Plant & Food RESEARCH
RANGAHAU AHUMĀRA KAI

www.plantandfood.com

PSA 3.7 Cleaning infected pollen

Everett K

February 2011

A report prepared for

Greg Clarke, Zespri, Mt Maunganui

Everett K

Plant & Food Research, Mt Albert

SPTS Client Report No.

PFR Client Rpt No. 42266

Name:	Kerry Everett
Qualifications:	PhD
Position:	Senior Scientist
Group:	BioProtection - Pathology & Applied Mycology
Team:	Horticultural Crop Diseases
Site:	Plant & Food Research Mt Albert 120 Mt Albert Road Sandringham Auckland 1025, New Zealand
Direct Dial:	+64-9-925 7133
Mobile:	+64-21-2268 133
Fax:	+64-9-925 7001
Email:	kerry.everett@plantandfood.co.nz

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

KEY PROJECT DETAILS

Project Title	Cleaning infected pollen
Project Protocol No./ Objective No.	PSA 3.7
Project Leader	Kerry Everett
Research Requested / Contracted by	ZESPRI Group Ltd
Date (Month, Year)	February 2011
Based on information as at	February 2011

RESEARCH QUESTION AND AIM

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

Experimental design

Table 1: Freeze/thaw cycles for Experiment 1

Treatment	Freeze	Thaw	Freeze	Thaw	Freeze	Thaw
1.1	1 h	1 h				
1.2	1 h	1 h	1 h	1 h		
1.3	1 h	1 h	1 h	1 h	1 h	1 h
2.1	2 h	2 h				
2.2	2 h	2 h	2 h	2 h		
2.3	2 h	2 h	2 h	2 h	2 h	2 h

Table 2: Freeze/thaw cycles for Experiment 2

Treatment	F	T	F	T	F	T	F	T	F	T	F	T	F	T
1	+	+												
2	+	+	+	+										
3	+	+	+	+	+	+								
4	+	+	+	+	+	+	+	+						
5	+	+	+	+	+	+	+	+	+	+				
6	+	+	+	+	+	+	+	+	+	+	+	+		
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+= 30 minutes

F = Freeze

T= Thaw

Table 3: Freeze/thaw cycles for Experiment 3

Treat-ment	12 hr	F	T	F	T	F	T	F	T	F	T	F	T	F	T	F	T
1		+	+														
2	+	+	+	+	+	+	+										
3	+	+	+	+	+	+	+	+	+								
4	+	+	+	+	+	+	+	+	+	+	+						
5	+	+	+	+	+	+	+	+	+	+	+	+	+				
6	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+		

+ = 1 hour except for column 1 (12 hours)

F = Freeze

T= Thaw

Methods and protocols

Pollen supplied by MAF Biosecurity and by PFR Te Puke was weighed out into 1.5ml Eppendorf tubes. There were three replicate tubes containing 0.03g of pollen each from three different pollen containers for each treatment.

1. Experiments 1, 2 and 3. Tubes containing pollen were placed in the freezer (-18°C) and then at ambient (c. 25°C) for the times described in Table 1, 2 and 3. After the final freeze thaw cycle, 400 µl of Oxoid nutrient broth was added to the dry pollen and vortexed once immediately after addition of nutrient broth, and again after 30 mins. After another 30 mins 10 µl was placed on King's medium B undiluted, and at a dilution of 1:10, 1:100, 1:1000 and 1: 10000 v/v with sterile deionised water. After 48 hours incubation at 25°C (for experiment 1 colonies were also counted after 24 hours) colonies were counted and the number of colony forming units per ml of nutrient broth per g of pollen was calculated and presented as a logarithmic function.
2. Experiment 4. An aliquot of 400 µl of 95% ethanol was added to the dry pollen and then vortexed. After 5, 10 and 20 minutes the pollen plus ethanol was centrifuged for 5 mins at 8500 rpm and the ethanol was removed, then 400 µl of Oxoid nutrient broth was added and the suspension was immediately vortexed. After 1 hour 10 µl was placed on King's medium B undiluted, and at a dilution of 1:10, 1:100, 1:1000 and 1: 10000 v/v with sterile deionised water. After 48 hours incubation at 25°C colonies were counted and the number of colony forming units per ml of nutrient broth per g of pollen was calculated and presented as a logarithmic function.
3. Experiment 5. An aliquot of 400 µl of 17.5M acetic acid (glacial), 5M acetic acid, 1 M acetic acid (the concentration in vinegar) and 0.1M acetic acid was added to three replicate Eppendorf tubes of pollen and immediately vortexed. After 30 minutes tubes were vortexed again, and after another 30 minutes tubes containing pollen and acetic acid were centrifuged for 5 mins at 8500 rpm and the acetic acid was removed, then 400 µl of Oxoid nutrient broth was added and

the suspension was immediately vortexed, then vortexed again after 30 minutes. After 1 hour in total 10 µl was placed on King's medium B undiluted. An aliquot of 10 µl of nutrient broth and 10 µl sterile deionised water were also placed on King's medium B after all other tubes to check for contamination. After 48 hours incubation at 25°C colonies were counted and the number of colony forming units per ml of nutrient broth per g of pollen was calculated and presented as a logarithmic function.

4. Experiment 6. An aliquot of 400 µl of 5M, 3M, 2M and 1M acetic acid was added to three replicate Eppendorf tubes of pollen and immediately vortexed. After 30 minutes tubes were vortexed again, and after another 30 minutes tubes containing pollen and acetic acid were centrifuged for 5 mins at 8500 rpm and the acetic acid was removed, then 400 µl of Oxoid nutrient broth was added and the suspension was immediately vortexed, then vortexed again after 30 minutes. After 1 hour in total 10 µl was placed on King's medium B undiluted. An aliquot of 10 µl of nutrient broth and 10 µl sterile deionised water were also placed on King's medium B after all other tubes to check for contamination. After 48 hours incubation at 25°C colonies were counted and the number of colony forming units per ml of nutrient broth per g of pollen was calculated and presented as a logarithmic function. The Eppendorf tubes containing 390 µl nutrient broth were centrifuged for 5 mins at 8500 rpm immediately after the 10 µl aliquots were removed. The nutrient broth was removed and the pollen was resuspended in 400 µl pollen germination media (10% sucrose and 100 mg/L boric acid). Following vortexing, an aliquot of 10 µl was placed in the centre of a microscope slide with a well and inverted on glass rods in a sealed plastic container with a damp paper towel (a hanging drop method). The plastic containers and the remaining pollen in germination media in Eppendorf tubes were then placed in the dark at 20°C for 24 hours. The glass slides were removed, a cover slip placed on the well, and examined with the compound microscope. A 100 µl aliquot of the pollen in Eppendorf tubes was placed on a glass slide, covered with a cover slip and examined with the compound microscope. Germination was recorded when the pollen tubes were the same length as the pollen grains, and 100 pollen grains per treatment per replicate were examined.
5. Controls. For each treatment except for freeze thaw experiment 2 and 3 there was an untreated control where three replicate Eppendorf tubes containing 0.03 g of pollen was amended with 400 µl of Oxoid nutrient broth and the suspension was immediately vortexed. After 30 minutes the tubes were vortexed again, and after 1 hour 10 µl was placed on King's medium B undiluted, and at a dilution of 1:10, 1:100, 1:1000 and 1: 10000 v/v with sterile deionised water. After 48 hours incubation at 25°C colonies were counted and the number of colony forming units per ml of nutrient broth per g of pollen was calculated and presented as a logarithmic function.

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

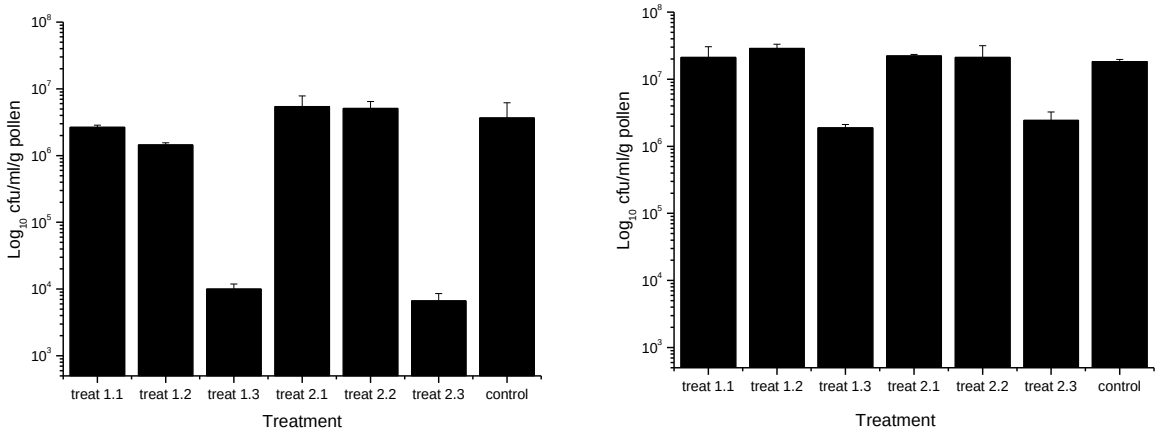

Figure 1. Experiment 1. Freeze thaw 1 hour and 2 hour cycles. See Table 1 for details of treatments. Left graph results were assessed 1 day after treatment, right graph 2 days after treatment.

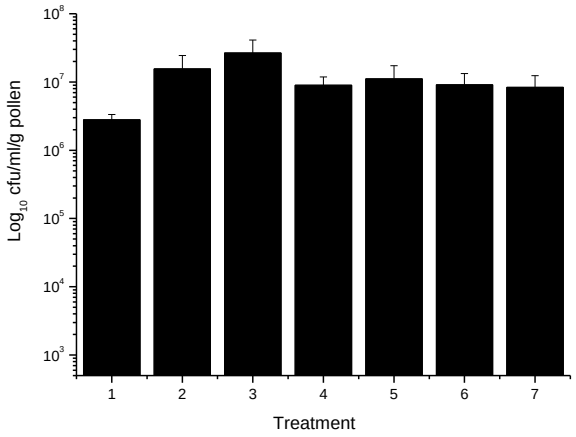

Figure 2. Experiment 2. Freeze thaw 30 minute cycles. See Table 2 for details of treatments.

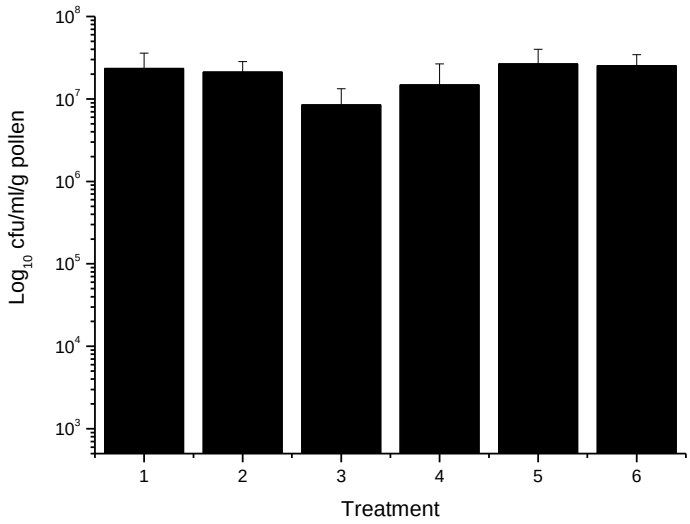

Figure 3. Experiment 3. Freeze thaw 1 hour cycles. See Table 3 for details of treatments.

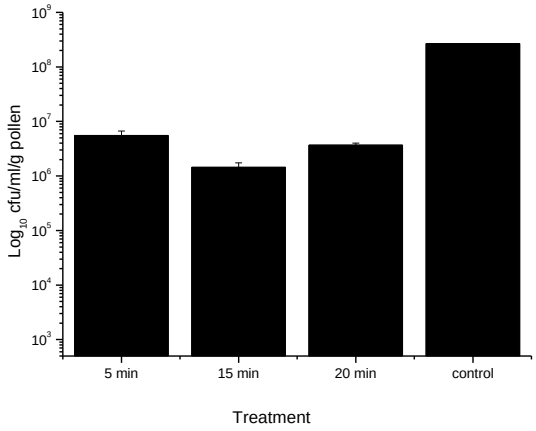

Figure 4. Experiment 4. Different durations in 95% ethanol.

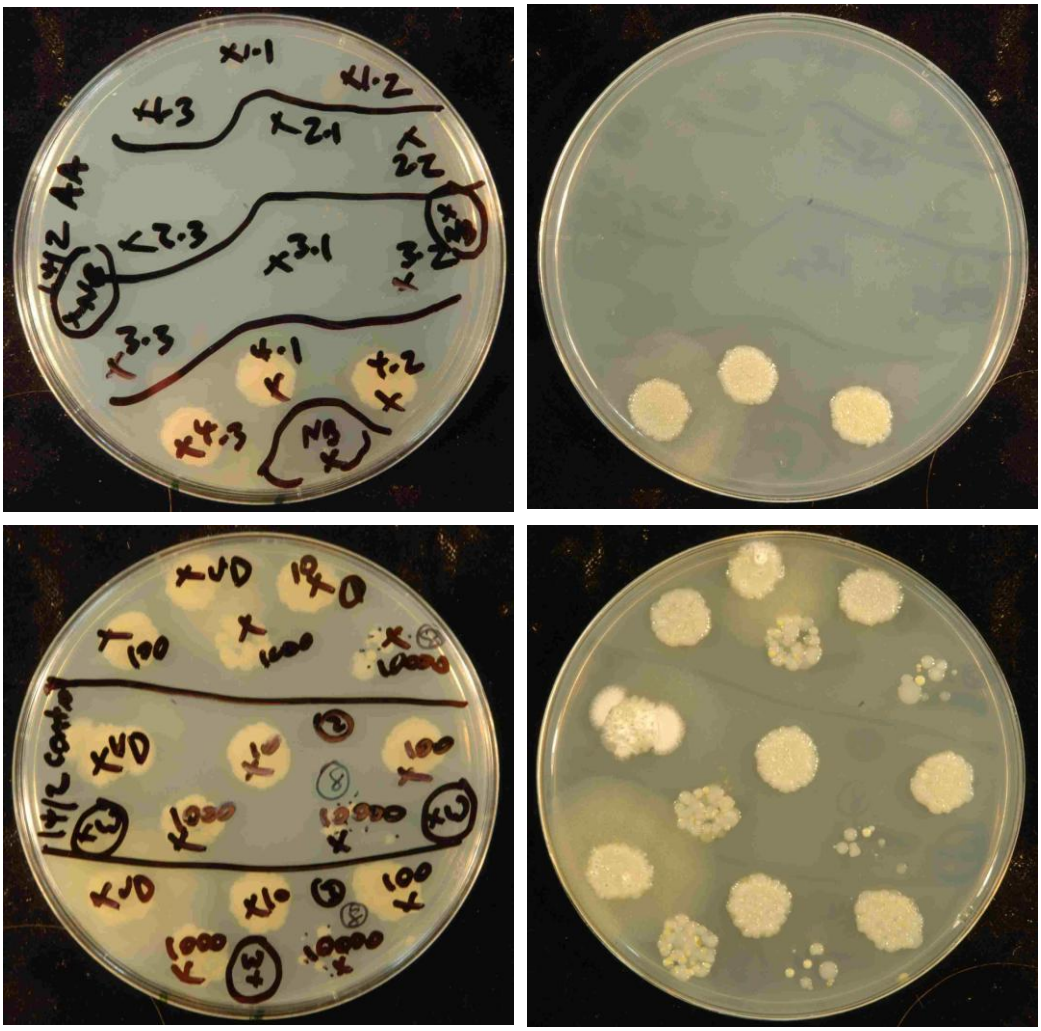

Figure 5. Experiment 5. Acetic acid for 1 hour at different molarities. Photographs on the left are the bottoms of Petri plates containing King's medium B and labels with treatments, photographs on the right are the top of the Petri plates showing bacterial colonies. Photographs were flipped so that the labels correspond exactly to the position of the bacterial colonies on the plates on the right.

1= Glacial Acetic acid 17.5M
2= Acetic acid 5M
3=Acetic acid 1M
4=Acetic acid 0.1M
UD = undiluted
10 = 1:10 v/v dilution with sterile deionised water (SDW)
100 = 1:100 v/v dilution with SDW
1000 = 1:1000 v/v dilution with SDW
10000= 1:10000 v/v dilution with SDW
NB= nutrient broth (control)
W = SDW (control)

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

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

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

RECOMMENDATIONS FOR INDUSTRY

CONCLUSIONS

.

FUTURE RESEARCH STEPS

REFERENCES

DISCLAIMER

LIMITED PROTECTION

CONFIDENTIALITY

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.

This report has been approved by:

Kerry Everett
Scientist
Date: January 2011

Bob Fullerton
Science Group Leader, Pathology and Applied Mycology
Date: January 2011