors that may be causes of disease. There is agreement between botanical and human or animal epidemiology that exposure to an infectious agent does not equal disease. However, the concept of a causal factor within plant pathology refers mostly to the identification of a single causal agent of disease (McIntyre & Sands 1977) such as Psa causing bacterial canker in kiwifruit and is proven using diagnostic techniques and fulfilling Koch's postulates. There are some diseases of complex aetiology, where it is recognised that several organisms are involved in disease, for example grape trunk diseases (Bertsch et al. 2013). Wallace (1978) provides a more generic view of complex aetiology in plant diseases with reference to multiple determinants of disease, for example several pathogens, soil salinity and nutrient deficiency. However, factors such as management are considered by plant pathologists to be incorporated into influencing either the amount of inoculum available (pathogen), the susceptibility of the host or contributing to favourable environmental conditions for disease development. In human or animal epidemiology, causes of disease refer to any factors that can contribute to the disease event occurring and these are referred to as separate or component causes of disease (Rothman 2012 Pg. 24). The measurement and investigation of causes of disease in human or animal populations requires the collection of observational data and both human and veterinary epidemiologists use four core observational study types, the randomised control trial (RCT), cohort studies, case-control studies and cross-sectional studies. These four study types are discussed in Section 3.5. Medical and veterinary epidemiologists have a shared language around the concepts of multicausality, study designs and the collection and analysis of observational data. The study of groups of animals in herds provides a key point of difference between medical and veterinary epidemiology, and the development of approaches for dealing with herds are likely to be applicable to orchards and fields in botanical epidemiology.

In conclusion, the development of modern botanical epidemiology appears to have occurred independently of medical or veterinary epidemiology and the use of observational study designs to investigate multiple factors of disease are largely absent from botanical epidemiology. This is possibly due, in part, to the lack of a key driver that occurs in human and medical epidemiology, that of the ethical issue of using experiments to apply risk factors or interventions that may cause harm to subjects, or conversely withholding treatment of diseased individuals. This is not a problem in plants. The other drivers and advantages in using observational studies in the study of disease are discussed in Section 3.5. The advanced methodology developed in botanical epidemiology for decision support and disease

management (Madden 2006; Madden et al. 2007) could provide valuable additional tools for use in veterinary and medical epidemiology. Likewise, the advanced research methods exploring observational data to measure disease and investigate multiple risk factors could be applied more within botanical epidemiology.

3.3 Cross-over of epidemiology and statistical techniques

In recent times, there have been several significant organised efforts to increase across discipline use of epidemiology techniques. In 1997 a symposium titled "Epidemiological concepts in human, veterinary and botanical ecosystems" was held in association with the annual conference of the American Phytopathological society (Rapport 1999). The intent of the symposium was to exchange epidemiology concepts and models that would bring the branches together (Nutter 1999b). It is interesting to note that Nutter, a botanical epidemiologist, proposed in his paper at the symposium that a broad definition for all epidemiology disciplines should be adopted and proposed the following:

> "*The study of the dynamic interaction of host and pathogen populations over time and space as affected by the environment*" (Nutter 1999a)

This is a narrower view of epidemiology than that defined within the medical or veterinary disciplines, and has a focus on pathogens rather than on causes of health-related events. Human and animal epidemiology includes infectious and non-infectious disease, e.g., cancer or hip dysplasia, or other health or production outcomes like pregnancy or milk production. In Rapport (1999)'s editorial, he voiced concern about what phytopathologists, medical and veterinary epidemiologists and ecologists had in common, and that a search for common ground proved difficult due to specialised languages within the communities which were barriers to communication. A second attempt to cross veterinary and medical epidemiology techniques into plant health was a symposium called "new applications of statistical tools in plant pathology" (Garrett et al. 2004). These tools included multivariable models (e.g. survival analysis) and meta-analysis. Garrett's keynote address (Garrett et al. 2004) noted that many of the statistical methods discussed were relatively new and little used in the field of plant pathology. The focus of Garrett and others at the symposium was on the application of these techniques to experimental study designs despite Sanogo and Yang suggesting observational studies could be beneficial to plant health (Sanogo & Yang 2004; Scherm & Ojiambo 2004). Garrett et al. (2004) and more recently Madden and Paul (2011), Savary et al. (2011) and Scherm et al. (2014) discuss using similar statistical methods to those that are applied to observational studies in humans and animals. Since this time the multivariable techniques

described by these authors have been used in a small number of observational studies of plant health (Dallot et al. 2004; Mila et al. 2004; Thebaud et al. 2006; Vicent et al. 2012) which were focused on risk factors contributing to disease and are detailed in Section 3.5. Several of these studies used multivariable logistic regression to model the binary outcome of diseased and not diseased (e.g. Mila et al. (2004); (Thebaud et al. 2006; Vicent et al. 2012) which is common in human and animal epidemiology studies. Multiple linear regression methods have been used by botanical epidemiologist since the 1970's for developing equations for predictive disease (Burleigh et al. 1972). In addition botanical epidemiologists have also adopted meta-analysis approaches to evidence synthesis which is consistent with human and animal epidemiology (Madden & Paul 2011; Ngugi et al. 2011).

These examples provide evidence of a desire to cross the disciplinary divide, but to date have failed to bring about wide adoption. It would appear that this failure is related to a difference in the definition of epidemiology and in the study approaches used, particularly observational studies. The adoption of analysis techniques used in veterinary and medical epidemiology for use in botanical epidemiology would be benefited by a greater understanding of the design of observational studies.

3.4 Measuring disease in a population

Across all epidemiology disciplines there are standardised ways of calculating and reporting disease frequency. While the same terms are used across the medical, veterinary and plant health disciplines they occasionally have different meanings. Studying disease in the population for any epidemiological discipline requires a definition of the outcome of interest. With plants the outcome is generally a measure of disease intensity (Madden 2006) which is a generic term for the amount of disease in a plant population and can be measured using incidence, prevalence or severity (Nutter 1999a). In humans and animals the outcome measure is typically incidence or prevalence. The use of disease intensity, particularly the severity measure, is a key difference in how plant epidemiologists refer to disease compared to medical and veterinary epidemiologists. In addition key disease measurement terms differ between medical, veterinary and plant pathology disciplines, in particular the use of signs and symptoms for describing disease, and incidence and prevalence as measures of the frequency of disease (Nutter 1999a) and these are discussed further (sections 3.4.1; 3.4.3; 3.4.4).

3.4.1 Signs and symptoms of disease

The terms signs and symptoms are used differently to describe disease. In humans symptoms and signs are defined in the Merriam-Webster online medical dictionary *(Merriam-Webster Inc. 2016b, a)* as:

> *"Sign: an objective evidence of disease especially as observed and interpreted by the physician rather than by the patient or lay observer."*
>
> *"Symptom: subjective evidence of disease or physical disturbance observed by the patient."*

In veterinary epidemiology the term 'clinical signs' is used to describe disease by Studdert et al. (2011) as:

> *"Any objective evidence of disease or dysfunction recognizable by the veterinarian."*

The term 'symptom' is not used in veterinary medicine because it is a subjective sensation perceived by a human patient only. The word symptomatic, however, is used in veterinary medicine to describe clinical signs as below (Studdert et al. 2011):

> *"Symptomatic: pertaining to or of the nature of a symptom. The word symptom is not used in veterinary medicine… because there is no comparable word relating to clinical sign, …, it is customary to use the word symptomatic …, that is, pertaining to or in the nature of a clinical sign."*

In contrast the definitions of sign and symptom in plant pathology are different and are consistent with their definition of causation where the evidence of disease is related to detection of the causal infectious agent (D'Arcy et al. 2001):

> *"Sign: an indication of disease from direct observation of a pathogen or its parts."*
>
> *"Symptom: an indication of disease by reaction of the host, e.g., canker, leaf spot, wilt."*

As this thesis studies a plant pathogen, the plant pathology definition of signs and symptoms are used.

3.4.2 Plant disease severity

Botanical epidemiologists use disease severity as a measure for disease intensity, in addition to incidence and prevalence in plant populations (discussed in Sections 3.4.3 and 3.4.4). Disease

severity is used to measure the amount of disease on individual plants, or plant parts, and it is then averaged to derive the mean severity of a population. Disease severity is generally measured as: the percentage of the plant area that is affected by the disease. It is assessed as the actual surface area of the host plant covered by the disease symptoms (such as leaf lesions) or assigning a categorical level (e.g. mild/moderate/severe) to describe severity (McRoberts et al. 2003). Disease severity can also be expressed as disease counts which may also be referred to as disease density. Disease counts are generally of the form of the number of lesions per leaf or other plant structure (McRoberts et al. 2003). The methods used by botanical epidemiologists to measure disease intensity are fully described in Madden et al. (2007) and by McRoberts et al. (2003).

3.4.3 Incidence

The term incidence is used in both botanical and medical/veterinary epidemiology; however, it does not have a consistent meaning across the disciplines.

Botanical epidemiology incidence:

> The number of diseased individuals within a defined population at a point in time (Nutter, 1999b).

Medical/Veterinary epidemiology incidence:

> The number of new cases of disease in a defined population within a defined period of time (Dohoo et al., 2009b Pg 75; Rothman, 2012 Pg 38).

Incidence in botanical epidemiology gives the proportion of diseased individuals within a population at a single point in time and is usually expressed as a percentage. This is a completely different meaning to incidence in medical and veterinary epidemiology in that incidence in plants does not relate to new cases and is at a point in time. Botanical disease incidence is almost the same as medical and veterinary disease prevalence.

Incidence in veterinary and medical epidemiology is expressed as an incidence risk or an incidence rate (Dohoo et al. 2009 Pg 75). Each of these is calculated differently and used for a different purpose when describing the frequency of disease.

Incidence risk is calculated by dividing the incidence count by the population at risk at the beginning of the time period. Note that the population at risk is the population that has not yet experienced the disease at the start of the period of interest and therefore excludes all existing cases in the population. For example, within 848 disease-free Te Puke orchards as of

1st September 2011, 86 developed Psa during September 2011 (10% incidence). The incidence risk can be interpreted as the probability that an individual 'unit' in the population will develop the event of interest over the time period. Therefore, in our example, Te Puke orchards had a 10% likelihood (incidence risk) of experiencing the disease in September 2011. An issue with incidence risk is that it assumes the study population remains stable, that is, that none of the study participants are lost from the study. For example, in a long-term observational study on use of copper sprays in kiwifruit orchards, some orchard owners may stop collecting data, withdraw from the study, remove the vines or sell the orchard.

When there are substantial losses of participants (loss to follow-up) it is better to use incidence rate rather than incidence risk to describe disease. The incidence rate divides the number of new cases by the actual time at risk (Dohoo et al. 2009). Using the time at risk allows us to include members of the population that may have dropped out of a study within the period of interest. Time at risk is calculated as the sum of the times at risk for every individual, that is, the time from enrolment until onset of disease, loss to follow-up or the end of the study. It is valuable in studies where the risk of losing subjects to follow-up is high because the data that have been collected can still contribute to the study (Vandenbroucke & Pearce 2012). It also allows subjects to be recruited into a study over time and calculates the contribution of subjects that are followed for different periods of time.

3.4.4 Prevalence

The term prevalence is also used in both botanical and medical/veterinary epidemiology, but with different meanings. In botanical epidemiology prevalence is the count of geographical sampling units where disease is present (e.g. fields, plots, regions, countries) divided by the number assessed (Nutter, 1999b). It is a broad scale equivalent of plant disease incidence and it is used infrequently. In contrast, in medical or veterinary epidemiology, prevalence is the number of new and existing cases of disease in the population, divided by the size of the population, at a given point of time (Dohoo et al. 2009 Pg 80; Rothman 2012 Pg 53).

Prevalence in botanical epidemiology is expressed as a percentage and is similar to plant epidemiology incidence but at a higher population resolution, normally the number of fields with disease (Madden et al. 2007), and can be associated with geographical units (Nutter 1999a). For example in the work of Mila et al. (2004), which investigated whether they could predict regional prevalence of soybean sclerotinia stem rot, they defined prevalence as the percentage of fields in which the disease was found and incidence as the percentage of infected plants in a field. Nutter (1999a) notes that botanical prevalence data can be used to

prioritise research and gives an example of disease prevalence recorded each year for a range of corn diseases. It is an interesting example as the host is an annual crop so the population at risk each season is naïve, and therefore at risk at the start of the season. In this case, the annual plant prevalence calculation is effectively the same as an animal/human incidence risk per 12 months.

There are some issues with using prevalence measures in observational studies. For example, lower disease prevalence (in the animal/human sense) in a population may be due to increased death rather than reduced disease. For example, the prevalence of Psa in 'Hort16A' orchards is now lower than it was in 2012, not due to a reduction in disease but due to the fact that disease was so severe that 'Hort16A' orchards were removed or grafted to other kiwifruit cultivars. Similarly, the reverse situation may occur and an increase in disease prevalence may in fact be due to a new treatment that does not eliminate disease but improves the life expectancy for individuals with disease. This issue of prevalence measures should be considered when investigating factors that might influence an individual's removal from the 'prevalent' group by increasing recovery, survivorship or death.

This thesis applies human and veterinary epidemiologic study types to a plant disease and therefore measures of disease are described using the human and veterinary definition of incidence and prevalence.

3.5 Study types

In experimental studies, the subjects are randomly assigned to a treatment group and compared to a control group. Experimental trials of this nature are very common in plant protection research, both in the laboratory and in the field (Aust & Kranz 1988; Garrett et al. 2004). The experimental study approach has the advantage that factors that may influence the association between the exposure and the outcome, called confounders, are controlled through randomisation (Lavori & Kelsey 2002; Martin 2008). Consequently, the results of experimental studies provide a strong degree of evidence towards causation.

Experimental studies can be split into laboratory based trials and clinical or field based trials. The advantage of a laboratory study is that the high level of control means that the results will give good evidence for causation in the modelled system. However, the disadvantage of laboratory trials is that the highly-controlled environment may mean the results do not represent causation in the real world. That is, the internal validity of the study is good in that there is confidence that the observed differences between groups can be attributed to the

effect of the intervention (Elwood 2007 Pg 80). However, the external validity of the results may be poor in that the results cannot be extrapolated to the wider population (Elwood 2007 Pg 81), for example, commercial production systems. To illustrate, the external validity of laboratory results for biological control agents are frequently not able to be demonstrated in the field (e.g. Fravel 1999; Froud & Stevens 2004; Murphy & Kay 2004). The most similar animal/human epidemiologic study to a plant field trial is the randomised control trial. They are similar in that the subjects are randomly assigned to intervention and control groups to control for potential confounding (Grimes & Schulz 2002b).

However an experimental study may not be feasible because: i) The factors under investigation are not easily manipulated in the field for practical, ethical or economic reasons e.g., soil type, frost, size of orchard and elevation; ii) The disease cannot be practically manipulated, such as controlled pathogens during a biosecurity incursion; iii) Interactions between multiple factors are of interest but are too complex to manipulate experimentally; and iv) a plant or animal health outbreak of unknown cause or origin is to be investigated (Thebaud et al. 2006; Dohoo et al. 2009; Froud et al. 2014). In these situations, an observational study can be used in the absence of experimental manipulation. Observational studies and large scale Randomised Control Trials (RCT's) allow understanding of how recommended management practices perform when applied by farmers, rather than researchers, and can be used to identify practical reasons why interventions may not work in the manner predicted by highly controlled experimental studies.

Observational studies are an effective way to describe the frequency of disease and to investigate multiple factors that may cause disease. While the focus is often on disease it is possible to apply this approach to, for example, high or low productivity or presence/absence of a plant pest. Observational studies are extensively used in human and veterinary research but are rare in plant protection research although a few examples do exist (Dallot et al. 2004; Thebaud et al. 2006; Everett et al. 2007; Vicent et al. 2012; Froud et al. 2014). Observational studies can also take advantage of data that has been collected for another purpose. For example, disease survey information, outbreak data and application of sprays as part of an audit. In an observational study the confounding and variability, which in an experimental study would be controlled through experimental design and randomisation, needs to be controlled during analysis.

There are a range of observational study designs to explore factors that increase or decrease the risk of disease. They include randomised control trials (RCT's), cohort and case-control

studies which investigate incident cases (new cases developing within a given time) and cross-sectional studies which investigate prevalent cases (Hudson et al. 2005; Sargeant et al. 2014). The distinction between using a study based on incident or prevalent cases is important when considering potential causation as the temporal order of cause and effect is missing in studies looking at prevalent (existing) cases (Sargeant et al. 2014). Temporality is discussed further in Section 3.6.4.

The four study types provide differing levels of confidence in the evidence towards causation with RCT's considered the highest of the four, followed by cohort, case-control and then cross-sectional (Figure 3-1). In RCT's as with experimental studies, the subjects are randomly assigned to receive the intervention or not. In comparison, in cohort, case-control and cross-sectional studies the researchers do not intervene in any way, leaving the situation to play out naturally (Petrie et al. 2002a). Observational studies often make use of actions or interventions that were going to be applied anyway and study an outcome that is naturally occurring in a population.

The design features and advantages and disadvantages of the four main study types are now described, along with examples of their use in plant studies and their potential to be applied to investigating Psa in kiwifruit.

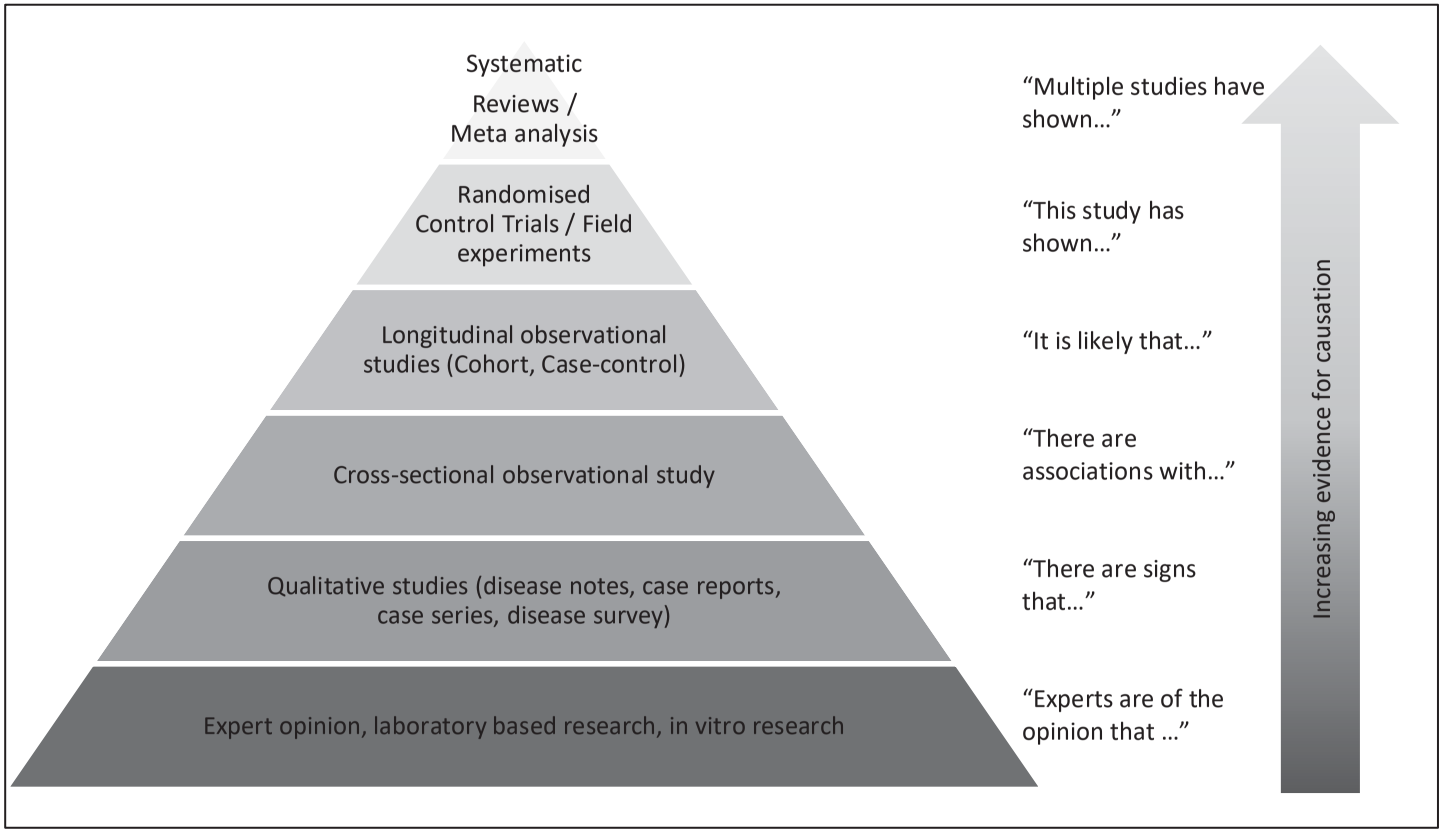

Figure 3-1 Evidence pyramid showing the different study types and the quality of evidence they provide. Modified from Holmes (2007) and Sargeant et al. (2014).

3.5.1 Randomised control trials

Randomised control trials (RCT's) in animals or humans or experimental field trials in plants may be more realistic than laboratory trials, if they are undertaken in the real world such as in the community or on commercial orchards or farms. These types of trials are restricted in the number of treatments or exposures that can be applied and are best used to assess the effect of interventions or variables that are easily manipulated (Petrie et al. 2002a; Martin 2008) . In these trials potential confounding such as farmer inputs and spatial heterogeneity can be controlled by robust randomisation to improve internal validity but also can match subjects or exclude subjects to manage confounders (Sargeant et al. 2014).

There are similarities between inoculated plant field trials and human or animal RCT challenge trials. For example, in plant field trials the plant is inoculated with the pathogen either before or after the intervention is applied to ensure that the intervention is tested against the disease. Similarly a typical challenge trial in veterinary research is where a preventative treatment is randomly applied and all animals are then exposed to the pathogen, to observe which develop disease, or for therapeutic treatments disease would be induced in all animals prior to randomly allocating the treatment (Sargeant et al. 2014).

In most plant based field trials all plants are generally managed the same way and all non-intervention factors (like edge-effects) are controlled for using randomisation and blocking designs (Aust & Kranz 1988; Katsantonis et al. 2007) which is similar to highly controlled RCT's and clinical trials. There are also similarities between the more frequently used plant experimental trials that rely on natural infection or development of an outcome and productive animal randomised control trials. In that the intervention is randomly applied, disease is naturally acquired and grower or farmer inputs are only controlled regarding the intervention (Sargeant et al. 2014).

In large scale RCT's used in veterinary and medical medicine the intervention is randomly allocated and there is little ability to control external factors, as would be attempted in an experimental trial for plants. These external factors include differences in farming practice, nutrition, environmental stressors or pollutants, or if the assigned treatment is actually administered. In an RCT variables known to impact on the outcome, that is confounders, can be managed by restricting the eligibility criteria for inclusion in the study (Concato et al. 2000; Sargeant et al. 2014).

The aim of RCT studies is for scientific vigour and their design increases the external validity, that is, to see if a preventative or therapeutic treatment is effective or better than the existing

treatment in the real world, for the average patient. In plants this would be equivalent to undertaking a large-scale trail where orchardists are randomly assigned to try a new intervention. For example, using a disease risk model to time sprays and leaving the 'untreated controls' to apply their existing protective spray protocols and then comparing disease or productivity outcomes.

There is potential to use RCT study designs more in plant health and good guidelines exist for use in medicine, the CONSORT statement (Begg et al. 1996), and veterinary research, the REFLECT statement (O'Connor et al. 2010), which could be applied to plants.

3.5.2 Cohort studies

A cohort study starts with the exposure(s) of interest and follows a cohort of subjects some of which are exposed to the risk and some of which are not through time to assess whether the exposed group develops more disease (Grimes & Schulz 2002a; Petrie et al. 2002a). Cohort studies can describe the frequency of disease in terms of incidence and identify risk factors that are associated with disease. The main disadvantages of a cohort study is that when studying rare disease, for example cancer, a large number of subjects are required to ensure that sufficient statistical power (Grimes & Schulz 2002b). The 'rare outcome' problem should not be an issue for plant protection studies because rare diseases have negligible economic impact and are unlikely to need detailed investigation, unless they are a phytosanitary issue for trade. Another problem with a cohort study is that there may be a long delay between exposure to a risk factor and the development of disease, such as smoking and lung cancer and the population needs to be followed for many years with the potential for subjects to withdraw from the study over time (Mann 2003). Long follow-up periods may not be an issue for plant studies as perennial plants are less likely to be removed and lost to follow-up although orchard ownership may change over time. There are analysis techniques that deal with varying times at risk for subjects lost to follow-up, such as survival analysis, which enables the data collected on the subject to be used even though they did not continue in the study (Cogger & Froud 2015). Although survival analysis does not remove the bias introduced by losses to follow-up as those that withdraw may differ fundamentally from those that remain.

Cohort studies are very rare in plant protection research. One example of the use of a cohort study in plant health is Dallot et al. (2004) although the terminology was different to that used in a veterinary or medical epidemiology it was evident that their description of "an exploratory study" was analogous to a cohort study. Dallot et al. (2004) undertook a study investigating the factors involved in the spread of plum pox virus in France on 11,883 peach or nectarine

trees in 19 orchard blocks and used multivariable survival analysis to control confounding. These techniques are discussed in Section 3.6.3.

In outbreak situations like Psa where good disease case data are available and production and management data is collected by the industry, a retrospective cohort study may be a very cost-effective approach for finding out how disease affects productivity. However, to investigate exposures that influence disease development that growers don't routinely report data on a cohort study would have ideally been set up during the initial stages of the response as the disease spread in the main kiwifruit growing regions. Orchards which had not yet experienced the outcome of interest would be recruited into the study and data on potential risk factors and disease development would be collected over time for a set period or until enough orchards had developed disease to enable analysis of results. Initiating a cohort study at the start of an outbreak could be considered in industry biosecurity preparedness planning. Long time frames to collect prospective cohort data can be a problem if industry practices that are measured at the start of a study change in response to the outbreak reducing the relevance of the findings.

3.5.3 Case-control studies

Case-control studies can be used to explore the association between one or more risk factors and a single disease outcome (Mann 2003). A case control study starts with a disease of interest and tries to identify exposures that increase the risk of having the disease. Individuals who develop the disease referred to as cases, are identified and recruited into the study. They are then compared with controls that is, a group of individuals that do not have the disease (Petrie et al. 2002a; Mann 2003; Sargeant et al. 2014). Note that in a case-control study the controls are a group in which the outcome of interest is absent, which is quite different from an experimental study where the exposure of interest is absent from the control group.

Case-control studies are most useful for situations where the pest or disease is rare (Mann 2003) because only a sample of the population is required for the controls. However, sampling controls requires a well-defined study base as they must be derived from the same population that gave rise to the cases (Petrie et al. 2002a). The main issue with case-control studies is they are susceptible to selection bias (see Section 3.6.1) and recall bias (Section 3.6.2).

In plant protection, case-control studies are likely to be useful in the initial stages of an outbreak investigation when the disease or pest is still establishing or for studying plant diseases that are both rare and affecting highly valued trees or crops, for example Dutch elm disease or Kauri dieback. The use of a case-control study for investigating kiwifruit bacterial

canker is less favourable than a cohort study would be, as the disease is very common, and, therefore the main advantage of using a case control study is not present. However future changes in the pathogen such as the development of bactericidal resistance in a small number of orchards may make this study design suitable for specific research questions. There is no evidence of a plant health case-control study example.

3.5.4 Cross-sectional studies

Cross-sectional studies are a snap-shot in time. Individuals in the sample are examined for the presence of an outcome of interest, that is, prevalent cases of disease, and their status regarding the presence or absence of specified exposures. Cross-sectional studies commonly involve surveys to collect data, which may range from simple one-page questionnaires addressing a single variable, to highly complex, multiple page questionnaires. Two examples of cross-sectional studies in plant health are from Thebaud et al. (2006) and Vicent et al. (2012). Thebaud et al. (2006) investigated orchard management risk factors for European stone fruit yellows from 69,000 trees in 225 orchards and used multivariable logistic regression to assess risk factors and ranked the importance of each variable. Vicent et al. (2012) investigated the relationship between a range of agronomic factors on Phytophthora branch canker of citrus from a random selection of 110 citrus orchards in Spain and used multivariable logistic regression with adjusted odds ratios to manage confounding. These papers highlighted the opportunity for observational studies in plant health and the need for further well-designed examples to be developed to aid people wishing to conduct observational studies in plant populations.

A disadvantage of cross-sectional studies is that researchers are limited in the degree to which they can use a cross-sectional study to identify risk factors because disease and exposure information are collected at the same time (Grimes & Schulz 2002b; Hammer et al. 2009). Collection of exposure and disease information simultaneously means we cannot distinguish the order of cause and effect and can result in flawed conclusions (Maselko et al. 2012; Engel & Wolff 2013; Shahar & Shahar 2013). For example, a cross sectional study looking at pet ownership and allergies and/or asthma in children showed that there was a lower risk of asthma or allergies in children in homes with pets. However, as noted by the authors, this could be due to selected avoidance or removal of pets from homes of children with asthma or allergies rather than a protection effect of pet ownership (Brunekreef et al. 1992).

A second disadvantage of cross-sectional studies is that they are susceptible to response bias. Response bias is a type of selection bias that occurs when those that choose to respond differ

either in their disease status or their exposure status to those that do not respond (Groves 2006; Hammer et al. 2009). Response bias is discussed in Section 3.6.1.

The advantage of cross-sectional studies is that they can estimate the prevalence of disease and generate hypothesis of potential risk factors for future research (Mann 2003). Also, when compared to case-control and cohort studies, cross-sectional studies are relatively quick and cheap to conduct as there is no on-going follow-up (Mann 2003).

The New Zealand Psa outbreak was ideally suited to a cross-sectional study to generate hypotheses on which factors should be investigated further, as disease was common and the kiwifruit industry urgently wanted to identify possible risk factors to prioritise experimental research for disease management.

As with RCT's there is potential to use cohort and cross-sectional study designs more in plant health and guidelines exist for their use too. The guidelines are the 'strengthening the reporting of observational studies in epidemiology' (STROBE) statements for medicine (Vandenbroucke et al. 2007)) and STROBE-Vet (O'Connor et al. 2016; Sargeant et al. 2016) for veterinary studies. The purpose of these guidelines is to provide guidance to authors on how to accurately report observational studies to improve critical appraisal and interpretation of the quality of the methods and results and these guidelines could be applied to observational studies in plants.

3.6 Error, bias, confounding and temporality

Jepsen et al. (2004) stated that there are four reasons for an association in an epidemiology study: chance, bias, confounding or cause. The principle aim of observational study design and analysis is to prevent, reduce and assess bias, confounding and chance in order to estimate an unbiased association between exposures and an outcome that may be causal (Jepsen et al. 2004). Types of bias that can problematic in observational studies are selection bias and information bias. In addition to bias both confounding and temporality are key issues to consider in the design and analysis of observational studies.

3.6.1 Selection bias

Selection bias is caused by either the methods used to select participants in a study or from factors that may influence participation (Jepsen et al. 2004; Rothman 2012). The bias arises when the study sample is not a representative selection of the population that the results are to be inferred to (Hammer et al. 2009). Selection of participants can be managed by well-defined selection criteria and randomisation of the population to be sampled. What is more

difficult to manage is bias caused by the participant selecting to participate or not. Some factors that influence participation in a study is interest in a specific disease. For example, people with a family history of the disease may be more likely to participate and also be more likely to get disease if there is a genetic component than those that don't participate. In surveys participant selection bias is referred to as response or non-response bias. This type of bias is of concern for measures of effects or when estimates of disease prevalence are extrapolated to a range of groups. For example, if the prevalence of disease was higher in high elevation orchards than in low elevation orchards, but more high elevation growers had responded to the survey, an estimate based on the survey results would inflate the estimated disease prevalence for low elevation orchards. A comparison of responders and non-responders in relation to any important factors that may influence response should be undertaken where possible to identify any limitations to the inference from the study (Mannetje et al. 2011).

3.6.2 Information bias

Information bias is when the information or measurement of an individual or group is incorrect with regard to either the outcome of interest or the exposure(s) of interest (Grimes & Schulz 2002b). In plant health, this could mean misclassification of a plant or orchard as diseased when it is not, or the collection of incorrect information such as what sprays are used and when. A common bias in case-control studies is recall bias. In that individuals that are positive for the outcome (e.g. Psa in kiwifruit orchards) are much more likely to recall potential exposures that could have caused the disease than non-cases which may not have given any prior thought to them at the time of questioning. Recall bias is an example of differential misclassification, in that one group is more likely to be classified as having an exposure than the other group as they could recall it better. Differential misclassification can lead to a bias towards or away from the null hypothesis and therefore is important to identify (Jepsen et al. 2004; Sargeant et al. 2014). In contrast, non-differential misclassification comes about by subjects being assigned to the wrong classification in either the exposure or the outcome group without any specific bias associated with either the outcome being present or a specific exposure occurring (Sargeant et al. 2014). An example of this would be in an obesity study, looking at exercise, where the scales are out by 10kg. This would lead to misclassification of some non-obese subjects as obese, independent of their exercise status. In these situations, the measurement is wrong independent of whether the subject is a case or a control or whether they are exposed or not. Non-differential misclassification bias is less concerning in a study than differential misclassification bias as it tends to dilute the measured effect and

pushes the point estimate towards the null hypothesis, that is, a lower effect is detected than would be if non-differential bias was absent (Jepsen et al. 2004; Sargeant et al. 2014) .

3.6.3 Confounding

Confounding is a form of bias that is a key concern for epidemiologists and one that it is important to consider when designing a study. Confounding is the confusion of effects between the exposure variable of interest and another variable that is closely correlated with it (Grimes & Schulz 2002b; Petrie et al. 2002b). To be a confounding variable there are three conditions that must be met:

1. The confounder must be associated with the outcome
2. The confounder must be non-causally associated with the exposure of interest (e.g. unevenly distributed within exposure groups)
3. The confounder must NOT be part of the causal pathway between the exposure of interest and the outcome.

An example of confounding from human health is the inclusion of smoking in almost all studies because smoking is associated with so many diseases. For example, if you were investigating the association between heavy drinking and throat cancer you are likely to get a much higher estimate of risk for heavy drinkers than the true value. This is because the result is confounded by the fact that heavy drinkers are also more likely to be smokers than the non-heavy drinkers they are being compared with. Smoking is also known to be causally associated with throat cancer, so smoking needs to be accounted for in the study design or analysis to remove its influence, so that a true value for the risk of heavy drinking on throat cancer can be determined.

The options to minimise confounding are (Grimes & Schulz 2002b):

1. Randomisation
2. Restriction
3. Matching
4. Analysis

Randomisation is used for experimental studies and RCT's but not for other observational studies. Restriction can be very effective and in the example given would mean restricting the data to only one cultivar. If restriction is used it is important to consider if the results are valid to the other cultivar. Matching can be used e.g. matching smokers with smokers and non-smokers with non-smokers. However matching should be used with care as matching can

introduce additional bias and variables used for matching cannot be explored as risk factors (Elwood 2007 pg 202). Control of confounding using analysis is generally achieved either by stratifying the results or using multivariable modelling. For our kiwifruit example the results would be given for the 'Hayward' group and for the 'Hort16A' groups separately. Multivariable modelling is the main technique used for controlling confounding by analysis (Grimes & Schulz 2002b).

There are many multivariable techniques that can be used for different outcome data types and structures. Linear and logistic regression are the most commonly used techniques for modelling observational data such as normal continuous productivity data or binary data such as disease presence or absence respectively. Other techniques such as survival analysis which can be used for time to event data, non-parametric models for non-normal count data and spatial analysis for spatial data are also used. Mixed effects models can also be applied for clustered observations (e.g. multiple observations from the one orchard) (Zuur et al. 2009). Dohoo et al. (2009) and Kabacoff (2011) provide information on multivariable linear regression, logistic and survival analysis. Information on extensions to logistic regression and survival analysis can be found in Hosmer et al. (2013) and Kleinbaum and Klein (2012). The chapters in this thesis provide examples of multivariable linear regression (Chapter 4) and multivariable logistic regression (Chapters 8 and 9) to control for the use of an observational study design without *a priori* randomisation to control confounder variables.

3.6.4 Adjustments for multiple comparisons

Typically, in plant pathology investigations involving big data or data mining, an adjustment of the p-value using a Bonferroni adjustment, or similar, is advised for multiple comparisons. However, this is not recommended for the type of study design and analysis methods utilised in observational studies (Rothman, 1990; Vandenbroucke et al., 2007). Adjustments are not required for several reasons. Where exposure variables are selected based on the potential for a biologically plausible association with the outcome, it is not reasonable to assume that for every comparison the null hypothesis will be true and to adjust for this. To make adjustments to P-values to reduce type 1 errors will increase type 2 errors and lead to poor interpretation of the results (Rothman, 1990). Type 1 errors are where the null hypothesis is rejected when it is true, that is, there is no effect. Type 2 errors are where the null hypothesis is accepted when it is false, that is, a true effect does exist (Jones et al. 2003). In addition, adjustment is not required when manual selection of variables is used rather than automated selection criteria, where an adjustment may be required. This is because automated selection criteria optimize

model fit without consideration of individual variables (Sainani 2014) and are not designed to test multiple relationships in the data, resulting in P-values that are too low and confidence intervals that are too narrow (Dohoo et al. 2009 Pg. 386). Automated selection criteria may also take away the researcher's insight into how the data can answer the research question because variables that are included on the basis of potential confounding or interaction will be discarded without due consideration of the impact of the removal on the coefficient values of remaining variables (Rothman et al. 2008).

3.6.5 Temporality issues in observational studies

Even when the effects of error, bias and confounding have been reduced in observational studies, a lack of time dimension in some study types can mean the interpretation of cause and effect is limited. This is most problematic in cross-sectional studies, but also for cohort or case-control studies using retrospective data. An important consideration for the study designs presented in this thesis is that of temporality and discussion of whether exposures happened prior to the onset of disease or whether the onset of disease influenced the exposure.

3.7 Conclusion

No matter what study design is applied, a single study is rarely sufficient to provide categorical proof of field efficacy or the contribution of a factor in causing disease (Hammer et al. 2009). To provide evidence of causation there are a range of research approaches that can be implemented, from expert opinion through to qualitative research, quantitative research, systematic reviews of a whole body of research and meta-analyses. If these approaches are pulled together, then evidence based decision making may be applied. Evidence based decision making is when a range of scientific evidence is considered before making an intervention as opposed to making intervention decisions on anecdotal information or expert opinion without supporting evidence (Simoneit et al. 2011; Sargeant et al. 2014). Synthesis of evidence involves considering all the results and the quality of multiple independent studies and meta-analyses before determining whether, together, they support the use of an intervention (Sargeant et al. 2014). The analysis technique of meta-analysis has arisen to provide a quantitative approach to the synthesis of evidence and is practised in plants (Garrett et al. 2004; Paul et al. 2010; Ngugi et al. 2011); animals (Holmes 2007; Simoneit et al. 2011) and human health (Concato et al. 2000).

As the Psa outbreak continues in New Zealand it is important to rapidly identify the risk factors that have the greatest impacts on infection and severity of disease in commercial orchards so that management changes can be put in place to reduce the impact while more traditional

experimental research is undertaken to find better ways to manage these prioritised risks. Observational studies are well suited to test hypothesis as to which risks are the most important in bacterial canker spread and expression and which to prioritise industry investment in. The use of observational study designs alongside traditional experimental research methods provides new ways to explore plant protection issues. One of the potential benefits of applying observational studies to plant health is that the alternative approach of experimental studies is almost always occurring in tandem. Qualitative studies are generally undertaken as repeat monitoring of a few plants or orchards and describing disease expression over time (normally available as commercial reports to the affected industry). Randomised field trials and laboratory experimental trials are common for environment factors or management factors and interventions as potential component causes of diseases. The ability to add quantitative observational studies to a wider range of component causes that don't easily lend themselves to experimental manipulation would add greatly to evidence based decision making in plant health.

However, it is important to understand the principles of observational study design so that the right study type can be used to answer the research question. The work within this thesis aims to provide a context of what the differences are between the disciplines and to improve the common understanding between them and ultimately allow greater uptake of observational study design techniques in plant health.

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CHAPTER 4

4 Kiwifruit bacterial canker in 'Hayward' kiwifruit: The effect of kiwifruit bacterial canker disease (*Pseudomonas syringae* pv. *actinidiae*) on 'Hayward' kiwifruit productivity

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Effect of kiwifruit bacterial canker disease on productivity of 'Hayward' kiwifruit using observational data and multivariable analysis

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4.1 Abstract

A virulent strain of *Pseudomonas syringae* pv. *actinidiae* biovar 3 (Psa), which causes bacterial canker in kiwifruit, was first recorded in New Zealand in November 2010. Psa has severely affected *Actinidia chinensis* var. *chinensis* cv. Hort16A kiwifruit productivity but its effect on green *Actinidia chinensis* var. *deliciosa* cv. Hayward kiwifruit productivity has been variable. An observational study design was used to quantify the effects of Psa infection on productivity (tray equivalents per hectare) of 'Hayward' kiwifruit harvested in 2012, using data captured by industry from 2599 orchards. A total of 939 orchards were Psa positive at the end of the study period. Multivariable linear regression was used to model 2012 productivity in the presence of Psa, while controlling for regional differences, elevation, 2011 productivity, harvest dates and application of agrichemicals. The model showed productivity was initially higher in the presence of Psa, and was not reduced until after one year of infection. The relationship between protective spray use and productivity was also quantified. It is likely that improved disease management has offset the impact of the disease and future research should consider a reassessment of the effects of disease after longer term exposure to Psa in New Zealand. The use of an observational cohort study to assess disease impacts using multivariable analysis in the 'real world' could have wider application in the field of plant epidemiology.

Keywords *Actinidia chinensis* var. *deliciosa,* observational cohort study, multivariable linear regression.

4.2 Introduction

Bacterial canker is a serious disease of kiwifruit vines that has had an increasing impact on kiwifruit worldwide since the 1980s, particularly in highly susceptible gold-fleshed cultivars (*Actinidia chinensis* var. *chinensis*), like 'Hort16A' (Ferrante & Scortichini 2009; Everett *et al*. 2011; Ferrante *et al.* 2012; Aitken & Hewett 2014). It is caused by *Pseudomonas syringae* pv. *actinidiae* (biovar 3) and both the pathogen and the disease are referred to by kiwifruit producers and researchers worldwide as 'Psa'. After it was first recorded in New Zealand on 5 November 2010, Psa severely affected 'Hort 16A', causing stem cankers, death and large-scale vine removal from orchards. Green-fleshed cultivars (*Actinidia chinensis* var. *deliciosa*), like 'Hayward' are less susceptible to Psa, but in New Zealand, in spring 2011, leaf spotting was common on 'Hayward' vines and more severe Psa symptoms, such as wilting and shoot dieback, were also being reported from orchards that had first become infected the previous season. Over this period, Psa was spreading both within affected regions and to new regions, and the severity of disease in individual orchards was highly variable. This variability was postulated to be due to the period of time an orchard had been infected, the disease mitigation measures applied and other orchard management factors and environmental conditions.

While the negative effects of Psa on 'Hort16A' were obvious, determining its effects on the productivity of 'Hayward' orchards was more complex, but was of considerable interest to the kiwifruit industry. An observational study using multivariable models was identified as an approach that could quantify productivity impacts in commercial orchards subjected to different management regimes and other factors affecting disease risk. There has been increased interest in applying multivariable models to experimental data (Garrett *et al.* 2004; Scherm *et al.* 2006), but the use of observational studies instead of experimental studies is rare in plant protection research, with only a small number of examples (Dallot *et al.* 2004; Thebaud *et al.* 2006; Everett *et al.* 2007; Vicent *et al.* 2012; Bouwmeester *et al.* 2016). These methods are, however, widely used in studies of animal health and production (Dohoo *et al.* 1984; Thrusfield 2007; Alarcon *et al.* 2011; Sova *et al.* 2013; Perera *et al*. 2014).

Observational studies are characterised by the collection of data in the real world without the application of an intervention or treatment. They are particularly useful where an experimental design is not feasible, for example where factors are not easily manipulated in the field, where results need to closely represent commercial reality for industry decision making and where there are multiple factors of interest or complex interactions affecting the

host-pathogen relationship (Thebaud et al. 2006; Dohoo et al. 2009e). Observational studies are also well suited to biosecurity outbreaks where little information exists on the factors that influence the disease (Wilesmith *et al.* 1988; van Engelsdorp *et al*. 2013; Froud & Cogger 2015). In an experimental approach, variability and factors that may influence the association between the exposure and the outcome, also called confounders, are controlled through randomisation. In contrast, in an observational study confounders are controlled using multivariable statistical models (Grimes & Schulz 2002b). Therefore, the confounders need to be identified during the study design and data collected to allow the confounding variables to be included in the model.

The aim of this study was to determine the effects of Psa on 'Hayward' productivity after Psa had been present in New Zealand orchards for two growing seasons (2010/11 and 2011/12). An observational cohort study design and multivariable analysis, similar to those commonly used in veterinary epidemiology, were used. A multivariable statistical model was constructed to quantify the relationship between 'Hayward' productivity and length of time an orchard had been exposed to Psa while accounting for the potential confounding factors such as protective sprays and differences in environmental conditions between growing regions. The study used retrospective data obtained from databases of productivity and agrichemical use held by the kiwifruit exporter Zespri International Ltd. (Zespri) and orchard disease information held by Kiwifruit Vine Health (KVH), the organisation that manages the response to Psa in New Zealand.

4.3 Methods

The multivariable model to describe the relationship between the length of time since an orchard became infected by Psa and orchard productivity was developed in four steps: i) variables were created to represent possible effects of agrichemicals on Psa and productivity, ii) potential confounders and their distribution among orchards were identified, iii) simple linear regression models were developed to examine relationships between potential confounders and the outcome (productivity), and iv) a multivariable model was developed to describe the relationship between time from first detection of Psa until harvest at the end of the 2011/2012 season and productivity in 'Hayward' kiwifruit, while controlling for the effects of the potential confounders.

4.3.1 Data extraction and management

Data were taken from orchards in all the growing regions in New Zealand (Figure 4-1). The criteria for inclusion of orchards were: i) Zespri registered orchard, ii) 'Hayward' fruit produced in the 2010/11 and the 2011/12 growing seasons, and iii) Complete productivity data for 2010/11 and 2011/12 growing seasons. Productivity and agrichemical data from Zespri were combined with Psa, orchard location and management data from KVH. Microsoft Access was used to merge the datasets and extract agrichemical data for orchards that met the inclusion criteria and time frame of the study. Both datasets have been described by Froud *et al.* (2014).

The outcome variable was 'Hayward' productivity in 2011/12, measured at harvest (late March to June 2012) in tray equivalents per hectare (te/ha) for each orchard. A tray equivalent is a single layer packing tray containing 18 to 36 kiwifruit with an average weight of 3.6 kg/tray for 'Hayward' kiwifruit (Mithraratne *et al.* 2010). The key factor of interest was the number of weeks between when Psa was confirmed in the orchard, and the 'Hayward' harvest date in the 2011/12 seasons. The date of first detection was based on data in the KVH database. The method used to confirm Psa positive detections changed during the outbreak. Cases were defined as either orchards with Psa confirmed by a diagnostic test, or by the visual observation of symptoms. The date of a positive diagnostic test, or the date visible signs of disease were reported, were recorded in the database as the date of confirmed infection.

Potential confounders were classified as orchard-related, production-related or spray-related. There were four orchard-related variables: i) elevation, ii) orchard size, iii) region, and iv) presence of other kiwifruit cultivars. Four production-related variables were: i) productivity in the 2010/11 season (te/ha), ii) harvest day in 2010/2011 season, iii) harvest day in 2011/2012 growing season, and iv) organic or conventional production system. Harvest day variables, which gave an indication of early or late harvest for 2011 and 2012, were constructed from the count of days between the start of the New Zealand 'Hayward' harvest for the season and the harvest date for each orchard. For those orchards with fruit harvested on more than one day the median harvest date was used in the calculations. Agrichemical data for the 2012 production season (11 March 2011 to 17 June 2012) included the first spray applied immediately after harvest in 2011 until the last spray applied while fruit were still present in the orchard in 2012 (228,065 spray events). Spray variables were created that grouped active ingredients for Psa management (Table 4-1) and those applied for other purposes (e.g., insecticides and foliar fertilisers). Spray data pertained to individual 'Hayward' blocks within an orchard. Productivity data pertained to all 'Hayward' blocks. Disease data pertained to a whole orchard, comprising multiple blocks in one locality. Differences in numbers of spray

applications between blocks within orchards were small so the spray data were aggregated by using the median number of applications per block in the analysis. The water source used for agrichemical spraying was categorised as: i) ground water (including water from bores and spring water that was not part of a water scheme), ii) surface water (including dam, tank, rivers and streams), iii) water scheme (including water taken from a rural or urban water scheme), and iv) mixed, where more than one water source category was used in an orchard.

Table 4-1 Classification of agrichemical and bio-fungicide active ingredients applied to 'Hayward' kiwifruit for Psa control during the 2012 growing season. The classification was based on use information contained in the agrichemical database (from Zespri data).

Spray category	Active ingredient
Copper	copper
Wound protection	didecyl dimethyl ammonium chloride
	tebuconazole
Antibiotics	streptomycin
Induced resistance (plant defence elicitors)	propiconazole with benzalkonium chloride and salicylic acid
	acibenzolar-S-methyl
	BioAlexin
	Mycorrcin
	Yeast culture
Bio-fungicides	*Bacillus subtillis*
	Bacillus amyloliquefaciens
	Pantoea agglomerans
	Ulocladium sp.
Biocides	benzalkonium chloride and copper sulphate
	chlorine dioxide
	dodine
	hydrogen peroxide
	peracetic acid
	miscellaneous experimental biocide products

4.3.2 Data analysis

Statistical analyses and graphics were undertaken using the R freeware statistical package version 3.0.1 (R Core Team 2013). The level of statistical significance was set at $P<0.05$. Continuous data were summarised using median and percentiles or mean and standard deviation. Initially, separate linear regression models were used to explore relationships between the outcome, which was 2012 productivity (te/ha), and the time that Psa was first detected or other orchard, spray and production variables. A Lowess smoothing line was fitted to visualise the relationship between 2012 productivity and time that Psa was first detected. For categorical variables with more than two levels (e.g. region), statistical significance was assessed using the partial F-test statistic. For several spray groupings, it was necessary to

recode the discrete count variables as categorical variables. Decisions about recoding were made from visual assessment of boxplots and scatterplots and the simple linear regression results. For agrichemical uses, where only a few active ingredients were applied, e.g., bud-break enhancers (max=2) and leaf drop promoters (max=3), the data were examined for evidence of a dose response e.g. did two applications have a greater effect than one? Where there were no differences in productivity between single and multiple applications, the variables were recoded to binary (Not used/Used). Where there was an obvious or significant "dose effect" on productivity, the variables were either left as continuous variables, where there were many applications e.g. copper (max=15), or converted to categorical variables when most orchards received only 1–4 applications. For example, herbicide applications were converted to a four-level categorical variable (Not used/1 spray/ 2 sprays/≥3 sprays). Productivity for 2011 showed a normal distribution and was scaled to a standardised unit for inclusion in the multivariable modelling.

The multivariable model was constructed in a five-step process. The first step was to construct a 'full' model that included weeks since Psa was detected, and any other variables that were associated with productivity at $P<0.20$. Exceptions were harvest days for the 2010/11 and 2011/12 seasons as these two variables were collinear and therefore only one could be included in the model. Harvest day for the 2010/11 season was included because the P-value was lower and the R^2 value was higher. The second step used an iterative manual backward elimination procedure to remove variables until either all remaining variables in the model were significantly associated with productivity, or the exclusion of the variable: i) altered the Beta co-efficient for weeks since Psa was detected by more than 20%, ii) changed the adjusted R^2 value by more than 5%, or iii) changed the AIC (Akaike information criterion) by more than four points. The significance of each coefficient was assessed using a t-test. For categorical variables with more than two levels, the statistical significance of all the levels in that group was assessed using the partial F-test. The third step was to determine if the continuous variables in the model had a linear relationship with productivity after accounting for the effects of other variables in the model. In a model with only one variable this could be done by visual inspection of a scatter plot. However, in a multivariable model the aim is to assess linearity in the presence of other variables, so the assumption of linearity was assessed through the inclusion of a quadratic term. If the quadratic was significant then the variable was deemed to have a non-linear relationship with the outcome and it was either converted to a categorical variable or the quadratic term retained was in the model, depending on which produced the highest adjusted-R^2. The fourth step was to ensure that no important factors

were excluded from the multivariable model. Each variable not included in the 'full' model or removed during model building was separately added back into the reduced model and retained if statistically significant in step four. Finally, in step five, we considered all biologically plausible two-way interactions via the inclusion of an interaction term. Any interactions that were significant, as determined by the partial F-test, were retained in the model. The adjusted R^2 value was used to assess the goodness of fit of the model as a whole.

No adjustments were made to p-values for the final model as they are not recommended where exposure variables are individually selected based on the potential for a biologically plausible association with the outcome (Rothman 1990; Vandenbroucke et al. 2007) and manual selection of model variables was applied rather than automated selection criteria (Dohoo et al. 2009c; Froud et al. 2015).

Standard model diagnostics for multivariable linear regression were performed (Kabacoff 2011). The distribution of the Studentised residuals were plotted and visually assessed. The square root of the standardised residuals was plotted against the fitted values and checked for a horizontal line of best fit with no apparent funnel or cone shapes formed by the points. Influential observations were assessed by plotting Cook's distance values against each variable. For Cook's distance, a cut-off for concern was set to 0.0002. This was calculated using 4/(n-k-1), where n is the number of observations (2599) and k is the number of coefficients in the final model (29).

Predicted productivity plots for some effects were constructed in R using the effects package (Fox 2003).

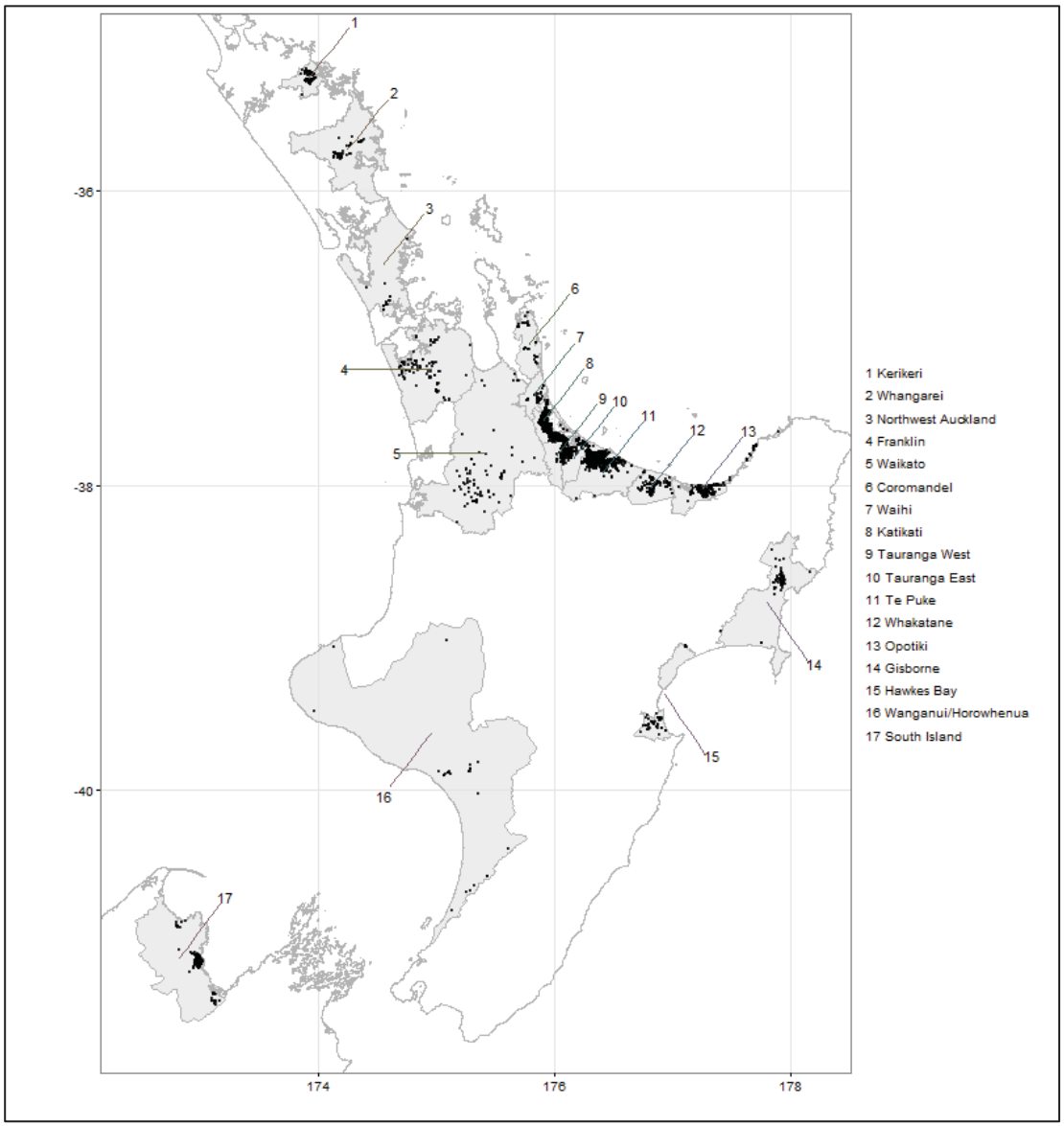

Figure 4-1. Map of New Zealand kiwifruit growing regions and kiwifruit orchard locations in 2012.

4.4 Results

There were 2953 Zespri registered orchards with 'Hayward' kiwifruit in 17 growing regions across the country. Of these 354 orchards were excluded from the dataset because of: missing outcome data (productivity in 2011/12, n=326), missing 2010/11 productivity data (n=25) or obvious data entry errors (n=3). The resulting data set contained 2,599 orchards from 17 growing regions. In 2011/12, the mean productivity was 8096 te/ha (SD 2551; Figure 4-2), while in 2010/2011 the mean was 8775 (SD 2551).

There were 934 known Psa-positive orchards in the final dataset of 2599 orchards. Of these, 71 were confirmed positive at the end of the 2010/11 season (3%) and 863 were confirmed

positive at the end of the 2011/12 season (33%). Psa positive orchards in 2010/11 were all located in Te Puke, whereas the Psa positive orchards at the end of the 2011/12 season were in Katikati (17), Te Puke (712), Tauranga West (9), Tauranga East (86), Waihi (14), Whakatane (58), Opotiki (32) and Franklin (6), with nine regions remaining Psa free. For those orchards that were infected, the median time to infection was 192 days (minimum=1; maximum=582). There was an initial apparent increase in productivity for infected orchards, followed by a gradual reduction, as shown by the Lowess smoothing line fitted to the relationship between 2012 productivity and time that Psa was first detected (Figure 4-3). The median planted area of 'Hayward' was 3 ha per orchard (Table 4-2) and median harvest date for both years was mid-May. The duration of harvest from the first to the last orchard was 86 and 84 days for 2011 and 2012, respectively, with the median harvest day 53 and 51 days after the start of annual harvest, respectively (Table 4-2). Mean productivity for 2012 was 8096 te/ha (SD 2541) which was lower than 2011 at 8775 (SD 2551).

The predominant Psa protectant spray was copper with 2165 of 2599 (83%) growers applying copper. On average three copper sprays were applied to 'Hayward' blocks during the season (Table 4-2). Very few growers used antibiotic sprays (287 of 2599; 11%) or biocide sprays (80 of 2599; 3%; Table 4-3). Insecticides and foliar fertilizers were the most commonly used non-Psa sprays (Table 4-3).

Univariable screening for an association between potential confounders or spray variables and productivity showed that all variables except for presence of *Actinidia chinensis* var. *chinensis* cv. Gold9 vines, frost protection sprays, biocide sprays and pesticide sprays were associated with productivity (P<0.20), and were therefore eligible for inclusion in the full multivariable model (Table 4-3 and Table 4-4).

The results of the multiple linear regression model showed there was a significant association between productivity (te/ha) and weeks of Psa infection, while accounting for confounders such as region and sprays (Table 4-5). However, the relationship was non-linear with effects on productivity only occurring after one year of infection (Figure 4-4). The model also showed the following: for each copper spray applied, productivity increased 45 te/ha (95% CI 12 to 79 te/ha), the use of wound protection sprays increased productivity by 294 te/ha (95% CI=119 to 470) and induced resistance sprays increase productivity by 236 te/ha (95% CI=74 to 398). Fungicides, bud-break enhancers and herbicide use also improved productivity. Several compounds not considered to be Psa protectants were also found to be associated with productivity (Table 4-5). The final model included an interaction between productivity in 2011

and elevation, that is, the effect of productivity in the previous seasons varied at the two different elevations (Figure 4-5).

Several variables had no significant association with productivity and were eliminated from the model. The most notable of these were foliar fertilisers and the presence of 'Hort16A' on the orchard. Diagnostics on the multivariable model showed no obvious violations of the key assumptions for linear regression on productivity. All observations had standardised residuals and Cook's distance values within acceptable limits (Dohoo et al. 2009e; Kabacoff 2011).

Table 4-2 Descriptive statistics for continuous variables considered as confounders in the relationship between time since Psa was detected and 2012 productivity. Data are from 2599 'Hayward' kiwifruit orchards.

Variable	Unit	Min	25th	Median	75th	Max
Harvest day in 2010/11	Day	0	39	53	64	86
Harvest day in 2011/12	Day	0	37	51	58	84
Copper sprays	Number sprays	0	1	3	5	15
Adjuvants	Number sprays	0	1	2	4	18
Area of 'Hayward'	ha	0	2	3	5	49

Table 4-3 Results of simple linear regression analyses describing the relationship between orchard layout and production factors and productivity in 2012, measured in tray equivalents per hectare (te/ha). Data from 2599 separate orchards with 'Hayward' kiwifruit.

Variable	Level	Number of orchards	%	Beta coefficient	95% CI's	P-value[a]
Elevation	≤80m	1931	74	Reference		
	>80m	668	26	-1062[b]	-1282 to -842[b]	<0.001
Productivity 2011	Productivity (per standardised unit)[c]	-		1547[c]	1469 to 1625	<0.001
Harvest day 2010/11	Time since start of 2011 harvest (days)	-		-25	-30 to -20	<0.001
Harvest day 2011/12	Time since start of 2012 harvest (days)	-		-24	-29 to -18	<0.001
Organic management	Conventional	2418	93	Reference		
	Organic	181	7	-2645	-3017 to -2273	<0.001
Region	Katikati	399	15	Reference		<0.001
	Te Puke	871	34	604	317 to 892	
	Tauranga West	242	9	1307	924 to 1690	
	Tauranga East	252	10	-183	-571 to 205	
	Waihi	41	2	-1101	-1882 to -321	
	Whakatane	144	6	159	-304 to 622	
	Opotiki	155	6	1641	1190 to 2091	
	Franklin	81	3	-61	-641 to 519	
	Waikato	71	3	-688	-1301 to -75	
	Coromandel	38	1	-1032	-1840 to -224	
	Poverty Bay	39	2	-1063	-1862 to -265	
	Hawkes Bay	33	1	-1143	-2005 to -281	
	Kerikeri	48	2	-1393	-2120 to -666	
	Auckland	19	1	-171	-1289 to 946	
	Whangarei	18	1	-1790	-2652 to -928	

Variable	Level	Number of orchards	%	Beta coefficient	95% CI's	P-value[a]
	Wanganui/Horowhenua	33	1	-1408	-2555 to -261	
	South Island	115	4	-1329	-1833 to -826	
Actinidia chinensis var. chinensis cv. Gold3	Not present	2296	88	Reference		
	Present	303	12	527	222 to 833	<0.001
A. chinensis var. chinensis x A. chinensis var. deliciosa cv. Green14	Not present	2483	96	Reference		
	Present	116	4	758	284 to 1233	0.002
A. chinensis var. chinensis cv. Hort16A	Not present	2190	84	Reference		
	Present	409	16	272	3 to 542	0.05
Other cultivar	Not present	2576	99	Reference		
	Present	23	1	762	-286 to 1810	0.152
Red cultivar	Not present	2571	99	Reference		
	Present	28	1	622	-329 to 1573	0.198
'Gold9' cultivar	Not present	2236	86	Reference		
	Present	363	14	143	-141 to 426	0.322

[a] Significance given for categorical variables using the partial F-test, and for all others using the t test statistic. [b] For orchards at elevations >80 meters above sea level average productivity is 1062 te/ha less than those at an elevation of ≤80m (95% CI:-1282 to -842); [c] Increasing 2010/11 productivity by one standard deviation from its mean will increase 2011/12 productivity by 1557 te/ha (95% CI: 1470 to 1625).

Table 4-4 Results of simple linear regression analyses describing the relationship between agrichemical spray factors and productivity in 2012, measured in tray equivalents per hectare (te/ha). Data from 2599 separate orchards with 'Hayward' kiwifruit.

Variable	Level	Number of orchards	%	Beta coefficient	95% CI's	P-value[a]
Copper sprays	Copper applied (per application)			259 [b]	221 to 297	<0.001
Wound protection sprays	Not used	1307	50			
	Used	1292	50	1521	1334 to 1708	<0.001
Leaf drop sprays	Not used	1603	62			
	Used	996	38	824	624 to 1023	<0.001
Foliar fertilisers	Not used	1229	47			
	Used	1370	53	605	410 to 800	<0.001
Induced resistance	Not used	1285	49			
	Used	1314	51	1182	991 to 1373	<0.001
Bio-fungicide	Not used	1688	65			
	Used	911	35	674	469 to 878	<0.001
Antibiotics	Not used	2312	89			
	Used	287	11	1022	711 to 1333	<0.001
General fungicides	Not used	1073	41			
	Used	1526	59	1348	1155 to 1541	<0.001
Bud-break enhancer	Not used	355	14			
	Used	2244	86	2439	2169 to 2709	<0.001
Adjuvants used	Adjuvants applied (per application)			193	153 to 234	<0.001
Insecticide use	Not used	55	2			
	1 spray	182	7	1135	372 to 1898	0.003
	2 sprays	604	23	1146	447 to 1844	0.001
	3 sprays	1048	40	1525	839 to 2211	<0.001
	4 sprays	401	15	1066	353 to 1779	0.003
	5 sprays	309	12	504	-222 to 1230	0.17
Water source for sprays	Ground Water	1374	53			

Variable	Level	Number of orchards	%	Beta coefficient	95% CI's	P-value[a]
	Surface water	186	7	-676	-1067 to -286	<0.001
	Water scheme	1007	39	51	-156 to 259	0.63
	Mixed water	32	1	-266	-1159 to 627	0.56
Herbicides	None used	2012	77	Reference		
	1 spray	234	9	361	16 to 706	0.04
	2 sprays	267	10	-292	-618 to 33	0.07
	≥ 3 sprays	86	3	309	-241 to 860	0.27
	Intercept			8114	8014 to 8213	
Fruit stain sprays	Not used	2523	97			
	Used	76	3	-594	-1176 to -12	0.05
Frost protection sprays	Not used	2464	95			
	Used	135	5	90	-353 to 532	0.69
Biocide sprays	Not used	2519	97			
	Used	80	3	272	-297 to 840	0.35
Pesticide (bird) sprays	Not used	2584	99			
	Used	15	1	-287	-1583 to 1010	0.66

[a] Significance given for categorical variables using the partial F-test, and for all others using the t test statistic. [b] Increasing copper use will increase 2011/12 productivity by 259 te/ha (95% CI 221 to 297) per application.

Table 4-5 Results of multiple linear regression describing the relationship between time since Psa was first detected (weeks) and 2012 productivity (tray equivalents per hectare; te/ha) while controlling for confounders. Data were from 2599 orchards with 'Hayward' kiwifruit. The model has an adjusted R^2 of 0.49 and 2567 degrees of freedom.

Variable	Level/Unit	Beta coefficient	95% CI's	P-value [a]
Intercept		6230	5850 to 6609	<0.0001
Time since detection	per week	14	0.6 to 27	0.04
Time since detection squared	per week^2	-0.2[b]	-0.5 to 0.0	0.04
Organic	Yes vs No	-145	-561 to 271	0.49
Elevation	>80 m vs <=80 m	-747	-951 to -543	<0.0001
Productivity in 2010/11	per standardised unit	1367	1277 to 1456	<0.0001
Elevation x 2011 productivity [c]		-549	-723 to -374	<0.0001
Harvest day 2010/11	per day since start	-17	-21 to -13	<0.0001
Copper	per spray	46	12 to 79	0.01
Wound protection sprays	Used vs not used	294	119 to 470	0.001
Induced resistance sprays	Used vs not used	235	74 to 398	0.004
General fungicides	Used vs not used	596	434 to 759	<0.0001
Bud-break enhancers	Used vs not used	1015	715 to 1316	<0.0001
Herbicides	1 spray vs not used	117	-135 to 369	0.36
	2 spray vs not used	-306	-552 to -60	0.01
	>=3 spray vs not used	-156	-561 to 250	0.45
Region	Te Puke vs Katikati	309	32 to 586	0.03
	Tauranga West vs Katikati	1102	810 to 1395	<0.0001
	Tauranga East vs Katikati	883	556 to 1210	<0.0001
	Waihi vs Katikati	790	153 to 1427	0.01
	Whakatane vs Katikati	621	256 to 987	0.001
	Opotiki vs Katikati	1385	1041 to 1728	<0.0001
	Franklin vs Katikati	57	-390 to 503	0.8
	Waikato vs Katikati	1854	1366 to 2343	<0.0001
	Coromandel vs Katikati	-591	-1209 to 26	0.06
	Poverty Bay vs Katikati	863	250 to 1475	0.01
	Hawkes Bay vs Katikati	531	-144 to 1205	0.12

Variable	Level/Unit	Beta coefficient	95% CI's	P-value [a]
	Kerikeri vs Katikati	-42	-611 to 527	0.88
	Auckland vs Katikati	116	-742 to 974	0.79
	Whangarei vs Katikati	216	-480 to 912	0.54
	Wanganui vs Katikati	318	-561 to 1197	0.48
	South Island vs Katikati	159	-255 to 573	0.45

The model is based on 2567 degrees of freedom and an adjusted R^2 of 0.49.

[a] P-value for the t-statistic, [b] Relationship between time since Psa was first detected and productivity was not linear (see Figure 4-4); [c] Interaction between elevation and productivity in 2010/11 means that the effect on 2012 productivity differs with elevation. Specifically, all other variables being constant, at elevations ≤ 80 meters when 2010/11 productivity increased by one standard deviation, productivity in 2011/12 increased by an average of 1367 te/ha while in orchards >80 meters the average increase in 2011/12 productivity is only 818 te/ha.

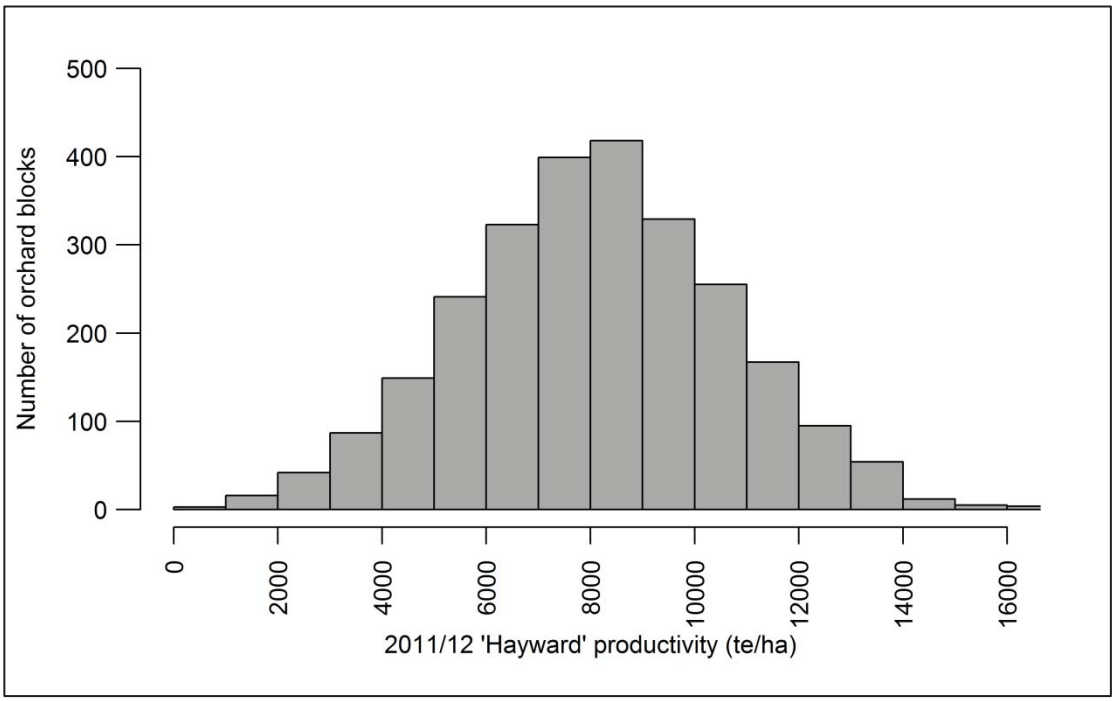

Figure 4-2. Histogram of 'Hayward' productivity in tray equivalents per hectare (te/ha) for the 2011/2012 growing season.

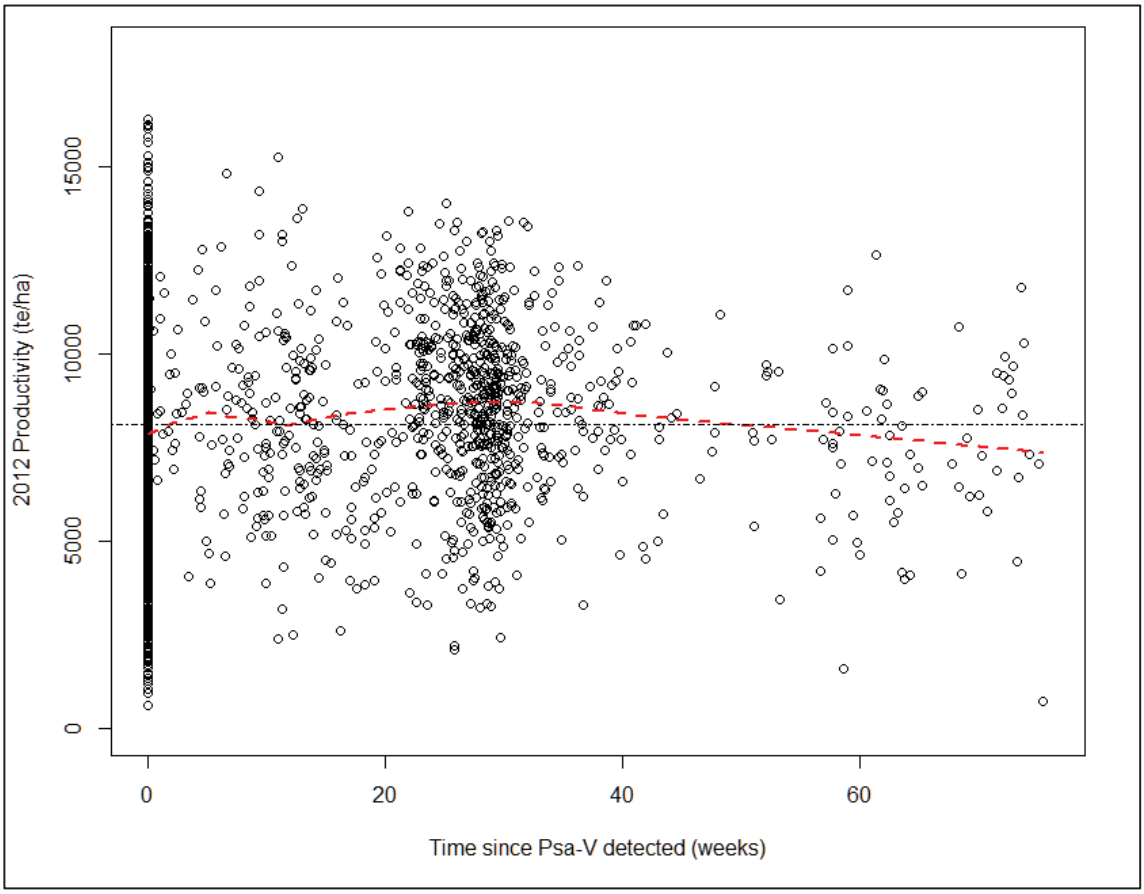

Figure 4-3 The relationship between 2012 productivity and time that Psa was first detected. The red line is a Lowess smoothing line fitted to the data and the grey hatched line shows the mean predicted 2012 productivity from the model.

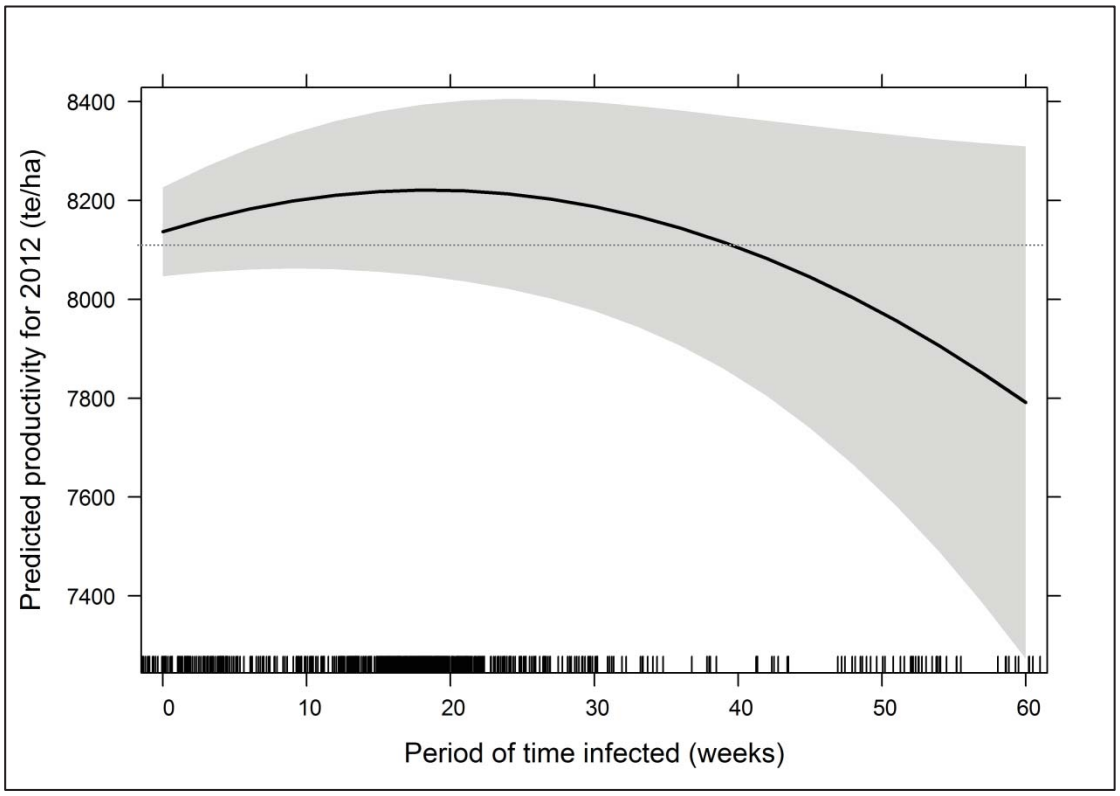

Figure 4-4 Predicted change in 'Hayward' kiwifruit productivity in relation to the time since Psa was first detected on an orchard from a multivariable linear regression model constructed with data from 2599 orchards with 'Hayward' kiwifruit. Grey line shows the mean predicted 2012 productivity from the model. Internal ticks on the x-axis show the spread of the modelled data.

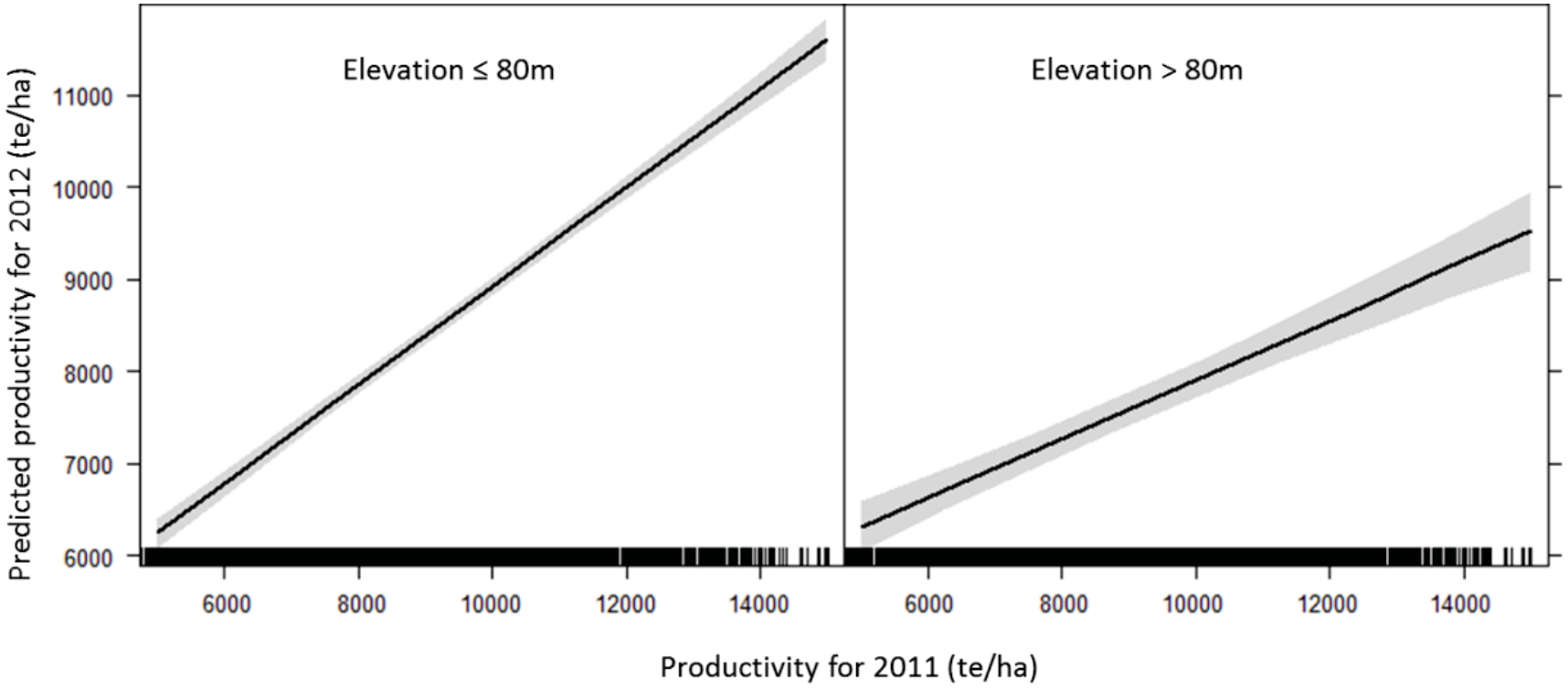

Figure 4-5 Predicted values for 2012 productivity with upper and lower confidence intervals fitted from a multivariable linear regression model against 2011 productivity for low elevation (≤80m) and for high elevation orchards (>80m) showing the interaction between these two exposure variables. Internal ticks on the x-axes show the spread of the modelled data. Model was constructed with data from 2599 orchards with 'Hayward' kiwifruit.

4.5 Discussion

The multivariable model showed that, after adjusting for all factors: region, elevation, 2011 productivity, day of harvest and use of protective sprays, the productivity of 'Hayward' kiwifruit orchards did not decline until an orchard had been infected for more than one year. Interestingly after accounting for all other factors, there also appeared to be an increase in production in infected orchards after Psa was first detected. Therefore, the one year delay before Psa affected overall productivity may have been related to the time taken for Psa to infect and become severe enough in 'Hayward' vines in individual orchards to cause an overall reduction in productivity. Alternatively, or in combination with the above scenario, improved canopy management actions taken following first detection of Psa in an orchard could have contributed to an initial increase in productivity. Orchard hygiene interventions may also have slowed the progress of disease through the orchard and therefore delayed the effect of the disease on productivity. It is also possible that the presence of the pathogen elicited a physiological response that initially improved productivity, as has been shown to occur with *Pseudomonas fluorescens* in blackberries (Garcia-Seco et al. 2013).

Our results showed that Psa caused a less noticeable decline in productivity in 'Hayward' than that reported by Froud et al. (2014) for 'Hort16A'. However, it is important to note that there was a reduction in 'Hayward' productivity after the disease had been present for about a year or more. At the time of this study only 36% of 'Hayward' orchards were infected and only 3% had been infected for over a year, consequently the current economic impact of Psa on 'Hayward' may be underestimated. Aitken and Hewett (2012) reported that the 2012 'Hayward' harvest was the highest recorded (just above pre-Psa 2009 and 2010 harvests), despite the arrival of Psa in New Zealand. This has been followed by small reductions in productivity in the 2013 and 2014 harvests (Aitken & Hewett 2015). More recently productivity in 2015 and 2016 has increased with the 2016 season higher than any previous season although the number of producing hectares has reduced by 22% from 10495 Ha of 'Hayward' in 2011 to 8151 Ha (Zespri International Ltd 2016a). The increased productivity may reflect a consolidation of growers who are able to manage Psa well. A further complication to determining the effects of Psa on 'Hayward' productivity is the development and application of new management tools for Psa in New Zealand orchards (Gaskin et al. 2012; Tyson et al. 2012b; Kiwifruit Vine Health Inc. 2013; Horner et al. 2015; Kiwifruit Vine Health Inc. 2015; Beresford et al. 2017) which had not been available to orchardists at the time of our study.

This analysis showed that several types of agrichemical remedies, namely copper, wound protection, induced resistance, fungicides and bud-break sprays improved productivity. The increase in productivity attributed to copper use supports the industry recommendations that a regular copper programme be maintained (Kiwifruit Vine Health Inc. 2015). While the model predicted an increase of 45 te/ha for each copper spray applied, this was based on data in which the maximum number of applications was 15 and we should not extrapolate beyond that number. In fact, only 25% of producers reported that they applied more than five copper sprays and predictions of expected benefits beyond five sprays are unlikely to be accurate. The results regarding Psa protective products that had a demonstrated productivity benefit (copper, induced resistance and wound protection sprays), compared with those that did not, should assist growers in making management decisions based on input costs.

There was a clear result that productivity in one season is an indicator (alongside elevation) of productivity in the following season. The remaining variation in the model was likely associated with intra-regional differences such as soil type and fertility, micro-climate, vine age and the management competence of individual growers.

It was not surprising that the use of both bud-break sprays and herbicides were associated with increased productivity, as both have known benefits for kiwifruit production. Fungicides, which are applied to control other kiwifruit pathogens, such as sclerotinia (*Sclerotinia sclerotiorum*) (Hoyte et al. 2007), also increased productivity. There was no evidence that applying other agrichemicals improved productivity, although biological products applied in an attempt to maintain productivity may still be beneficial (Monchiero et al. 2015; Mowat et al. 2015). Insecticide applications are mostly aimed at maintaining market access and their use would not be expected to increase crop productivity. Neither bio-fungicides, leaf drop sprays nor foliar fertilisers affected productivity. The use of fruit stain, biocide and bird repellent sprays was very low; thus, an association was unlikely to have been detected. The use of adjuvants was not able to be assessed fully as the link between the adjuvant and the specific spray it was applied with was lost during data aggregation to median counts per orchard. However, Gaskin (2012) has reported copper efficacy being improved when adjuvants are added, and therefore it would be useful to test the efficacy of protectants applied with and without adjuvants against Psa disease severity using similar analysis techniques in the future.

This observational study design using multivariable analysis was able to resolve the effect of individual factors amongst many and to quantify the relationship between ‘Hayward’ productivity and length of time an orchard had been exposed to Psa, while accounting for

many potential confounding factors, in 'real world' commercial orchards. Although observational studies have the disadvantage of no direct evidence for causality (Ioannidis 2016), they have the advantage that the results quantify the relative importance of a wide range of factors that can't be simultaneously controlled for in experimental studies (Grimes & Schulz 2002a; Rochon et al. 2005; Thrusfield 2007). Observational studies also overcome the low external validity that experimental studies can suffer from with scaling effects when extrapolating to the wider population (Van der Plank 1963; Englund & Cooper 2003). Epidemiology is defined as the study of disease within the population and this type of study is a practical way to investigate disease at the population level. There is potential to make greater use of this type of study both to investigate biosecurity outbreaks and to investigate other pests, diseases or management factors that influence productivity in horticultural crops.

While this study shows that Psa can have an effect on productivity of 'Hayward' kiwifruit one year or more after introduction into a block, the results also indicate that new management practices and improved orchard management can off-set the effects of the disease to some extent. The statistical model developed in this study is limited in its generalisability to 'Hayward' cultivar kiwifruit and to the season that was modelled, however it provided timely information for the management and understanding of an emerging outbreak. Future investigations should focus on assessing the economic impact of this disease on new cultivars after an extended period of exposure to Psa and continuing to improve disease management practices to reduce the effects of longer term exposure to the disease.

4.6 Acknowledgements

We would like to thank KVH for provision of Psa data and Zespri for the provision of productivity and spray data, in particular thanks to Greg Clark, John White and Mike Steele from Zespri and Rachel Windner from KVH. This project was funded by the Kiwifruit Vine Health and Zespri Psa research programme under contract number V11348.

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CHAPTER 5

5 Kiwifruit bacterial canker in 'Hayward' kiwifruit: Design of a quantitative questionnaire for kiwifruit growers

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STATEMENT OF CONTRIBUTION TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Karyn Froud

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Please indicate either:

- The percentage of the Published Work that was contributed by the candidate: 90%

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- Describe the contribution that the candidate has made to the Published Work:

  Designed, collected data, analysed data, wrote paper and complied edits

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Kiwifruit bacterial canker in 'Hayward' kiwifruit: Design of a quantitative questionnaire for kiwifruit growers

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5.1 Abstract

Longer term effects of *Pseudomonas syringae* pv. *actinidiae* biovar 3 (Psa) on 'Hayward' kiwifruit (*Actinidia chinensis* var. *deliciosa*) production are unclear and there is uncertainty about what impact orchard activities could have on disease prevalence. The aim of the present study was to determine the validity of the data obtained from a cross-sectional observational study using a quantitative postal questionnaire on disease and risk factor prevalence from commercial growers of 'Hayward'. The questionnaire was sent to 1669 growers and 442 responded (26%), a response rate similar to that of other agriculture surveys in New Zealand. Non-responses were analysed against a range of factors to assess response bias. There was a higher response rate from organic growers, and those affiliated with specific packhouses. There were no differences between responders and non-responders according to the period of time their orchard had been infected with Psa or to orchard productivity. We conclude that a postal questionnaire was an effective way to obtain quantitative disease, risk factor and hygiene data from commercial producers.

Keywords *Actinidia deliciosa,* risk factors, 'Hayward', cross-sectional, quantitative survey, postal, non-response bias.

5.2 Introduction

Bacterial canker in kiwifruit is a serious disease caused by *Pseudomonas syringae* pv. *actinidiae* biovar 3 (Psa), which was first reported in New Zealand in November 2010 (Everett et al. 2011). Psa affects kiwifruit plants by causing leaf spotting, shoot and cane dieback and stem cankers. The whole vine may be affected in severe cases leading to death and/or removal of the vine from the orchard. The disease had an immediate and major effect on the productivity of gold-fruiting *Actinidia chinensis* var. *chinensis* 'Hort16A' vines, which have now largely been

removed and replaced with a more resistant gold cultivar ('Zesy002'), commonly called Gold3. However, green-fruiting *A. chinensis* var. *deliciosa* 'Hayward' vines in New Zealand are continuing to be grown in the presence of the disease (Ferrante & Scortichini 2009; Ferrante et al. 2012; Vanneste 2012) with limited impact of Psa on productivity (Froud et al. 2014).

Experimental studies are commonly used to investigate the epidemiology of plant diseases. However, they may not always be feasible where multiple risk factors are involved (Thebaud et al. 2006; Froud et al. 2014). When investigating multiple risk factors, observational studies offer several advantages (Dohoo et al. 2009e). Observational studies provide insights on the performance of recommended management practices in animal health when applied by farmers, rather than researchers. This approach can be used to identify practical reasons why interventions may not work in the manner predicted by experimental studies. This type of study design has been rare in plant protection research although a few examples do exist (Dallot et al. 2004; Thebaud et al. 2006; Everett et al. 2007; Vicent et al. 2012; Froud et al. 2014; Cogger & Froud 2015).

We chose to use a cross-sectional study based on an industry survey, to investigate Psa prevalence, risk and hygiene factors in 'Hayward' orchards. Cross-sectional studies can be used to quickly identify which potential risk factors are important in disease developing in a population. They are also useful for determining the prevalence of pests, pathogens and possible risk factors for disease (Dohoo et al. 2009e; Rothman 2012).

There are challenges to developing a survey that will result in a high response rate of valid, unbiased data. This paper describes the development, design and distribution of a questionnaire to kiwifruit growers. It discusses survey design principles and tests for response bias. Response bias is a form of selection bias, as it relates to those who respond to the survey compared with those who did not.

5.2.1 Development of the questionnaire

The questionnaire was developed in a three-step process:

Step 1. A workshop to identify key risk factors

Risk factors were identified from information provided by technical experts in Zespri International Limited (Zespri) and Kiwifruit Vine Health (KVH) plus two kiwifruit grower representatives. This allowed the development of a causal web diagram, which was used to visualise postulated relationships between risk factors and to identify any potentially confounding variables on which data would need to be collected (Figure 5-1) (Dohoo et al.

2009e; Rothman 2012). Collection of data for potential confounding variables is essential when an observational study is used to investigate risk factors, as the data are not randomised to address differences in the source population, as they would be in an experimental trial (Froud & Cogger 2015).

Step 2. Development of the draft questionnaire

The questionnaire was developed following the principles outlined in Dohoo et al. (2009d). A key concern when using a cross-sectional study is the temporality of factors; that is, did exposure to risk precede the development of disease? In this study, the growers were being asked to describe their experience of the disease in their orchards in February 2013. Therefore, the questions were structured to focus on factors that existed or occurred prior to February 2013. In addition disease onset data specific to the orchard block of interest would be requested from the grower. The questionnaire was then circulated to Zespri and KVH technical experts to seek consensus on its content and to modify where necessary before pre-testing.

Step 3. Pre-testing of the questionnaire

Pre-testing aimed to identify any questions that may have been confusing, ambiguous or misleading and also to determine if the layout and instructions given to respondents were suitable. Pre-testing also aimed to identify additional questions and additions to existing closed question options. Pre-testing also enabled us to determine if there was any reluctance to answer specific questions and to estimate the time required to complete the questionnaire.

The pre-testing methodology involved face-to-face interviews with 10 growers in the Eastern Bay of Plenty in January 2013. The growers were given information about the purpose of the pre-test and then asked to review the questionnaire cover letter. Growers were asked to complete the questionnaire for a specific block on their orchard. The grower and researcher discussed each question during completion to determine whether the language was suitable, the intent of the question was obvious, the tick-box answers were exhaustive and clear and whether interpretation was consistent between the researchers and respondents. The first nine growers interviewed received the draft questionnaire while a tenth grower received a modified version based on feedback from the initial nine growers. The last section of the draft questionnaire required the grower to provide detailed orchard management and spray diary information. Five growers were asked to fill out this last section of the questionnaire and the remaining growers were asked to comment on whether they felt they could answer the questions accurately, based on the records they routinely keep. All growers were asked if the

list of vine management activities was clear and to note any omissions. The time taken by each grower to complete each question was also recorded. Overall the grower interviews for pre-testing the questionnaire took between 60 and 90 min.

Growers involved in the pre-testing found that the questions were easily interpreted and they only suggested minor changes to the language. All growers were keen to participate and nine out of 10 growers stated they would very likely complete the questionnaire if it arrived in the post. Of these 10 growers, eight did actually fill in and return the postal questionnaire. Pre-test participants estimated that if they completed the questionnaire as a postal survey, it could be completed in 45 to 60 min if they had vine management records to hand.

There is evidence that more people will participate in a survey if it is much shorter than 45-60 min (Edwards et al. 2002). Therefore, there was a risk that the time to complete this questionnaire would reduce the response rate. We considered that a monetary incentive (a Zespri-funded $20 fuel voucher for each respondent that completed and returned the questionnaire) and the personal interest by growers in Psa would compensate for the rather long time required to complete the questionnaire. Both of these factors are proven to increase response rates (Edwards et al. 2002).

Questionnaires were sent to all Zespri registered growers that met our eligibility criteria (Figure 5-2) rather than a sample, which also aimed to maximise the number of questionnaires returned for analysis. Pre-testing was an important part of the process that ensured that the majority of questions would elicit answers that were appropriate for subsequent analysis.

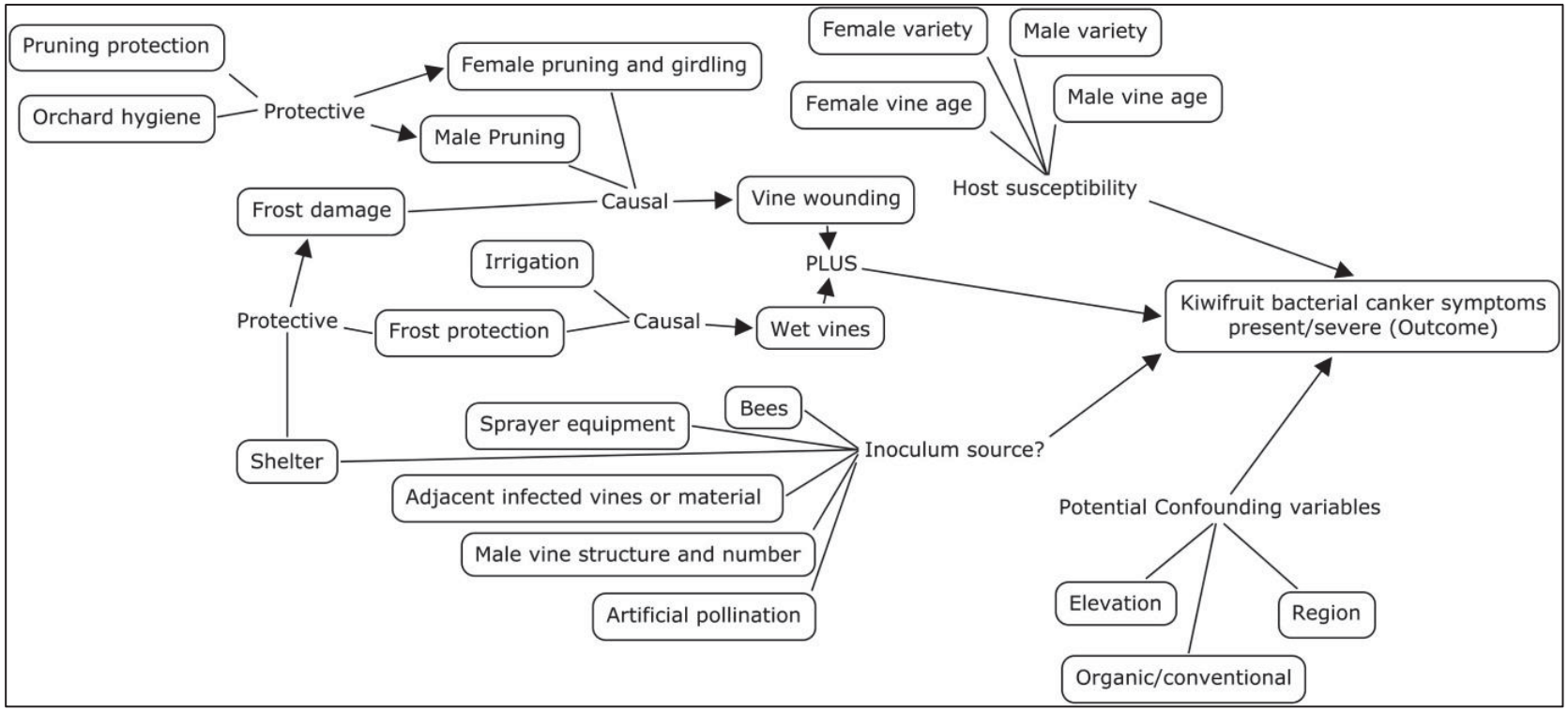

Figure 5-1 Postulated causal diagram of factors that could increase or decrease the risk of kiwifruit bacterial canker symptoms in 'Hayward' kiwifruit blocks and how these factors may be associated with each other or with potentially confounding variables.

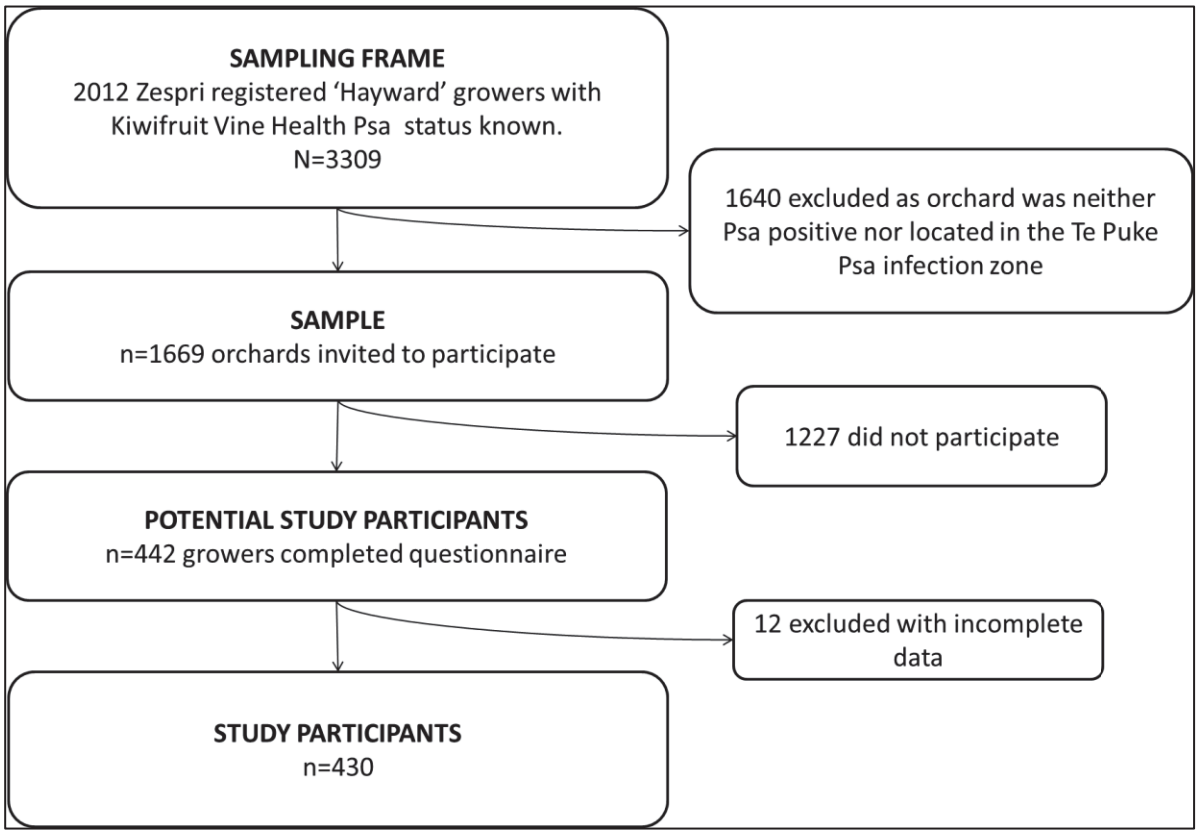

Figure 5-2 Sampling plan showing selection of a sampling frame and the eligibility criteria for inclusion in the study.

5.2.2 Content of the questionnaire

Growers were required to report detailed information about the occurrence of disease symptoms and management of vines at the level of a 'Hayward' block rather than the cultivar or orchard level. A single block was selected as there is less variability in the management of vines within a block when compared with how different blocks of the same cultivar within an orchard are managed. Asking growers to respond for each of the 'Hayward' blocks in the orchard would have generated clustered data and significantly increased the time taken to complete the survey which could have risked a reduced participation rate and/or data quality.

A block was randomly selected within each orchard from the sampling frame using an algorithm written in the 'R' freeware statistical package version 3.0.1. Random selection of blocks was to avoid selection bias such as orchardists' selecting their 'most average', best or worst block depending on what they thought was most relevant to the study.

The final questionnaire comprised a covering letter and the questionnaire proper in three sections. The covering letter explained briefly the purpose of the survey and indicated the randomly selected block on which the grower would report. The cover letter contained only

the minimum information to enable respondents to answer the questionnaire accurately. The research aim was not included in the letter to avoid response bias where respondents answer in a way that they consider helpful for the study.

The first section of the questionnaire focused on the disease with questions relating to the presence of symptoms between March 2012 and February 2013. The second section sought information on host, environment and orchard management factors. The third section was optional for growers to complete. It aimed to collect detailed temporal information on vine and management activities between March 2012 and February 2013. Answers in the third section needed to be accurate as they were to be used to combine daily risk exposures with daily disease prediction model data. There was some concern that time required to answer the third section might discourage some growers from returning the survey. To reduce that possibility the following note was included at the start of section 3:

"Thank you for filling in the survey to this point. We are aware that all growers may not have access to the required information to fill out the following section (section 3) accurately. If you do not have this information available please return your survey following completion of section 1 and 2 and leave section 3 blank. If you do have access to the information that would allow you to complete section 3 please do so as this is a valuable part of this survey."

5.2.3 Distribution of the questionnaire

Zespri provided block and production data for all registered 'Hayward' growers producing kiwifruit for export in 2012 and KVH provided data on Psa status and date of detection in orchards. Orchards that were recorded in both data sets formed our sampling frame (Figure 5-2), which comprised 3,309 kiwifruit growing operations in 17 regions throughout New Zealand. To be eligible for inclusion in the study, an orchard had to have producing 'Hayward' vines as of harvest in 2012 and either be in the Te Puke Psa infected region or classified as infected as of 1st January 2013 from another growing region. A decision was made to include all orchards in Te Puke as 98.5% of orchards in the region were confirmed as infected as of 1 January 2013. The method used to define which orchards were infected (cases) changed during the outbreak. Initially, cases were defined by the Ministry for Primary Industries as orchards with Psa confirmed by a diagnostic test. Later in the epidemic, when the number of infected orchards had increased substantially, KVH provided the case definition as orchards with Psa confirmed either by a diagnostic test or through the observation of visual symptoms of bacterial canker and confirmed by a technical representative of Zespri, KVH, a packhouse or similar industry body. The symptoms accepted as evidence of Psa during the growing season

were blackened canes or shoots with die-back and/or stem wilting. In winter diagnostic symptoms were weeping cankers with or without red or white exudate. The date of a positive diagnostic test, or the date visible signs of disease were reported, were recorded in the database as the date of confirmed infection. The KVH dataset also contained data regarding the elevation and the main packhouse for each orchard.

The options for distribution of the survey were telephone survey, online survey or postal survey. Phone-based surveys are known to improve response rates (Mannetje et al. 2011). However, for this study it was not considered economically viable because of the large sample size and the length of the questionnaire. If the targeted population is generally familiar with online technology and has easy internet access then there are advantages in using an online format. These are improved data quality, completeness of responses to individual questions, and the ability to restrict access to irrelevant questions (Lonsdale et al. 2006; Kongsved et al. 2007; de Bernardo & Curtis 2013). However, online distribution of the survey was not considered practical as rural internet access can be problematic in the Bay of Plenty and the complexity of Section 3 regarding timing of crop management activities would have made it difficult to configure. Using a postal format has been shown to increase response rates compared with internet-based surveys, especially for older respondents. Postal surveys also reduce response bias, as all eligible participants can access the survey (Kongsved et al. 2007; Borkan 2010; Partin et al. 2015). Therefore, a postal survey was selected to disseminate the questionnaire. If there were insufficient responses from the postal survey, a second round of postal questionnaires to non-responders was planned and if necessary follow-up phone surveys.

To maximise the response rate to the postal questionnaire, Zespri placed advanced notice of the survey in the industry newsletter in late February 2013, offered a $20 fuel voucher to growers on receipt of their completed survey by the due date, and included a post-paid return envelope.

The questionnaire was sent by Zespri to 1669 eligible ‘Hayward’ growers on 14 March 2013 with the request to return them before 12 April 2013. A reminder to complete the surveys was disseminated by Zespri in early April by email and included requests to packhouses to encourage their growers to return their surveys.

5.2.4 Response to the questionnaire

The response rate for the questionnaire was 26% (442 from 1669 eligible recipients). This rate of return is consistent with most kiwifruit industry questionnaires (M. Jopling, Zespri Ltd,

personal communication) and also typical of New Zealand farmer response rates to epidemiology and on-farm hygiene questionnaires (Greer & Teulon 2003; Van Toor & Teulon 2006; Neumann et al. 2013; Rosanowski et al. 2013b). Twelve respondents were excluded from further analysis either because they did not provide information about the disease status of the block (n=9) or other key information was missing (n=3). Therefore, the final dataset comprised data from 430 'Hayward' kiwifruit blocks.

5.2.5 Response bias

Non-responders can influence the validity of a study of this kind. With more than 70% of growers not returning the survey, bias in the results could occur if those who did not respond differed in a systematic way from the rest of the grower population. This type of bias is of concern when estimates of disease prevalence are extrapolated to a range of groups. For example, if the prevalence of severe symptoms was higher in high elevation orchards than in low elevation orchards, but more high elevation based growers had responded to the survey, any estimate based on such survey results would inflate the estimated disease prevalence on low elevation orchards. However, the primary objective of the present study was to collect data to identify risk factors associated with the introduction of Psa onto an orchard, and with severity of symptoms. A response bias is less likely to impact identifying risk factors than it would be in estimating disease prevalence in the population. This is because observational studies to determine factors that alter risk are based on biological processes and as such do not need to be based on a statistically representative population (Rothman et al. 2008a). For example, if a study conducted in 'Hayward' vines in one location showed that a management technique decreased disease, it need not necessarily be repeated in another location except to demonstrate a potential causal relationship or to determine if the size of the effect depends on other factors that may vary between the regions.

To analyse the potential for response bias, a range of factors for which information existed in the Zespri and KVH data sets were compared with whether a 'Hayward' grower responded or did not respond in order to identify any differences between factors that were common to non-responders. Response status was assigned based on those who responded (coded as 1) and those who did not respond (coded as 0). Data included the following variables: region, kiwifruit cultivar 'Hort16A' presence on the orchard, the main contracted packhouse for the orchard, organic or conventional management, orchard size (Ha), productivity in 2011 and 2012 (tray equivalents per hectare) and the number of days since Psa had been detected on the orchard.

For continuous data, box plots were constructed to visualise the relationship between response status and the orchard size (Ha), productivity in 2011 and 2012 (tray equivalents per hectare), and the number of days since Psa was first detected on the orchard. For each continuous variable, the significance of its relationship with response status was assessed using a two-sample t-test. Comparison of categorical variables was visualised in two-way tables and the relationship between response status and the region, pack-house, presence of 'Hort16A' or organic/conventional management was tested using Chi-squared tests or Fisher's exact test with simulated P-values (where sample sizes were small). Odds ratios for response status for all variables were calculated using simple logistic regression and exponentiation of the log odds.

The response status was not significantly associated with elevation, productivity in 2011 or 2012, days since Psa was first detected or the presence of 'Hort16A' kiwifruit vines. In contrast, organic producers were more likely to respond than those using conventional management (35% vs 25%; P=0.04).

When all of the regions were compared, there was a significant difference in response status between regions (P=0.002) with the response in Te Puke (the main kiwifruit growing region) being lower than elsewhere. However, there was no significant difference in response status between any of the larger growing regions (P=0.09) after regions with less than 50 eligible orchards were excluded. There was a significant difference in the response pattern according to the affiliation of a grower with a particular packhouse (P<0.001), which was not related to the numbers of affiliated growers supplying a packhouse. Of those packhouses with >50 supply orchards there were two with particularly high response rates (40% and 35%) and two packhouses with particularly small response rates (16% and 17%). The higher response rate for growers affiliated with two large pack-houses was likely linked to the efforts the packhouses made using grower newsletters and emails to encourage growers to respond. There was also a statistically significant difference in numbers of responders according to mean orchard size (4.4 ha for responders' vs 4.9 ha for non-responders; P=0.02) but the difference between orchard size was only half a hectare which is unlikely to be biologically important with an overall mean orchard size of 4.8 ha. If there was a greater difference in orchard size between those that responded and those that did not, there could be concern that large orchard management practices differ from much smaller orchards and therefore the results of the survey would not be valid for large orchards.

The higher response rates from organic growers and those who used particular pack-houses suggest some non-response bias may have been present.

5.2.6 Item omission

From the 430 orchard blocks included in the analysis, the item omission rate was very low with 0.23% to 1.16% of respondents not answering a question where an answer was expected. The questions not answered were always among eight questions, and of these the two that were most frequently left blank were the age of female and male vines (4/430 and 5/430 of respondents left these blank respectively). All other omitted questions had only one, two or three blanks among the respondents' surveys. On inspection of the data, missing values appeared to be missing at random with only one grower neglecting to answer two questions. This indicates that individual questions were easily interpreted. Although the numbers of omitted answers were very small there was a trend of more omitted answers towards the end of the questionnaire, indicating that the long period of time required to complete the questionnaire was close to exceeding grower tolerances.

5.3 Conclusion

This study showed that a postal questionnaire was an effective way to obtain disease, risk factor, and orchard hygiene data for a cross-sectional study on plant health. The use of a causal web to visualise factors in the study and to identify potential confounders and possible interactions between variables is recommended when designing observational studies (Dohoo et al. 2009e; Froud & Cogger 2015). In this study, visualising the causal web aided the development of the individual questions to ensure that data were collected from potential confounding variables. The collection of confounder data in this observational study will be important when the effects of orchard based environmental risk factors for disease are investigated in future analysis of these data.

The pre-testing of the questionnaire ensured that there was a clear understanding of the meaning of the questions by the respondents, providing confidence in the results obtained. If the researcher and the respondent have a different interpretation of a question, then interpretation and validity of results can be fundamentally biased.

This study obtained a typical response rate for this industry despite its length. We attribute this to growers' personal interest in Psa, the encouragement of some packhouses for their growers to complete the questionnaire, and the reward of the fuel voucher. If the cross-sectional study been investigating a less devastating disease, the length of the questionnaire

may have reduced the response rate below that sufficient for robust analysis (Edwards et al. 2002; Rolstad et al. 2011). The questionnaire described in this paper was very long and this may have influenced our overall response rate, and the slightly higher item omission rate near the end of the questionnaire.

The availability of industry data allowed us to compare respondents in the context of all potential participants in a survey and identify the potential for response bias between responders and non-responders (Groves 2006; Groves & Peytcheva 2008; Mannetje et al. 2011). The higher response rates from organic growers and those who used particular packhouses suggest some non-response bias may have been present and care will need to be taken in the future interpretation of the data set to address this.

Non-response may have two implications in a study of this type; firstly, if the aim is estimating the prevalence of disease for a population, where bias is known to be present it is important to present stratified results (i.e. report separate disease prevalence rates for conventional and organic growers). The second issue of non-response is the potential for bias around estimates of risk factors if there is a correlation between the outcome variable of interest (in our study this is presence of Psa) or key potential risk factors for the disease that could be associated with non-response (Mannetje et al. 2011). In this study, there was no difference between responders and non-responders associated with the time period that the disease had been present, a factor which could have affected the validity of future results. The data collected from this survey describe adequately the prevalence of Psa symptoms (Froud et al. 2015, Chapter 6) and the range of specific grower management practises, stratified for organic and conventional growers. The data can also be used to investigate risk factors that are associated with the introduction and severity of disease in commercial orchards and will also be useful to determine the relationship between weather risk, vine management wounds, protective spray applications and kiwifruit bacterial canker development.

5.4 Acknowledgements

Thank you to KVH for Psa detection data and survey review, to Greg Clark and Shane Max (Zespri International Limited), Jenny Natusch and Richard Klas (kiwifruit growers) for assistance with survey development. Thanks to Tracy McCarthy, Clare Morris, Madeleine Jopling and others (Zespri) for administering the questionnaire, the incentive programme and data entry. We would also like to thank the ten Whakatane region growers who pre-tested the questionnaire, and for their very valuable feedback. This project was funded by the Zespri and

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CHAPTER 6

6 Kiwifruit bacterial canker in 'Hayward' kiwifruit: Orchardist-observed prevalence of symptoms

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Note: The scientific name for 'Hayward' and 'Hort16A' kiwifruit changed during the writing of this thesis from *Actinidia deliciosa* and *Actinidia chinensis to Actinidia chinensis* var. *deliciosa* and *Actinidia chinensis* var. *chinensis*. In addition, the common usage of Psa changed to Psa or Psa biovar3 rather than Psa-V. This paper was published using the former nomenclature and therefore this chapter is presented with the original terms.

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STATEMENT OF CONTRIBUTION TO DOCTORAL THESIS CONTAINING PUBLICATIONS

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We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Karyn Froud

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8/12/2016
Date

Orchardist-observed prevalence of symptoms of kiwifruit bacterial canker disease in 'Hayward' kiwifruit blocks in New Zealand

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Keywords: *Actinidia deliciosa,* 'Hayward', cohort, questionnaire, observational study.

6.1 Abstract

In November 2010, a virulent strain of *Pseudomonas syringae* pv. *actinidiae* biovar 3, the cause of bacterial canker in kiwifruit, was first recorded in New Zealand. The disease caused by this pathogen is commonly referred to as Psa-V in New Zealand. Initially the impacts of Psa-V were most severe in the gold-fleshed kiwifruit cultivar 'Hort16A' (*Actinidia chinensis*). More recently there have been reports of symptoms affecting the green-fleshed cultivar 'Hayward' (*Actinidia deliciosa*). In 2013, a study was undertaken of Psa-V in 'Hayward' orchards to investigate relationships between disease expression observed by orchardists and environmental, management and vine-related factors. This paper presents initial results from that study on the Psa-V symptoms observed in the field by orchardists. Questionnaires sent to the owners of 1669 randomly selected 'Hayward' blocks from different orchards were returned for 26.4% (442/1,669) of the blocks and 430 of these were suitable for analysis. Eighty-four percent (363/430) of respondents reported observing Psa-V symptoms in the selected block between March 2012 and February 2013. The most common symptom reported on female vines was leaf spot (76%), cane die-back (31%) and green shoot wilting (30%). In the same blocks the most common symptoms reported on male vines were leaf spot (70%), cane die-back (46%), red exudate (39%), and green shoot wilting (32%). Bud drop was reported from 41% of female vines and 33% of male vines, although this symptom can be caused by other pathogens. Although these results indicate a high prevalence of severe Psa-V symptoms in 'Hayward' blocks, most growers reported low numbers of vines being affected within the blocks.

6.2 Introduction

In November 2010, a virulent strain of *Pseudomonas syringae* pv. *actinidiae* biovar 3 (Vanneste et al. 2013), the cause of bacterial canker in kiwifruit, was first recorded in New Zealand. The disease caused by this pathogen is commonly referred to as Psa-V in New Zealand (Everett et al. 2011). Psa-V affects kiwifruit plants by causing leaf spotting, shoot and cane die-back and stem cankers and, in severe cases, the whole vine is affected, leading to death and/or removal from the orchard. Psa-V has had a major impact on 'Hort16A' vines (*Actinidia chinensis*) and in 2011 started to be reported more frequently to be affecting 'Hayward' vines (*Actinidia deliciosa*) in New Zealand, as has previously been observed in Italy (Ferrante & Scortichini 2009; Ferrante et al. 2012; Vanneste 2012).

Leaf spotting, which is a less damaging symptom to the vine, occurs commonly in 'Hayward' orchards within Psa-V infected regions of New Zealand. Reports of more severe symptoms such as leaf wilt and cane die-back were being received by industry in the spring of 2011 from concerned 'Hayward' growers. In addition, there were reports that male vines of various cultivars, used as pollinators of 'Hayward', were more severely affected and may be contributing to increased disease symptoms in the female 'Hayward' vines. The prevalence of severe symptoms of Psa-V in commercial 'Hayward' orchards was unknown. Severe symptoms have been observed in Italy on this cultivar (Ferrante et al. 2012).

The aim of this research was to describe the prevalence of Psa-V symptoms in commercial 'Hayward' kiwifruit orchards. This study is part of a larger study that aims to identify factors associated with the likelihood of Psa-V being introduced into an orchard in a recently infected region and factors associated with severe symptoms of disease (disease severity) within orchards already infected with Psa-V.

6.3 Methods

We used an observational study design which involved the administration of questionnaires to 'Hayward' kiwifruit growers based on their Psa-V exposure.

The questionnaire was drafted in consultation with technical experts from Zespri Group Limited (Zespri), Kiwifruit Vine Health (KVH) and two 'Hayward' growers. It was pre-tested in interviews with 10 growers and then finalised following feedback from pre-testing.

A Psa-V infection status dataset was provided by KVH which could be linked to other industry data. Zespri provided production data for 2012. Data from KVH and Zespri were merged into a

single data set and this formed the sampling frame from which eligible orchards and blocks were selected.

To be eligible for inclusion in the study orchards had to:

- Have producing 'Hayward' vines as of harvest in 2012
- Either be in the Te Puke region (which had 98.5% of orchards confirmed with Psa-V by 1 March 2013) or
- Be located within another growing region and have tested positive for Psa-V before 1 January 2013.

For each eligible orchard, one 'Hayward' block was randomly selected from the database using the R statistical package v. 3.0.1 (R Core Team 2013).

The questionnaire was administered by mail by the Zespri Grower Services team and sent to 1669 eligible 'Hayward' growers on 14 March 2013. Growers were asked to return their completed questionnaires before 12 April 2013. To maximise the return of questionnaires from growers, Zespri offered a $20 fuel voucher to growers on receipt of their completed survey. Copies of the questionnaire and the cover letter are in Appendix 1 and 2.

A database form to enable rapid entry of the questionnaire data was designed using EpiData software (Lauritsen & Bruus 2013) and data were entered directly by Zespri staff into an EpiData database. Data were extracted from EpiData into MS® Excel and stored in MS® Access.

Of the 442 survey forms returned to Zespri for data entry, 12 were removed because of missing information for key variables, leaving 430 observations (blocks) in the final dataset.

A binary variable for block Psa-V status at the end of the study (February 2013) was coded as 1 if respondents answered "yes" to the question "Do you have any visible Psa-V symptoms in the block as of February 2013 (including old spotting/symptoms)?" and 0 if they answered "no".

Psa-V symptoms (Table 6-1) were described as binary variables for female and male vines. Growers were concerned that bud drop might be caused by Psa-V, so this was included in the questionnaire along with known Psa symptoms. Each binary variable was coded 0 if no vines (or buds) were affected in the block and 1 if some were affected. Growers were asked if Psa-V symptoms in female vines were more or less severe than those in male vines and responses were coded as a categorical variable.

MS Excel and R were then used to assess the completeness and validity of the aggregated data set.

6.4 Results

The response rate was 26.4% (442 from 1669 eligible recipients); of these, 430 had completed the disease severity data which was the key outcome data for analysis. This return rate is what was expected for the study group (Zespri Grower Services, pers. comm.).

Overall, 84% of our survey respondents reported Psa-V symptoms from either male or female vines in the block on which they were asked to report. There were slightly more blocks with only female vines showing symptoms (78%) than blocks with symptoms only on males (76%), and 62% reported symptoms on both sexes. Of the blocks where symptoms were observed, when asked whether disease symptoms on female vines within 'Hayward' blocks were more or less severe than those on male vines, 18% stated that females were worse, 39% stated that male vines were worse than females and 21% stated they were the same.

The two most distinctive Psa-V symptoms (i.e. symptoms that are unlikely to be caused by other agents) are cane die-back and green shoot wilt. For these two symptoms, males had a higher prevalence of both symptoms, particularly cane die-back - with 46% in males compared with 31% in females (Table 6-1).

The most common symptoms reported on female vines were leaf spot, cane die-back, green shoot wilt and red exudate. In the same blocks the most common symptoms reported on male vines were leaf spotting, cane die-back, stem cankers and green shoot wilt (Table 6-1).

While it has not been established that bud-drop is caused by Psa-V, those blocks with Psa-V symptoms reported bud drop on female vines in 41% of blocks and bud-drop on male vines in 33% of blocks. Bud drop was not reported in the 67 blocks that did not report other symptoms of Psa-V.

Table 6-1 Percentage of randomly selected 'Hayward' kiwifruit blocks with various symptoms attributed to *Pseudomonas syringae* pv. *actinidiae* (Psa-V) that were reported for the period March 2012 and February 2013 (n=430).

Visible symptoms	Symptom observed by grower	
	Female vines	Male vines
Leaf-spot	75.6%	69.8%
Green shoot wilt	30.5%	31.9%
Cane die-back	31.4%	45.8%
Stem canker	8.6%	23.3%
Red exudate	17.9%	39.1%
White exudate	3.0%	9.5%
Bud drop[1]	41.2%	32.8%
Other symptoms	1.6%	2.1%

[1] It is not confirmed that bud drop is caused by Psa-V.

6.5 Discussion

It was valuable to undertake a pre-test in this study and it was an important step in the questionnaire design process. Analysis of results can be fundamentally biased if the researcher and the respondent have different interpretations of a question.

The results showed that there was a higher prevalence of severe Psa-V symptoms in males than in females. This could have consequences for pollination in the future, as growers may regard the retention of male vines in orchards as presenting a higher risk than using artificial pollination.

Bud drop was widely reported from symptomatic blocks. Research programmes to understand bud drop and its management are underway.

The next stages of this research will investigate a range of host, environment and management factors that may contribute to either the initial introduction of Psa-V into an orchard or that may be associated with the expression of severe symptoms, and to explore the relationship between weather and timing of key orchard management operations (e.g. girdling, pruning) and protective spray applications. The most distinctive symptoms of Psa-V in 'Hayward' kiwifruit, compared with symptoms of other possible pathogens, are cane die-back and green shoot wilt. It is these two symptoms that will be used for future analyses. The results from these studies will be used to guide strategies for the management of Psa-V.

6.6 Acknowledgements

Thank you to KVH for Psa-V detection data and survey review, to Shane Max (Zespri Group Ltd), Jenny Natusch and Richard Klas (kiwifruit growers) for assistance with survey development. Thanks to Tracy McCarthy, Clare Morris, Madeleine Jopling and others (Zespri Group Ltd) for administering the questionnaire, the incentive programme and data entry. We would also like to thank the ten Whakatane region growers who pre-tested the questionnaire, and for their very valuable feedback. This project was funded by the Zespri and Kiwifruit Vine Health Psa research and development programme under contract number V11367.

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CHAPTER 7

7 Kiwifruit bacterial canker in 'Hayward' kiwifruit: Management practices, environmental features and disease onset of *Pseudomonas syringae* pv. *actinidiae* in 'Hayward' kiwifruit orchards in New Zealand

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Management practices, environmental features and disease onset of *Pseudomonas syringae* pv. *actinidiae* in 'Hayward' kiwifruit orchards in New Zealand

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7.1 Abstract

Kiwifruit bacterial canker caused by *Pseudomonas syringae* pv. *actinidiae* biovar 3 (Psa) may threaten the long-term productivity of 'Hayward' kiwifruit (*Actinidia chinensis* var. *deliciosa*) in New Zealand. This paper describes results from a 2013 survey on orchard and management factors that could affect the risk of Psa in kiwifruit orchards. In the blocks we studied, the median age of vines was 30 years for females and 25 years for males. Key factors that were common were frost damage, reported by 25% of growers, girdling, used by 65% of growers and artificial pollination, used by 36% of growers. Post-pruning sprays were used by 75% of growers and most growers also applied protective sprays and used orchard hygiene practices. The disease was present in 84% of surveyed kiwifruit blocks and 75% reported the first appearance of disease in spring. This study has quantified the current practices and layout of commercial orchards and provides information on management operations that are used widely within the New Zealand kiwifruit industry which could be manipulated to reduce the effects of Psa on kiwifruit production.

Keywords: Psa, Biovar 3, *Actinidia chinensis* var. *deliciosa,* cross-sectional, observational study, questionnaire

7.2 Introduction

Bacterial canker is a serious disease of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae* biovar 3 (Psa) that was first reported in New Zealand in November 2010 (Everett et al. 2011). Bacterial canker has had a major impact on the highly susceptible kiwifruit cultivar 'Hort16A' (*Actinidia chinensis* var. *chinensis*). However, the long-term impact on 'Hayward' (*Actinidia chinensis* var. *deliciosa*) is unclear (Ferrante & Scortichini 2009; Ferrante et al. 2012; Vanneste 2012; Froud et al. 2014). Maintaining the productivity of 'Hayward' in the presence of Psa is an important aim for the New Zealand kiwifruit industry. Understanding whether improvements could be made to orchard management to control kiwifruit bacterial canker, first requires an understanding of the range of management practices that are currently used in commercial kiwifruit orchards and their frequency of use. Effective decision making on research investment during an outbreak of a new disease or pest ideally requires the affected industry to quantify the number of orchards that would benefit from knowledge of growing practices that increase or decrease disease risk. Likewise, to assess the impact of industry changes to manage a new disease outbreak, it is useful to quantify the uptake of disease management and hygiene measures in commercial orchards.

When the multiple factors that might influence disease are studied simultaneously, an observational study provides a more effective alternative to single- or limited-factor experimental designs (Thebaud et al. 2006; Dohoo et al. 2009e). Observational studies, such as cross-sectional, cohort and case-control studies, are often used in veterinary and medical research, but rarely in plant protection research (Froud & Cogger 2015). This study used a cross-sectional design and questionnaire to obtain survey data from growers on the prevalence of disease and the frequency of environmental, host and management factors in commercial orchards. Disease prevalence data informs industry of the size of the disease problem, and frequency data indicates how many growers are undertaking management activities which could be manipulated to reduce the impact of disease.

This study aims to quantify: a) the orchard features of a typical block; b) the current management practices; c) the uptake of Psa management recommendations; and d) the disease onset and symptoms of Psa-infected commercial orchards. This will identify orchard and management factors that are used widely within the New Zealand kiwifruit industry and which could be modified or controlled to reduce the effects of Psa on kiwifruit production. This paper is the third in a series and is part of a larger study to identify risk factors. The first two papers in the series described the design and dissemination of the grower survey

questionnaire (Froud et al. 2016, Chapter5) and the prevalence of the disease (Froud et al. 2015, Chapter 6) in 'Hayward' kiwifruit orchards in New Zealand.

7.3 Methods

7.3.1 Study design and data collection

The survey used a quantitative questionnaire which was sent to growers in March 2013 (Froud et al. 2016, Chapter 5). The study required growers to report detailed information on the occurrence of symptoms of disease and management of vines at the level of a single 'Hayward' kiwifruit block. Technical experts from Zespri International Ltd (Zespri), Kiwifruit Vine Health (KVH) and two kiwifruit growers assisted with the identification of factors for inclusion in the study. The questionnaire is provided in Appendix 1 and 2 and a summary of the topics covered in the questionnaire is presented in Table 7-1.

The questions were constructed to obtain quantitative data using closed questions with growers asked to select answers from a range of options. Three question formats were used: i) select all that apply (e.g. select all forms of pollination used); ii) select one possible answer from a list (e.g. did you use artificial pollination); and iii) select a rating based on a defined scale (e.g. canopy density ratings). The questionnaire was reviewed by industry technical experts and pre-tested with 10 growers to identify questions that were confusing, ambiguous or misleading, to determine if the layout and instructions given to respondents were appropriate and to identify additions to multiple choice answer options.

Table 7-1 Topics covered in the mail-out questionnaire used to collect information from 'Hayward' (Actinidia chinensis var. deliciosa) blocks located in orchards affected by *Pseudomonas syringae* pv. *actinidiae* biovar 3 (Psa)

Question number	Factor category	Information sought
1-9	Disease status	Description of disease prevalence and symptoms on female and male vines
10, 11, 18–21	Orchard layout	Adjacent land: Current use, whether kiwifruit had been cut out, if kiwifruit present, the variety grown and Psa status
16, 22	Orchard layout	Block shape and type of shelter
17	Vine management	Whether organic or conventional management
23–26	Vine management	Pollination methods used in 2011/12 and 2012/13 and, if artificial pollination was used, the source of pollen
27–29	Vine management	Type of frost protection used and severity of frost damage
30	Vine management	Type of irrigation used

31	Vine management	Canopy density rating (1-5) based on the Zespri Kiwigreen Manual 1. Open canopy with more than 30% gaps and grass cover 2. Open canopy with less than 30% gaps and grass cover 3. Closed canopy with little grass cover 4. Dense canopy with grass cover in patches only 5. Dense canopy with no gaps and no grass cover
32, 33	Vine management	Girdling of female vines in 2011/12 and 2012/13 growing seasons
34, 35	Vine management	Girdling of male vines in 2011/12 and 2012/13 growing seasons
36, 37	Vine management	Management of vine pruning and Psa infected material
38, 39, 42, 43	Orchard layout	Female vine age, variety and ratio to male vines
40–42	Orchard layout	Male vines including variety, age and layout in orchard
45–47	Disease management	Whether weather information was used when planning orchard activities and the source of the weather information
48, 49	Disease management	Who sprays crops for disease and reasons why spraying might have been delayed
50, 51	Disease management	Sprayer information including ownership, number of orchards sprayer is used in, and calibration
53, 54	Disease management	Use of disease hygiene measures for equipment and when pruning and girdling

To be eligible for inclusion in the study, an orchard had to have producing 'Hayward' vines at harvest in 2012 and be classified as infected with Psa on 1 January 2013. Further details on inclusion criteria are given in Froud et al. (2016). Zespri provided block and production data for all registered 'Hayward' growers producing kiwifruit for export in 2012, and KVH provided data on Psa status and date of first detection in orchards. Orchards that were recorded in both data sets formed the sampling frame. The block that was to be reported on was randomly selected from the sampling frame using an algorithm written in the 'R' freeware statistical package version 3.0.1.

The questionnaire was sent by Zespri Grower Services to 1669 eligible 'Hayward' growers in March 2013 and 442 responded. Data were entered into a database using a purpose built form designed to minimise data entry errors created using the EpiData software (Lauritsen & Bruus 2013).

7.3.2 Data analysis

Microsoft Access was used to combine the questionnaire data with the industry data sets. MS Excel and the 'R' freeware statistical package version 3.0.1 were used to assess the completeness and validity of the aggregated dataset.

For the majority of orchard description questions, answers were coded as either present or not present, or used or not used. Where the grower could choose between multiple levels these answers were coded as multi-level categorical variables. For example, frost damage was a categorical variable with levels of no damage, minor damage, moderate damage or severe damage. For variables that were very similar, such as adjacent land-use categories of gully, bush and forest, and for those for which there were few observations, and it was appropriate biologically to combine categories, the data were combined into new variables. For these, the new variable is presented in the results table, below the component variables. In addition, continuous variables were visually assessed using boxplots and histograms and those that were not normally distributed were recoded as multi-level categorical variables or binary variables, such as the percentage of blocks with frost damage.

The distribution of continuous variables was explored using histograms. Non-normally distributed data were summarised using quartiles. Means and standard deviation were used to summarise normally distributed variables. Categorical data was summarised using counts and percentages in each level.

7.4 Results

442 responses to the grower survey were received, with 12 responses excluded from further analysis either because they did not provide information about the disease status of the block (n=9), or other key information was missing (n=3). Therefore, the final dataset for this study comprised data for 430 blocks from 'Hayward' kiwifruit orchards. The regional distribution of survey respondents is shown in (Table 7-2). There were 205/430 (48%) responses from growers from the Te Puke area which is both the largest growing area for kiwifruit and also the first region in which Psa (biovar 3) was detected in New Zealand. Forty-two of the 430 respondents were organic growers with the remainder using conventional growing systems: these two growing systems are described in Carey et al. (2009).

Table 7-2 Number and percentage of respondents by region out of 430 'Hayward' orchards from Psa infected regions at 1 January 2013.

Growing region/area	Number of respondents	% of respondents	Date Psa first reported in region
Franklin	2	0.005	21/11/2011
Waikato	4	1	20/8/2012
Coromandel	8	2	31/8/2012
Waihi	5	1	19/9/2011
Katikati	64	15	26/9/2011
Tauranga East	50	12	5/8/2011
Tauranga West	37	9	18/10/2011
Te Puke	205	48	5/11/2010
Whakatane	29	7	29/9/2011
Opotiki	24	6	20/10/2011
Poverty Bay	1	0.002	20/11/2012
Hawkes Bay	1	0.002	10/10/2012

7.4.1 Orchard layout — female and male vine age

The median age of female vines in the selected blocks was 30 years (25th percentile =20; 75th percentile = 33 years) compared with 25 years for male vines (25th percentile =11; 75th percentile = 31 years; Figure 7-1). All the female vines were *A. chinensis* var. *deliciosa*, and dominated by the 'Hayward' cultivar (375 of 430; 87%). A strain of 'Hayward' known as the "Kramer" clone was also present (48 of 430; 11%), and 18 of 430 (4%) growers did not know which of the two were in the block. Eleven blocks had both 'Hayward' and the "Kramer" clone present. Only one block had no male vines and the dominant male cultivar of *A. chinensis* var. *deliciosa* was 'Chieftain' (365 of 430; 85%) followed by 'Matua' (94 of 430; 22%), 'M series' (92 of 430; 22%) and 'M56' (76 of 430; 18%). It was also common to have more than one male cultivar present in blocks (164 of 430; 38%). The dominant male vine planting system was 'opposing female', where individual male vines are placed in the same rows as female vines (249 of 430; 58%), with the remainder using a strip male system (180 of 430; 42%) in which a strip of male vines are planted in rows between female rows. The ratio of male to female vines in the opposing females planting system ranged from 1:1 to 1:10, with the majority having a 1:4 (84 of 245; 34%), 1:6 (65 of 245; 27%), 1:8 (33 of 245; 13%) or 1:5 ratio (29 of 245; 12%). The ratio of male to female rows in the strip male systems varied from 1:1 to 1:8, however the majority were either 1:1 (148 of 180; 82%) or 1:2 (23 of 180; 13%). Of the growers using the strip male system, 112 of 180 (62%) trained male leaders and canes over 2 to 3 bays. Only 53 of the 180 (29%) growers that used the strip male system also used artificial pollination, compared with 100 of the 249 (40%) growers that used the opposing female system. In

addition, the median age of strip male vines was 15 years compared with 28 years for male vines in opposing female systems.

While the majority of the randomly selected blocks from the Zespri database were structured as single blocks (364 of 430; 85%), 66 of 430 (15%) blocks were made up as a composite of several smaller blocks within the same orchard and which are managed in the same manner.

7.4.2 Orchard layout — adjacent land use

Growers were asked what the land adjacent to the block was used for and could select any land use options that applied. Most blocks were immediately adjacent to either another 'Hayward' block on their orchard and/or a neighbour's orchard, with "adjacent to a farm paddock" the next most common adjacent land-use (Table 3). The results showed that 74 of 430 (17%) blocks were adjacent to gullies which are potential harbours of Psa-infected wilding kiwifruit vines (Kiwifruit Vine Health Inc. 2015). Additionally, 27% of blocks were immediately adjacent to kiwifruit that had been cut out because of Psa infection, of which 82 of 116 (71%) had been 'Hort16A' vines (Table 7-3).

Table 7-3 Description of the land use immediately adjacent to 430 'Hayward' kiwifruit blocks, along with adjacent kiwifruit cultivars on the same orchard or neighbouring orchards. Each block could have multiple types of adjacent land use.

Adjacent land use	Number of blocks	% of blocks
Kiwifruit, same orchard	360	84
Kiwifruit, neighbour's orchard	227	53
Cut out kiwifruit block	39	9
Paddock/farmland	153	36
Residential buildings	63	15
Kiwifruit packhouse	7	2
Road	76	18
Other horticulture crop	36	8
Waterway/stream/lake	22	5
Gully	74	17
Forestry	15	3
Native bush/forest	32	7
Combined variable of gully, bush or forest	93	22
Orchard buildings	22	5
Commercial buildings	3	1
Other crop packhouse	2	0
Estuary/coastland	5	1
Other adjacent land use	5	1
'Hayward' adjacent — same orchard	318	74
'Hort16A' adjacent — same orchard	24	6
'G3' adjacent — same orchard	41	10
'G9' adjacent — same orchard	20	5
'G14' adjacent — same orchard	8	2
'Hayward' adjacent — neighbours orchard	181	42
'Hort16A' adjacent — neighbours orchard	49	11
'G3' adjacent — neighbours orchard	28	7
'G9' adjacent — neighbours orchard	12	3
'G14' adjacent — neighbours orchard	6	1

7.4.3 Orchard layout — Type of shelter and orchard elevation

The most common shelter species was Japanese cedar (*Cryptomeria japonica*) on 66% of blocks, followed by she-oak (*Casuarina* sp.) on 41% of blocks (Table 7-4). Willow and poplar shelters were also relatively common. There were 193 blocks with a single shelter belt type and it was common to have a mix of shelter species with 105 having two species, 78 with three species and 43 with four or more species (maximum of seven types). Fast track shelter, a white windbreak cloth used under the canopy within blocks, was present in 34 of 430 (8%) blocks.

Table 7-4 Shelter belt types adjacent to 430 'Hayward' kiwifruit blocks. Each block could have multiple types of adjacent shelter species.

Shelter type	Number of blocks	% of blocks
Japanese cedar (*Cryptomeria japonica*)	282	66
She-oak (*Casuarina* sp.)	178	41
Willow (*Salix* sp.)	64	15
Artificial shelter	55	13
Pine (*Pinus* sp.)	44	10
Cypress (*Cupressus* sp.)	35	8
Poplar (*Populus* sp.)	32	7
Gum (*Eucalyptus* sp.)	12	3
None specified	11	3
Italian alder (*Alnus cordata*)	9	2

The frequency distribution of orchard elevation was highly skewed towards low elevation with a median of 39 m above sea level (first quartile 19 m; third quartile 109 m).

7.4.4 Vine management — type of frost protection and frost damage

A total of 166 of the 430 (39%) growers used frost protection in their blocks with overhead water the most common type (82 of 166; 49%), followed by wind machines (n=37), ThermoMax™ (a biodynamic plant spray, 22 of 166; 13%) and nitrogen foliar sprays (12 of 166; 7%). Use of under-vine sprinklers was uncommon (n=8) as was the use of helicopters, burners and fans (4 growers each). Frost damage was reported from 107 of 430 (25%) orchard blocks, half of which had used no frost protection (54 of 107; 50%). A summary of the severity and extent of frost damage is given in Table 7-5.

Table 7-5 Severity of frost damage observed by growers in spring 2012, and a description of how much of the block was affected by frost in 430 'Hayward' kiwifruit blocks.

Frost damage	Level	Number of blocks	% of blocks
Severity of damage	No frost damage	323	75
	Minor damage (leaves singed)	77	18
	Moderate damage (whole leaves affected)	20	5
	Severe damage (whole shoots affected)	10	2
	Combined variable of moderate or severe damage	30	7
Estimate of vines damaged	No vines damaged in block	324	75
	A few isolated vines with frost damage (1–5%)	61	14
	Less than a quarter of vines with frost damage (6–25%)	32	7
	Less than half the vines with frost damage (26–50%)	7	2
	More than half the vines with frost damage (51–75%)	1	0
	Most/all of the vines with frost damage (76–100%)	5	1

Only 135 of the 430 (31%) growers used irrigation in their 'Hayward' block, with most using under-vine sprinklers (88 of 430; 20%) and drip-line irrigation (36 of 430; 8%), and only 18 (4%) using overhead irrigation.

7.4.5 Vine management — pollination system

Introduction of bees was the most common pollination method (384 of 430; 89%) followed by artificial pollination (Table 7-6) and more than one method of pollination was often used. Fewer orchards used artificial pollination in the 2011/12 season (85 of 430; 20%) than in the 2012/13 season (153 of 430; 36%).

Table 7-6 Methods of pollination used for 430 selected 'Hayward' kiwifruit blocks during the 2011/12 or the 2012/13 flowering period (October) and a description of the source and application method for artificial pollination users in 2011/12 (n=85) and 2012/13 (n=153).

Pollination method	Number of blocks	% of blocks	Number of observations[1]
Used bee hives for pollination 2011/12	378	88	430
Used bee hives for pollination 2012/13	384	89	430
Used artificial pollination 2011/12	85	20	430
Used artificial pollination 2012/13	153	36	430
Used natural wind/bees only for pollination 2011/12	40	9	430
Used natural wind/bees only for pollination 2012/13	20	5	430
Used wind blower for pollination 2011/12	10	2	430
Used wind blower for pollination 2012/13	10	2	430
Used both bees and artificial pollination 2011/12	74	17	430
Used both bees and artificial pollination 2012/13	130	30	430
Only used bee hives for pollination 2011/12	296	69	430
Only used bee hives for pollination 2012/13	254	59	430
Only used artificial pollination 2011/12	11	3	430
Only used artificial pollination 2012/13	23	5	430
Artificial pollination source 2011/12- own flower	26	31	85
Artificial pollination source 2011/12- commercial	59	69	85
Artificial pollination source 2012/13 - own flower	42	27	153
Artificial pollination source 2012/13 - commercial	113	74	153
Dry application method for artificial pollination 2011/12	75	88	85
Wet application method for artificial pollination 2011/12	10	12	85
Dry application method for artificial pollination 2012/13	137	90	153
Wet application method for artificial pollination 2012/13	17	11	153

[1] Number of observations relates to either all orchard blocks in the study or just those that used artificial pollination in the 2011/12 or 2012/13 season.

7.4.6 Vine management

Most of the 430 growers reported an open canopy, that is 94 (22%) reported a canopy score of 1 and 252 (59%) reported a canopy score of 2. Of the growers that reported a closed canopy, 63 (15%) reported a score of 3 and 18 (4%) reported a dense canopy with a score of 4. A single grower reported a very dense canopy rating of 5.

Girdling involves cutting into the cambium of the vine to increase fruit size and dry matter. Girdling of female vines was common with 279 of 430 (65%) growers girdling in 2011/12, split into 105 growers girdling in spring, 115 in summer and 59 in spring and summer. This was very similar in the 2012/13 season with 286 of 430 (67%) blocks with female vines girdled, split into 105 spring, 116 summer and 65 spring and summer. Girdling of male vines was very rare with 12 of 430 (3%) blocks girdled in 2011/12 and 14 of 430 (3%) in 2012/13. Growers were also asked about disease protection measures used during girdling. Of the 290 growers that girdled in either year, 197 (68%) stated that they dip their girdling equipment in disinfectant between vines, a further 41 (14%) dip their equipment between blocks and the remaining 51 of 290 (18%) do not sanitise their equipment.

7.4.7 Disease management

396 of the 430 (92%) growers stated that they use weather data to plan vine management activities and 377 of 430 (88%) growers use weather data to manage disease spraying. The KVH Psa-V Risk Model, which was available from early 2012, was used by 92 of 430 (21%) growers to time vine management actions and disease sprays on their orchard blocks.

There were several vine management hygiene measures routinely used by growers (Table 7-7). Most growers (365 of 430; 85%) did not allow pruners to work during wet weather. Of the 33 growers (8%) that stated that they did not clean their pruning equipment, 18 used their own equipment within their orchards. Over half the growers (241 of 430; 56%) either cleaned their pruning equipment between individual vines or between bays, which typically consist of two vines.

Table 7-7 Disease hygiene measures used routinely for pruning equipment used by 430 'Hayward' kiwifruit growers with Psa infected orchards. Growers could select all that applied.

Routinely used hygiene measures	Number of blocks	% of blocks
Use orchards own equipment	107	25
Do not routinely clean equipment	33	8
Clean equipment between vines	125	29
Clean equipment between bays	169	39
Clean equipment between blocks	131	30
Clean equipment daily	166	39
Clean equipment on arrival at orchard	236	55

Growers were asked how they managed pruned material both from seasonal vine pruning, and for shoots, canes and leaders cut out from diseased vines (infected leaves and buds were excluded). Their responses are given in Table 7-8. For seasonal pruning 390 of 430 (91%) growers removed or mulched prunings within two weeks and only 66 of 430 (15%) left them on the ground for more than two weeks. There were 109 of 430 (25%) blocks with no diseased shoots, canes, leaders or vines. Of those with disease, 113 of 321 (35%) growers did not cut out diseased material. Those growers with diseased plant material that did cut out either removed diseased material from the orchard (153 of 321) or mulched it immediately (43 of 321) and five growers selected both options (Table 7-8).

Table 7-8 Management of kiwifruit vine pruning material for normal vine management (n=430 orchard blocks) and also for blocks (n=321) that reported Psa infected shoots, canes, leaders or vines between March 2012 and February 2013. Growers could select all answers that were applicable.

Variable	Number of blocks	% of blocks	No. observations
Vine prunings collected and removed from block	3	1	430
Vine prunings mulched immediately after pruning	264	61	430
Vine prunings mulched within 2 weeks of pruning	123	29	430
Vine prunings mulched within 1 month of pruning	28	7	430
Vine prunings left on the ground beneath vines	38	9	430
No diseased shoots, canes, leaders or vines in block	109	25	430
Diseased material cut-out and removed from block	153	48	321
Diseased material cut-out and mulched immediately	43	13	321
Diseased material cut-out and mulched within 2 weeks	14	4	321
Diseased material cut-out and mulched within 1 month	5	2	321
No diseased material was cut-out of block	113	35	321

The use of protective sprays for disease prevention during pruning, were also investigated and the results are given (Table 7-9). Three growers did not answer this question. A combined total of 329 of 427 (77%) growers sprayed vines after pruning and 54 of these growers also applied a pre-pruning spray.

Table 7-9 Application of protective sprays to manage Psa risk during pruning for 427 'Hayward' kiwifruit growers with Psa infected orchards. Growers could select all that applied.

Variable	Number of blocks	% of blocks
No pruning protection measures	16	4
Special pre-pruning protective spray applied	63	15
Pruning protection measures pruning follow-up back pack spray	40	9
Pruning protection measures spray full block at end of pruning	258	60
Pruning protection measures instant wound protection with hand spray	142	33
Pruning protection measures pruned rows sprayed at end of day	54	13

7.4.8 Disease management — spraying

Questions about the use of protectant sprays to control Psa included who applied sprays, what equipment was used and how regularly it was calibrated (Table 7-10). The most significant reasons for delays in applying sprays against Psa once a decision to spray had been made are also detailed in Table 7-10. The most notable reason for delaying protective sprays was unfavourable wet weather (339 of 428; 79%). Of the growers who used spray contractors, 132 of 228 (58%) stated that the availability of spray contractors was a significant reason for delayed sprays. There was a clear increase in the number of growers using their own equipment in their block after Psa was first detected in New Zealand (November 2010; Figure 7-2).

Table 7-10 Description of Psa protective spray variables for 430 'Hayward' orchard blocks. Growers could select all that applied.

Psa protective spray variables	Number of blocks	% of blocks	No. observations[1]
Disease spraying done by owner	176	41	430
Disease spraying done by contractor	228	53	430
Disease spraying done by orchard manager	36	8	430
Disease spraying done by orchard worker	24	6	430
Own sprayer (exclusive to orchard) mostly used to apply disease sprays on block	131	31	422
Own sprayer (multiple orchards) mostly used to apply disease sprays on block	75	18	422
Combined variable — Own sprayer used[2]	195	46	422
Contractors equipment mostly used to apply disease sprays on block	229	54	422
Own sprayer calibrated last 6 months	70	36	192[3]
Own sprayer calibrated last 12 months	85	44	192
Own sprayer calibrated last 24 months	20	10	192
Own sprayer not calibrated recently	17	9	192
Sprays delayed due to unfavourable wet weather	339	79	428
Sprays delayed due to risk of spray drift	121	28	428
Sprays delayed due to orchard workers working in the block	50	12	428
Sprays delayed due to spray contractor availability	136	32	428
Sprays delayed due to withholding periods	41	10	428
Sprays delayed due to incompatible spray usage	46	11	428
Sprays delayed due to spray equipment availability	18	4	428

[1] The number of observations reflects the full data set and includes a subset of the blocks where the growers use their own spray equipment (n=206). [2] There were 11 growers that selected own sprayer exclusive to orchard and own sprayer used on multiple orchards. [3] Three growers that use their own spray equipment did not answer the calibration question.

7.4.9 Disease status and onset

The grower-observed prevalence of kiwifruit bacterial canker in selected 'Hayward' orchard blocks was 84% (363/430). However, within blocks grower estimates of the percentage of vines showing disease symptoms was low, especially for symptoms other than leaf spotting on female vines (Table 7-11). Further details of disease prevalence and a summary of symptoms are reported in Froud et al. (2015, Chapter 6).

Table 7-11 Number of 'Hayward' blocks in which a symptom was observed out of 430 'Hayward' orchards from Psa infected regions as of 1 January 2013, along with the percentiles of male or female vines showing the specific symptom within the blocks where the symptom was observed.

Symptom	Female vines					Male vines				
	Number of blocks	Percentiles				Number of blocks	Percentiles			
		25	50	75	Max		25	50	75	Max
Leaf-spot	325	5	20	55	100	299	5	20	70	100
Green-shoot wilt	131	2[a]	5	10	100	137	5	10	30	100
Cane dieback	135	1	5	10	100	197	2	10	30	100
Stem canker	135	1	5	10	90	100	5	10	30	100
Red exudate	77	1	2	10	100	168	2	10	30	100
White exudate	13	2	10	20	100	41	5	10	20	100

[a]25th percentile of vines showing leaf-spot symptoms within the 131 blocks where leaf-spot was observed.

Most growers reporting disease symptoms in their blocks (347/363; 96%) gave an estimation of the month that they first saw symptoms in their selected block (Figure 7-3). The spring period (September to November) had the highest frequency of growers first detecting symptoms in their blocks. The median number of months that Psa had been detected by the end of the study was 12 months (25th quartile was 4 months and 75th quartile was 18 months). The first report of Psa in New Zealand was made in November 2010 and 36 growers estimated that disease in their blocks was also first observed in spring 2010 with one estimating disease onset 9 months earlier in January 2010. The majority of these growers (n=25) were reporting on orchards within the Te Puke region.

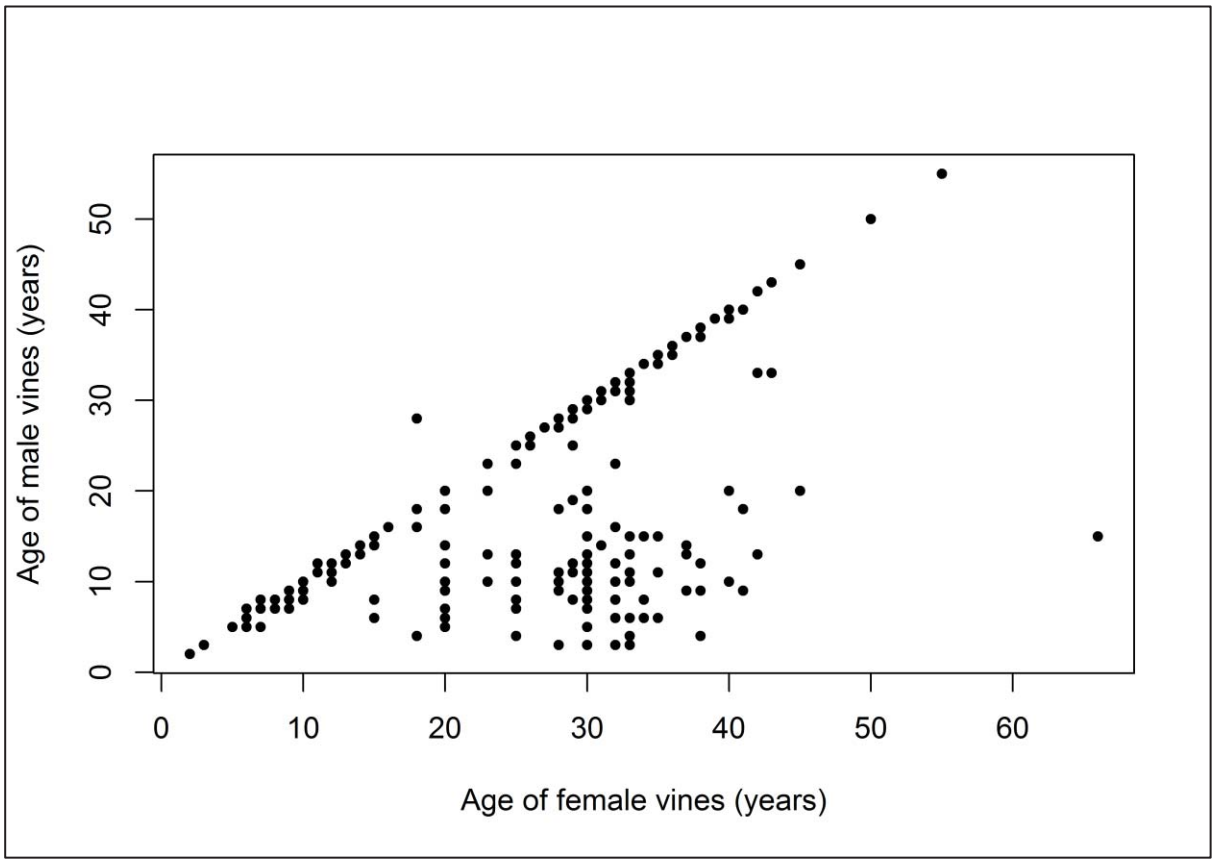

Figure 7-1 Age of female vines in 'Hayward' kiwifruit orchards compared with age of male vines.

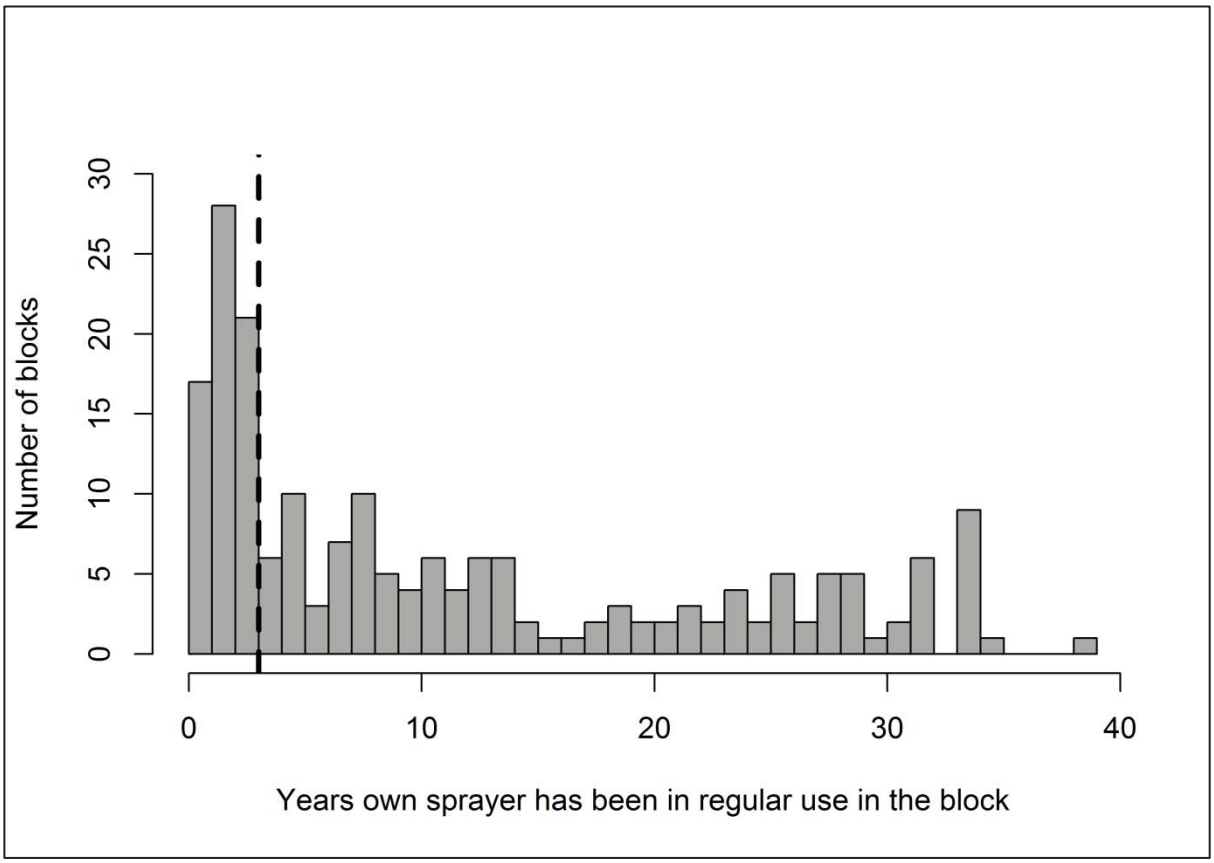

Figure 7-2 Period of time (years) during which growers (n=194/430) have regularly used their own spray equipment in their selected 'Hayward' block as of 2013 (years = 0). The dashed line indicates the first official detection of Psa in New Zealand in 2010 (3 years prior to the survey).

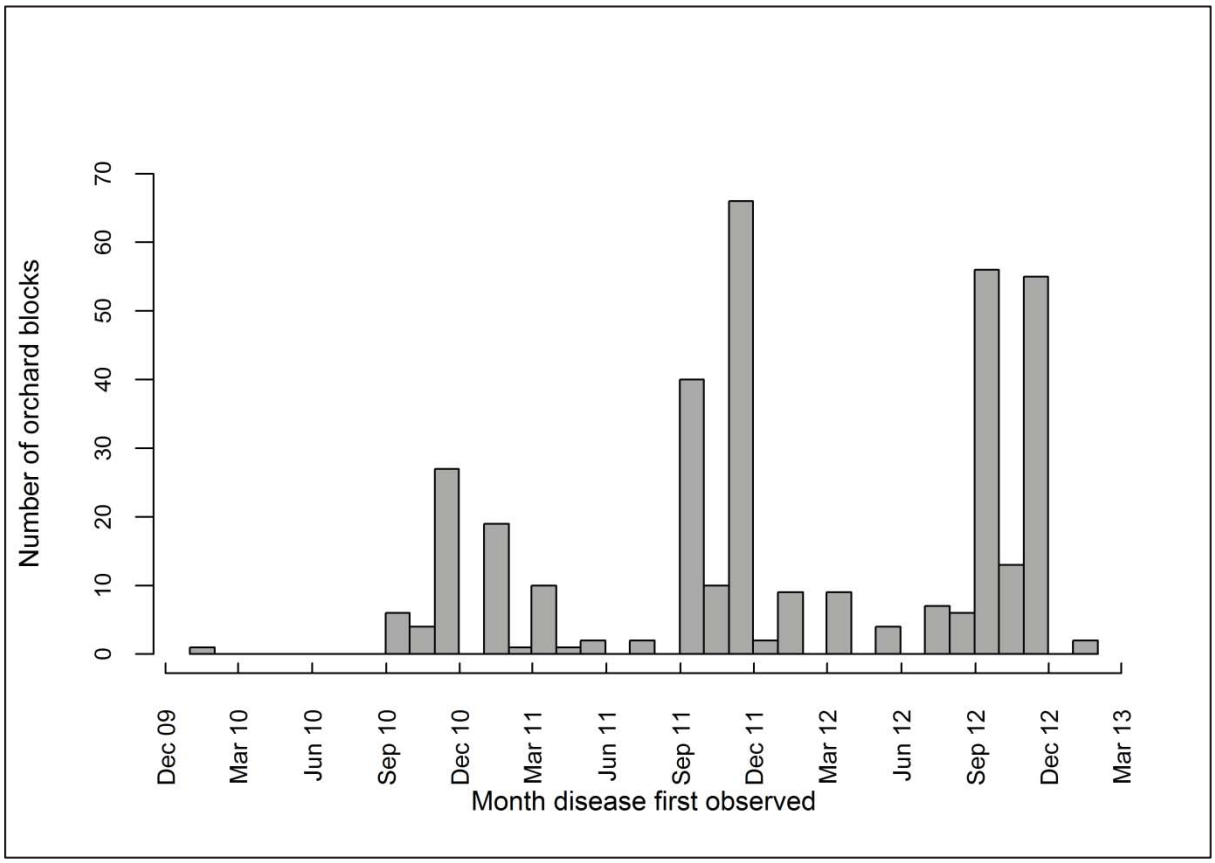

Figure 7-3 The grower estimated date of the first appearance of kiwifruit bacterial canker symptoms in their selected 'Hayward' blocks.

7.5 Discussion

The purpose of this study was to quantify the orchard features of a typical block within a Psa infected orchard and to describe the current practices used by commercial 'Hayward' growers. The study identified that use of girdling, use of artificial pollination and frost damage were common in orchards. The study also aimed to quantify the uptake of Psa management recommendations. It showed that most growers used post-pruning sprays, disease protection and hygiene practices. The final aim of the study was to describe the disease prevalence. It was shown that Psa was present in 84% of surveyed blocks, mostly recorded as leaf-spotting symptoms and 75% of growers reported the first onset of disease in spring.

7.5.1 Typical 'Hayward' orchard blocks

The results show that the typical 'Hayward' kiwifruit block in a Psa infected orchard is at low elevation with 30-year-old 'Hayward' female vines and 25-year-old 'Chieftain' male vines with Japanese cedar (*Cryptomeria japonica*) and she-oak (*Casuarina* sp.) shelter belts. The difference in vine age between males and females was explained by industry representatives

(G. Clark, Zespri Ltd. personal communication), as being the result of growers replacing their male vines to improve pollination outcomes, including conversion to strip male growing systems (Doyle et al. 1989). The variation in age of both male and female vines will provide an opportunity to further explore the relationship between vine age and disease, as other researchers have shown differing effects of vine age on disease prevalence (Li et al. 2001; Vanneste et al. 2011b; Zhang et al. 2013).

Although 'Hayward' was the dominant female cultivar, the "Kramer" clone a strain of 'Hayward', which is very similar but considered to be less vigorous (Ferguson 1999), was present in 11% of blocks. Further research to ascertain if the "Kramer" clone vines are more or less susceptible to disease, due to lower vigour, could be useful given that breeding for resistance is an important target for kiwifruit grown in a Psa environment (Fraser et al. 2015; Hoyte et al. 2015; Tanner 2015). Male pollinator cultivars were quite diverse with over a third of blocks using more than one variety, however 'Chieftain' was dominant in most blocks and further research into the susceptibility of this cultivar is warranted.

Shelter species diversity was similar to that reported by Perley et al. (2006) and willow and poplar shelters were relatively common despite not being recommended for kiwifruit shelters as their roots can invade the kiwifruit root zone and compete with the crop for soil moisture and nutrients (Hughes et al. 1994), although growers can compensate for this by root pruning. During the initial period of the Psa outbreak in New Zealand there were concerns that artificial shelter was increasing disease risk. The survey results show that only 13% of blocks used artificial shelter, however anecdotally there has been an increase in the use of artificial shelter in recent years and therefore further investigation of this potential risk factor may be warranted.

7.5.2 Frequency of potential Psa risk factors

In this study, we found that pollination was dominated by the use of beehives with 89% of growers introducing them into their blocks. It is known that bees can become contaminated with Psa when foraging in infected orchards (Pattemore et al. 2014) and beehives can be moved between two or three orchards during the pollination period. KVH has developed protocols for beehive hygiene aimed at eliminating orchard to orchard spread (Pentreath 2011; Kiwifruit Vine Health Inc. 2016e) which is important for such a high frequency practice.

The results showed that artificial pollination was used by a fifth of growers in spring 2011 and a third of growers in spring 2012. Research has confirmed the ability of pollen to harbour Psa (Gallelli et al. 2011; Vanneste et al. 2011c; Everett et al. 2012d) and trials in Italy have provided

evidence of the transmission of Psa via contaminated pollen (Stefani & Giovanardi 2011; Tontou et al. 2014). Given the frequency of its use, and the role that artificial pollination has in improving fruit size for 'Hayward' kiwifruit, the New Zealand kiwifruit industry has prioritised research into the association between disease and artificial pollination use, along with the development of protocols for use and research into ways to clean pollen (Everett et al. 2012d; Miller et al. 2015; Kiwifruit Vine Health Inc. 2016d).

Girdling was undertaken on female vines by two-thirds of the growers in the study. Girdling is carried out in spring to increase fruit size and in summer to increase dry matter resulting in better yields (Patterson & Currie 2011), and growers are now girdling in spring to reduce Psa bud browning (Stephen Hoyte, Plant and Food Research, personal communication). However, girdling has also been identified as a potential risk factor for Psa as it results in a large, slow healing wound (Snelgar et al. 2012a; Snelgar et al. 2012b; Tyson et al. 2012a), although there is only limited evidence of girdling being strongly associated with transmission of Psa and it is based on experiments that involved artificial inoculation (Snelgar et al. 2012b). Given the frequency of girdling, clarifying the association between girdling and disease could lead to a change in the practice that could influence the management of disease within the kiwifruit industry. In contrast, male vine girdling is very low at 3% and further research into the risk of male vine girdling is not warranted.

Frost damage was reported in a quarter of the blocks, half of which had frost protection in place. Frost has been shown to be important in disease development in Italy where they have found that frost damage allows direct entry of the pathogen into the vine through the damaged tissue (Ferrante & Scortichini 2014). Although the exact mechanism by which frost promotes kiwifruit bacterial canker remains unclear it is postulated that cell rupture during freezing is likely to be important in aiding bacterial movement and access to nutrients. Our results indicate that frost damage is a frequent event and therefore improvements in frost management could be important in disease management, in particular investigating why frost protection efforts were not effective in blocks.

One third of growers use irrigation with most using under-vine sprinklers or dripline systems. Although growers and industry raised concerns that overhead irrigation was putting their orchards and neighbouring orchards at risk of disease, this practice is uncommon with only 4% of growers using overhead irrigation. Consequently, changes to overhead irrigation would not substantially alter the burden of disease within the wider kiwifruit industry.

7.5.3 Uptake of Psa management recommendations

The KVH Psa-V weather risk model had an uptake of a fifth of growers as of March 2013. The risk model was developed in early 2012 to assist with spray timing in relation to periods of high infection risk related to cool, wet weather (McKay et al. 2012; Beresford et al. 2017). Those growers reflect the early adopters of the new technology (Gent et al. 2013). There is potential to increase use of the model, especially from 2016 as the model is now more user friendly and accessible (Kiwifruit Vine Health Inc. 2016c). Nearly 80% of growers said they had to delay applying protective sprays because of wet weather. The wider adoption of the weather risk model to plan sprays prior to forecast infection events could improve the timing of protective sprays and reduce delays in getting protection on the vines. The increase in numbers of growers using their own sprayers also allows greater flexibility in spray timing and provides more opportunity to apply protective sprays based on forecast infection risk. The other main reason given for delaying protective sprays was contractor availability, which is an aspect that growers should consider actively managing if they are concerned about the kiwifruit bacterial canker situation in their blocks.

This study also found that the majority of growers now undertake some vine management hygiene practices. Anecdotally, vine management hygiene practices were not the industry norm prior to the Psa outbreak, which suggests a fundamental change to orchard management in the time since Psa was first detected in New Zealand. Over 90% of growers now report using the recommended pruning equipment hygiene practices (Kiwifruit Vine Health Inc. 2016b) between orchards and between blocks. However, within a block, equipment hygiene practices were applied just over half of the time, except for girdling equipment where more than two-thirds of growers routinely dipped their girdling equipment in disinfectant between vines. One plausible reason for the difference in practice between blocks and within individual blocks (between vines) is that equipment hygiene may be considered less important after a block or orchard has become infected. While this is an understandable view, the prevalence of severe kiwifruit bacterial canker symptoms (i.e. all symptoms excluding leaf spotting) reported by growers on vines in this study was very low (a median of 5% of female vines and 10% of male vines showing symptoms) and implementing pruning hygiene measures between vines or bays could improve disease management.

Three quarters of growers were using post-pruning sprays which is recommended for Psa management in the orchard (Kiwifruit Vine Health Inc. 2015). However there remains some uncertainty about the efficacy of post-pruning sprays which have not been proven in experimental research to date (Kiwifruit Vine Health Inc. 2015). The KVH guidelines for

management of infected material is removal, followed by burial or burning as the preferred options (Kiwifruit Vine Health Inc. 2015, 2016a). Just under half of the growers were complying with that guideline. A further 19% used mulching which is also recommended but is the least-preferred option. The results showed that 35% of growers did not cut out symptomatic material, although symptoms on canes and stems such as cankers and dieback were uncommon in this study and growers would not be expected to cut out material that only had leaf spotting. While the KVH disposal protocol does not cover non-diseased pruning waste, there is likely to be asymptomatic infected material in pruned waste (Tyson et al. 2014b) and currently 12% of growers do not remove or mulch prunings. This is likely to be increasing their risk of disease as Tyson et al. (2012b) showed that Psa can remain in infected pruning debris on the orchard floor.

7.5.4 Disease onset and prevalence

The prevalence of disease in the selected blocks was high (84%), which was expected given that the sampled orchards were confirmed Psa-positive by KVH, an eligibility criterion for inclusion in the study. The first observation of symptoms during spring by most growers with symptomatic blocks is consistent with other studies that show a spring increase in disease symptoms (Rosanowski et al. 2013a) particularly on newly developing leaves which are highly susceptible (Serizawa & Ichikawa 1993b; Tyson et al. 2015). One grower cited January 2010 for the observation of disease symptoms in his selected block which was 10 months prior to the first report of Psa in New Zealand. This date is within the likely establishment period of Psa in New Zealand (Ministry for Primary Industries 2011). This property was close (less than 1.5 km) to the first orchards to report the disease and was well within the modelled local spread distance of Psa in New Zealand (Rosanowski et al. 2013a). Thirty-six growers indicated that they had first seen symptoms during the 2010 spring period. The distribution of 12 early reports from regions outside Te Puke in spring 2010 suggests that some growers may have reported symptoms similar to those caused by Psa biovar 3 which may have been caused by other pathogens known to cause leaf spotting in New Zealand such as *Pseudomonas syringae* pv. *actinidifoliorum* (previously and commonly known as Psa biovar 4 or Psa-LV), *Pseudomonas viridiflava, Pseudomonas* sp. or *Pseudomonas syringae* pv. *syringae* (Young et al. 1997; Vanneste et al. 2013; Cunty et al. 2015). Alternatively it could indicate that Psa had spread to a greater extent than was appreciated at the time of the first report in New Zealand (Everett et al. 2011). While this latter scenario is possible, extensive surveillance and sampling outside the initial infected zone of Te Puke, did not detect Psa biovar 3 until spring 2011 (Richardson et al. 2012; Rosanowski et al. 2013a). It is possible that some growers misclassified

their symptoms as Psa rather than other pathogens when estimating when symptoms were first observed.

The cross-sectional study design of this survey was an effective way to obtain industry disease, risk factor and hygiene prevalence data. It also provides an example for other industries on how crop management data that could provide useful insight for research funding, particularly following a biosecurity incursion and will provide a resource for further research on the effects of Psa on kiwifruit production. While the focus of this research was to identify factors that may be associated with disease, this quantitative data could also be used to investigate other associations such as crop management and productivity outcomes, using the methodology described in Froud et al. (2015) and Froud and Cogger (2015) which offers a different approach to previous attempts of modelling management actions in kiwifruit (Doyle et al. 1989).

This is the first comprehensive study of the features and management practices in a large sample of commercial kiwifruit orchards in New Zealand, either before or after the arrival of Psa. These data can focus attention on factors that are used widely within the industry that can be manipulated to reduce the impacts of Psa on kiwifruit production. Future work using the results from this survey will investigate risk factors that are associated with kiwifruit bacterial canker in commercial operations and recommend interventions to reduce the impact of disease across the industry.

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CHAPTER 8

8 Kiwifruit bacterial canker in 'Hayward' kiwifruit: Risk factors for the development of disease in a block

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Risk factors for kiwifruit bacterial canker disease development in 'Hayward' kiwifruit blocks

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8.1 Abstract

In November 2010 *Pseudomonas syringae* pv. *actinidiae* biovar 3 (Psa), the cause of kiwifruit bacterial canker was first recorded in New Zealand. Kiwifruit bacterial canker is a severe disease and has caused significant loss in susceptible cultivars. This study examined risk factors relating to disease management, vine management and orchard layout that were associated with disease symptoms observed by orchardists in *Actinidia chinensis* var. *deliciosa* 'Hayward' orchards. A cross-sectional study using data collected via a questionnaire investigated orchard blocks that were symptom-free in March 2012. The outcome we modelled was detection of disease in the block during the study period from March 2012 to February 2013, and multivariable logistic regression was used to identify potential risk factors. Data from 194 growers were included and comprised 53 orchard blocks which remained disease free and 141 which became diseased. This cross-sectional study identified four factors that were associated with Psa symptom development. The associated factors identified in this study are not necessarily causal, but our results can be used by the kiwifruit industry to help prioritise research needs to identify processes involved in the development of kiwifruit bacterial canker in kiwifruit orchards. Priority for further research is the relationship between the timing of copper sprays, callus tissue formation and Psa mobilisation. A second priority is to determine the biological mechanism for the association between girdling and a reduction in disease risk. After accounting for other factors in the model, there were still significant differences between kiwifruit growing regions in the probability bacterial canker would develop in the block. Use of a cross-sectional study provided a new way to investigate plant disease risk factors and this type of study could be more extensively used, especially during incursions of unwanted organisms.

Keywords: Observational, survey, cross-sectional, multivariable logistic regression, confounding, temporality.

8.2 Introduction

Pseudomonas syringae pv. *actinidiae* biovar 3 (Psa) causes kiwifruit bacterial canker disease, which was first detected in New Zealand in late 2010 (Everett et al. 2011) and resulted in severe economic losses to the kiwifruit industry. There was an estimated 20% volume loss in the first 24 months predominantly affecting the gold-fleshed cultivar 'Hort16A' (*Actinidia chinensis* var. *chinensis*) which had to be removed from infected regions and replaced with more tolerant cultivars (Tanner 2015). Psa continues to cause concern for growers of the green-fleshed 'Hayward' (*Actinidia chinensis* var. *deliciosa*) in New Zealand and internationally, with uncertainty around its long term effect on this widely grown cultivar (Ferrante & Scortichini 2009; Ferrante et al. 2012; Vanneste 2012). Psa causes leaf spotting, shoot wilt, cane dieback and stem cankers and, in severe cases, may lead to death of the vine or the removal of vines from the orchard.

While Psa inoculum, favourable weather and a susceptible host are essential for infection, there are many other host, environmental and management factors that can alter the likelihood of disease developing. Potential risk factors for Psa have been reviewed by Froud et al. (2015, Chapter 2) and include vine age, frost, elevation, girdling, pruning and use of artificial pollination. Artificial pollination, pruning management and stem girdling are of concern to the kiwifruit industry because these practices are required to produce high quality fruit. In addition, the efficacy of many of the recommended Psa orchard hygiene and disease management practices (e.g. equipment sanitisation, post-pruning copper sprays), was unknown in commercial orchards.

An understanding of relationships between disease outcomes and risk factors in plant pathology often focuses on experimental studies involving only one or two factors. However, an experimental approach has limitations when a wide range of interacting risk factors must be considered. Experimental studies involving multiple factors are complex and require considerable time and other resources, and factor interactions can be difficult to interpret. Furthermore, some factors may be difficult to manipulate, for example frost and elevation, and experimental systems may not be able to accurately represent naturally infected vines in the orchard situation. Also, control options may need to be examined under real-world conditions because of interactions with other factors that may alter the risk of infection. It may be possible to overcome these limitations by using an observational study that utilises data collected from commercial orchards to better understand the factors that alter the risk of disease expression.

Observational studies have a long history of use in human (Rothman 2012) and veterinary health (Dohoo et al. 2009e) to understand the distribution of, and the factors contributing to, disease. There is also the potential for observational studies to be used for plant health, particularly in relation to identifying risk factors. The type of observational study design depends on the research question. Ideally, a longitudinal study such as a cohort study would be used to obtain the strongest evidence for a causal link between exposures (factors) of interest and a disease outcome. In a cohort study a sample of the population which is free of the disease, is selected for investigation and then data about exposures to possible risk factors and disease development are collected prospectively over time (Petrie et al. 2002b). In the New Zealand Psa outbreak, this type of study could have been set up in the early stages of the incursion, e.g., early in 2011, to collect data as the disease spread through the main kiwifruit growing regions. However, cohort studies require large sample sizes, can be expensive and take a long time to gather sufficient data. They also run the risk that industry practices that are measured at the start of a study change in response to the outbreak and are no longer valid or used at the end. When disease spreads rapidly, as in the New Zealand Psa outbreak, a cross-sectional study is an alternative approach, because it collects outcome and exposure data at a single point in time (often using questionnaires) with the aim of identifying exposures that are associated with an increased or decreased risk of disease development. This can be used to generate hypotheses about which factors should be investigated further, using either experimental studies or more comprehensive observational studies to determine causal relationships.

The aim of this cross-sectional study was to identify disease management, vine and orchard layout factors associated with the development of kiwifruit bacterial canker into an orchard block. The outcome of development of kiwifruit bacterial canker refers to the first development of disease in blocks, not the introduction of the pathogen, as Psa can be asymptomatic within kiwifruit tissue for long periods (Vanneste et al. 2011a; Abelleira et al. 2015). The study used observational data from commercial orchards, collected by means of a questionnaire. In addition, the paper illustrates the methodology used in a cross-sectional study and discusses the advantages and disadvantages of this type of epidemiological study, including its usefulness for hypothesis generation during disease outbreaks and the risk of over-interpretation of the results.

8.3 Methods

8.3.1 Study design

The cross-sectional study utilised a data set collected from kiwifruit growers via a questionnaire. It posed 54 questions concerning the prevalence of kiwifruit bacterial canker in relation to disease management, vine and orchard layout factors in randomly selected 'Hayward' blocks within Psa infected orchards over the period 1 March 2012 to 28 February 2013. The questionnaire was drafted in consultation with Zespri International Limited (Zespri), Kiwifruit Vine Health (KVH) and 'Hayward' growers and its development has been described by Froud et al. (2016) .

The questionnaire was sent by Zespri to 1669 'Hayward' growers and 442 completed survey forms were returned. Where questions were not answered for particular exposure variables, the missing value was left blank. A summary of the sample plan and the sampling frame is given in Figure 8-1.

8.3.2 Inclusion criteria for analysis

The aim of the analysis was to identify factors associated with the recent development of disease in an orchard block, so data were therefore limited to the 194 blocks reported to be free of symptoms on 1 March 2012. The date of Psa development in each block was determined from the response to the question:

Knowing what you do now about Psa symptoms in your orchard, when do you think is the earliest you saw symptoms that on reflection probably were Psa in the block even if they tested negative?

Where a grower who reported symptoms of Psa did not answer the question about the earliest date, they were excluded from the dataset. In addition, observations from smaller growing regions where less than 10 growers completed the survey were excluded from the dataset (Coromandel (n=7), Waihi (n=3), Hawkes Bay (n=1), Poverty Bay (n=1), Waikato (n=4), and Franklin (n=2) (Figure 8-1). MS Excel and the 'R' freeware statistical package version 3.0.1 were used to assess the completeness and validity of the aggregated dataset and missing or unusual values were checked with Zespri.

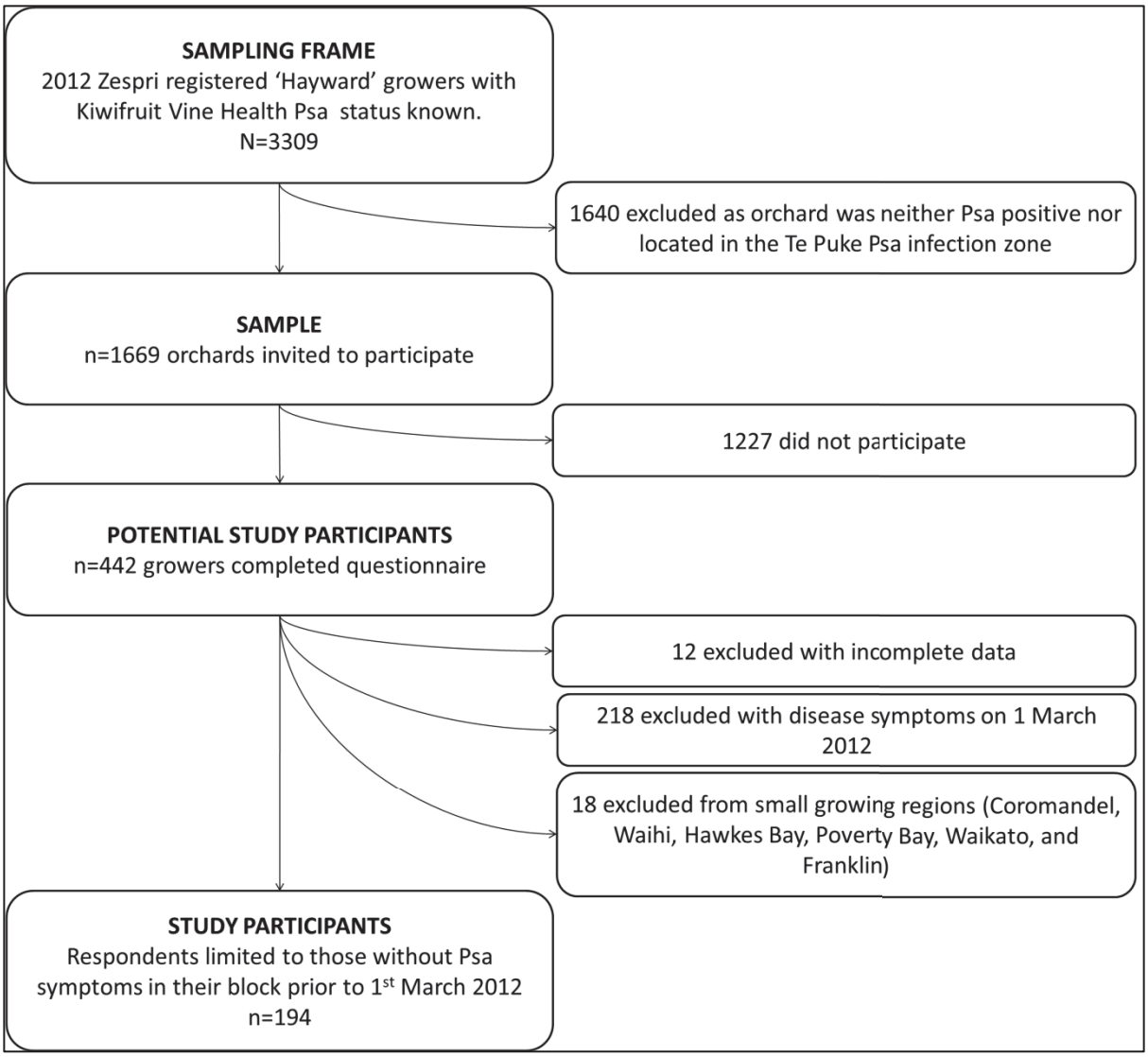

Figure 8-1 Sampling plan showing selection of a sampling frame and the inclusion criteria for the study of factors affecting development of bacterial canker in orchard blocks of 'Hayward' kiwifruit.

8.3.3 Classification of outcome variable

The Psa status of each block in February 2013 was described by a binary outcome variable that used the 'yes' and 'no' answers from the question below if the 'not sure' option had not been selected.

Do you have any visible Psa-v symptoms in the block as of Feb 2013 (including old spotting/symptoms)?

No ☐ *Yes* ☐ *Not sure* ☐

8.3.4 Classification of exposure variables

For orchard description questions that allowed for more than one answer, possible answers were converted to one or more new binary variables that coded not present or present, or not used or used. For example, answers to the question:

What pollination methods did you use in this block during last seasons (2011/12) flowering period? Please select all relevant methods.

Natural wind/bees ☐ *Introduced bees* ☐ *Wind blow flowers* ☐

Artificial pollination ☐ *Other (please specify)* ☐ ……

Note: Wind blow flowers refers to the practice of blowing male vines with a wind blower to release pollen into the orchard

These were converted to five binary variables: 1) only used natural wind and bees, 2) used bee hives, 3) used bee hives only, 4) used artificial pollination, and 5) used wind blow flowers only**.** Where the answers were mutually exclusive, then a series of binary variables were converted to a categorical variable with multiple levels. For example, frost damage could be no damage, minor damage, moderate damage or severe damage. For variables with few observations and where it made biological sense, categories were combined into new variables, e.g. mild, moderate and severe frost damage were combined into any frost damage versus no frost damage in the 2012/13 season.

Variables that were very similar were combined into a new aggregated variable. For example, the variable "blocks routinely sprayed just after pruning" was constructed by combining: "used a follow-up backpack sprayer after pruning", "sprayed pruned rows at the end of the day" and "applying a full block spray at the end of pruning". Excluded from the combined "blocks routinely sprayed just after pruning" variable was the variable, "instant wound protection with hand sprayers", as this may have been interpreted to include wound protectant compounds applied as paints or gels.

Selection of the reference category for modelling the data was considered for each multilevel category, based on which level was the most appropriate to compare with other levels. In the case of the regions, Katikati was selected as it was closest to the mean production and elevation of the whole dataset (Dohoo et al. 2009f).

8.3.5 Data analysis

Data analysis was conducted using 'R' statistical package version 3.3.1 (R Core Team 2016) and the level of statistical significance was set at $P \leq 0.05$. Continuous variables were visually assessed using boxplots and histograms and those that were not normally distributed were recoded as multi-level categorical variables or binary variables. Descriptive statistics for

continuous exposure variables were given as medians and 25th and 75th quartiles, where data were non-normal/skewed, and means with standard deviation, where data were normally distributed. Descriptive statistics were calculated for the numbers and percentage (of total respondents) of observations for each binary or multi-level categorical exposure variable. Nominal data were presented as counts and percentages.

Univariate screening using separate, unmatched, logistic regression procedures was used to determined associations between Psa status of blocks and each explanatory variable. Statistical significance was assessed using the log-likelihood ratio test statistic. Temporality of disease development (March 2012 to February 2013) in relation to the timing of artificial pollination (November 2012) was investigated by sub-setting the data into disease-free plus those that developed disease prior to flowering (n=144) and disease-free plus those that developed disease at or after flowering (November 2012; n=106). Logistic regression for each of these subsets determined whether there was a difference in the association with the Psa status of the block.

Explanatory variables associated with the outcome at $P \leq 0.20$ were considered for inclusion in a multivariable logistic regression model of the full dataset (n=194). Screening explanatory variables at a very liberal P-value of 0.20 allows for the inclusion of variables that may not be statistically significant prior to controlling for other factors that may be confounding their association with the outcome (Dohoo et al. 2009c). Prior to inclusion in the model, the problem of correlation between exposures (multi-collinearity; (Marill 2004)) was addressed. An example of potential collinearity occurred for the variables indicating use of frost protection and the degree of frost damage because these two variables were highly correlated. Where there was obvious collinearity, only one of the related categorical variables was selected based on importance for the system being modelled. In this case frost damage was biologically important for disease development and was included in the modelling.

A preliminary main effects model was built using a backward procedure in which all eligible variables, excluding those that were considered collinear, were included and were then removed from the model using manual backward elimination until all the remaining variables were significantly associated ($P \leq 0.05$) with the outcome using the Log-likelihood ratio test statistic (Dohoo et al. 2009b). The model was then extended to include a fixed effect coding for the region the orchard was in, and variables were reassessed for elimination if they were no longer significantly associated with the outcome. Variables not significant in the final model were separately added back to the model and retained if the P-value for the log-likelihood ratio test statistic was ≤0.05.

Interaction, which is also referred to as effect measure modification, is when the effect of one predictor variable on the outcome differs with different values of a second predictor variable (Rothman 2012). All biologically plausible two-way interactions were considered for inclusion in the model and retained if the log-likelihood ratio test statistic was significant. The fit of the model was assessed using the deviance test on the covariate patterns, the Hosmer-Lemeshow test and the le Cessie-van Houwelingen-Copas-Hosmer unweighted sum of squares test (Kabacoff 2011). Overdispersion can be an issue in logistic regression and is where the variance is much larger in one group than expected for a binomial distribution. Overdispersion was checked by visual inspection of a plot of residuals against the half-normal quantiles (Kabacoff 2011) and the calculated dispersion parameter, that is the residual deviance divided by the degrees of freedom (Zuur et al. 2009). Leverage, caused by observations with unusual combinations of predictor variables having a disproportionate influence on the model results, was assessed visually by plotting the Pearson's residuals against the logit and calculating the Hat-statistic and plotting Hat-values against the Studentized residuals (Kabacoff 2011).
No adjustments were made to p-values for the final model as they are not recommended where exposure variables are individually selected based on the potential for a biologically plausible association with the outcome (Rothman 1990; Vandenbroucke et al. 2007) and manual selection of model variables was applied rather than automated selection criteria (Dohoo et al. 2009c; Froud et al. 2015).
The logistic regression coefficients were presented as adjusted odds ratios in the final model. The use of odds ratios is appropriate if the outcome is rare because then the odds ratio is similar to the relative risk in the population. If disease prevalence is high, as in this study, the odds ratio provides an over-estimate of the relative risk. Therefore, the logistic regression coefficients were also converted to predicted probabilities for visual presentation and discussion of the results.

8.4 Results

Of the 194 blocks classified as having no Psa symptoms on 1 March 2012 (Figure 8-1), 141 had symptoms reported on 28 February 2013. Of these, 54 orchardists first detected disease in their blocks in September 2012, corresponding with the typical time for bud-break and first leaf emergence of 'Hayward', and a further 46 detected the disease in November 2012 when flowering typically occurs. In total, disease was first observed in 88 orchards prior to November (flowering) in 2012 and in a further 48 during or after November 2012. The remaining 53 blocks were free of symptoms at the end of the study period.

The univariate screening identified variables associated with risk of disease that had a log-likelihood test statistic P-value ≤0.20 (Table 8-1). Factors with a P-value > 0.20 that were not included in the multivariable model included organic management, being adjacent to a block from which kiwifruit had been removed because of disease, fast-track (a type of internal shelter) or artificial shelter, application of artificial pollination in spring 2011, different male cultivars present in the block and pruning or girdling equipment hygiene. The binary frost damage variable (of 'no frost' or 'any frost') was eliminated during the model building process as it was not significant. Elevation and region were both associated with differences in disease risk (*P*=0.13 and <0.001 respectively) (Table 8-1), however, most of the variability in elevation data was because of the elevations of orchards in Tauranga East (130 m) and Te Puke (60 m) being much higher than those in the other four regions (Katikati, Opotiki, Tauranga West and Whakatane) which were all between 10 and 30 m (Figure 8-2). It was expected that elevation would be collinear with region and therefore both could not be included in the final model. However, because region could account for other unmeasured factors, such as climate and soil, region, rather than elevation, it was included in the final model.

The multivariable model identified factors associated with the risk of disease symptoms in the block (Table 8-2). The risk of disease was greater in blocks where artificial pollination was used and when Psa protective block sprays were routinely applied immediately after pruning, and less when female vines were girdled in the summer Table 8-2. The predicted probability of disease decreased with increasing male vine age as shown in Figure 8-3. Tauranga East and Te Puke had a higher risk of symptoms developing than Katikati, the reference region.

The two subsets of data that were used to assess the timing of disease development compared to timing of artificial pollination use had unadjusted odds ratios that showed a similar (higher) risk for disease development for both data subsets. For orchards that developed disease prior to flowering (and therefore prior to artificial pollination) the risk of developing disease was 2.26 (CI's 1.03 to 5.28; *P*=0.05) times higher when artificial pollination was used than when it was not. Likewise, for those orchards that developed disease at or after flowering the risk of developing disease was 2.40 (CI's 1.01 to 6.02; *P*=0.05) times higher in orchards where artificial pollination was used than when it was not.

The chi-squared test statistic of 7.04 with 8 degrees of freedom for the Hosmer-Lemeshow goodness-of-fit test (*P*=0.53), and the le Cessie-van Houwelingen-Copas-Hosmer unweighted sum of squares goodness-of-fit test (*P*=0.62) showed that the model was a good fit for the data and the dispersion parameter was close to one (1.01). This indicated that overdispersion was not a problem in the model. Inspection of diagnostic plots showed no unusual observations.

There were three data points associated with influential patterns, which were checked for data entry errors. No errors were detected so they were retained in the model.

Table 8-1 Univariate association between management, vine and environment related variables, and risk of development of bacterial canker in 'Hayward' kiwifruit blocks. Data were from 194 valid respondents to a mail-out survey of 430[a] 'Hayward' blocks that were in orchard properties classified as infected with Psa.

		Number (%) blocks		Odds Ratio (OR)	OR 95% CI[b]	P-value for LRT[c]
Variable	**Level**	**Psa absent**	**Psa Present**			
Blocks routinely sprayed just after pruning	No	26 (13)	42 (22)	Ref [d]		0.01
	Yes	27 (14)	99 (51)	2.27 [e]	1.19–4.36 [f]	
Region	Katikati	24 (12)	32 (16)	Ref		<0.001
	Tauranga East	3 (2)	27 (14)	6.75	2.07–30.60	
	Tauranga West	13 (7)	17 (9)	0.98	0.40–2.43	
	Te Puke	6 (3)	42 (22)	5.25	2.02–15.56	
	Whakatane	2 (1)	11 (6)	4.13	0.99–28.29	
	Opotiki	5 (3)	12 (6)	1.8	0.58–6.29	
Used artificial pollination spring 2012	No	43 (22)	89 (46)	Ref		0.01
	Yes	10 (5)	52 (27)	2.51	1.20–5.67	
Used bee hives only for pollination spring 2012	No	12 (6)	58 (30)	Ref		0.01
	Yes	41 (21)	83 (43)	0.42	0.20–0.84	
Willow shelter	No	51 (26)	122 (63)	Ref		0.03
	Yes	2 (1)	19 (10)	3.97	1.10–25.50	
Age of male vines in block	(years)	-	-	0.97	0.94–1.00	0.04
Block irrigated	No	29 (15)	96 (49)	Ref		0.09
	Yes	24 (12)	45 (23)	0.57	0.30–1.08	
Any frost canopy damage in 2012/13 season	No	45 (23)	103 (53)	Ref		0.07
	Yes	8 (4)	38 (20)	2.08	0.94–5.11	
Girdled female vines in summer 2011/12	No	26 (13)	89 (46)	Ref		0.08
	Yes	27 (14)	52 (27)	0.56	0.30–1.06	
Cypress shelter block	No	51 (26)	136 (70)	Ref		0.10
	Yes	3 (2)	16 (8)	3.04	0.82–19.70	
Elevation	<20m	28 (14)	61 (31)	Ref		0.13

Variable	Level	Number (%) blocks		Odds Ratio (OR)	OR 95% CI[b]	P-value for LRT[c]
		Psa absent	Psa Present			
	21–80 m	18 (9)	43 (22)	1.10	0.54–2.25	
	>80 m	7 (4)	27 (14)	2.43	1.01–6.53	
Age of female vines in block	(years)	-	-	0.98	0.94–1.01	0.16
Block is adjacent to a gully or bush	No	45 (23)	107 (55)	Ref		0.16
	Yes	8 (4)	34 (18)	1.79	0.80–4.42	
Used commercial pollen for artificial pollination	No	44 (23)	103 (53)	Ref		0.14
	Yes	9 (5)	38 (20)	1.80	0.83–4.26	
Used artificial pollination spring 2011	No	45 (23)	112 (58)	Ref		0.38[g]
	Yes	8 (4)	29 (15)	1.46	0.64–3.63	

[a] Data limited to 194 blocks that did not have symptoms of Psa present in March 2012 that were in the six main growing regions (Katikati, Opotiki, Tauranga East, Tauranga West, Te Puke and Whakatane).

[b] 95% Confidence Interval; [c] Significance of Likelihood ratio test statistic; [d] Reference category.

[e] Interpretation: When growers routinely sprayed vines with Psa protectants just after pruning the risk of Psa disease expression is 2.27 times greater than when growers do not routinely spray just after pruning before adjusting for other factors.

[f] Interpretation: We are 95% confident that the increased risk of disease expression associated with growers routinely spraying blocks just after pruning, before adjusting for other factors is between 1.19–4.36.

[g] Artificial pollination was not within the $P<0.2$ screening range, however it was included in the results table because of its interest to the study design and interpretation of results.

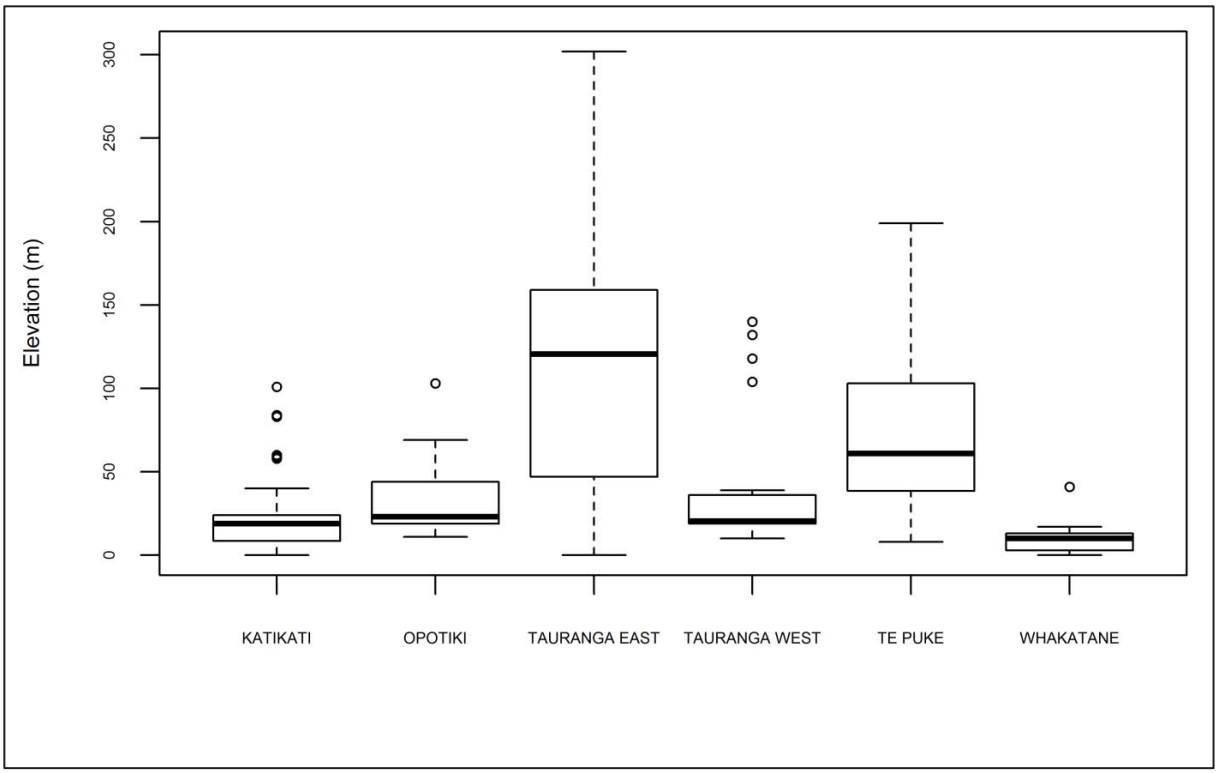

Figure 8-2 Boxplots of the variability in orchard elevation above sea level within each main kiwifruit growing region included in the study of factors affecting development of bacterial canker in orchard blocks of 'Hayward' kiwifruit.

Table 8-2 Results of a multivariable logistic regression model describing the relationship between kiwifruit bacterial canker symptoms in an orchard block and a range of exposure variables. Region was included in the model to account for spatial clustering. Data were from 194 growers who were disease free selected from respondents to a mail-out survey of 430[a] 'Hayward' blocks that were in orchards classified as infected with Psa or located in Te Puke.

Variable	Odds Ratio (OR)	OR 95% CI[b]	P-value[c]
Used artificial pollination in spring 2012			0.003
No	Ref.[d]		
Yes	3.67[e]	1.51–9.70[f]	
Blocks routinely sprayed just after pruning			0.005
No	Ref.		
Yes	2.87	1.38–6.13	
Age of male vines in block (years)	0.96	0.93–0.997	0.03
Used summer vine girdling in 2011/12			0.03
No	Ref.		
Yes	0.43	0.20–0.91	
Region			0.002
Katikati	Ref.[g]		
Tauranga West	0.98	0.36–2.67	
Tauranga East	6.73	1.91–32.39	
Te Puke	5.15	1.86–16.30	
Whakatane	2.11	0.45–15.56	
Opotiki	1.13	0.31–4.52	

[a] Data limited to the 194 blocks that did not have symptoms of Psa present in March 2012 and that were located in Katikati, Opotiki, Tauranga East, Tauranga West, Te Puke and Whakatane; [b] 95% Confidence Interval; [c] Significance of Likelihood ratio test statistic, where $P<0.05$ is considered significant; [d] Reference; [e] Interpretation: After accounting for other variables in the model, artificial pollination when compared with no artificial pollination, increased the risk of disease development, with the odds of development 3.67 times higher in blocks that used artificial pollination; [f] Interpretation: We are 95% confident that the increased risk of disease expression associated with artificial pollination is between 1.51–9.70; [g] Katikati was the reference region in the model and both Tauranga East and Te Puke had a significantly higher risk of disease than Katikati.

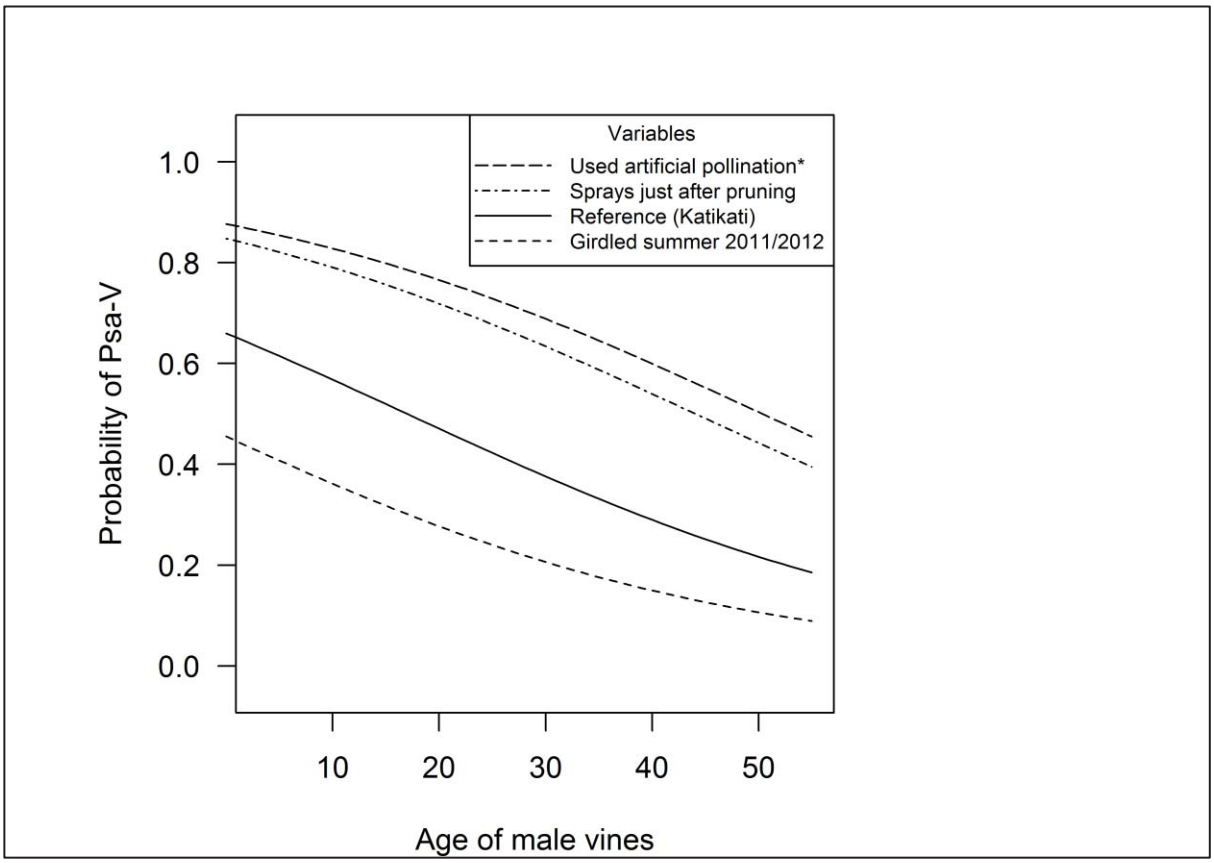

Figure 8-3 The predicted probability that, within a Psa infected kiwifruit orchard, a kiwifruit block that was non-symptomatic on 1 March 2012 would develop symptoms of kiwifruit bacterial canker within the study period ending on 28 February 2013. The probability of Psa being detected is equivalent to the reference line for the Katikati region across the male vine age range. Risk factors above this line (i.e. used artificial pollination and routinely use post pruning sprays) increase the risk of symptoms developing and factors below the line (summer girdling) reduce the probability of symptoms developing in the blocks.

*Artificial pollination in spring 2012. Most infection occurred prior to artificial pollination and this variable was probably a proxy for another unmeasured variable that was associated with disease development.

8.5 Discussion

The specific purpose of this study was to identify disease management, vine and orchard layout factors associated with the development (first expression of symptoms) of bacterial canker in disease free 'Hayward' kiwifruit blocks within orchards that already had blocks affected by bacterial canker. There was also an additional, more general, aim to explore the use of cross-sectional study design and multivariable analysis in a crop disease context for identifying risk factors and generating hypotheses that could guide further research. There have been few previous studies of plant diseases using this approach (Dallot et al. 2004; Thebaud et al. 2006; Zewde et al. 2007; Vicent et al. 2012; Froud et al. 2014; Froud et al. 2015, Chapter 6).

An important concern in observational studies is the potential presence of confounders. Rothman (2012) defines confounding as:

> *… the confusion, or mixing, of effects: this definition implies that the effect of the exposure is mixed together with the effect of another variable, leading to a bias.*

Confounding is typically controlled in observational studies by using multivariable regression. For this study, because the outcome was binary, multivariable logistic regression was used (Hosmer Jr et al. 2013). Results from a multivariable logistic regression model can be presented as either adjusted odds ratios or as predicted probabilities. An odds ratio is a good estimate of risk when the outcome is rare, but overestimates risk when the outcome is common (Grant 2014). In this study disease was observed in 77% of the blocks and therefore the odds ratio would have been an overestimate of the relative risk for the explanatory variables. Because of this, results were also presented graphically on the probability scale and the focus was on whether there was an increase or decrease in the risk compared with the reference region (Katikati), rather than the magnitude of the change.

Cross-sectional studies do not provide causal evidence about relationships between exposures and the outcome, but can indicate that causality may exist. An important consideration for all observational studies, but particularly for cross-sectional studies, is temporality, i.e., that a potential cause must precede the effect (Rothman et al. 2008b; Dohoo et al. 2009a; van Engelsdorp et al. 2013). The design of a cross-sectional study that collects both exposure and outcome data simultaneously cannot distinguish the order of cause and effect and can result in spurious conclusions from results with the potential for reverse-causality (Maselko et al. 2012; Engel & Wolff 2013). Generally the date of detection of disease is not recorded in cross-sectional studies, making it difficult to assess temporality (Shahar & Shahar 2013), but the design of this study enabled us to consider some aspects of temporality.

The study identified two variables that were associated with an increased risk of disease developing in 'Hayward' orchard blocks, namely, the application of artificial pollen in spring 2012, and the practice of routinely spraying Psa protectants on vines immediately after pruning. The risk of kiwifruit bacterial canker was reduced by summer girdling. The disease risk was inversely associated with the age of male vines (i.e. the risk decreased when the vines were older). Furthermore, after adjusting for these factors, there were significant differences between the regions.

8.5.1 Artificial pollination

Although artificial pollination, which was applied during November 2012, was significantly associated with an increased probability of disease development, the detection date reported by many growers was earlier than the time that pollination occurred. In addition, the bivariate analyses of the separate pre-flowering and flowering/post-flowering subsets both showed a similar association between artificial pollination and with disease development. Although pollen is known to harbour Psa (Gallelli et al. 2011; Vanneste et al. 2011c; Everett et al. 2012d; Tontou et al. 2014), which could allow artificial pollination to introduce Psa into kiwifruit blocks, the most likely reason for the association with artificial pollination is that another unidentified factor was strongly associated with both the use of artificial pollination and disease development. Such a factor might be, for example, growers with high numbers of symptomatic vines in the rest of the orchard, who perceive a high risk of Psa introduction into disease free blocks, and are more likely to apply artificial pollination to maximise productivity of the remaining healthy vines. It is also plausible that growers who had a high proportion of kiwifruit vines exhibiting kiwifruit bacterial canker symptoms would use artificial pollination to augment diseased male pollinator vines, which may confound this association. A further consideration making it unlikely that a causal association would be found between artificial pollination and the appearance of symptoms is that in spring 2011 the use of artificial pollination in our surveyed blocks was lower (20%) than in 2012 (36%) (Froud et al. In prep.) and therefore any causal association would have been difficult to detect with the limited power of our study. Longitudinal observational studies or experimental studies are needed to determine whether artificial pollination enhances the risk of disease development in disease free blocks. A study of this kind is recommended as a priority for the kiwifruit industry.

8.5.2 Practice of routinely spraying blocks immediately after pruning

The routine application of Psa protective sprays after pruning was associated with a higher predicted probability of disease development in the block. Growers were not asked to specify the type of protective spray used, however, based on the subset of growers that answered in-

depth vine management questions in an additional section of the questionnaire (Froud et al. 2016, Chapter 5) copper compounds predominated, with some use of plant defence elicitor chemicals and foliar fertilisers as 'Psa protective sprays' (unpublished results). It is possible that the association observed in this study was the result of another unmeasured confounding factor. For example, if growers who had visible bacterial canker in adjacent blocks, and therefore were more likely to develop symptoms in our surveyed blocks, took a risk-averse approach they might be more likely to protect pruning wounds in asymptomatic blocks with copper sprays leading to a confounded result. Alternatively, there is anecdotal evidence that growers who prune during a high-risk weather event, i.e., cold and wet conditions, are more likely to apply a post-pruning spray to mitigate the risk. There are some biologically plausible reasons for the observed association. Some of the compounds found in copper spray mixes can inhibit callus formation (Mercer 1983; Manivel & Handique 1984; Doster & Bostock 1988; Taddei et al. 2007), which may keep the wound open to infection for longer. Water runoff from post-pruning sprays may enable the mobilisation of bacteria and carry it into the pruning wound. At present there is not sufficient evidence that post-pruning sprays are beneficial (Kiwifruit Vine Health Inc. 2015) and further research is recommended to assess the efficacy of post-pruning protection and determine the relationship between wound protectant compounds, callus tissue formation and Psa mobilisation. In 2012, the use of hand-applied wound protectants (paints and gels) were not common and were not included in the survey. Recent unpublished research has shown that these products have efficacy against Psa infection into wounds (Everett et al. 2016). Any future observational studies should clearly distinguish between hand-applied wound protectants (which may include copper compounds) and sprayer application of copper to protect pruned blocks.

8.5.3 Presence of old male vines

Our results indicated that the presence of older male kiwifruit vines had a lower risk of disease development in blocks and this finding is consistent with other research (Vanneste et al. 2011b). There was no significant association with female vine age which has a different age distribution than male vine age, due to the replacement of male vines to newer cultivars over time (Doyle et al. 1989). There was also no association between different male cultivars and the development of disease, which would indicate that male age is more important than male cultivar. The age of male vines cannot be manipulated by growers. However, the association of higher risk with younger blocks suggests that a different approach to disease management may be required in blocks with younger male vines than in older blocks with lower risk.

8.5.4 Summer girdling

The association found between girdling in the summer of 2011/12 and lower risk of disease development is contrary to the results of Snelgar et al. (2012a) on 'Hort16A' kiwifruit vines. In experimental field trials they observed higher Psa infection rates on girdled vines than on non-girdled vines. A biologically plausible reason for our finding may be the result of an elicited increase in resistance in the vines that were girdled (Schilmiller & Howe 2005). However, spring girdling was not associated with either higher or lower risk of disease expression and it is possible that any effect of spring girdling in eliciting a resistance response may have been offset by high-risk weather events at the time of girdling. Girdling and post-pruning sprays were included as an interaction term but this was not significant, which is consistent with Snelgar et al. (2012b) who found that protective sprays did not reduce Psa infection of girdle wounds.

Possible confounders relating to the lower risk of disease development with summer girdling were: 1) that growers of orchards where Psa was detected but was at low prevalence within blocks may have been more likely to girdle their vines because of a perceived lower disease risk, and 2) that because it is recommended to apply girdling only to un-stressed vines (Currie et al. 2008), there could be a higher number of stressed vines (i.e. diseased plants) in our un-girdled group than in our girdled group. This relationship will be further explored in future research into the risk factors associated with the presence of severe symptoms of kiwifruit bacterial canker.

8.5.5 Regional effects

The between-region differences in risk of disease development are likely to be related to unmeasured factors, such as climate and elevation differences, but may also be related to the length of time the pathogen has been present in a region. Cogger and Froud (2015) found differences in time to Psa confirmation between different regions during the New Zealand outbreak. They showed that while the Te Puke region was severely affected with 10% of orchards infected after 6 months, orchards in the Whakatane and Tauranga East regions had a much faster rate of disease occurrence on naive orchards following first detection in the region, with 41% and 27% of orchards infected in the first 6 months respectively. Orchards in both Te Puke and Tauranga East are located over a much wider range of elevation than those in the other regions, and higher elevations may have contributed to increased risk. Li et al. (2001) found that in China the prevalence of kiwifruit bacterial canker disease was greater above 750 m elevation than at lower elevations, and suggested that lower temperatures at the higher elevations may favour the disease. Studies in New Zealand on blossom blight

(*Pseudomonas viridiflava*) in kiwifruit also found a link between more severe disease at higher elevations in Te Puke (Pennycook & Triggs 1991). Elevation was excluded from our multivariable model as it was considered to be collinear with region as orchards in four of the six regions had very little variation in elevation. High elevation could be important for disease development but there are few orchards at high elevations in New Zealand (the highest at 302 m) and therefore investigating elevation effects further is likely to be of little value for understanding disease in the majority of orchards.

8.6 Conclusion

The factors identified in this study that affected risk of bacterial canker symptoms in blocks were artificial pollination and protective spraying of blocks immediately after pruning (increased risk), and summer girdling and greater age of male vines (decreased risk). The implications of these findings for orchard risk management and the design of further research have been described. While the significant risk factors in a well-designed cross-sectional study may not be causal, as long as the results are interpreted with caution around temporality and potential confounding (Rothman & Greenland 2005; Shahar & Shahar 2013) they should be interpreted as factors that contribute significantly to an increased or decreased prevalence of disease (Maes et al. 2001). These methods can be applied to complex real-world situations during a pest or disease outbreak and can allow scientists and industry managers to establish research priorities (Mann 2003). The statistical model developed in this study is limited in its generalisability to 'Hayward' cultivar kiwifruit and to the regions that were modelled, however it provided timely information for the management of an emerging outbreak. The use of a cross-sectional design in this study provided a new way to investigate plant disease risk factors and this type of study could be more extensively used, especially during incursions of unwanted organisms. Wider adoption of these types of study in plant protection research is likely to occur as the principles of observational study design become better understood from studies such as this one.

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CHAPTER 9

9 Kiwifruit bacterial canker in 'Hayward' kiwifruit: Risk factors associated with severe symptoms of disease in a block

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Hypothesis generation of potential risk factors for severe kiwifruit bacterial canker disease in New Zealand 'Hayward' kiwifruit orchards

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9.1 Abstract

Pseudomonas syringae pv. *actinidiae* biovar 3 (Psa) is the causal agent of kiwifruit bacterial canker and was first recorded in New Zealand in November 2010. In this study, we investigated risk factors for the development of severe bacterial canker in blocks of naturally infected commercial *Actinidia chinensis* var. *deliciosa* 'Hayward' cultivar orchards. Severe disease was classified as 5% or more vines in a block showing shoot wilt and/or cane dieback. An observational cross-sectional study of 331 growers with Psa symptoms present in 'Hayward' blocks between March 2012 and February 2013 was conducted. Data on symptoms, orchard layout and orchard practices were collected via a questionnaire and analysed to identify potential risk factors for severe disease, using multivariable analysis. Results showed that the probability of severe disease increased with time after Psa was first detected in the block and was highest when frost damage occurred; poplar, cypress or pine shelter belts were present and when artificial pollination was used. The risk of severe bacterial canker was lower when spring girdling of female vines was undertaken. Increased disease over time has the potential to affect industry wide productivity. The biological process for frost promoting Psa is reasonably well understood. Shelter belts are not easily changed in established orchards but when there is an opportunity to change then species other than poplar, pine and cypress should be considered. Both artificial pollination and girdling are commonly used management practices and further research is required to understand the biological mechanisms of their relationship with an increased or reduced probability of severe disease.

Keywords: Observational study, multivariable logistic regression, odds ratio, confounding, temporality.

9.2 Introduction

Pseudomonas syringae pv. *actinidiae* (Psa) biovar 3 causes kiwifruit bacterial canker, a disease which was first detected in New Zealand in late 2010 (Everett et al. 2011). Kiwifruit bacterial canker exhibits mild symptoms of leaf spotting or more serious symptoms of shoot wilt and cane dieback, stem cankers and flower wilting (Everett et al. 2011). The serious symptoms can lead to bud or flower drop, shrivelled fruit and vine death. Shoot wilting and cane dieback, caused by bacteria blocking the xylem (Nardozza et al. 2015), is only observed with Psa biovar 3 (Vanneste et al. 2013). Presence of serious symptoms in commercial orchards is likely to be a complex interaction between time infected and other on-orchard factors.

Prior to the arrival of Psa in NZ, kiwifruit production was dominated by two cultivars, green fruiting 'Hayward' (*Actinidia chinensis* var. *deliciosa*) and gold fruiting 'Hort16A' (*Actinidia chinensis* var. *chinensis*). 'Hort16A' is no longer commercially viable in Psa infected regions and has been replaced with less susceptible cultivars (Tanner 2015). 'Hayward' orchards have remained productive (Aitken & Hewett 2013, 2015; Zespri International Ltd 2016a) although researchers in New Zealand, Italy and France have reported an increase in the presence of the serious symptoms (Ferrante & Scortichini 2009; Ferrante et al. 2012; Vanneste 2012; Vanneste et al. 2013). Assessments of the impacts of Psa on 'Hayward' productivity indicate that productivity initially increased in the presence of Psa and did not start to decrease until one year after infection was first detected in a block (Chapter 4).

Following the outbreak of Psa in New Zealand the kiwifruit industry and researchers have proposed many hypotheses about which factors could alter the prevalence of systemic disease symptoms in Hayward orchards including vine age, frost, shelter, elevation, adjacent land use, artificial pollination, pruning management and the practice of girdling (Froud et al. 2015, Chapter 2). Many of these factors are difficult, if not impossible to manipulate in a traditional experiment (e.g. frost and elevation). In addition, there is value in assessing the impact of multiple practices simultaneously under commercial growing conditions and with natural disease development.

Observational studies, which are frequently used in human and veterinary research, are an effective way to investigate multiple factors of interest involving the host, disease and environment (including management) in real-world situations and can complement the experimental approach (Martin 2008; Froud et al. 2014). Observational studies are uncommon in plant protection research (Dallot et al. 2004; Thebaud et al. 2006; Everett et al. 2007; Vicent et al. 2012; Froud et al. 2014; Cogger & Froud 2015; Froud et al. 2015, Chapter 6). A key difference between observational and experimental studies is that extraneous factors, called

confounders, are not able to be managed through randomisation and are typically controlled for at the analysis stage using multivariable statistical models (Dohoo et al. 2009e). Confounding is the confusion or mixing of effects between measured and/or unmeasured variables (Rothman 2012).

This study is a cross-sectional observational study aimed to investigate the time an orchard had been infected with Psa, along with orchard layout and vine or disease management factors that may be associated with a higher or lower risk of severe disease in 'Hayward' kiwifruit blocks. The outcomes of the research aimed to indicate the potential for productivity impacts over time and to identify factors which may be important in the development of systemic symptoms, to be prioritised for further research.

9.3 Methods

9.3.1 Study design

A cross-sectional study of 'Hayward' kiwifruit blocks from Psa infected orchards was made using data collected via a mail out questionnaire. The questionnaire design and implementation are described in detail in Froud et al. (2016). Briefly, the questionnaire was drafted in consultation with technical experts from Zespri International Limited (Zespri), Kiwifruit Vine Health (KVH) and two 'Hayward' growers. It was pre-tested in interviews with 10 growers and then finalised. The questionnaire (Appendix 2) investigated the disease history of the block from March 2012 to February 2013 and considered disease management, vine and orchard layout factors. The questionnaire was sent by Zespri to 1669 'Hayward' growers and growers were assigned a single randomly selected block to respond about. Zespri received 442 completed surveys. Questions were constructed to obtain quantitative data using closed questions with growers asked to answer from a range of options. There were three question formats used: i) select all that apply (e.g. select all forms of pollination used); ii) select one possible answer from a list (e.g. did you use artificial pollination), and iii) rating based on a defined scale (e.g. canopy density ratings). Missing values were left blank when questions were not answered for exposure variables. Data from the KVH Psa database of orchard Psa status, location and elevation were added to the survey data.

9.3.2 Inclusion criteria for analysis

Data for this paper were limited to 331 blocks in which any kiwifruit bacterial canker symptoms had been reported by growers as of March 2013 (Figure 9-1). Where a grower did not answer the question about the earliest date Psa symptoms were observed, they were excluded from the dataset (n=17) or if the orchard was in a region where less than 10 growers

completed the survey they were excluded from the dataset (Coromandel five growers; Waihi five growers, Waikato three growers and Franklin one grower; Figure 9-1). The resulting data set comprised of data from six regions namely Tauranga West, Tauranga East, Te Puke, Katikati, Whakatane and Opotiki.

9.3.3 Classification of outcome variable

For each block, a binary outcome variable was generated that coded for the presence of "severe" kiwifruit bacterial canker in the block as of 28 February 2013. A block was classified as "severe" if the systemic symptoms of either green-shoot wilt or cane die-back were present in 5% or more of the female vines in the block. If neither of these symptoms was present, or less than 5% of vines were affected, the block was classified as "not severe". The definition of green-shoot wilt and cane die-back as being "severe" symptoms was because these are characteristic of Psa biovar 3 systemic infection and are potentially more damaging than some other Psa symptoms, e.g., leaf spotting. The selection of 5% or more was based on the results from Chapter 7 (unpublished data), which found that where these symptoms were recorded by growers the median value was 5% of vines in the block with green-shoot wilt or cane dieback symptoms (25^{th} percentiles were 2% and 1% respectively and 75^{th} percentiles were 10% for both).

9.3.4 Classification of key exposure variables

When the answers to orchard description questions allowed for more than one answer, each possible answer was converted to a separate binary variable that coded not present or present, or not used or used. Where the answers were mutually exclusive, then nominal or ordinal variables were constructed. For example, frost damage could be no damage, minor damage, moderate damage or severe damage. For variables with few observations, and it was appropriate, biologically, to combine categories, the data were combined into new variables. Frost damage was recoded to a binary variable of 'no frost damage' and 'frost damaged' which combined minor, moderate or severe damage. The months since Psa was first observed in the selected orchard block as of 28 February 2013 were based on the answer to the question:

> *Knowing what you do now about Psa symptoms in your orchard, when do you think is the earliest you saw symptoms that on reflection probably were Psa in the block even if they tested negative?*

9.3.5 Data analysis

Data analysis was conducted using 'R' statistical package (R Core Team 2013) and the level of statistical significance was set at $P \leq 0.05$. Microsoft Access was used to combine the questionnaire data with the KVH Psa database variables. Microsoft Excel and the 'R' freeware statistical package version 3.0.1 were used to assess the completeness and validity of the aggregated dataset and missing or unusual values were checked with Zespri or against the industry datasets.

Continuous variables were visually assessed using histograms. Descriptive statistics for continuous exposure variables were given as medians and 25th and 75th quartiles, where data were non-normal/skewed, and means with standard deviation, where data were normally distributed. Descriptive statistics for each binary or multi-level categorical exposure variable were calculated for the number and percentage of total respondents, stratified against the outcome of severe disease or not. Nominal data were presented as counts and percentages stratified against the outcome.

Univariate screening using separate, unmatched, logistic regression procedures was used to determine the association between severe kiwifruit bacterial canker in the block and each exposure variable. Statistical significance was assessed using the log-likelihood ratio test statistic. Explanatory variables associated with the outcome at $P \leq 0.20$ were considered for inclusion in a multivariable logistic regression model of the full dataset (n=331). Screening explanatory variables at the relatively high P-value of 0.20 allowed for the inclusion of variables that may not have been statistically significant prior to controlling for other factors and which may have been confounded with the outcome (Dohoo et al. 2009c). Only exposure variables associated with the outcome at $P \leq 0.20$ are presented in the results.

Selection of the reference category for modelling the data was considered for each multilevel category, based on which level was the most appropriate to compare with other levels (Dohoo et al. 2009f). In the case of the regions, Katikati was selected as it was closest to the mean production and elevation of the whole dataset.

A preliminary main effects model was built in a six-step process. In step one all variables eligible for inclusion, excluding those that were considered collinear, were included in a full model. Prior to inclusion in the model, consideration was given to the problem of correlation between exposures (multi-collinearity; (Marill 2004)) and if exposures were collinear then only one was included in the model. An example of potential collinearity in our research was the collection of data on variables that indicated the use of frost protection and the degree of frost damage sustained in kiwifruit orchards. These two variables were highly correlated as frost protection was only used where there was a risk of frost damage. Where there was obvious

collinearity only one of the related categorical variables was selected based on importance for the system being modelled, in this case frost damage was considered to be biologically important for disease development and was included in the modelling. Step two removed non-significant (P<0.05) variables from the model using an iterative manual backward procedure until all the remaining variables were significantly associated with the outcome using the log-likelihood ratio test statistic. The model that contained only those variables with a statistically significant association with the outcome was termed the preliminary main effects model. Step three added variables not included in the preliminary main effects model back separately to the model and retained if the *P*-value for the log-likelihood ratio test statistic was less than 0.05. Step four tested variables that were considered collinear with variables in the preliminary main effects model by replacing them in the model and the final variable was retained based on significance values of the log-likelihood ration test statistic. Step five considered all biologically plausible two-way interactions for inclusion in the model and they retained if the log-likelihood ratio test statistic was significant. The resulting model was then called the final main effects model. Step six extended the model to include a random effect coding for region. The purpose of this variable was to account for unmeasured variables that may also have been clustered in space. Once the random effect was introduced all the fixed effects variables from the preliminary main effect model were retained even if they were no longer significantly associated with the outcome and this became the final mixed effects model.

No adjustments were made to p-values for the final model as they are not recommended where exposure variables are individually selected based on the potential for a biologically plausible association with the outcome (Rothman 1990; Vandenbroucke et al. 2007) and manual selection of model variables was applied rather than automated selection criteria (Dohoo et al. 2009c; Froud et al. 2015).

The logistic regression coefficients were presented as adjusted odds ratios in the final mixed effects model. The use of odds ratios is normally appropriate if the outcome is rare as the odds ratio is similar to the relative risk in the population. However, if disease prevalence is common, as it is in our study, the odds ratio provides an over-estimate of the effect. Therefore, for visual presentation and discussion of the results of the logistic regression coefficients for the key variables of interest were converted to predicted probabilities. As region was added as a random effect, we calculated the average marginal predicted probability which provides the average change in probability across all six regions (UCLA Statistical Consulting Group 2014).

The fit of the final mixed effects model was tested by comparing it with a partial model containing all the fixed effects variables (i.e. excluding the random effect variable of region)

using the log-likelihood ratio test statistic. Residuals of the final mixed effects model were visualised by plotting the Pearson residuals against the fitted values and uneven variances for each variable were checked visually by plotting the Pearson residuals against the values for each variable (Zuur et al. 2009) and, if detected, then examining observation numbers within each group in two-by-two tables. As diagnostics of mixed effects models are limited, the fit of the model was also assessed with region included as a fixed effect rather than a random effect, using the deviance test on the covariate patterns, the Hosmer-Lemeshow test and the le Cessie-van Houwelingen-Copas-Hosmer un-weighted sum of squares test (Kabacoff 2011). Over-dispersion was checked by visual inspection of a plot of residuals against the half-normal quantiles and calculating the dispersion parameter. Leverage was assessed visually by plotting the Pearson's residuals against the logit. Outliers were checked to see if they were a result of data entry errors and assessed against the KVH or Zespri data where possible. One outlier with a value of months since Psa was first observed of 39 months (6 months earlier than any other observations) was excluded from analysis as the information was contrary to KVH records of not detected test results.

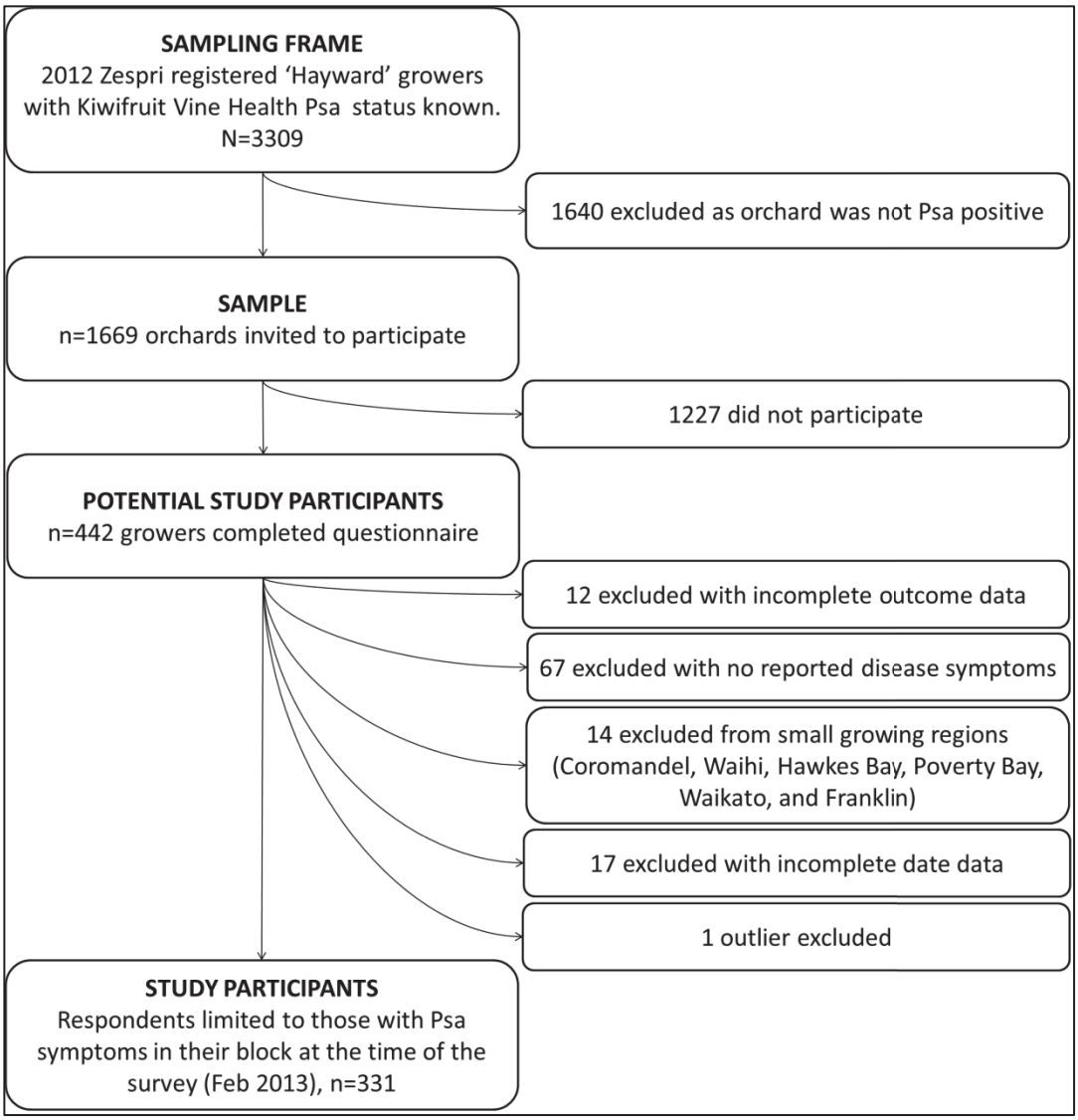

Figure 9-1 Sampling plan showing selection of a sampling frame and the eligibility criteria for inclusion in the study.

9.4 Results

Of the 331 blocks in our study group, 94 (28%) were considered to have severe kiwifruit bacterial canker that is more than 5% of the female vines showing green-shoot wilt or cane die-back and the remaining 237 (72%) were considered not severe. The median number of months Psa had been confirmed on orchards was 16 months (25th quartile 6 months and 75th quartile was 18 months) with a maximum of 33 months infected.

The univariable screening for an association between severe kiwifruit bacterial canker and disease period, orchard layout and management exposure variables showed that 19 variables were eligible for inclusion in the multivariable analysis based on an association at $P<0.20$ (Table 9-1 and Table 9-2). The relationship between exposure variables and severe kiwifruit bacterial canker with $P>0.20$ are not presented.

Results of the final multivariable mixed effects model found that there was a significant association between the length of time kiwifruit bacterial canker had been detected in the block and an increased risk of severe kiwifruit bacterial canker being present (Table 9-3). The predicted probability of severe kiwifruit bacterial canker being present steadily increased for every additional month an orchard block had Psa detected when controlling for all other variables (i.e. adjusting all other variables to not used/not present) and was significantly higher when frost damage was present Figure 9-2. In addition, frost damage, the presence of poplar in shelter belt plantings and using artificial pollination all increased the likelihood that severe symptoms of kiwifruit bacterial canker would be observed. In contrast the model showed that girdling female vines significantly reduced the risk of severe symptoms of kiwifruit bacterial canker being present in the block (Table 9-3).

The majority of growers only had one or two shelter species in their blocks and therefore shelter species were considered to be potentially collinear because the presence of one species would make the presence of another species unlikely. All potentially eligible shelter species for inclusion in the model were tested in the final model and cypress, pine and poplar were all significantly associated with the serve symptoms being present. When tested in the final model the adjusted coefficient of cypress was 2.51 (95% CI: 1.05 to 6.0; *P*=0.04) and for pine was 2.37 (95% CI: 1.02 to 5.49; *P*=0.04). Poplar was retained in the final model with an adjusted coefficient of 2.46 (95% CI: 1.02-5.95; *P*=0.04).

The fit of the final mixed effects model was better than the partial model and diagnostic plots of the Pearson residuals showed all but one observation were within -3 and +3. This observation was checked and was retained in the model. The variance was not uniform for frost or female girdling which may have affected how well the model fitted the data. On inspection of the data in two-by–two tables neither of these variables had low numbers of observations in any group and so both variables were retained in the model. For the model prior to the inclusion of the random effect both the Hosmer-Lemeshow test and the le Cessie-van Houwelingen-Copas-Hosmer unweighted sum of squares test indicated that the model was a reasonable fit for the data. Furthermore, the dispersion parameter did not indicate over-dispersion was a problem. Influential observations were checked to ensure that they were not the result of data entry errors and all of them were retained in the model. Four influential covariate patterns were explained by a cluster of observations having spring 2011 or 2012 detection dates, which is when disease is most commonly detected in the orchard.

Table 9-1 Univariate association between disease period, frost and orchard-related factors and risk of 5% or more female vines showing severe symptoms of kiwifruit bacterial canker in a 'Hayward' block. Data were from 331 growers who had disease in their blocks, selected from respondents to a mail-out survey of 430 'Hayward' blocks that were in orchards classified as infected with Psa.

		Number (%) of blocks		Odds Ratio (OR)	OR 95% CI[a]	*P*-value[b]
Variable	**Level**	**Not Severe**	**Severe**			
Number of months with Psa	Per month	-	-	1.06	1.03-1.09	<0.001
Frost damage in spring 2012	No damage	192 (58)	53 (16)	Ref. [c]		<0.001
	Yes	45 (14)	41 (12)	3.30 [d]	1.96-5.58 [e]	
Frost damage in spring 2012	No damage	192 (58)	53 (16)	Ref.		<0.001
	Minor damage	31 (9)	30 (9)	3.51	1.95-6.33	
	Moderate or severe damage	14 (4)	11 (3)	2.85	1.20-6.63	
Frost protection was used in the block	No	149 (45)	49 (15)	Ref.		0.07
	Yes	88 (27)	45 (14)	1.55	0.96-2.52	
	No	131 (40)	63 (19)	Ref.		0.05
Shelter for block is casuarina	Yes	106 (32)	31 (9)	0.61	0.37-1.00	
Shelter for block is cypress	No	220 (66)	82 (25)	Ref.		0.12
	Yes	17 (5)	12 (4)	1.89	0.85-4.11	
Shelter for block is pine	No	218 (66)	80 (24)	Ref.		0.07
	Yes	19 (6)	14 (4)	2.01	0.94-4.17	
Shelter for block is willow	No	205 (59)	73 (21)	Ref.		0.05
	Yes	32 (9)	21 (6)	1.84	0.99-3.38	
Shelter for block is poplar	No	224 (68)	81 (24)	Ref.		0.02
	Yes	13 (4)	13 (4)	2.77	1.22-6.27	
Closed/dense canopy (n=329 observations)	No (rating 1,2)	196 (60)	84 (26)	Ref.		0.08
	Yes (rating3,4,5)	40 (12)	9 (3)	0.53	0.23-1.09	
Elevation	Less than 20m	86 (26)	35 (11)	Ref.		0.08
	20m to 80m	74 (22)	19 (6)	0.63	0.33-1.18	
	Greater than 80m	77 (23)	40 (12)	1.28	0.74-2.21	

[a] 95% Confidence Interval; [b] Significance of Likelihood ratio test statistic; [c] Reference level; [d] Interpretation: When there was frost damage in spring 2012 the risk of severe kiwifruit bacterial canker being present is 3.30 times greater than when no frost damage occurred. [e] Interpretation: We are 95% confident that the higher risk of severe disease when there was frost damage is between 1.96 and 5.58.

Table 9-2 Univariate association between vine and disease management-related factors and risk of 5% or more female vines showing severe symptoms of kiwifruit bacterial canker in a 'Hayward' block. Data were from 331 growers who had disease in their blocks, selected from respondents to a mail-out survey of 430 'Hayward' blocks that were in orchards classified as infected with Psa.

Variable	Level	Number (%) of blocks		Odds Ratio	OR 95% CI[a]	*P*-value[b]
		Not severe	Severe			
Female vines were girdled in spring 2012	No	134 (40)	67 (20)	Ref.[c]		0.01
	Yes	103 (31)	27 (8)	0.52[d]	0.31-0.87[e]	
Female vines were girdled in spring 2011	No	135 (41)	67 (20)	Ref.		0.01
	Yes	102 (31)	27 (8)	0.53	0.31-0.89	
Management type	Conventional	217 (66)	80 (24)	Ref.		0.09
	Organic	20 (6)	14 (4)	1.9	0.90-3.9	
Used artificial pollination in spring 2012	No	152 (46)	48 (15)	Ref.		0.03
	Yes	85 (26)	46 (14)	1.71	1.06-2.78	
Used artificial pollination in spring 2011	No	187 (56)	77 (23)	Ref.		0.54[f]
	Yes	50 (15)	17 (5)	0.83	0.44-1.50	
Used bee hives only for pollination in spring 2012	No	100 (30)	49 (15)	Ref.		0.10
	Yes	137 (41)	45 (14)	0.67	0.41-1.08	
Used bee hives only for pollination in spring 2011	No	83 (25)	25 (8)	Ref.		0.14
	Yes	154 (47)	69 (21)	1.49	0.88-2.56	
Management of pruning material (n=330 observations)	Pruning material mulched immediately	136 (41)	62 (19)	Ref.		0.09
	Pruning material mulched within 1 month	75 (23)	27 (8)	0.79	0.46-1.34	
	Pruning material left on ground	26 (8)	4 (1)	0.34	0.10-0.91	
Disease spraying done by orchard manager	No	214 (65)	90 (27)	Ref.		0.08
	Yes	23 (7)	4 (1)	0.41	0.12-1.11	
Pruning hygiene practices	Equipment cleaned between vines	63 (19)	31 (9)	Ref.		0.04
	Equipment cleaned between bays	70 (21)	24 (7)	0.71	0.39-1.30	
	Equipment cleaned between blocks	26 (8)	18 (5)	1.48	0.70-3.11	
	Equipment cleaned daily	39 (12)	12 (4)	0.67	0.28-1.58	
	Equipment cleaned on arrival in orchard	23 (7)	2 (1)	0.19	0.03-0.83	
	Equipment not cleaned	16 (5)	7 (2)	0.98	0.79-1.18	

[a] 95% Confidence Interval; [b] Significance of Likelihood ratio test statistic; [c] Reference level; [d] Interpretation: When vines were spring girdled the risk of severe kiwifruit bacterial canker being present is 48% less than when vines were not spring girdled. [e] Interpretation: We are 95% confident that the decreased risk of severe disease when vines are spring girdled is between 13% and 69% less. [g] Not significant but of interest.

Table 9-3 Results of multivariable logistic regression model describing the relationship between severe kiwifruit bacterial canker (5% or more female vines showing systemic symptoms) and time infected or other orchard factors in a 'Hayward' block. Data were from 331 growers who had disease in their blocks, selected from respondents to a mail-out survey of 430[a] 'Hayward' blocks that were in orchards classified as infected with Psa.

Variable	Odds Ratio (OR)	OR 95% CI[b]	*P*-value[c]
Time kiwifruit bacterial canker symptoms had been present in the block (months)	1.05	1.01-1.09	0.007
Vines damaged by frost in spring 2012			<0.001
No	Ref.[d]		
Yes	3.71[e]	2.10-6.54[f]	
Female vines girdled in spring 2012			0.05
No	Ref.		
Yes	0.57	0.32-1.00	
Poplar in shelter belt plantings			0.05
No	Ref.		
Yes	2.46	1.02-5.95	
Used artificial pollination in spring 2012			0.02
No	Ref.		
Yes	1.85	1.08-3.07	
Region [g]			

The model is based on 324 degrees of freedom. [a] Data limited to 331 blocks located in Tauranga West, Tauranga East, Te Puke, Katikati, Whakatane and Opotiki and in which any symptoms of kiwifruit bacterial canker had been observed as of March 2013. [b] 95% Confidence Interval; [c] Significance of Likelihood ratio test statistic; [d] Reference level; [e] Interpretation: The risk of severe kiwifruit bacterial canker being observed was 3.71 times higher in frost damaged blocks compared to blocks with no frost damage. [f] Interpretation: We are 95% confident that the increased risk of severe disease when frost damage occurred is between 2.10 and 6.54; [g] Variance of 0.15 (SD=0.38) around the intercept due to the random effect of region.

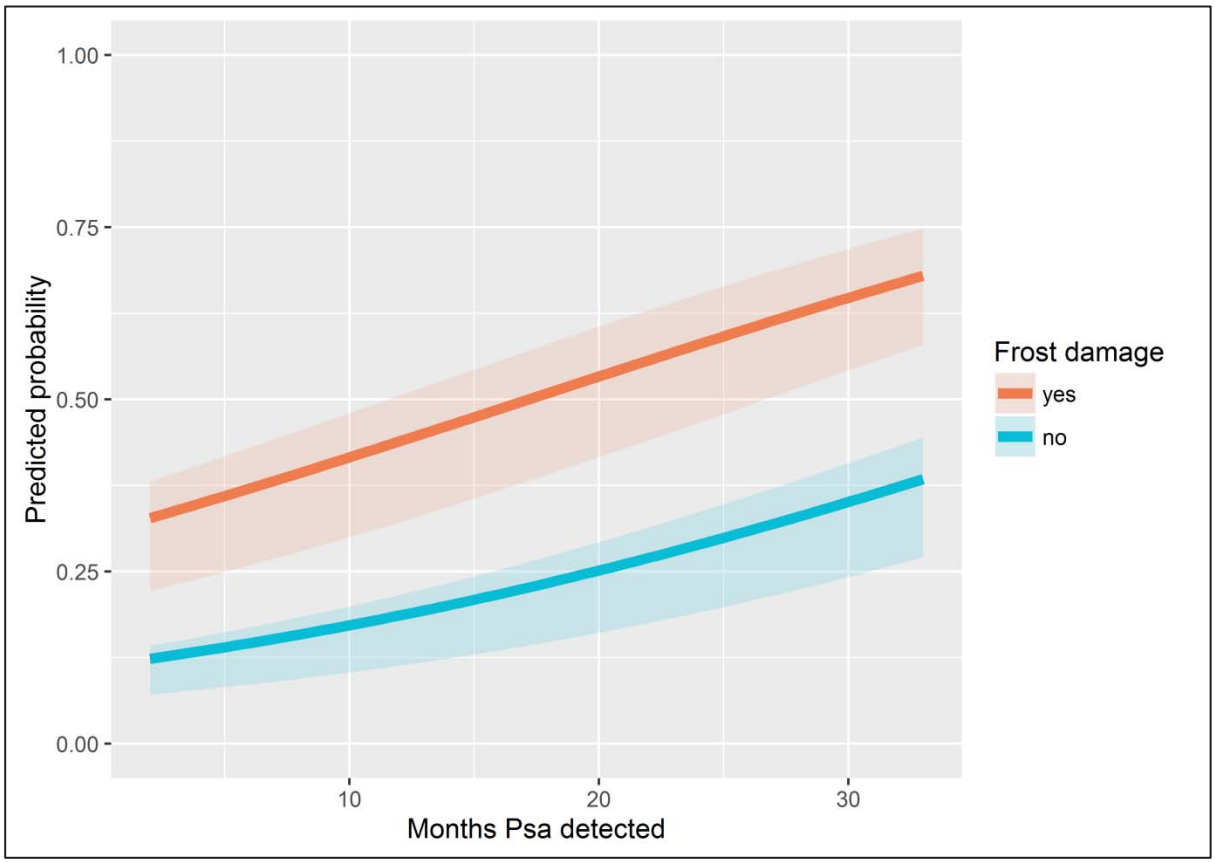

Figure 9-2 Plot of the average marginal predicted probability across all regions, that is, the average change in probability of severe kiwifruit bacterial canker (shoot wilt or dieback on 5% or more female vines) in a 'Hayward' block after adjusting for all other factors, across the months the orchard has shown Psa symptoms. The average probability of severe symptoms being detected is equivalent to the reference line for no frost with an increased probability of severe symptoms in blocks that reported frost damage. Shaded areas around the lines show the upper and lower quartiles which shows where 50% of the predicted values lie.

9.5 Discussion

In this study, which investigated risk factors associated with severe kiwifruit bacterial canker disease in 'Hayward' kiwifruit blocks, nearly 30% of the blocks surveyed showed severe disease. Risk of severe disease increased with time after Psa was first detected in a block and was greater when frost damage occurred. It was also greater when poplar, cypress or pine shelter belts were present and when artificial pollination was used. The risk of severe bacterial canker was smaller when spring girdling of female vines was undertaken. The use of multivariable analysis allowed measured confounders, including those associated with differences between growing regions, to be controlled.

The prevalence of severe bacterial canker was higher than expected in 'Hayward' which has been regarded as susceptible to leaf infection but not to the more severe symptoms (Hoyte et al. 2015). This cultivar has been expected to remain free of serious effects of the disease (Greer & Saunders 2012). While the number of orchards with severe disease was high, the blocks that were affected typically had a low within block prevalence of systemic symptoms (median of 5% of vines), that is, while many orchards had disease not many vines were affected.

The number of months that kiwifruit bacterial canker symptoms had been observed in the block was positively associated with an increased risk severe disease in the blocks. These results are consistent with other research in New Zealand (Vanneste et al. 2013; Froud et al. 2014) and studies in Italy (Kay 2011, 2012), which showed that the impact of disease increased over time. The results are consistent with another observational study in New Zealand, which found that production losses were not observed until at least 12 months after Psa was first found in an orchard (Chapter 4, unpublished data). Shoot-wilt and cane dieback symptoms are caused when the bacterium spreads systemically within the vine and are caused by a proliferation of bacterial cells blocking the vascular tissue (Vanneste et al. 2011b; Nardozza et al. 2015). These systemic symptoms can take up to 12 months to occur after first exposure to Psa in naturally infected 'Hayward' orchards (Vanneste et al. 2013). Tyson et al. (2014b) showed using experimental inoculation of 'Hort16A' that systemic movement of Psa is initially slow and increases over time, which supports our finding that risk of severe disease in orchards increased over time. A key consideration when using a cross-sectional study design is that data on both the outcome and the potential risk factors are collected at a single point in time and therefore the temporal direction of cause and effect cannot be proven for time-changing variables (Ioannidis 2016). Although the increasing risk of severe disease over time that was found in this study is well supported by other research, this relationship should be considered as significantly associated with an increased risk of disease, rather than causal when interpreting the results.

The association found between frost damage and higher risk of severe kiwifruit bacterial canker is consistent with evidence from Ferrante and Scortichini (2014), who found that frost causes plant membrane damage, allowing Psa bacterial entry to the vine, and also promotes increased multiplication of Psa in inoculated shoots. Kiwifruit bacterial canker symptoms are also strongly linked to low temperature which is associated with Psa bacterial population growth (Serizawa & Ichikawa 1993c).

Girdling in spring was found to be associated with a reduced likelihood of severe kiwifruit bacterial canker and the relationship was strong for girdling in both spring 2011 and spring

2012. While this is contradictory to research in New Zealand that found higher Psa infection rates for girdled vines in field experiments (Snelgar et al. 2012a), it is consistent with the finding of Chapter 8 (unpublished data) that risk of Psa introduction was lower when there was summer girdling of female vines. Fruit set of 'Hayward' vines suffering from Psa bud rot has also responded well to spring girdling (Ryan & Jeffery 2014) and this practice is now recommended to manage bud rot in New Zealand 'Hayward' orchards (Zespri International Ltd 2016b). An explanation for this reduction in risk may be due to the long-term effects of girdling in eliciting an immune response increasing the health of vines, which has been shown in other plant systems (Schilmiller & Howe 2005). However, it is possible that the association was confounded by growers of orchards without systemic symptoms being more likely to girdle their vines because of low perceived disease risk. Girdling is not recommended for stressed vines (Currie et al. 2008), therefore there could be a higher number of stressed vines (i.e. diseased) in our un-girdled group compared with our girdled group, which may have confounded the observed relationship.

The presence of poplar, cypress or pine shelter was found to increase the risk of severe kiwifruit bacterial canker. There is little in common between these species, however cypress and pine were traditionally recommended for tall boundary shelter, rather than internal shelters, (Hathaway 1990) and their association may be confounded by the presence of unmeasured edge effects of blocks that are on the boundary of a property. Poplars are deciduous and late in coming into leaf in spring (Hathaway 1990). Therefore, the lack of shelter protection in early spring may increase the risk of vines being damaged, thereby providing points were Psa can enter the vine. Temporality of data collection is unlikely to be an issue with shelter as it does not typically change over time and disease has only been present in New Zealand for a short period. There is, however, no evidence that shelterbelt species provide an alternative host for Psa (Vanneste et al. 2015) and therefore any association is unlikely to be a direct biological process. It is uncommon to replace shelter species and therefore this research can only indicate that poplar, cypress and pine may be problematic and growers replacing or developing new shelter should consider avoiding these species.

The results of this study showed a higher risk of severe bacterial canker when artificial pollination was used in spring 2012. In contrast, there was no association with artificial pollination use in spring 2011 and the risk of severe disease (P=0.90). Pollen is known to harbour Psa (Gallelli et al. 2011; Vanneste et al. 2011c; Everett et al. 2012d; Tontou et al. 2014) and Psa inoculum may be introduced into the orchard via contaminated pollen during the high-risk spring period when vines have young leaves, flowers and canes that are more susceptible to infection and systemic spread of the bacteria. However, Tontou et al. (2014), observed a 12

month delay between the application of infected pollen and the observation of leaf spotting. It is unknown whether systematic symptoms could develop rapidly enough between spring 2012 and the time of block assessment in March 2013 for this to be a direct causal relationship, especially given the possible temporal issues with disease appearance in a cross-sectional study. The increased production due to artificial pollination may put additional stress on already diseased vines making them more susceptible to developing systemic symptoms of bacterial canker. It is also plausible that growers who had a high proportion of kiwifruit vines exhibiting kiwifruit bacterial canker symptoms would use artificial pollination to optimise the productivity of their vines which may confound this association.

There were differences in risk between growing regions. There were a range of unmeasured climatic and soil differences between areas that could be the reason for these differences. In addition, as kiwifruit bacterial canker is an emerging outbreak, growing region was important to include as a potential confounder as there are differences in the periods of time that each region had been exposed to Psa and this could affect the amount of additional inoculum available for development of disease into an orchard block.

In addition to confounding and temporality, these survey based data could have suffered from measurement bias through inaccurate assessment of symptoms. However, Psa biovar 3 is the only pathogen known to cause the systemic symptoms of wilt and dieback that were used to form our outcome variable. Relying on growers to assess symptoms and to provide an estimate of percentages of vines affected will generate some measurement bias and variability in the results and this should be taken into account when extrapolating the results. The importance of this pathogen to New Zealand growers has resulted in considerable exposure to the different disease symptoms through multiple print and television media channels including industry resources such as a symptom guide, weekly updates, videos and a dedicated website (kvh.org.nz) and therefore misclassification of symptoms is expected to be minimal.

This study was limited to kiwifruit growing regions that had been exposed to Psa for some time, however the findings of an increased risk of developing systemic symptoms over time and in association with frost are likely to hold true for other growing regions in New Zealand and internationally. It is hypothesised that systemic spread and blocking of vascular tissue resulting in shoot wilt and cane dieback will also impact fruit production and that an increase in systemic symptoms over time may reduce the productivity of ‘Hayward’ in a Psa environment. The extrapolation of these results to other kiwifruit cultivars should be done with caution given the variation in susceptibility of cultivars to Psa biovar 3 (Hoyte et al. 2015) and also due to differences in the stages of crop development when frost typically occurs in New Zealand.

The factors identified in this study that affected risk of developing severe disease were the period of time infected, frost damage, shelter belt species and artificial pollination (increased risk) and spring girdling reduced risk. The implications of these findings for orchard risk management and the design of further research have been identified. The statistical model developed in this study is limited in its generalisability to 'Hayward' cultivar kiwifruit and to the regions that were modelled, however it provided timely information for the management of an emerging outbreak. The direct link between occurrence of systemic symptoms and loss of productivity is unknown and warrants further research. Research into the impact of frost and the main biological process associated with frost and systemic symptoms may influence which frost protection technologies are appropriate in a Psa environment. Girdling appears to reduce the presence of severe kiwifruit bacterial canker, however, the biological mechanism for this is unclear and warrants further investigation as girdling could have the potential to improve Psa management. Further studies are needed to determine whether, and by what mechanism, artificial pollination contributes to severe kiwifruit bacterial canker and this is recommended as a research priority for the kiwifruit industry. While the significant risk factors in a well-designed cross-sectional study may not be causal, as long as the results are interpreted with caution around temporality and potential confounding (Rothman & Greenland 2005; Shahar & Shahar 2013), they should be interpreted as factors that contribute significantly to an increased or decreased prevalence of disease (Maes et al. 2001). These methods can be applied to complex real-world situations during a pest or disease outbreak and can allow scientists and industry managers to establish research priorities (Mann 2003) and there is potential for observational studies to be more extensively used in plant protection research.

9.6 Acknowledgements

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CHAPTER 10

10 General Discussion

This thesis applied observational studies to investigate the impact of Psa in commercial orchards to: 1) quantify a change in productivity associated with disease; 2) determine the prevalence of disease in orchards; 3) identify factors that altered the initial development of disease and 4) identify factors that affect the presence of severe disease. Aim 1 used retrospective industry data from 2599 'Hayward' orchards and aims 2-4 used data collected from 430 'Hayward' growers using a cross-sectional survey, which was sent to all Psa confirmed orchards.

10.1 Change in 'Hayward' productivity associated with Psa

The changes in productivity associated with Psa infection (Chapter 4) were determined from retrospective industry data from commercial orchards subjected to different management regimes and infection periods. That study demonstrated how existing data sources can allow rapid analysis and provide important information for assessing the impact of an emerging plant disease epidemic. The results showed that, after adjusting for the factors of region, elevation, 2011 productivity, day of harvest and use of protective sprays, the productivity of 'Hayward' kiwifruit orchards did not decline until an orchard had been infected for more than one year. There also appeared to be an initial increase in productivity after Psa was detected, which may have been from improved canopy management post-detection or an elicited physiological response.

The initial increase in productivity with a one year delay before Psa negatively affected productivity may have been related to the time for Psa to infect and become severe in enough 'Hayward' vines in individual orchards to cause a detectable reduction in productivity. The cross-sectional survey of growers showed that blocks that were affected typically had a low within block prevalence of systemic symptoms (Median = 5% of vines), that is, while many orchards had disease not many vines were affected (Chapters 5 and 6). The decrease in production after one year is consistent with the results from Chapter 9 which found the probability of severe disease increased with the time since disease was first detected. Severe disease was defined as 5% or more female vines with shoot wilt or cane dieback. The economic assessment of Psa on the kiwifruit industry assumed no impact on 'Hayward' (Greer & Saunders 2012) however, the results from this study do not support that assumption.

The research in Chapter 4 was undertaken in the early stages of the Psa outbreak when Psa had been detected in only 36% of 'Hayward' orchards and there were only 3% of orchards in which Psa had been present for more than a year. The start of the New Zealand Psa epidemic was most likely nine to 18 months prior to the first detection in November 2010 (Ministry for

Primary Industries 2011; Rosanowski et al. 2013a). Recent industry productivity data has indicated small reductions in productivity in the 2013, and 2014 harvests (Aitken & Hewett 2015). However productivity in 2015 and 2016 has increased with the 2016 season higher than any previous season, although the number of 'Hayward' producing hectares has reduced by 22% (Zespri International Ltd 2016a). The increased productivity may reflect a consolidation of growers who are able to manage Psa well and/or the effects of new management tools for Psa in New Zealand orchards (Gaskin et al. 2012; Tyson et al. 2012b; Kiwifruit Vine Health Inc. 2013; Horner et al. 2015; Kiwifruit Vine Health Inc. 2015; Beresford et al. 2017). Alternatively, it could reflect a period of excellent growing conditions and conditions less favourable for Psa multiplication and disease development.

The extensive databases available from Zespri, on productivity and agrichemical applications, and KVH on the industry wide Psa outbreak were fundamental in our ability to undertake a rapid assessment of productivity effects from Psa. These databases are less likely to be available in other plant based industries, where crops are not primarily grown for export, or where they are not managed under a single marketing structure. From a biosecurity preparedness viewpoint, other plant industries should consider the minimum requirements for data to assist in outbreak investigation, for example pest and disease survey data, production data, spray data and plant material movements. Plant based industries should consider implementing consistent data fields so that data aggregation is feasible in the event of an outbreak. Despite the extensive data available from KVH and Zespri there were issues with data structure in the industry databases that required extensive re-coding and data cleaning. It would be useful for researchers and industry to work together to set-up data management protocols in the event of an outbreak to minimise such issues. In outbreak situations where good quality data is not immediately available, then prospective surveys with pre-agreed data fields could be developed as part of biosecurity preparedness.

In the productivity study, it was unfortunate that spray data and productivity data were not both available at the block level and that spray data had to be aggregated as mean sprays of each spray type. While the analysis was still effective the data were unable to fully utilised, for example, it was not possible to use the date of spray application to associate sprays with weather risk events.

10.2 Questionnaire

The cross-sectional survey (Chapter 5) showed that a postal questionnaire was an effective way to obtain disease, risk factor, and orchard hygiene data for a plant health study. Factors to

be included in the study were discussed at a workshop involving industry experts and two representative growers. Draft factors for inclusion were visualised using a causal web, which was an effective tool to identify potential confounders and interactions between variables. Visualising the causal web also aided the development of the individual questions.

The draft questionnaire was pre-tested on 10 'Hayward' growers. Pre-testing the questionnaire ensured a clear understanding between researchers and respondents. This is an important step as interpretation and validity of results can be fundamentally biased if there is an undetected difference in interpretation of questions (Dohoo et al. 2009d Pg 66). A valuable additional feature of pre-testing is the opportunity to gather insight into how growers record data (calendars, computers, notebooks, accounting packages, etc.) and to obtain an indication of how robust grower recall data will be. It also gives an opportunity to fully understand how grower practices are performed and what growers see as priorities for research.

The survey (Chapter 5) obtained a typical response rate for the kiwifruit industry (26%), despite its length (Appendix 2) and was consistent with other studies in New Zealand (Greer & Teulon 2003) and a similar prevalence study on potato scab in Canada (24%) (Hill & Lazarovits 2005). If the cross-sectional survey was investigating a less devastating disease than Psa, the length of the questionnaire may have reduced the response rate below that sufficient for robust analysis (Edwards et al. 2002; Rolstad et al. 2011).

The availability of industry data allowed us to compare respondents in the context of all potential participants in the survey and identify the potential for response bias between responders and non-responders. Non-response may have two implications in a study of this type: firstly, if the aim is estimating the prevalence of disease for a population, then it is important to present stratified results if bias is known to be present (Groves 2006; Groves & Peytcheva 2008). The second implication of non-response is the potential for biased estimates of risk factors if there is a correlation between the outcome variable of interest (presence of Psa) or key potential risk factors for the disease that could be associated with non-response (Mannetje et al. 2011), such as low productivity. In this study, there was no difference between responders and non-responders associated with the time period that the disease had been present, or productivity factors which could have affected the validity of the risk factor analysis results in Chapters 8 and 9.

10.3 Symptoms associated with Psa in commercial orchards

Prior to the work presented in Chapter 6 the prevalence of Psa symptoms in commercial 'Hayward' kiwifruit orchards was unknown, however, reports of green shoot-wilt and cane die-back were being received by industry in the spring of 2011. The results of the disease survey in Chapter 6 provided industry with an insight into the prevalence of disease as of March 2013. The first onset of symptoms was reported in spring by most growers, which was consistent with other studies (Serizawa & Ichikawa 1993b; Rosanowski et al. 2013a; Tyson et al. 2015). The prevalence of disease based on any symptoms in the selected blocks was high (84%), which was expected given that the sampled orchards were confirmed Psa-positive by KVH, an eligibility criterion for inclusion in the study. However, the prevalence of systemic symptoms of bacterial canker, namely green shoot-wilt and cane dieback, in the 430 surveyed 'Hayward' blocks was higher than expected on both male (51%) and female vines (42%). The prevalence of systemic symptoms was concerning as 'Hayward' had been regarded as susceptible to leaf infection but not to the more severe symptoms (Hoyte et al. 2015) and this cultivar was anticipated to remain free of serious effects of the disease (Greer & Saunders 2012). The higher prevalence of systemic Psa symptoms in male vines could have consequences for pollination in the future and may act as an inoculum source within blocks. The total number of orchards with any systemic symptoms of disease was 57% but only 28% of blocks had 5% or more of the female vines with systemic symptoms, that is, while many orchards had disease not many of the productive female vines were affected.

The results presented in Chapter 6 also indicated that bud drop was an issue and was reported from female vines in 41% of blocks and in male vines from 33% of blocks. This was the first time the prevalence of bud drop had been reported in association with Psa and it was unclear if the cause was Psa or frost damage. Subsequent work has shown that Psa infection can cause bud browning and bud drop (Tyson et al. 2014a) and research programmes to understand bud drop and its management are underway.

Some growers may have reported symptoms similar to Psa biovar 3, but which may have been caused by other pathogens known to cause leaf spotting in New Zealand such as *Pseudomonas syringae* pv. *actinidifoliorum* (previously and commonly known as Psa biovar 4 or Psa-LV), *Pseudomonas viridiflava, Pseudomonas* sp. or *Pseudomonas syringae* pv. *syringae* (Young et al. 1997; Vanneste et al. 2013; Cunty et al. 2015). These may have been misclassified in our disease prevalence estimates and in defining the outcome variable in Chapter 8. However, Psa biovar 3 is the only pathogen known to cause the systemic symptoms of wilt and dieback that

were used to form our outcome variable in Chapter 9. Relying on growers to assess symptoms and to provide an estimate of percentages of vines affected will have generated some measurement bias and variability in the results, which must be taken into account when extrapolating the results. The importance of this pathogen to New Zealand growers has resulted in considerable exposure to the different disease symptoms through multiple print and television media channels including industry resources such as a symptom guide, weekly updates, videos and a dedicated website (kvh.org.nz) and therefore misclassification of symptoms is expected to be minimal.

10.4 Orchard management practices in commercial orchards

Chapter 7 is the first comprehensive study of the features and management practices in a large sample of commercial kiwifruit orchards in New Zealand. The typical 'Hayward' kiwifruit block in a Psa infected orchard is at low elevation with 30-year-old 'Hayward' female vines and 25-year-old 'Chieftain' male vines with Japanese cedar (*Cryptomeria japonica*) and she-oak (*Casuarina* sp.) shelter belts. Artificial pollination was used by a fifth of growers in spring 2011 and a third of growers in spring 2012. Girdling was undertaken on female vines by two-thirds of the growers in the study but on only 3% of male vines. Frost damage is a frequent event and was reported in a quarter of the blocks.

Over 90% of growers reported using pruning equipment hygiene practices and nearly 75% of growers were using post-pruning sprays which is recommended for Psa management in the orchard (Kiwifruit Vine Health Inc. 2015). Nearly 80% of growers said they had to delay applying protective sprays because of wet weather, however only 20% of growers had adopted the KVH Psa-V weather risk model at the time of our survey. Increased use of the KVH Psa-V weather risk model to plan sprays prior to forecast infection events could improve the timing of protective sprays and reduce delays in getting protection on the vines.

10.5 Potential risk factors for disease development and presence of severe bacterial canker

The aim of Chapters 8 and 9 was to identify factors that altered the initial development of disease and factors that impact on the presence of severe of disease to assist the kiwifruit industry to prioritise future research. The factors associated with the initial development of any bacterial canker symptoms in 194 asymptomatic 'Hayward' kiwifruit blocks (Chapter 8) and which were associated with severe disease of 5% or more female vines with green shoot wilt or cane dieback in 331 symptomatic blocks (Chapter 9) were investigated.

Chapter 8 identified two variables that were associated with an increased probability of initial disease development in asymptomatic 'Hayward' orchard blocks, namely, the application of artificial pollen in spring 2012, and the practice of routinely spraying Psa protectants on vines immediately after pruning. The probability of kiwifruit bacterial canker was reduced in association with summer girdling. The probability of disease decreased as male vines were older, after adjusting for these factors, there were significant differences between the regions.

Chapter 9 showed that the probability that severe disease would be present in a block increased with the period since Psa was first detected, when artificial pollination was used, when frost damage occurred and when poplar, cypress or pine shelter belts were present. The risk of severe bacterial canker was lower in association with spring girdling of female vines. There were also significant regional differences in the probability of severe disease. The most important factors identified in Chapters 8 and 9 for prioritised research were artificial pollination, sprays after pruning, period infected, frost, girdling and regional effects.

10.5.1 Artificial pollination

Use of artificial pollination increased the probability of both disease development (Chapter 8) and severe disease (Chapter 9). Pollen is known to harbour Psa (Gallelli et al. 2011; Vanneste et al. 2011c; Everett et al. 2012d; Tontou et al. 2014) and as such could have been an entry point of inoculum into blocks. However, the blocks in our study were already exposed to Psa inoculum from adjacent blocks and it is unclear if any additional inoculum on pollen would have had time to initiate the disease symptoms observed by growers or would have been more important than naturally occurring inoculum. Generally, the date of detection of disease is not recorded in cross-sectional studies, making it difficult to assess temporality (Shahar & Shahar 2013), but the design of the questionnaire (Chapter 5) enabled us to consider some aspects of temporality. The temporality of the association with disease was problematic in both Chapter 8 and 9. In Chapter 8 the relationship between artificial pollination application and disease detection was an issue as some growers reported symptoms developing prior to the application of pollen in their blocks. In Chapter 9 there was limited time to develop severe symptoms between artificial pollination in spring 2012 and assessment of severe disease in February 2013. Consistent with insufficient time to develop symptoms, Tontou et al. (2014), observed a 12 month delay between the application of infected pollen and the observation of leaf spotting and did not observe any systemic symptoms developing within 12 months.

Due to observed issues with temporality, non-causal factors that are strongly associated with artificial pollination could be the reason for the association with both disease development

and severe disease. One non-causal reason for the association is that the increased productivity of blocks which used artificial pollination may put additional stress on vines that are infected with Psa, making them more likely to develop disease symptoms. It is also plausible that growers who had a high proportion of kiwifruit vines exhibiting kiwifruit bacterial canker symptoms would use artificial pollination to either augment diseased male pollinator vines or optimise the productivity of their vines which may confound this association.

10.5.2 Protective sprays

The routine application of protective sprays immediately after pruning was associated with an increased probability of initial disease development (Chapter 8) but not with presence of severe disease (Chapter 9). The majority of protective sprays used after pruning in New Zealand are copper mixes which have been shown to inhibit callus formation in other plant systems (Mercer 1983; Manivel & Handique 1984; Doster & Bostock 1988; Taddei et al. 2007). This may keep pruning wounds open to infection for longer leading to an increased probability of disease developing, as observed in Chapter 8. In addition, water runoff from protective sprays may enable the mobilisation of bacteria and transfer bacteria onto fresh pruning wounds. The mobilisation of Psa inoculum in post-pruning sprays could have increased infection if there was limited inoculum present in the block. The observed association between post-pruning sprays and disease development may also have been due to a confounding factor, for example, growers who pruned when there was a high-risk weather event may have been more likely to apply a post-pruning spray to mitigate this risk. At present, there is limited evidence of the efficacy of post-pruning sprays in controlling Psa. However there is evidence that some hand-applied post-pruning gels and paints are beneficial if applied carefully (Everett et al. 2014; Cornish et al. 2015) and these may be a more effective alternative until more is known about the best use of post-pruning sprays.

10.5.3 Period infected with Psa

The number of months that kiwifruit bacterial canker symptoms had been observed in the block was positively associated with an increased probability of severe disease in the blocks (Chapter 9). These results are consistent with other research in New Zealand (Vanneste et al. 2013) and studies in Italy (Kay 2011, 2012), that showed a higher impact of disease over time. The association with severe disease and the period infected are also compatible with the results of the productivity study (Chapter 4), where productivity losses were not observed until the orchards had been confirmed with Psa for over one year. Other studies have shown that systemic symptoms of kiwifruit bacterial canker can take up to 12 months to occur from first

exposure to Psa in naturally infected 'Hayward' orchards (Vanneste et al. 2013) and systemic movement of Psa in 'Hort16A' initially moves slowly after experimental inoculation and increases over time (Tyson et al. 2014b). The delay in disease expression is consistent with an increased probability of severe disease developing over time. However, as with other associations found in these studies the use of a cross-sectional study design and collection of data at a single point in time means that although the period the block had been symptomatic increased the risk of severe disease is supported by other research, this relationship should be considered as associated rather than causal.

The results in Chapter 9 were limited to kiwifruit growing regions that had been exposed to Psa for some time, however the findings of an increased risk of developing systemic symptoms over time are likely to hold true for extrapolation to other growing regions in New Zealand and internationally. It is hypothesised that systemic spread and blocking of vascular tissue resulting in shoot wilt and cane dieback will also impact fruit production and that an increase in systemic symptoms over time may reduce the productivity of 'Hayward' in a Psa environment. However, the extrapolation of these results to other kiwifruit cultivars should be done with caution given the variation in susceptibility of cultivars to Psa biovar 3 (Hoyte et al. 2015).

10.5.4 Frost damage

The presence of frost damage was associated with a higher probability of severe disease (Chapter 9) but not with the initial development of disease (Chapter 8). Ferrante and Scortichini (2014) found that frost causes plant membrane damage allowing Psa bacterial entry to the vine and promotes increased multiplication of Psa in inoculated shoots. The results in Chapter 9 are consistent with this, in that our study of severe disease was set in an environment where inoculum was readily available to multiply as all the blocks were symptomatic. This is in contrast to the study of disease development where symptomatic vines were absent at the start of the study period, and therefore inoculum availability would have been lower. Kiwifruit bacterial canker symptoms are also strongly linked to low temperature, which is associated with increased Psa bacterial multiplication (Serizawa & Ichikawa 1993c).

10.5.5 Girdling

Girdling was associated with a reduced probability of disease development (Chapter 8) and severe disease (Chapter 9), indicating a protective effective. This is contrary to the results of Snelgar et al. (2012a) who found higher Psa infection rates on 'Hort16A' vines, but is consistent with improved fruit-set in 'Hayward' vines suffering from Psa bud rot when spring girdling was applied (Ryan & Jeffery 2014). Girdling is now recommended to manage bud rot in New

Zealand 'Hayward' orchards (Zespri International Ltd 2016b). Our results are also consistent with a protective effect arising from an elicited immune response in girdled vines, or an increase in the health of vines that were girdled, which has been shown in other plant systems (Schilmiller & Howe 2005). Uncertainty arising from use of a cross-sectional study design means that it is also possible that the association was observed because orchards without systemic symptoms were more likely to girdle due to a perceived low disease risk. Another non-causal reason for the associations is that girdling is not recommended for stressed vines (Currie et al. 2008) and therefore, there could have been more stressed vines (i.e. diseased) in our un-girdled group compared with our girdled group. The association between girdling and a reduced risk of disease indicates that girdling may be an important factor for disease management in the future. The frequency of use of girdling on female vines was 65% of growers and only 3% on male vines and therefore there is potential to increase usage of girdling if it is proven to be protective in future research, especially in male vines.

10.5.6 Regional effects

There were differences in the risk of both the initial development of disease and presence of severe disease between growing regions. One possible reason for this is that each region had Psa present for varying periods of time which may have affected available inoculum in the orchards. Other possible causes of the differences between regions include the density of orchards within a region and climatic and soil differences between areas.

10.6 Future industry research needs for Psa

Chapter 4 showed a reduction in productivity after one year, however this was early in the outbreak and future research should focus on assessing the economic impact of this disease on both 'Hayward' and new cultivars after an extended period of exposure to Psa and continued improvement in disease management. There is also an opportunity to incorporate the KVH Psa-V weather risk model (Beresford et al. 2017) into this assessment to account for climatic effects between different regions and harvest years.

The severe disease data collected in this thesis could be used to assess the effect of systemic symptoms on productivity for the 2013 harvest using industry data. Experimental research is recommended to investigate the biological mechanism between systemic disease and production loss. Experimental studies could be complimented by the use of a prospective cohort observational study with field measurements of systemic symptoms, using disease incidence or a botanical epidemiology measure of disease intensity. This would be used to assess the effect of systemic symptoms on productivity outcomes, in commercial orchards.

The cross-sectional survey and analysis was successful in generating a range of hypotheses about potential risk factors that are recommended for further research, namely artificial pollination, post-pruning sprays, period infected, frost, girdling and regional effects. Research into the safe application of girdling, and into determining the biological mechanism of why girdling appeared protective in our study, may increase the use of this management tool for Psa beyond the current use to reduce bud drop. In addition, male vines were found to have a higher prevalence of systemic symptoms of Psa in our study and male girdling was very uncommon. It may be possible to reduce the effect of disease in male vines by increasing the use of male girdling and this should be researched further.

The temporal issues with timing of artificial pollination application and the association with both the initial development of disease and severe disease indicate that another factor is involved. It is recommended that a more in depth survey around artificial pollination use is undertaken to identify any closely related factors, such as male pollination failure, that may have confounded the relationship we observed between artificial pollination and an increased risk of disease. In addition, it would be beneficial to investigate whether physiological stress from using artificial pollination on diseased vines impacts disease expression. Research into the impact of frost and the main biological process associated with frost and systemic symptoms may influence which frost protection technologies are most appropriate in a Psa environment. Finally, research into understanding the relationship between copper sprays, callus tissue formation and Psa mobilisation is recommended to ensure that protective spray applications are optimally timed.

10.7 Application of observational studies in plant health

The observational studies presented in this thesis have shown the utility of the approach to determine the effect of disease on productivity to describe the prevalence of disease and identify risk factors for disease in real world situations. The results presented in Chapter 4 exploring impact of Psa on productivity could not have been undertaken using an experimental field trial. Prior to the arrival of Psa, Te Puke was one of the higher producing regions for 'Hayward', in addition to this, as Psa arrived in each region there were shifts in spray patterns with increases in foliar fertilisers and copper sprays and a reduction in other management sprays. There are also other pests and diseases that affect kiwifruit productivity that may have changed due to changes in protective spray or management practices. It would be extremely difficult to control all of these factors in an experimental field trial.

The use of a cross-sectional design in this thesis provided a new way to investigate plant disease risk factors and this type of study could be more extensively used, especially during incursions of unwanted organisms. Results of observational studies can quantify the relative importance of a wide range of factors that can't be simultaneously controlled for in experimental studies (Grimes & Schulz 2002a; Rochon et al. 2005). An important consideration for cross-sectional studies, is temporality, where the potential cause must precede the effect (Rothman et al. 2008b; van Engelsdorp et al. 2013). By design, a cross-sectional study collects both exposure and outcome data simultaneously and cannot distinguish the order of cause and effect, which can result in spurious conclusions (Engel & Wolff 2013). This thesis applied observational studies to complex real-world commercial orchards during a disease outbreak and provided industry managers with a reduced range of risk factors as research priorities.

There is potential for greater use of observational studies in plant health, particularly using cross-sectional studies for biosecurity outbreak situations. There is also potential to expand the results of this thesis and to undertake prospective cohort studies on the risk factors identified in this study.

10.8 Concluding statement

The results of this study support previous research that systemic symptoms of Psa are more likely to develop over time. This may result in a delayed impact in productivity in the absence of effective disease management. The use of a quantitative questionnaire was very effective in obtaining disease prevalence and risk factor data for analysis. The study quantified the prevalence of disease in 'Hayward' blocks and confirmed that male vines were more affected, which may have future implications in pollination. Hypothesis generation of risk factors for prioritised research identified that girdling required more research into potential use as a protective measure against Psa and identified that artificial pollination, frost and the effect of copper spray applied immediately after pruning all required prioritised research into the underlying biological mechanisms for their association with disease.

Wider adoption of these types of study in plant protection research is likely to occur as the principles of observational study design become better understood from studies such as this one.

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Appendix 1 - Questionnaire cover letter

Karyn Froud
Biosecurity Scientist
11 Pine St
Mt Eden 1041, Auckland
Telephone:027 514 4159
karyn.froud@orcon.net.nz

Dear Grower,

Your orchard KPIN has been selected to participate in a Kiwifruit Vine Health funded study to learn more about Psa-v in Hayward orchards. The research is a collaboration between Massey University, Plant and Food Research, ZESPRI and Karyn Froud & Associates. We hope you will participate because this information will help us get a fuller picture of Psa-v disease progression and could help to identify strategies to reduce the impact of Psa-v disease on kiwifruit production.

What do you need to do?

We would like you to answer a simple questionnaire that has three sections. All three sections are asking questions about a specific Hayward block on your property. We have randomly selected one of the blocks associated with your KPIN and written the block name or number on the front of the questionnaire. Note the number is the identifier that is used when you submit your spray diary to ZESPRI.

The last section of the survey will require you to provide dates for key activities so you may find it useful to have your spray diary with you. Information in each section of the survey is important so we appreciate your time in completing them all. When you have completed the questionnaires please return them to me using the prepaid envelope.

What should I do if I don't have Psa?

Your information is **very** valuable to us as it could help us identify strategies that will help other producers manage the disease or keep the disease out so please fill in the questionnaire.

I would like to provide information for a different block?

We appreciate that your blocks may vary in management and/or severity of symptoms. However, we would ask that you limit your answers to the block we have selected. The reasons for this are to make sure we get a good representation of kiwifruit blocks across all growers and don't limit ourselves to looking only at the very best or worst which could lead to our recommended strategies being flawed.

Will this information be treated confidentially?

Please be assured that your responses will be held in the strictest confidence and will not be given or sold to any third party. When the results are written for publication, no identifying information will be used.

What to do if I have concerns about this project?

If you require any further information please contact me via the phone on 027 514 4159 or by email on karyn.froud@orcon.net.nz.

Yours Sincerely

Karyn Froud

Appendix 2 - Questionnaire

KPIN: mail merge KPIN **BLOCK: mail merge block number**

Managing Psa-v in Hayward blocks survey

Please complete this survey for the specified Hayward block on your property

Orchard KPIN	_ _ _ _
Hayward block	_ _ _ _

Note: to avoid selection bias which will affect the quality of our results we have randomly assigned blocks that we want information about. **Please make sure that your responses refer to that block only.**

If you have any concerns, comments or questions about the content of this research that you wish to raise with the researchers, please contact Karyn Froud, Biosecurity Scientist

Telephone 027 514 4159, email: karyn.froud@orcon.net.nz

Please fill in all of the sections with as much accuracy as possible. **Please return the completed forms to us in the postage paid envelopes by Friday 12th 2013.**

Completed forms can be posted to:

ZESPRI Grower Contact Centre, PO Box 4043, Mount Maunganui

KPIN: mail merge KPIN **BLOCK: mail merge block number**

SECTION 1 – PSA-V HISTORY OF THE BLOCK

Please answer all questions below for your selected Hayward block. All questions refer to the last 12 months from March 2012 – February 2013 unless stated otherwise

Current 2012/13 growing season

1. Do you have any visible Psa-v symptoms in the block as of Feb 2013 (including old spotting/symptoms)?

No ☐ Yes ☐ Not sure ☐

2. Which of your vines (if any) are currently showing Psa-v symptoms (including old spotting/symptoms)?

Both males and females ☐ No symptoms ☐
Males only ☐ Not sure ☐
Females only ☐

MALES 2012/13

3. Please select the Psa-v symptoms you have on your MALE vines by ticking all symptoms that are or were present in the block from March 2012 to February 2013; AND giving an estimated percentage of the MALE vines (or buds) showing these symptoms (*Select as many as are applicable*)

No Psa-v symptoms ☐
Leaf spotting ☐ Approx. _____ % of male vines are affected in the block
Green shoot wilting ☐ Approx. _____ % of male vines are affected in the block
Cane dieback ☐ Approx. _____ % of male vines are affected in the block
Stem cankers/cracking ☐ Approx. _____ % of male vines are affected in the block
Red exudate/ooze ☐ Approx. _____ % of male vines are affected in the block
White exudate/ooze ☐ Approx. _____ % of male vines are affected in the block
Bud drop ☐ Approx. _____ % of male BUDS were affected in the block
Other symptoms (specify) ☐ …… Approx. _____ %

4. If you have cut out any Psa-v infected MALE canes, leaders or vines between March 2012 and February 2013 can you please estimate the percentage of canopy that has been removed? (*include all material removed since the onset of disease in your block*)

None removed ☐
I estimate _____ % of the MALE canopy has been removed from the block

FEMALES 2012/13

5. Please select the Psa-v symptoms you have on your FEMALE vines by ticking all symptoms that are or were present in the block from March 2012 to February 2013; AND giving an estimated percentage of the FEMALE vines (or buds) showing these symptoms (*Select as many as are applicable*)

No Psa-v symptoms ☐
Leaf spotting ☐ Approx. _____ % of female vines are affected in the block
Green shoot wilting ☐ Approx. _____ % of female vines are affected in the block
Cane dieback ☐ Approx. _____ % of female vines are affected in the block
Stem cankers/cracking ☐ Approx. _____ % of female vines are affected in the block
Red exudate/ooze ☐ Approx. _____ % of female vines are affected in the block
White exudate/ooze ☐ Approx. _____ % of female vines are affected in the block
Bud drop ☐ Approx. _____ % of female BUDS were affected in the block
Other symptoms (specify) ☐ …… Approx. _____ %

KPIN: mail merge KPIN **BLOCK: mail merge block number**

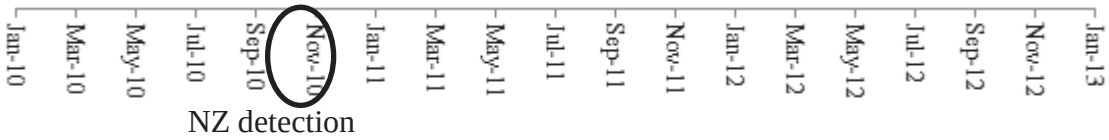
6. **If you suffered bud drop in your FEMALE vines please indicate the estimated percentage of crop loss for the block**
   No bud drop ☐
   Approx. _____ % of the crop was lost due to assumed Psa-v related bud drop

7. **Do your female vines have Psa-v symptoms that are more or less severe than the male vines?**
   No female symptoms ☐ About the same ☐
   Females more severe than males ☐ Females less severe than males ☐

8. **If you have cut out any Psa-v infected FEMALE canes, leaders or vines between March 2012 and February 2013 can you please estimate the percentage of canopy that has been removed?** (*include all material removed since the onset of disease in your block*)
   None removed ☐
   Approx. _____% of the FEMALE canopy has been removed from the block

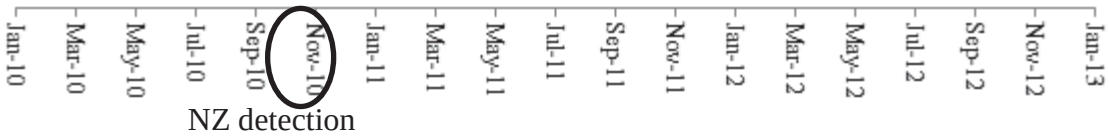
PSA-V ARRIVAL

9. **Knowing what you do now about Psa-v symptoms in your orchard, when do you think is the earliest you saw symptoms that on reflection probably were Psa-v in the block even if they tested negative?** *(Please circle the earliest suspected time in the timeline below)*
   No Psa-v in block ☐



10. **Are there any Psa-v infected kiwifruit blocks immediately adjacent to the block (over the shelter) including neighbours?**
    No ☐ Yes ☐ Not sure ☐

11. **If there are Psa-v infected kiwifruit blocks adjacent to the block, including neighbours, can you indicate when you think that those blocks became infected?** *(Please circle the earliest suspected time in the timeline below)* No Psa-v adjacent blocks ☐



LAST YEAR'S GROWING SEASON 2011/12

12. **Did you have Psa-v symptoms in the block a year ago as of Feb 2012?**
    No ☐ Yes ☐ Not sure ☐

13. **If you had Psa-v in Feb 2012, were the Psa-v symptoms more or less severe than Feb 2013 (current year)?**
    No symptoms in Feb 2012 ☐ Symptoms less severe back in Feb 2012 ☐
    About the same ☐ Symptoms more severe back in Feb 2012 ☐

THE 2010/11 GROWING SEASON - TWO YEARS AGO

14. **Did you have Psa-v symptoms in the block two years ago as of Feb 2011?**
    No ☐ Yes ☐ Not sure ☐

15. **If you had Psa-v in Feb 2011, were the Psa-v symptoms more of less severe than Feb 2012 (last year)?**
    No symptoms in Feb 2011 ☐ Symptoms **less** severe back in Feb 2011 ☐
    About the same ☐ Symptoms **more** severe back in Feb 2011 ☐

KPIN: mail merge KPIN **BLOCK: mail merge block number**

SECTION 2 - BLOCK INFORMATION

Please answer all questions below for your selected Hayward block. All questions refer to the last 12 months from March 2012 – February 2013 unless stated otherwise

16. How is the selected Hayward block structured on the orchard?

It is a single block ☐ It is made up of several blocks ☐

17. Is the block managed organically or conventionally?

Organic ☐ Conventional ☐

18. What is the land immediately adjacent to each side of the block currently used for? Please select as many uses as relevant *(i.e. for each landuse on the other sides of the shelter belts in the block)*

Kiwifruit same orchard	☐	Kiwifruit neighbours orchard	☐
Cut-out kiwifruit block	☐	Other horticulture crop	☐
Gully	☐	Waterway/stream/lake	☐
Forestry	☐	Native Bush/forest	☐
Paddock/ farmland	☐	Orchard buildings	☐
Residential buildings	☐	Commercial buildings	☐
Kiwifruit Packhouse	☐	Other crop packhouse	☐
Road	☐	Estuary/coastland	☐
Other (please specify)	☐ …….		

19. If you selected "Kiwifruit same orchard" above please select the relevant varieties

Not selected ☐ Hayward ☐ Hort 16a ☐ G3 ☐
G9 ☐ G14 ☐ Other variety *(please specify)* ☐ …….

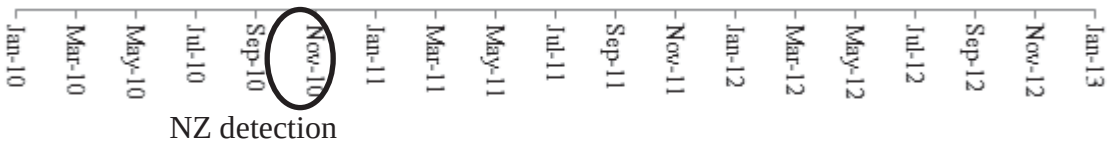
20. If you selected "kiwifruit neighbours orchard" above, select the relevant varieties

Not selected ☐ Hayward ☐ Hort 16a ☐ G3 ☐
G9 ☐ G14 ☐ Other variety *(please specify)* ☐ …….

21. If an immediately adjacent kiwifruit block has recently been cut out or grafted to a new variety due to Psa-v infection, please select the original variety below and indicate on the timeline when it was removed.

Not applicable ☐ Hayward ☐ Gold 16a ☐ Not sure ☐
New variety (please specify) ☐ …….

Approximate removal date:



22. What shelter do you use in this block? *Please select as many as are applicable*

Artificial shelter	☐	Italian alder (*Alnus)*	☐
Fast track shelter	☐	Willow	☐
Sheoak (*Casuarina*)	☐	Japanese cedar (*Cryptomeria)*	☐
Cypress	☐	Gum (*Eucalyptus)*	☐
Pine	☐	Poplar	☐
Other (please specify)	☐ ……		

23. What pollination methods did you use in this block during the current crop's flowering period (2012/13)? *Please select all relevant methods.*

Natural wind/bees ☐ Introduced bees ☐ Wind blow flowers ☐
Artificial pollination ☐ Other (please specify) ☐ ……

Note: Wind blow flowers refers to the practice of blowing male vines with a wind blower to release pollen into the orchard

KPIN: mail merge KPIN **BLOCK: mail merge block number**

24. If you used artificial pollination in the block during the current crop's flowering period (2012/13), please indicate BOTH the source and application method.

Not used ☐ Own flower/pollen ☐ Commercial flower/pollen ☐
Dry application ☐ Wet application ☐

25. What pollination methods did you use in this block during last seasons (2011/12) flowering period? *Please select all relevant methods.*

Natural wind/bees ☐ Introduced bees ☐ Wind blow flowers ☐
Artificial pollination ☐ Other (please specify) ☐ ……

Note: Wind blow flowers refers to the practice of blowing male vines with a wind blower to release pollen into the orchard

26. If you used artificial pollination in the block during last seasons (2011/12) flowering period please indicate BOTH the source and application method.

Not used ☐ Own flower/pollen ☐ Commercial flower/pollen ☐
Dry application ☐ Wet application ☐

27. What type of frost protection did you use on this block for this current season 2012/13? *Please select as many as are relevant to the block.*

No frost protection	☐	Overhead water	☐
Helicopter	☐	Diesel burners	☐
Wind machines	☐	Fans	☐
Nitrogen foliar sprays	☐	Under-vine sprinklers	☐
Thermomax	☐	Other (please specify)	☐ ……

28. Please indicate the level of frost damage in this block in spring 2012.

No frost damage ☐ Moderate damage (whole leaves affected) ☐
Minor damage (leaves singed) ☐ Severe damage (whole shoots affected) ☐

29. How much of the Block was frost damaged in spring 2012?

No vines damaged	☐	More than half the vines (>50%)	☐
A few isolated vines (1-5%)	☐	Most of the vines (>75%)	☐
Less than a quarter of vines (<25%)	☐	All of the vines (95-100%)	☐
Less than half the vines (>25%)	☐		

30. What type of irrigation do you use on this block this season? *(please tick as many as are applicable)*

No irrigation	☐	Overhead water	☐
Drip-line irrigation	☐	Under-vine sprinkler irrigation	☐
Other (please specify)	☐ ……		

31. As of Feb 2013 how would you describe your canopy density? *Please select the best description for the block (descriptions are based on the Kiwigreen Manual canopy rating system).*

1. Open canopy with more than 30% gaps and green grass cover ☐
2. Open canopy with less than 30% gaps and green grass cover ☐
3. Closed canopy with little green grass cover ☐
4. Dense canopy with green grass cover in patches only ☐
5. Dense canopy with no gaps and no green grass cover ☐
6.

32. Have you, or do you intend to, girdle your FEMALE vines in this block this season?

No ☐ Yes in spring/early summer ☐ Yes in summer ☐ Not sure ☐

33. Did you girdle your FEMALE vines in this block last season (2011/12 growing season)?

No ☐ Yes in spring/early summer ☐ Yes in summer ☐ Not sure ☐

KPIN: mail merge KPIN **BLOCK: mail merge block number**

34. Have you, or do you intend to, girdle your MALE vines in this block this season?
No ☐ Yes in spring/early summer ☐ Yes in summer ☐ Not sure ☐

35. Did you girdle your MALE vines in this block last season (2011/12 growing season)?
No ☐ Yes in spring/early summer ☐ Yes in summer ☐ Not sure ☐

36. Over the Mar 2012 to Feb 2013 period what did you do with your normal vine management pruned kiwifruit plant material?
Left on ground beneath vines ☐
Mulch immediately after pruning complete ☐
Mulch within 2 weeks of pruning ☐
Mulch within 1 month of pruning ☐
Collect and remove from block ☐
Other (please specify) ☐ ……

37. Over the Mar 2012 to Feb 2013 period what did you do with any Psa-v diseased shoots, canes, leaders or vine material removed from vines? (do not include leaves or buds)
No Psa-v diseased material in block ☐
No Psa-v diseased material cut-out of block ☐
Diseased material cut-out and removed from block ☐
Diseased material cut-out and mulched immediately ☐
Diseased material cut-out and mulched within 2 weeks ☐
Diseased material cut-out and mulched within 1 month ☐
Other (please specify) ☐ ……

38. Please write the year the majority of your FEMALE vines in this block were planted.
Year FEMALES planted _ _ _ _ Or give the estimated age …… years

39. Please select what variety(s) the FEMALE vines in this block are.
Hayward ☐ Kramer ☐ Not sure ☐

40. Please write the year the majority of your current MALE vines in this block were grafted.
Year MALES grafted _ _ _ _ **Or** give the estimated age …… years

41. Please select what variety(s) the MALE vines in the block are.
No males ☐ Matua ☐ Chieftain ☐ M56 ☐ M. series ☐
Other (please specify) ☐ ……

42. What system of male to female vines do you have in the block?
Strip males ☐ Opposing female ☐ No males ☐

43. If you chose opposing female above what ratio of male to female vines do you have in the block? *(For example a ratio 1 male: 6 females means there is 1 male to every 6 females)*
Not opposing female ☐
There is _____ male to every _____ females in this block.

44. If you chose strip male above, please select the layout of males to females in this block AND state the ratio of male rows to female rows in this block *(select all relevant options)*
Not strip male ☐ There are females in the male rows ☐
One male per bay ☐ Males are stretched over 2-3 bays ☐
There is _____ male row to every _____ female row(s) in this block.

KPIN: mail merge KPIN **BLOCK: mail merge block number**

45. Do you use weather information to MANAGE DISEASE SPRAYING on your orchard?

No ☐ Yes ☐

46. Do you use weather information to PLAN VINE MANAGEMENT on your orchard?

No ☐ Yes ☐

47. What source or sources of weather information do you mostly use to make day to day disease spray and vine management decisions? *(select all relevant options)*

None used	☐	Spray consultant	☐
KVH weather forecasting	☐	KVH Psa-v risk model	☐
NIWA	☐	Metservice	☐
MetVUW	☐	Radio/TV	☐
Packhouse information	☐	Harvest NZ	☐
Look outside	☐	Other (please specify)	☐……

48. Who does the disease spraying on your orchard?

Orchard owner	☐	Orchard manager	☐
Spray contractor	☐	Orchard worker	☐

49. What are the most significant reasons for delays in applying Psa disease sprays once a decision to apply them has been made?

Unfavourable wet weather	☐	Withholding periods	☐
Risk of spray drift (wind)	☐	Incompatible spray usage	☐
Orchard workers working in block	☐	Spray equipment availability	☐
Spray contractor availability	☐	Other (please specify)	☐……

50. What equipment do you currently use to apply most disease sprays in this block?

Own sprayer used exclusively on this KPIN ☐ Own sprayer used on several KPIN's ☐

Contractor's equipment ☐ Other (please specify) ☐……

51. If you answered "own sprayer" above can you please indicate how long it has been in regular use in your block? Note if you have recently returned to using your own equipment use the recent date below.

We have used our own sprayer to do most disease spraying in this block since ……(month) of ……..(year) Do not use own sprayer ☐

52. If you use your own sprayer to apply most of your disease sprays has it been calibrated recently?

Calibrated within last 6 months	☐	Calibrated within 12 months	☐
Calibrated within 24 months	☐	Not calibrated recently	☐
Do not use own sprayer	☐		

53. Which Psa-v hygiene measures do you routinely use on pruning equipment? *Please select all relevant items.*

Do not clean equipment	☐	Clean equipment on arrival at orchard	☐
Use orchards own equipment	☐	Clean equipment between vines	☐
Clean equipment between bays	☐	Clean equipment between blocks	☐
Clean equipment daily	☐	Other *please specify*	☐……

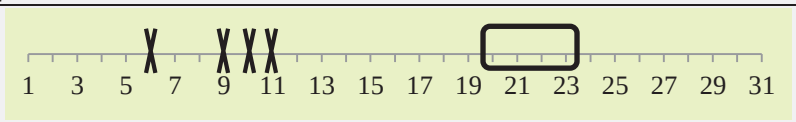
54. When undertaking pruning in the block do you undertake any of the following protection measures for disease? *Please select all relevant items.*

No prevention measures	☐	Workers put off during rain/wet spells	☐
Special pre-pruning protective spray	☐	Instant wound protection with hand spray	☐
Follow-up backpack sprayer	☐	Pruned rows sprayed at the end of day	☐
Full block spray at end of pruning	☐	Dip girdling equipment between vines	☐
Dip girdling equipment between blocks	☐	Other (*please specify)*	☐ ……

SECTION 3 – MONTHLY VINE AND MANAGEMENT ACTIVITIES

Please select what activities from the lists were undertaken in this **block** for the specified month. Please place a cross on the exact date(s) or circle the approx. dates when the activity was undertaken on the monthly timeline.

E.g. if an activity occurred on the 6th of March, the 9th, 10th and 11th of March and approximately the 20-23rd of March you would complete it as shown below.

☑ *Example activity*	

We will be matching activity dates with local weather data so accurate dates are really important.

Month: March 2012

☐ **Female** pruning - general	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Female** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Frost Protection	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Fruit picking	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Fruit thinning	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Girdling	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Grafting	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Irrigation	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** pruning - general	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male root** pruning	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Psa spray application *Specify (e.g. Cu, KeyStrepto, Elicitor)*	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31

Month: April 2012

☐ **Female** pruning - general	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Female** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Frost Protection	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Fruit picking	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Fruit thinning	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Girdling	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Irrigation	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** pruning - general	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Psa spray application *Specify (e.g. Cu, KeyStrepto, Elicitor) ……*	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31

Month: May 2012

☐ **Female** pruning - general	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Female** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Frost Protection	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Fruit picking	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Fruit thinning	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Girdling	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Irrigation	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** pruning - general	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Psa spray application *Specify (e.g. Cu, KeyStrepto, Elicitor) ……*	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Winter defoliant spray	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31

Month: June 2012

☐ **Female** pruning – winter	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Females** - tying down vines	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Female** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Frost Protection	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Fruit picking	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Grafting	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Irrigation	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** pruning - general	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Psa spray application *Specify (e.g. Cu, KeyStrepto, Elicitor)*	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Winter defoliant spray	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31

Month: July 2012

☐ **Female** pruning – winter	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Females** - tying down vines	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Female** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Grafting	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** pruning - general	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Psa spray application *Specify (e.g. Cu, KeyStrepto, Elicitor)*	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31

Month: August 2012

☐ **Female** pruning – winter	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Females** - tying down vines	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Female** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Girdling	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Grafting	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Hi-cane application	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** pruning - general	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Psa spray application *Specify (e.g. Cu, KeyStrepto, Elicitor)*	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31

Month: September 2012

☐ **Female** pruning – winter	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Female** pruning – zero leaf?	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Females** - tying down vines	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Female** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Frost Protection	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Girdling	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Grafting	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** pruning - general	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male root** pruning?	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Psa spray application *Specify (e.g. Cu, KeyStrepto, Elicitor)*	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31

Month: October 2012

☐ Bud thinning	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Female** pruning – tip squeezing	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Female** pruning – zero leaf	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Female** pruning – general	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Female** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Frost Protection	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Girdling	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Grafting	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Irrigation	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** pruning – tip squeezing	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** pruning – zero leaf	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** pruning - general	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male root** pruning?	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Psa spray application *Specify (e.g. Cu, KeyStrepto, Elicitor)*	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31

Month: November 2012

☐ Artificial pollination	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Beehives introduced	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Bud thinning	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Female** pruning – tip squeezing	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Female** pruning – zero leaf	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Female** pruning – general	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Female** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Frost Protection	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Girdling	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Grafting	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Irrigation	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** pruning – tip squeezing	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** pruning – zero leaf	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** pruning - general	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male root** pruning?	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Psa spray application *Specify (e.g. Cu, KeyStrepto, Elicitor)* ……	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31

Month: December 2012

☐ Artificial pollination	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Beehives introduced	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Female** pruning – tip squeezing	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Female** pruning – zero leaf	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Female** pruning – general	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Female** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Fruit thinning	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Girdling	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Grafting	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Irrigation	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** pruning – tip squeezing	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** pruning – zero leaf	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** pruning - general	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male root** pruning?	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Psa spray application *Specify (e.g. Cu, KeyStrepto, Elicitor)* ……	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31

Month: January 2013

☐ **Female** pruning – general	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Female** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Fruit thinning	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Girdling	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Grafting	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Irrigation	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** pruning – tip squeezing	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** pruning – zero leaf	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** pruning - general	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male root** pruning?	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Psa spray application *Specify (e.g. Cu, KeyStrepto, Elicitor)* ……	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31

Month: February 2013

☐ **Female** pruning – general	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Female** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Fruit thinning	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Girdling	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Grafting	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Irrigation	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** pruning – tip squeezing	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** pruning – zero leaf	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** pruning - general	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male root** pruning?	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ **Male** Psa-v diseased leaders or vines removed	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
☐ Psa spray application *Specify (e.g. Cu, KeyStrepto, Elicitor) ……*	1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31

Are there any comments you would like to make?

Comments: