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

PROJECT DETAILS

Project Title	3.1 Is Psa associated with pollen available in New Zealand
Project Protocol No./ Objective No.	
Project Leader	Joel Vanneste
Research Requested / Contracted by	ZESPRI
Date (Month, Year)	
Based on information as at	18 December 2010

KEY QUESTION AND AIM

We have found that at least one sample of pollen collected from Italy in an infected orchard contained some live cells of Psa. Presence of Psa on pollen has some important economic consequences knowing that most kiwifruit orchards are artificially pollinated and that Psa is a potential epiphytic bacterium. Results from Italy might not be extrapolated to New Zealand because of the differences in kiwifruit pollen collection methods used in Italy (vacuumed from open flowers in the field) and in New Zealand (pollen milled from flowers collected as closed flowers in the field). Therefore, the aim of this study was to determine whether Psa was associated with kiwifruit pollen collected in New Zealand, or with kiwifruit pollen collected overseas and present in New Zealand.

METHODOLOGY

Methods and Protocols

Only lots of pollen available in New Zealand were analysed in this study. These lots were from different geographic origins (including some pollen from Chile) and different years. Several methods for detection of Psa in pollen samples were used: PCR on washings, PCR on plating from washings and PCR on DNA extracted from the pollen.

PCR on washings:

Weigh out 500mg of pollen in a 28ml sample bottle. Add 10ml of sterile water to the pollen and vortex every 5 minutes for half an hour until the pollen is rehydrated.

Dispense 1ml of the hydrated pollen in a micro-centrifuge tube (if one wants to increase the level of detection several 1 ml aliquots can be taken from the same sample).

Spin down pollen debris for 1.5 min at 13,000rpm in a micro-centrifuge.

Carefully remove 300µl of supernatant and place into a clean 1.5ml micro-centrifuge

tube.

Use 10 µl of the supernatant as template for PCR.

Take 100µl of the supernatant and place into 900 µl of sterile water (1:10 dilution).

Use 10 µl of this dilution as template for PCR.

PCR on platings:

Follow the steps 1 to 4 as above, then plate 100 µl on a King's B plate supplemented with cycloheximide. After 24 hours incubation at 28°C, select colonies which morphologically could be Psa or take a swipe and confirm identification by PCR.

PCR on DNA extracted from the pollen:

Weigh out 500mg of pollen in a 28ml sample bottle. Add 10ml of sterile water to the pollen and vortex every 5 minutes for half an hour until the pollen is rehydrated.

Dispense 1ml of the hydrated pollen in a micro-centrifuge tube.

Spin down pollen debris for 5 min at 14,000rpm in a micro-centrifuge.

Remove supernatant and take as much of the pellet and extract the DNA using the ZR Fungal/Bacterial DNA miniprep ™ extraction kit (Zymo Research, USA).

Take 5 µl of the final volume of 100 µl for the PCR.

PCR for identification of Psa was carried out using the primers Psa F1/R2 (Rees George et al. 2010) as described in Vanneste et al. 2010.

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

Results are presented in Table 1. In several cases we obtained an amplicon of the right size after PCR, but no culture of Psa has been isolated from pollen samples collected from New Zealand or collected overseas and available in New Zealand. Therefore, we cannot conclude that live cells of Psa were present on the samples of pollen analysed in this study.

Table 1: Detection of an amplicon of 280 bp after PCR using the Psa F1/R2 primers.

Sample No.	Source	Description	PCR assay		
			on pollen washing	on colonies or bacteria harvested from plates	on DNA extracted from pollen sample
10763	KiwiPollen	New Season pollen 6/11/2010	-	-	ND
10	KiwiPollen	New Season	-	ND	ND

764		pollen 6/11/2010			
10 765	KiwiPollen	New Season pollen 6/11/2010	-	ND	ND
10 766	KiwiPollen	New Season pollen 6/11/2010	-	ND	ND
10 767	KiwiPollen	New Season pollen 6/11/2010	-	-	ND
10 768	KiwiPollen	New Season pollen 6/11/2010	-	-	ND
10 769	KiwiPollen	New Season pollen 6/11/2010	-	ND	ND
10 770	KiwiPollen	New Season pollen 6/11/2010	-	-	ND
10 771	KiwiPollen	Quad duster 1 pollen	-		ND
10 772	KiwiPollen	Quad duster 2 pollen	-	-	ND
10 773	KiwiPollen	French pollen	-	-	ND
10 774	KiwiPollen	French pollen	-	-	ND
10 775	KiwiPollen via Te Puke P&FR	KiwiPollen vial TP Nov 18.2010 # 1	-	No Psa like colonies	+
10 776	KiwiPollen via Te Puke P&FR	KiwiPollen vial TP Nov 18.2010 # 2	-	No Psa like colonies	+
10 777	KiwiPollen via Te Puke P&FR	KiwiPollen vial TP Nov 18.2010 # 3	-	No Psa like colonies	+
10 778	KiwiPollen via Te Puke P&FR	KiwiPollen vial TP Nov 18.2010 # 4	-	No Psa like colonies	+
10 779	KiwiPollen via Te Puke P&FR	KiwiPollen vial TP Nov 18.2010 # 5	-	No Psa like colonies	+
10 780	KiwiPollen via Te Puke P&FR	KiwiPollen vial TP Nov 18.2010 # 6	-	No Psa like colonies	-
10 781	KiwiPollen via Te Puke P&FR	KiwiPollen vial TP Nov 18.2010 # 7	-	No Psa like colonies	+
10 782	KiwiPollen via Te Puke P&FR	KiwiPollen vial TP Nov 18.2010 # 8	-	No Psa like colonies	-
10 783	KiwiPollen via Te Puke P&FR	KiwiPollen vial TP Nov 18.2010 # 9	-	No Psa like colonies	+
10 784	KiwiPollen via Te Puke P&FR	KiwiPollen vial TP Nov 18.2010 # 10	-	No Psa like colonies	+
10 785	KiwiPollen via Te Puke P&FR	KiwiPollen vial TP Nov 18.2010 # 11	-	No Psa like colonies	+
10 793	DMS Te Puna, Matthew Greenbank	Chilean pollen	-	ND	+
10 794	DMS Te Puna, Matthew Greenbank	Chilean pollen	ND	ND	ND
10 795	DMS Te Puna, Matthew Greenbank	Chilean pollen	-	ND	ND
10 796	DMS Te Puna, Matthew Greenbank	Chilean pollen	ND	ND	ND

10797	DMS Te Puna, Matthew Greenbank	New Zealand pollen	-	ND	+
10798	DMS Te Puna, Matthew Greenbank	New Zealand pollen	ND	ND	ND
10759	MAF IDC	Pollen from Chile (KiwiPollen) 13	ND	Weak banda	ND
10760	MAF IDC	Pollen from Chile (KiwiPollen) gp4	ND	Very faint banda	ND
10761	MAF IDC	Pollen from Chile (KiwiPollen) 17	ND	-	ND
10961	P&F Research	kiwifruit pollen Ltd (?) used for germplasm collection	ND	ND	-

a Those results were obtained by harvesting bacterial growth from plates were no isolated colonies could be found (overgrowth). No bacteria which would give a strong amplicon using the Psa F1/R2 primers could be isolated.

ND.= Not Done

RECOMMENDATIONS FOR INDUSTRY

To determine as soon as possible whether any pollen sample from New Zealand or available in New Zealand represents a risk of spreading the pathogen or the disease in this country or in any country where this pollen would be used.

REFERENCES:

Rees-George J, Vanneste JL, Cornish DA, Pushparajah IPS, Yu J, Templeton MD, Everett KR 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.

Vanneste JL, Yu J, Cornish DA 2010. Molecular characterisations of Pseudomonas syringae pv. actinidiae strains isolated from the recent outbreak of bacterial canker on kiwifruit in Italy. New Zealand Plant Protection. 63: 7-14.

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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.

This report has been approved by:

Joel Vanneste, Bacteriology team

Date: XX December 2010

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Date: XX December 2010