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

KEY PROJECT DETAILS

Project Title	Can Psa be removed from propagation material? (5.6 Remediation of budwood Phase 1)
Project Leader	Kerry Everett
Research Requested / Contracted by	ZESPRI

RESEARCH QUESTION AND AIM

There is evidence that canes of vines infected with *P.s.* pv. *actinidiae* (Psa) become systemically infected. Canes are used for propagating new varieties of kiwifruit, and existing varieties. It would be useful to find a method to ensure that propagating material is free of Psa, especially if a valuable irreplaceable new variety becomes infected by Psa. This research note reports on the first step (phase 1), which is to identify the thermal death point of the bacterial cells. Phase 2 will identify the thermal death point of the bacterial cells in budwood and the survival of budwood at temperatures that are lethal to bacterial cells.

METHODOLOGY (Include brief details of experimental design, methodology and protocols)

The thermal death point was determined in vitro for three isolates of Psa (KEP1 and KEP2 isolated from KPIN 2389 and isolate KEP3 from KPIN 7668, also known as RP2. A bacterial suspension of 10^5 cfu/ml was treated by placing in an Eppendorf tube then floating in a water bath at 26.3, 35, 40, 45, 50, 55 and 60°C. At each temperature, a 100-µl aliquot was removed after 5, 15, 35, 75, 155, 315 and 635 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 were placed at 25°C for 24 hours and colonies counted. There were three isolate replicates each in an Eppendorf tube for each time:temperature combination.

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

1) A three-way analysis of variance using MINITAB® release 15 with isolate (replicate), time and temperature as factors showed that isolate was not significant, and that time and temperature were significant ($P<0.0001$).
2) Raw data showed that 50°C was a lethal temperature for Psa, even after only 5 min of exposure (Figure 1).
3) Any time:temperature combination greater than 45°C for 5 minutes killed Psa (Figure 2).
4) Modelling the thermal response using the matrix function of the graphics package Origin® 7.5 (Figure 3) suggested that 42.5°C for longer than 150 minutes was the lowest time:temperature combination that could be used to kill the bacterial cells.

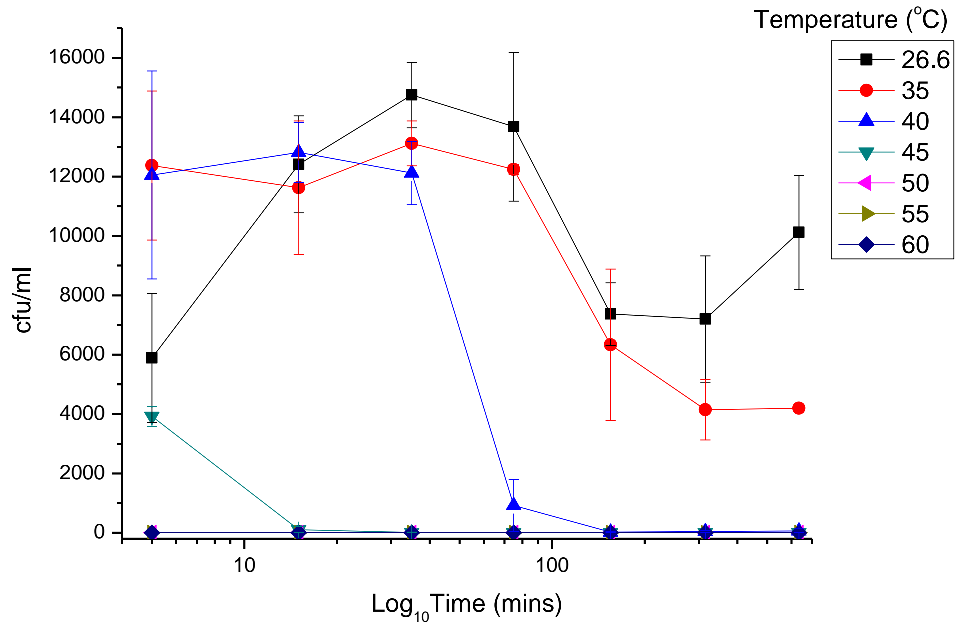

Figure 1. Mean colony-forming units per ml ± standard errors of three *Pseudomonas syringae* pv. *actinidiae* (Psa) isolate replicates (KEP1, KEP2 and KEP3) after exposure to different temperatures for different lengths of time then placing on King's medium B for 24 hours at 25°C.

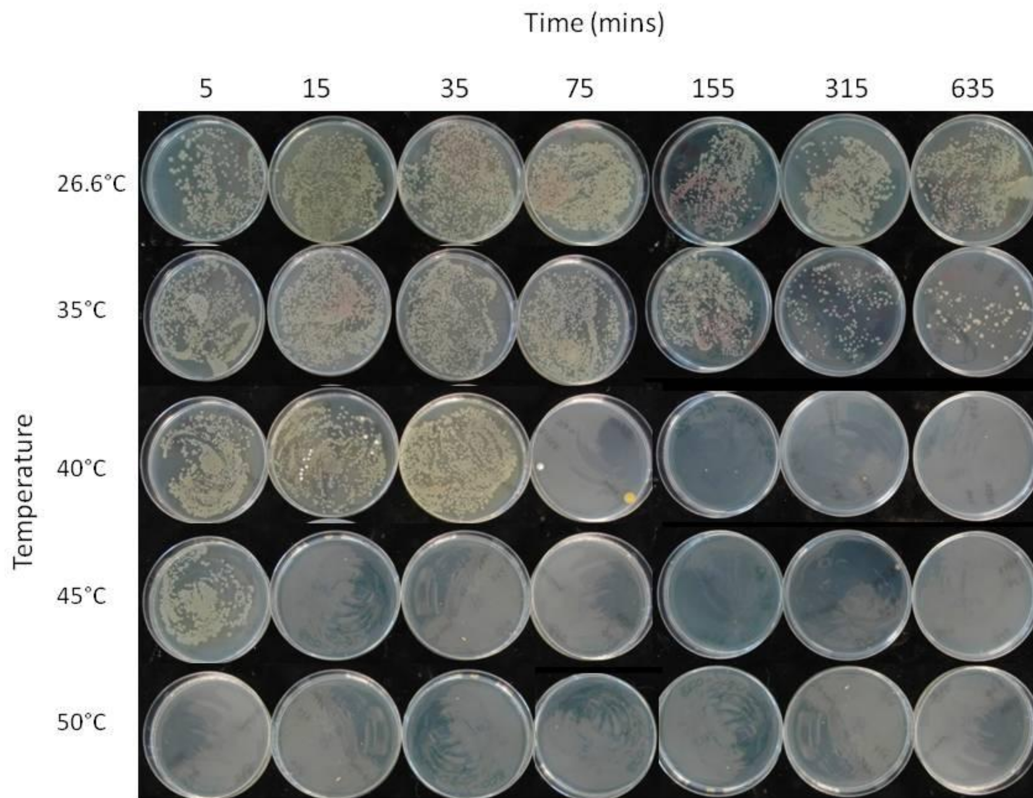

Figure 2. The number of colonies for each time:temperature combination for *Pseudomonas syringae* pv. *actinidiae* isolate KEP3 after 24 hours at 25°C on King's medium B.

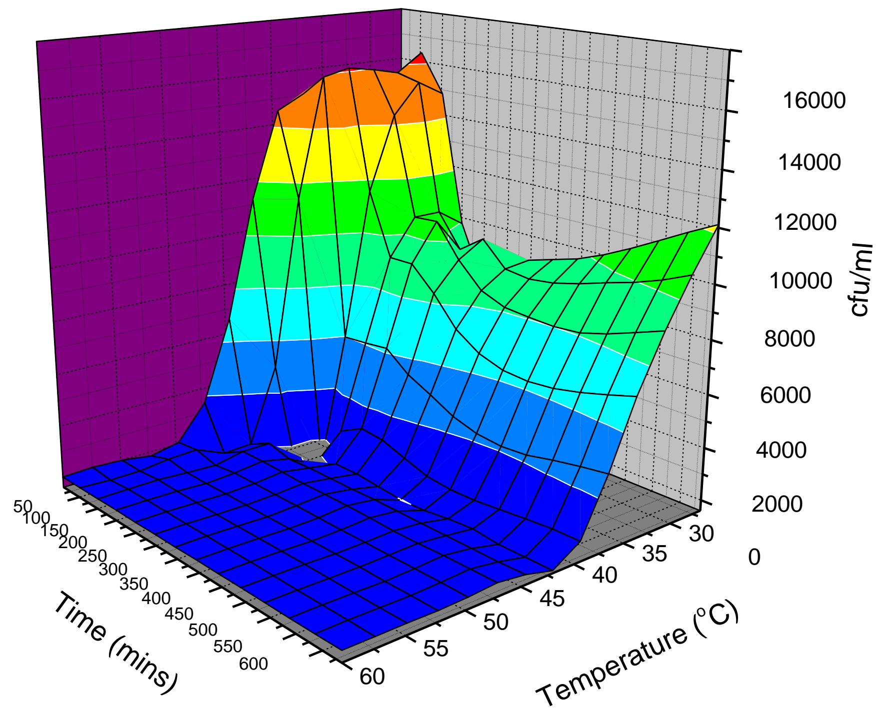

Figure 3: Model of the time:temperature combinations necessary to kill bacterial cells of *Pseudomonas syringae* pv. *actinidiae*, using the matrix function of the graphics package Origin® 7.5.

RECOMMENDATIONS FOR INDUSTRY

For elimination of bacteria in infected tissue, temperatures of 50°C for 1 hour at the centre of infected wood, fruit or leaves should result in 100% mortality of Psa.

CONCLUSIONS

Temperatures of 45°C for 15 minutes or longer or at 50ºC or higher for 5 minutes or longer will kill Psa cells. Modelling suggested that 42.5ºC for 100 minutes was sufficient to kill Psa cells but this needs to be confirmed experimentally, and the effect on the budwood is yet to be determined.

FUTURE STEPS

The time:temperature combinations capable of killing bacterial cells inside budwood without affecting the health of the budwood need to be determined. Temperatures between 40°C and 50°C for times less than 60 minutes will be investigated in more detail.

REFERENCE

King EO, Ward MK, Raney DE 1954. Two simple media for the demonstration of pyocyanin and fluorescin Journal of Laboratory and Clinical Medicine 44: 301-307.

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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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