EPIDEMIOLOGY AND MANAGEMENT OF WALNUT BLIGHT IN TASMANIA

by

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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

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<td>AFLP</td>
<td>Amplified fragment length polymorphism</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AUDPC</td>
<td>Area under the disease progress curve</td>
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<tr>
<td>BOX</td>
<td>BOX elements</td>
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<tr>
<td>BS</td>
<td>Brilliant cresyl blue starch medium</td>
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<tr>
<td>cfu</td>
<td>Colony forming unit</td>
</tr>
<tr>
<td>CHEF</td>
<td>Contour-clamped homogenous electric field electrophoresis</td>
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<tr>
<td>CRV</td>
<td>Critical risk value</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EBDC</td>
<td>Ethylene-bisdithiocarbamate</td>
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<tr>
<td>EIL</td>
<td>Economic injury level</td>
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<tr>
<td>ERIC</td>
<td>Enterobacterial repetitive intergenic consensus</td>
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<tr>
<td>FN</td>
<td>False negative</td>
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<tr>
<td>FNP</td>
<td>False negative proportion</td>
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<tr>
<td>FP</td>
<td>False positive</td>
</tr>
<tr>
<td>FPP</td>
<td>False positive proportion</td>
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<tr>
<td>GC-FAME</td>
<td>Gas chromatography-fatty acid methyl ester</td>
</tr>
<tr>
<td>GYCA</td>
<td>Glucose-yeast extract-calcium carbonate</td>
</tr>
<tr>
<td>MI</td>
<td>Moisture intensity</td>
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<tr>
<td>MLSA</td>
<td>Multilocus sequence analysis</td>
</tr>
<tr>
<td>NA</td>
<td>Nutrient agar</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PNB</td>
<td>Percent new blight</td>
</tr>
<tr>
<td>PTB</td>
<td>Packing tissue brown</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
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<tr>
<td>pv.</td>
<td>Pathovar</td>
</tr>
<tr>
<td>pvs.</td>
<td>Pathovars</td>
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<tr>
<td>REP</td>
<td>Repetitive extragenic palindromic</td>
</tr>
<tr>
<td>RH</td>
<td>Relative humidity</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>SAUDPC</td>
<td>Standardised area under the disease progress curve</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>Sodium dodecyl sulfate polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>SDW</td>
<td>Sterile distilled water</td>
</tr>
<tr>
<td>SQ</td>
<td>Succinate quinate medium</td>
</tr>
<tr>
<td>TB</td>
<td>Modified Tween medium</td>
</tr>
<tr>
<td>TP</td>
<td>True positive</td>
</tr>
<tr>
<td>TPP</td>
<td>True positive proportion</td>
</tr>
<tr>
<td>TN</td>
<td>True negative</td>
</tr>
<tr>
<td>TNP</td>
<td>True negative proportion</td>
</tr>
<tr>
<td>WMAR</td>
<td>Weighted mean absolute rate</td>
</tr>
<tr>
<td>YDC</td>
<td>Yeast dextrose calcium carbonate agar</td>
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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 10⁹ 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 ($T_{min}=1^\circ C; T_{max}=35^\circ C$) 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
Abstract

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.