PLANT & FOOD RESEARCH Pseudomonas syringae pv actinidiae (Psa) RESEARCH NOTE

PROJECT DETAILS

Project Title	5.6 Budwood remediation - Interim report
Project Protocol No./ Objective No.	
Project Leader	Kerry Everett
Research Requested / Contracted by	ZESPRI
Date (Month, Year)	March 2011

KEY QUESTION AND AIM

Canes of vines infected with *P.s.* pv. *actinidiae* are becoming systemically infected and showing signs of dieback and other secondary symptoms. Canes are used for propagating new varieties of kiwifruit, and existing varieties. It is essential to find a method to ensure that propagating material is free of Psa, especially if a valuable irreplaceable new variety becomes infected by Psa.

Aim: To kill all Psa bacterial cells on or in propagating material.

METHODOLOGY

EXPERIMENTAL DESIGN

Kiwifruit canes will be harvested from an infected orchard in February (dormant wood) and in early June/July (cuttings and graftwood).

The thermal death point will be determined 'in vitro' for three isolates of Psa. A bacterial suspension of 10^5 cfu/ml will be treated by placing in an eppendorf tube then floating in a water bath at 30C, 35C, 40C, 45C, 50C, 55C and 60C. At each temperature a 100 ul aliquot will be removed after 5, 10, 20, 30 and 60 minutes, placed onto King's medium B (KMB) (King et al., 1954) in Petri plates and spread with a sterilised bent glass rod. Petri plates will be placed at 28C for 24 hours and any growth recorded. There will be three replicate eppendorf tubes per isolate/time/temperature combination.

On the basis of these 'in vitro' results a protocol for treating kiwifruit graftwood/cuttings was evaluated using dormant cuttings to test the impact of the treatment on the viability of the wood.

Kiwifruit cane material harvested in early June from an infected orchard will be sampled by isolating immediately after harvest, and will then be treated by placing in a water bath at 10 different time/temperature combinations. Following

treatment, this material will be sampled by isolating, and replicate canes will be processed as per normal for grafting and for rooting, and compared with untreated material.

Isolations will be made using two methods. The first will be by washing the outside of the canes by placing bacterial saline (BS) and the cane in a plastic bag followed by shaking/massaging for 2 mins. A 100 ul aliquot will be spread on KMB in Petri plates and incubated for 24 hours at 28C. Any growth will be recorded, and isolates identified by PCR using the method of Rees-George et al. (2010). For the second method the canes will be surface sterilised (Everett et al., 2003) then dissected and the internal tissue will be crushed in sterile distilled water then spread onto KMB. Any resultant bacterial colonies will be identified by PCR using the method of Rees-George et al. (2010).

Rationale:

Removal of live bacteria from the inside of propagation material will be difficult using chemicals. Heat or cold treatment are probably the only viable options to do that. Heat treatment is routinely used for removing viruses and bacteria from grape cuttings, and could be applied to removing Psa from kiwifruit cuttings. Cold treatment will be easier to implement as it is part of current practice and should also be tested.

KEY RESULTS (all results must be auditable in terms of access to raw data if required)

1) Result of the in vitro trails have been reported previously

FUTURE RESEARCH STEPS

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RECOMMENDATIONS FOR INDUSTRY

REFERENCES

Everett, K.R., Rees-George, J., Parkes, S.L. and Johnston, P.R. 2003. Predicting avocado fruit rots by quantifying inoculum potential in the orchard before harvest. 5th World Avocado Congress. 19-24th October. Granada-Malaga, Spain. pp. 601-606. p. 601-606.

King, E.O., Ward, M.K. and Raney, D.E. 1954. Two simple media for the demonstration of pyocyanin and fluorescin Journal of Laboratory and Clinical Medicine 44:301-307.

Rees-George, J., Vanneste, J.L., Cornish, D.A., Pushparajah, I.P.S., Yu, J., Templeton, M.D. and Everett, K.R. 2010. Detection of *Pseudomonas syringae* pv. *actinidiae* using Polymerase Chain Reaction (PCR) primers based on the 16S-23S rDNA intertranscribed spacer region and comparison with PCR primers based on other gene regions. Plant Pathology 59:453-464.

This report has been prepared by The New Zealand Institute for Plant & Food Research Limited (Plant & Food Research), which has its Head Office at 120 Mt Albert Rd, Mt Albert, Auckland.

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