Pathogen eradication using the pistachio dieback bacterium as a model

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Pistachio nuts

Dedicated to my parents,

Vu Tu Hung

&

Thanh Thi Yen My

with all my love, respect and gratitude

Table of contents

Abstract

Pistachio dieback is a bacterial disease involving internal staining, trunk and limb lesions, decline, dieback and, in some instances, death of trees. The causal agent is *Xanthomonas translucens* pv. *pistaciae* (*Xtp*), a vascular pathogen that provides a local model to assess the effectiveness of existing eradication strategies for systemic bacterial pathogens of woody perennials which are likely to be introduced into Australia. Burning and burial are two accepted means of disposal of diseased plant material for eradication purposes. However, there is little or no information on the survival of bacterial pathogens following burning or burial of infected wood. The aim of the project was to evaluate the efficacy of burning and burial as means of safe disposal of diseased wood.

Burning of pistachio wood, naturally infected with *Xtp*, was conducted twice in field conditions. Controlled laboratory experiments with pure cultures of *Xtp* and with naturally and artificially infected pistachio wood were performed to support the results of the burns. Viable *Xtp* was detected in some non-burned wood, but not in charcoal, ash or partially burned wood. In liquid culture, 65°C was lethal to *Xtp* whereas survival at 60^{o}C or less varied with culture medium and duration of exposure. In infected wood *Xtp* survived exposure to 40 - 55°C for at least 60 min *in vitro* but was killed by exposure to 60°C for 15 min or more. These data corroborated the burning experiment.

Survival of *Xtp* in infected pistachio wood placed on the soil surface or buried 10 cm deep was evaluated in an open environment at the University of Adelaide, Waite Campus orchard, South Australia. The experiment was conducted from August 2008 to March 2011 using naturally infected pistachio wood segments and mulched wood, and was partially repeated in 2010 over 5 months using naturally infected pistachio wood segments and artificially infected twigs. Viable *Xtp* was isolated from branch segments and from

mulched wood buried for 31 and 23 months, respectively. Viable *Xtp* was not detected in branch segments placed on the soil surface at any time, but was detected in one mulched wood sample at 20 months and in artificially infected twigs for up to 3 months after the placement of wood on the soil surface. Prevailing dry weather conditions during the study might have contributed to the quick decline in *Xtp* population in the wood on the soil surface. Infrequent isolation of *Xtp* from buried materials might have been due to the entry of the pathogen into a dormant state, such as viable but nonculturable, in response to changing environments during burial.

The ability of copper to induce *Xtp* to become viable but nonculturable was investigated. Copper induced nonculturability in *Xtp* at 0.05 mM but this effect was not obvious at 0.005 or 0.01 mM. *Xtp* exhibited some ability to adapt to the presence of copper at 0.05 mM and there was some indication that spontaneous mutants existed in the *Xtp* population prior to exposure to copper. Further research is required to confirm the existence of the viable but nonculturable state in *Xtp* as well of spontaneous copper-resistant mutants in the population.

In conclusion, burning is an appropriate eradication technique to dispose of infected debris, providing the pathogen is exposed to a temperature of 60°C or greater for at least 15 minutes. Decomposition of woody material and loss of viability of the pathogen were slow and influenced by environmental conditions. In addition, the pathogen might enter a nonculturable state or evolve in response to changing conditions during burial and become a possible source of inoculum for new infections. Overall, knowledge gained from this study provides information to support and extend existing eradication response strategies for newly introduced or emerging pathogens.

Declaration

I, Vu Thanh Tu Anh, certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Vu Thanh Tu Anh

Statement of the contributions to jointly authored paper

T. A. Vu Thanh, M. R. Sosnowski, D. Giblot-Ducray, C. Taylor and E. S. Scott, 2012. Effect of burning and high temperature on survival of *Xanthomonas translucens* pv. *pistaciae* in infected pistachio branches and twigs. Doi: 10.1111/j.1365-3059.2012.02596.x. Presented in Chapter 4.

Author contributions: TAVT and MRS designed and conducted the first burning experiment. TAVT designed and conducted the second burning experiment and *in vitro* temperature experiments, analysed the data and drafted/constructed the manuscript. ESS, MRS and DGD contributed to the research ideas, design, interpretation of experiments and the editing of the manuscript. CT liaised with local authorities and pistachio growers for materials and assisted with the burning experiments. All co-authors contributed to the final version of the manuscript.

The paper presented in this thesis is in submission form according to the instructions to authors of the journal available on the following website: http://www.wiley.com/bw/submit.asp?ref=0032-0862&site=1. *This thesis has been prepared according to the University of Adelaide's specifications for 'combination conventional/publication format'.*

The following authors agree that the statement of the contributions to jointly authored paper accurately describes their contribution to the research manuscript and give consent to its inclusion in this thesis.

T. A. Vu Thanh ----------

M. R. Sosnowski ----------

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Vu Thanh, T. A., Giblot-Ducray, D., Sosnowski, M. and Scott, E. 2009. Effect of burial and burning on survival of pistachio dieback bacteria. Microbial Ecology Workshop: Concepts and techniques for disease control, a workshop of the 17th Australasian Plant Pathology Society Conference. Newcastle, New South Wales, Australia. 27 September, p 16.

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Sosnowski, M. R., Emmett, R. W., Vu Thanh, T. A., Wicks, T. J. and Scott, E. S., 2009. Eradication of *Elsinoe ampelina* by burning infected grapevine material. Proceedings of the 17th Australasian Plant Pathology Society Conference. Newcastle Civic Centre, Newcastle, New South Wales, Australia. 29 September - 1 October, p 208.

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Abbreviations

ABSPA	antibiotic benlate sucrose peptone agar
ANOVA	analysis of variance
BOM	Bureau of Meteorology
bp	base pair
BSPA	benlate sucrose peptone agar
CFU	colony forming units
dNTPs	deoxynucleotide triphosphates
EPP	emergency plant pest
h, min, s	hour, minute, second
LB	Luria-Bertani broth
LSD	least significant difference
NA	nutrient agar
NA+A	nutrient agar plus antibiotics
NB	nutrient broth
PCR	polymerase chain reaction
PVP	polyvinylpyrrolidone
SDW	sterile distilled water
SPA	sucrose peptone agar
SPB	sucrose peptone broth
SPC	spread plate count