UNIVERSITY
OF TASMANIA

EPIDEMIOLOGY AND MANAGEMENT OF WALNUT BLIGHT IN TASMANIA

by

Michael David Lang

B Agr Sc (Hons)

School of Agricultural Science

Submitted in fulfilment of the requirements for the

Degree of Doctor of Philosophy

University of Tasmania March, 2012

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgment is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

This thesis may be made available for loan and limited copying and communication in accordance with the Copyright Act 1968.

The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

Michael David Lang
University of Tasmania
March, 2012

Signed:

Date:

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF EQUATIONS

ACKNOWLEDGEMENTS

I would firstly like to express my sincere gratitude to Kathy Evans for her guidance and assistance throughout this project.

A special thank you to Mostyn and Gaye Veith and to Leigh Titmus of Walnuts Australia for their permission in allowing trials to be conducted in their orchards.

My sincere thanks to Walnuts Australia staff, namely Rodney 'Kouj' Jones, Julie Sulcz and Dumi Mhlangha, and to my former colleagues at Agronico Pty Ltd, namely Julian Shaw, James Hills, James Kirkham, Mark McGee, Odin Franssen, Britta Matthews, Patrick Lyons, Stuart McKay and Robyn Tait for their assistance in conducting and maintaining field trials.

I am extremely grateful for the support and advice provided by Steve Sibbett from the University of California, who was always more than generous with sharing his extensive expertise and knowledge on all walnut related topics. I would also like to thank Sarah Pethybridge of Botanical Resources Australia, Jason Scott of the Tasmanian Institute of Agriculture and Henry Ngugi of the Pennsylvania State University for their advice.

I would like to acknowledge my indebtedness to Walnuts Australia, Tasmanian Institute of Agriculture, Agronico Pty. Ltd. and Horticulture Australia Limited for their financial support.

My greatest thanks, however, are reserved to my wife Julie Siebert, who has not only provided counsel and support during the PhD, but also assisted with a myriad of tasks associated with this project and concomitant work related programmes.

ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
AUDPC	Area under the disease progress curve
BOX	BOX elements
BS	Brilliant cresyl blue starch medium
cfu	Colony forming unit
CHEF	Contour-clamped homogenous electric field electrophoresis
CRV	Critical risk value
DNA	Deoxyribonucleic acid
EBDC	Ethylene-bisdithiocarbamate
EIL	Economic injury level
ERIC	Enterobacterial repetitive intergenic consensus
FN	False negative
FNP	False negative proportion
FP	False positive
FPP	False positive proportion
GC-FAME	Gas chromatography-fatty acid methyl ester
GYCA	Glucose-yeast extract-calcium carbonate
MI	Moisture intensity
MLSA	Multilocus sequence analysis
NA	Nutrient agar
PCR	Polymerase chain reaction
PNB	Percent new blight
PTB	Packing tissue brown

pv.	Pathovar
pvs.	Pathovars
REP	Repetitive extragenic palindromic
RH	Relative humidity
RNA	Ribonucleic acid
SAUDPC	Standardised area under the disease progress curve
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SDW	Sterile distilled water
SQ	Succinate quinate medium
TB	Modified Tween medium
TP	True positive
TPP	True positive proportion
TN	True negative
TNP	True negative proportion
WMAR	Weighted mean absolute rate
YDC	Yeast dextrose calcium carbonate agar

ABSTRACT

Walnut blight, caused by the bacterium *Xanthomonas arboricola* pv. *juglandis*, is a major factor limiting walnut production worldwide. Knowledge of disease epidemiology in Tasmania was developed as a basis for designing an improved crop protection strategy. The aims of this project were to verify *X. arboricola* pv. *juglandis* as the causal organism of walnut blight, establish the impact of natural infections on crop yield, determine the critical environmental factors associated with the temporal development of walnut blight, and to refine current crop protection using identified weather factors to time copper-based biocides, in Tasmania.

Studies of the pathogenicity and growth on semi-selective media of up to 37 bacterial isolates from Tasmania demonstrated that *X. arboricola* pv. *juglandis* is the cause of walnut blight in commercial walnut orchards and home gardens. Determining the pathogenicity of *X. arboricola* pv. *juglandis* on Franquette fruit required inoculating half full-size diameter fruit with 10^9 cfu/ml. Pathogenic isolates metabolized quinate and hydrolysed starch; they were identified as *X. arboricola* by MLSA and GC-FAME and named *X. arboricola* pv. *juglandis* based on the host and pathovar concept of taxonomy.

Walnut blight can lead to the premature drop of fruits in Tasmania. The incidence and severity of disease on fruits, and subsequent reduction in crop yield, were similar for cultivars Vina and Franquette. There was a strong inverse relationship between crop yield and the standardised area under the disease progress curve (SAUDPC) for incidence and SAUDPC for severity for 10 site-years. The monomolecular model with K = 1 described temporal disease incidence (R^2 values from 88 to 99%) and temporal fruit size (R^2 values from 96 to 99%) for the 10 site-years, and allowed crop yield to be predicted according to disease incidence at various fruit sizes. It was predicted that nearly two fruit dropped prematurely for every fruit that was diseased when fruit were 25% full-size diameter. The rate of fruit loss at 50% fruit size, or larger, was approximately half of that at 25% fruit size. Some diseased fruits were predicted to remain on trees until harvest when infected at larger fruit sizes.

A formulation of copper hydroxide and mancozeb, Mankocide® DF, applied between budburst and half fruit size, reduced disease incidence and increased crop yield in

two of three site-years. Disease incidence at Forth in 2004−05 was adequately controlled with two or more copper-based sprays, applied at budburst and 7 days after budburst, with a corresponding crop yield of 77% in comparison to 50% yield with non-treatment. In 2005−06 at Forth, crop yield was predicted to increase linearly by 2% with every spray, when nine sprays were applied at 7 day intervals. However, in a year with low disease incidence i.e., less than 11% incidence irrespective of treatment, no significant relationship between the number of spray applications and crop yield occurred. As such, with disease incidences of 10% or less at half fruit size, multiple sprays are predicted to reduce economic gain as the cost of spraying outweighs the return from increased yield.

The development of walnut blight differed markedly between years in Tasmania. In 2005–06, the wettest year of the study, nearly 100% of Vina and Franquette fruits developed disease, and disease progression was best described by the logistic or Gompertz models (R^2 values from 88 to 98%). In contrast, the linear and monomolecular models best described disease progression in 2004–05 and 2006–07, the two drier years of the study (R^2 values from 93 to 99%). Daily moisture intensity was defined as the total daily rainfall divided by duration of surface wetness; this variable, when cumulated for the period 17 to 24 adjusted-calendar-days (Tmin= 1^oC; Tmax = 35^oC) before a disease assessment, accounted for 83% of the variance in the percentage of fruits developing symptoms of walnut blight between assessments. Daily rainfall, days with rainfall and minimum temperature were also significantly related to disease development of fruits. In half of the epidemics studied, disease incidence of individual fruits within fruit clusters increased exponentially relative to the increase in disease incidence of fruit clusters. It is postulated that bacterial masses emerging from substomatal cavities may be transported in rain splash and serve as secondary inoculums to adjacent fruit within a cluster.

A rain-intensity-based model was developed for predicting the optimum time to apply copper-based sprays. Mankocide® DF, timed according to the model, provided similar control of walnut blight with the same or fewer numbers of sprays than those timed by commercial operations at Forth and Swansea in 2008–09. At Forth, nearly

100% disease incidence was observed on non-treated fruits, and five or more sprays were required to significantly reduce the rate of disease progression. With two budburst sprays only, 93% of fruits were diseased near harvest; in comparison, less than 40% of fruits had blight lesions when sprays were timed according to the rain-intensity model only, combined weekly (two or four sprays from budburst) and model regimens, and a commercial weekly spray schedule (eight sprays from budburst). At Swansea, a near 60% of non-treated fruits were diseased at harvest; however, the rain-intensity model provided the same level of disease control as a weekly-based spray regime i.e., less than 20% disease incidence at harvest, even though up to three less sprays were applied. These results support the continued development and validation of the rainfall-intensity-based model for timing crop protection sprays in Tasmania.

INTRODUCTION

Commercial production of walnuts (*Juglans regia*) in Australia is rapidly expanding to satisfy the demand of the Australian consumer, and to supply northern hemisphere markets with fresh, high quality walnuts in the off-season (Australian Nut Industry Council (ANIC), 2009). As such, import substitution and export opportunities are predicted to generate AUD 26 million of industry value, and lead to AUD 31 million in exports, by 2012 (ANIC, 2009). A major walnut production area in Australia, with 650 ha of newly planted orchards, is Tasmania; these orchards represent approximately one quarter of the area under walnut cultivation in Australia (ANIC, 2009).

A major disease limiting production of walnuts is walnut blight, caused by *Xanthomonas arboricola* pv. *juglandis* (Teviotdale and Schroth, 1998). The disease can occur in both seedling and mature walnut trees and can affect both vegetative and reproductive stages; the flowers and fruits are, however, highly susceptible to infection (Miller and Bollen, 1946). Flower and early fruit infections can cause blossoms and developing fruit to drop prematurely, whereas infections later in fruit growth can stain the shell of the developing nut which reduces the quality of the in-shell product (Teviotdale and Schroth, 1998). In Tasmania, walnut blight has led to 70% crop loss when crop protection strategies were not effectively implemented (Lang *et al*., 2006); if continued unchecked, these losses may compromise the Tasmanian walnut industry.

The current management strategy for walnut blight in Tasmania is based on multiple applications of copper-based bactericides for protecting susceptible plant tissue; however, multiple sprays have led to an increase in the frequency of copper tolerant strains of the walnut blight pathogen in French and Northern Californian walnut orchards (Gardan *et al*., 1993; Lee *et al*., 1993) and may have potential long-term detrimental effects on crop yield in walnut orchards (Radix and Seigle-Murandi, 1993; Bunemann *et al*., 2006). Importantly, multiple copper-based sprays do not always control the disease in Tasmania (Lang *et al.*, 2006). As such, a more sustainable strategy for the control of walnut blight in Tasmania is required.

The epidemiology of walnut blight has not been studied in Tasmania. The lack of knowledge of disease development, and how this is affected by environmental and host factors, is a potential limiting factor to the development of management strategies for the control of walnut blight in Tasmania. Thus, the aims of this project were to:

1) verify *X. arboricola* pv. *juglandis* as the causal organism of walnut blight, and outline the diagnostic process used to identify and characterise a broad sample of isolates from symptomatic walnut fruit, in Tasmania (Chapter 5),

2) establish the impact of natural *X. arboricola* pv. *juglandis* infections on crop yield, and quantify the economic cost and benefit for adopting various crop protection programs, in Tasmania (Chapters 6 and 7), and

3) determine the critical environmental factors associated with the temporal development of walnut blight, and to develop a weather-based predictive model for timing crop protection sprays to achieve more economically and environmentally sustainable outcomes, in Tasmania (Chapters 8 and 9).

The thesis is divided into four parts: (i) a review of the literature, (ii) general materials and methods, (iii) results of experiments with discussion (five chapters), and (iv) a general discussion of the findings and recommendations for future research. Selected information from the literature review may also be found in the introductory sections of experimental chapters to allow a self-contained report of each study.

LITERATURE REVIEW

3.1 INTRODUCTION

Chapter 3 reviews the literature on the causal organism of walnut blight, *Xanthomonas arboricola* pv. *juglandis*, and on the development and management of walnut blight. Section 3.2 provides a brief overview of the taxonomy and nomenclature of *Xanthomonas*, prior to describing the geographical distribution and symptom expression of walnut blight. Section 3.3 reviews methods for differentiating *X. arboricola* pv. *juglandis* from other xanthomonads and plant pathogenic bacteria. Section 3.4 describes the disease cycle of walnut blight, the relationship between disease cycles and epidemics, and the factors that influence the development of disease, prior to reviewing the description and analysis of walnut blight epidemics. Section 3.5 investigates factors involved in controlling walnut blight, with particular focus on cultural and chemical methods that reduce pathogen inoculums. Finally, Section 3.6 summarises key research areas that will provide greater knowledge of the causal organism and the factors involved in the development and management of walnut blight in Tasmania.

3.2 CAUSAL ORGANISM

3.2.1 Nomenclature

The causal organism of walnut blight was first isolated from walnut orchards in California by Pierce (1896) and described and named as *Pseudomonas juglandis* (Pierce, 1901). Changing systems of nomenclature led to the pathogen being reclassified as *Bacterium juglandis* (Pierce) Smith (Smith, 1905), *Phytomonas juglandis* (Pierce, 1901) Bergey *et al.* (Bergey *et al.*, 1930) and *X. juglandis* (Pierce, 1901) Dowson (Dowson, 1932). Until 1974, the use of a single phenotypic property, host specificity, determined species in the genus *Xanthomonas*. Hence, a xanthomonad isolated from a new host plant was commonly classified as a new species. The pathovar concept of taxonomy was introduced in 1980 so that plant pathologists were able to name organisms pathogenic to particular hosts (Dye *et al.*, 1980). As such, nearly all *Xanthomonas* species were named as pathovars of *X. campestris* including the causal organism of walnut blight, *X. campestris* pv. *juglandis* (Pierce, 1901) Dye (Dye *et al.*, 1980). In the mid 1990's, phylogenetic

taxonomy of the genus *Xanthomonas* revealed groupings that were not consistent with a purely phenotypic classification. Using DNA homology and phenotypic data, Vauterin *et al.* (1995) proposed fourteen new *Xanthomonas* species including *X. arboricola* pv. *juglandis* (Pierce, 1901) Vauterin *et al.* for the walnut blight pathogen. However, the proposal of Vauterin *et al.* (1995) has not been universally accepted; for example Schaad *et al.* (2000) proposed *X. juglandis* pv. *juglandis.*

The rejection of *X. arboricola* pv. *juglandis* by Schaad *et al.* (2000) was based on their interpretation of rules within the International Code of Nomenclature of Bacteria (Sneath, 1992). Although Schaad *et al.* (2000) accepted the elevation of *X. arboricola* pv. *juglandis* to the rank of species they did not accept *X. arboricola* as it was not derived from the earliest legitimate pathovar epithet. However, because of the findings of Lee *et al.* (1992) and Vauterin *et al.* (1995), and the frequent use of *X. arboricola* in published literature, this review will refer to the causal organism of walnut blight as *X. arboricola* pv. *juglandis.*

3.2.2 Geographical distribution

Walnut blight is widely distributed with its range closely corresponding to areas of walnut cultivation (Miller and Bollen, 1946). The disease has been reported in Europe, Asia, Africa, the Western Hemisphere and Oceania (Table 3.1). In Australia, symptoms resembling those of walnut blight have been reported in New South Wales, South Australia, Tasmania, Victoria and Western Australia (Rodway, 1912; Cole, 1916; Osborn and Samuel, 1922; Anonymous, 1926; Dowson, 1932; Adam and Pugsley, 1934; Goss and Doepel, 1961). However, identification of *X. arboricola* pv. *juglandis* as the causal organism in Australia is yet to be confirmed.

Table 3.1. The geographical distribution of walnut blight (CABI/EPPO, 2001).

Region	Country
Europe	Austria; Bulgaria; Denmark; former USSR; former Yugoslavia; France; Germany; Greece; Italy; Netherlands; Poland; Portugal; Romania; Slovenia; Spain; Switzerland; Ukraine; United Kingdom.
Asia	Azerbaijan; China; Georgia; India; Iran; Iraq; Israel; Lebanon; Uzbekistan.
Africa	South Africa; Zimbabwe.
Western hemisphere	Argentina; Bermuda; Canada; Chile; Mexico; United States of America; Uruguay.
Oceania	Australia, New Zealand.

3.2.3 Hosts and alternate-hosts of X. *arboricola* pv. *juglandis*

Species of the walnut genus *Juglans* are hosts of *X. arboricola* pv. *juglandis*. Walnuts which have had natural occurrences of walnut blight include Persian or English walnut, *J. regia* (Smith *et al.,* 1912; Rudolph, 1933; Tamponi and Donati, 1990; du Plessis and van der Westhuizen, 1995; Maria *et al*., 1997), the Northern Californian black walnut, *J. hindsii*, and Paradox hybrids where *J. regia* is crossed with either *J. hindsii* or the Southern Californian black walnut, *J. californica* (Smith *et al.,* 1912; Rudolph, 1933). The Japanese walnut, *J. ailantifolia*, has also hosted the walnut blight pathogen naturally (Anonymous, 1935).

With artificial inoculation, the disease has been produced in the Black walnut species (*J. hindsii, J. californica*) and Eastern black walnut (*J. nigra*), Butternut species (*J. cinerea*), Paradox hybrids, and Royal hybrids where *J. nigra* is crossed with either *J. hindsii* or *J. californica* (Smith, 1914; Smith *et al.,* 1912; Smith, 1915). Miller and Bollen (1946) have also artificially caused the disease in *J. nigra* and *J. hindsii*. Similarly, Belisario *et al.* (1997) and du Plessis and van der Westhuizen (1995) artificially caused the disease in *J. hindsii* and *J. regia* cultivars.

Walnut blight can occur in both seedling and mature walnut trees and can affect both vegetative and reproductive tissues (Miller and Bollen, 1946). The presence of *X. arboricola* pv. *juglandis* has been recorded on plant surfaces other than walnut.

Esterio and Latorre (1982) consistently isolated *X. arboricola* pv. *juglandis* from symptomless broad-leaved herbaceous plants and grasses from beneath trees in commercial walnut orchards in Chile during winter, spring, summer and autumn. Esterio and Latorre (1982) then inoculated walnut twigs grown under glasshouse conditions to demonstrate the pathogenicity of the collected isolates. While Esterio and Latorre (1982) did not differentiate between epiphytic and endophytic bacteria, the ability of *X. arboricola* pv. *juglandis* populations to survive in all four seasons suggests that the weed species provided protection to the bacterial population. The ability of *X. campestris* pv. *vesicatoria* to multiply on detached almond and walnut leaves, the multiplication of *X. arboricola* pv. *pruni* on tomato leaves (Timmer *et al.*, 1987), and the isolation of *X. arboricola* pv. *pruni* from tissues inside walnut leaves (du Plessis and van der Westhuizen, 1995) indicates that bacterial survival does not depend upon inciting disease of a specific host plant. These observations suggest that *X. arboricola* pv. *juglandis* could reside within or on other plant species, in addition to walnuts, in Tasmania.

3.2.4 Disease symptoms on walnut

The symptoms of walnut blight have been reported on all succulent tissue including buds, shoots, leaves, flowers and fruits (Rudolph, 1933; Miller and Bollen, 1946; Mulrean and Schroth, 1982; Lindow *et al.*, 2004; Chevallier *et al.*, 2010). The disease only affects current season growth, has not been detected on the roots of walnut trees, rarely causes appreciable defoliation even in years that are favourable for disease development, and has never been reported to kill a mature tree (Teviotdale and Schroth, 1998). Rudolph (1933) and Miller and Bollen (1946) prepared exhaustive studies of the symptoms of walnut blight. A summary of their descriptions of blight symptoms on the shoots, buds, leaves, flowers and fruits of walnuts, including how these symptoms develop, is presented in Appendix 2. A summary of symptoms of other diseases or disorders of walnut that have symptoms similar to walnut blight is then presented in Appendix 3.

3.3. IDENTIFICATION OF *X. ARBORICOLA* PV. *JUGLANDIS*

3.3.1. Cell morphology

Xanthomonas arboricola pv. *juglandis* is a Gram-negative rod bacterium, occurring commonly alone or in pairs, and uncommonly in chains and filaments (Pierce, 1896; Smith *et al.*, 1912; Miller and Bollen, 1946; Bradbury, 1986). Cells isolated from Californian, Oregon and Washington orchards in the USA, were arranged singly or in pairs, with average length and diameter of 1.86 μm and 0.58 μm respectively (Miller and Bollen, 1946). However, Miller and Bollen (1946) also detected cells up to 3.8 μm in length. In previous studies of *X. arboricola* pv. *juglandis* isolated from Californian orchards, cells mostly occurred singly, although they were often found in pairs or more rarely in shorter or longer chains of four to eight individuals (Pierce, 1896; Smith *et al.*, 1912). In these two studies, cell length varied from 1.0 to 3.0 μm with an average diameter of between 0.3 to 0.5 μm. While pairs of cells are typical of some *Xanthomonas* strains, it is not always obvious whether cells are an elongated single cell or paired cells (Swings *et al.*, 1993), thus cell length may be dependent upon whether the bacterium had divided or had elongated but not yet divided.

Xanthomonas arboricola pv. *juglandis* has been described as actively motile by a single polar flagellum (Pierce, 1896; Miller and Bollen, 1946; Bradbury, 1986). However, Kamoun and Kado (1990) found on culture media that five different *X. campestris* pathovars switched from non-motile, non-chemotactic, extracellular polysaccharide producing colonies to motile, chemotactic colonies that did not produce extracellular polysaccharide and visa versa. Using scanning electron microscopy, du Plessis (1984) also observed that flagella were never present on *X. arboricola* pv. *pruni* cells in plum tissue. In contrast, cells with flagella were present when grown in artificial media. The same observations were noted on *X. arboricola* pv. *pruni* colonies collected from infected peach leaves (Miles *et al.*, 1977). As some xanthomonads can be either motile or non-motile, further studies may identify non-motile strains of *X. arboricola* pv. *juglandis*.

3.3.2 Colony morphology

Xanthomonas arboricola pv. *juglandis* produces a water-insoluble yellow pigment in culture media, such as yeast dextrose calcium carbonate agar (YDC) (Bradbury, 1986). On YDC, the bacterial colonies are large, domed, smooth, mucoid and yellow (Lelliot and Stead, 1987). This bacterial species grows readily on nutrient agar (NA) with colonies appearing within 48 h at 25 to 28°C (Bradbury, 1986).

3.3.3 Xanthomonadins

Most *Xanthomonas* strains form xanthomonadins when cultivated on common growth media such as glucose-yeast extract-calcium carbonate (GYCA) medium and NA medium (Swings *et al.,* 1993). Xanthomonadins are brominated, aryl-polyene, yellow, water soluble pigments that are associated exclusively with the outer cell wall of xanthomonads (Stephens and Starr, 1963). As xanthomonadins are unique to the genus they allow differentiation between xanthomonads and other plant-associated yellow pigmented bacteria such as those from the genus *Pseudomonas, Erwinia* and *Flavobacterium* (Starr *et al.,* 1977). However, no consistent pattern of xanthomonadin content correlates within *Xanthomonas* taxonomy at the subgeneric level (Starr, 1981); for example, the major pigment formed by *X. arboricola* pv. *juglandis,* xanthomonadin 1, occurs as a variable component of some but not all pigment complexes of *Xanthomonas* spp.

3.3.4 Starch hydrolysis

Starch hydrolysis has been observed in *X. arboricola* pv. *juglandis* strains isolated from walnut orchards in California, Oregon and Washington (Pierce, 1896; Smith *et al.,* 1912; Miller and Bollen, 1946; Mulrean and Schroth, 1981). Small, round, pale blue, opalescent colonies with intact margins surrounded by an opaque zone, due to starch hydrolysis, on the semi-selective medium brilliant cresyl blue starch (BS) (Mulrean and Schroth, 1981), were characteristic phenotypic features of *X. arboricola* pv. *juglandis* isolated from walnut buds and catkins in Californian orchards (Mulrean and Schroth, 1981). BS medium has also been effective in the isolation and positive reaction to starch hydrolysis of *X. arboricola* pv. *juglandis* strains collected from leaf and twig lesions from northern, central and southern Italy

(Scortichini *et al.,* 2001). In contrast, six of eleven *X. arboricola* pv. *juglandis* isolates collected from nursery trees in South Africa did not show starch hydrolysis on BS medium (du Plessis and van der Westhuizen, 1995). In du Plessis and van der Westhuizen's (1995) study, the opaque zone surrounding colonies was not present after 7 days incubation at 27^{o}C. However, four of the five isolates examined by Miller and Bollen (1946) required 13 days incubation at 28^{o}C before starch hydrolysis was indicated fully; in contrast, the remaining isolate required only 2 days incubation before starch hydrolysis was fully indicated. Thus, the isolates in du Plessis and van der Westhuizen's (1995) study may have required a longer incubation period for starch hydrolysis to be indicated.

In addition to *X. arboricola* pv. *juglandis* (synonym: *X. campestris* pv. *juglandis*), BS medium can efficiently recover *X. campestris* pv. *malvacearum, X. campestris* pv. *phaseoli, X. campestris* pv. *pruni and X. campestris* pv. *vesicatoria* (Mulrean and Schroth, 1981). Furthermore, a frequent contaminant of isolations from walnut buds on BS media was *Pseudomonas syringae* (Mulrean and Schroth, 1981); however, these bacteria did not readily hydrolyse starch and thus were easily distinguishable from *X. arboricola* pv. *juglandis.*

3.3.5 Quinate metabolism

The metabolism of quinic acid to gallic acid, protocatechuic acid and pyrogallol on succinate quinate (SQ) medium by *X. arboricola* pv. *juglandis and X. arboricola* pathovars *corylina* and *pruni* separated these pathovars from nearly 350 strains representing all known genetic groups of xanthomonads (Lee *et al*., 1992). Furthermore, all 114 strains of *X. arboricola* pv. *juglandis* isolated from bud and twig cankers of different walnut cultivars grown in California metabolised quinic acid indicating that this phenotypic property was stable for this population. However, one of three strains of *X. campestris* pv. *celebensis* and one of four strains of *X. campestris* pv. *carotae* were also able to metabolise quinic acid, suggesting that, with further examination, a greater number of *Xanthomonas* strains may be identified that metabolise quinate.

3.3.6 Carbon utilisation

Carbon is the major element of macromolecules, and on a dry weight basis can consist of approximately 50% of a bacterial cell (Madigan *et al.*, 1997). Different carbon sources can restrict the growth of xanthomonads thus their growth patterns on media containing different carbon compounds are a useful criterion for differentiation of taxa (Swings *et al.*, 1993). The metabolic activity of xanthomonad in response to 95 different carbon sources with the Biolog GN microplate system (Biolog, Inc., Hayward, California) has been used to analyse the carbon utilization of the *Xanthomonas* genus. Examination of 183 strains of the genus *Xanthomonas* indicated that *X. arboricola* pathovars *juglandis, corylina, poinsetticola, populi* and *pruni* were distinguished from all other xanthomonads by metabolic activity on dextrin, glycogen, Tween 80, N-acetyl-D-glucosamine, cellobiose, L-fucose, D-galactose, gentiobiose, maltose, D-psciose, D-trehalose, monomethylsuccinate, acetic acid, DL-lactic acid, succinic acid, bromosuccinic acid, succinamic acid, L-glutamic acid, glycyl-L-glutamic acid and glycerol. These strains did not utilize N-acetyl-D-galactosamine, L-arabinose, D-mannitol, B-methyl-D-glucoside, D-raffinose, L-rhamnose, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucuronic acid, p-hydroxyphenylacetic acid, a-ketovaleric acid, quinic acid, D-saccharic acid, glucuronamide, L-phenylalinine, thymidine and glucose-6-phosphate (Vauterin *et al.*, 1995).

3.3.7 Nitrogen utilization

Xanthomonas arboricola pv. *juglandis* can utilize a relatively wide variety of nitrogen sources; furthermore, obligate aerobic bacteria such as *X. arboricola* pv. *juglandis* can utilise nitrate instead of oxygen as an electron acceptor in anaerobic conditions (Janse, 2006). Miller and Bollen (1946) found that *X. arboricola* pv. *juglandis* utilized peptone, aspartic acid, a-alanine, 1-leucine, sodium ammonium phosphate, allantoin, tyrosine, uric acid and brucine whereas hippuric acid, d-glutamic acid and sodium nitrite did not support growth. The nitrate reduction test is useful for distinguishing *Xanthomonas* species that do not reduce nitrate, from the many yellow saprophytic bacteria that do (Bradbury, 1970).

3.3.8 Fatty acids

Xanthomonads have an inner and outer membrane structure typical of Gram negative bacteria (dos Santos and Dianese, 1985). The major component of the innermost or cytoplasmic membrane is a class of complex lipids, the phospholipids (Madigan *et al.*, 1997), whereas the outer membrane contains lipopolysaccharide, protein and phospholipids in an approximate ratio of 2:2:1 (Dawes and Sutherland, 1976). Lipids can account for 20% of the weight of bacteria with the main constituent being the water-insoluble organic fatty acids (Lechevalier and Lechevalier, 1988).

An extensive analysis of the whole cell fatty acid content of nearly 1000 strains of the genus *Xanthomonas* by gas-liquid chromatography identified 21 different fatty acids within *X. arboricola* pv. *juglandis* (Yang *et al.*, 1993). Based on fatty acid profiles, 31 major clusters were defined. *X. arboricola* pv. *juglandis* (synonym: *X. campestris.* pv. *juglandis*) was included in the second largest cluster with *X. campestris* pathovars *aberrans, armoraciae, barbareae, campestris, carotae, cassiae, celebensis, corylina, eucalypti, guizotiae, incanae, manihotis, papavericola, phaseoli, poinsetticola, pruni, raphani, vesicatoria, vitians* and *zinniae.* While a total of 65 fatty acids were isolated, the fatty acids 11:0 iso, 11:0 iso 3OH, 12:0 3OH, 13:0 iso 3OH, 15:0 iso, 16:1 cis 9, 16:0, 17:1 iso F, and 17:0 iso were present in more than 99% of the strains and were therefore considered to be the most common within this genus. Furthermore, the methyl branched fatty acid 11:0 iso, and the branched chain fatty acids 11:0 iso 3OH and 13:0 iso 3OH, were characteristic for the entire *Xanthomonas* genus. Yang *et al.* (1993) suggested that this characteristic could differentiate *Xanthomonas* from other bacteria.

Fatty acid analysis depends on saponifying the fatty acids with sodium hydroxide, converting them to their methyl esters, and then separating and quantifying each fatty acid by gas-liquid chromatography (Onderdonk and Sasser, 1995). Analysis of an unknown sample results in an automatic comparison of the composition of an unknown strain to a database containing profiles of more than 100,000 strains of over 1,500 bacterial species from culture collections around the world (Sasser, 2006). Identification is based on comparison with the profile library, and indicates how

closely the bacterium compares to fatty acid profiles in the library collection. However, sample identification is not definitive as the analysis assumes that species of microorganisms have a normal distribution for their fatty acid profiles and that the mean of the population for fatty acid percentages characterises the group.

3.3.9 Proteins

Proteins are classed as either catalytic or structural and have major roles in cell function. Catalytic proteins, or enzymes, operate a variety of chemical reactions that occur within cells, whereas structural proteins are integral to the structures of cells in membranes, walls and to components within the cytoplasm.

In an extensive study of the whole cell protein content of more than 300 strains of *Xanthomonas*, Vauterin *et al.* (1991) used sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to identify two discrete groups within *X. arboricola* pv. *juglandis* strains, with a similarity between the groups of less than 80%. The two groups were correlated to strain origin with one group from the Netherlands and the other from the United Kingdom. The protein content of the United Kingdom group had 90% similarity to *X. campestris* pathovars *vesicatoria II, pruni, poinsetticola,* and *malvacearum,* whereas the Netherlands isolates were most related to *X. campestris* pv. *campestris* with approximately 85% similarity. Only eight isolates of *X. arboricola* pv. *juglandis* were examined in Vauterin *et al.*'s (1991) study, so the inclusion of more isolates may have increased the number of protein profiles identified.

3.3.10 DNA restriction analysis

DNA restriction techniques are based on specific sequences of double stranded DNA being recognized and cleaved by restriction endonucleases with the pool of restriction fragments of varying length separated by gel electrophoresis. The resulting banding pattern, such as those generated by contour-clamped homogenous electric field (CHEF) electrophoresis, is a genomic fingerprint of the organism.

Using CHEF electrophoresis of total genomic DNA, du Plessis and van der Westhuizen (1995) identified six clusters with distinctly different banding patterns

from 16 pathogenic *X. arboricola* pv. *juglandis* isolates collected from infected walnut nursery trees in South Africa. Three isolates of *X. arboricola* pv. *pruni* were also investigated producing reproducible DNA fragment banding patterns similar to each other but distinctly different from the banding patterns of the *X. arboricola* pv. *juglandis* isolates. Similarly, Lazo *et al.* (1987) using restriction fragment length polymorphism techniques analysed the banding patterns of 93 strains belonging to 26 pathovars of *X. campestris* and concluded that this method was suitable for differentiating the pathovars studied.

3.3.11 REP-PCR

Scortichini *et al.* (2001) used repetitive extragenic palindromic (REP) polymerase chain reaction (PCR) for genomic fingerprinting. By using enterobacterial repetitive intergenic consensus (ERIC), BOX elements (BOX) and repetitive extragenic palindromic (REP) primer sets, Scortichini *et al.* (2001) established that 61 strains of *X. arboricola* pv. *juglandis* collected from New Zealand, Romania, the Netherlands, Hungary, Romania, Italy, Portugal, Spain, France, USA, United Kingdom, Greece and Iran could be divided into three major groups. The first two groups were 85% genetically similar, whereas the third group was 78% genetically similar to the other strains. Each of the three major groups could be divided into two subgroups according to the country of origin, although the overall genetic similarity of the strains from one country was always greater than that of strains from other countries. The ERIC, BOX and REP primer sets gave reproducible genomic PCR profiles consisting of a total of 36 reproducible, clearly resolved bands of approximately 100-1700 bp. ERIC and BOX primers were more discriminative in differentiating *X. arboricola* pv. *juglandis* strains with 14 and 13 bands, respectively, in comparison to only 9 bands for the REP primer. The genomic PCR profiles of *X. arboricola* pv. *juglandis* strains were clearly differentiated from strains of *X. arboricola* pv. *fragariae*, the cause of leaf blight of strawberry, and from representative strains of *X. arboricola* pathovars *pruni* and *corylina* (Scortichini and Rossi, 2003).

3.3.12 AFLP-PCR

Amplified fragment length polymorphism (AFLP) analysis has also proved to be reproducible, reliable and sensitive enough to distinguish genomic variability among

X. arboricola pv. *juglandis* isolates. For example, differences among banding patterns of 66 isolates of *X. arboricola* pv. *juglandis* collected from England, France, Italy, the Netherlands, Romania, USA and New Zealand were detected using AFLP techniques (Loreti *et al.,* 2001). The AFLP technique identified 83 fragments between 200 and 600 bp, 76 of which were polymorphic. Visual inspection of banding patterns revealed that *X. arboricola* pv. *juglandis* could be differentiated from *X. arboricola* pathovars *pruni* and *corylina, X. campestris* pv. *campestris, X. fragariae, X. hortorum* and *X. axonopodis* pv. *vesicatoria.*

3.3.13 MLST

Multilocus sequence analysis (MLSA) provided a robust method for differentiation of 119 validated strains of various *Xanthomonas* species (Young *et al.,* 2008). *X. arboricola* pv. *juglandis, X. arboricola* pv. *celebensis, X. arboricola* pv. *corylina* and *X. arboricola* pv. *populi* were clearly differentiated from all other species, except for one strain of *X. vesicatoria*, by the congruence of three house-keeping gene sequences, tonB-dependent receptor (*fyaA*), DNA gyrase subunit B (*gyrB*) and RNA polymerase sigma factor (*rpoD*), and the structural gene, chaperone protein dnaK (*dnaK*). Based on the data set provided, the method provided a relatively simple method for identifying strains as members of known species, or of indicating their status as members of new species.

3.4 DISEASE DEVELOPMENT

3.4.1 The disease cycle

The disease cycle can be delineated into a sequence of events; the overwintering of the pathogen and the inoculation, penetration and infection of the plant. When there is only one infection cycle per season the disease is monocyclic, whereas it is polycyclic if there is more than one infection cycle per season (Agrios, 1997). Following is a review of the different stages in the development of walnut blight.

3.4.1.1 Overwintering of the pathogen

While xanthomonads do not form special survival structures they can survive for long periods of time in perennial plants (Schuster and Coyne, 1974). In northern

Californian orchards, Miller and Bollen (1946) were able to successfully recover *X. arboricola* pv. *juglandis* for up to 18 months from dead tissue and discolored marginal areas of twig lesions and infected leaves, and for up to 8 months from old infected nut mummies. Smith *et al.* (1912) and Rudolph (1933) also isolated *X. arboricola* pv. *juglandis* from lesions on walnut twigs grown during the preceding season. However, the importance of buds as an overwintering site in comparison to other infected plant tissue was highlighted by Miller and Bollen (1946), where they were able to isolate viable *X. arboricola* pv. *juglandis* cells for up to 5 years after infection of the detached buds.

In addition to overwintering in diseased tissue, *X. arboricola* pv. *juglandis* cells survive on walnut buds and leaves without expressing disease symptoms. In the Central Valley of Chile, Esterio and Latorre (1982) consistently recovered pathogenic *X. arboricola* pv. *juglandis* isolates from symptomless walnut buds, twigs, catkins and mummified nuts in winter and early spring. Similarly, in northern California the major overwintering sites for *X. arboricola* pv. *juglandis* were the symptomless walnut buds and catkins in the early-blooming varieties Eureka, Payne, Ashley and Serr, the middle-blooming varieties Hartley and Machette, and the late-blooming variety Franquette (Mulrean and Schroth, 1982). However, in Mulrean and Schroth's (1982) study, significantly more buds were colonised with *X. arboricola* pv. *juglandis* than catkins i.e., nearly 70% colonisation of buds in comparison to less than 45% colonisation of catkins. In a further study undertaken in northern California, approximately 70% of buds of the middle-blooming variety Chandler harboured *X. arboricola* pv. *juglandis* in winter in comparison to 60% of buds in spring, suggesting that buds provided a major overwintering site for *X. arboricola* pv. *juglandis* (Lindow *et al.*, 2004).

The colonisation and overwintering of *X. arboricola* pv. *juglandis* in walnut buds and catkins can occur both epipyhytically and internally. In Mulrean and Schroth's (1982) study on the ecology of *X. arboricola* pv. *juglandis* on walnuts in northern California, 90% of colonised buds and 45% of colonised catkins had both epiphytic and internal populations of the pathogen. When buds had both internal and epiphytic populations present, internal populations of *X. arboricola* pv. *juglandis* were usually

ten-fold greater. Furthermore, in the nine cultivars examined there was no significant difference in colonisation between fruitful or vegetative buds. Mulrean and Schroth (1982) suggested that the ability of *X. arboricola* pv. *juglandis* to colonise buds both internally and epiphytically enhanced survival, as internal populations enabled the pathogen to survive environmental stresses whereas epiphytic populations provided a ready inoculum source when environmental conditions and host phenology were favorable for disease development.

While buds have been found to be the major overwintering sites for *X. arboricola* pv. *juglandis* populations (Miller and Bollen, 1946; Mulrean and Schroth, 1982), there is a high degree of spatial segregation of the walnut blight pathogen within buds (Lindow *et al.*, 2004). Walnut buds comprise of the outermost bud scales, followed by cataphylls to the innermost embryonic leaves. The embryonic leaves surround a flower meristem at the apex of the bud. Lindow *et al.* (2004) examined the spatial distribution of *X. arboricola* pv. *juglandis* cells within the reproductive buds of cultivar Ashley, and observed that approximately 80%, 70% and 40% of bud scales, cataphylls and embryonic leaves respectively, were colonised with *X. arboricola* pv. *juglandis* cells. The highest populations of *X. arboricola* pv. *juglandis* prior to budburst in spring were detected in the bud scales. However, no significant difference in population density was detected between the outermost and innermost bud scales, with approximately 1,000 cells per bud scale. As with the bud scales, there was no significant difference in the population density of *X. arboricola* pv. *juglandis* between the outermost and innermost of the four cataphylls per bud. However, significantly less *X. arboricola* pv. *juglandis* cells were present on cataphylls, i.e., 100 to 300 cells per cataphyll, in comparison to the population observed on bud scales. With contaminated embryonic leaves, less than 100 cells per leaf were observed prior to budburst. Lindow *et al.* (2004) suggested that as the interior of the bud is probably relatively moist, this location offered protection for *X. arboricola* pv. *juglandis* cells from environmental stresses.

3.4.1.2 Inoculation

Inoculation is defined as the arrival or transfer of a pathogen onto a host, and the inoculums as the pathogen or its propagules that cause infection (Agrios, 1997).

Bacterial inoculum consists of whole individuals of the bacterium. Inoculum that survives over the winter and causes primary infections is termed primary inoculum. Inoculum that is produced from primary infections can cause secondary infections, and is termed secondary inoculum.

As previously discussed, principal overwintering sites of *X. arboricola* pv. *juglandis* are the vegetative and reproductive walnut buds (Miller and Bollen, 1946; Esterio and Latorre, 1982; Mulrean and Schroth, 1982), and that within buds there is a high degree of spatial segregation of *X. arboricola* pv. *juglandis* populations (Lindow *et al.*, 2004). Lindow *et al.* (2004) reported that prior to budburst, embryonic leaves were seldom contaminated with *X. arboricola* pv. *juglandis*, however, within 3 weeks of budburst nearly all leaves were infected with the pathogen. Similarly, fruits became colonised with *X. arboricola* pv. *juglandis* shortly after their formation. Observation of the pattern of colonisation of embryonic and developing walnut leaves by *X. arboricola* pv. *juglandis* led Lindow *et al.* (2004) to suggest that leaves become inoculated shortly after emergence from the bud and that moisture was the most logical mechanism for moving the inoculum.

3.4.1.3 Penetration

The penetration of plant surfaces by plant pathogens can occur via direct penetration, wounds or natural openings of the host tissue. As bacteria lack active mechanisms for penetrating plant surfaces, entry can only occur through wounds and natural openings and is favoured by external conditions such as the availability of water and the structure of plant openings (Rudolph, 1993).

Natural openings within the surface of plant leaves, stems and fruits include hydathodes, lenticels and nectarines and stomata. Stomata act as a common entry point as the significantly larger diameter of stoma in comparison to bacterial cells enables bacteria suspended in a film of water over a stoma to penetrate the substomatal cavity (Agrios, 1997). The importance of stomata as penetration sites for *X. arboricola* pv. *juglandis* in walnut flowers, fruits and leaves was identified by Miller and Bollen (1946). In their study, infection of cultivar Franquette pistillate flowers in the pre-bloom and bloom period occurred primarily through stomata

located in the bracteole lobes or adjacent tissue, and in the post-bloom period principally through the stomata on the sides of the fruit. Miller and Bollen (1946) suggested that the scarcity of lateral infections on pre-bloom and bloom flowers was because of significantly fewer stomata on the sides of flowers prior to bloom in comparison to post-bloom flowers. The importance of stomata as infection sites for *X. arboricola* pv. *juglandis* in walnut fruit was supported by Garcin *et al.*'s (2001) study where they observed, with scanning electron microscopy, the infection of flower sepals of cultivar Franquette was quickest where stomata were the most numerous.

Other wounds that can facilitate penetration of bacteria include breakage of trichomes, pruning or mowing of plants, insect feeding, leaf fall or transplanting (Rudolph, 1993). While stomata were the principal entry points for *X. arboricola* pv. *juglandis* into walnut flowers, fruits and leaves in Miller and Bollen's (1946) study, they also observed that access to walnut tissue occurred occasionally with mechanical injuries such as limb rubbing and hail damage. Furthermore, Goodman (1982) suggested that entry of *X. arboricola* pv. *juglandis* into dormant buds may have occurred through leaf traces. The transmission of *X. arboricola* pv. *pruni* in plum nursery trees with pruning (Goodman and Hattingh, 1988) suggests that entry of *X. arboricola* pv. *juglandis* into walnut trees may also occur with management practices such as pruning and hedging.

3.4.1.4 Infection

Infection is defined as the establishment of a parasite within a host plant (Agrios, 1997), and involves the growth and reproduction, or colonization, of the pathogen via the invasion of, and acquisition of nutrients from, susceptible host cells.

Walnut blight is predominantly a disease of the parenchymatous tissues with vascular tissues invaded, if at all, only in the later stages of attack (Miller and Bollen, 1946; Garcin *et al.*, 2001). Upon entry via stomata, *X. arboricola* pv. *juglandis* cells multiply and colonise the sub-stomatal cavity prior to progressing intercellularly between palisade tissue and spongy parenchyma cells (Miller and Bollen, 1946; Garcin *et al.*, 2001); as the infection progresses, the bacteria multiply rapidly in the

intercellular spaces, often until the entire parenchyma between two adjacent veins is completely invaded.

The intercellular space of plants contains all the nutrients essential for the initial stages of bacterial multiplication (Novacky and Ullrich-Eberius, 1982); however, one of the earliest changes in infected walnut tissue is increased membrane permeability, as pectin- and other cell wall-degrading enzymes degenerate cell walls (Miller and Bollen, 1946; Garcin *et al.*, 2001). Similarly, the transmembrane potential of cotton cotyledons rapidly changed when infected with *X. campestris* pv. *malvacearum* (Novacky and Ullrich-Eberius, 1982). Necrosis occurs as cell membranes become leaky, accumulate phenolic substances and disintegrate (Garcin *et al.*, 2001), although Miller and Bollen (1946) suggested that osmotic pressure may also lead to cell death, as plasmolysis of the cell protoplast was observed in tissues infected with *X. arboricola* pv. *juglandis.*

Infection of xanthomonads may also involve entry into the vascular tissue (Rudolph, 1993); however, the systemic movement of *X. arboricola* pv. *juglandis* in walnut tissue has not been reported. In contrast, systemic movement in the vascular bundles has been reported for *X. arboricola* pv. *pruni* in peach trees (du Plessis, 1984, 1990). Thus, it is possible that *X. arboricola* pv. *juglandis* could move systemically within the plant, although the mode of entry from the parenchyma into the vascular tissue would need to be established.

3.4.1.5 Dissemination

Almost all dissemination of plant pathogens is carried out passively by such agents as air, water and insects (Agrios, 1997). Bacterial multiplication in the intercellular space often leads to a physical pressure so that bacterial masses emerge from the substomatal cavities and serve as secondary inoculum. For example, bacterial cells were shown, with scanning electron microscopy, to be disseminated from the stomata of Franquette fruits after inoculation with *X. arboricola* pv. *juglandis* (Garcin *et al.*, 2001). Similarly, Miles *et al*. (1977) observed masses of *X. arboricola* pv. *pruni* being pressed out of the stomata of peach leaves 9 days after inoculation. Furthermore, Egel *et al.* (1991) established that external populations of *X. campestris*

pv. *citri* on leaf surfaces were a result of internal bacteria being exuded from lesions onto the moist leaf surface.

In Californian orchards, *X. arboricola* pv. *juglandis* from lesions on leaves and fruits of the current season growth were the source of secondary infections (Miller and Bollen, 1946). The intensity of secondary infections was found to be dependent upon the number of primary infections in trees and the extent of rainfall during the latter part of the infection period. Similarly, Mulrean and Schroth (1982) found that twigs infected during spring in Californian orchards were a source for secondary inoculum within the growing season. Both Miller and Bollen (1946) and Mulrean and Schroth (1982) demonstrated that the incidence of secondary infections were traceable to specific periods of rainfall. Furthermore, Miller and Bollen's (1946) findings from their 14 year study of walnut blight in northern Californian orchards, led them to suggest that rainfall was the most significant, if not the only, agent in the dissemination of inoculum under conditions in the Pacific Northwest region of the United States. The importance of rainfall was also noted in a field study in northern California, where Adaskaveg *et al.* (2000) reported a significant increase of disease incidence on fruits in trees that were irrigated with overhead sprinklers, to simulate rainfall, for 6 h per week in comparison to non-irrigated trees.

When a lesion is hit by a raindrop, bacteria are easily suspended into the rain splash and transported through the air and deposited onto a new host (Stall *et al.*, 1993). Thus, wind driven rain splash or water splash of sprinkler irrigation may be an important factor in the dispersal of *X. arboricola* pv. *juglandis* in Tasmania.

As with walnut buds, another major overwintering site of *X. arboricola* pv. *juglandis* are catkins. Catkins consist of many individual staminate, or male, flowers that are formed the season prior to flowering. Each flower can contain up to forty pollen bearing stamens and anthers; upon maturity each anther releases hundreds of pollen grains that are dispersed by wind (Polito, 1998). To determine whether pollen was a source of walnut blight infection under Californian conditions, Ark (1944) dusted pistillate flowers and the abaxial surface of leaves with pollen collected from either diseased or healthy catkins. Of the 50 flowers dusted with pollen from healthy

catkins no blight symptoms were apparent on the developed nuts. In comparison, 30 of the 50 flowers dusted with pollen from contaminated catkins were infected. Similarly, seven of ten leaves had blight lesions within one month of being dusted with pollen from contaminated catkins, whereas blight lesions were not observed on any leaves after dusting with pollen from healthy catkins. This led Ark (1944) to suggest that aerial dissemination of infected pollen from diseased catkins could transmit the bacterium to pistillate flowers and cause infection when the environmental conditions were favourable for disease development. However, Miller and Bollen (1946) observed that there was little or no correlation between the peak of disease development and the time of most pollen shedding, taking into account the lag time between pollen shedding and symptom development, and suggested that while infected pollen from diseased catkins may disseminate the pathogen it was not significant factor in the development of the disease under Californian conditions.

Insects can act as vectors of diseases by the transferral of inoculum to host plants through punctures caused by feeding and oviposition. In Californian walnut orchards, the walnut aphid, *Chromaphis juglandicola*, and walnut blister mite, *Eriophyes tristriatus erineus nalepa*, have been reported to spread *X. arboricola* pv. *juglandis* (Smith *et al.*, 1912; Rudolph, 1943). Similarly, in southern Spain the presence of walnut aphids led to higher disease incidence on walnut leaves (Arquero *et al.*, 2006). Furthermore, the New Zealand flower thrips, *Thrips obscuratus*, vectored *X. arboricola* pv. *pruni* in a New Zealand nectarine orchard leading to an increase in disease incidence (McLaren *et al.*, 2003).

In the Pacific Northwest region of the United States, however, Miller and Bollen (1946) were not able to isolate *X. arboricola* pv. *juglandis* from the bodies of walnut aphids collected from leaves that contained numerous blight lesions. Additionally, they were unable to infect healthy leaves with walnut aphids that had previously fed on diseased leaves (Miller and Bollen, 1946). Furthermore, they observed only a few species of flies on diseased fruits that came into contact with the exudates from lesions, and that the peak of development of all the common insect pests of walnuts did not correspond to the development of walnut blight. These observations led

Miller and Bollen (1946) to conclude that insects did not disseminate, to any great significance, *X. arboricola* pv. *juglandis* in the Pacific Northwest region.

3.4.2 Factors that affect disease development

The development of plant diseases caused by the genus *Xanthomonas* is markedly influenced by the environment, affecting both pathogen development and the susceptibility of the host plant (Stall *et al.*, 1993). Following is a review of the pathogen, host and environmental factors that influence the development of walnut blight.

3.4.2.1 Pathogen virulence

The capacity for a bacterium to penetrate, establish and multiply in a host plant is determined in part by virulence factors or aggressins (Janse, 2006). Virulence factors include enzymes that make plant tissue and cells available for colonisation by the bacterium.

Smith *et al.* (1912) reported that *X. arboricola* pv. *juglandis* produced a cellulose dissolving enzyme. In contrast, Miller and Bollen (1946) grew *X. arboricola* pv. *juglandis* strains on a cellulose agar medium for up to 43 days and did not detect measurable levels of cellulase by any of the strains. They suggested that a cellulolytic enzyme may be produced by the pathogen in invaded tissues within the host, or by the host under the stimulus of bacterial invasion.

In contrast to cellulase, both Smith *et al.* (1912) and Miller and Bollen (1946) found proteolytic enzymes produced by *X. arboricola* pv. *juglandis*. However, Miller and Bollen (1946) found differences between isolates: four isolates hydrolysed pectin within 4 days at 28^{o}C but one isolate took 24 days to show activity. Miller and Bollen (1946), using microscopy, observed that the cell walls of carrot pieces inoculated with *X. arboricola* pv. *juglandis* were more swollen, and the intercellular spaces larger, than those in controls. From this, Miller and Bollen (1946) suggested that an exo-enzyme, of the nature of proto-pectinase, is produced by *X. arboricola* pv. *juglandis* which acts on the middle lamella and other pectin like substances

present in cell walls, softening and partially dissolving them, thus facilitating the intercellular penetration by the bacteria.

Hydrolysis of starch has been recorded in *X. arboricola* pv. *juglandis* strains isolated from orchards in California, Oregon and Washington (Pierce, 1896; Smith *et al.*, 1912; Miller and Bollen, 1946; Mulrean and Schroth, 1981). Miller and Bollen (1946) found diastase (synonym: amylase), the catabolic enzyme responsible for starch hydrolysis, produced in relatively large quantities when the organism was grown on potato dextrose agar or starch agar. In contrast to strains isolated from American orchards, six of the eleven isolates collected from nursery trees in South Africa did not show starch hydrolysis (du Plessis and van der Westhuizen, 1995). In their study, Miller and Bollen (1946) only examined three isolates of *X. arboricola* pv. *juglandis* for starch hydrolysis. Further examination may highlight differences in the rate of starch hydrolysis amongst *X. arboricola* pv. *juglandis* strains.

3.4.2.2 Quantity of inoculum

Most xanthomonads are very competent when initiating an infection and, theoretically, one cell in a favourable location in a susceptible plant can multiply to a population that will cause necrosis (Stall *et al.*, 1982). In Californian orchards, a direct relationship was observed between increasing mean populations sizes of *X. arboricola* pv. *juglandis* on individual walnut buds in late winter and early spring and the subsequent incidence of disease to fruits in early summer (Lindow *et al.*, 2004); in these studies, a population density of 1.0×10^5 cells per bud led to between 25% and 45% of fruits with disease in 7 of 10 years; however, as few as 1.0×10^2 cells per bud still led to between 15% and 25% disease incidence. In contrast, Mulrean and Scroth (1982) reduced populations of *X. arboricola* pv. *juglandis* on young shoots in Californian orchards from 1.1×10^8 cells to 1.9×10^5 cells per shoot, with four copper-based sprays applied during the dormant season, without affecting the subsequent disease incidence of fruits or crop loss. Similarly, the incidence of disease on Franquette fruits in early summer was significantly different between two orchards in France, even though populations of *X. arboricola* pv. *juglandis* in dormant buds were similar (Giraud *et al.*, 2010). Thus, a greater understanding is

required on the relationship between inoculum load, host phenology and infection of walnut tissue in relation to environmental conditions.

3.4.2.3 Host susceptibility

Factors that influence the proportion of cells that lead to disease symptoms include the defense mechanism of plants, such as the production and accumulation of phenolic compounds (Radix *et al*., 1993), and other resistance mechanisms against bacterial pathogens.

Disease resistance has been reported in cultivar Serr (Robinson, 1969), and in one year old *Juglans nigra, J. cinerea* and *J. sieboldiana* nursery trees after being sprayed to runoff with a bacterial suspension of *X. arboricola* pv. *juglandis* (Belisario *et al.*, 1997). Furthermore, Belisario *et al.* (1997) found that cultivar Franquette was amongst the most resistant of the cultivars tested. In Spanish orchards, leaves of cultivar Franquette had significantly less blight incidence in comparison to cultivar Vina, which led to the authors concluding that Vina was more susceptible to blight than Franquette (Aleta *et al.,* 2001). However, when Aleta *et al.* (2001) inoculated one month old fruits with a suspension of *X. arboricola* pv. *juglandis* both cultivars Franquette and Vina were considered to be highly susceptible to walnut blight. Similarly, when cultivar Serr and its two parent cultivars in Californian orchards were artificially inoculated with *X. arboricola* pv. *juglandis*, disease rapidly occurred and spread naturally to un-inoculated parts of the plant (Mulrean and Schroth, 1982). All these results indicate that different plant cultivars and plant tissue may vary in their susceptibility to *X. arboricola* pv. *juglandis* and that the response depends on the interaction of the plant genotype with the environment in which a cultivar is being grown.

Various polyphenols, in particular juglone and its glucoside, have been implicated in reducing the susceptibility of walnuts to infections of *X. arboricola* pv. *juglandis* (Radix *et al.,* 1998). The greater quantity of juglone around the circumference of fruits in cultivar Parisienne, in comparison to Franquette, led Radix *et al.* (1998) to suggest that increased juglone content reduced susceptibility of Parisienne in French orchards. Further studies found that the permeability of soil induced variations in the

polyphenol content of walnut tissues. Only minimal numbers of walnut cultivars have, at present, been studied for polyphenol content; further studies examining the interaction of polyphenolic type and/or quantity among different cultivars on blight susceptibility is warranted.

The geographical region in which a cultivar is grown does not appear to alter the susceptibility of walnuts to walnut blight. Woeste *et al.* (1992) found *J. regia* cultivars collected from China, Afghanistan, Japan, France and the United States of America to be susceptible to blight after inoculation with *X. arboricola* pv. *juglandis.* In South Africa, du Plessis and van der Westhuizen (1995) identified *X. arboricola* pv. *juglandis* from diseased leaf and shoot material sampled from three *J. regia* cultivars and *J. regia* seedling rootstock. Arquero *et al.* (2006) assessed disease symptoms on eight Californian varieties grown on *J. hindsii* and Paradox rootstocks in southern Spain. Similarly, Belisario *et al.* (1997) artificially caused the disease in the *J. regia* varieties Feltre and Malizia and cultivar Decollatura from Southern Italy, and the *J. regia* cultivars Payne and Serr from the United States of America. These observations indicate that *J. regia* cultivars grown in Tasmania may be susceptible to walnut blight.

3.4.2.4 Host morphology

As previously discussed, stomata have been identified as a pre-eminent site for infection of walnut tissue (Miller and Bollen, 1946; Garcin *et al.*, 2001). However, the morphology of walnut stomata and their relationship to bacterial infection has not been studied. In peach cultivars, susceptibility to infection by *X. arboricola* pv. *pruni* was significantly increased with a wide stomatal aperture, such that water congestion of the substomatal chamber and neighbouring intercellular space was favored (Matthee and Daines, 1969). Similarly, a difference in structure between stomata of mandarin and grapefruit appeared to determine field resistance of mandarin for *X. axonopodis* pv. *citri* (Janse, 2006). These reports suggest that morphological differences in walnut stomata will influence infection by *X. arboricola* pv. *juglandis*.

3.4.2.5 Rainfall and free moisture

The importance of rainfall in the development of walnut blight has been discussed previously (*Section 3.4.1.5*); however, the duration of surface wetness of host tissues is also critical for disease development. In field and glasshouse trials in northern Californian, as little as 5 min of continuous wetting was necessary for infection of fruits when stomata were wide open and the tissues water congested (Miller and Bollen, 1946). The wetting periods required for infection increased with increasing fruit age, a smaller amount of water in host tissues and with the extent of stomatal opening. In field studies conducted in California, however, a minimum of 12 to 24 h of surface wetness was required for disease development in fruits (Adaskaveg *et al.*, 1995). Differences in the cultivars and developmental stages used by Miller and Bollen (1946) and Adaskaveg *et al.* (2004) may have contributed to the differences reported, as these factors appear to alter the susceptibility of fruits, as discussed in *Section 3.4.2.3*.

In California, the increased frequency and duration of dews has been associated with high numbers of *X. arboricola* pv. *juglandis* in buds and catkins, and the high incidence of blighted fruits and leaves of cultivar Marchette, in comparison to other late-blooming cultivars (Mulrean and Schroth, 1982). However, cultivars in this study were not located within the same orchard; thus, factors other than free moisture may have influenced disease incidence.

3.4.2.6 Temperature

In glasshouse trials examining the effects of temperature on the infection of walnut tissue by *X. arboricola* pv. *juglandis*, Miller and Bollen (1946) observed that temperature at the time of, and following, inoculation was not important in the infection of fruits, and that temperatures ranging from 5^{o}C to 27^{o}C seemed to only hasten the appearance of lesions. The infection of walnut leaves, however, was influenced by temperature with the incidence of infected leaves increasing as temperatures increased from 4^{o}C to 30^{o}C (Miller and Bollen, 1946). In citrus tissue, the multiplication of *X. citri* occurs in two phases, initial growth and secondary growth (Koizumi, 1977). Initial growth occurred at temperatures of 6^{o}C causing mild

decomposition of the cell walls with the establishment of bacteria within the plant. Secondary growth in inoculated citrus leaves began with lesion appearance and occurred after the degeneration of the plasmalemma and tonoplast adjacent to causal bacteria. Based on the relationship between symptom appearance and secondary growth, the effective temperature for secondary growth began at 13^{o}C (Koizumi, 1977). In contrast, the dynamic of *X. arboricola* pv. *juglandis* multiplication *in planta* in relation to temperature is not well understood.

3.4.2.7 Relative humidity

The effect of relative humidity on the multiplication of *X. arboricola* pv. *juglandis* has not been studied; however, an increase in relative humidity within the tree canopy has been implicated for increased disease incidence on fruits in southern Spain (Arquero *et al.,* 2006). Bacterial populations tend to increase at high humidity when plants are wet (Rudolph, 1993); for example, populations of *X. campestris.* pv. *vesicatoria*, the causal pathogen of bacterial spot in tomatoes, increased 10- to 100-fold on tomato leaves at a relative humidity greater than 90%, fluctuated wildly at relative humidity between 50 and 65%, and declined to very low levels at a relative humidity from 10 to 25% (Timmer *et al.,* 1987). Similarly, *X. arboricola* pv. *pruni* multiplied on detached tomato leaves under high humidity (Timmer *et al.,* 1987).

Periods of continuous high humidity and a film of free water on plant surfaces can also induce water congested areas in intercellular spaces. Water congestion appears to have important physiological effects on plant tissue that increase susceptibility to bacterial plant pathogens (Bennet and Carter, 1990). For example, epiphytic *X. arboricola* pv. *pruni* was found to persist year round on the surfaces of peach and plum trees; however, symptoms failed to develop after inoculation without first inducing water congestion (Shepard and Zehr, 1994).

3.4.3 Epidemic development

Knowledge of the temporal development of disease can provide information on or generate hypotheses about the relative importance of primary and secondary inoculum, the mechanisms of pathogen dispersal, and the effects of environmental factors on epidemics.

3.4.3.1 Description of temporal development of disease

Plant disease epidemics are typically described as monocyclic, polycyclic or polyetic (Agrios, 1997). In monocyclic diseases, a pathogen completes only one generation (disease cycle) per growing year, whereas, in polycyclic diseases the pathogen goes through more than one generation per growing year. In polyetic diseases the pathogen completes a disease cycle over several years. The disease progress curve (Campbell and Madden, 1990), when fitted to observed data for disease incidence or severity, represents the integration of host, pathogen and environmental effects occurring during an epidemic, with various parameters being derived for describing the epidemic.

Models used to generate and analyse the disease progress curve include the exponential, monomolecular, logistic and Gompertz models (Campbell and Madden, 1990). Monocyclic disease is typically described by the monomolecular model, where the rate of change in disease is greatest at the time of the first disease assessment and decreases from that point onwards (Madden, 1980). Polycyclic disease can be typically described by either the exponential or logistic models (Madden, 1980). The exponential model describes a situation where the rate of infection is lowest at the time of initial assessment and increases with increasing levels of secondary inoculum production over time (Madden, 1980). The logistic curve is an extension of the exponential curve where the rate of disease spread increases initially, but then becomes limited by reducing levels of available healthy host tissue (Madden, 1980). The Gompertz model also provides a curve shape that represents polycyclic disease (Campbell, 2003); however, in the Gompertz model the rate at which the disease spreads approaches the inflexion point more rapidly but then declines more slowly than the logistic model.

3.4.3.2 Temporal progression of walnut blight

To date, only one study, by Adaskaveg *et al.* (2000) in California, has quantified the temporal progression of walnut blight. In their study, the development of walnut blight was monitored on fruits of cultivar Ashley and Vina every 7 to 10 days between mid spring and early summer for 4 years in two commercial orchards

located in Butte and Tehama counties in California. The resultant disease incidence data were then compared to linear, monomolecular, exponential, logistic and Gompertz models, according to procedure described by Campbell and Madden (1990), to best describe the disease progress curves. Within the 4 years, disease progress was best described by the monomolecular model in four Ashley orchards and three Vina orchards, and the Gompertz model in two Vina orchards and one Ashley orchard. This result suggests that monocyclic disease is the most common type of epidemic in Californian orchards, although analyses of more epidemics over a longer period of time would confirm their relative frequency.

3.5 DISEASE MANAGEMENT

Methods to control plant disease may exclude the pathogen from the host, improve the resistance of the host, directly protect plants from pathogens, and/or eradicate or reduce pathogen inoculum (Agrios, 1997). The decision process to determine which control method, or combinations of control methods, is best suited for a particular disease must be based on knowledge of the pathogen life cycle and the interaction between host and pathogen (Lozano and Wholey, 1974). Hence, methods used to control walnut blight have generally focused on reducing pathogen inoculum and protecting plants from infection.

3.5.1 Copper and non-copper treatments

The most effective bactericide treatments for reducing populations of *X. arboricola* pv. *juglandis* are those products containing metallic copper (Miller and Bollen, 1946; Mulrean and Schroth, 1982). The most widely used copper products contain copper oxychloride, copper hydroxide, cuprous oxide or Bordeaux mixture (Mew and Natural, 1993). Copper is an essential element for enzymes involved in cell respiration (Garcia-Horsman *et al.,* 1994); however, above a certain concentration copper damages DNA and lipid membranes and becomes toxic to the cell (Hoshino *et al.,* 1999; Muller *et al.,* 2000; Finney and O'Halloran, 2003). Alternative chemicals to copper for controlling walnut blight have not been identified, with bactericidal sanitation treatments and antibiotics proving to be either ineffective or unreliable against walnut blight (Miller and Bollen, 1946; Belisario and Zoina, 1995; Lang *et al.,* 2006).

3.5.2 Amendments to copper sprays

Copper applied alone does not always provide effective control of walnut blight. The toxicity of copper is reduced by the low solubility of copper bactericides (Arman and Wain, 1958), and by copper ions forming complexes with organic compounds on plant surfaces (Menkissoglu and Lindow, 1991). However, the addition of ethylene-bisdithiocarbamate fungicides (EBDC's), such as maneb and mancozeb, to copper has increased control of walnut blight in comparison to applications of copper alone. In Tasmanian orchards, Lang *et al.* (2006) found blight incidence was significantly reduced when copper-based products were combined with mancozeb. Similarly, Buchner *et al.* (2001) reported that the addition of EBDC's increased the efficacy of copper-based products for the control of walnut blight in northern California. The toxicity of copper has also been enhanced by the addition of EBDC's for the control of *X. campestris* pv. *vesicatoria* in pepper and bacterial speck caused by *Pseudomonas syringae* pv. *tomato* (Conlin and McCarter, 1983; Marco and Stall, 1983). The chelating ability of EBDC's is thought to increase copper toxicity as copper ions are prevented from complexing with other organic substances (Cooksey, 1990). The addition of iron, in the form of ferric chloride, to copper hydroxide has also shown to enhance the toxicity of copper to *X. arboricola* pv. *juglandis*, although the incidence of walnut blight was not significantly reduced (Lee *et al.*, 1993). These findings indicate the importance of combining amendments with copper-based products to increase the toxicity of copper to the walnut blight pathogen.

3.5.3 Timing of copper-based sprays

Reducing or excluding the initial inoculum is a suitable strategy for the control of monocyclic epidemics. Blight incidence of fruits in monocyclic epidemics was entirely dependent upon the amount of initial inoculum in seven of ten orchards in California (Adaskaveg *et al.*, 2000). Similarly, Ninot *et al.* (2002) found that populations of *X. arboricola* pv. *juglandis* in Spanish orchards were highly variable in spring and relatively constant during summer, leading them to suggest that chemical treatment to reduce population growth is best concentrated during spring rather than summer. Nino *et al.* (2002) found that three sprays applied during the two to three week period of budburst did not significantly increase disease incidence and

severity on fruits in comparison to a total of seven sprays i.e., three budburst sprays and four sprays applied at weekly intervals after the budburst period. In northern Californian orchards, disease incidence on fruits was significantly reduced after application of two sprays in which copper-based sprays were applied one and two weeks after budburst (Lindow *et al.,* 2004). Furthermore, during a 14-year study conducted in northern Californian orchards, a maximum of three sprays applied during the budburst to early post-blossom period often provided satisfactory control of blight (Miller and Bollen, 1946). These results suggest that bactericide sprays applied during budburst may reduce pathogen inoculum, protect primary infection courts and reduce disease incidence during monocyclic epidemics.

Bacterial populations generally increase exponentially under conditions favourable for multiplication (Stall *et al.,* 1993), and a direct relationship has been observed between the numbers of *X. arboricola* pv. *juglandis* cells in buds and the mean number of diseased fruits (Lindow *et al.,* 2004). In a study conducted over 5 years in Californian orchards, wet spring seasons led to polycyclic epidemics and increased the severity of disease (Adaskaveg *et al.,* 2000). Thus, multiple applications of copper are generally applied after the budburst period in an attempt to reduce the rate of disease increase. However, high rainfall may limit copper persistence on trees (Ninot *et al.,* 2002). Rainfall of 40 to 90 mm per week reduced copper levels on sour cherry leaves by more than one half, leaving sub-lethal levels for the control of *Pseudomonas syringae* pv. *morsprunorum* (Olsen and Jones, 1983). Therefore, to improve control of walnut blight, the persistence of copper on walnut tissues after rainfall warrants investigation.

In polycyclic epidemics, the inoculum can be multiplied many times during the growing season, requiring further control applications during the growing season. However, copper tolerant strains of the walnut blight bacterium are present in French and Northern Californian walnut orchards that have a history of high copper use (Gardan *et al.,* 1993; Lee *et al.,* 1993); these bacteria have been found to accumulate copper ions in the periplasm and outer membrane of the cell (Cooksey, 1990). Furthermore, high levels of copper within soils can lead to crop losses in walnuts (Radix and Seigle-Murandi, 1993): concentrations of less between 80 to 110 mg

copper per kg of soil in orchards have led to decline in soil biota (Martin, 1986; Paoletti *et al.*, 1995). Ninot *et al.* (2002) found that application of 14 kg of copper per hectare for 3 years led to an 80% increase of copper in the top 10 cm of soil in comparison to application at 7 kg per hectare. Hence, the use of intensive copper spray regimes may have potential long-term detrimental effects on plant performance and the frequency of copper tolerant strains of *X. arboricola* pv. *juglandis.*

In northern Californian walnut orchards, temperature and extended wetness periods have been identified as critical environmental factors in walnut blight epidemics (Adaskaveg *et al.*, 2000); this knowledge has been used to develop XanthoCast™, a model that utilises wetness period duration and temperature for calculating the cumulated daily risk of disease. In field trials conducted over 9 years, copper-based spray programmes timed according to host phenology i.e., the budburst period, and XanthoCast™ predictions after the budburst period, have predicted the same number, or a reduced the number, of sprays when compared to a calendar-based spray regime, while providing similar disease control (Adaskaveg *et al.*, 2009). These results indicate the potential for reducing the number of copper sprays in Tasmania.

3.5.4 Biological methods

Xanthomonad phages are abundant in nature and occur together with host bacteria in soil and lesions of diseased plants (Hayward, 1964; Stolp and Starr, 1964). In New Zealand walnut orchards, bacteriophages were readily isolated from the top 2.5 cm of soil underneath the trees where *X. arboricola* pv. *juglandis* was isolated (McNeil *et al.*, 2001). As a consequence, the potential use of bacteriophages as bio-control agents against *X. arboricola* pv. *juglandis* have been trialed in New Zealand orchards; however, further research is required for this method to be implemented into an integrated disease management strategy (Jenkins *et al.*, 2010).

3.6 SUMMARY

Studies on the development and management of walnut blight have not been conducted in Tasmania. The causal organism of walnut blight has yet to be confirmed in Tasmania, and the temporal progression of walnut blight and the environmental factors that influence the development of disease have not been

studied. The current tactic of applying multiple prophylactic applications of copper-based sprays can sometimes result in control failures and there is a lack of viable, alternative management methods. Understanding the critical epidemiological factors associated with the development and expression of walnut blight will help refine current copper-based spray practices to economically and environmentally sustainable levels in Tasmania. Such knowledge will also aid identification and development of novel control measures.

GENERAL MATERIALS AND METHODS

4.1 TERMINOLOGY

At maturity the walnut consists of the hull, shell and edible kernel (Pinney *et al*., 1998). In this study, the term "fruit" refers to all three parts, whereas the shell and kernel is termed the "nut".

Walnut harvest commences when both the kernel and hull are mature. Maturation of the kernel is attained when the packing tissue around the kernel halves has turned brown (PTB), whereas hull maturity is defined by the cracking and separation of 95% or more of the hull from the shell (Olson *et al*., 1998). In this study, PTB alone is used as the predictor of harvest.

4.2 STUDY SITES

Commercial walnut orchards are established in four growing regions in Tasmania, namely the North coast, East coast, Tamar Valley and Coal Valley (Appendix 4). This study was conducted in commercial orchards in the North coast and East coast that were located near the townships of Forth (latitude, longitude; 41^{o}10'45"S, 146^{o}16'41"E) and Swansea (latitude, longitude; 42^{o}03'41"S, 148^{o}03'24"E) respectively.

These regions were selected as mean daily rainfall was distinctly higher, and mean daily minimum and maximum temperatures lower, at the North coast than the East coast for the months of spring (using data from the Australian Government, Bureau of Meteorology, http://www.bom.gov.au/climate/data/; furthermore, these regions were established earlier and have greater area of walnuts planted than the Tamar Valley and Coal Valley.

4.3 ORCHARD PLANTINGS AND DESIGN

Trees in commercial orchards were Persian, or English walnut (*Juglans regia*) grafted onto Northern Californian black walnut (*J. hindsii*) rootstocks. The walnut cultivars included the early- to mid-leafing Vina, the mid leafing Howard and the late leafing Franquette (Hendricks *et al*., 1998). The orchards had intra- and inter-row tree spacing's of 4 and 7 m respectively. Trees within the experimental sites had

canopies of approximately 50 m^3 when in full leaf and were grown under similar cultural management at all site-years; however, severe water restrictions created by drought meant that trial sites were not irrigated at Swansea in 2006–07.

4.4 DEPICTION OF BUDBURST

Walnut buds open and shoots begin to elongate over a period of 2 to 3 weeks (Hills and Lang, 2004; Lindow *et al*., 2005, 2006; Buchner *et al*., 2009); this period can vary among cultivars and weather conditions (Lindow *et al*., 2005, 2006; Buchner *et al*., 2009). Budburst in walnuts is defined when buds lengthen and the exterior of the basal leaves are distinguishable (Germain *et al*., 1999); this stage is termed *Cf* (Appendix 5). In stage *Cf 2*, the scales and bracts separate and the first leaves begin to separate (Germain *et al*., 1999). To account for variation in temporal development of buds, budburst in this study was defined when 5% of terminal buds attained stage *Cf 2*, and is consistent with the definition used by Tasmanian walnut growers.

4.5 DISEASE ASSESSMENTS

Disease incidence and severity was assessed between six and ten times each year on the fruits of six non-treated single-tree plots per cultivar per site. Assessments were conducted on the early- to mid-leafing Vina and the late-leafing Franquette (Hendricks *et al*., 1998) at 1 to 2 week intervals for the first 3 months of fruit growth, and then at 4 to 5 week intervals until PTB. In Tasmania, budburst and PTB in Vina occurs typically in October and April respectively, whereas these stages occur approximately 3 weeks later in Franquette.

In 2004−05, approximately 4 weeks after terminal budburst, 45 clusters per tree, containing two to three fruits per cluster, were arbitrarily selected from terminal and lateral shoots from 1.0 to 2.5 m above ground level, and were tagged and numbered. The selected clusters were approximately equidistant to each other around the tree. Each fruit was assessed for the presence or absence of walnut blight, and the severity of blight lesions, at 1 to 2 week intervals for the first 3 months of fruit growth and then at 4 to 5 week intervals until kernel maturity.

Premature drop of fruit of <10 mm in diameter caused a loss of sample at Forth in 2004−05; hence, in 2005−06, 2006−07 and 2008–09, disease incidence was assessed on 100 fruits, from 40 to 45 arbitrarily selected clusters, from 30 to 40 days after budburst, at which time fruits were at least 10 mm in diameter. For these initial assessments, when diseased fruits were observed, clusters were tagged and numbered at that time, and included in all subsequent assessments. After fruits had attained 10 mm diameter, a total of 40 to 45 clusters inclusive of previously tagged clusters, were arbitrarily selected, individually tagged and numbered and individual fruit or clusters assessed for disease incidence and severity until kernel maturity.

Disease incidence was assessed as the presence or absence of water-soaking and/or black lesions resembling blight symptoms on the hull of fruits. Disease severity was assessed for each fruit in the cluster using Horsfall and Barrett's (1945) scale, as the percentage of fruit hull covered by water-soaking and/or black lesions resembling blight symptoms. The Horsfall and Barrett (1945) scale defined disease severity into one of 12 classes i.e., 0, 0–3, 3–6, 6–12, 12–25, 25–50, 50–75, 75–88, 88–94, 94–97, 97–100 and 100%; mid-point values were used to derive disease severity of individual fruit, prior to calculating the mean severity per tree.

4.6 FRUIT SIZE ASSESSMENTS

Fruit size was assessed at the same time as disease assessments. The diameter of fruits within tagged clusters was used as an estimate of fruit size, with the maximum diameter of each fruit, on a line perpendicular to the stem and blossom end of the fruit, recorded with a ruler at each assessment.

4.7 ENVIRONMENTAL SENSORS

Surface wetness, ambient temperature and rainfall were measured at both orchards at each site-year. Relative humidity (RH) was measured at both orchards in 2004−05 and 2005−06 and at Swansea in 2006−07. At each site, one temperature sensor and one RH sensor (Gemini Data Loggers (UK) Ltd, Chichester, UK) were mounted in a Stevenson screen (Hastings Data Loggers Pty Ltd, NSW, Australia) located under the tree canopy, 1 m above ground level. At each site-year, one perspex cylindrical surface wetness sensor (Hastings Data Loggers Pty Ltd, NSW, Australia, as per

Young *et al*., 1979) was mounted horizontally within the tree canopy, 1.5 m above ground level. One tipping bucket rainfall sensor per site-year (Rain Collector II, Davis Instruments, Hayward, USA) was mounted 1.5 m above ground level and positioned in a level site, without surrounding vegetation, within 50 m of Stevenson screens. All environmental sensors were connected to data loggers (Gemini Data Loggers (UK) Ltd, Chichester, UK) with measurements recorded at 1 and 2 min intervals at Forth and Swansea, respectively.

4.7 STATISTICAL PROGRAMMES

SAS/STAT® version 9.2 (SAS Institute Inc., Cary, NC, USA) was used for linear regressions. All other statistical analyses i.e., two-sample comparisons, chi-square tests and one-way and multifactor analysis of variance were conducted with STATGRAPHICS *Plus* (Statpoint Technologies Inc., Warrenton, Virginia, USA).

CHARACTERISATION AND PATHOGENICITY OF *XANTHOMONAS ARBORICOLA* STRAINS ASSOCIATED WITH WALNUT BLIGHT

5.1 INTRODUCTION

Walnut blight, caused by *Xanthomonas arboricola* pv. *juglandis*, is one of the most important diseases of Persian (English) walnut (*Juglans regia*), with a range that closely corresponds to areas of walnut cultivation (Vauterin *et al.*, 1995). The pathogen attacks flowers, shoots, leaves, buds and fruits of all commercial cultivars (Teviotdale and Schroth, 1998). All above ground host tissue within the current-seasons growth is susceptible to infection; however, after host organs pass through one growing season they are not susceptible to further infection (Miller and Bollen, 1946; Teviotdale and Schroth, 1998).

The first visible symptoms of walnut blight of fruits consist of small, circular or irregularly circular water soaked spots (Teviotdale and Schroth, 1998). As the pathogen invades the surrounding tissue the lesion increases in size with the tissues turning black as the infected areas age. However, the initial black necrotic lesions of walnut blight may also resemble walnut anthracnose, caused by *Gnomonia leptostyla* (Miller *et al*., 1940), which is considered to be the most harmful fungal disease in walnut orchards in Mediterranean countries (Belisario, 1992). In early fruit growth in Italian and French orchards, symptoms of brown apical necrosis, caused by *Fusarium* spp. and *Alternaria* spp. in association with *X. arboricola* pv. *juglandis*, have also been confused with walnut blight symptoms (Belisario *et al*., 2002; Moragrega and Ozaktan, 2010). Symptoms of walnut blight have also been confused with plant disorders or damage; for example, on leaves that have been poisoned by excessive levels of alkali or boron in the soil, subjected to sunburn or wind damage (Rudolph, 1933), and with frost damage on staminate flowers (Miller and Bollen, 1946).

Walnut blight was first reported in Tasmania in the early 20th century by Rodway (1912), who observed the appearance of small black spots on the surface of fruits and leaves of walnut trees located in home gardens in the southern region of Tasmania. In the last decade, commercial production of walnuts has expanded rapidly in the northern, southern and eastern regions of Tasmania, and the presence of walnut fruit with symptoms of blight has been reported in northern and eastern growing regions of Tasmania (Lang *et al*., 2006; 2008). However, the presence of *X. arboricola* pv.

juglandis in samples of symptomatic walnut tissue from any growing region has not been confirmed. Thus, the objectives of this study were to identify and characterise the causal agent of putative walnut blight symptoms in Tasmania.

5.2 EXPERIMENTAL PROCEDURE

5.2.1 Origin and storage of bacterial isolates

Walnut fruits, shoots and leaves from a commercial orchard at Forth, Tasmania, with symptoms resembling descriptions of those induced by *X. arboricola* pv. *juglandis* (Miller and Bollen, 1946) were collected from cultivars Vina, Howard and Franquette (Table 5.1). One diseased leaf, shoot and fruit, from each of 10 arbitrarily selected trees per cultivar, were removed from trees using secateurs rinsed in 70% ethanol, placed in separate resealable polythene bags, and stored at 4^{o}C until isolation of bacteria. Within 4 h of collection, the necrotic lesion on each fruit, shoot and leaf was wiped with 70% ethanol and a small piece of tissue, approximately 4 mm^2, was excised aseptically from the margin of the lesion, suspended in 2 ml of sterile distilled water in a 5 mm McCartney bottle and agitated for 60 min on a platform mixer (RaTek Instruments, Melbourne, Australia) at 100 rpm. After agitation, the bottle was rested for 15–20 min, and then a loop full of suspension streaked onto nutrient agar (NA; Fluka, Buchs, Switzerland) in a Petri plate and incubated at 28^{o}C for 48 h. Single bacterial colonies were then selected, transferred onto NA and incubated at 28^{o}C for 48 h. After incubation, isolates were transferred to Microbank™ tubes (Pro-Lab Diagnostics, Ontario, Canada) and stored at −80^{o}C.

Table 5.1. Bacterial isolates collected from diseased walnut fruits, shoots and leaves of three cultivars from a commercial orchard at Forth, Tasmania. Isolates were collected from 10 arbitrarily selected trees per cultivar on 23 April 2004.

Isolate code and tree no.		Cultivar	Host tissue
FL	1,5,6,8,10	Franquette	Leaf
FF	1,2,3,4,5,6,7,8,10	Franquette	Fruit
FS	1,4	Franquette	Shoot
HL	3,5,6,7,8,10	Howard	Leaf
HF	1,2,4,5,7	Howard	Fruit
HS	1,10	Howard	Shoot
VL	1,2,3,4,6	Vina	Leaf
VF	1,3,4,5,6,7,9,10	Vina	Fruit
VS	2,3,4,5,6	Vina	Shoot

Symptomatic walnut fruit were also collected from nine commercial walnut orchards from four growing regions, and from three home gardens, in Tasmania (Table 5.2). Fruits were removed from trees and stored until isolation of bacteria, as previously described. Bacteria were isolated within 48 h of collection by Usha Saravanan (Saravanan, 2007) and stored at −80^{o}C using methods described above.

Table 5.2. Bacterial isolates collected from diseased fruits of four cultivars from commercial orchards and from unknown cultivars from home gardens in different geographic regions within Tasmania.

Isolate code and fruit no.		Region	Location	Cultivar [z]	Date of isolation (2005)
Commercial orchards					
US	1	North coast	Forth	Vina	02-Feb
US	2	North coast	Forth	Franquette	02-Feb
US	5	Tamar Valley	Sidmouth	Serr	17-Feb
US	9	Tamar Valley	Rowella	Chandler	17-Feb
US	10,11,12	Tamar Valley	Rowella	Franquette	17-Feb
US	13,14,15	Tamar Valley	Rowella	Chandler	17-Feb
US	19,22	North coast	Forth	Vina	18-Feb
US	23	North coast	Forth	Franquette	18-Feb
US	25,26,28	North coast	Penguin	Vina	01-Mar
US	31,33	North coast	Howth	Chandler	01-Mar
US	39,41,43,44	North coast	Penguin	Vina	02-Mar
US	48,49	East coast	Swansea	Vina	17-Mar
US	50,53	East coast	Swansea	Serr	17-Mar
US	56	East coast	Cranbrook	Vina	18-Mar
US	62	East coast	Cranbrook	Serr	18-Mar
US	63,64,65	Coal Valley	Penna	Vina	24-Mar
US	71,74,75	Coal Valley	Richmond	Chandler	24-Mar
Home gardens					
US	16	Tamar Valley	Rosevale	−	17-Feb
US	25	North coast	Penguin	−	22-Feb
US	35	North coast	Kindred	−	01-Mar

[z] A dash signifies that the cultivar is unknown.

5.2.2 Phenotyping of bacterial isolates

Isolates from 2004 (Table 5.1) were recovered from −80^{o}C storage and grown on NA. Three semi-selective media for *X. arboricola* pv. *juglandis*, brilliant cresyl blue starch (BS) (Mulrean and Schroth, 1981), modified Tween (TB) (Schaad *et al*., 2001) and succinate quinate (SQ) (Lee *et al.,* 1992) were used to characterise the phenotype of bacterial colonies that had grown on NA for 48 h at 28^{o}C, including reference *X. arboricola* pv. *juglandis* strains (Table 5.3). Loopfuls of inoculum were streaked on BS, TB and SQ medium and incubated at 28^{o}C. The presence or absence of starch hydrolysis on BS and TB media, and quinate metabolism on SQ medium, were recorded 4 and 7 days after the start of incubation. Starch hydrolysis was recorded as positive when bacterial colonies were surrounded by an opaque zone and white halo on BS and TB, respectively. Quinate metabolism was recorded as positive when a diffusion of deep-green colour surrounded colonies on SQ. Colony colour was also characterized on SQ media, and colony growth on BS, TB and SQ media characterised qualitatively, in terms of size of colonies, as strong, moderate or weak.

Table 5.3. Reference isolates of plant pathogenic bacteria, from the New South Wales (NSW) Plant Pathology Herbarium, Orange, Australia (DAR), used in this study. WA is Western Australia.

Isolate code & reference no.		Genus and species	Host	Location
DAR	33423	*Xanthomonas arboricola* pv. *juglandis*	Walnut	NSW
DAR	41287	*Xanthomonas arboricola* pv. *pruni*	Peach	NSW
DAR	73873	*Xanthomonas arboricola* pv. *juglandis*	Walnut	Tasmania
DAR	76585	*Pseudomonas syringae* pv. *syringae*	Walnut	Tasmania
DAR	77325	*Erwinia rhapontici*	Barley grain	WA

Fatty acid methyl ester (GC-FAME) profiles of a subset of bacterial isolates from 2004, selected from each of the five putative groups identified on semi-selective media, were then determined using the Sherlock® Microbial Identification System (Microbial ID, Inc. (MIDI), Newark, DE, USA). GC-FAME analyses were performed by Dorothy Noble (Plant Pathology Herbarium, former NSW Agriculture,

Australia). In summary, bacteria were cultured in trypticase soy broth with 1.5% Bacto™ agar for 1 day at 28°C. Fatty acids were extracted following the sample preparation procedures described in the Microbial Identification System Handbook (Anon., 2005) and analysed using a Hewlett-Packard 6890 gas chromatograph. Profiles of each isolate were then compared and matched, according to a similarity index, to fatty acid composites of known reference strains.

Isolation and phenotyping of 37 bacterial isolates from 2005 (Table 5.2), using BS and SQ media and GC-FAME, were performed by Usha Saravanan (Saravanan, 2007) and Dorothy Noble (Plant Pathology Herbarium, former NSW Agriculture, Australia), using methods described above.

Multilocus sequence analysis (MLSA) of ten bacterial isolates, isolated in 2004 or 2005 and putatively identified by GC-FAME as *Xanthomonas* sp., and reference strains of *X. arboricola* pv. *juglandis and X. arboricola* pv. *pruni* was then conducted using the housekeeping gene, RNA polymerase sigma factor (*rpoD*). MLSA was coordinated by Dr. Jacky Edwards (Crop Health Services, Agriculture Victoria, Australia). The identification of each isolate was then determined by comparing similarities of *rpoD* sequences with those already held in the basic local alignment search tool (BLASTN) database.

5.2.3 Pathogenicity tests

Pathogenicity studies to test Koch's postulates were undertaken on detached walnut fruit. Three experiments were conducted in different years using Franquette fruit collected from one commercial orchard and selected trees that had not been treated with copper bactericide, and that had no visible symptoms of walnut blight.

In the first year, three-quarter full-sized healthy fruits were detached, washed for 30 s in 70% ethanol, rinsed for 60 s in sterile distilled water (SDW), washed for 30 s in 10.5 g/l sodium hypochlorite, and then rinsed twice in SDW, each time for 60 s. Each fruit was then placed in the base of a 225 ml clear plastic cup, lined at the base with absorbent paper moistened with SDW. Bacterial isolates and reference strains (Tables 5.1, 5.2 and 5.3) were recovered from −80°C on NA for 48 h at 28°C. A 10

µl droplet of bacterial suspension, photo-metrically adjusted to 2.1–3.2 x 10^7 cfu per ml in SDW (Appendix 6), was placed on the surface of the fruit towards the apical, or blossom end, and the surface of the fruit wounded by inserting a sterilized 0.4 mm diameter stainless steel entomological pin (Asta E157 Size 0) to a depth of 2 mm, through the droplet of bacterial suspension. After inoculation, the cup was covered with plastic cling wrap and the fruit incubated for 2 weeks at 25^{o}C.

In the second and third years, half full-sized healthy fruits were detached and washed as previously described, and then placed in the base of 15 ml clear plastic ice cube trays, lined at the base with absorbent paper moistened with SDW. Bacterial isolates and reference strains were restored as previously described, and suspensions photo-metrically adjusted to 1.2–4.3 x 10^9 and 1.9–2.9 x 10^9 cfu per ml in the second and third years, respectively. In the second year, inoculum was diluted in SDW from 10^9 to 10^6 cfu per ml. Fruits were wound inoculated to a depth of 2 mm using the method previously described, placed in sealed snap-lock resealable polythene bags, and then incubated at 25^{o}C for 2 weeks.

Fruits were inspected for symptom expression for up to 14 days after inoculation. Symptoms were recorded as positive or negative, with positive symptoms associated with the development of water soaking or water soaking and black lesions emanating from the point of inoculation. Lesion development was recorded as the size, or diameter, of the lesion surrounding the inoculation point.

After the second experiment, isolates and reference strains were isolated from necrotic lesions 14 days after inoculation, grown on TB and SQ media and the presence or absence of starch hydrolysis and quinate metabolism recorded, respectively, as previously described.

5.3 RESULTS

5.3.1 Isolate profiles

Nearly all bacteria isolated from fruits, shoots and leaves of Vina, Howard and Franquette (Table 5.1) were able to hydrolyse starch on BS and TB media, and

metabolise quinate on SQ medium (Table 5.4); however, quinate metabolism was not observed until 7 days incubation with the two *X. arboricola* pv. *juglandis* reference isolates and eight isolates from 2004. Six bacteria isolated from leaves and shoots, and one isolated from fruit, did not hydrolyse starch within 7 days i.e., putative Groups C, D and E, and had distinctly different colony colour on SQ media i.e., pale yellow and bright yellow, to all other isolates. The growth of all bacteria was weak on BS media; in comparison, the growth of isolates that were positive for starch hydrolysis was moderate on TB media. Growth of all isolates was moderate or strong on SQ media.

Fatty acid profiling of five bacterial isolates that hydrolysed starch and metabolised quinate within 4 days (Group A) were identified as *X. arboricola* pv. *juglandis* and as *X. arboricola* pv. *juglandis* or *X. arboricola* pv. *pruni* (Table 5.5). The two reference *X. arboricola* pv. *juglandis* strains, included within a subset of eight isolates that hydrolysed starch within 4 days and metabolised quinate within 7 days (Group B) were identified as *X. arboricola* pv. *juglandis* and as *X. arboricola* pv. *juglandis or X. arboricola* pv. *pruni*. The three isolates that did not hydrolyse starch were putatively identified as belonging to the genera *Pantoea* or *Pseudomonas*.

Multilocus sequence analysis (MLSA) putatively identified five bacterial isolates as *X. arboricola* pv. *juglandis* and five as *X. arboricola* spp. (Table 5.5). The two reference *X. arboricola* pv. *juglandis* strains (DAR33423 and DAR73873) were identified by MLSA as *X. arboricola* pv. *juglandis*, and the reference *X. arboricola* pv. *pruni* strain (DAR41287) as *X. arboricola* pv. *pruni*.

Of the five isolates identified as *X. arboricola* pv. *juglandis* by MLSA, two were putatively identified by GC-FAME as *X. arboricola* pv. *juglandis* and three as either *X. arboricola* pv. *pruni* or as *X. arboricola* pv. *juglandis or X. arboricola* pv. *pruni* (Table 5.5). The two isolates identified as *X. arboricola* spp. by MLSA were putatively identified by fatty acid profiling as either X. *fragariae* or *X. axonopodis*.

Table 5.4. Putative isolate group (A-E) for isolates listed in Table 5.1 and two *X. arboricola* pv. *juglandis* reference strains, according to colony growth and presence (+) or absence (−) of starch hydrolysis on brilliant cresyl blue starch (BS) and modified Tween (TB) media, and colony growth and colour and presence or absence of quinate metabolism on succinate quinate medium (SQ), after 4 and 7 days incubation (di) at 28°C.

Medium	Phenotype	di	Putative isolate [Z] group				
			A[Y]	B[X]	C[W]	D[V]	E[U]
BS	Starch hydrolysis	4&7	+	+	−	−	−
	Colony growth	4&7	weak	weak	weak	weak	weak
TB	Starch hydrolysis	4&7	+	+	−	−	−
	Colony growth	4&7	moderate	moderate	weak	weak	weak
SQ	Quinate metabolism	4,7	+,+	−,+	+,+	+,+	−,+
	Colony growth	4&7	strong	strong	moderate	moderate	moderate
	Colony colour	4&7	yellow	yellow	bright yellow	pale yellow	pale yellow
Total no. of isolates per group			32	10	4	2	1

[Z] Description of isolates and reference strains in Tables 5.1 and 5.3 respectively. [Y] Group A = FL 1,6,8,10; FF 1-5,7,8,10; FS 1; HL 7; HF 2,4,5,7; HS 10; VL 1-4; VF:1,3,4,7,9,10; VS 3-5. [X] Group B = FF 6; HL 5,6,8; HF 1; VL 6; VF 6; VS 6; DAR33423; DAR73873. [W] Group C = FL 5; FS 4; VF 5; VS 2. [T] Group D = HL 3; HS 1. [U] Group E = HL 10.

Table 5.5. Putative identification of reference strains (Table 5.3) and bacterial isolates, collected in 2004 (Table 5.1), with GC-FAME (Analysis 1) and of reference strains and bacterial isolates, collected in 2004 and 2005 (Tables 5.1 and 5.2), with GC-FAME and multilocus sequence analysis (MLSA) (Analysis 2).

Analysis 1			**Analysis 2**		
Isolate code and reference strain [Z]	**Putative isolate group (Table 5.4)**	**GC-FAME** [Y]	**Isolate code and reference strain** [Z]	**GC-FAME** [Y]	**MLSA** [Y]
FF 3	A	*Xaj*	US 10	*Xaj/Xap*	*Xa*
FF 7	A	*Xaj*	US 16	*Xaj/Xap*	*Xaj*
FS 4	C	*Pa*	US 22	*Xf/Xaxon*	*Xa*
HF 4	A	*Xaj*	US 23	*Xaj/Xap*	*Xa*
HF 5	A	*Xaj/Xap*	US 33	*Xf/Xaxon*	*Xa*
HL 3	D	*Pss*	US 48	*Xap*	*Xa*
HL 10	E	*Psm*	US 56	*Xaj*	*Xaj*
VF 3	A	*Xaj*	US 65	*Xap*	*Xaj*
DAR33423	B	*Xaj*	US 71	*Xap*	*Xaj*
DAR73873	B	*Xaj/Xap*	VF 3	*Xaj*	*Xaj*
–	–	–	DAR33423	*Xaj*	*Xaj*
–	–	–	DAR41287	*Xap*	*Xap*
–	–	–	DAR73873	*Xaj/Xap*	*Xaj*

[Z] Description of isolates and reference strains in Tables 5.1 and 5.3 respectively. [Y] *Xaj* = *Xanthomonas arboricola* pv. *juglandis*, *Xap* = *X. arboricola* pv. *pruni*, *Xa* = *Xanthomonas arboricola* spp., *Xf/Xanon=X. fragariae* or *X. axonopodis*, *Pss* = *Pseudomonas syringae* pv. *syringae*, *Psm* = *P. syringae* pv. *mendocina*, *Pa* = *Pantoea agglomerans*.

5.3.2 Pathogenicity tests

Water soaking and black lesions around wound inoculations constituted a positive pathogenicity test (Fig. 5.1); in contrast, localised dried black lesions without water-soaking and dried localized damage without lesion development indicated that an isolate was not pathogenic to walnut fruit.

Fig. 5.1. Development of water soaking and black coloured lesion (left), localised dried black lesions without water-soaking (centre) and dried localised damage (right), 14 days after wound inoculation of half size Franquette fruits with putative *Xanthomonas arboricola* pv. *juglandis* (US 23, left), a reference *X. arboricola* pv. *pruni* strain (DAR 41287, centre) and sterilized distilled water (right).

Reference *X. arboricola* pv. *juglandis* strains and *X. arboricola* isolates, except for US 48, developed lesions on three-quarter full-sized diameter Franquette fruit within 14 days of wound inoculation (Table 5.6). Of seven fruit inoculated with US 22 and US 33, seven and three developed lesions, respectively. These isolates were putatively identifed as *Xanthomonas fragariae* or *X. axonopodis* by GC-FAME, or as *X. arboricola* by multilocus sequence analysis (MLSA). No lesions developed on fruits inoculated with reference strains of *X. arboricola* pv. *pruni*, *Pseudomonas agglomerans* or *Erwinia rhapontici*. Only five isolates, US 11, 22, 23, 64 and 75, and one *X. arboricola* pv. *juglandis* reference strain, DAR 33423, developed lesions in all seven fruit. Mean lesion size ranged from 2.0 to 3.8 mm, with a maximum diameter of 4.0 mm.

All *X. arboricola* isolates, except the reference *X. arboricola* pv. *pruni* strain, DAR41287, caused lesions of ≥2 mm diameter to develop within 14 days of inoculation of half size Franquette fruit with 10^9 cfu per ml (Table 5.7). In the second experiment, the number of fruit with lesions, and the size of lesions, was greater with inoculation at 10^9 cfu per ml in comparison to 10^6 cfu per ml. Inoculation with US 48 led to lesions in six half-sized fruits; in comparison, no three-quarter sized fruits developed lesions (Table 5.6). Seven and four fruits inoculated with US 22 and 33

respectively, putatively identifed as *X. fragariae* or *X. axonopodis* by GC-FAME or as *X. arboricola* by MLSA, developed lesions (Table 5.7). Lesions developed in four fruits inoculated with the *X. arboricola* pv. *juglandis* reference strain; in contrast, no lesions developed on fruits inoculated with reference bacterial strains pathogenic to crops other than walnuts. In the third experiment, the numbers of half-sized fruits with lesions were equivalent to the second experiment.

Bacteria were isolated from necrotic lesions 14 days after inoculation of half size Franquette fruits with *X. arboricola* isolates and the *X. arboricola* pv. *juglandis* reference strain, DAR73873. All isolates from these lesions were able to hydrolyse starch and metabolise quinate on TB and SQ media respectively, within 7 days incubation.

Table 5.6. Putative identification of isolates collected in 2005 (Table 5.2), strain VF3 (Table 5.1) and reference strains (Table 5.3) with GC-FAME and multilocus sequence analysis (MLSA), and the number and mean diameter of lesions and/or water-soaking on the fruit surface ≥ 2 mm diameter, 14 days after pin-prick inoculations with 2.1−3.2 × 10^7 cfu per ml on three-quarter size Franquette fruit.

Isolate code and reference strain [Z]	GC-FAME[Y]	MLSA[Y]	Fruit with lesions (n = 7)	Lesion diam. ± SE (mm)	Isolate code[Z]	GC-FAME[Y]	Fruit with lesions (n = 7)	Lesion diam. ± SE (mm)	Isolate code [Z]	GC-FAME[Y]	Fruit with lesions (n = 7)	Lesion diam. ± SE (mm)
US 10	*Xaj/Xap*	*Xa*	5	3.4 ± 0.9	US 1	*Xap*	2	2.0 ± 0.0	US 35	*Xap*	4	2.5 ± 0.5
US 16	*Xaj/Xap*	*Xaj*	6	3.3 ± 0.4	US 2	*Xap*	5	2.8 ± 0.2	US 39	*Xap*	4	2.5 ± 0.3
US 22	*Xf/Xaxon*	*Xa*	7	3.0 ± 0.3	US 5	*Xaj/Xap*	3	2.0 ± 0.0	US 41	*Xap*	4	2.5 ± 0.3
US 23	*Xaj/Xap*	*Xa*	7	3.1 ± 0.3	US 9	*Xap*	2	3.5 ± 1.5	US 43	*Xap*	2	2.0 ± 0.0
US 33	*Xf/Xaxon*	*Xa*	3	2.3 ± 0.3	US 11	*Xap*	7	2.9 ± 0.4	US 44	*Xap*	3	2.0 ± 0.0
US 48	*Xap*	*Xa*	0	−	US 12	*Xap*	5	3.8 ± 0.4	US 49	*Xap*	6	2.7 ± 0.2
US 56	*Xaj*	*Xaj*	5	2.2 ± 0.2	US 13	*Xap*	6	2.7 ± 0.5	US 50	*Xap*	4	3.8 ± 0.8
US 65	*Xap*	*Xaj*	4	2.3 ± 0.3	US 14	*Xap*	3	2.0 ± 0.0	US 53	*Xap*	6	2.8 ± 0.5
US 71	*Xap*	*Xaj*	1	4.0 ± 0.0	US 15	*Xap*	2	3.5 ± 0.3	US 62	*Xap*	4	2.5 ± 0.3
VF 3	*Xaj*	*Xaj*	6	2.5 ± 0.2	US 19	*Xap*	4	3.0 ± 0.7	US 63	*Xaj/Xap*	4	3.3 ± 0.8
DAR33423	*Xaj*	*Xaj*	7	2.9 ± 0.4	US 25	*Xap*	5	2.8 ± 0.4	US 64	*Xap*	7	2.9 ± 0.5
DAR73873	*Xaj/Xap*	*Xaj*	5	3.0 ± 0.4	US 26	*Xap*	4	2.0 ± 0.0	US 74	*Xap*	6	3.0 ± 0.5
DAR41287	*Xap*	*Xap*	0	−	US 28	*Xap*	5	2.4 ± 0.2	US 75	*Xaj/Xap*	7	3.1 ± 0.5
DAR76585	*Pss*	−	0	−	US 31	*Xap*	2	2.5 ± 0.5	SDW	−	0	−
DAR77325	*Er*	−	0	−	US 35	*Xap*	4	2.5 ± 0.5				

[Z] Description of isolates in Tables 5.1 and 5.2 and reference strains in Table 5.3, SDW = sterlilized distilled water. [Y]*Xaj = Xanthomonas arboricola* pv. *juglandis*, *Xap = X. arboricola* pv. *pruni*, *Xa = Xanthomonas arboricola*, *Xf/Xanon=X. fragariae* or *X. axonopodis*, *Pss=Pseudomonas syringae* pv. *syringae*, *Er=Erwinia rhapontici*.

Table 5.7. Putative identification of isolates collected in 2005 (Table 5.2), strain VF3 (Table 5.1) and reference strains (Table 5.3) with GC-FAME and multilocus sequence analysis (MLSA), and the number and mean diameter of lesions and/or water-soaking on the fruit surface ≥ 2 mm diameter, 14 days after pin-prick inoculations with 1.2−4.3 × 10^6 or 1.2−4.3 × 10^9 cfu per ml (Experiment 2) and 1.9−2.9 × 10^9 cfu per ml (Experiment 3) on half size Franquette fruit.

Isolate code[Z]	GC-FAME[Y]	MLSA[Y]	Experiment 2				Experiment 3	
			10^6 cfu per ml		10^9 cfu per ml		10^9 cfu per ml	
			Fruit with lesions (n = 7)	Lesion diam. ± SE (mm)	Fruit with lesions (n = 7)	Lesion diam. ± SE (mm)	Fruit with lesions (n = 7)	Lesion diam. ± SE (mm)
US 10	*Xaj/Xap*	*Xa*	3	3.5 ± 0.4	7	5.9 ± 0.5	7	5.7 ± 0.5
US 16	*Xaj/Xap*	*Xaj*	3	5.3 ± 1.2	3	5.9 ± 0.7	2	5.5 ± 0.5
US 22	*Xf/Xaxon*	*Xa*	5	5.3 ± 0.5	7	5.7 ± 0.3	5	4.6 ± 0.2
US 23	*Xaj/Xap*	*Xa*	2	2.0 ± 0.0	7	5.2 ± 0.9	5	4.0 ± 0.3
US 33	*Xf/Xaxon*	*Xa*	0	−	4	3.5 ± 0.3	3	3.3 ± 0.3
US 48	*Xap*	*Xa*	5	2.8 ± 0.4	6	6.0 ± 0.4	6	5.2 ± 0.3
US 56	*Xaj*	*Xaj*	0	−	4	4.0 ± 0.9	5	3.6 ± 0.5
US 65	*Xap*	*Xaj*	1	3.0	7	3.9 ± 0.3	6	3.7 ± 0.3
US 71	*Xap*	*Xaj*	2	3.0 ± 1.0	7	4.7 ± 0.7	5	5.2 ± 0.6
VF 3	*Xaj*	*Xaj*	0	−	5	2.0 ± 0.0	2	3.0 ± 1.0
DAR73873	*Xaj*	*Xaj*	1	2.8	4	2.8 ± 0.3	3	2.7 ± 0.3
DAR41287	*Xap*	*Xap*	0	−	0	−	0	−
DAR76585	*Pss*	−	0	−	0	−	0	−
DAR77325	*Er*	−	0	−	0	−	0	−
SDW	−	−	0	−	0	−	0	−

[Z] Description of isolates in Tables 5.1 and 5.2 and reference strains in Table 5.3. [Y] *Xa=Xanthomonas arboricola, Xaj=Xanthomonas arboricola* pv. *juglandis, Xap=X. arboricola* pv. *pruni, Xf/Xanon=X. fragariae* or *X. axonopodis, Pss=Pseudomonas syringae* pv. *syringae, Er=Erwinia rhapontici*

5.4 DISCUSSION

This study demonstrated that *X. arboricola* pv. *juglandis* is the cause of walnut blight in Tasmania. Pure cultures of *X. arboricola* pv. *juglandis* isolated successfully from commercial walnut orchards and home gardens from different regions in Tasmania produced water-soaking and necrotic lesions in detached fruit of cultivar Franquette. These inoculations simulated natural infection, because initial symptoms consisted of small, circular or irregularly circular water-soaked areas, which then increased in size with the tissues turning black, as the infected areas aged (Rudolph, 1933; Miller and Bollen, 1946; Mulrean and Schroth, 1982; Arquero *et al*., 2006). Moreover, *X. arboricola* pv. *juglandis* was isolated from inoculated fruit, thus satisfying Koch's postulates. In contrast, fruit inoculated with reference strains of *X. arboricola* pv. *pruni* and the genus *Pseudomonas, Pantoea* and *Erwinia* did not produce visible disease symptoms.

In this study, reference *X. arboricola* pv. *juglandis* strains and bacteria isolated from different walnut tissues and cultivars that were putatively identified as *X. arboricola* pv. *juglandis*, were shown to metabolize quinate. Similarly, Lee *et al*. (1992) reported that all 114 strains of *X. arboricola* pv. *juglandis*, isolated from bud and twig cankers from different walnut cultivars and locations in California, metabolized quinate within 4 to 6 days of incubation. From nearly 350 strains representing all known genetic groups of xanthomonads, only xanthomonads of DNA homology group 6, which includes *X. arboricola* pv. *juglandis*, *X. arboricola* pv. *pruni, X. arboricola* pv. *corylina* and *X. arboricola* pv. *celebensis*, were positive for this characteristic (Lee *et al*., 1992). The metabolism of quinate by isolates from Tasmania further signifies this characteristic for xanthomonads of DNA homology group 6.

Non-xanthomonad bacteria such as *Pseudomonas syringae* pv. *syringae* can also use quinate for growth, producing a yellowish colour change on succinate quinate (SQ) medium (Lee *et al.,* 1992). Similarly, bacteria isolated from diseased walnut leaf tissue, and putatively identified as *P. syringae* pv. *syringae* by GC-FAME, expressed quinate metabolism on SQ medium in this study. Putative *P. syringae* pv. *syringae*

isolated from diseased fruits from other growing regions in Tasmania also utilised quinate for growth (Saravanan, 2007). In contrast to *X. arboricola* pv. *juglandis* isolates, however, *P. syringae* pv. *syringae* cannot hydrolyse starch on brilliant cresyl blue starch (BS) medium (Mulrean and Schroth, 1981), as was observed in this study.

The presence or absence of starch hydrolysis helped differentiate bacterial isolates from a single orchard in Tasmania into two putative groups (Table 5.4). While all isolates identified as *X. arboricola* pv. *juglandis* hydrolysed starch in this study, this characteristic was not evident amongst 2 of 67 isolates with 5 days incubation at 28^{o}C, from diseased fruit from across a broad geographical range in Tasmania (isolates US 04 and US 63; Saravanan, 2007). Similarly, 6 of 11 *X. arboricola* pv. *juglandis* isolates collected from nursery trees in South Africa did not hydrolyze starch with 7 days incubation (du Plessis and van der Westhuizen, 1995). However, four of five strains isolated from Californian walnut orchards required 13 days incubation at 28^{o}C prior to starch hydrolysis being observed, whereas the remaining isolate required only 2 days incubation (Miller and Bollen, 1946). Thus, the time taken for hydrolytic activity to be detected for some *X. arboricola* pv. *juglandis* isolates collected in Tasmania may be greater than reported here.

In general, growth of *X. arboricola* pv. *juglandis* was weak on BS media in this study, and made the isolation and characterization of isolates difficult in comparison to modified Tween (TB) and SQ media. Similarly, Saravanan (2007) found the growth of bacteria isolated from different growing regions in Tasmania was weak on BS medium in comparison to growth on SQ and glucose yeast extract calcium carbonate agar. Furthermore, BS medium was found to be ineffective for isolation and diagnosis of *X. arboricola* pv. *juglandis* in South Africa (du Plessis and van der Westhuizen, 1995), possibly because of an absence of hydrolytic activity (Scortichini *et al.,* 2001). Given the relatively slow rate of growth of xanthomonads on BS media reported here, it was concluded that the BS medium was less useful than TB and SQ media for selecting *Xanthomonas* spp. from walnut blight lesions.

Development of the pathogenicity assay using detached fruit of cultivar Franquette required selecting fruit at a suitable stage of development and an appropriate inoculum concentration. Fewer than half of the three-quarter full-sized fruits inoculated with *X. arboricola* pv. *juglandis* developed lesions; furthermore, half-sized fruit appeared to be more susceptible to lesion development than larger fruit. Similarly, studies conducted in the greenhouse and in the field over a 10-year period in California showed that walnut fruits inoculated at half full-size were more susceptible to disease than when inoculated at three-quarter full-size, and that the reduced susceptibility manifested itself by a longer incubation period and with more restricted development of lesions (Miller and Bollen, 1946). Inoculum concentrations required to incite lesions by all *X. arboricola* pv. *juglandis* isolates in this study were similar to previous pathogenicity studies in walnuts i.e., 10^8 and 10^9 cfu per ml (Mulrean and Schroth, 1981; Woeste *et al*., 1992), suggesting that the assay for Franquette needs 10^9 cfu and half-size fruit.

In this study, inoculation of detached walnut fruits by isolates from Tasmania identified by MLSA as *X. arboricola*, and by GC-FAME as *X. arboricola* pv. *pruni*, or as *X. fragariae* or *X. axonopodis*, led to the development of water soaking and lesion development. An extensive analysis of the whole cell fatty acid content of nearly 1,000 strains of the genus *Xanthomonas* by gas-liquid chromatography identified 21 different fatty acids within *X. arboricola* pv. *juglandis* (Yang *et al.*, 1993). Based on fatty acid profiles, 31 major clusters were defined, with *X. arboricola* pv. *juglandis* (synonym: *X. campestris* pv. *juglandis*) included in the second largest cluster with other *X. campestris* pathovars such as *carotae, celebensis, corylina, manihotis, phaseoli* and *vitians.* In the GC-FAME analyses of isolates from Tasmania, six *X. arboricola* pathovars were co-listed with *pruni* as the putative identification, and *juglandis* was often co-listed with *pruni* and/or *corylina* (Saravanan, 2007). However, isolates identified as *X. arboricola* pv. *pruni* and *X. fragariae* or *X. axonopodis* by GC-FAME were also identified as *X. arboricola* pv. *juglandis* and *X. arboricola*, respectively, by MLSA. Fatty acid analysis depends upon the diversity of fatty acid profiles for a particular bacterial taxon in the Sherlock® database. Given that the database probably contained few representative isolates from Australia, let alone Tasmania, then it was not surprising that bacterial

isolates demonstrated to be pathogenic on walnuts in this study were not always identified as pathovar *juglandis* by GC-FAME. Hence, isolates from walnuts named as *X. arboricola* pv. *pruni,* or as *X. fragariae* or *X. axonopodis* by GC-FAME analysis were designated as *X. arboricola* pv. *juglandis* based on the pathovar concept of taxonomy, where pathogenic organisms are named according to a particular host (Dye *et al.,* 1980).

In summary, the frequent association of *X. arboricola* pv. *juglandis* with symptomatic walnut tissue, and the development of lesions in healthy fruit after inoculation with *X. arboricola* pv. *juglandis*, confirmed *X. arboricola* pv. *juglandis* as the causal agent of walnut blight in Tasmania. However, the separation of *X. arboricola* pathovars using phenotypic features remains unresolved; hence, an approach that incorporates genotyping or DNA sequence analysis, as reviewed previously (Chapter 3), may assist more accurate characterization of *X. arboricola* pv. *juglandis* strains in Tasmania and elsewhere.

YIELD OF WALNUT FRUITS IS INVERSELY RELATED TO THE INCIDENCE OF WALNUT BLIGHT ON IMMATURE FRUITS OF VARIOUS SIZES

6.1 INTRODUCTION

Xanthomonas arboricola pv. *juglandis*, the causal organism of walnut blight, can cause significant crop losses in walnut orchards. The disease leads to the highest yield reduction of all foliar diseases of walnut in most of the European walnut production countries (Parveaud *et al*., 2010), and is considered one of the most serious above-ground diseases of walnuts in California (Teviotdale and Schroth, 1998). In Tasmanian orchards, there is potential for almost all walnut fruit to drop prematurely when conditions are suitable for disease to develop, and when crop protection is not implemented (Lang *et al.*, 2006).

The premature drop of fruits differs, in part, with the age at which host organs are infected. In Californian and European orchards, infections early in fruit growth almost always lead to premature fruit drop, whereas those fruit infected later are less likely to do so (Miller and Bollen, 1946; Belisario *et al.*, 1997; Teviotdale and Schroth, 1998; Arquero *et al*., 2006). In field and glasshouse trials in California, fruits that were inoculated with *X. arboricola* pv. *juglandis* prior to half size invariably dropped prior to harvest, whereas mostly all of those inoculated at, or after, half fruit size did not drop prematurely (Miller and Bollen, 1946). The mechanism responsible for the premature drop of immature fruits is not well understood; however, the increased susceptibility of immature fruits to drop may occur because of the greater period of time that pectinolytic enzymes, secreted by *X. arboricola* pv. *juglandis*, have to degrade infected tissues and cause cell apoptosis or necrosis, in comparison to older fruit (Garcin *et al*., 2001). As such, Miller and Bollen (1946) suggested that the development and control of epidemics of walnut blight in California was required between the time bacteria came into contact with flowers and when fruits were about half size.

In Tasmania, fruit drop associated with walnut blight has only been reported for one location and cultivar (Lang *et al*., 2006). Furthermore, the relationship between the degree of disease at a particular stage of fruit development and crop yield has not been reported in previous studies from other walnut growing regions. Hence, the aims of this chapter were to determine the impact of natural *X. arboricola* pv.

juglandis infections on crop yield from two growing regions and cultivars in Tasmania, and to quantify the relationship between disease incidence or severity and crop yield. A further aim was to predict disease incidence at various fruit sizes for quantifying the relationship between disease incidence on fruit of a specific size and crop yield.

6.2 EXPERIMENTAL PROCEDURE

6.2.1 Orchards and tree selection

Disease progression was monitored in Vina for three years, 2004−05, 2005−06 and 2006−07 and in Franquette in 2005−06 and 2006−07, at Forth and Swansea. For each cultivar, location and year, or site-year, six single-tree plots were selected prior to budburst. At Forth, Vina plots were the non-treated control trees within bactericide efficacy trials containing 60 to 90 trees. At Swansea, Vina plots were arbitrarily selected within sites containing 16 to 20 non-treated trees. Single tree plots of Franquette were arbitrarily selected from single polleniser tree rows, containing between 15 to 24 non-treated trees that were adjacent to Vina plots at Forth, and within 150 m of Vina sites at Swansea.

6.2.2 Assessments

6.2.2.1 Disease incidence and severity

Disease incidence and severity in non-treated trees was assessed between six and ten times each year, at 1 to 2 week intervals for the first 3 months of fruit growth and then at 4 to 5 week intervals for the following 3 months until kernel maturity, on the fruits of six single-tree plots, as per methods described in the General Materials and Methods (Chapter 4).

6.2.2.2 Fruit size

The diameter of fruits within tagged clusters was used as an estimate of fruit size. The maximum diameter of each fruit, on a line perpendicular to the stem and blossom end of the fruit, was recorded with a ruler at each assessment.

6.2.3 Data analyses

6.2.3.1 Effect of a walnut blight epidemic on crop yield

Prior to analyses, disease incidence and severity was adjusted for the premature drop of diseased fruits by carrying the disease incidence and severity value at the assessment prior to fruit drop through to each subsequent assessment. In this chapter, disease incidence and severity calculated from diseased fruits attached to trees in addition to diseased fruits that had dropped prior to each assessment is termed "gross" disease, whereas disease values from fruits that were attached to trees only is termed "observed" disease.

Estimated crop yield was calculated as the percentage of fruits, from tagged clusters (refer to Section 4.5), that remained on trees at kernel maturity. Fruit that dropped prematurely that did not have visible symptoms of walnut blight at assessments were removed from the data set prior to calculating gross disease incidence and severity.

The standardized area under the disease progress curve (SAUDPC) (Campbell and Madden, 1990) for gross disease incidence and gross mean disease severity was calculated for each single-plot tree per trial site. The SAUDPC was calculated by trapezoidal integration and then standardised by dividing the interval (days) between the appearance of disease symptoms and kernel maturity.

Analysis of variance (ANOVA) was used to determine significant differences in the SAUDPC of disease incidence and severity, the observed disease incidence and severity at kernel maturity, and the crop yield in Franquette and Vina, between locations and between years. Linear regression was then used to identify the relationship between the mean SAUDPC for disease incidence or severity and crop yield, and between the gross disease incidence or severity at kernel maturity and crop yield, using data from all 10 site-years.

6.2.3.2 Relationship between disease incidence and severity

Linear regression was used to determine the relationship between the mean SAUDPC of disease incidence and the log transformed mean SAUDPC of disease severity for

Vina and Franquette. Regression lines were then compared to determine if parameter estimates (slope, intercept) of corresponding linear relationships for Vina and Franquette were significantly different at $P < 0.05$.

6.2.3.3 Disease progress model

Disease progress curves for gross disease incidence only were evaluated using the monomolecular model, according to the approach described by Campbell and Madden (1990). The monomolecular model was selected so that equations for temporal disease incidence could be substituted into monomolecular models describing temporal fruit size, and then used to predict the effect of disease incidence at various fruit sizes on crop yield (Section 6.2.3.4). In subsequent chapters, disease progress curves were evaluated using the linear, monomolecular, exponential, logistic or Gompertz models (Campbell and Madden, 1990) to identify the model of best fit.

Disease progress data for the model included the disease assessment when disease symptoms were first apparent, and all subsequent assessments. As linearized transformations of the monomolecular, logistic and Gompertz models are undefined with disease incidence of 100% (Campbell and Madden, 1990), disease incidences of 100% were reduced to 99.9%, and converted to proportions prior to analysis. The predicted disease incidence ($\hat{y}_d$) at any time during an epidemic was then calculated according to Eq. 6.1:

$$\hat{y}_d = K\,[1 - B_d \exp^{(-rMd \times t)}] \qquad \text{(Eq 6.1)}$$

where K = the asymptote that provided the best fit for describing disease progression, $B_d = \ln(K/(K-y_d))$, the linearised form of disease incidence, r_{Md} = the rate parameter for disease incidence, and t = the time in days from budburst until maximum, or 100% disease incidence was first observed. The optimum K for each epidemic was determined from various values of K by calculating the linear regression for $\ln(K/(K-y_d)$ as a function of t and then selecting the regression that had the highest coefficient of determination (R^2) and $P < 0.05$. Asymptotes examined for each epidemic were; K_1 = disease incidence of 1.0, K_2 = the highest observed disease incidence (expressed as a proportion) plus 1.0×10^{-5}, $K_3 = (K_1 + K_2)/2$, K_4 =

$(K_1+K_3)/2$, $K_5 = (K_1+K_4)/2$, $K_6 = (K_1+K_5)/2$, $K_7 = (K_1+K_6)/2$, $K_8 = (K_1+K_7)/2$ and $K_9 = (K_1+K_8)/2$.

6.2.3.4 Effect of disease incidence at various fruit sizes on crop yield

Temporal fruit size was analysed using the monomolecular model as described by Campbell and Madden (1990). Prior to analysis, the size of fruits at each assessment was calculated as a proportion of the final fruit size. The predicted size of fruits ($\hat{y}_f$) was then estimated by

$$\hat{y}_f = 1[1-B_f \exp^{(-rMf\times t)}] \qquad \text{(Eq. 6.2)}$$

where $B_f = \ln(1/(1- y_f))$, the linearised form of proportional fruit size, and r_{Mf} = the rate parameter for proportional fruit size. The relationship between the proportional fruit size as a function of the number of days from budburst was determined for each cultivar and site-year using linear regression, with a test significant if $P < 0.05$.

The time in days from budburst for fruits to attain 0.25, 0.50, 0.75 and 1.0 proportion of final fruit size, $t_{(0.25\text{-}1.0)}$, was then predicted for each cultivar and site-year by rearranging eq. 6.2. Thus, the calculation for $t_{(0.25\text{-}1.0)}$ was given by

$$t_{(0.25\text{-}1.0)} = \{[\ln(-\hat{y}_f + 1) / B_f] \times (1 / -r_{Mf})\} \quad \text{(Eq. 6.3)}$$

Disease incidences at 0.25, 0.50, 0.75 and 1.0 proportion of final fruit size, $\hat{y}_{d(0.25\text{-}1.0)}$, for each cultivar and site-year were then predicted by substituting $t_{(0.25\text{-}1.0)}$ for t in equation 6.1. Hence, the formula for $\hat{y}_{d(0.25\text{-}1.0)}$ was given by

$$\hat{y}_{d(0.25\text{-}1.0)} = K_{optimum}[1- B_d \exp^{(-rMd\times t(0.25\text{-}1.0))}] \qquad \text{(Eq. 6.4)}$$

The mean predicted disease incidence at 0.25, 0.50, 0.75 and 1.0 fruit size at each site-year for a particular cultivar were then used as independent variables and regressed against mean percentage crop yield to derive the predicted change in crop yield per 1% increase in disease incidence, and the predicted yield loss at the predicted value of 100% disease incidence. Linear regression was significant if $P < 0.05$. To test whether a single regression model could explain predicted change in crop yield with increasing disease incidence, for Vina and Franquette and at different

fruit sizes, comparison of regression lines analysis was then conducted to determine if parameter estimates (slope, intercept) of corresponding linear relationships were significantly different at $P < 0.05$.

6.3 RESULTS

6.3.1 Description of walnut blight epidemics and fruit drop

Disease incidence and severity varied significantly between years. Nearly all fruits had disease symptoms in 2005−06; in comparison, less than one-half and one-quarter of Franquette and Vina fruits, respectively, were diseased in 2006−07 (Table 6.1; Figs. 6.1 and 6.2). In 2005−06 at Forth, disease incidence of 100% was reached in Vina prior to the premature drop of diseased fruit, hence gross and observed disease values were identical (Fig. 6.1). Gross incidence in Vina and Franquette, measured as the standardised area under the disease progress curve (SAUDPC), was 78 percent per day or more in 2005−06 when averaged over sites; in comparison, 26 percent per day or less occurred in 2006−07 (Table 6.1). Disease incidence and severity in 2004−05 were intermediate between those observed in 2005−06 and 2006−07.

Premature drop of fruits with symptoms of disease led to a reduction in observed disease incidence relative to gross disease incidence, from 60 days after budburst in all epidemics with less than 100% disease incidence (Figs. 6.1 and 6.2). The greatest differences between observed and gross disease incidence at kernel maturity were 21% in Franquette, at Swansea in 2006−07, and 22% in Vina at Forth in 2005−06. At Forth in 2004−05, premature drop of diseased Vina fruit occurred from 37 days after budburst and led to a 16% difference between observed and gross incidence midway through the season.

Among all epidemics, gross disease severity was greatest in Vina at Forth in 2005−06, with 29% of the hull surface, on average, covered by lesions at kernel maturity (Figs. 6.1 and 6.2). Mean disease severity was less than 11% on Vina fruits in all other epidemics, and less than 10% on Franquette fruits at the site-years studied. Premature drop of diseased fruits led to a reduction in the observed disease severity on fruits in the last half of the season in 5 of the 10 epidemics.

Table 6.1. Mean percent gross disease incidence and severity at kernel maturity, the mean standardised area under the disease progress curve (SAUDPC, percent per day (%-day)) for gross disease incidence and severity, and the mean crop yield (percentage of tagged fruit remaining at kernel maturity) for non-bactericide treated Vina or Franquette fruits at 10 site-years.

Cultivar	Factor	Kernel maturity				SAUDPC				Crop yield (%)[Z]	
		Incidence (%)[Z]		Severity (%)[Z]		Incidence (%-day)[Z]		Severity (%-day)[Z]			
Vina	Location										
	Forth	71.1	a	13.6	a	55.1	a	8.3	a	40.9	a
	Swansea	65.7	a	5.2	b	49.0	a	3.3	b	59.9	b
	Year										
	2004−05	81.3	b	6.9	b	54.3	b	3.8	b	57.6	b
	2005−06	100.0	a	20.5	a	85.9	a	13.1	a	9.0	a
	2006−07	24.0	c	0.8	c	15.9	c	0.4	c	84.7	c
Franquette	Location										
	Forth	70.4	a	5.7	a	53.6	a	3.5	a	38.4	a
	Swansea	75.1	a	4.6	a	50.3	a	2.2	b	47.1	a
	Year										
	2005−06	97.5	a	8.3	a	77.5	a	4.8	a	16.1	a
	2006−07	48.0	b	2.0	b	26.4	b	0.9	b	69.3	b

[Z] For each location and year for a particular cultivar, means within columns followed by different letters are significantly different at $P < 0.05$.

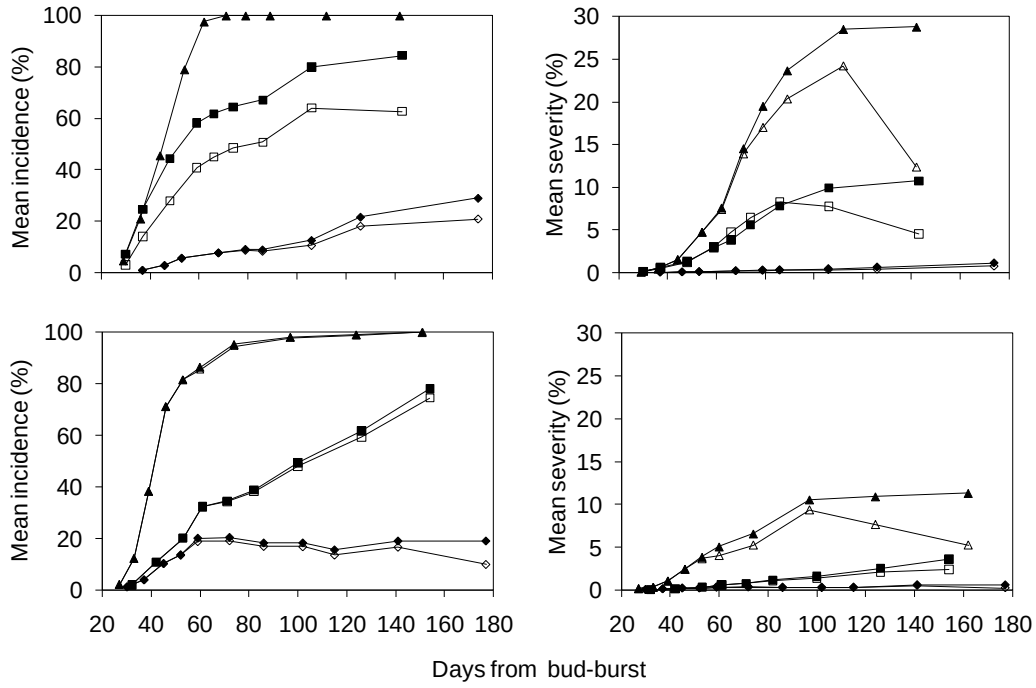

Fig. 6.1. Temporal progression of gross (closed symbols) and observed (open symbols) disease incidence and severity on non-bactericide treated Vina fruits at Forth (top left and right) and Swansea (bottom left and right) in 2004−05 (squares), 2005−06 (triangles) and 2006−07 (diamonds). Budburst occurred on the 18-Oct-04, 19-Oct-05 and 5-Oct-06 at Forth, and the 8-Oct-04, 8-Oct-05 and 25-Sep-06 at Swansea. Each data point represents the mean of six replicates.

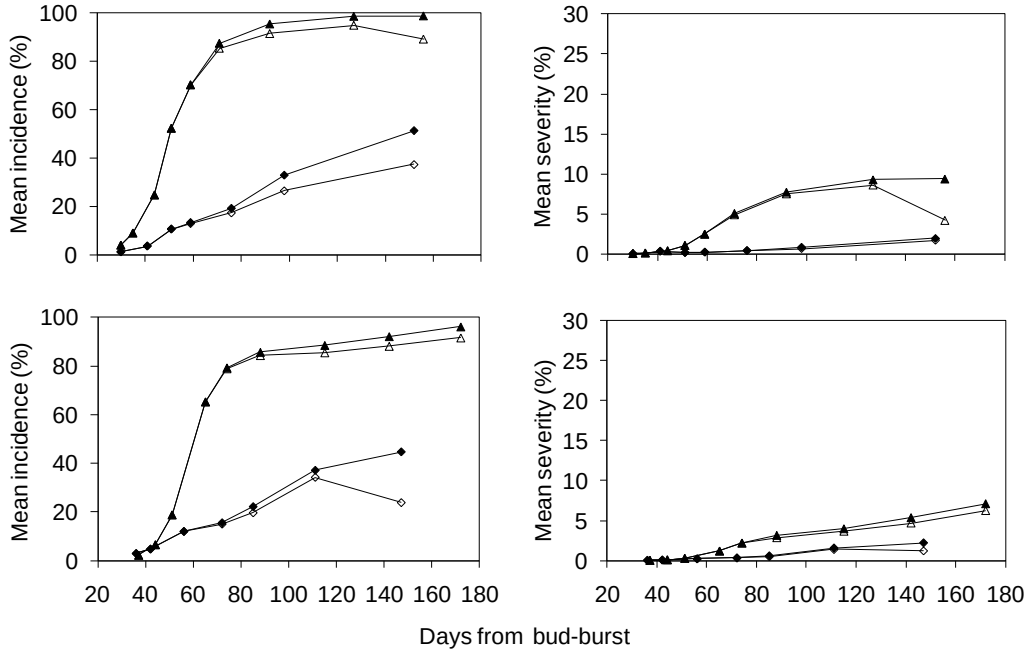

Fig. 6.2. Temporal progression of gross (closed symbols) and observed (open symbols) disease incidence and severity on non-treated Franquette fruits at Forth (top left and right) and Swansea (bottom left and right) in 2005−06 (triangles) and 2006−07 (diamonds). Budburst occurred on the 8-Nov-05 and 1-Nov-06 at Forth, and the 25-Oct-05 and 17-Oct-06 at Swansea. Each data point represents the mean of six replicates.

6.3.2 Relationship between disease incidence and severity

The SAUDPC of gross disease incidence and log transformed gross disease severity were significantly related in Vina and Franquette (Table 6.2, Fig. 6.3), with incidence accounting for 96% of the variance in severity for each cultivar. Comparison of the regression lines for Vina and Franquette found no significant difference between slopes ($P = 0.08$) and intercepts ($P = 0.37$). With the SAUDPC data pooled from Vina and Franquette, the relationship between disease incidence and severity was still significant, with incidence accounting for 92% of the variance in log transformed severity for the 10 site-years.

Table 6.2. Parameters of simple linear regression of the standardised area under the disease progress curve (SAUDPC) of gross disease incidence on log transformed gross disease severity of non-bactericide treated Vina or Franquette fruits at 10 site-years.

Cultivar	n	Intercept	Slope	R^2	*P-value*
Vina	6	− 1.602	0.049	95.9	< 0.001
Franquette	4	− 0.956	0.032	96.5	< 0.05
Pooled	10	− 1.396	0.043	92.4	< 0.0001

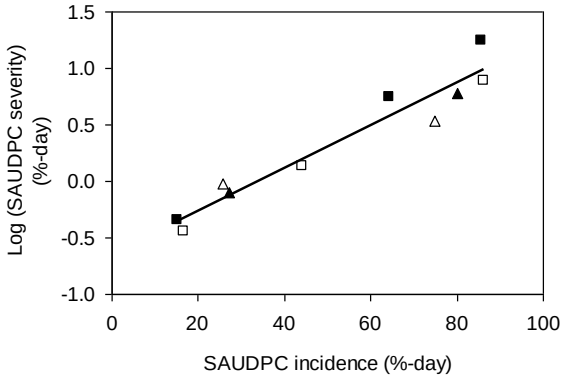

Fig. 6.3. Relationship between the standardised area under the disease progress curve (SAUDPC) of gross disease incidence and log transformed gross disease severity, of non-bactericide treated Vina (squares) and Franquette (triangles) fruits, for three and two years respectively, at Forth (closed symbols) and Swansea (open symbols). Each data point represents the mean of six replicates. Summary statistics and model parameters of regression are provided in Table 6.2.

6.3.3 Effect of walnut blight epidemics on crop yield

Crop yield varied significantly among site-years. In Vina, significantly fewer nuts were produced in 2005−06 than in 2004−05 and 2006−07; that is, fruit present at kernel maturity as a percentage of fruit tagged previously were 9, 58 and 85% respectively (Table 6.1). In Franquette, significantly fewer nuts were produced in 2005−06 than in 2006−07 i.e., 16 and 69% respectively. Crop yield in Vina varied significantly between locations, with significantly fewer nuts at Forth in comparison to Swansea i.e., 41 and 60%, respectively.

Crop yield was inversely related to gross disease incidence and log transformed gross disease severity when measured as either the SAUDPC or as disease at kernel maturity (Table 6.3). However, the SAUDPC accounted for greater variance in crop yield than disease at kernel maturity i.e., 91−100% and 71−98% respectively. There was no significant difference between the crop yield of Vina and Franquette when regressed against the SAUDPC for incidence ($P = 0.83$ and $P = 0.33$ for slope and intercept respectively) or SAUDPC severity ($P = 0.16$ and $P = 0.10$ for slope and intercept respectively). When data were pooled for Vina and Franquette, the SAUDPC for disease incidence or log transformed severity accounted for more than 90% of the variance in crop yield (Table 6.3; Fig. 6.4); these models predict that a 1 percent per day increase in disease incidence reduces crop yield by 1%, and a log (0.1) percent per day increase in severity reduces crop yield by 6%.

Table 6.3. Parameters of simple linear regressions of the standardised area under the disease progress curve (SAUDPC) of gross disease incidence and log transformed gross disease severity, or gross disease incidence and severity at kernel maturity, on crop yield of non-bactericide treated Vina fruits in 2004−05, 2005−06 and 2006−07, and Franquette fruits in 2005−06 and 2006−07, and pooled Vina and Franquette fruits, at Forth and Swansea.

Disease measurement	**Cultivar**	**Disease descriptor**	**n**	**Intercept**	**Slope**	***R*^{2}**	***P-value***
SAUDPC	Vina	Incidence	6	108.561	− 1.117	90.9	< 0.01
		Log (severity)	6	71.259	− 52.072	91.7	< 0.01
	Franquette	Incidence	4	97.610	− 1.057	95.1	< 0.05
		Log (severity)	4	66.400	− 75.988	99.8	< 0.001
At kernel maturity	Vina	Incidence	6	111.078	− 0.887	70.9	< 0.05
		Log (severity)	6	84.545	− 52.594	84.5	< 0.01
	Franquette	Incidence	4	120.503	− 1.070	92.3	< 0.05
		Log (severity)	4	99.059	− 91.597	98.3	< 0.01
SAUDPC	Pooled	Incidence	10	104.362	− 1.097	90.8	< 0.0001
	Pooled	Log (severity)	10	66.271	− 55.641	89.5	< 0.0001

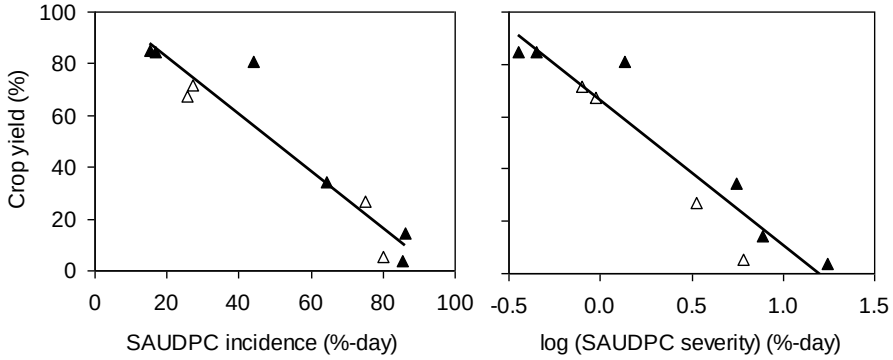

Fig. 6.4. Relationship between crop yield and the standardized area under the disease progress curve (SAUDPC) of gross disease incidence (left) and SAUDPC of log transformed gross disease severity (right) on non-bactericide treated fruits of Vina (closed symbols) and Franquette (open symbols) for 10 site-years. Each data point represents the mean of six replicates. Model parameters are listed in Table 6.3.

6.3.4 Disease progress model

The progression of gross disease incidence was described well by the monomolecular model in eight of ten epidemics, with days from budburst accounting for between 96 and 99% of the variance in disease incidence (Table 6.4). The results for linear regressions for the remaining two epidemics produced R^2 values of 88% in Vina at Forth in 2005−06, and 93% in Vina at Swansea in 2006−07. The asymptote that best fit disease progression was in the range K = 0.97–1 for eight epidemics, with the other two epidemics having a K of 0.88 or 0.40. The model also obtained R^2 values in the range 88 to 99% for all epidemics using K = 1.As such, application of K = 1 was considered suitable to predict disease incidence at any particular time during an epidemic for examining the effects of disease incidence at various fruit sizes on crop yield.

Table 6.4. Parameters from regression of $\ln(K/(K-y_d)$ (from Eq. 6.1 where y_d is gross disease incidence on non treated fruits) as a function of t_d (time in days from budburst) for data from 10 site-years, with asymptotes that provided the best fit (K=optimum), and asymptotes equal to 1 (K=1, in parenthesis).

Cultivar	Location	Year	n	Asymptote K=optimum (K=1)	Intercept K=optimum (K=1)	Slope K=optimum (K=1)	R^2 K=optimum (K=1)	*P*-value K=optimum and K=1
Vina	Forth	2004−05	9	0.88 (1)	−0.629 (−0.198)	0.027 (0.016)	0.98 (0.94)	< 0.0001
		2005−06	6	1	−4.941	0.143	0.88	< 0.01
		2006−07	9	1	−0.086	0.002	0.96	< 0.0001
	Swansea	2004−05	9	1	−0.405	0.012	0.99	< 0.0001
		2005−06	10	1	−1.400	0.055	0.99	< 0.0001
		2006−07	6	0.40 (1)	−0.513 (−0.154)	0.018 (0.006)	0.93 (0.93)	< 0.01
Franquette	Forth	2005−06	9	0.99 (1)	−2.409 (−2.409)	0.066 (0.066)	0.99 (0.96)	< 0.0001
		2006−07	7	1	−0.199	0.006	0.99	< 0.0001
	Swansea	2005−06	9	0.97 (1)	−1.122 (−1.122)	0.032 (0.032)	0.96 (0.92)	< 0.0001
		2006−07	7	1	−0.175	0.005	0.98	< 0.0001

6.3.5 Effect of disease incidence at various fruit sizes on crop yield

The monomolecular model was appropriate for describing the increase in fruit size from budburst to kernel maturity, with R^2 values in the range 96 to 99% for the 10 site-years (Table 6.5). Temporal fruit size was similar between Vina and Franquette, with the number of days from budburst to reach 25, 50, 75 and 100% full-size in the range of 39 to 45, 55 to 67, 80 to 92 and 107 to 136 days respectively. Reduced irrigation to Vina fruits at Swansea in 2006−07, due to drought, reduced the size of fruits; when growth was not limited, the maximum mean diameter of fruits was 43 and 42 mm in Vina and Franquette respectively.

The models for fruit size and disease progression allowed estimation of disease incidence at a certain fruit size. There was a strong inverse relationship between predicted disease incidence at various fruit sizes and crop yield, with linear regressions producing R^2 values of between 93 and 99% for the 10 site-years (Table 6.6). When fruits were predicted to be 25% fruit size, every 1% increase in disease incidence reduced the predicted yields by 2%. In comparison, a reduction in yield of 1%, or less, was predicted for every 1% increase in incidence at larger fruit sizes. The smallest fruit size approaching 100% yield in the absence of disease was Vina at 50% fruit size, where crop yield was predicted to be 97% by the equation:

$$\hat{y}_v = -1.01\ d_{i(50)} + 97.01 \qquad \text{(Eq. 6.5)}$$

where $\hat{y}_v$ is the predicted crop yield of Vina, and $d_{i(50)}$ is the cumulated disease incidence at 50% of final fruit size.

Table 6.5. Parameters from regression of the linearised form of the monomolecular model, $\ln(1/(1-y_d)$ (from Eq. 6.2 where y_f is the size of non treated Vina and Franquette fruits) as a function of t (time in days from budburst) for 10 site-years. Regressions describing temporal fruit size all had *P*−values of < 0.0001. Also presented are the maximum fruit diameter and the predicted number of days from budburst for fruits to reach 25% (FS_{25}), 50% (FS_{50}), 75% (FS_{75}) and 100% (FS_{100}) maximum fruit diameter (from Eq. 6.3).

Cultivar	Site	Year	n	Intercept	Slope	R^2	Maximum fruit diam. (mm)[Z]		FS_{25} (days)	FS_{50} (days)	FS_{75} (days)	FS_{100} (days)
Vina	Forth	2004−05	9	2.36	−39.58	97.3	45.3	c	41	60	82	111
		2005−06	10	2.29	−35.31	99.2	41.5	b	39	60	85	121
		2006−07	9	2.33	−42.11	97.0	44.3	c	45	67	92	127
	Swansea	2004−05	9	2.34	−38.04	96.5	45.2	c	40	59	81	111
		2005−06	10	2.26	−30.60	98.3	40.0	b	36	55	80	119
		2006−07	11	2.21	−28.01	98.7	32.5	a	35	55	85	136
Franquette	Forth	2005−06	9	2.29	−36.42	98.4	43.6	b	41	61	87	124
		2006−07	7	2.33	−35.80	97.1	43.3	b	38	56	78	107
	Swansea	2005−06	9	2.311	−39.17	95.2	40.0	a	43	64	89	125
		2006−07	7	2.37	−41.00	96.5	42.1	b	42	61	82	110

[Z] For each cultivar, means within column followed by different letters are significantly different at $P < 0.05$

Table 6.6. Parameters from regression of the linearised form of the monomolecular model for predicted disease incidence at 25, 50, 75 and 100% fruit size[Z] as a function of crop yield (%) using data from six or four site-years for non-bactericide treated Vina or Franquette fruits, respectively. Each linear model is presented in Figure 6.5 and was used to derive the predicted change in crop yield per 1% increase in disease incidence and the predicted yield loss at the predicted value of 100% disease incidence.

Cultivar	Fruit size (%)	n	Intercept	Slope	R^2	*P-value*	Change in crop yield (%) per 1% increase in disease incidence	Crop yield (%) at 100% disease incidence
Vina	25	6	91.77	− 1.74	99.1	< 0.0001	1.7	0
	50	6	97.01	− 1.01	96.3	< 0.001	1.0	0
	75	6	108.80	− 1.05	93.2	< 0.01	1.0	3.8
	100	6	123.64	− 1.09	93.2	< 0.01	1.1	14.6
Franquette	25	4	76.52	− 1.79	99.4	< 0.01	1.8	0
	50	4	83.52	− 1.04	99.3	< 0.01	1.0	0
	75	4	90.62	− 0.90	98.2	< 0.01	0.9	0.6
	100	4	101.09	− 0.91	95.9	< 0.01	0.9	10.1

[Z] Refer to equation 6.4

The slope of the linear regression at 25% fruit size was significantly greater than the slope at 50% fruit size for either cultivar (Vina, $P = 0.001$; Franquette, $P < 0.01$) (Fig. 6.5). At 25% fruit size, the maximum predicted disease incidence for zero crop yield was less than 52% irrespective of cultivar; in comparison, maximum predicted disease incidence was approximately 100% in Vina and greater than 80% in Franquette, for larger fruit sizes.

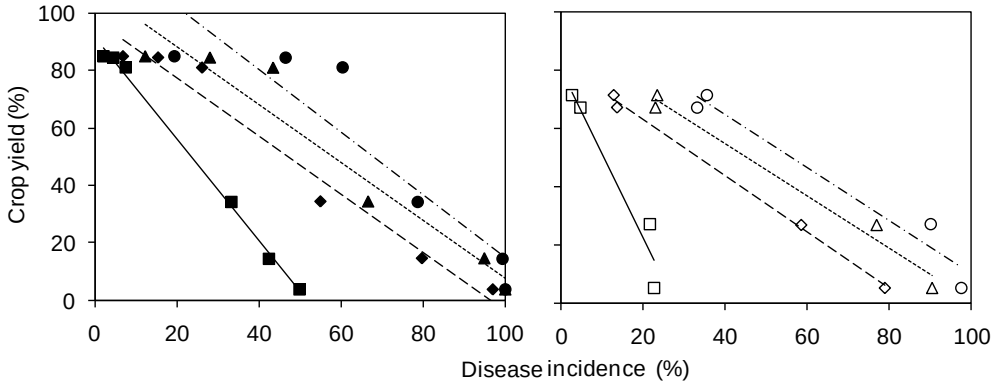

Fig. 6.5. Relationship between crop yield and predicted disease incidence in Vina (left; solid symbols) and Franquette (right; open symbols) at 25% (square symbol, solid line), 50% (diamond symbol, dashed line), 75% (triangle symbol, dotted line) and 100% (circle symbol, dashed and dotted line) fruit size using data for 10 site-years. Each data point represents the mean of six replicates. Model parameters are listed in Table 6.6.

Linear regressions between the predicted disease incidences at 75% fruit size and crop yields of Vina and Franquette were not significantly different in intercept ($P = 0.10$) and slope ($P = 0.57$) (Table 6.7). Therefore, Vina and Franquette data from this fruit size were pooled so that a single model could be used to define the relationship between predicted disease incidence and crop yield. Using a common slope and intercept, the model was described by,

$$\hat{y}_{vf} = -0.96\, d_{i(75)} + 100.96 \qquad R^2 = 91\% \qquad P < 0.0001 \qquad \text{(Eq. 6.6)}$$

where $\hat{y}_{vf}$ = predicted crop yield of Vina and Franquette nuts, and $d_{i(75)}$ is the predicted disease incidence at 75% of final fruit size, which is predicted to be

between 80 to 92 days after budburst. The model predicted that for every 1% increase in disease incidence the predicted yield was reduced by 1% (Fig 6.6).

Table 6.7. Analysis of variance comparing the slopes and intercepts of the linear regressions for Vina and Franquette for a particular fruit size from Table 6.6.

Terms	***P−value***			
	25% fruit diameter	**50% fruit diameter**	**75% fruit diameter**	**100% fruit diameter**
Intercepts	< 0.001	< 0.05	0.10	0.29
Slopes	0.72	0.83	0.57	0.59

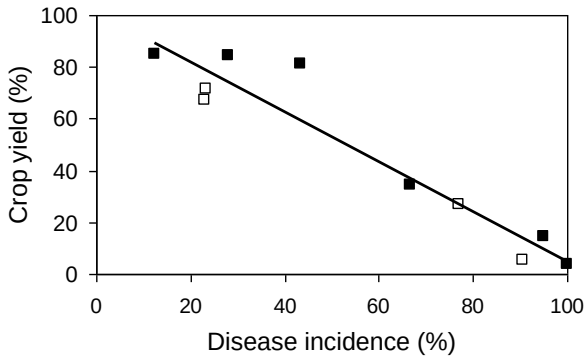

Fig. 6.6. Relationship between crop yield and predicted disease incidences at 75% fruit size on non-bactericide treated Vina (solid symbols) and Franquette (open symbols) fruits using data from 10 site-years. Each data point represents the mean of six replicates.

6.4 DISCUSSION

The strong inverse relationship between walnut blight incidence and crop yield suggests that walnut blight can lead to the premature drop of fruits in Tasmania. This finding is supported by the presence of disease symptoms on fallen fruit and the decline in observed disease incidence or severity in the later stages of the epidemic. This finding is also consistent with reported crop losses associated with walnut blight

in other growing regions of the world (Miller and Bollen, 1946; Belisario *et al.*, 1997; Teviotdale and Schroth, 1998; Arquero *et al*., 2006).

The incidence and severity of disease on fruits, and subsequent reduction in crop yield, were similar for Franquette and Vina in Tasmania. The susceptibility of Franquette to blight in Tasmania is in contrast to reports from Spanish orchards, where fully developed Franquette fruits had significantly less incidence of blight in comparison to Vina, and as such, were considered to have low and high disease susceptibility respectively (Aleta *et al*., 2001). Similarly, in evaluations of natural walnut blight infections on fruits conducted over 5 years in California, Franquette was one of six of 15 genotypes that were considered to be the least susceptible to blight infection (Adaskaveg *et al*., 2009). However, one month old Franquette fruits within Spanish orchards were considered to be highly susceptible to blight when inoculated with a suspension of *X. arboricola* pv. *juglandis* (Aleta *et al*., 2001; Moragrega *et al*., 2008a), and over 50% of three month old Franquette fruits developed lesions when inoculated with suspensions of *X. arboricola* pv. *juglandis* isolated from Tasmanian orchards (Chapter 5). In California, increasing mean population sizes of *X. arboricola* pv. *juglandis* in individual walnut buds prior to budburst were positively correlated to the incidence of disease in fruits, of cultivars Ashley, Vina and Hartley, three months after budburst (Lindow *et al*., 2004). These results suggest that disease incidence observed for Franquette in Tasmania may involve processes that increase *X. arboricola* pv. *juglandis* populations during early fruit development, given that inoculated fruits are highly susceptible to developing disease.

In this study, premature drop of diseased Vina and Franquette fruits was more often pronounced approaching, and in, the latter half of the season i.e., from January to April, in comparison to the first half of the season. In contrast, fruit drop of diseased Chico and Chandler fruits in Spain occurred from one month after flower receptivity until the end of vegetative growth (Arquero *et al*., 2006), and in French orchards natural infections to Franquette fruits in the first half of fruit growth led to 71% of infected fruits dropping within the first half of the season (Chevallier *et al*., 2010). Similarly, in Californian orchards fruit drop occurred shortly after flowers had been

dusted with pollen from catkins infected with *X. arboricola* pv. *juglandis* (Ark, 1944). However, in apical necrosis, a disease of walnut fruit found in European orchards caused by *X. arboricola* pv. *juglandis* in association with *Fusarium* spp. and *Alternaria* spp., diseased fruits of cultivars Hartley, Chandler, Serr, Lara and Vina usually dropped from the beginning of July, or mid-way through the season, even though fruits are usually infected at an early growth stage (Moragrega and Ozaktan, 2010). While the timing at which premature fruit drop occurs differed between these studies, and may depend upon environmental factors such as rain or temperature, as suggested by Moragrega and Ozaktan (2010), a common finding was that early infections to fruit led to their premature drop.

The relationship between walnut blight and crop yield in Tasmania was explained well using the standardised area under the disease progress curve (SAUDPC) as the descriptor of disease. Estimation of yield from SAUDPC models has mostly been applied to epidemics that are of relatively short duration and late in the crop growth cycle, and where yield is accumulated in a short period of time (Campbell and Madden, 1990); however, the stronger relationship between SAUDPC and crop yield, in comparison to cumulated disease at kernel maturity and crop yield, suggests that walnut blight infections during earlier stages of fruit growth may have been an important factor in reducing crop yield.

The monomolecular, exponential and logistic models serve as the basis for detailed epidemiological studies (Madden *et al*., 2007). The monomolecular model describes an epidemic where the rate of change in disease is greatest at the time of the first disease assessment and decreases from that point onwards (Madden, 1980). The monomolecular model has also been used to describe the general growth of plants over time (Hunt, 1982). While the monomolecular model was not developed specifically for plant pathology or physiology (Campbell and Madden, 1990), the model provided a good description of the temporal change in disease incidence, in eight of ten epidemics, and fruit size, in all ten site-years, observed in this study. As such, the monomolecular model was used in this study so that crop yield could be predicted according to disease incidence at various fruit sizes.

The greatest rate of yield loss in Tasmania was predicted when Vina and Franquette fruit were diseased at 25% of the final size; at this size, nearly two fruit prematurely dropped for every fruit that was diseased, suggesting that disease may have spread to adjacent fruit within clusters. The rate of fruit loss at 50% fruit size, or larger, was approximately half of what was predicted at 25% size. As such, premature fruit drop was reduced to the extent that at 75 and 100% fruit size some diseased fruits were predicted to remain on trees until harvest, suggesting that infections later in fruit growth do not always lead to premature fruit drop. For example, in field and glasshouse trials in California, inoculation of fruits during the flower bloom period nearly always led to fruits dropping prematurely; however, fruit inoculated between half- and three-quarter sizes remained on trees until harvest, with lesions confined to the outer portion of the fleshy hull (Miller and Bollen, 1946). These results suggest that fruits in Tasmania may remain on trees until harvest if infected at larger fruit sizes.

In this study, the relationship between disease incidence and yield was well described by single-point models, where non-treated yield of Vina and Franquette fruits was related to disease incidence levels at various fruit sizes. A limitation of a single-point model is that the relationship between crop yield and disease epidemic is based on only a small portion of the disease progress curve (Campbell and Madden, 1990); however, between 93 and 99% of the variance in the crop yield of Vina and Franquette was explained by disease incidence. Further tests and validation of these models are required across a full range of cultivars and growing regions, and to determine the effect of copper sprays on disease incidence and crop yield.

COPPER-BASED SPRAYS TIMED STRATEGICALLY REDUCE THE INCIDENCE OF WALNUT BLIGHT AND INCREASE CROP YIELD AND ECONOMIC RETURN

7.1 INTRODUCTION

The current management strategy for walnut blight in Tasmania is to protect susceptible fruits, from budburst to shell formation, with application of copper-based biocides at 7- to 14-day intervals for up to 10 applications per year (Permit number PER9391, Australian Pesticides and Veterinary Medicines Authority, ACT, Australia). In Tasmania, this strategy means that copper-based sprays may be applied in early- to mid-leafing cultivars such as Vina from mid-October to mid-January, as these times correspond to budburst and shell formation respectively. However, multiple copper sprays do not always control the disease (Lang *et al.*, 2006), and copper tolerant strains of *Xanthomonas arboricola pv. juglandis* have been identified in Tasmanian orchards (Saravanan, 2007). Long term use of copper-based biocides can severely impair soil microbial function (Bunemann *et al*., 2006), and may have potential long-term detrimental effects on crop yield in walnut orchards (Radix and Seigle-Murandi, 1993). Effective alternatives to copper in Tasmania have not been identified, with bactericidal sanitation treatments, chemical elicitors of induced resistance, and antibiotics proving to be either ineffective or unreliable (Lang *et al*., 2006). Hence, a strategy for optimising the number and timing of copper-based sprays for adequate disease control is required.

Disease forecast systems can assist growers in determining when to apply disease management techniques, by using information about the weather, crop, or pathogen to predict the outbreak or increase in intensity of a disease (Campbell and Madden, 1990). In northern Californian walnut orchards, temperature and extended wetness periods have been identified as critical environmental factors in walnut blight epidemics (Adaskaveg *et al.*, 2000); this knowledge has been used to develop XanthoCast™, a model that utilises wetness period duration and temperature for calculating the daily and cumulated daily risk of disease. In field trials in California, XanthoCast™ reduced the number of biocide sprays, when compared to a calendar-based spray regime, while providing similar disease control (Adaskaveg *et al.*, 2006). These results suggest scope for reducing the number of copper sprays in Tasmania.

This study aimed to compare the effectiveness of copper-based spray programs, where the timing of sprays was varied according to crop phenology and a weather-based spray models for the control of walnut blight, and to quantify the economic cost and benefit for adopting various spray programs in mature walnut orchards in Tasmania.

7.2 EXPERIMENTAL PROCEDURE

7.2.1 Orchard

A total of three trials were conducted in cultivar Vina at a commercial orchard at Forth (Chapter 4), from October to March in 2004−05, 2005−06 and 2006−07. All trees had canopies of approximately 50 m^3 when in full leaf and were grown with similar cultural management.

7.2.2 Effect of copper sprays on disease incidence and crop yield

Three trials examined the timing of copper sprays on disease incidence and crop loss. Trials were designed as randomised complete blocks, with four replicates in 2004−05 and six replicates in 2005−06 and 2006−07. Trials in 2004−05 were conducted in a 60 tree site, two rows wide by 30 trees long, and in 2005−06 and 2006−07 in 90 tree sites, three rows wide by 30 trees long.

Single-tree plots were randomly allocated to one of up to ten treatments, with treatments timed according to calendar, weather and combined calendar and weather-based spray regimes (Table 7.1). Calendar timed copper-based spray treatments were applied at budburst (Day 0), or 1 or 2 weeks after budburst (Days 7 and 14 respectively). Sprays were then applied at 7 day intervals for a maximum of six, nine and eight applications in 2004−05, 2005−06 and 2006−07, respectively.

The weather-based spray regime was timed according to the predictive model, Xanthocast™ (Adaskaveg *et al*., 2000), a model that utilises wetness period duration and temperature for calculating the daily and cumulated daily risk of disease (Appendix 7). In 2005−06, sprays timed according to the weather were applied according to Xanthocast™ only, whereas in 2006−07 Xanthocast™ sprays were

timed from 28 days after budburst, and followed four calendar timed sprays that were applied at 0, 7, 14 and 21 days after budburst (Table 7.1). Xanthocast™-timed sprays were not trialled in 2004−05.

Table 7.1. Number of copper-based spray treatments, timed according to calendar (7 day interval), weather (model) and combined calendar and weather-based (calendar then model) spray regimes in 3 years at Forth. A dash indicates that the particular treatment was not undertaken in that year.

Days from budburst in which sprays applied (day 0 = budburst) [Z]	Decision on spray timing	Number of sprays		
		2004−05	2005−06	2006−07
non-treated	–	0	0	0
0	at burdburst	1	1	1
0–7	7 day interval	2	2	2
0–14	7 day interval	3	3	3
0–21	7 day interval	4	4	4
0–28	7 day interval	–	5	–
0–X [Y]	7 day interval	6	9	8
7–X [Y]	7 day interval	5	8	7
14–X [Y]	7 day interval	–	7	6
4,16,40, 50	model	–	4	–
0–21 then 37, 60	7 day interval then model	–	–	4 then 2

[Z] Budburst (day 0) = 18-Oct-04, 19-Oct-05 and 5-Oct-06. [Y]X = 35, 56 and 49 days in 2004−05, 2005−06 and 2006−07 respectively.

7.2.3 Copper-based sprays

Spray application consisted of the copper hydroxide and mancozeb formulation, Mankocide® DF (Du Pont (Australia) Limited, North Sydney, NSW), applied at 500 g/100 l with a spray volume of 1,000 l/ha. All Mankocide® DF treatments were prepared in rainwater, and were applied using a calibrated air sheer backpack mister

(Stihl SR 400; Stihl Incorporated, Virginia Beach, United States). Surfactants or penetrants were not included in any treatment.

7.2.4 Assessment of disease incidence

Disease incidence in non-treated trees was assessed nine or ten times each year, on the fruits of six single-tree plots, as per methods described in the General Materials and Methods (Chapter 4). Trees consisted of non-treated plots within each trial, in addition to two non-treated trees adjacent to the trial site in 2004−05.

For the purposes of modelling yield gain in relation to the number of copper-based spray applied, the disease incidence in copper treated trees was evaluated when fruits had reached half full-size diameter, termed half fruit size. The diameter of fruits within tagged clusters was used as an estimate of fruit size, as per methods described in Section 4.6. This disease assessment time was based on findings from Chapter 6 that blight incidence on Vina fruits at half fruit size accounted for 96% of the variance in non-treated crop yield. In 2004−05 and 2005−06, assessments per single-tree plot were conducted on up to 120 fruits located from four limbs facing in North, South, East and West directions. In 2006−07, assessments per single-tree plot were conducted on up to 120 fruits within 40 fruit clusters with between two and three fruits per cluster at half fruit size; clusters were arbitrarily selected from around the tree canopy.

7.2.5 Environmental sensors

Surface wetness duration and ambient temperature for determining Xanthocast™ spray scores, and rainfall sensors were positioned at trials sites as per methods described in the General Materials and Methods (Chapter 4). Data were collected at 1 min intervals for each trial.

7.2.6 Data analysis and model development

7.2.6.1 Gross and observed disease incidence

Temporal disease progression on fruits from non-treated plots was calculated each year as gross disease i.e., diseased fruits attached to trees in addition to diseased

fruits that had dropped prior to each assessment, and observed disease i.e., fruits that were attached to trees only. The area under disease progress curve (AUDPC) was calculated for gross and observed disease for each year, as per methods described by Campbell and Madden (1990). Differences between the AUDPC of gross and observed disease for each year were determined using independent-sample t-test procedures, with mean separation at $P < 0.05$.

The temporal progression of gross disease incidence, only, from non-treated plots was analysed from the time of symptom expression until the highest observed disease incidence, or until 100% disease incidence. For each epidemic, disease incidence over time, measured as the number of days from budburst, was modeled using linear, monomolecular, exponential, logistic or Gompertz models (Campbell and Madden, 1990) to identify the model of best fit. Prior to analysis, disease incidences of 100% were reduced to 99.9%. All disease incidences were converted to proportions (y) and the mean value for six plots transformed using the equations,

$$B_e = \ln (y) \qquad \text{(eq. 7.1)},$$

$$B_m = \ln [1 / (1-y)] \qquad \text{(eq. 7.2)},$$

$$B_l = \ln [y / (1-y)] \qquad \text{(eq. 7.3) and}$$

$$B_g = -\ln [-1n (y)] \qquad \text{(eq. 7. 4)},$$

where B_e, B_m, B_l and B_g are the linear forms of the exponential, monomolecular, logistic or Gompertz models respectively (Campbell and Madden, 1990). Values for B_e, B_m, B_l or B_g for each epidemic were then plotted against time and linearity evaluated by linear regression.

The model that best described temporal progression of disease incidence for each epidemic was selected after examination of the following statistics from linear regression: *F*-values for linearity, the coefficient of determination and mean square error of back-transformed predicted disease incidence, the standard error for the estimate of the parameter estimates, and of plots of the standardized residuals versus predicted values (Campbell and Madden, 1990).

7.2.6.2 Effect of copper sprays on disease incidence and crop yield

Observed disease incidence was calculated as the percent of diseased fruits of those fruits assessed at half fruit size. Observed crop yield was calculated as the percent of fruits at half fruit size that remained on trees at PTB fruit stage (Chapter 4). In one replicate in 2004−05, plots with treatment of copper-based sprays from budburst for 1, 2, 4 or 6 weeks had 90%, or more, non-symptomatic fruit drop prior to half fruit size. These plots were not considered to be representative of the orchard, and the raw data removed from the data set. To enable a complete data set to be analysed for this year, the means of each treatment were calculated from the three remaining replicates and then substituted for the raw data. To determine treatment effects, disease incidence at half fruit size and crop yield were analysed by one-way ANOVA with blocks, with mean separation at $P < 0.05$.

7.2.6.3 Relationship between the number of copper-based sprays and crop yield

The observed crop yield of copper- and non-treated fruits were regressed against the number of copper-based sprays applied from budburst, for each year. Linear regression was then used to determine whether there was a significant relationship between dependent and independent variables.

7.2.6.4 Yield gain with regard to copper-based sprays and disease incidence

To predict the increase in crop yield with copper-based sprays, observed non-treated crop yields were subtracted from crop yields with sprays applied from budburst to determine the observed yield increase, or yield gain. The relationship between yield gain and two independent variables, the number of copper sprays from budburst to half fruit size and disease incidence at half fruit size, was then determined by multiple regression analysis, with separate regressions carried out for data from each year.

Yield increases were calculated for 2004−05 and 2005−06 trials. Data from 2006−07 was excluded as crop yield was not significantly different between copper-based spray treatments and non-treatment.

7.2.6.5 Economic threshold for crop loss associated with walnut blight

To predict crop yields at various disease incidences and numbers of copper-based sprays, the increase in yields were predicted with one to ten sprays at various disease incidences, and then added to the predicted crop yields of non-treated fruits at half fruit size. Non-treated half full-size Vina fruits were used as a reference for predicting crop yields as per results of Chapter 6. Crop yield was predicted in Vina at half fruit size by the equation:

$$\hat{y}_v = -1.01\ d_{i(50)} + 97.01 \qquad \text{(Eq. 6.5)}$$

where $\hat{y}_v$ is the predicted crop yield of Vina, and $d_{i(50)}$ is the cumulated disease incidence at 50% of final fruit size.

To estimate the economic threshold for control measures associated with walnut blight, predicted crop income and control cost after applying single and multiple copper sprays were then estimated for a mature walnut orchard in Tasmania. Assumptions included in the model were a crop yield of 5,000 kg per ha of in-shell walnuts, a revenue of AUD 5.00 per kg of in-shell walnuts, and costs of AUD 150.00 per ha per application of Mankocide® DF.

7.3 RESULTS

7.3.1 Gross and observed disease incidence

Premature drop of fruits led to a reduction in observed disease incidence relative to gross disease incidence in 2004−05 and 2006−07 (Fig. 7.1). In 2005−06, disease incidence of 100% was reached prior to the premature drop of diseased fruit, hence gross and observed disease values were identical. A significant difference between the AUDPC of observed and gross disease incidence occurred in 2004−05 (Table 7.2), with a 17% difference between observed and gross incidence 59 days after budburst. There was no significant difference between the two methods of disease assessment in 2005−06 or 2006−07. Temporal disease progression on non-treated Vina fruits at Forth was described by the linear and monomolecular models in two years and the logistic model in one year (Table 7.3).

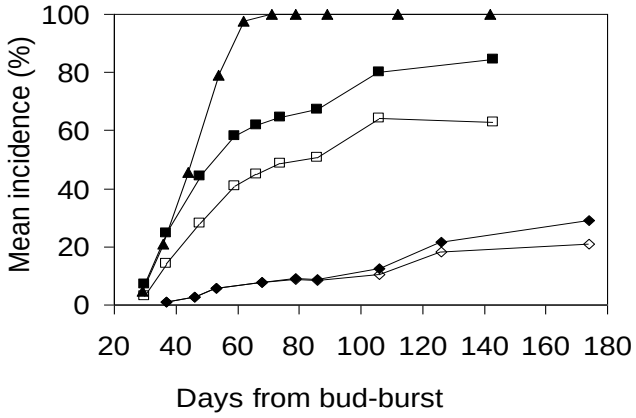

Fig. 7.1. Temporal progression of gross (closed symbols) and observed (open symbols) disease incidence on non-treated Vina fruits at Forth in 2004−05 (squares), 2005−06 (triangles) and 2006−07 (diamonds). Budburst occurred on the 18-Oct-04, 19-Oct-05 and 5-Oct-06. Each data point represents the mean of six replicates. Fig. 7.1 is reproduced, in part, from Fig 6.1 (Section 6.3.1).

Table 7.2. Summary statistics[Z] of the standardised area under the disease progress curve (SAUDPC, percent per-day (%-day)) of observed and gross disease incidence on non-treated Vina fruits in 2004−05, 2005−06 and 2006−07 at Forth.

SAUDPC (%-day) statistics and tests	**2004−05**		**2005−06**		**2006−07**	
	Observed disease	**Gross disease**	**Observed disease**	**Gross disease**	**Observed disease**	**Gross disease**
Group statistics						
Mean	52.4	72.9	104.3	104.6	13.9	16.1
Std. Dev	14.5	16.5	0.7	0.6	5.1	4.7
Std. Error	5.9	6.7	0.3	0.3	2.1	1.9
n	6	6	6	6	6	6
Equality of means[Y]						
t value	2.30		0.83		0.77	
df	10		10		10	
P > t (two-tail)	0.05		0.43		0.45	

[Z]data in Table 7.2 reported, in part, in Table 6.1 (Section 6.3.1); however, different statistical tests were incorporated in Table 7.2 to those reported previously. [Y] t-test assuming equal variances, equality of variances; 2004−05, $P = 0.79$; 2005−06, $P = 0.86$; 2006−07, $P = 0.89$.

Table 7.3. Model of best fit for describing temporal progression of walnut blight incidence in Vina, in 3 years at Forth.

Year	n	Model	R^{*2Z}	R^{2Y}	*P-level*	Intercept	Slope ($\times 10^2$)
2004−05	9	Monomolecular	94.2	94.4	< 0.0001	− 0.198	1.569
2005−06	6	Logistic	97.7	91.3	0.0029	− 11.183	26.062
2006−07	9	Linear	96.8	96.8	< 0.0001	− 0.065	0.200

[Z] coefficient of determination for agreement between gross disease incidence and back-transformed predicted disease incidence. [Y] coefficient of determination for agreement between gross disease incidence and transformed predicted disease incidence

7.3.2 Effect of copper sprays on disease incidence and crop yield

In 2004−05, trees that had two or more copper sprays from budburst, applied at 7 day intervals from budburst or 7 days after budburst, had less than 7% disease incidence of fruits; in comparison, budburst only treatment and non-treatment had 24% and 43% incidence respectively (Table 7.4). Two or more copper sprays significantly increased crop yield in comparison to non-treatment.

In 2005−06, between four and nine copper sprays, applied at 7 day intervals from budburst, significantly reduced blight incidence in comparison to three or less applications (Table 7.4); however, disease control was not satisfactory with at least 47% disease incidence. When sprays were applied beyond 28 days, disease incidence was significantly reduced when the initial spray was applied at budburst or 7 days after budburst in comparison to when the spray was delayed until 14 days after budburst. There was no significant difference in disease incidence and crop yield between sprays timed according to the Xanthocast™ predictive spray model and non-treatment.

Disease incidence in 2006−07 was less than 11% irrespective of treatment (Table 7.4). There was no significant difference in disease incidence between calendar-timed sprays and the combined calendar and Xanthocast™ sprays. Crops yielded more than 75% fruits, irrespective of whether trees were non-treated or had single or multiple sprays.

Table 7.4. Mean percent of Vina fruits with blight at half fruit size and crop yield after single and multiple copper-based sprays, timed according to a calendar (7 day interval) and/or Xanthocast™ (model) (Adaskaveg *et al*., 2000) at Forth. Initial sprays were applied at budburst (Day 0), or 7 or 14 days after budburst (Days 7 and 14 respectively), and then at 7 day intervals for up to 35, 56 and 49 days after budburst in 2004−05, 2005−06 and 2006−07.

Days from budburst when sprays applied (day 0 = budburst) [Z]	Decision on spray timing	No. of sprays	2004−05 [Y] (monomolecular) [X]				2005−06 [Y] (logistic) [X]				2006−07 [Y] (linear) [X]			
			Blight (n=4) (%)		Yield (n=4) (%)		Blight (n=6) (%)		Yield (n=6) (%)		Blight (n=6) (%)		Yield (n=6) (%)	
Non-treated	–	0	42.8	c	50.4	a	79.8	cd	3.0	a	7.7	abc	85.0	bc
0	at burdburst	1	23.5	b	63.6	ab	83.9	d	7.5	ab	9.3	bc	88.6	c
0–7	7 day interval	2	3.9	a	77.4	b	81.2	c	11.7	ab	5.3	a	79.9	abc
0–14	7 day interval	3	6.1	a	75.3	b	69.9	bc	9.8	ab	–	–	–	–
0–21	7 day interval	4	3.6	a	84.2	b	51.4	a	13.2	b	6.1	ab	81.9	abc
0–28	7 day interval	5	–	–	–	–	47.2	a	10.7	ab	–	–	–	–
0–X [W]	7 day interval	6–9 [V]	6.5	a	82.7	b	46.7	a	24.5	c	5.1	a	81.0	abc
7–X [W]	7 day interval	5–8 [U]	5.4	a	80.8	b	54.7	a	15.5	bc	7.1	ab	79.1	ab
14–X [W]	7 day interval	6 or 7 [T]	–	–	–	–	65.9	b	23.3	c	10.9	c	74.9	a
4,16,40, 50	model	4	–	–	–	–	76.3	bcd	9.5	ab	–	–	–	–
0–21 + 37, 60	7 day interval + model	6	–	–	–	–	–	–	–	–	5.9	ab	81.8	abc

[Z] Budburst (day 0) = 18-Oct-04, 19-Oct-05 and 5-Oct-06 [Y]Means within columns followed by different letters are significantly different at $P < 0.05$: [X] Model of best fit i.e., monomolecular, logistic and linear models (Chapter 7, Table 7.2): [W] X = 35 days in 2004−05; X = 56 days in 2005−06; X = 49 days in 2006−07: [V] 6 sprays in 2004−05; 9 sprays in 2005−06; 8 sprays in 2006−07: [U] 5 sprays in 2004−05; 8 sprays in 2005−06; 7 sprays in 2006−07: [T] 7 sprays in 2005−06; 6 sprays in 2006−07.

7.3.3 Environmental factors and disease incidence

Rainfall of 135, 371 and 22 mm occurred within 70 days of budburst in 2004−05, 2005−06 and 2006−07 respectively (Table 7.5). In 2004−05, less than 1 mm of rainfall was recorded within 6 days of budburst; however, 96 mm occurred between 7 and 27 days. In 2005−06, 241 mm rainfall occurred within 27 days of budburst, whereas 7 mm occurred over the same time period in 2006−07. In 2005−06, a further 130 mm rainfall from 28 to 70 days; in comparison, 39 and 15 mm rainfall was recorded in 2004−05 and 2006−07 respectively.

Table 7.5. Cumulative daily rainfall for 3 years, and Xanthocast™ scores for 2 years in the specified periods from budburst (Day 0) to 70 days after budburst at Forth.

Days from budburst [Z]	2004−05	2005−06		2006−07	
	Rainfall (mm)	Rainfall (mm)	Xanthocast™ (score)	Rainfall (mm)	Xanthocast™ (score)
0–6	0	107	74	0	3
7–13	26	29	5	6	1
14–20	47	92	39	1	0
21–27	23	13	15	0	0
0–27	96	241	133	7	4
28–70	39	130	39	15	36
0–70	135	371	172	22	40

[Z] Budburst = 18-Oct-04, 19-Oct-05 and 5-Oct-06

Xanthocast™ attained a cumulative score of 172 within 70 days of budburst in 2005−06 (Table 7.5). The Xanthocast™ model predicted four sprays within 70 days of budburst in 2005−06 and two sprays from 28 to 70 days of budburst in 2006−07 (Fig. 7.2); however, for each prediction the spray threshold of 5 was exceeded prior to the spray being applied. In 2005−06, rainfall of 15 mm or more was recorded on days when, and/or on days immediately prior to, spray thresholds of 5 being attained; hence, sprays were delayed until spray index scores of 45, 23, 9 and 6 were attained. In 2006−07 spray index scores of 9 and 6, and precipitation of 6 and 2 mm per day, were recorded prior to sprays being applied.

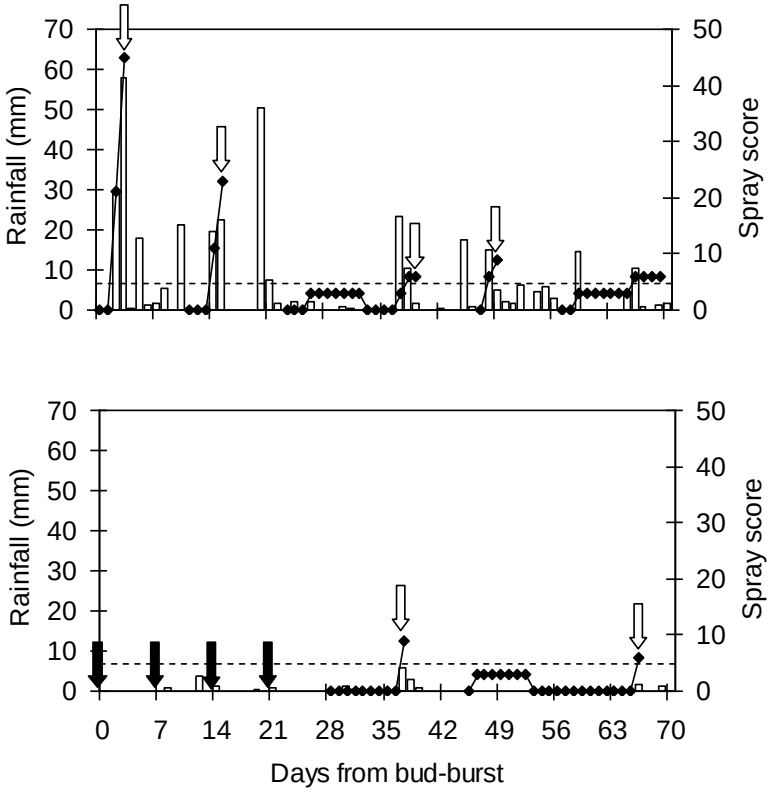

Fig. 7.2. Rainfall (mm) and Xanthocast™ score (Spray score) per day, for predicting the timing of copper sprays, from budburst (Day 0) to 70 days after budburst in 2005−06 (top) and 2006−07 (bottom) at Forth. Budburst occurred on the 19-Oct-05 and 5-Oct-06. The dashed line represents a threshold of 5 for the Xanthocast™ score. Clear block arrows represent copper-based sprays timed according to Xanthocast™. Solid block arrows represent sprays timed according to a 7 day calendar regime.

7.3.4 Relationship between the number of copper sprays and crop yield

The importance of copper-based sprays between budburst and half fruit size were identified, with a significant relationship between crop yield and the number of copper-based sprays applied from budburst in 2 of 3 years (Fig. 7.3, Table 7.6). In 2004−05, crop yield was significantly related to the square root of the number of copper-based sprays; with 5 sprays, a yield of 84% was predicted in comparison to 52% with non-treatment. In 2005−06, crop yield was predicted to increase linearly by 2% with every spray. No significant relationship between the copper-based sprays and crop yield occurred in 2006−07.

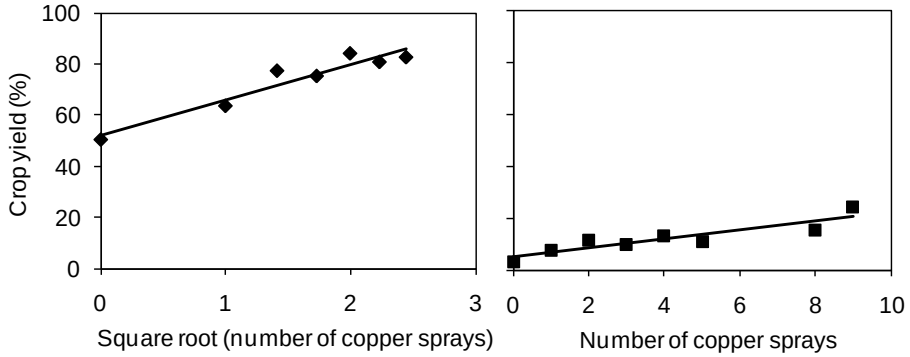

Fig. 7.3. Mean percent crop yield and number of copper-based sprays, applied from 5% terminal budburst, in 2004−05 (left) and 2005−06 (right) at Forth. Data points represent the mean of 4 and 6 replicates in 2004−05 and 2005−06 respectively. Summary statistics and model parameters of regressions are provided in Table 7.5.

Table 7.6. Parameters and statistics of the linear equations fitting the mean percent crop yield with single and multiple copper-based sprays, applied from 5% terminal budburst, in 3 years at Forth.

Year	n	Crop yield (y)	Copper sprays (x)	Intercept	Slope	*P*	R^2
2004−05	6	y	√(x)	51.557	14.459	< 0.01	91.7
2005−06	7	y	x	4.372	2.075	< 0.01	87.7
2006−07	5	y	√(x)	86.065	− 1.923	0.31	33.5

7.3.5 Yield increase with regard to copper-based sprays and disease incidence

Copper-based sprays increased crop yields by between 13 and 34% in 2004−05 (Table 7.7). Yield gains of ≤ 22% occurred in 2005−06. The number of copper-based sprays and disease incidence at half fruit size was positively correlated to increased yield, accounting for 93% of the variance in increased yield (Table 7.8). The equation was:

$$YI = 35.804 - 17.715 \times \log_{10}(DI) + 1.493 \times CS \quad \text{(Eq. 7.5)}$$

where YI = yield increase, DI = percent disease incidence on fruits at half full-size, and CS = the number of copper-based sprays applied from budburst till half fruit size. A yield increase of 38% above non-treated yield was predicted with 5% disease incidence and 10 sprays were applied (Fig. 7.4); in comparison, yield with copper-based sprays were predicted to increase by only 2% when all fruits were diseased and only one copper spray was applied.

Table 7.7. Mean percent disease incidence on half size Vina fruits (Blight) and crop yield (Yield) with non-treatment and after single and multiple copper-based sprays, and increase in crop yield (Yield increase) with various numbers of copper-based sprays from budburst to half fruit size in 2 years at Forth.

Days from budburst when sprays applied (day 0 = budburst)[Z]	2004−05 (n=4)				2005−06 (n=6)			
	No. of copper sprays	Blight (%)	Yield (%)	Yield increase (%)	No. of copper sprays	Blight (%)	Yield (%)	Yield increase (%)
Non-treated	0	42.8	50.4	–	0	79.8	3.0	–
0	1	23.5	63.6	13.2	1	83.9	7.5	4.5
0–7	2	3.9	77.4	27.0	2	81.2	11.7	8.7
0–14	3	6.1	75.3	24.9	3	69.9	9.8	6.8
0–21	4	3.6	84.2	33.8	4	51.4	13.2	10.2
0–28	–	–	–	–	5	47.2	10.7	7.7
0–35	6	6.5	82.7	32.3	–	–	–	–
0–56	–	–	–	–	9	46.7	24.5	21.5

[Z] Budburst = 18-Oct-04 and 19-Oct-05

Table 7.8. Parameters from analysis of variance (ANOVA) and multiple regression of the change in yield of harvestable nuts due to treatment (YI, Eq. 7.5), in cultivar Vina at Forth in 2004−05 and 2005−06.

Multiple Regression Analysis						
Dependent variable: YI						
Parameter	**Estimate**		**Standard error**	**t-statistic**	**P-value**	
Constant	35.804		2.821	12.693	0.0000	
Log_{10}(DI)	−17.715		1.659	−10.681	0.0000	
CS	1.493		0.385	3.879	0.0047	
ANOVA						
Source	SS	Df	Mean square	F-Ratio	P-value	R^2 (adj.)
Model	1106.770	2	553.387	66.15	0.0000	92.9
Residual	66.921	8	8.365			
Total (Corr.)	1173.70	10				

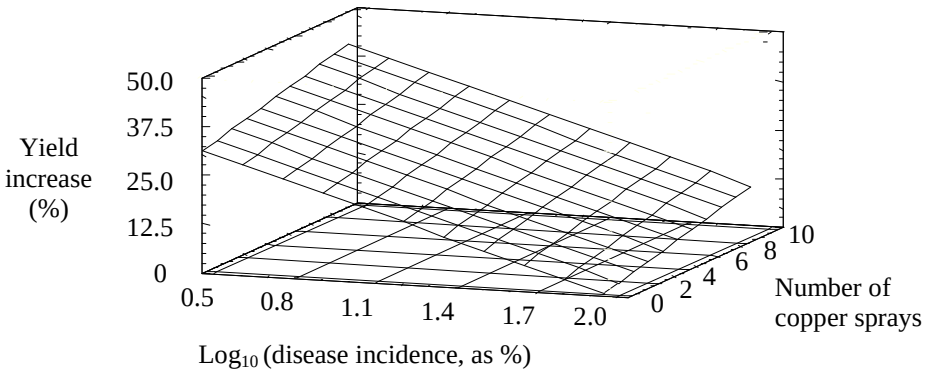

Fig. 7.4. Estimated response surface of the mean increase in crop yield with copper-based sprays applied from budburst and mean percent disease incidence at half fruit size at Forth in 2004−05 and 2005−06.

7.3.6 Economic threshold for crop loss associated with walnut blight

Predicted crop yields increased with each copper-based application when disease incidence on half size fruits was 25%, or more (Fig. 7.5). With a single copper-based spray, yields of 84, 54 and –2% were predicted with 25, 50 and 100% disease incidence respectively (Fig. 7.5, Table 7.9). Yields were predicted to increase by up to 14% when 10 copper-based sprays were applied i.e., 97, 67 and 12% with 25, 50 and 100% incidence respectively. With disease incidence of 10%, or less, one copper-based spray increased yields to 97%; further sprays did not increase yields as maximum non-treated yield is predicted to be 97.0 % (Table 7.9).

With 25% or more disease incidence, multiple sprays are predicted to increase crop incomes in comparison to a single spray (Fig 7.6). Crop income, relative to control costs, is maximized with 10 sprays i.e., estimated income of AUD 6292.00, 5159.00 and 3825.00 per ha at 25, 50 and 100% incidence respectively. With 10% disease, or less, multiple sprays are predicted to reduce economic gain than a single spray only, as spraying costs outweigh the return from increased yield.

With 0.5% incidence, an economic gain of AUD 1.00 per ha is predicted when costs associated with one copper-based spray are included (Fig. 7.6, Table 7.9); however, further sprays result in a net income loss as control costs are greater than crop income derived from yield gains. At 5% incidence, income is greater than costs, irrespective of the number of sprays (Fig. 7.6); however, economic gain is reduced with each spray, from AUD 1133.00 with one spray to AUD 8.00 with 10 sprays.

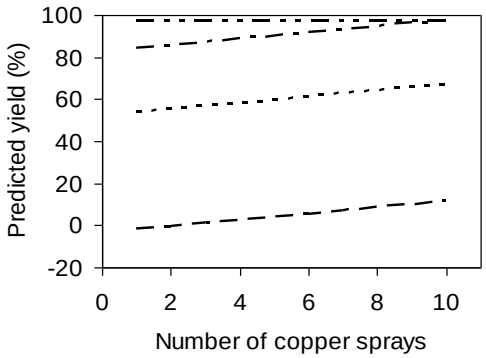

Fig. 7.5. Predicted yields for disease incidences at half fruit size of 10% (dash and double dots), 25% (dash and single dots), 50% (dots) and 100% (dash), after 1 to 10 copper-based sprays applied to Vina between budburst and half fruit size.

Table 7.9. Predicted yields of non-treated Vina fruits with disease incidence from 0.5 to 100% at half fruit size (non-treated yield), the predicted yield gain above non-treated yield, predicted yield with copper-based spray (%), gross gain (kg/ha) and net gain (AUD/ha) of Vina nuts with one copper-based spray (copper-spray) applied at budburst at Forth.

Disease incidence (%)	Non-treated yield (%)[Z] A	Predicted yield increase (%)[Y] B	Sum of A and B (%)	Predicted yield with copper-spray (97.0% max[W]) C	Predicted yield gain with copper-spray (%) D=C−A	Predicted yield gain with copper-spray (kg/ha)[V] E=[(D×0.01) ×5000]	Predicted gross gain with copper-spray[U] (AUD/ha) F=(E×5)	Predicted net gain with copper-spray[T] (AUD/ha) G=[F−(125 ×1)]
0.5	96.5	42.6	139.1	97.0	0.5	25	126	1
0.75	96.3	39.5	135.8	97.0	0.8	38	189	64
1	96.0	37.3	133.3	97.0	1.0	50	252	127
5	92.0	24.9	116.9	97.0	5.0	252	1258	1133
10	86.9	19.6	106.5	97.0	10.1	503	2517	2392
25	71.8	12.5	84.4	84.4	12.5	627	3133	3008
50	46.7	7.2	53.9	53.9	7.2	360	1800	1675
100	−3.7	1.9	−1.8	−1.8	1.8	93	466	341

[Z] Refer to Equation 6.5: [Y] Refer to Equation 7.5: [W] Refer to Equation (6.5); where maximum non-treated yield is predicted to be ≥97.0%, yield with copper-based spray does not exceed 97.0%. [V] Predicted yield gain (kg/ha) assumes a yield of 5,000 kg/ha of in-shell product. [U] Predicted gross gain (AUD/ha) assumes a revenue of AUD 5.00 kg of in-shell product. [T] Predicted net gain (AUD/ha) assumes copper-based spray costs of AUD 125.00/ha/application

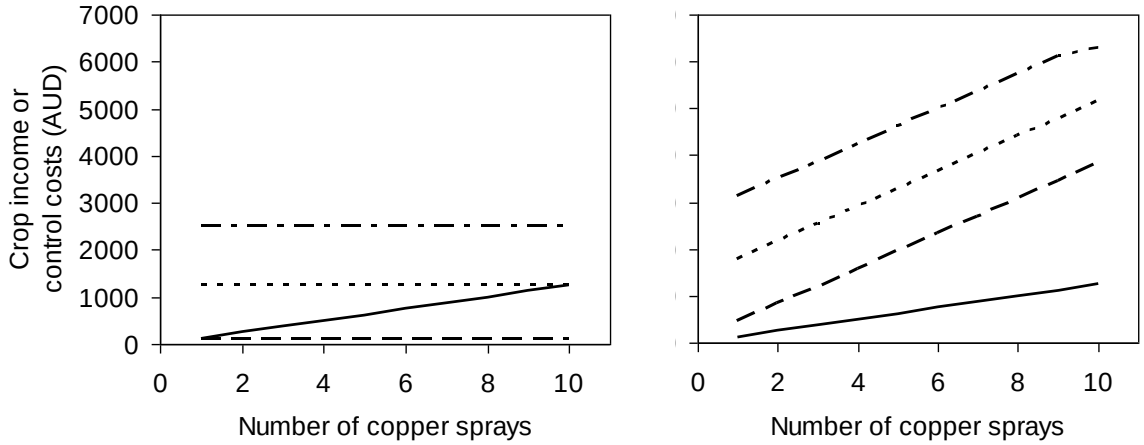

Fig. 7.6. Predicted increase in crop income for in-shell walnuts at various disease incidences, and control costs for walnut blight, with single and multiple copper-based sprays at Forth. (Left) Predicted increase in crop income with disease incidence of 0.5% (dashes), 5% (dots) and 10% (dash and dots) at half fruit size and control costs per copper application from budburst (solid line); (Right) Predicted increase in crop income with disease incidence of 25% (dash and dots), 50% (dots) and 100% (dash) at half fruit size and control costs per copper application from budburst (solid line). Models and assumptions for estimating crop incomes and control costs are provided in Table 7.9.

7.4 DISCUSSION

The strong relationship between applications of copper-based sprays and increased crop yields suggests that management of walnut blight is economically sustainable in Tasmania. However, crop loss can also be brought about by other harmful biotic and abiotic agents (Campbell and Madden, 1990), such as the fungal disease Anthracnose, caused by *Gnomonia leptostyla* (Miller et al., 1940), brown apical necrosis, caused by the fungi of the genus *Fusarium* and *Alternaria* (Belisario *et al*., 2002; Moragrega and Ozaktan, 2010), and sunburn, wind damage and frosts (Rudolph, 1933; Miller and Bollen, 1946). Hence, assessments at several times during the growing season in this study provided greater clarity between losses associated with walnut blight and losses caused by other harmful organisms and environmental stresses.

In this study, disease incidence in an epidemic where disease progress was defined by the monomolecular model was adequately controlled with, at most, two copper-based sprays, applied at budburst and 7 days after budburst. Similarly, in two trials conducted in California, three or less sprays applied at 7 day intervals from budburst significantly reduced disease incidence in comparison to non-treatment (Buchner *et al*., 2004). Further in-season sprays, applied for up to 6 weeks after budburst, did not reduce disease incidence in comparison to three early-season sprays. In previous trials conducted over a 15 year period in northern California, disease was also satisfactorily controlled with one to three sprays applied during the budburst to early post-blossom period (Miller and Bollen, 1946). Furthermore, three early-season sprays provided similar disease control in comparison to seven early- and mid-season sprays in Spain (Nino *et al*., 2002). While these latter studies did not describe the temporal progression of disease, they suggest that copper-based sprays, applied from the bud burst to early post-bloom period, may adequately control epidemics that have a slow rate of development.

The timing of copper-based sprays appears to be critical for controlling walnut blight in Tasmania. For example, in an epidemic where disease progression was defined by the monomolecular model, a single budburst spray significantly reduced disease

incidence compared to non-treatment, but did not provide the same level of disease control as compared to two sprays from budburst. In contrast, applying from three to six sprays from budburst did not provide any greater control than two sprays from budburst. Furthermore, in epidemics defined by monomolecular and logistic models, delaying the initial copper spray until 14 days after budburst provided less control of disease compared to when sprays were applied from budburst. Thus, host, pathogen and environmental factors during the budburst period, and shortly after the budburst period, appear to be critical for the development of walnut blight in Tasmania.

The primary overwintering location for *X. arboricola* pv. *juglandis* is the interior of reproductive walnut buds (Miller and Bollen, 1946; Esterio and Latorre, 1982; Mulrean and Schroth, 1982). Over 10 years in Californian orchards, Lindow *et al*. (2004) observed that increased *X. arboricola* pv. *juglandis* populations within buds at budburst increased the likelihood of disease incidence of walnut fruits; however, populations of *X. arboricola* pv. *juglandis* were greatly reduced on fruits developed from buds where copper-based biocides were applied immediately after budburst, in comparison to fruits where buds had sprays applied from 3 to 6 days prior to budburst (Lindow *et al*., 2005, 2006). Copper-based biocides are not able to penetrate sufficiently into unopened walnut buds, (Lindow *et al*., 2000). Furthermore, walnut budburst is asynchronous, with fruitful buds opening over a period of 2 or more weeks (Hills and Lang, 2004; Lindow *et al*., 2005, 2006; Buchner *et al*., 2007, 2008). As initial copper sprays in Tasmania were applied when only 5% of terminal buds had burst, a single budburst spray may have had little benefit in reducing inoculum potential in a large proportion of reproductive buds.

After budburst, the movement of inoculum to the interior of the bud will influence the likelihood of infection to flowers and fruit. Prior to budburst the innermost embryonic leaves and meristimatic tissue of walnut buds are seldom contaminated with bacterial cells (Lindow *et al*., 2004). However, bacterial cells are easily dispersed from infection sites in free moisture and rain splash droplets (Butterworth and MacCartney, 1991), thus moisture during the budburst period, and shortly thereafter, is a logical mechanism for moving the inoculum from outermost scales and cataphylls of the walnut bud to the leaves and fruits. Early-season copper-based

sprays may have shielded susceptible tissue from the transfer of inoculum, and provided adequate protection to fruit in an epidemic where disease progression was defined by the monomolecular model.

In contrast, multiple copper-based applications did not provide adequate control when temporal disease was defined by the logistic model (Table 7.4). Nearly 250 mm rainfall was recorded within 4 weeks of budburst during the development of a "logistic" epidemic; in contrast, less than half this amount was recorded when epidemics were defined by monomolecular and linear models. In copper-based spray rate trials at Forth, conducted concurrently to this study, significantly more disease occurred in an epidemic defined by the logistic model when copper-based sprays were applied at one quarter of the rate recommended for the walnut industry in Tasmania in comparison to industry recommendations (Lang *et al*., 2010). On sour cherry leaves, rainfall from 40 to 90 mm per week reduced copper levels by more than one half, leaving a sub-lethal dose of copper for the control of *Pseudomonas syringae* pv. *morsprunorum* (Olsen and Jones, 1983). Thus, copper on walnut fruits may have been depleted to sub-lethal levels by heavy rainfall in Tasmania in an epidemic that was defined by the logistic model.

In this study, the increases in crop yield and economic returns associated with increased copper-based sprays justified multiple applications in an epidemic described by the logistic model. However, epidemics described by the monomolecular and linear models, the economic benefit of multiple copper-based sprays was inconclusive, as yield returns did not always recover the costs of crop protection. Furthermore, in this study protective copper-based sprays were not applied between half- and three-quarter fruit size i.e., from 60 to 84 days after budburst. As infections to fruit during this period may potentially lead to yield loss (Chapter 5), appropriately times copper-based sprays may have protected fruit and increased crop yields. Thus, the ability to successfully forecast disease and predict the optimum time for applying copper-based sprays in an epidemic described by the monomolecular and linear models would maximize orchard efficiencies and economic returns.

In spray trials, the spray threshold of Xanthocast™ used to predict the time of applying copper-based biocides was often exceeded by continuous rainfall in an epidemic where temporal disease was described by the logistic model (Fig. 7.2); hence, copper-based sprays were often applied up to 2 days later than predicted by the model. The delay between the predicted and actual time of applying sprays may have reduced control, as application of copper-based biocides prior to rainfall provides significantly better control of walnut blight in contrast to spray application after rainfall (Olsen and Buchner, 2002).

Disease forecasting systems that are developed in one climate may not be suitable for predicting the timing of sprays in a new location, especially if weather conditions are markedly different between locations. For example, Shtienberg *et al*. (2003) found that the fire blight warning system, BIS95, that was developed for the control of blossom blight in the low temperature environment of England (Billing, 1996), was not suitable for predicting fire blight in the warmer and drier climate of Israel. Xanthocast™, which was developed for the warm, dry environments associated with northern Californian walnut orchards, did not provide sufficient warning for the timing of protectant sprays in the wetter climate of Tasmania; thus, adaptation of the Xanthocast™ model may be required if it is to be effective in Tasmania.

In summary, this study determined that walnut blight can be controlled with minimal applications of a copper-based biocide during the budburst period, and shortly thereafter, in an epidemic described by the monomolecular model. During epidemics described by the logistic model, copper applications need to be timed according to pathogen activity and weather to maximize the persistence of copper, so that susceptible tissue remains protected against *X. arboricola* pv. *juglandis*. The results justify the development of a weather-based model for timing of copper-based sprays for optimal control of walnut blight, while minimizing the use of copper, in Tasmanian walnut orchards.

RAINFALL IS THE PRINCIPAL ENVIRONMENTAL VARIABLE INVOLVED IN THE DEVELOPMENT OF WALNUT BLIGHT ON FRUITS

8.1 INTRODUCTION

Walnut blight, caused by the bacterium *Xanthomonas arboricola* pv. *juglandis*, can develop when virulent and active populations of the pathogen interact with susceptible hosts under favorable environmental conditions. A period of surface wetness on fruits and leaves has been implicated in the development of walnut blight in California (Miller and Bollen, 1946; Adaskaveg *et al*., 2000; Lindow *et al*; 2004), and an increase in relative humidity (RH) within the tree canopy suggested as the reason for disease development on fruits in southern Spain (Arquero *et al*., 2006); in contrast, no correlation between disease incidence on fruits and rainfall or RH was observed in French orchards (Chevallier *et al*., 2010).

The disease progress curve (Campbell and Madden, 1990) represents the integration of host, pathogen and environmental effects occurring during an epidemic. Moreover, it allows analysis of the impact of the factors influencing epidemic development. In northern Californian walnut orchards, the temporal progression of walnut blight on Ashley and Vina fruits was most often described by monocyclic disease processes, where infection periods for disease are initiated from an overwintering or primary inoculum source (Adaskaveg *et al*., 2000); in contrast, polycyclic epidemics occurred only with extensive wetness periods during spring, with the development of new infections producing inoculum for further infections.

While the disease progress curve allows situational analysis after the event, the practical application of temporal analyses is to identify factors correlated to the rate of disease progress and select those that can be measured during the growing season to support crop protection decisions. Therefore, the aims of this chapter were to determine the critical environmental factors associated with the development and expression of walnut blight in Tasmania.

8.2 EXPERIMENTAL PROCEDURE

8.2.1 Orchards, tree selection and disease assessment

The orchards, trees and methods used for disease assessment are the same as those described in Chapter 6 (Sections 6.2.1 and 6.2.2.1).

8.2.2 Environmental sensors and variables

Surface wetness, ambient temperature, relative humidity (RH) and tipping bucked rainfall sensors were positioned at trials sites, as per methods described in the General Materials and Methods (Chapter 4). Data were collected at 1 and 2 min intervals at Forth and Swansea, respectively.

Environmental variables used for analyses of disease development were daily minimum, maximum and mean ambient temperatures, daily rainfall, the number of days with greater than 0.2 mm of rainfall, mean daily RH, the number of hours per day with RH of 90% or more, and the duration of surface wetness per day for values between 10 and 100%. Mean daily ambient temperature and RH were calculated by averaging all measurements taken at intervals of 1 or 2 min. Daily moisture intensity was calculated from the daily rainfall divided by the duration of surface wetness during and after rainfall.

8.2.3 Data analyses

8.2.3.1 Temporal progression of disease

Temporal gross disease incidence values from non-treated plots for each site-year was calculated from diseased fruits attached to trees in addition to diseased fruits that had dropped prior to each assessment. The temporal progression of disease incidence were then compared to linear, monomolecular, exponential, logistic or Gompertz models (Campbell and Madden, 1990), and the most appropriate model for describing disease progression selected, as per methods described in Chapter 7.

Disease progress was also modeled for each single tree (non-treated) plot, using Eqs. 7.2 to 7.4. Weighted mean absolute rate (WMAR) values were calculated for each

plot at each location using methods described by Campbell and Madden (1990). The WMAR (ρ) for each replicate disease progress curve was calculated as $\rho = r / (2m + 2)$, where r is the rate parameter calculated from the growth model and m is the assumed shape parameter that corresponds to that model i.e., m = 0 for monomolecular, 1 = Gompertz and 2 = logistic. The WMAR means for each location were then compared using analysis of variance, with mean separation at $P < 0.05$.

8.2.3.2 Relationship between disease increase and each environmental variable

The rate of change in disease incidence from one disease assessment to the next was estimated for each epidemic and plotted against time. The rate of change was estimated using the equation,

$$PNB = [(Y_{i+1} - Y_i) / (t_{i+1} - t_i)] \text{ (eq. 8.1)}$$

where PNB (percentage new blight) is the percentage of fruits per day with blight symptoms that were not present at the previous assessment, and Y_{i+1} at t_{i+1} represents the disease assessment immediately following the previous assessment of Y_i at t_i. Fruits with no disease symptoms that abscised prematurely were removed from the data set.

The relationship between PNB and an environmental variable was examined by cumulating the latter for different adjusted-calendar-day periods prior to each disease assessment. One adjusted-calendar-day was defined as a continuous or non-continuous period of 24 h when the temperatures were between 1°C and 35°C. These temperatures reflect the lower and upper thresholds of growth of *X. arboricola* pv. *juglandis* colonies, isolated from Californian orchards, under controlled conditions (Miller and Bollen, 1946), To illustrate the calculation, if the temperature dropped below 1°C for 2 h in a 24 h period, then one adjusted-calendar-day was equivalent to a total time of 26 h. Each environmental variable for each site-year and cultivar was cumulated for an interval of 8 adjusted-calendar-days prior to disease assessment, for seven specific intervals according to the series 1 to 8, 5 to 12, 9 to 16, and so on, until 25 to 32 adjusted-calendar-days prior to the assessment. An interval of 8 adjusted-calendar-days was selected in favour of 1 to 7 and 9 day intervals as this period provided the strongest relationship between PNB and each environmental

variable. Environmental data were cumulated for each interval prior to each assessment, with the final assessment occurring when fruits were predicted to reach half size i.e., between 55 and 67 days after budburst (Chapter 6, Table 6.5). Fruits of this size were more susceptible to infection with inoculation of *X. arboricola* pv. *juglandis* than older fruit (Chapter 5, Tables 5.6 and 5.7).

A series of linear regressions between PNB and each environmental variable were calculated for each 8-day interval to determine which 8-day interval best described the relationships, by recording the frequency of $P < 0.0001$, $0.0001 \geq P < 0.001$, $0.001 \geq P < 0.01$ to $0.01 \geq P < 0.05$ for the series of linear regressions associated with a particular 8-day interval. Linear regressions were assessed using the statistics described previously for the disease progress curves. A two-way table was constructed showing the frequency of each *P*-value for each 8-day interval, and the frequency then tested by chi-square statistical procedures. A significant chi-square test indicated an association between an 8-day interval and the frequency of significant relationships between PNB and each environmental variable.

Once statistically significant relationships were identified for each cultivar, analyses were then conducted to determine if parameter estimates (slope, intercept) of corresponding linear relationships for Vina and Franquette were significantly different at $P < 0.05$. If not, then linear models were developed by pooling the data for both cultivars.

8.3 RESULTS

8.3.1 Disease progression in relation to the weather.

Disease incidence was greatest in 2005−06 with nearly all fruits developing blight within 85 days of budburst (Fig. 8.1). The rate of change in disease incidence, or percent new blight per day (PNB), in Vina at Swansea increased from less than 2% to nearly 5% per day within 39 days of budburst in 2005−06 (Fig 8.2); at Forth, PNB increased from 2.3 to 3.3% per day within 49 days of budburst. In Franquette, PNB was estimated to be between 3 and 4% per day within 58 days of budburst at Forth and Swansea. Markedly more rainfall occurred in 2005−06 than other years, with up

to 389 and 178 mm within 85 days of budburst of Vina and Franquette, respectively (Table 8.1). For a specific cultivar in 2005−06, the number of days with rainfall was greater at Forth than Swansea; however, approximately half of all days within 85 days of budburst had rainfall, at either location.

Disease incidence was least in 2006−07, with less than 30% and 50% incidence in Vina and Franquette respectively (Fig. 8.1). Disease incidence increased with time in all epidemics except for Vina at Swansea, where maximum incidence occurred 72 days after budburst. PNB was predicted to be less than 1% per day, irrespective of location (Fig. 8.2). Markedly less rainfall occurred in 2006−07 than in other years (Table 8.1). In 2006−07, average minimum temperatures were generally lower and budburst was 1 to 2 weeks earlier than other years.

In 2004−05, the incidence of fruits with disease and the quantity, and number of days, of rainfall was in between those of other years (Fig. 8.1; Table 8.1). Disease incidence and rainfall was greater at Forth than at Swansea i.e., 85 and 75% incidence respectively, and 148 and 126 mm rainfall respectively. PNB was less than 1% per day at Swansea, and from 63 days after budburst at Forth (Fig. 8.2).

Maximum daily temperatures, for each cultivar and year, were approximately 5^{o}C higher at Swansea than at Forth; however, maximum temperatures never reached the upper developmental threshold of 35^{o}C for all 10 site-years (Table 8.1). The lower developmental threshold of 1^{o}C was exceeded in Vina at Swansea in all years, and in Vina and Franquette at Forth and Swansea, respectively, in 2006−07.

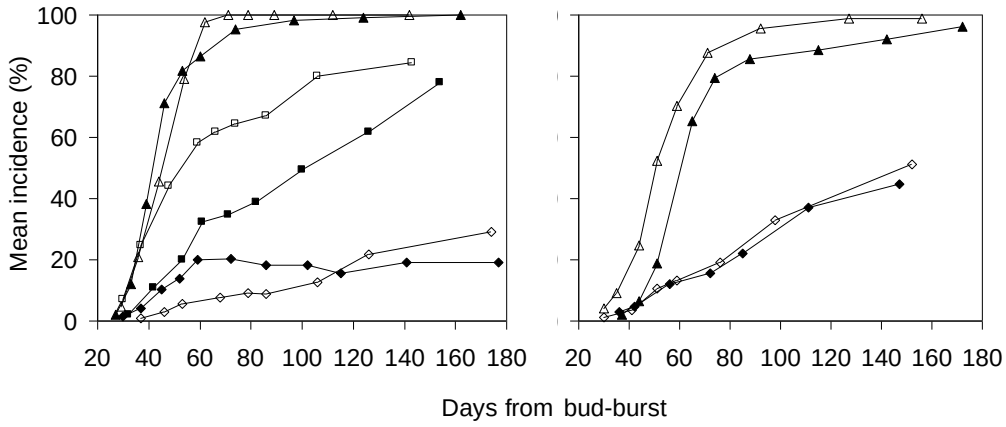

Fig. 8.1. Progression of disease incidence on non-treated Vina (left) and Franquette (right) fruits at Forth (open symbols) and Swansea (closed symbols) in 2004−05 (squares), 2005−06 (triangles) and 2006−07 (diamonds). Budburst occurred in Vina on the 18-Oct-04, 19-Oct-05 and 5-Oct-06 at Forth and the 8-Oct-04, 8-Oct-05 and 25-Sep-06 at Swansea, and in Franquette on the 8-Nov-05 and 1-Nov-06 at Forth and the 25-Oct-05 and 17-Oct-06 at Swansea. Each data point represents the mean of six replicates. Fig. 8.1 is reproduced, in part, from Figs. 6.1 and 6.2 (Section 6.3.1).

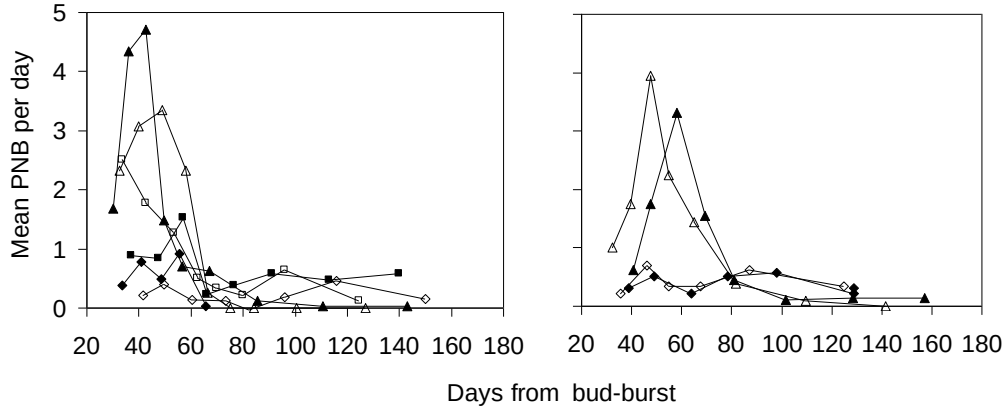

Fig. 8.2. Estimated rate of change in disease incidence, or the percent new blight (PNB) per day, on non-treated Vina (left) and Franquette (right) fruits at Forth (open symbols) and Swansea (closed symbols) in 2004−05 (squares), 2005−06 (triangles) and 2006−07 (diamonds). Each data point represents the mean of six replicates.

Table 8.1. Summary of rainfall and temperature data at Forth and Swansea from budburst to 85 days after budburst in Vina and Franquette for the years cited. Weather data recorded were cumulated daily rainfall and days with ≥ 0.2 mm rainfall, and the mean, minimum and maximum daily temperature.

Cultivar	Location	Year	Budburst	Total rainfall (mm)	Number of rain days (≥0.2 mm)	Temp. per day (°C)		
						Mean	Min.	Max.
Vina	Forth	2004−05	18-Oct-04	148	25	14.0	4.4	25.4
		2005−06	19-Oct-05	389	47	14.4	4.4	24.1
		2006−07	05-Oct-06	41	18	12.5	− 0.2	27.9
	Swansea	2004−05	08-Oct-04	126	23	13.5	− 0.9	29.8
		2005−06	08-Oct-05	239	37	14.0	0.8	29.8
		2006−07	25-Sep-06	21	13	12.4	− 0.2	27.9
Franquette	Forth	2005−06	08-Nov-05	150	43	14.6	4.1	24.7
		2006−07	01-Nov-06	62	14	15.2	1.1	25.8
	Swansea	2005−06	25-Oct-05	178	38	15.5	1.6	31.0
		2006−07	17-Oct-06	62	14	13.9	0.8	30.6

8.3.2 Modelling temporal disease progression.

The model of best fit for describing progression of disease incidence on fruit from budburst to harvest varied according to site-year (Table 8.2). The monomolecular or linear model was selected for epidemics in 2004−05 and 2006−07, with days after budburst accounting for between 93 and 99% of the variance in disease incidence. In 2005−06, the Gompertz and logistic models accounted for between 88 and 98% of temporal variance in incidence of disease.

The predicted time to reach 50% disease incidence varied dramatically between years, with 148 days, or more, required in 2006−07 (Table 8.2). In contrast, less than 43 and 67 days were required for Vina and Franquette, respectively, in 2005−06. In 2004−05, 50% disease incidence in Vina was predicted 57 and 98 days after budburst at Forth and Swansea respectively.

The weighted mean absolute rate (WMAR) increase in disease incidence in 2005−06 was 0.037 and 0.015 per day in Vina and Franquette, respectively, significantly more than in other years (Table 8.3). There was no difference in the WMAR between locations in either Vina or Franquette.

Table 8.2. Model of best fit for describing temporal progression of walnut blight incidence and the predicted number of days from budburst to reach 50% disease incidence (inc.) in Vina and Franquette at 10 site-years.

Cultivar **Site**	**Year**	**n**	**Model** [Z]	R^{*2Y}	R^{2X}	***P-level***	**Intercept**	**Slope ($\times 10^2$)**	**Predicted days to 50% inc.**
Vina									
Forth	2004−05	9	Monomolecular	94.2	94.4	< 0.0001	− 0.198	1.569	57
	2005−06	6	Logistic	97.7	91.3	0.0029	− 11.183	26.062	38
	2006−07	9	Linear	96.8	96.8	< 0.0001	− 0.065	0.200	−
Swansea	2004−05	9	Monomolecular	92.9	92.2	< 0.0001	− 0.405	1.120	98
	2005−06	10	Gompertz	96.2	96.0	< 0.0001	− 2.618	6.946	43
	2006−07	6	Monomolecular	93.9	93.0	0.0019	− 0.154	0.571	148
Franquette									
Forth	2005−06	9	Gompertz	97.1	92.8	< 0.0001	− 2.110	4.825	51
	2006−07	7	Monomolecular	98.8	99.1	< 0.0001	− 0.199	0.597	149
Swansea	2005−06	9	Gompertz	88.4	84.9	0.0004	− 1.803	3.220	67
	2006−07	7	Monomolecular	98.3	98.2	< 0.0001	− 0.175	0.530	164

[Z]data in Table 8.2 was reported, in part, in Table 6.4 (Section 6.3.4) and Table 7.2 (Section 7.3.1); however, linear, exponential, monomolecular, logistic and Gompertz models were analysed for all site-years in Table 8.2 in contrast to those reported previously. [Y] coefficient of determination for agreement between observed and back-transformed predicted disease incidence. [X] coefficient of determination for agreement between observed and transformed predicted disease incidence.

Table 8.3. Weighted mean absolute rate (WMAR) of increase in walnut blight on non-copper treated Vina and Franquette fruits at Forth and Swansea, and Vina and Franquette in the years specified.

Cultivar	Factor	n	WMAR [z] (ρ value)	
Vina	*Location*			
	Forth	18	0.018	a
	Swansea	18	0.014	a
	Year			
	2004−05	12	0.008	a
	2005−06	12	0.037	b
	2006−07	12	0.003	a
Franquette	*Location*			
	Forth	12	0.011	a
	Swansea	12	0.008	a
	Year			
	2005−06	12	0.015	b
	2006−07	12	0.005	a

[z] For each cultivar, means within location or year followed by different letters are significantly different at $P < 0.05$

8.3.3 Relationship between PNB and each environmental variable

A significant association ($X^2 = 34.11$, df = 18, $P = 0.01$) between 8-day intervals and the frequency of various *P* values from linear regressions between PNB and environmental variables was present in Franquette (Table 8.4). The 17 to 24 and 21 to 28 adjusted-calendar-day intervals in Franquette had the greatest frequency of significant regressions i.e., four each, whereas the 25 to 32 intervals had the least. In Vina, 8-day intervals and the frequency of various *P* values were not independent ($X^2 = 11.55$, df = 12, $P = 0.48$); however, a total of three regressions were significant at $0.001 \geq P < 0.01$ for each of the 13 to 20, 17 to 24 and 25 to 32 day intervals.

When Vina and Franquette data were combined, the 17 to 24 adjusted-calendar-day interval had the greatest frequency of regressions significant at $P < 0.05$, and was the only period where regressions were significant at $P < 0.0001$ (Table 8.4). Hence, the 17 to 24 adjusted-calendar-day interval was selected to model the relationship between environmental factors and PNB.

Table 8.4. Count and percent frequency (in parenthesis) of various *P* values from linear regressions between percent new blighted (PNB) fruit per day and environmental variables cumulated for intervals of 1−8, 5−12, 9−16,… 25−32 adjusted-calendar-days prior to disease assessment for10 site-years.

Cultivar	**Adjusted-calendar-day intervals before disease assessment**							**Row**
P−values	**1−8**	**5−12**	**9−16**	**13−20**	**17−24**	**21−28**	**25−32**	**total**
Franquette								
$0.01 \geq P < 0.05$	2 (11)	0	3 (16)	1 (5)	1 (5)	0	1 (5)	8
$0.001 \geq P < 0.01$	0	2 (11)	0	2 (11)	0	2 (11)	0	6
$0.0001 \geq P < 0.001$	0	0	0	0	0	2 (11)	0	2
$P < 0.0001$	0	0	0	0	3 (16)	0	0	3
Total (P < 0.05)	*2*	*2*	*3*	*3*	*4*	*4*	*1*	*19*
Vina								
$0.01 \geq P < 0.05$	1 (6)	1 (6)	0	0	1 (6)	0	0	3
$0.001 \geq P < 0.01$	1 (6)	0	1 (6)	3 (18)	3 (18)	2 (12)	3 (18)	13
$0.0001 \geq P < 0.001$	0	0	0	0	0	0	1 (6)	1
Total (P < 0.05)	*2*	*1*	*1*	*3*	*4*	*2*	*4*	*17*
Franquette + Vina (Total P < 0.05)	*4*	*3*	*4*	*6*	*8*	*6*	*5*	*36*

For the 17 to 24 adjusted-calendar-day interval, regressions for the six site-years in Vina were significant for moisture intensity ($P = 0.010$, $R^2 = 33.0\%$), rainfall ($P = 0.043$, $R^2 = 22.0\%$), minimum temperature ($P = 0.003$, $R^2 = 42.5\%$) and mean temperature ($P = 0.003$, $R^2 = 41.3\%$). Large studentized residual values were identified with rainfall data from Swansea in 2005−06, indicating potential outlying and influential observations; these data are highlighted as star symbols in Figs. 8.3

and 8.4. When comparing regressions with and without data from Swansea in 2005−06, there was no significant difference in the slopes and intercepts for moisture intensity ($P = 0.86$, $P = 0.33$ respectively), rainfall ($P = 0.72$, $P = 0.34$ respectively), minimum temperature ($P = 0.60$, $P = 0.40$ respectively) and mean temperature ($P = 0.48$, $P = 0.77$ respectively). Hence, data from five site-years was used to model environmental factors and PNB in Vina.

Moisture intensity accounted for greater than 84% of the variance of PNB per day in Vina and Franquette (Table 8.5). Daily rainfall and the number of days when rainfall occurred were also significant factors in disease development, accounting for 60 to 69% and 31 to 71% of the variance in PNB per day, respectively.

Mean and minimum daily temperatures were significantly correlated to PNB per day in Vina, accounting for 36 and 55% of the variance respectively (Table 8.5). Minimum temperature also accounted for 25% of the variance in PNB in Franquette.

Maximum daily temperature, mean daily RH, the duration per day with RH of 90 to 100% and the duration per day of surface wetness were not significantly correlated to development of new disease in Vina and Franquette (Appendix 8, Table A8.1).

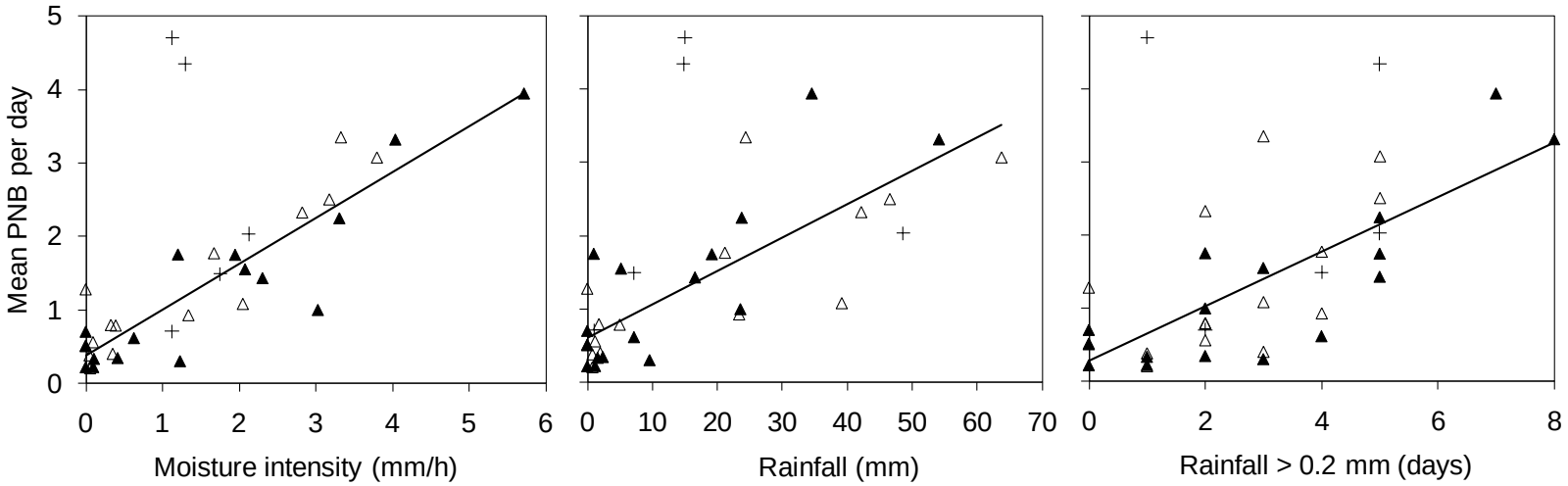

Fig. 8.3. Mean percent new blight (PNB) per day on Vina (open symbols) and Franquette (closed symbols) fruits as a function of the moisture intensity (mm rainfall/h of surface wetness during and after rainfall), rainfall (mm) and days with ≥ 0.2 mm rainfall, cumulated from 17 to 24 adjusted-calendar-days prior to PNB for 10 site-years. Linear models represent pooled Vina and Franquette data for nine-site years, after removal of Vina data from Swansea in 2005−06 (crosses). Each data point represents the mean of six replicates. Model parameters are listed in Table 8.5.

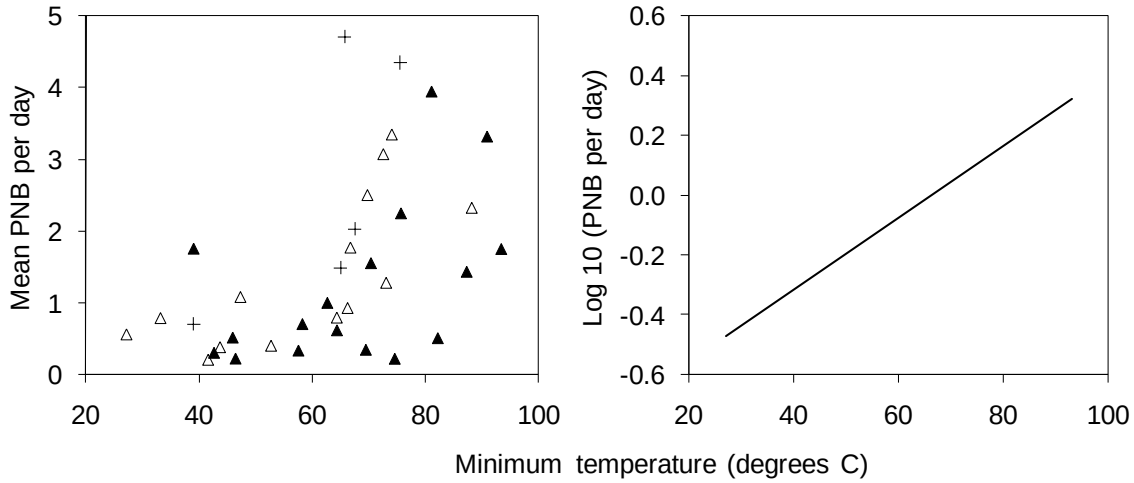

Fig. 8.4. Mean percent new blight (PNB) per day on Vina (open symbols) and Franquette (closed symbols) fruits as a function of the minimum temperature (^{o}C), cumulated from 17 to 24 adjusted-calendar-days prior to PNB for 10 site-years. Linear model represents pooled Vina and Franquette data for nine-site years, after removal of Vina data from Swansea in 2005−06 (crosses). Each data point represents the mean of six replicates. Model parameters are listed in Table 8.5.

Table 8.5. Regression parameters for percent new blight per day (PNB) of Vina and Franquette fruits, and pooled Vina and Franquette fruits, as a function of environmental variables cumulated from 17 to 24 adjusted-calendar-days (Tmin = 1°C Tmax = 35°C) prior to disease assessments for nine site-years.

Cultivar	Environmental variable	n	PNB (y)	Intercept	Slope	R^2	*P−value*
Vina	Mean temp. (°C)	14	$\log_{10}(y)$	− 1.9252	0.0189	35.8	< 0.05
	Minimum temp. (°C)	14	$\log_{10}(y)$	− 0.8892	0.0154	55.4	< 0.01
	Rainfall (mm)	14	y	0.6530	0.0379	60.2	< 0.01
	Rainfall ≥ 0.2 mm (days)	14	y	0.3674	0.3872	31.2	< 0.05
	Moisture intensity (mm/h)	14	y	0.4427	0.6808	85.0	< 0.0001
Franquette	Minimum temp. (°C)	17	$\log_{10}(y)$	− 0.8795	0.0119	25.2	< 0.05
	Rainfall (mm)	17	y	0.5107	0.0604	69.0	< 0.0001
	Rainfall ≥ 0.2 mm (days)	17	y	0.1689	0.3731	70.9	< 0.0001
	Moisture intensity (mm/h)	17	y	0.3020	0.5988	83.7	< 0.0001
Pooled	Minimum temp. (°C)	31	$\log_{10}(y)$	− 0.8007	0.0120	30.4	< 0.01
	Rainfall (mm)	31	y	0.6043	0.0455	61.4	< 0.0001
	Rainfall ≥ 0.2 mm (days)	31	y	0.2735	0.3738	53.4	< 0.0001
	Moisture intensity (mm/h)	31	y	0.3797	0.6242	82.5	< 0.0001

There was no significant difference in the slopes and intercepts of regressions between Vina and Franquette for moisture intensity ($P = 0.12$, $P = 0.46$ respectively), rainfall ($P = 0.44$, $P = 0.11$ respectively), rain days ($P = 0.39$, $P = 0.93$ respectively) and minimum temperature ($P = 0.09$, $P = 0.61$ respectively). Hence, data from Vina and Franquette was pooled to model environmental factors against PNB.

Of the four environmental variables modeled, moisture intensity accounted for the highest variance in PNB per day i.e., 83% (pooled data) (Table 8.5). The equation is:

$$PNB = [(0.6242 \times MI) + 0.3799] \text{ (eq. 8.2)},$$

where MI = daily rainfall (mm) / daily surface wetness during and after rainfall (h) cumulated for the previous 17 to 24 adjusted-calendar-days. The maximum moisture intensity cumulated from 17 to 24 days was 6 and 4 mm/h in Franquette and Vina respectively (Fig 8.3).

Daily rainfall and the number of days when rainfall occurred were also significant factors in disease development, accounting for 61 and 53% of the variance in PNB per day, respectively (Table 8.5). The maximum quantity of rainfall cumulated was 64 mm for Vina and 54 mm for Franquette (Fig 8.3). Franquette had a greater range in the number of days where rainfall occurred i.e., from 0 to 8 days in comparison to 0 to 5 days in Vina.

The maximum increase in PNB per day was similar in Vina and Franquette (Fig 8.3); however, PNB was observed three times in Franquette, and once in Vina without factors associated with rainfall being recorded.

Minimum daily temperatures in Vina and Franquette accounted for 30% of the variance in PNB per day (Table 8.5), with cumulated minimum temperatures ranging between approximately 25 and 95^{o}C for both cultivars (Fig. 8.4).

Multiple regressions of independent variables from these models were not attempted because of the high level of correlation between the independent variables (Table 8.6).

Table 8.6. Pearson product moment correlations between + 1 and − 1, and the *P* value of the statistical significance of the estimated correlations (in parenthesis), between each pair of variables of pooled Vina and Franquette data for nine site-years. For each pair of variables, 31 pairs of data values were used to compute each coefficient.

	Constant	**Moisture intensity (mm/h)**	**Rainfall (mm)**	**Days with rainfall (≥ 0.2 mm)**	**Minimum temp. (^{o}C)**
Constant	1.00	–	–	–	–
Moisture intensity (mm/h)	0.91 (*P*<0.0001)	1.00	–	–	–
Rainfall (mm)	0.78 (*P* <0.001)	0.85 (*P* <0.0001)	1.00	–	–
Days with rainfall (≥ 0.2 mm)	0.73 (*P* <0.0001)	0.81 (*P* <0.0001)	0.73 (*P* <0.0001)	1.00	–
Minimum temp. (^{o}C)	0.56 (*P* <0.01)	0.54 (*P* <0.01)	0.48 (*P* <0.01)	0.47 (*P* <0.01)	1.00

8.3.4 Relationship between fruit and fruit cluster disease incidence

A significant relationship between percent disease incidence of individual fruits and fruit clusters occurred at all site-years. In 2004−05 at Forth, the rate at which percent disease increased in Vina fruits and clusters was similar, such that 95% disease incidence of fruits was predicted with 100% disease incidence of clusters (Table 8.7). At Swansea, the rate of disease incidence of fruits increased with increasing disease of clusters, such that 89% disease incidence of fruits was predicted with 100% of clusters diseased. In 2005−06, in three of four epidemics the disease incidence of individual fruits increased exponentially with increasing disease incidence of clusters i.e., in both cultivars at Forth and in Vina at Swansea, such that a near 100% disease incidence of fruits were predicted. The rate at which percent disease increased in Franquette fruits and clusters was similar at Swansea in 2005−06. In 2006−07, the percent disease incidence of individual fruits increased at a rate of between 0.6% and 0.9% for every 1.0% increase in disease incidence of clusters; at theses four sites, the maximum disease incidence predicted in fruits was from 57 to 71% and from 82 to 85% in Vina and Franquette respectively.

Table 8.7. Summary of regressions between percent disease incidence of fruits and clusters, between 39 and 46 days after budburst, or approximately 10 mm fruit size, and harvest at 10 site-years.

Cultivar ***Location***	**Year**	**n**	**Predicted disease incidence of fruit (Y)**	**Intercept**	**Slope**	R^2	***P*-value**	**Predicted fruit incidence at 100% cluster incidence**
Vina								
Forth	2004−05	9	Y	− 8.4037	1.0389	99.5	< 0.0001	95
Forth	2005−06	4	ln(Y)	2.7472	0.01823	98.9	< 0.01	97
Forth	2006−07	7	Y	− 2.9598	0.7389	99.9	< 0.0001	71
Swansea	2004−05	8	√(Y)	1.7954	0.07632	99.7	< 0.0001	89
Swansea	2005−06	8	ln(Y)	2.3967	0.02200	99.6	< 0.0001	99
Swansea	2006−07	4	Y	− 0.5480	0.5707	98.5	< 0.01	57
Franquette								
Forth	2005−06	6	ln(Y)	2.9475	0.01638	99.2	< 0.0001	98
Forth	2006−07	5	Y	− 3.8125	0.8609	99.8	< 0.0001	82
Swansea	2005−06	7	Y	− 13.9035	1.0991	99.7	< 0.0001	96
Swansea	2006−07	5	Y	− 3.5029	0.8847	99.8	< 0.0001	85

8.4 DISCUSSION

The development of walnut blight was markedly different between the years, with the linear, monomolecular, logistic and Gompertz models describing the temporal progression of disease incidence on fruit in Tasmania. These results suggest potential differences among epidemics in the timing and/or extent of bacterial multiplication and dispersal which warrant further investigation. Interestingly, in one epidemic (Fig. 8.1, Swansea 2006−07), disease progression increased rapidly after budburst and then reduced with time; this anomaly, however, may have been because of misdiagnosis of frost damage to fruits, as symptoms of walnut blight have been confused with damage caused by frosts during the flowering period of staminate flowers, and shortly thereafter (Miller and Bollen, 1946).

This study found that factors related to rainfall and temperature were the most important environmental variables involved in fruits becoming diseased in Tasmania. Increasing moisture intensity, quantity of rainfall, the number of days with rainfall or minimum temperatures, were strongly correlated to increased disease incidence in Vina and Franquette in the two locations and three growing years studied. Rainfall has also been implicated with the development of walnut blight in California (Miller and Bollen, 1946), and in diseases caused by other *Xanthomonas* species, such as sugarcane leaf scold (Champoiseau *et al*., 2009), leaf blight of onion (Schwartz *et al*., 2003) and citrus bacterial spot (Gottwald *et al*., 1992). In contrast, relative humidity and the duration of surface wetness of host tissue were not related significantly to disease development of walnut fruits in Tasmania.

The causal pathogen of walnut blight, *X. arboricola* pv. *juglandis*, primarily overwinters in buds and catkins (Miller and Bollen, 1946; Esterio and Latorre, 1982; Mulrean and Schroth, 1982), as reviewed in Chapter 3. In Californian orchards, Lindow *et al*. (2004) observed that prior to budburst the outermost scales and cataphylls of buds were uniformly colonised with *X. arboricola* pv. *juglandis* cells, whereas the innermost embryonic leaves and meristematic tissue were seldom colonised. In contrast, within 3 weeks of budburst nearly all leaves were infected with the pathogen, and fruits were colonised with *X. arboricola* pv. *juglandis* shortly

after their formation, suggesting that centripetal movement of *X. arboricola* pv. *juglandis* from the outermost portion of dormant buds leads to the colonisation of internal bud parts during shoot development. As bacteria are easily suspended and transported onto healthy host tissue with rain splash (Stall *et al.*, 1993), the movement of inoculum from bud to shoots, flowers and fruit will influence the likelihood of infection for a given amount of inoculum within the bud. Thus, factors such as rainfall are likely to promote dispersal of bacteria to healthy walnut tissues.

Increasing minimum temperature was positively correlated to the development of new disease symptoms in Vina and Franquette fruit in Tasmania. Furthermore, mean daily temperature was also correlated to an exponential increase of new disease in Vina. In contrast to these findings, in studies conducted in glasshouses with temperatures ranging from 5^{o}C to 27^{o}C, the temperature at the time of, and following, inoculation of Franquette fruits with *X. arboricola* pv. *juglandis* did not affect the percent infection of fruits (Miller and Bollen, 1946); however, the infection of walnut leaves was influenced by temperature, with the incidence of infected leaves increasing as temperatures increased from 4^{o}C to 30^{o}C. In citrus leaves, the multiplication of *Xanthomonas citri* occurs in two phases; initial and secondary growth (Koizumi, 1976a, b). Initial growth occurs at 6^{o}C, causing mild decomposition of the cell walls with the establishment of bacteria within the plant, whereas secondary growth begins at 13^{o}C and occurs after the degeneration of the plasmalemma and tonoplast adjacent to causal bacteria (Koizumi, 1977). Thus, the increase of new disease in walnut fruits may have occurred as a function of the influence of temperature on *X. arboricola* pv. *juglandis* multiplication *in planta*, although this relationship has not been studied in walnuts.

In this study, the period that best described the relationship between the development of new disease in fruits and environmental conditions was 17 to 24 adjusted-calendar-days prior to disease assessment, when factors such as rainfall or temperature were conducive to disease development. Presumably this interval represents a potential time when bacteria are dispersed to healthy tissue, followed by multiplication of the bacterium in the infection court before new symptoms become visible. Similarly, in Californian orchards the development of walnut blight was

correlated to favorable environmental conditions 15 to 21 days prior to the appearance of disease in non-copper treated Ashley and Vina fruits (Adaskaveg *et al*., 1999). However, new disease in Tasmania was predicted on some occasions when rainfall was not recorded in the 17 to 24 day period, and may have been the consequence of rainfall occurring before or after this period. These models were developed empirically using data collected from orchards in Tasmania; as such, examining the effect of environmental conditions on disease development under controlled conditions may define these relationships further.

This study found that the incidence of disease was greater in fruit clusters than on individual fruits in the early stages of an epidemic. However, in half of the epidemics, as the epidemic progressed the rate of increase in disease incidence was greater in individual fruits than in clusters, such that at high levels of disease the incidence of disease in fruits and clusters were similar. On a spatial scale, walnut fruits are generally in contact with each other within a cluster, and except for infrequent situations where fruit clusters are in contact, fruits are always closer within clusters than between clusters. Bacterial multiplication in the intercellular space of host tissues often leads to a physical pressure so that bacterial masses emerge from the substomatal cavities, and serve as secondary inoculum. For example, bacterial cells were shown, with scanning electron microscopy, to be disseminated from the stomata of Franquette fruits after inoculation with *X. arboricola* pv. *juglandis* (Garcin *et al*., 2001). Similarly, Miles *et al*. (1977) observed masses of *X. arboricola* pv. *pruni* being pressed out of the stomata of peach leaves 9 days after inoculation. Hence, the disease status of fruits within a cluster may influence the disease status of adjacent fruits within a cluster.

In summary, the incidence of walnut blight can vary markedly between cultivars, locations and years in Tasmania, with the development of damaging epidemics when conditions are favourable. Central to the approach for identifying and quantifying key factors involved in the development of epidemics has been temporal analyses of disease progression. As such, moisture intensity has been identified as a key factor in the development of walnut blight in Tasmania, and can be used to develop a moisture-based model for timing crop protection in Tasmanian walnut orchards.

DEVELOPMENT OF A RAINFALL-BASED PREDICTIVE MODEL FOR TIMING COPPER-BASED SPRAYS TO CONTROL WALNUT BLIGHT

9.1 INTRODUCTION

Outbreaks of walnut blight, caused by *Xanthomonas arboricola* pv. *juglandis*, are strongly influenced by weather conditions, the phenological development of cultivars, and the population of *X. arboricola* pv. *juglandis* overwintering in walnut buds. In Tasmania, rainfall from budburst to half fruit size has been identified as a critical factor for the development of walnut blight epidemics (Chapter 8).

The current management strategy for walnut blight is based on application of copper-based biocides for protecting susceptible leaves and fruit, especially when growth of shoots is rapid in spring. In spray timing trials in Tasmania, two copper-based sprays, applied one week apart from 5% terminal budburst provided sufficient control of blight in an epidemic where temporal disease incidence was defined by the monomolecular model (Chapter 7); however, multiple budburst and in-season spray applications were required in an epidemic defined by the logistic model. The results suggested scope for reducing the number of copper-based sprays, by utilizing weather and crop phenology for calculating the optimum time to apply crop protection strategies.

In California, the walnut blight forecasting system, XanthoCast™ (Adaskaveg *et al.,* 2000), utilises wetness period duration and temperature for calculating the daily and cumulated daily risk of disease for determining when to apply disease protection. However, field trials where copper-based sprays were timed according to XanthoCast™ did not adequately control disease in Tasmania (Chapter 7), possibly because surface wetness rather than rainfall was used to determine the threshold disease risk for spray applications. While XanthoCast™ worked well in the region in which it was developed, different model parameters will be required for walnuts grown in Australia. Hence, the aim of this study was to develop, and commence validation of, a moisture-based predictive model for timing crop protection sprays for the control of walnut blight in Tasmanian orchards.

9.2 EXPERIMENTAL PROCEDURE

9.2.1 Model development.

The economic threshold for crop loss associated with walnut blight in a mature orchard in Tasmania has been reported previously (Chapter 7). In summary, net income gain is predicted when disease incidence on half size fruits was predicted to be 0.5% after one copper-based spray; however, further sprays result in net income loss as control costs are greater than crop income derived from yield gains.

Percentages for disease incidence from here on relate to the predicted percentage at half fruit size. To determine an action threshold at which management should implement copper-based sprays, risk algorithms and economic injury levels (EIL), between 0.5 and 1.0% incidence, were used to retrospectively predict the need or otherwise for crop protection at Forth and Swansea in 2004−05, 2005−06 and 2006−07. These predictions were then compared to observed requirements for crop protection for these site-years, and the EIL that was most appropriate for predicting the requirement for crop protection, selected as the management action threshold. Following is a more detailed explanation of this process.

Moisture intensity was incorporated into a risk algorithm to predict the need for crop protection, as this factor was directly related to percentage new blighted fruit per day (PNB), from budburst to half fruit size (Chapter 8). The equation is:

$$PNB = [(0.6242 \times MI) + 0.3799] \text{ (eq. 8.2)},$$

where MI = daily rainfall (mm) / daily surface wetness during and after rainfall (h) cumulated for the previous 17 to 24 adjusted-calendar-days (Tmin = 1^{o}C, Tmax = 35^{o}C) for data pooled for nine site-years for Vina and Franquette (Chapter 8). The PNB model was then used to define the critical risk value (CRV) to predict the need for crop protection measures. The equation of the CRV is:

$$CRV = [(0.6242 \times \sum MI_{8\text{-days}}) + 0.3799] \text{ (eq. 9.1)}$$

where CRV is calculated by summing MI values for the previous 8 days. The CRV for 8-day intervals for each site-year, from budburst onwards, was then compared to

an EIL of 0.5, 0.75, and 1.0% incidence, and classified either as a week that required crop protection (CRV ≥ EIL) or a week that did not require protection (CRV < EIL).

Results from spray timing trials for three site-years (2004−05 to 2006−07) at Forth were used to ascertain if a protective copper-based spray had been required, in any given week, in epidemics where temporal disease incidence was defined by linear, monomolecular and logistic models, to reduce blight incidence significantly below that observed in non-treated control (Chapter 7). Those weeks where crops required protection were called *cases* and those weeks that did not were called *controls*.

The predicted requirement for crop protection for each 7-day interval at Forth and Swansea, based on the CRV at each EIL, was then compared to the observed requirement for crop protection as defined above. The predicted requirement for crop protection was classified as a true positive (TP), true negative (TN), false positive (FP) or false negative (FN) prediction, using methods described by Madden *et al*. (2007). Each category was then expressed as a proportion (P), and the overall accuracy for each EIL (Madden *et al*., 2007), described by:

TPP = TP / (number of *cases*) (eq. 9.2)

TNP = TN / (number of *controls*) (eq. 9.3)

FPP = 1 − TNP (eq. 9.4)

FNP = 1 − TPP (eq. 9.5)

Overall accuracy (%) = {[TPP × proportion of *cases*] + [TNP × proportion of *controls*]} × 100 (eq. 9.6)

Disease incidence at half fruit size leads to premature fruit drop in Tasmania (Chapter 6); hence, economic losses are incurred when crop protection is not predicted when protection is required (FNP). However, applying crop protection when it is not required (FPP) incurs control costs that are not compensated with increased yield. Thus, an EIL that minimised FNP and FPP was used as an arbitrary criterion for selecting the management action threshold.

9.2.2 Model verification

Verification of the threshold CRV for management action consisted of comparing the efficacy of various spray times in field trials at Forth and Swansea in 2008-09 (Table 9.1). To enhance the ease of application of CRV for disease management, equation 9.1 was multiplied by 133.33 so that crop protection sprays are applied at a CRV of 100. Hence, the equation of the forecaster is:

$$CRV_{100} = \{[(0.6242 \times \sum RI_{8\text{-days}}) + 0.3799] \times 133.33\} \text{ (eq. 9.7)}$$

where CRV_{100} is calculated by summing RI values from the previous 8 days (as per equation 9.1).

Table 9.1. Number of copper-based spray treatments, timed according to calendar (7 day interval), weather (model) and combined calendar and weather-based (calendar then model) spray regimes at Forth and Swansea. A dash indicates that the particular treatment was not undertaken at that site.

Days from budburst in which sprays applied (day 0 = budburst) [Z]	Decision on spray timing	Number of sprays	
		Forth	Swansea
non-treated	–	0	0
0	at burdburst	–	1
0–7	7 day interval	2	2
0–14	7 day interval	–	3
0–21	7 day interval	4	–
7–14	7 day interval	–	2
X	7 day interval	8	5
0 then X [Y]	7 day interval then model	–	6
0–7 then X [Y]	7 day interval then model	9	7
0–14 then X [Y]	7 day interval then model	–	8
0–21 then X [Y]	7 day interval then model	9	–
7–14 then X [Y]	7 day interval then model	–	7
0– X [Y]	commercial	9	8

[Z] Budburst (day 0) = 18-Oct-08 and 10-Oct-08 at Forth and Swansea respectively. [Y] X = 61 and 68 days in Forth and Swansea respectively

Trial plots were selected from an area of commercial hedgerow orchard with uniform tree growth. All trees had canopies of approximately 50 m^3 when in full leaf and were grown under similar cultural management.

Treatments of the copper-mancozeb formulation, Mankocide® DF (Du Pont (Australia) Limited, North Sydney, NSW), were applied to single-tree plots of cultivar Vina, replicated six times, that were arranged randomly in whole blocks. Initial sprays commenced at budburst, where specified, with the total number of applications varying per treatment.

Mankocide® DF was applied at a rate of 500 g/100 l in a spray volume of 1,000 l/ha using a calibrated air sheer backpack mister (Stihl SR 400; Stihl Incorporated, Virginia Beach, United States). All Mankocide® DF treatments were prepared in rainwater; surfactants or penetrants were not included in any treatment.

9.2.3 Disease assessments and environmental sensors

Disease incidence was assessed on the fruits of each single-tree plot, and rainfall and surface wetness duration for determining CRV were positioned at trials sites, as per methods described in the General Materials and Methods (Chapter 4). Data were collected at 1 min intervals for each trial.

9.2.4 Data analysis

For each plot of non-treated fruits, cumulative disease incidence was calculated from budburst until harvest at each site and then fitted to linearised forms of the linear, exponential, monomolecular, logistic and Gompertz models (Campbell and Madden, 1990). The most appropriate model for describing disease progression was selected by methods described in Campbell and Madden (1990) (Chapter 7).

The standardized area under the disease progress curve (SAUDPC) for cumulative disease incidence per plot, and the weighted mean absolute rate (WMAR) for non-treated plots at each location, were calculated and subjected to ANOVA as described in Chapter 8.

Observed crop yield was calculated as the percent of tagged fruits that remained on trees at packing tissue brown fruit stage. To determine treatment effects, crop yields were analysed by one-way ANOVA with blocks, with mean separation at $P < 0.05$.

9.3 RESULTS

9.3.1 Model development

At an EIL of 0.5% the need for spraying was correctly predicted for 26 of 32 weeks (Tables 9.2 and 9.3); however, on 17 occasions sprays were predicted when they were not required. At an EIL of 0.75%, the requirement for crop protection was correctly predicted on 24 occasions, and not predicted when required on eight occasions i.e., seven occasions for epidemics defined by linear and monomolecular models, and on one occasion for an epidemic defined by the logistic model. At an EIL of 1.0%, 21 sprays were correctly predicted when required; hence, on 11 occasions crop protection was not predicted when required.

The overall accuracy for correctly predicting the need for applying, or not applying, crop protection increased from 62 to 75% (Table 9.4) as the EIL increased from 0.5 to 1.0%. The proportion of false positive (FPP) predictions i.e., crop protection is predicted when it is not required, was greatest at 0.5% and least at 1.0%; in contrast, the proportion of false positive (FNP) predictions was least at 0.5% and greatest at 1.0%. The FPP and FNP predictions at 0.75% were between those of 0.5 and 1.0%.

As a management action threshold, an EIL of 1.0% incidence was considered to be inappropriate as 0.34 proportions of predictions were false negatives (FNP) (Table 9.4); similarly, an EIL of 0.5% was considered inappropriate as 0.61 proportions of predictions were false positives (FPP). Hence, an EIL of 0.75% incidence was selected as the action threshold for a mature walnut orchard in Tasmania.

After sprays were applied, daily MI data and 8-day CRV_{100} were calculated as previously described; however, there was a minimum of 7 days between spray applications, irrespective of whether a CRV of 100 was reached beforehand, to comply with permit regulations for the use of copper-based biocides in Australian

walnut orchards (Permit number PER9391, Australian Pesticides and Veterinary Medicines Authority, ACT, Australia).

Table 9.2. Critical risk value (CRV) for predicting the need for crop protection (0 = not required (no), 1 = required (yes)), at economic injury level's (EIL) of 0.5, 0.75 and 1.0%, and the observed need for crop protection (Spray needed), for each 7 day interval from budburst, based on epidemics defined by monomolecular and linear models (2004−05 and 2006−07) and logistic and Gompertz models (2005−06) at Forth and Swansea in each of 3 years.

Site (Year)	CRV[Z] per 7 day periods after budburst (day 0 = budburst)		CRV (%) ≥ EIL[Y] 0 = no 1 = yes			Spray needed[Y] 0 = no 1 = yes
	7-day period	CRV (%)	0.5 (%)	0.75 (%)	1.0 (%)	
Forth						
(2004−05)	0-6	0.42	0	0	0	1
	7-13	1.53	1	1	1	1
	14-20	2.37	1	1	1	1
	21-27	1.57	1	1	1	0
	28-34	0.58	1	0	0	0
	35-41	0.38	0	0	0	0
	42-48	0.66	1	0	0	0
	49-55	0.93	1	1	0	0
	56-62	0.92	1	1	0	0
	63-70	0.38	0	0	0	0
(2005−06)	0-6	3.46	1	1	1	1
	7-13	2.41	1	1	1	1
	14-20	3.47	1	1	1	1
	21-27	1.42	1	1	1	1
	28-34	0.55	1	0	0	1
	35-41	3.79	1	1	1	1
	42-48	2.87	1	1	1	1
	49-55	4.33	1	1	1	1
	56-62	2.05	1	1	1	1
	63-70	2.29	1	1	1	1
(2006−07)	0-6	0.76	1	1	0	1
	7-13	0.58	1	0	0	1
	14-20	0.48	0	0	0	1
	21-27	0.42	0	0	0	0
	28-34	0.59	1	0	0	0
	35-41	1.05	1	1	1	0
	42-48	0.43	0	0	0	0
	49-55	0.38	0	0	0	0
	56-62	0.38	0	0	0	0
	63-70	0.45	0	0	0	0

Site (Year)	**CRV[Z] per 7 day periods after budburst (day 0 = budburst)**		**CRV (%) ≥ EIL[Y] 0 = no 1 = yes**			**Spray needed[Y] 0 = no 1 = yes**
	7-day period	**CRV (%)[Y]**	**0.5 (%)**	**0.75 (%)**	**1.0 (%)**	
Swansea						
(2004−05)	0-6	0.38	0	0	0	1
	7-13	1.22	1	1	1	1
	14-20	1.65	1	1	1	1
	21-27	0.91	1	1	0	0
	28-34	1.27	1	1	1	0
	35-41	0.91	1	1	0	0
	42-48	0.38	0	0	0	0
	49-55	1.03	1	1	1	0
	56-62	0.63	1	0	0	0
	63-70	0.77	1	1	0	0
(2005−06)	0-6	2.64	1	1	1	1
	7-13	1.09	1	1	1	1
	14-20	1.24	1	1	1	1
	21-27	1.66	1	1	1	1
	28-34	1.21	1	1	1	1
	35-41	0.91	1	1	0	1
	42-48	1.29	1	1	1	1
	49-55	3.18	1	1	1	1
	56-62	1.44	1	1	1	1
	63-70	0.98	1	1	0	1
(2006−07)	0-6	0.38	0	0	0	1
	7-13	0.38	0	0	0	1
	14-20	0.41	0	0	0	1
	21-27	0.62	1	0	0	0
	28-34	0.44	0	0	0	0
	35-41	0.55	1	0	0	0
	42-48	0.59	1	0	0	0
	49-55	0.89	1	1	0	0
	56-62	0.38	0	0	0	0
	63-70	0.38	0	0	0	0

[Z] Refer to equation 9.1. [Y] Refer to section 9.2.1

Table 9.3. The numbers of true positive (TP), false positive (FP), false negative (FN) and true negative (TN) predictions at economic injury levels of 0.5, 0.75, and 1.0% incidence at Forth and Swansea in 2004−05, 2005−06 and 2006−07.

Economic injury level (%)	Predicted status	True status	
		Spray required (cases)	No spray required (controls)
0.5	Spray required	26 (TP)	17 (FP)
	No spray required	6 (FN)	11 (TN)
0.75	Spray required	24 (TP)	10 (FP)
	No spray required	8 (FN)	18 (TN)
1.0	Spray required	21 (TP)	4 (FP)
	No spray required	11 (FN)	24 (TN)
Number of cases and controls		32	28

Table 9.4. The true positive proportion (TPP), false positive proportion (FPP), false negative proportion (FNP), true negative proportion (TNP) and percent overall accuracy of TPP and TNP predictions at economic injury levels of 0.5, 0.75 and 1.0% incidence at Forth and Swansea in 2004−05, 2005−06 and 2006−07.

Economic injury level (%)	TPP[Z]	TNP[Y]	FPP[X]	FNP[W]	Overall accuracy[V] (%)
0.5	0.81	0.39	0.61	0.19	62
0.75	0.75	0.64	0.36	0.25	70
1.0	0.66	0.86	0.14	0.34	75

[Z] Refer to equation 9.2. [Y] Refer to equation 9.3. [X] Refer to equation 9.4. [W] Refer to equation 9.5. [V] Refer to equation 9.6

9.3.2 Model verification

9.3.2.1 Temporal progression of disease incidence

Disease incidence was greatest in Forth with nearly all fruits developing blight prior to harvest (Fig. 9.1). The timing of disease onset was sooner, and the progression of blight incidence more rapid, at Forth than at Swansea. The incidence of disease was best described by the monomolecular model at Swansea and the Gompertz model at

Forth, with time accounting for between 89 and 97% of the variance in disease incidence (Table 9.5). The rate of disease development was significantly different between locations, with WMAR values of 2.4×10^{-2} and 0.056×10^{-2} at Forth and Swansea respectively.

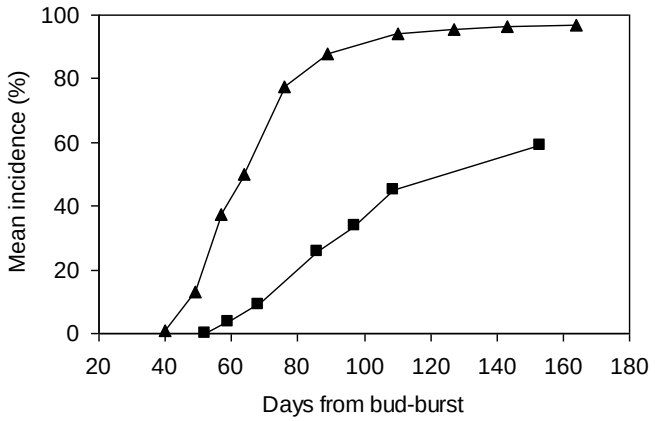

Fig. 9.1. Mean percentage disease incidence in non-treated Vina fruits from budburst at Forth (triangles) and Swansea (squares) in 2008-09. Budburst occurred on the 10-Oct-08 and 18-Oct-08 at Swansea and Forth respectively. Each data point represents the mean of six replicates.

Table 9.5. Summary of linear regression statistics used in the evaluation of linear, exponential, monomolecular, logistic and Gompertz models for appropriateness of describing progress of disease incidence, and the weighted mean absolute rate (WMAR) of disease increase, on non-treated Vina fruits at Forth and Swansea in 2008-09.

Location	n	Model	WMAR ($\times 10^2$) [Z]		R^{2} [Y]	Intercept	Slope ($\times10^2$)	Days 50% [X]
Forth	10	Gompertz	0.056	a	88.7	−2.811	4.478	71
Swansea	7	Monomolecular	2.354	b	97.1	−0.447	0.885	129

[Z] Means followed by different letters are significantly different at $P < 0.05$. [Y] Coefficient of determination. [X] Number of days (predicted) to 50% disease incidence. Disease incidence (y, expressed as a proportion) in the Gompertz and monomolecular models were transformed with the equations $y = -\ln[-\ln(y)]$ and $y = \ln[1/(1-y)]$ respectively.

9.3.2.2 Timing of copper-based sprays at Forth

Eight or more applications of copper-based sprays significantly reduced the progression of disease incidence in comparison to four or less sprays and non-treatment (Table 9.6). With non-treatment and with sprays applied two times from budburst, nearly all fruit were diseased approaching harvest (Fig. 9.2); in comparison, less than 40% of fruits had blight lesions when copper-based spray was timed according to the rainfall-based model only, a combined weekly and rainfall-based regime, and the weekly commercial spray schedule.

Four or more applications of copper significantly increased crop yield in comparison to two copper sprays and non-treated fruits (Table 9.6). Less than 28% of harvestable nuts were produced with non-treatment and with two sprays; in comparison, further weekly applications significantly increased crop yield with 60% nuts produced with four sprays. Crop yield on trees sprayed according to commercial and rainfall-based regimes were not significantly different from each other.

Table 9.6. Mean standardised area under the disease progress curve (SAUDPC) and mean percentage harvestable fruits (Crop yield) after treatment with copper between budburst and half fruit size at Forth in 2008-09. Initial treatments were applied from budburst (day 0) and timed according to commercial (Commercial), calendar (7 day interval) and/or a rainfall-based model (model).

Decision on spray timing	Days from budburst in which sprays applied (day 0 = budburst) [Z]	No. of sprays	SAUDPC (%−day) [Y]		Crop yield (%) [Y]	
Non-treated	−	0	71.5	a	21.7	a
7 day interval	0,6	2	64.8	a	27.5	a
7 day interval	0,6,14,20	4	38.5	b	60.4	b
Model	6,18,25,32,40,47,54,61	8	14.6	c	82.6	c
7 day interval then model	0,6 then 18,25,32,40,47,54,61	9	14.5	c	82.3	c
7 day interval then model	0,6,14,20 then 28,36,44,51,58	9	25.1	c	68.2	bc
Commercial	0,6,14,20,32,40,47,55,61	9	20.9	c	74.4	bc

[Z] Budburst sprays (day 0) were applied on the 18-Oct-08. [Y] Means followed by different letters are significantly different at $P < 0.05$.

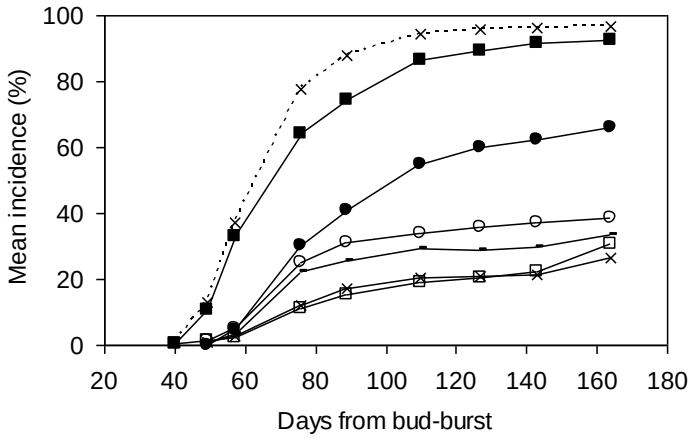

Fig. 9.2. Mean percentage disease incidence from budburst in non-treated Vina fruits (cross with dashed line) and in Vina fruits with copper applied according to the model only (cross with solid line), commercial (dash with solid line), weekly (closed symbols) and weekly and model (open symbols) spray regimes, at Forth in 2008-09. Treatments from budburst according to weekly spray regimes were applied a total of two (square) and four (circles) times, with or without subsequent sprays applied according to the model. Budburst occurred on the 18-Oct-08. Each data point represents the mean of six replicates.

9.3.2.3 Timing of copper-based sprays at Swansea

Copper-based treatments that were timed according to the rainfall-based model significantly reduced disease progression in comparison to non-treatment and budburst only sprays (Table 9.7). Nearly 60% of fruits had disease symptoms at harvest when non-treated or with up to three sprays during budburst (Fig. 9.3). With copper-based sprays timed according to the model only, a combined weekly and model spray regime, and a weekly commercial spray schedule, less than 20% of fruits had blight lesions at harvest.

A crop yield of 80%, or more, was produced with copper-based sprays timed according to the rainfall-based model only, a combined weekly and model spray regime and a weekly commercial spray schedule (Table 9.7); however, there was no significant difference in yield, irrespective of treatment.

Table 9.7. Mean standardised area under the disease progress curve (SAUDPC) and mean percentage harvestable nuts (yield) after treatment with copper between budburst and half fruit size at Swansea in 2008-09. Initial treatments were applied from budburst (day 0) and timed according to commercial (Commercial), calendar (7 day interval) and/or a rainfall-based disease model (model).

Decision on spray timing	Days from budburst in which sprays applied (day 0 = budburst) [Z]	No. of sprays	SAUDPC (%−day) [Y]		Crop yield (%) [Y]	
Non-treated	−	0	27.4	b	89.8	a
7 day interval	0	1	26.5	b	86.2	a
7 day interval	0,7	2	26.7	b	87.0	a
7 day interval	0,7,14	3	25.9	b	87.1	a
7 day interval	7,14	2	24.9	b	86.8	a
Model	28,35,42,52,68	5	2.9	a	89.8	a
7 day interval then model	0 then 28,35,42,52,68	6	4.0	a	91.9	a
7 day interval then model	0,7 then 28,35,42,52,68	7	3.4	a	89.1	a
7 day interval then model	0,7,14 then 28,35,42,52,68	8	3.1	a	89.8	a
7 day interval then model	7,14 then 28,35,42,52,68	7	3.2	a	90.2	a
Commercial	0,7,14,28,35,42,60,68	8	3.7	a	88.6	a

[Z] Budburst sprays (day 0) were applied on the 10-Oct-08. [Y] Means followed by different letters are significantly different at $P < 0.05$.

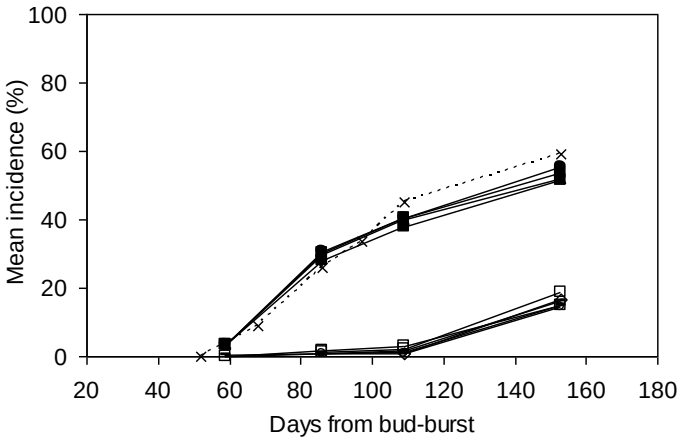

Fig. 9.3. Mean percentage disease incidence from budburst in non-treated Vina fruits at Swansea in 2008-09. The six lines in a group with the lowest disease incidences represent the commercial spray regime and treatments that utilized the rainfall-based model. The five lines in a group with the highest disease incidences represent non-treated fruits and weekly budburst sprays only. Budburst occurred on the 10-Oct-08. Each data point represents the mean of six replicates.

9.3.2.4 Predicted timing of copper-based sprays

At Forth, the rainfall-based model predicted a total of eight sprays between 7 and 62 days of budburst (Fig. 9.4); in contrast, nine sprays were applied when two sprays were applied from budburst followed by application of the model. A total of nine sprays were also applied with four budburst sprays followed by the model regime, with the second last spray applied 2 days after the optimal time. The last of the four budburst sprays i.e., 20 days after budburst, was applied prior to rain intensity of 3 mm/h; on all other days in which rainfall occurred, rain intensity was less than 1 mm/h.

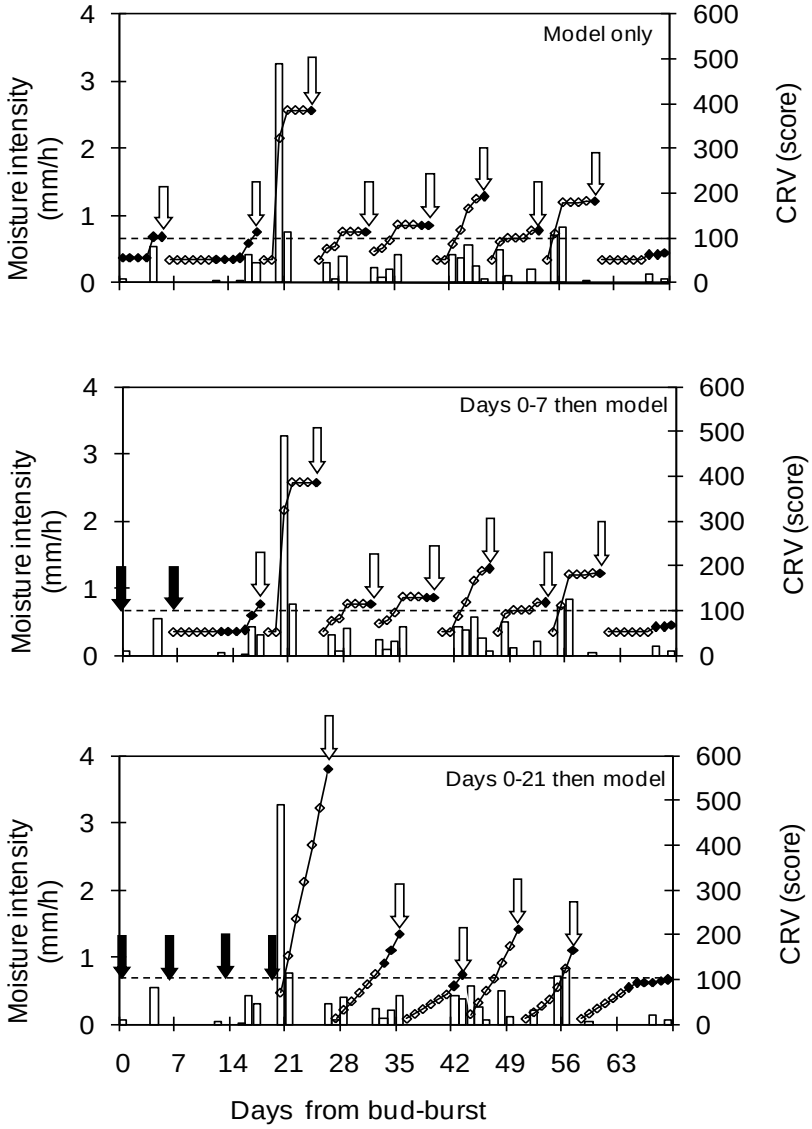

Fig. 9.4. Moisture intensity per day (mm/h, bars), and critical risk value (CRV) per day (diamonds), for predicting the timing of copper-based sprays from budburst (Day 0) to 70 days after budburst at Forth in 2008-09. The dashed line represents the CRV threshold of 100. Solid block arrows represent sprays timed according to a 7 day calendar regime. Clear block arrows represent sprays timed according to the CRV. Sprays were not re-applied until 7 days after the previous sprays were applied (open diamonds), irrespective of whether the CRV threshold of 100 was exceeded or not. Solid block arrows represent sprays timed according to a 7 day calendar regime.

The rainfall-based model predicted five sprays within 70 days of budburst at Swansea; however, the threshold of 100 was not reached until 28 days after budburst (Fig. 9.5). Copper-based sprays were not applied until 4 and 8 days after model predictions at days 53 and 68 respectively. Threshold values of 354 and 388 were reached prior to sprays being applied 53 and 68 days after budburst respectively. A rain intensity of 5 mm/h occurred 69 days after budburst, and rain intensities of 1 mm/h, or more, occurred on five occasions between 28 and 66 days.

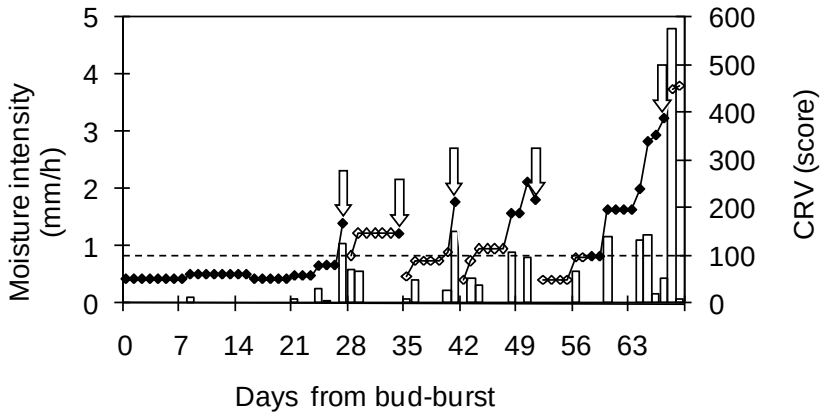

Fig. 9.5. Moisture intensity per day (mm/h, bars), and critical risk value (CRV) per day (diamonds), for predicting the timing of copper-based sprays from budburst (Day 0) to 70 days after budburst at Swansea in 2008-09. The dashed line represents the CRV threshold of 100. Clear block arrows represent sprays timed according to the CRV. Sprays were not re-applied until 7 days after sprays were applied (open diamond), irrespective of whether the CRV threshold of 100 was exceeded or not.

9.3.3 Rainfall and moisture intensity

From budburst to 20 days after budburst there was a total of 53 mm rainfall and 8 mm/h moisture intensity at Forth (Table 9.8); in comparison, there was less than 1 mm and 1 mm/h of rainfall and moisture intensity, respectively, for the same period at Swansea. In contrast, more rainfall and moisture intensity had cumulated from budburst to 70 days after budburst at Swansea in comparison to Forth.

Table 9.8. Weekly rainfall and moisture intensity (MI) cumulated from budburst (day 0) to 10 weeks after budburst (day 70) at Forth and Swansea in 2008-09.

Days from budburst [z]	Forth		Swansea	
	Rainfall (mm)	MI (mm/h)	Rainfall (mm)	MI (mm/h)
0−6	2.4	2.7	0	0
7−13	2.6	2.9	0.4	0.1
14−20	53.4	7.7	0.6	0.2
21−27	56.4	9.0	11.2	1.7
28−34	64.0	10.5	13.0	1.9
35−41	66.8	10.9	41.2	3.8
42−48	86.0	12.8	55.0	5.4
49−55	104.0	14.5	74.2	6.8
54−62	106.8	15.1	81.4	7.9
63−70	108.0	15.4	114.6	15.8

[z] Budburst (day 0) = 18-Oct-08 and 10-Oct-08 at Forth and Swansea respectively.

9.4 DISCUSSION

In this study, sprays timed according to the rainfall-based model, developed empirically using Tasmanian data, provided similar control of walnut blight with fewer numbers of sprays than those timed by commercial operations. The timing of copper-based sprays in Tasmanian walnut orchards is aimed at minimising the number of false negative predictions i.e., where sprays are not predicted when they are required; for this parameter, the rainfall-based model provided appropriate thresholds for timing crop protection in two mature walnut orchards in Tasmania.

The rainfall-based model in this study was based on the relationship between moisture intensity and fruits with newly-developed symptoms of blight (Chapter 8). Factors associated with rainfall have been included in other predictive spray models for bacterial diseases, such as daily rainfall for the control of *Pseudomonas syringae* pv. *tomato*, the causal agent of bacterial speck of tomato (Jardine and Stephens, 1987)

and the previous days rainfall for the control of fire blight, caused by *Erwinia amylovora* (Steiner, 1990; Steiner and Lightner, 1996; Holtz *et al*., 2002). However, to the author's knowledge, moisture intensity has not been explored previously as an explanatory variable for predicting the time for applying crop protection in a bacterial phytopathosystem.

The temporal incidence of walnut blight was markedly different between locations in 2008-09, with epidemics defined by monomolecular and Gompertz models at Swansea and Forth respectively. Greater rainfall and moisture intensity during the budburst and shoot elongation period at Forth, in comparison to Swansea, support the findings from previous studies on the development of walnut blight in Tasmania (Chapter 8), where factors related to rainfall were found to be the most important environmental variables involved in the development of disease in fruits.

At Forth in 2008-09, a total of nine copper-based sprays, timed according to a commercial schedule or a combination of budburst and weather-based regime, significantly improved disease control compared to four, or fewer, sprays. However, the same level of disease control and crop yield was achieved with eight sprays, timed according to the rainfall-based model only. The major distinction between spray regimes at Forth was that crop protection was not predicted until 6 days after budburst with the weather-based model only; with all other spray programmes, the initial spray was applied at budburst. As such, the model correctly predicted that there was no need to apply crop protection at budburst i.e., a true negative prediction. Of interest, in a previous epidemic at Forth defined by the logistic model, delaying the initial spray until 7 days after budburst did not affect disease incidence or crop yield, indicating that in this previous epidemic the initial spray was also not required (Chapter 7). These results suggest that the rainfall-based model may potentially reduce the number of sprays applied in epidemics where disease progression is defined by logistic and Gompertz models, in comparison to a schedule-based regime, by predicting the optimal time to begin crop protection.

At Swansea in 2008-09, the rainfall-based model did not time the first copper-based spray until 28 days after budburst, although this apparent late time of application did not adversely affect crop yield in comparison to sprays applied at budburst. A crop yield of 90% was recorded when sprays were timed according to the model only; however, this was no greater than yield with non-treatment. Thus, sprays timed according to the model were not required, or falsely predicted, and as such, the costs associated with applying copper-based sprays would not have been compensated with increased yield. Notwithstanding this fact, the model predicted three fewer sprays than the commercial spray programme, while providing the same level of disease control and crop yield.

Sprays timed according to the rainfall-based model were delayed on two occasions at Swansea because of unsuitable weather for applying sprays. As such, sprays were applied up to 8 days after the critical risk value of 100 was attained. However, the delay in spray application did not appear to affect crop yield, given that crop yields were no different to yields in non-treated trees, and that crop yields at this site were similar to, or higher than, maximum crop yields in other epidemics defined by linear and monomolecular models (Chapter 7). These results concur with the number of false positive predictions, and suggest that the action threshold may overestimate the requirement for applying crop protection. To reduce the proportion of false positive predictions, the economic injury level (EIL) could be raised; however, raising the EIL would increase the proportion of false negative predictions. This would disregard a major criterion for determining the action threshold, as false negative predictions need to be minimised as the value of the walnut crop in Tasmania is high relative to potential crop loss associated with walnut blight.

Factors associated with rainfall intensity, or rainfall rate, are important in the development of disease in other plant pathosystems. In pea bacterial blight, caused by *Pseudomonas syringae* pv. *pisi,* rainfall rate and wind exerted the greatest influence on the spread and transfer of inoculum in field plots in the United Kingdom (Roberts, 1997). The duration of rainfall intensity affected the dispersal of *X. axonopodis* pv. *citri*, the causal organism of citrus canker, with the greatest dispersal

of bacteria from the inoculum source within the first 5 min of rainfall (Bock *et al*., 2005); in contrast, the quantity of bacteria dispersed within 10 and 60 min of the start of rainfall was one-third and one-tenth, respectively, of that dispersed within the first 5 min period. Higher rain intensity also increased the numbers of *Pseudomonas syringae* pv. *tomato* in the canopy of tomato plants when the pathogen was established within the plant prior to the rain event (Pietrarelli *et al*., 2006); however, higher rain intensity reduced epiphytic survival of *Pseudomonas syringae* pv. *tomato*. Survival of the fungal pathogen, *Colletotrechum acutatum*, the causal organism of strawberry anthracnose, increased with rainfall intensity from 15 to 30 mm/h; however, fruit infections decreased with higher intensity as spores were removed from potential infection sites by run-off (Madden *et al*., 1996). Clearly, many factors associated with rainfall intensity affect plant-bacteria pathosystems, thus further studies of the survival and infection of walnut host tissue by *X. arboricola* pv. *juglandis*, under field and controlled conditions, will enhance the development of the rainfall-based model.

In summary, this study determined that walnut blight can be controlled with treatment of copper-based sprays, timed according to moisture intensity during the budburst period and shortly thereafter, in epidemics where disease progression is defined by the monomolecular model. During an epidemic defined by the Gompertz model, further sprays are needed to account for the level of pathogen activity. The moisture intensity-based model indicated the timing of copper-based sprays, irrespective of the epidemic type, and when used for the timing of sprays at two sites in Tasmania in a single growing season, provided a level of disease control that was equivalent to a current commercial program. Further improvements to the prototype model will be achieved with testing and validation at multiple sites and seasons across the full range of cultivars and environmental conditions experienced in Australian walnut orchards.

GENERAL DISCUSSION AND RECOMMENDTATIONS FOR FURTHER RESEARCH

10.1 GENERAL DISCUSSION

This study investigated the epidemiology and management of walnut blight, caused by *Xanthomonas arboricola* pv. *juglandis*, in Tasmania, Australia. Bacterial strains isolated from Tasmanian orchards were characterised and their pathogenicity determined. Crop loss associated with diseased fruits was quantified at various fruit sizes. The critical environmental factor involved in the development of blight on fruits was identified, and a rainfall-based model developed and tested for predicting the timing for applying crop protection.

10.1.1 Characterisation and pathogenicity of *Xanthomonas arboricola* strains associated with walnut blight

X. arboricola pv. *juglandis* was confirmed as the causal agent of walnut blight in Tasmania (Chapter 5), and this finding concurs with those from other walnut growing regions of the world (CABI/EPPO, 2001). The detached fruit assay verified *X. arboricola* pv. *juglandis* as the causal pathogen, based on the pathovar concept of taxonomy (Dye *et al.*, 1980), as isolate identification by semi-selective media and GC-FAME was resolved to species level only. The detached-fruit assay was validated for cultivar Franquette; however, the method needs be tested for identifying the pathogenicity of bacteria isolated from fruit of other walnut cultivars.

Even though *X. arboricola* pv. *juglandis* was isolated frequently from symptomatic walnut tissue, *Pantoea agglomerans* was also isolated from a Franquette shoot (Chapter 5) and from a Vina fruit in a separate study on the copper sensitivity of *X. arboricola* pv. *juglandis* strains isolated from commercial orchards in Tasmania (Saravanan, 2007). This isolation of this bacterium and the presence of *Fusarium semitectum* and *Alternaria alternata* from Chandler fruits from a commercial orchard at Swansea (Alessandra Belisario, Plant Pathology Senior Scientist, CRA − Plant Pathology Research Centre, Rome, Italy, pers. comm., 2006) may indicate the presence of apical necrosis (synonym: brown apical necrosis), a disease that has only recently been reported in orchards in Spain, Italy and France (Belisario *et al*., 2002; Arquero *et al*., 2006; Moragrega *et al*., 2008b) and Shandong Province, China (Yang *et al*., 2011).

In China, the bacterial pathogen, *Pantoea agglomerans*, was identified as the causal agent of apical necrosis (Yang *et al*., 2011), whereas fungal pathogens of the genus *Fusarium* and *Alternaria* have been isolated from symptomatic walnut fruits in Spain, Italy and France (Belisario *et al*., 2002; Arquero *et al*., 2006; Moragrega *et al*., 2008b). In Spanish orchards, *X. arboricola* pv. *juglandis* is considered the main causal organism, with *Fusarium* and *Alternaria* species acting as secondary or opportunistic pathogens (Moragrega and Ozaktan, 2010); however, in Italian and French orchards *X. arboricola* pv. *juglandis* was only isolated sporadically from apical lesions, and the most common organisms associated with lesions were *F. semitectum*, *F. semitectum* var. *majus* and *F. avenaceum* (Belisario *et al*., 2002); *A. alternata* was also frequently isolated from diseased fruit from Italy and France. As apical necrosis has reduced yield in cultivar Lara by up to 20% in a year with high disease incidence in northern Italy (Belisario *et al*., 2002), studies are warranted to confirm the presence or otherwise of this disease in Tasmania.

10.1.2 Yield of walnut fruits is inversely related to the incidence of walnut blight on immature fruits of various sizes

An important finding of this study was that the incidence and severity of blight on fruits were similar for Franquette and Vina (Chapter 6); this finding is in contrast to other growing regions where Franquette are considered to be less susceptible to blight than Vina (Aleta *et al*., 2001; Adaskaveg *et al*., 2009). Rainfall is important for the spread and transfers of plant pathogenic bacteria (Roberts, 1997; Bock *et al*., 2005; Pietrarelli *et al*., 2006), and may trigger the multiplication of epiphytic bacterial populations that lead to the appearance of disease (Hirano and Upper, 1990). As rainfall-related factors were strongly correlated to the development of new disease in Franquette in Tasmania (Chapter 8), the difference in the susceptibility of Franquette between Tasmania and other growing regions may reflect a difference in rainfall conditions at budburst, and shortly thereafter, between the cultivars and growing regions. Research into walnut blight in Tasmania has focused initially on controlling the disease in Vina, as one of the first commercial orchards established in Tasmania was planted with this cultivar and walnut blight caused crop losses of 50%, or more, in some years (Hills and Lang, 2004). The susceptibility of Franquette to infection with *X. arboricola* pv. *juglandis* in this study suggests that knowledge of

the blight susceptibility of other commercial cultivars grown in Tasmania, relative to Vina, is required.

The premature drop of diseased fruits in Tasmania confirmed the importance of managing walnut blight from budburst until at least half fruit size, irrespective of the epidemic type (Chapter 6). Walnut blight epidemics varied between years in Tasmania, with the linear, monomolecular, logistic and Gompertz models best fitting disease progression in Vina and Franquette, depending upon the year and location (Chapters 8 and 9); however, temporal disease incidence and temporal fruit size were well described by the monomolecular model, and allowed crop yield to be predicted according to disease incidence at various fruit sizes (Chapter 6). Model development was based on assessments conducted at 4 to 5 week intervals in the latter half of fruit growth (Chapter 4); however, the period between kernel maturity and fruit drop in Vina can be as short as 7 days at Swansea (Lang and Evans, 2010). Thus, more frequent assessments to determine the time of fruit drop near kernel maturity would enhance the crop yield models developed in this study.

Application of survival analysis, a statistical technique that analyses the occurrence and timing of events in a population (Scherm and Ojiambo, 2004), such as the time to death of an individual measured from the time of disease onset, may also increase the understanding of premature drop of walnuts. Survival analysis has successfully identified the "time-to-abscission" of blueberry leaves affected by Septoria leaf spot in southeastern United States (Ojiambo and Scherm, 2005), the postincubation "time-to-death" of papaya due to yellow crinkle disease in Australia (Esker *et al.*, 2006) and management options for ray blight in pyrethrum fields in Australia (Pethybridge *et al*., 2010). Thus, studies of the survival of fruit of various sizes may provide greater clarity as to when fruits can "survive" to harvest after infection with *X. arboricola* pv. *juglandis*, and thus determine when crop protection strategies are no longer required.

10.1.3 Copper-based sprays timed strategically reduce the incidence of walnut blight and increase crop yield and economic return

If rainfall facilitates the transfer of overwintering inoculum from the outer bud scales and cataphylls of buds, to the inner embryonic leaves and meristematic tissue of buds, then either the transfer of this inoculum onto susceptible tissue must be prevented, or the susceptible tissue must be protected. Emerging, susceptible leaves and developing fruit must also be protected, especially when shoot growth is rapid in spring. Copper-based sprays applied from budburst reduced the incidence of walnut blight in Vina fruits in three of five epidemics in Tasmania (Chapters 7 and 9); in two other epidemics, applying sprays did not provide any greater control than non-treatment, as only 8% or less of non-treated fruits were diseased at half fruit size. Thus, strategically timed sprays have provided significant protection of walnut tissues in Tasmania in the year that they were applied. In Californian orchards, however, up to 10^5 colony forming units per bud of *X. arboricola* pv. *juglandis* have been reported in the buds on newly developing shoots within one month of budburst (Lindow *et al*., 2004); these populations may potentially influence the incidence of walnut blight in the following year, as shoot and fruit growth is initiated from these buds. Thus, while sprays applied during the budburst period were beneficial for disease control within the current season in Tasmania, further sprays may be required to prevent possible colonisation of buds and epidemics for the following year.

Strategically timed copper-based sprays provided a significant level of crop protection in Tasmania for epidemics defined by monomolecular, logistic and Gompertz models (Chapters 7 and 9), where the greatest rates of disease increase in non-treated fruit were generally within 60 days after budburst. Copper damages the DNA and lipid membranes of cells, and becomes toxic to cells above a certain concentration (Hoshino *et al*., 1999; Muller *et al*., 2000; Finney and O'Halloran, 2003). In Californian orchards, products containing metallic copper have significantly reduced populations of *X. arboricola* pv. *juglandis* overwintering in walnut buds (Mulrean and Schroth, 1982) and epiphytic *X. arboricola* pv. *juglandis* populations on walnut leaves (Lee *et al*., 1993). Similarly, populations of other xanthomonads have been significantly reduced when treated with copper biocides

i.e., *Xanthomonas campestris* pv. *vesicatoria*, the causal agent of bacterial leaf spot of pepper and tomato (Jones *et al*., 1991; Martin *et al*., 2004). Thus, multiple applications of copper-based sprays may have reduced potential inoculum load in Tasmania, although the relationship between inoculum load and infection of walnut tissue in Tasmania is yet to be quantified.

10.1.4 Rainfall is the principle environmental variable involved in the development of walnut blight on fruits

Moisture intensity, calculated as the daily rainfall divided by the duration of surface wetness during and after rainfall in the period from 17 to 24 adjusted-calendar-days prior to new disease being observed, was strongly correlated to the development of new disease in both Franquette and Vina fruits. An empirical approach was used for model development in this study, where the relationship between new disease and environmental conditions was developed from observations or measurements collected in orchards. Empirical models describe an observed process, and as such, are extremely useful in the initial stages of exploring relationships between two or more variables in epidemic development (Madden *et al*., 2007); however, the utility of empirical models may be limited by inadequacies in observed data. For example, only a small proportion of a host population are typically observed in characterizing epidemics, and as such, the estimates of disease can be imprecise depending upon the spatial heterogeneity of the disease and the sample size (Madden *at al.*, 2007). Hence, examining the effect of rainfall and other environmental factors on disease development with greater temporal and spatial resolution, including studies involving inoculation under controlled conditions, may define these relationships more precisely.

Analysis of the spatiotemporal progression of disease incidence of fruit clusters may further describe the influence of rainfall-related factors on the development of walnut blight in Tasmania. In Californian orchards, the colonisation of walnut buds with *X. arboricola* pv. *juglandis* was not influenced by the position of the bud along the shoot, whether the bud was lateral or terminal, or whether the bud was fruitful or vegetative (Mulrean and Schroth, 1982). Although Mulrean and Schroth (1982) did not report on the spatiotemporal progression of disease during the year, these results

suggest that the buds containing populations of *X. arboricola* pv. *juglandis* were randomly distributed within the tree canopy in the initial stages of an epidemic. Ordinary runs analysis is a statistical technique that identifies the disease pattern and spread as either random or aggregated (Madden *et al*., 1982). It has been used to describe aggregations or clusters of citrus canker (*X. campestris* pv. *citri*) associated with wind-driven rainfall in nurseries and in citrus groves in Argentina (Gottwald *et al*., 1989) and the shoot blight phase of fire blight on apple, caused by *Erwinia amylovora*, in the United States (Biggs *et al*., 2008). This analytical approach might prove useful for informing sampling strategies for data underlying empirical models.

In this study, the duration of surface wetness was used for calculating the moisture intensity (Chapter 8). Surface wetness was used for measuring the duration of rainfall as it was observed that the surface-wetness sensor better represented the duration of free water on walnut fruits from rainfall, than that recorded by the tipping-bucket rainfall sensor. This observation was particularly evident when rainfall was light and/or intermittent where fruit surfaces would become wet, and remain wet between each period of rainfall and shortly thereafter. The wetness sensor would record the entire period of these rainfall events, whereas the rainfall sensor would only record each 0.2 mm accumulation of rainfall; hence, the rainfall sensor under-represented the duration of free water on fruits.

The duration of free water, as measured by surface-wetness sensors, was a critical component for defining moisture intensity and predicting walnut blight epidemics in Tasmania (Chapters 8 to 9). However, surface-wetness duration varies with the type of crop, its developmental stage, and the position, angle and geometry of individual plant parts (Sutton *et al*., 1984; Penrose and Nicol, 1996; Sentelhas *et al*., 2005; Batzer *et al*., 2008). Furthermore, surface-wetness sensors vary in design and fabrication and can vary in their measurement of surface wetness (Henshall and Beresford, 1997; Lau *et al*., 2000; Sentelhas *et al*., 2004, 2007), thus making the duration of surface wetness difficult to define. In order to better understand the effect of moisture intensity on the development of walnut blight, and improve the accuracy of predicting epidemics, a better understanding of the relationship between the

duration of free water on fruit surfaces and the duration estimated by surface-wetness sensors is required.

In this study, cylindrical surface wetness sensors were positioned 1.5 m above ground level, in the middle of the tree canopy, and facing in a north-south direction (Chapter 4); this position was selected as it was as close as practicable to the location of fruits within the canopy, without leaves interfering with the sensor, and it was assumed that the drying of the sensor from wind and sunlight within the canopy would better approximate the drying of fruits, in contrast to the drying of the sensor in an exposed position. The frequency and duration of free water, as measured by surface-wetness sensors, can vary with sensor placement within tree canopies, as observed in apple tree canopies in New Zealand (Penrose and Nicol, 1996). Variation in measurements with different sensor design, fabrication and placement is yet to be established in walnuts. A simple method to reduce variation associated with in-canopy measurements of surface-wetness duration is the placement of more sensors at different spatial locations within the tree canopy, and using the mean data for measuring surface wetness (Penrose and Nicol, 1996), or locating sensors outside of crop canopies (Zhang and Gillespie, 1990; Henshall and Beresford, 1997; Sentelhas *et al*., 2004, 2005). Both approaches require that data are calibrated to data collected from cylindrical surface wetness sensors used in this study, so that the model algorithms used for the timing of crop protection are accurately supported.

10.1.5 Development of a rainfall-based predictive model for timing copper-based sprays to control walnut blight

The empirical rainfall-based model developed in this study was based on the correlation of moisture intensity to disease incidence, where pathogen activity was estimated indirectly as the development of new disease in Vina and Franquette fruits (Chapter 9). In Tasmania, copper-based sprays timed according to the rainfall-based model provided similar control of walnut blight with fewer numbers of sprays than those timed by commercial operations. Furthermore, the model delayed the initial sprays until after budburst without reducing the control of the disease.

The risk algorithm that formed the basis of the rainfall-based model was developed from models that predicted the increase in crop yield with copper-based sprays relative to non-treatment (Chapters 6 and 7) and the development of new disease per day on fruits as a function of moisture intensity (Chapter 8). Furthermore, the risk algorithm was modeled on a critical risk value that was based on an estimated economic injury level of 0.75% disease incidence (Chapter 9). However, the predictions underlying the rainfall-based model will be influenced by the effectiveness of increasing crop yield with copper-based sprays, and the yet-to-be determined accuracy and precision required for determining moisture intensity. The predictions for applying crop protection will also be affected by the production capacity of the orchard and factors out of the control of those involved in crop production i.e., cost of copper-based sprays, application costs and crop income. Notwithstanding these matters, the rainfall-based model is ready to be tested and validated at multiple sites and seasons across the full range of cultivars and environmental conditions experienced in Australian walnut orchards.

10.2 RECOMMENDATIONS FOR FURTHER RESEARCH

Further research is required across the range of walnut cultivars and climates in which walnuts are grown commercially in Australia to:

1. *Verify the presence or otherwise of apical necrosis in Tasmania. If present, identify the fungal and/or bacterial pathogens associated with this disease.*

This study confirmed *X. arboricola* pv. *juglandis* as the causal organism of walnut blight in Tasmania. However, fungal and bacterial pathogens that cause apical necrosis, a recently reported disease of walnuts in Europe and China, were isolated from symptomatic shoots and fruits as part of this study; further studies are warranted to confirm the presence or otherwise of this disease in Tasmania.

2. *Characterise a greater range, and determine the copper sensitivity, of X. arboricola* pv. *juglandis isolates from Tasmanian orchards.*

This study identified two phenotypic groups of *X. arboricola* isolates from one commercial orchard in Tasmania. However, in a concurrent study on the copper

sensitivity in Tasmanian populations of *X. arboricola* pv. *juglandis*, Saravanan (2007) suggested that exposure to copper-based sprays through commercial orchard practices has selected for bacterial isolates that can tolerate higher doses of copper than those that have never been exposed to copper. This finding highlights the need to reduce inputs of copper in commercial walnut production in Tasmania, and that copper sensitivity of the walnut blight pathogen is monitored in all growing regions in Tasmania.

3. *Describe and analyse the spatiotemporal progression of walnut blight incidence in other cultivars and regions.*

This study provides a description of the temporal progression of blight incidence in Vina and Franquette fruit in Tasmania. However, monitoring disease progression has not been conducted in other cultivars and in other walnut growing regions in Australia. In the cool-temperate environment of Tasmania, the major walnut cultivars grown are Ashley, Chandler, Howard, Lara, Serr, Tulare and Vina (www.websterltd.com.au/walnuts/varieties); plantings of these cultivars have recently expanded into the semi-arid environment of the Riverina region of New South Wales, Australia (Australian Nut Industry Council, 2009). Spatiotemporal analysis of disease progress curves will enable similarities and differences between cultivars and regions to be identified, and enhance the analyses already provided.

4. *Quantify rainfall-based and other environmental variables, and other host and pathogen factors influencing development of walnut blight of fruit.*

This study has confirmed that moisture intensity is a significant factor in the development of blight incidence on fruit; however, this factor does not fully explain the variance in the percentage of newly blighted fruit per day in Tasmania. Temperature and surface-wetness duration have been implicated in the development of walnut blight in California (Adaskaveg *et al*., 2000), and relative humidity in Spanish orchards (Arquero *et al*., 2006); furthermore, increasing population sizes of *X. arboricola* pv. *juglandis* on individual walnut buds at budburst led to a subsequent increase in disease incidence to fruits in early summer in Californian orchards (Lindow *et al.,* 2004); these factors need to be varied in controlled environments to

quantify their impact on bacterial growth and colonization, and on the incidence and extent of symptom development, of host tissues.

5. *Determine the time and duration when walnut buds on developing walnut shoots are susceptible to colonisation with X. arboricola* pv. *juglandis.*

In Tasmania, copper-based sprays applied during the budburst period were beneficial for disease control in the current season; however, *X. arboricola* pv. *juglandis* have been reported in the buds on newly developing shoots within one month of budburst (Lindow *et al*., 2004). Thus, research is required to determine the stage/s of bud development susceptible to colonisation and to model the duration for all buds complete a particular phenological stage. With this knowledge, it may be possible to ascertain whether or not further sprays may prevent possible colonisation of buds and reduce inoculum for the following year. The effect of any reduction in inoculum on epidemic development over a range of early season conditions also needs to be determined.

6. *Verify the relationship between disease incidence and crop yield, and examine the survival of fruits after infection with X. arboricola* pv. *juglandis.*

This study identified that the incidence of walnut blight on fruits was strongly inversely related to crop yield in Tasmania, and that fruits required protection from budburst until at least half fruit size when conditions were suitable for disease to develop. However, more frequent assessments in the latter half of the growing year to determine the timing of fruit drop, and trials to determine the survival, or "time-to-drop", of fruits, after inoculation with *X. arboricola* pv. *juglandis* at various sizes would enhance the models developed in this study.

7. *Determine if concentrations of copper-based sprays are reduced on the surfaces of fruits with rainfall and/or with fruit growth, and examine the efficacy of mixtures of copper-based sprays with a penetrant at budburst.*

This study has shown that multiple copper-based sprays do not provide adequate control in some epidemics; this result may have been caused by a reduction in copper, to sub-lethal concentrations, with heavy rainfall or with rapid growth of

walnut tissue in early fruit growth. Furthermore, one budburst spray only was not as effective in controlling disease as two budburst sprays in epidemics defined by monomolecular, logistic and Gompertz models. In California, the addition of an organosilicant penetrant to copper-based sprays 1 week after budburst reduced the numbers of *X. arboricola* pv. *juglandis* in comparison copper-based sprays applied alone (Lindow *et al*., 2000). Hence, trials examining the persistence of copper-based sprays and efficacy of penetrants are warranted in Tasmania.

8. *Confirm the efficacy of the rainfall-based model and the need for regional calibration.*

This study provided initial results on the timing of copper-based sprays for managing walnut blight in Vina in two growing regions of Tasmania. As surface wetness is a critical component of calculating rain intensity i.e., the variable that forms the basis of the rainfall-based model, detailed investigations into the different design, fabrication and placement of surface wetness sensors are necessary. Such a study could be part of an analysis of regional climatic data to provide a regional risk assessment for walnut blight. For this to be feasible, however, a source of leaf wetness data, or a means of calculating leaf wetness based on measured variables, is needed. Further research is required in walnut growing regions in Tasmania and mainland Australia to test and improve the rainfall-based model in a greater range of cultivars and environmental conditions.

REFERENCES

Adam DB, Pugsley AT, 1934. Bacterial plant diseases in Victoria. *Journal of the Department of Agriculture of Victoria* **32**, 304–311.

Adaskaveg JE, Kirkpatrick BC, Buchner R, Olsen B, 1995. *Epidemiology and management of walnut blight*. Available from http://walnutresearch.ucdavis.edu/1995/1995_237.pdf

Adaskaveg JE, Buchner R, Olsen B, Adams CJ, Thompson D, Forster H, 1999. *Epidemiology and management of walnut blight*. Available from http://walnutresearch.ucdavis.edu/1999/1999_267.pdf

Adaskaveg JE, Buchner R, Olsen B, Adams CJ, Thompson D, Forster H, 2000. *Epidemiology and management of walnut blight*. Available from http://walnutresearch.ucdavis.edu/2000/2000_329.pdf

Adaskaveg JE, Forster H, Thompson D, Driever G, Buchner R, Olsen B, Pickel C, Prichard T, Grant J, 2004. *Epidemiology and management of walnut blight*. Available from http://walnutresearch.ucdavis.edu/2004/2004_315.pdf

Adaskaveg JE, Forster H, Thompson D, Driever G, Connell J, Buchner R, 2005. *Epidemiology and management of walnut blight*. Available from http://walnutresearch.ucdavis.edu/2005/2005_291.pdf

Adaskaveg JE, Forster H, Thompson D, Driever G, Connell J, Buchner R, 2006. *Epidemiology and management of walnut blight*. Available from http://walnutresearch.ucdavis.edu/2006/2006_203.pdf

Adaskaveg JE, Forster H, Thompson D, Enns J, Connell J, Buchner R, 2009. *Epidemiology and management of walnut blight*. Available from http://walnutresearch.ucdavis.edu/2009/2009_241.pdf

Agrios GN, 1997. *Plant Pathology, 4th Edition.* Academic Press, New York.

Aleta N, Ninot A, Moragrega C, Llorente I, Montesinos E, 2001. Blight sensitivity of Spanish selections of *J. regia*. *Acta Horticulturae* **544**, 353–361.

Anonymous, 1926. Black spot on walnuts. *Garden and Field* **51**, 13.

Anonymous, 1935. Bacterial blight of Japanese walnut. *United States Department of Agriculture, Bureau of Plant Industry* **90**, 57.

Anonymous, 2005. *MIS Operating Manual for Sherlock® Microbial Identification System*. Midi Inc., Newark, Delaware, USA. Available from: www.midiinc.com/media/pdfs/MIS-Manual-6.0.pdf.

Ark PA, 1944. Pollen as a source of walnut bacterial blight infection. *Phytopathology* **34**, 330–334.

Arman P, Wain RL, 1958. Studies on the copper fungicides – the role of leaf exudates in the solution of copper from Bordeaux mixture. *Annals of Applied Biology* **46**, 366-374.

Arquero O, Lovera M, Rodriguez R, Salguero A, Trapero A, 2006. Characterisation and development of necrotic lesions of walnut tree fruits in southern Spain. *Acta Horticulturae* **705**, 457–464.

Australian Nut Industry Council (ANIC), 2009. *Australia's nut industry − growing for success.* ANIC, Berri, South Australia, Australia.

Batzer JC, Gleason ML, Taylor SE, Koehler KJ, Monteiro JEBM, 2008. Spatial heterogeneity of leaf wetness duration in apple trees and its influence on performance of a warning system for sooty blotch and flyspeck. *Plant Disease* **92**, 164–170.

Belisario A, 1992. Phytopathological problems of walnut. *Informatore Agrario* **48**, 51–53.

Belisario A, Zoina A, 1995. Occurrence of Persian (English) walnut blight and its control in the nursery. *European Journal of Forest Pathology* **25**, 224–231.

Belisario A, Zoina A, Pezza L, Luongo L, 1997. Susceptibility of species *Juglans* to pathovars of *Xanthomonas campestris*. *European Journal of Forest Pathology* **29**, 75–80.

Belisario A, Maccaroni M, Corazza L, Balmas V, Valier A, 2002. Occurrence and etiology of brown apical necrosis on Persian (English) walnut fruit. *Plant Disease* **86**, 599–602.

Bennet EL, Carter CE, 1990. Influences of water congestion and *Xanthomonas campestris.* pv. *pruni* on proteins of peach leaves. *Phytopathology* **80**, 1023.

Bergey DH *et al*, 1930. *Manual of Determinative Bacteriology, 5th Edition.* Williams & Wilkins, Baltimore, USA.

Biggs AR, Turechek WW, Gottwald TR, 2008. Analysis of fire blight shoot infection epidemics on apple. *Plant Disease* **92,** 1349–1356.

Billing E, 1996. BIS95, an improved approach to fire blight risk assessment. *Acta Horticulturae* **411**, 121–126.

Bock CH, Parker PE, Gottwald TR, 2005. Effect of simulated wind-driven rain on duration and distance of dispersal of *Xanthomonas axonopodis* pv. *citri* from canker-infected citrus trees. *Plant Disease* **89,** 71–80.

Bradbury JF, 1970. Isolation and preliminary study of bacteria from plants. *Review of Plant Pathology* **49**, 213–218.

Bradbury JF, 1986. *Xanthomonas* Dowson 1932. In: *Guide to Plant Pathogenic Bacteria*. CAB International Mycological Institute, Slough, UK.

Buchner RP, Adaskaveg J.E, Olson WH, Lindow SE, 2001. Walnut blight (*Xanthomonas campestris* pv. *juglandis*) control investigations in northern California, USA. *Acta Horticulturae* **544**, 369–378.

Buchner RP, Olson WH, Adaskaveg J, Lindow S, Pickel C, Gilles CK, Miller S, Humphreys L, 2004. *Walnut blight control investigations 2004*. Available from http://walnutresearch.ucdavis.edu/2004/2004_339.pdf

Buchner RP, Lindow SE, Adaskaveg JE, Gilles CK, Koutsoukis R, 2007. *Walnut blight control investigations Tehama 2007*. Available from: http://walnutresearch.ucdavis.edu/2007/2007_203.pdf

Buchner RP, Lindow SE, Adaskeveg JE, Pickel C, Gilles CK, Koutsoukis R, Goldman-Smith S, 2008. *Walnut blight control investigations Tehama 2008*. Available from: http://walnutresearch.ucdavis.edu/2008/2008_195.pdf

Buchner RP, Lindow SE, Adaskeveg JE, Gilles CK, Koutsoukis R, 2009. *Walnut blight control investigations Tehama 2009*. Available from: http://walnutresearch.ucdavis.edu/2009/2009_259.pdf

Bunemann EK, Schwenke GD, Van Zwieten L, 2006. Impact of agricultural inputs on soil organisms – a review. *Australian Journal of Soil Research.* **44**, 379–406.

Butterworth J, McCartney HA, 1991. The dispersal of bacteria from leaf surfaces by water splash. *Journal of Applied Bacteriology*. **71,** 484–496.

CABI/EPPO, 2001. *Xanthomonas arboricola* pv. *juglandis*. *Distribution Maps of Plant Diseases, Map No. 133*. CAB International, Wallingford, UK.

Campbell CL, 2003. Disease progress in time: modelling and data analysis. In: *The Epidemiology of Plant Diseases* (Editor: D.G. Jones), Kluwer Academic Publishers, Dordrecht, Netherlands.

Campbell CL, Madden LV, 1990. *Introduction to Plant Disease Epidemiology*, Wiley-Interscience, New York.

Champoiseau P, Rott P, Daugrois JH, 2009. Epiphytic populations of *Xanthomonas albilineans* and subsequent sugarcane stalk infection are linked to rainfall in Guadeloupe. *Plant Disease* **93**, 339–346.

Chevallier A, Bray O, Prunet JP, Giraud M, 2010. Factors influencing walnut blight symptoms emergence and development *Acta Horticulturae* **861**, 473–478.

Cole CF, 1916. The walnut – diseases. *Journal of Agriculture, Victoria* **13,** 154–161.

Conlin KC, McCarter SM, 1983. Effectiveness of selected chemicals in inhibiting *Pseudomonas syringae* pv. *tomato* in vitro and in controlling bacterial speck. *Plant Disease* **67**, 639–644.

Cooksey DA, 1990. Genetics of bactericide resistance in plant pathogenic bacteria. *Annual Review of Phytopathology* **28**, 201–219.

Dawes IW, Sutherland IW, 1976. *Microbial Physiology*, Blackwell Scientific, Oxford, UK.

dos Santos RMDB, Dianese JC, 1985. Comparative membrane characterisation of *Xanthomonas campestris* pv. *cassavae* and *X. campestris* pv. *manihotis. Phytopathology* **72**, 581–587.

Dowson WJ, 1932. Notes on some bacterial plant diseases in Tasmania. *Journal of Pomology and Horticultural Science* **10**, 301–305.

du Plessis HJ, 1984. Scanning electron microscopy of *Xanthomonas campestris* pv. *pruni* in plum petioles and buds. *Journal of Phytopathology* **109**, 277–284.

du Plessis HJ, 1990. Systemic invasion of plum seed and fruit by *Xanthomonas campestris* pv. *pruni* through stalks. *Journal of Phytopathology* **130**, 37–45.

du Plessis HJ, van der Westhuizen TJ, 1995. Identification of *Xanthomonas campestris* pv. *juglandis* from (Persian) English walnut nursery trees in South Africa. *Journal of Phytopathology* **143**, 449–454.

Dye DW, Bradbury JF, Goto M, Hayward AC, Lelliot RA, Schroth MN, 1980. International standards for naming pathovars of phytopathogenic bacteria and a list of pathovar names and pathotype strains. *Review of Plant Pathology* **59**, 153–168.

Egel DS, Graham JH, Riley TD, 1991. Population dynamics of strains of *Xanthomonas campestris* differing in aggressiveness of Swingle citumelo and grapefruit. *Phytopathology* **81**, 666–671.

Esker PD, Gibb KS, Padovan A, Dixon PM, Nutter FW Jr, 2006. Use of survival analysis to determine the postincubation time-to-death of papaya due to yellow crinke disease in Australia. *Plant Disease* **90**, 102–107.

Esterio MA, Latorre BA, 1982. Potential sources of inoculum of *Xanthomonas juglandis* in walnut blight outbreaks. *Journal of Horticultural Science* **57**, 69–72.

Finney LA, O'Halloran TV, 2003. Transition metal speciation in the cell: insights from the chemistry of metal ion receptors. *Science* **300**, 931–936.

Garcia-Horsman JA, Barquera B, Rumbley R, Ma J, Gennis RB, 1994. The super family of heme-copper respiratory oxidases. *Journal of Bacteriology* **176**, 5587–5600.

Garcin A, El-Maataoui M, Tichadou S, Prunet J P, Ginibre T, Penet C, 2001. Walnut blight, new knowledge for an old disease: summary of research (1995-2000) *Infos-Ctifl* **171**, 27–30.

Gardan L, Brault T, Germain E, 1993. Copper resistance of *Xanthomonas campestris* pv. *juglandis* in French walnut orchards and its association with conjugative plasmids. *Acta Horticulturae* **311**, 259–265.

Germain E, Prunet J, Garcin A, 1999. *Le noyer.* Ctifl, Paris, France.

Giraud M. Prunet JP, Chevallier A, Ramain S, Thiriaud V, Santrac J, Bray O, 2010. Study of *Xanthomonas arboricola* pv. *juglandis* population dynamics in French walnut orchards over three years. *Acta Horticulturae* **861**, 439–443.

Goodman RN, 1982. The infection process. In: *Phytopathogenic Prokaryotes, Volume 2*, (Editors: M.S. Mount and G.H. Lacy), Academic Press, New York.

Goodman CA, Hattingh MJ, 1988. Mechanical transmission of *Xanthomonas campestris* pv. *pruni* in plum nursery trees. *Plant Disease* **72**, 643.

Goss OM, Doepel RF, 1961. Bacterial blight or black spot of walnuts. *Journal of Agriculture, Western Australia* **4**, 833–834.

Gottwald TR, Timmer LW, McGuire RG, 1989. Analysis of disease progress of citrus canker in nurseries in Argentina. *Phytopathology* **79**, 1276–1283.

Gottwald TR, Graham JH, Richie SM, 1992. Relationship of leaf surface populations of strains of *Xanthomonas campestris* pv. *citrumelo* to development of citrus bacterial spot and persistence of disease symptoms. *Phytopathology* **82**, 625–632.

Hayward AC, 1964. Bacteriophage sensitivity and biochemical group in *Xanthomonas malvacearum. Journal of General Microbiology* **35**, 287–298.

Hendricks LC, Coates WW, Elkins RB, McGranahan GH, Phillips HA, Ramos DE, Reil WO, Snyder RG, 1998. Selection of varieties. In: *Walnut production manual*. (Editor: D.E. Ramos) University of California, Oakland, USA. pp. 84–89.

Henshall WR, Beresford RM, 1997. Performance of wetness sensors used in plant disease forecasting. In: *Proceedings of the Fiftieth New Zealand Plant Protection Conference*, Lincoln University, Canterbury, New Zealand, pp: 107–111.

Hills JL, Lang MD, 2004. *Investigation into the control of bacterial blight in walnuts: Project NT99003*. Horticulture Australia Limited, Sydney.

Hirano SS, Upper CD, 1990. Population biology and epidemiology of *Pseudomonas syringae*. *Annual Review of Phytopathology* **28,** 155–177.

Holtz BE, Hoffman EW, Teviotdale BL, 2002. Predicting the occurrence of fire blight in the San Joaquin Valley of California. *Acta Horticulturae* **704**, 167–174.

Horsfall JG, Barratt RW, 1945. An improved grading system for measuring plant diseases. *Phytopathology* **35**, 655.

Hoshino N, Kimura T, Yamaji A, Ando T, 1999. Damage to the cytoplasmic membrane of Escheridia coli by catechin-copper complexes. *Free Radical Biology and Medicine* **27**, 1245–1250.

Hunt R, 1982. *Pant Growth Curves: The Functional Approach to Plant Growth Analysis*. University Park Press, Baltimore, USA.

Janse JD, 2006. *Phytobacteriology – Principles and Practice*. CABI Publishing, Wallingford, UK.

Jardine DJ, Stephens CT, 1987. A predictive system for timing chemical applications to control *Pseudomonas syringae* pv. *tomato*, causal agent of bacterial speck. *Phytopathology* **77,** 823–827.

Jenkins T, Marsh C, Lang MD, Vanestre J, Walter M, Obanar F, 2010. Walnut blight sustainable management research in New Zealand. *Acta Horticulturae* **861**, 479–487.

Jones JB, Woltz SS, Jones JP, Portier KL, 1991. Population dynamics of *Xanthomonas campestris* pv. *vesicatoria* on tomato leaflets treated with copper bactericides. *Phytopathology* **81,** 714–719.

Kamoun S, Kado CI, 1990. Phenotypic switching affecting chemotaxis, xanthan production, and virulence in *Xanthomonas campestris. Applied and Environmental Microbiology* **56**, 3855–3860.

Koizumi M, 1976a. Behaviour of *Xanthomonas citri* (Hasse) Dowson in the infection process. I. Multiplication of the bacteria and histological changes following needle prick inoculation. *Annals of the Phytopathological Society of Japan* **42**, 407–416.

Koizumi M, 1976b. Behaviour of *Xanthomonas citri* (Hasse) Dowson in the infection process. II. Multiplication of the bacteria and histological changes following rubber-block press of infiltration inoculation. *Annals of the Phytopathological Society of Japan* **42**, 517–525.

Koizumi M, 1977. Relation of temperature to the development of citrus canker lesions in the spring. *Proceedings of the International Society of Citriculture* **3**, 924–928.

Lang MD, Evans KJ, 2010. *Advancing hull split to maximize yield and quality of walnuts: Project WN09000*. Horticulture Australia Limited, Sydney, Australia.

Lang MD, Hills JL, Evans KJ, 2006. Preliminary studies toward managing walnut blight in Tasmania. *Acta Horticulturae* **705**, 451–456.

Lang MD, Evans KJ, Pethybridge SJ, 2008. Temporal progression of walnut blight, caused by *Xanthomonas arboricola* pv. *juglandis*, in Australia. In: *9th International Congress of Plant Pathology*, Torino, Italy. (Editors: A. Porta-Puglia and P. Gonthier). *Journal of Plant Pathology* **90**, S2.360.

Lang MD, Evans KJ, Pethybridge SJ, 2010. Timing bactericides strategically for management of walnut blight in Tasmania, Australia. *Acta Horticulturae* **861**, 465–71.

Lau YF, Gleason ML, Zriba N, Taylor SE, Hinz PN, 2000. Effects of coating, deployment angle, and compass orientation on performance of electronic wetness sensors during dew periods. *Plant Disease* **84,** 192–197.

Lazo GR, Roffey R, Gabriel DW, 1987. Pathovars of *Xanthomonas campestris* are distinguishable by restriction fragment-length polymorphism. *International Journal of Systematic Bacteriology* **37**, 214–221.

Lechevalier H, Lechevalier MP, 1988. Chemotaxonomic use of lipids – an overview. In: *Microbial Lipids* (Editors: C. Ratledge and S.G. Wilkinson). Academic Press, London, UK, pp. 869–902.

Lee YA, Hildebrand DC, Schroth MN, 1992. Use of quinate metabolism as a phenotypic property to identify members of *Xanthomonas campestris* DNA homology group 6. *Phytopathology* **82**, 971–973.

Lee YA, Schroth M N, Henderson M, Lindow SE, Wang XL, Olson B, Buchner RP, Teviotdale B, 1993. Increased toxicity of iron-amended copper-containing bactericides to the walnut blight pathogen *Xanthomonas campestris* pv. *juglandis Phytopathology* **83**, 1460–1465.

Lelliot RA, Stead D, 1987. *Methods for Diagnosis of Bacterial Diseases of Plants.* Blackwell Scientific Publications, Oxford, UK.

Lindow SE, Teviotdale B, Buchner R, Olsen B, Sibbett S, Beede B, Haxo A, Legac J, Light L, 2000. *Epidemiological approaches to the control of walnut blight disease*. Available from: http://walnutresearch.ucdavis.edu/2000/2000_303.pdf

Lindow SE, Buchner R, Olsen B, Koutsoukis R, 2004. *Epidemiological approaches to the control of walnut blight disease*. Available from: http://walnutresearch.ucdavis.edu/2004/2004_291.pdf

Lindow SE, Buchner R, Connell J, Koutsoukis R, 2005. *Epidemiological approaches to the control of walnut blight disease*. Available from: http://walnutresearch.ucdavis.edu/2005/2005_263.pdf

Lindow SE, Buchner R, Koutsoukis R, 2006. *Epidemiological approaches to the control of walnut blight disease*. Available from: http://walnutresearch.ucdavis.edu/2006/2006_221.pdf

Loreti S, Gallelli A, Belisario A, Wajnberg E, Corazza L, 2001. Investigation of genomic variability of *Xanthomonas arboricola* pv. *juglandis* by AFLP analysis. *European Journal of Plant Pathology* **107**, 583–590.

Lozano C, Wholey DW, 1974. The production of bacteria-free planting stock of cassava, *Manihot seculenta* Crantz. *World crops* **27**, 115–117.

Madden L V, 1980. Quantification of disease progression. *Protection Ecology* **2**,159–176.

Madden LV, Louie R, Abt JJ, Knoke JK, 1982. Evaluation of tests for randomness of infected plants. *Phytopathology* **72**, 195–198.

Madden LV, Yang XS, Wilson LL, 1996. Effects of rain intensity on splash dispersal of *Colletotrichum acutatum*. *Phytopathology* **86**, 864–874.

Madden LV, Hughes G, van den Bosch F, 2007. *The study of plant disease epidemics*. American Phytopathological Society, Minnesota, USA.

Madigan MT, Martinko JM, Parker J, 1997. *Biology of Microorganisms, 8th Edition*. Prentice Hall Inc., New Jersey, USA.

Marco GM, Stall RE, 1983. Control of bacterial spot of pepper initiated by strains of *Xanthomonas campestris* pv. *vesicatoria* that differ in sensitivity to copper. *Plant Disease* **67**, 779–781.

Maria P, Donatella C, Gennaro C, 1997. Susceptibility of thirty two walnut varieties to *Gnomonia leptostyla* and *Xanthomonas campestris* pv. *juglandis*. *Acta Horticulturae* **442**, 379–384.

Martin NA, 1986. Toxicity of pesticides to *Allolobophora caliginosa* (Oligochaeta: Lumbrisidae). *New Zealand Journal of Agricultural Research* **29**, 699–706.

Martin H L, Hamilton V A, Kopittke R A, 2004. Copper tolerance in Australian populations of *Xanthomonas campestris* pv. *vesicatoria* contributes to poor field control of bacterial spot of pepper. *Plant Disease* **88**, 921–924.

Matthee FN, Daines RH, 1969. The influence of nutrition on susceptibility of peach foliage to water congestion and infection by *Xanthomonas pruni*. *Phytopathology* **59**, 285–287.

McLaren GF, Fraser JA, Alspach PA, 2003. New Zealand flower thrips and their impact on nectarine fruit diseases. *New Zealand Plant Protection* **56**, 157–163.

McNeil DL, Romero S, Kandula J, Stark C, Stewart A, Larsen S, 2001. Bacteriophages: a potential biocontrol agent against walnut blight (*Xanthomonas campestris* pv. *juglandis*). *New Zealand Plant Protection* **54**, 220–224.

Menkissoglu O, Lindow SE, 1991. Chemical forms of copper on leaves in relation to the bactericidal activity of cupric hydroxide deposits on plants. *Phytopathology* **81**, 1258–1263.

Mew TW, Natural MP, 1993. Management of *Xanthomonas* diseases. In: *Xanthomonas* (Editors: J.G. Swings and E.L. Civerolo), Chapman and Hall, London, pp: 341–362.

Miles WG, Daines RH, Rue JW, 1977. Pre-symptomatic egress of *Xanthomonas pruni* from infected peach leaves. *Phytopathology* **67**, 895–897.

Miller PW, Bollen WB, 1946. *Walnut Bacteriosis and its Control: Technical Bulletin No. 9*, United States Department of Agriculture, Oregon State College, Corvallis, USA.

Miller PW, Bollen WB, Simmons JE, Gross HH, Barss HP, 1940. The pathogen of filbert bacteriosis compared with *Phytomonas juglandis*, the cause of walnut blight. *Phytopathology* **30**, 713–733.

Moragrega C, Ozaktan H, 2010. An update on apical necrosis of Persian (English) walnut. *Journal of Plant Pathology* **92**, S1.67–71.

Moragrega C, Llorente I, Montesinos E, Rovira M, Aleta N, 2008a. Susceptibility of walnut cultivars and Spanish selections to bacterial blight (*Xanthomonas arboricola* pv. *juglandis*). In: *9th International Congress of Plant Pathology, Torino, Italy*. (Editors: A. Porta-Puglia and P. Gonthier). *Journal of Plant Pathology* **90**, S2.362.

Moragrega C, Aleta N, Rovira M, Matias J, 2008b. *Disease progress and microorganisms associated to brown apical necrosis of walnut. Files of the COST 873 WG1-4 Meeting: Active research to combat bacterial diseases of stone fruits and nuts. Athens 2008*. Available from: http://www.cost873.ch/_uploads/_files/Moragrega_BANSpain.pdf

Muller J, Sigel RKO, Lippert B, 2000. Heavy metal mutagenesis: insights from bioinorganic model chemistry. *Journal of Inorganic Biochemistry* **79**, 261–265.

Mulrean EN, Schroth MN, 1981. A semi-selective medium for the isolation of *Xanthomonas campestris* pv. *juglandis* from walnut buds and catkins. *Phytopathology* **71**, 336–339.

Mulrean EN, Schroth MN, 1982. Ecology of *Xanthomonas campestris* pv. *juglandis* on Persian (English) walnuts. *Phytopathology* **72**, 434–438.

Ninot A, Aleta N, Moragrega C, Montesinos E, 2002. Evaluation of a reduced copper spraying program to control bacterial blight of walnut. *Plant Disease* **86**, 583–587.

Novacky A, Ullrich-Eberius CI, 1982. Relationship between membrane potential and ATP level in *Xanthomonas campestris* pv. *malvacearum* infected cotton cotyledons. *Physiological Plant Pathology* **21**, 237–249.

Ojiambo PS, Scherm H, 2005. Survival analysis of time to abscission of blueberry leaves affected by Septoria leaf spot. *Phytopathology* **95**, 108–113.

Olsen BD, Jones AL, 1983. Reduction of *Pseudomonas syringae* pv. *morsprunorum* on Montmorency sour cherry with copper and dynamics of the copper residues. *Phytopathology* **73**, 1520–1525.

Olsen WH, Buchner RP, 2002. Leading edge of plant protection for walnuts. *HortTechnology* **12**, 615–618.

Olsen WH, Labavitch JM, Martin MC, Beede RH, 1998. Maturation, harvesting and nut quality. In: *Walnut production manual*. (Editor: D.E. Ramos), University of California, Oakland, USA. pp. 273–276.

Onderdonk AB, Sasser M, 1995. Gas-liquid and high-performance chromatographic methods for the identification of microorganisms. In: *Manual of Clinical Microbiology, 6th edition* (Editors: Murray, Baron, Pfaller, Tenover and Yolken), American Society of Microbiology, D.C. pp: 123–129.

Osborn TGB, Samuel G, 1922. Some new records of fungi for South Australia. *Transactions of the Royal Society of South Australia* **46**, 166–181.

Paoletti MG, Schweigl U, Favretto MR, 1995. Soil macro-invertebrates, heavy metals and organochlorines in low and high input apple orchards and coppiced woodland. *Pedobiologia* **39**, 20–33.

Parveaud CE, Ramain S, Verhaeghe A, 2010. The relationship between walnut blight expression and tree water status. *Acta Horticulturae* **861**, 435–438.

Penrose LJ, Nicol HI, 1996. Aspects of microclimate variation within apple tree canopies and between sites in relation to potential *Venturia inaequalis* infection. *New Zealand Journal of Crop and Horticultural Science* **24,** 259–266.

Pethybridge SJ, Ngugi HK, Hay FS, 2010. Use of survival analysis to assess management options for ray blight in Australian pyrethrum fields. *Plant Pathology* **59**, 480–491.

Pierce NB, 1896. Bacteriosis of walnuts. *California Fruit-Grower* **19**, 243.

Pierce NB, 1901. Walnut bacteriosis. *The Botanical gazette* **31**, 272–273.

Pietrarelli L, Balestra GM, Varvaro L, 2006. Effects of simulated rain on *Pseudomonas syringae* pv. *tomato* populations on tomato plants. *Journal of Plant Pathology* **88**, 245–251.

Pinney K, Labavitch JM, Polito VS, 1998. Fruit growth and development. In: *Walnut production manual*. (Editor: D.E. Ramos), University of California, Oakland, USA. pp. 139–143.

Polito VS, 1998. Floral biology: flower structure, development and pollination. In: *Walnut production manual*. (Editor: D.E. Ramos), University of California, Oakland, USA. pp: 127–132.

Radix P, Seigle-Murandi F, 1993. Hazards of copper accumulation in soils of walnut orchards. *Fresenius Environmental Bulletin* **2**, 565–569.

Radix P, Bastien C, Jay-Allemand C, Charlot G, Seigle-Murandi F, 1998. The influence of soil nature on polyphenols in walnut tissue – a possible explanation of differences in the expression of walnut blight. *Agronomie* **18**, 627–637.

Roberts SJ, 1997. Effect of weather conditions on local spread and infection by pea bacterial blight (*Pseudomonas syringae* pv. *pisi*). *European Journal of Plant Pathology* **103,** 711–719.

Robinson RA, 1969. Disease resistance terminology. *Review of applied mycology* **48**, 593–606.

Rodway L, 1912. Bacterial black spot of walnut. *The Agricultural Gazette*. pp. 85–86.

Rudolph BA, 1933. Bacteriosis (blight) of the English walnut in California and its control. *California Agricultural Experiment Station Bulletin* **564**, 3–86.

Rudolph BA, 1943. A possible relationship between the walnut erimose mite (*Eriophyes tristriatus erineus*) and walnut blight. *Science* **98**, 430–431.

Rudolph K, 1993. Infection of the plant by *Xanthomonas.* In: *Xanthomonas* (Editors: J.G. Swings and E.L. Civerolo), Chapman and Hall, London, pp: 193–245.

Saravanan U, 2007. *Copper sensitivity in Tasmanian populations of the walnut blight pathogen Xanthomonas arboricola* pv. *juglandis.* MSc. thesis. University of Tasmania, Australia.

Sasser M, 2006. *Bacterial identification by gas chromatographic analysis of fatty acid methy esters (GC-FAME).* Available from: DOI: http://www.midi-inc.com/pdf/MIS_Technote_101.pdf

Schaad NW, Vidaver AK, Lacy GH, Rudolph K, Jones JB, 2000. Evaluation of proposed amended names of several psuedomonads and xanthomonads and recommendations. *Phytopathology* **90**, 208–213.

Schaad NW, Jones JB, Chun W, 2001. *Laboratory guide for identification of plant pathogenic bacteria; 3rd edition*. The American Phytophathological Society, Minnesota, USA.

Scherm H, Ojiambo PS, 2004. Applications of survival analysis in botanical epidemiology. *Phytopathology* **94**, 1022–1026.

Schuster ML, Coyne DP, 1974. Survival mechanisms of phytopathogenic bacteria. *Annual Review of Phytopathology* **12**, 199–221.

Schwartz HF, Otto KL, Gent DH, 2003. Relation of temperature and rainfall to development of *Xanthomonas* and *Pantoea* leaf blights of onion in Colorado. *Plant Disease* **87,** 11–14.

Scortichini M, Rossi MP, 2003. Genetic Diversity of *Xanthomonas arboricola* pv. *fragariae* strains and comparison with some other *X. arboricola* pathovars using repetitive PCR genomic fingerprinting. *Journal of Phytopathology* **151**, 113–119.

Scortichini M, Marchesi U, di Prospero P, 2001. Genetic diversity of *Xanthomonas arboricola* pv. *juglandis* (synonyms: *X. campestris* pv. *juglandis; X. juglandis* pv. *juglandis*) strains from different geographical areas shown by repetitive polymerase chain reaction genomic fingerprinting. *Journal of Phytopathology* **149**, 325–332.

Sentelhas PC, Monteiro JEBA, Gillespie TJ, 2004. Electronic leaf wetness duration sensor: why it should be painted. *International Journal of Biometeorology* **48**, 202–205.

Sentelhas PC, Gillespie TJ, Batzer JC, Gleason ML, Monteiro JEBA, Pezzopane JRM, Pedro Junior MJ, 2005. Spatial variability of leaf wetness duration in different crop canopies. *International Journal of Biometeorology* **49**, 363–370.

Sentelhas PC, Gillespie TJ, Santos EA, 2007. Leaf wetness duration measurement: comparison of cylindrical and flat plate sensors under different field conditions. *International Journal of Biometeorology* **51**, 265–273.

Shepard DP, Zehr EI, 1994. Epiphytic persistence of *Xanthomonas campestris* pv. *pruni* on peach and plum. *Plant Disease* **78**, 627–629.

Shtienberg D, Schwarz H, Oppenheim D, Zilberstaine M, Herzog Z, Manulis S, Kritzman J, 2003. Evaluation of local and imported fire blight warning systems in Israel. *Phytopathology* **93**, 356–363.

Smith EF, 1905. *Bacteria in Relation to Plant Diseases*. Carnegie Institute of Washington, USA, pp. 171.

Smith CO, 1914. Walnut blight experimental work. *American Nut Journal* **2**, 46.

Smith CO, 1915. Walnut blight or bacteriosis. *California State Department of Agricultural Monthly Bulletin* **4**, 254–258.

Smith RE, Smith CO, Ramsey HJ, 1912. Walnut culture in California - walnut blight. *California Agricultural Experiment Station Bulletin* **231**, 119–398.

Sneath PHA, 1992. *International Code of Nomenclature of Bacteria and Statutes of the International Committee on Systematic Bacteriology and Statutes of the Bacteriology and Applied Microbiology Section of the International Union of Microbiological Societies.* American Society of Microbiology, Washington, DC.

Stall RE, Hall CB, Hicks JR, 1982. Development of the cell collapse phase of the hypersensitive reaction in pepper in atmospheres varying in humidity, oxygen, and carbon dioxide. *Journal of Phytopathology* **103**, 211–217.

Stall RE, Gottwald TR, Koizumi M, Schaad NC, 1993. Ecology of plant pathogenic xanthomonads. In: *Xanthomonas* (Editors: J.G. Swings and E.L. Civerolo), Chapman and Hall, London, pp: 265–290.

Starr MP, 1981. The genus *Xanthomonas*. In: *The Prokaryotes, Volume 1* (Editors: M.P. Starr, H. Stolp, H.G. Truper, A. Balows and H.G. Schlegel), Springer Verlag, Berlin, Germany, pp. 742–763.

Starr MP, Jenkins CL, Bussey LB, Andrews AG, 1977. Chemotaxonomic significance of the xanthomonadins, novel brominated arylpolyene pigments produced by bacteria of the genus *Xanthomonas. Archives of Microbiology* **113**, 1–9.

Steiner PW, 1990. Predicting apple blossom infections by *Erwinia amylovora* using the MARYBLYT model. *Acta Horticulturae* **273**, 139–148.

Steiner PW, Lightner GW, 1996. *Maryblyt™ 4.3, a predictive program for forecasting fire blight disease in apples and pears.* University of Maryland, MD, USA.

Stephens WL, Starr MP, 1963. Localisation of carotenoid pigment in cytoplasmic membrane of *Xanthomonas juglandis. Journal of Bacteriology* **86**, 1070–1074.

Stolp H, Starr M, 1964. Bacteriophage reactions and speciation of phytopathogenic xanthomonads. *Journal of Phytopathology* **51**, 442–478.

Sutton JC, Gillespie TJ, Hildebrand PD, 1984. Monitoring weather factors in relation to plant disease. *Plant Disease* **68,** 78–84.

Swings J, Vauterin L, Kersters K, 1993. The bacterium *Xanthomonas* In: *Xanthomonas* (Editors: J.G. Swings and E.L. Civerolo), Chapman and Hall, London, pp: 121–156.

Tamponi G, Donati GP, 1990. Walnut cultivar susceptibility to *Xanthomonas juglandis*. *Acta Horticulturae* **284**, 301–302.

Teviotdale BL, Schroth MN, 1998. Bark, fruit and foliage diseases. In: *Walnut production manual* (Editor: D.E. Ramos), University of California, Oakland, USA. pp: 242–246.

Timmer LW, Marois JJ, Achor D, 1987. Growth and survival of xanthomonads under conditions nonconductive to disease development, *Phytopathology* **77**, 1341–1345.

Vanderplank JE, 1963. *Plant diseases: epidemics and control*. Academic Press, NewYork.

Vauterin L, Swings J, Kersters K, 1991. Grouping of *Xanthomonas campestris* pathovars by SDS-PAGE of proteins. *Journal of General Microbiology* **137,** 1677–1687.

Vauterin L, Hoste B, Kersters K, Swings J, 1995. Reclassification of *Xanthomonas*. *International Journal of Systematic Bacteriology* **45**, 472–489.

Woeste KE, McGranahan GH, Schroth MN, 1992. Variation among Persian walnuts in response to inoculation with *Xanthomonas campestris* pv. *juglandis*. *Journal of the American Society for Horticultural Science* **117**, 527–531.

Yang P, Vauterin L, Vancanneyt M, Swings J, Kersters K, 1993. Application of fatty acid methyl esters for the taxonomic analysis of the genus *Xanthomonas*. *Systematic and Applied Microbiology* **16**, 47–71.

Yang KQ, Qu WW, Liu X, Liu HX, Hou LQ, 2011. First report of *Pantoea agglomerans* causing brown apical necrosis of walnut in China, *Plant Disease* **95**, 773.

Young K, Galbreath NH, Hewett EW, 1979. A system of regulating sprinkling during evaporative cooling of apple buds to delay bloom. *Journal of Agricultural Engineering Research* **24**, 209–213.

Young JM, Park DC, Shearman HM, Fargier E, 2008. A multilocus sequence analysis of the genus Xanthomonas. *Systematic and Applied Microbiology* **31**, 366–377.

Zhang Y, Gillespie TJ, 1990. Estimating maximum droplet wetness duration on crops from nearby weather station data. *Agricultural and Forest Meteorology* **51**, 145–158.

APPENDICES

A1.1. Journal papers

Lang MD, Evans KJ, 2010. Epidemiology and status of walnut blight in Australia. *Journal of Plant Pathology* **92**, S1.49–55.

Lang MD, Evans KJ, 2010. Timing bactericides strategically for management of walnut blight in Tasmania, Australia. *Acta Horticulturae* **861**, 465–471.

A1.2. Conference abstracts

Lang MD, Evans KJ, Hills JL, 2005. Towards sustainable management of walnut blight in Tasmania. In: *Proceedings of the 15th Biennial Australasian Plant Pathology Society Conference,* APPS, Geelong, Australia, p. 101.

Lang MD, Evans KJ, Pethybridge SJ, 2007. Strategic application of copper for managing walnut blight in Tasmania. In: *Proceedings of the 16th Biennial Australasian Plant Pathology Society Conference, Back to Basics: Managing Plant Disease*, APPS, Adelaide, Australia, p. 38.

Lang M.D, Evans KJ, Pethybridge SJ, 2008. Temporal progression of walnut blight, caused by *Xanthomonas arboricola* pv. *juglandis*, in Australia. In: *9th International Congress of Plant Pathology, Torino, Italy*. A. Porta-Puglia and P. Gonthier, eds. *Journal of Plant Pathology* **90**, S2.360.

Lang MD, Evans KJ, Pethybridge SJ, 2009. Epidemiology of walnut blight, caused by *Xanthomonas arboricola* pv. *juglandis*, in Tasmania, Australia, In: *Proceedings of the 17th Biennial Australasian Plant Pathology Society Conference, Plant Health Management: An Integrated Approach,* APPS, Newcastle, Australi, p. 45.

Lang MD, Hills JL, Evans KJ, 2009. Timing bactericides strategicaly for management of walnut blight in Tasmania, Australia. In: *Proceedings of the 6th International Walnut Symposium*, Australian Walnut Industry Association, Melbourne, Australia, p. 63.

Lang, MD, Evans KJ, Pethybridge SJ, 2009. Epidemiology of walnut blight, caused by *Xanthomonas arboricola* pv. *juglandis*, in Tasmania, Australia. In: *Proceedings of the 6th International Walnut Symposium*, Australian Walnut Industry Association, Melbourne, Australia, p. 70.

Saravanan U, Lang MD, Evans KJ, 2009. Copper tolerant strains of the walnut blight bacterium identified in Tasmania, Australia. In: *Proceedings of the 6th International Walnut Symposium*, Australian Walnut Industry Association, Melbourne, Australia, Abstract 161, p. 31.

A1.3. Conference reports

Lang MD, Evans KJ, 2009. Epidemiology of walnut blight in Tasmania, Australia. Annual Meeting of COST Action 873, Cetera, Italy, October 26–29, 2009. Available from: http://www.cost873.ch/5_activites/meeting_detail.php?ID=24

A1.4. Industry papers

Lang MD (Editor), 2007. *Sustainable management of walnut blight: Project NT04013.* Horticulture Australia Limited, Sydney, Australia, 80 pp.

Lang MD, Evans KJ, 2007. Optimizing spray applications for managing walnut blight in Tasmania. *Australian Nutgrower* **21**: 34–38.

Lang MD, Evans KJ, 2009. Characters of the walnut blight bacterium in Tasmania. *Australian Nutgrower* **23**: 35–39.

Lang MD, Evans KJ, 2011. *A predictive model for managing walnut blight effectively with less copper: Project WN08003*. Horticulture Australia Limited, Sydney, Australia, 28 pp.

Following are descriptions of the symptoms of walnut blight on the shoots, buds, leaves, flowers and fruits of walnuts, including how these symptoms develop, as prepared in exhaustive studies by Rudolph (1933) and Miller and Bollen (1946).

A2:1. Symptoms on shoots

The first visible disease symptoms on shoots consist of dark green, translucent or water soaked circular areas of a few millimetres in diameter. Lesions enlarge as bacteria invade surrounding tissues with the greatest development commonly occurring along the length of the shoot. As the lesions develop, the central areas turn dark brown or black with a narrow translucent or water soaked band surrounding the lesion. The width of the translucent or water soaked band varies according to the development of the disease. In young tender tissue the band may vary from one to several millimetres in width. However, the band may disappear entirely when the disease ceases to be active.

When fully developed, stem lesions are typically irregularly oval, depressed in elevation, have well defined margins and are dark brown to black in colour. The lesion may only involve the outer portion of the bark or it may extend inwards so that all tissues are destroyed. Lesions can range in size from a few millimetres to several centimetres. In severe infections the entire tip of the shoot may be girdled causing the distal portion of the shoot to die. More commonly the infection spreads so slowly that the tip of the shoot grows away from the lesion. Such lesions may never attain a diameter on more than a few millimetres.

The stems of shoots are most commonly infected when the tissues are young and succulent. As the stem ages and their tissues become more lignified the susceptibility to infection diminishes. Thus, in comparison to the apical end of the shoot the basal end is less susceptible to infection. As older dying tissue dries, the epidermis may crack and exude decomposed cellular products and bacterial slime that dries on the tissue surface as a white flaky precipitate. Stem lesions generally become inactive within the first season as shoots mature and become more resistant to infection. Callus formation around the edges of lesions is frequently evident later in the season.

A2:2. Symptoms on buds

Leaf buds and staminate and pistillate flower bearing buds on young shoots are susceptible to the disease. Disease symptoms consist of small, dark green, water soaked areas in the outer bud scales or bracts that turn dark brown as the lesions develop. Buds infected early in the growing season generally die by the end of the season as the pathogen invades the inner parts of the bud. However, if the infection is localized in the bud bracts, the bud may not die, and leaves, staminate and pistillate flowers arising from these buds in the following spring may be infected with bacteria.

A2:3. Symptoms on leaves

All parts of a leaf can be infected by the pathogen, including the lamina, rachis, petiole, midrib, lateral veins and veinlets. Lesions on the tender margins of young growing leaflets generally occur from infections from the previous season within the leaf bud. The basal leaves which occupy the outermost position in dormant buds are infected more often than the apical leaves (Mulrean and Schroth, 1982). In contrast, lesions on the lamina, rachis, petiole, midrib and veins typically occur later in spring and originate from infections that occur in the current season.

Disease symptoms on the margins of leaflets generally appear as circular to irregularly circular, pale yellowish-green, water-soaked, translucent areas which later turn a dark brown as the tissue dies. The presence of lesions on the margin usually causes distortion and malformation of the leaflets as healthy tissue grows away from infected areas.

Lesions on the leaf lamina are often miscellaneously scattered over the leaf surface although they often are grouped near the apical end of the leaflet in positions where water may accumulate. Isolated leaf lesions rarely measure more than 4 mm in diameter, although several smaller lesions may coalesce to for a single larger lesion. When fully developed lesions on the leaf lamina are angular to irregularly circular and reddish to dark brown in color. Lesions on the lamina do not usually distort the shape of the leaf.

Fully developed lesions that are on the midribs, veins, rachis and petiole are commonly dark brown or black and slightly depressed. From the veins the bacteria frequently invade the parenchymatous tissue forming reddish to brown lesions on the lamina. In severe infections, the petiole or rachis may be killed leading to the death of the leaf or leaflet.

A2:4. Symptoms on staminate (male) flowers and catkins

Disease symptoms appear on infected staminate flowers after staminate buds have broken dormancy in spring, and catkins are partially to completely elongated. Individual florets of the catkin initially appear water soaked and wilted which contrasts with the green turgid appearance of healthy florets. The infected florets quickly turn black and die. A single floret may be affected or all the florets on the catkin may show disease symptoms. Similarly the rachis may be infected at any point along its length. In most cases local areas in the catkin are attacked and as a result the catkin becomes distorted and deformed as growth is discontinued in diseased areas.

A2:5. Symptoms on pistillate (female) flowers and fruits

Infections that occur during the pre-bloom or blooming periods are predominantly located at the apical or blossom end of the pistillate flower. Initial symptoms consist of small, circular or irregularly circular water soaked areas in the bracts, bracteoles or involucre. As the pathogen invades the surrounding tissue the lesion increases in size with the tissues turning black as the infected areas age. A narrow water-soaked zone peripheral to the discolored areas often occurs when the disease is active. When bacterial activity ceases this zone usually disappears and the line between diseased and healthy tissue becomes sharply defined. The elevated lesion becomes depressed as the lesion ages and droplets containing decomposed cellular materials and bacteria may ooze from lesions during high humidity.

Infections that occur after bloom of the pistillate flower are predominantly located on the sides of the fruit. While lateral infections may occur from the pre-bloom period and often accompany apical-end infections, they predominate after the pollination period. When fully developed, the lateral lesions appear as depressed, circular to irregularly circular black areas with well defined margins. The size and extent of the

lesion depends upon the age of the fruit at the time of infection and can range from a few millimetres in diameter to over half the surface area of the nut.

Although lesions that occur from infections during the pre-bloom or blooming periods are generally visible on the exterior of the fruit, the interior may be infected without the disease being outwardly noticeable. Infections that occur before the completion of pollination almost invariably end with the fruit being shed from the tree prior to the fruit reaching maturity. Infections that occur between the completion of the blooming period and fruits that are approximately one half full-size diameter frequently penetrate to the newly formed shell tissue and rot the developing ovary or kernel. With increasing shell hardness the shell may be perforated and the developing kernel blackened and shriveled. If infection occurs after the fruits are half full-size diameter the lesions are usually confined to the outer portion of the fleshy hull although the bacteria may occasionally succeed in reaching the shell. If the bacteria reach the shell then the infected hull tissue usually adheres to the shell until maturity and a brown colored stain remains on the shell surface when the lesions are scraped off.

Symptoms of walnut blight have been confused with leaves poisoned by excessive levels of alkali or boron in the soil, with leaves with sunburn and wind damage, with catkins that have had frosts or prolonged periods of rain during flowering or when staminate buds begin to grow (Rudolph, 1933; Miller and Bollen, 1946), and on fruit with the initial symptoms of the fungal disease Anthracnose caused by *Gnomonia leptostyla* (Miller *et al.*, 1940). Furthermore, in early fruit development in Italian and French orchards, symptoms of brown apical necrosis, caused by fungal pathogens of the genus *Fusarium* and *Alternaria*, in association with *X. arboricola pv. juglandis*, have also been confused with walnut blight symptoms (Belisario *et al.*, 2002; Moragrega and Ozaktan, 2010).

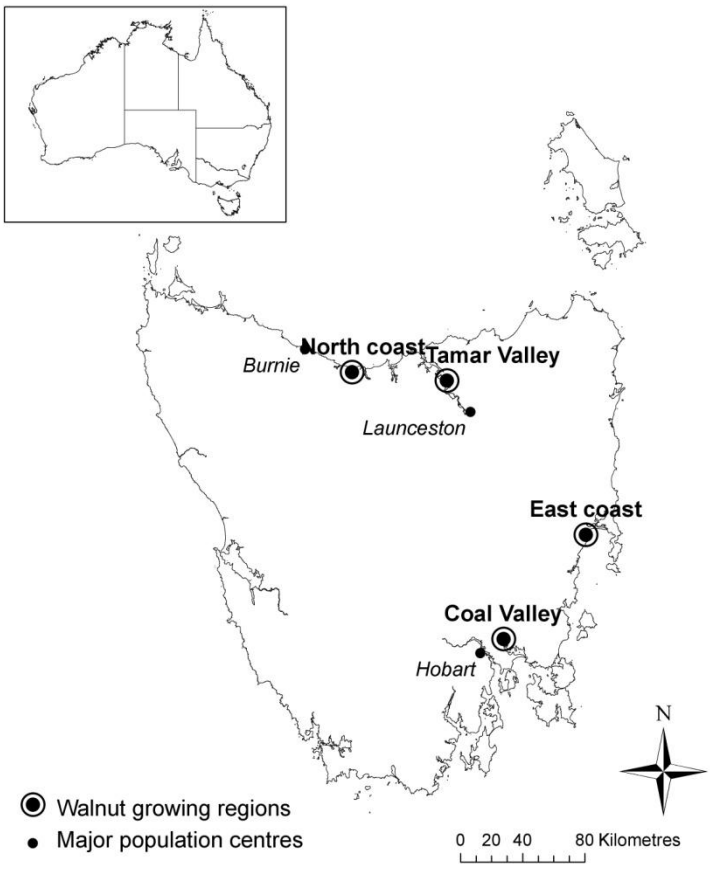

Fig. A4.1. Location of the major walnut growing regions in Tasmania, Australia.

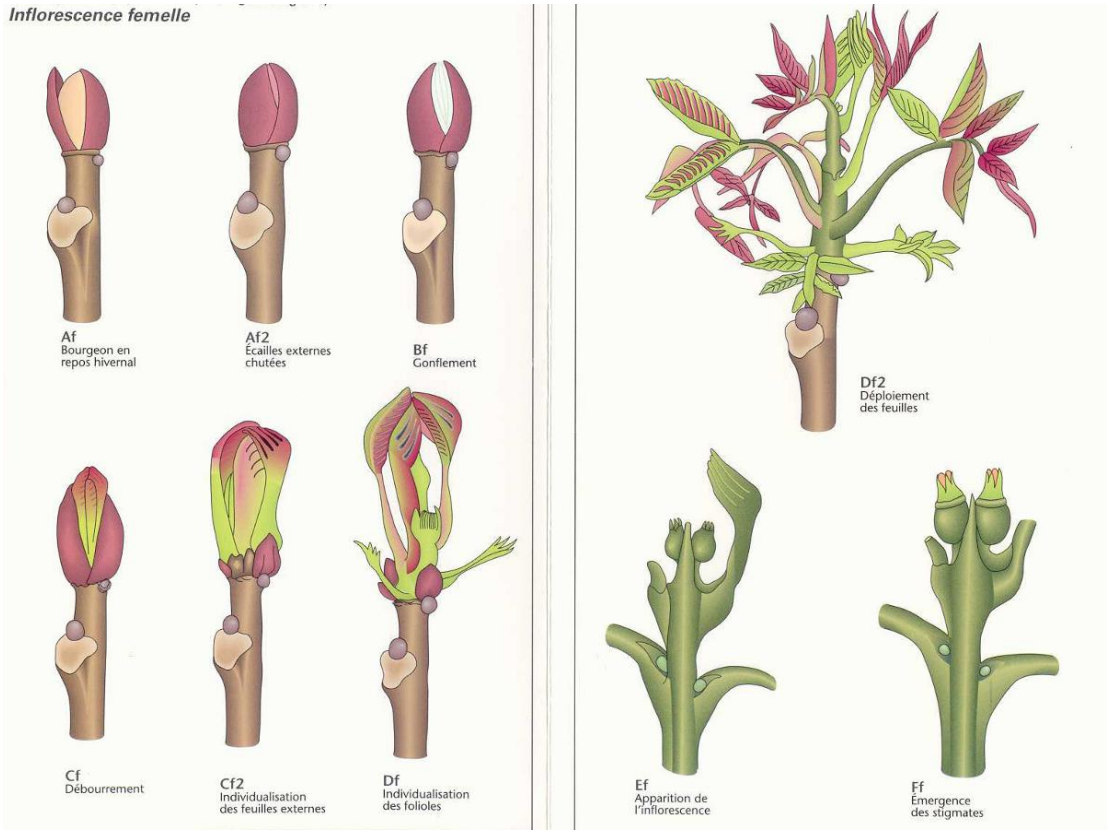

Fig. A5.1. Phenological stages of walnut buds and pistillate flowers, from Af to Ff (adapted from Germain *et al*. 1999); Af – during winter the bud is covered with scales and is dormant; Af2 – as early as July the harder outside scales fall and the bud is enveloped by other semi-membranous scales; Bf – white bud (the bud inflates, the external scales loosen and a whitish down appears under the ends of the scales); Cf – budburst (the bud lengthens and the exterior of the basal leaves are distinguishable); Cf2 – the scales and bracts separate and the first leaves begin to separate; Df – the first leaves are separate and their leaflets are well individualized; Df 2 – the first leaves are completely deployed and the basal leaves are more or less oblique; Ef – appearance of the female flowers; Ff – appearance of the stigmas. In Tasmania, stage Cf in Vina normally occurs in October, and takes 2–3 weeks to progress to stage Ff; these stages occur approximately 3 weeks later in Franquette.

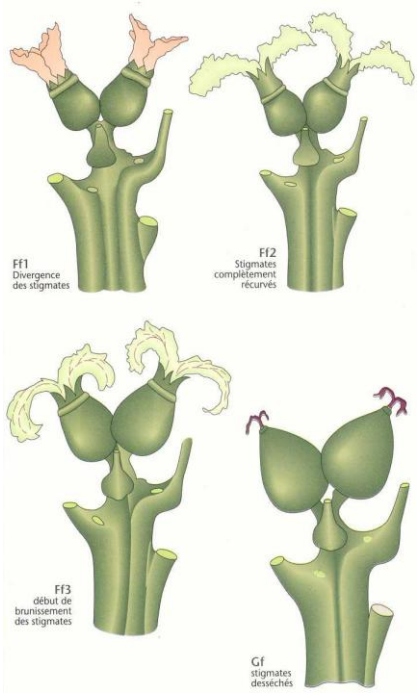

Fig. A5.2. Phenological stages of walnut pistillate flowers, from Ff1 to Gf (adapted from Germain *et al*. 1999); Ff1 – the stigmas are yellow to orange colour, with optimum receptivity i.e., full female flowering; Ff2 – the stigmas are a green to pale yellow colour and are completely re-curved; Ff3 – the stigmas begin to turn a fine brown color; Gf – the stigmas dry out and become necrotic. In Tasmania, stage Ff1 in Vina normally occurs in November, and takes 10–14 days to progress to stage Gf; these stages occur approximately 3 weeks later in Franquette.

The reference *X. arboricola* pv. *juglandis* strain DAR33423 (Plant Pathology Herbarium, NSW Agriculture, Australia) was recovered from −80°C storage and grown on glucose yeast extract calcium carbonate agar. After 48 h incubation at 26°C, five suspensions of *X. arboricola* pv. *juglandis* were prepared by transferring loopfuls of inoculum into 10 mm McCartney bottles containing 5 ml sterile distilled water (SDW). Suspensions were agitated for 10 min on a RaTek platform mixer (RaTek Instruments Pty. Ltd., Melbourne, Australia) at 100 rpm. After agitation, the absorbance of each suspension was determined with a spectrophotometer (Unicam Helios Gamma, ThermoFisher Scientific, England), at a wavelength of 620 nm.

A 0.5 ml solution from each suspension was then serially diluted a total of six times, into 4.5 ml SDW in McCartney bottles. Suspensions were agitated for 10 min on a RaTek platform mixer at 100 rpm between each serial dilution. A 0.5 ml sample from each diluted suspension was then plated onto nutrient agar (NA; Fluka, Buchs, Switzerland) in a Petri plates and incubated at 26°C for 4 days. The numbers of active bacterial colonies were then counted, and the colony forming units (cfu) per ml calculated. The equation was given by:

$$cfu = [(absorbance + 1.22 \times 10^{-2}) / 7.18 \times 10^{-7}] \quad \text{(Eq. A6.1)}$$

where cfu is the predicted colony forming units of *X. arboricola* pv. *juglandis* per ml of bacterial suspension at an absorbance of 620 nm.

This equation was then used to determine the cfu of a suspension of *X. arboricola* pv. *juglandis* after measuring its absorbance at 620 nm. The suspension was then adjusted to the desired range in cfu using SDW.

Xanthocast™ (Adaskaveg *et al*., 2000), a temperature-mediated surface wetness accumulation model: as interpreted by Michael Lang

1. Each day, determine the number of hours in each of three temperature ranges, from 6 to <12°C, from 12 to 17°C and from >17 to 27°C.

2. Each day, determine the number of hours fruits are wet for each temperature range, with the duration of fruit "wetness" estimated with surface wetness sensor values between 10 and 100%.

3. Each day, add one "point" for every 8, 4 and 1 hrs duration of surface wetness for the 6 to <12°C, 12 to 17°C and >17 to 27°C temperature categories respectively.

4. Multiply the points for the 12 to 17°C category by 3 then sum the points across each temperature category to get a "daily disease index".

5. Sum each daily disease index for a period of 7 days or until a threshold of 5 is reached. Apply a bactericide as soon as possible after a threshold of 5 is reached.

6. If a threshold of 5 is not reached within 7 days, then sum the daily disease index over a total of 7 days by adding the most recent daily disease index and removing the oldest daily disease index. Apply a bactericide as soon as possible after a threshold of 5 is reached.

7. Seven days after applying the bactericide, begin cumulating the daily disease index again until a threshold of 5 is reached, as previously described.

Table A8.1: Regression parameters for percent new blight per day (PNB) of Vina and Franquette fruits as a function of environmental variables cumulated 17 to 24 adjusted-calendar-days (Tmin = 1°C Tmax = 35°C) prior to disease assessments for nine site-years.

Cultivar	Environmental variable	n	PNB (y)	Intercept	Slope ($\times 10^2$)	R^2	*P−value*
Vina	Maximum temp. (°C)	14	y	5.977	− 3.031	9.6	0.28
	Mean RH (%)	9	ln (y)	− 0.040	0.083	2.2	0.68
	RH 90-100% (h)	9	ln (y)	− 0.046	0.919	9.1	0.40
	Surface wetness (h)	14	y	2.346	− 1.684	18.5	0.12
Franquette	Mean temp. (°C)	17	ln (y)	− 1.261	0.911	1.3	0.66
	Maximum temp. (°C)	17	ln (y)	1.954	− 1.323	3.2	0.49
	Mean RH (%)	13	ln (y)	1.121	− 0.210	1.1	0.73
	RH 90-100% (h)	13	ln (y)	− 1.732	3.911	16.7	0.17
	Surface wetness (h)	17	ln (y)	0.170	− 0.669	4.8	0.40

Brilliant cresyl blue-starch (BS) medium (Mulrean and Schroth, 1981)

Potato starch (Difco)	10.0 g
$K_2HPO_4 \cdot 3H_2O$	3.0 g
KH_2PO_4	1.5 g
$(NH_4)_2SO_4$	2.0 g
L-methionine	0.25 g
Nicotinic acid	0.25 g
L-glutamate	0.25 g
Brilliant cresyl blue	0.01 g
Methylene green	0.01 g
Bacto agar (Difco)	15.00 g
Sterile distilled water	1,000 ml

Adjust pH of the medium to between 6.8 and 7.0 before autoclaving with 8.0 ml/l of 1 N NaOH. The medium may be stored in bottles and re-melted when needed.

Succinate-quinate (SQ) medium (Lee *et al*., 1992)

Succinic acid (disodium salt, hexahydrate)	10.0 g
Quinic acid	5.0 g
K_2HPO_4	1.5 g
$(NH_4)_2SO_4$	1.0 g
Yeast extract	0.5 g
Agar	15.0 g
Sterile distilled water	1,000 ml

Adjust pH of the medium to between 7.2 and 7.5 with 10.0 ml/l of 10 N NaOH before autoclaving for 20 min at 121^oC. After autoclaving, cool the medium to about 50^oC and then add 7.5 ml of autoclaved 20% $MgSO_4.7H_20$.

Modified Tween (TB) medium (Schaad *et al*., 2001)

Bacto-peptone (Difco)	10.0 g
KBr	10.0 g
CCl_2 (anhydrous)	2.0 g
Boric acid	0.3 g
Bacto agar (Difco)	15.0 g
Tween 80	20.0 ml
Sterile distilled water	1,000 ml

Autoclave and then 15-30 min before pouring add aseptically prepared aqueous solutions of cyclohexamide and antibiotics:

Cyclohexamide	1.0 ml

Prepare by adding 1.0 g cyclohexamide to 10 ml 50% ethanol

Cephalexin	10.0 ml

Prepare by adding 100.0 mg cephalexin to 10 ml sterile distilled water (SDW)

5-Fluorouracil	0.66 ml

Prepare by adding 50.0 mg 5-fluorouracil to 10 ml 50% ethanol

Tobramycin	0.33 ml

Prepare by adding 10.0 mg tobramycin to 10 ml SDW