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

PROJECT DETAILS

Project Title	2.6 Presence of Psa within tissue of wilting canes of kiwifruit Expt 1
Project Protocol No./ Objective No.	
Project Leader	Joel Vanneste
Research Requested / Contracted by	ZESPRI
Date (Month, Year)	December 2010

KEY QUESTION AND AIM

To ensure that the recommendations to cut vines 10 cm above the graft union when shoot die-back has been observed are supported by some scientific data. Such a drastic measure is needed only if Psa is present in the cane tissue. This project aims to determine whether Psa can be detected in the cane tissues of vines showing secondary die-back symptoms.

METHODOLOGY

1) Experimental Design

Plant material was obtained from the first orchard where Psa was observed: this orchard is identified as PR1 or as KPIN 9287. Three samples from three separate vines showing die-back symptoms were supplied by ZESPRI staff. Each sample consisted of 1 leaf, and 4 pieces of cane, the cane pieces being numbered 1…4, with 1 closest to the die-back.

Another experiment with canes sampled from another orchard where presence of Psa has been confirmed will be reported separately (Expt 2).

2) Methods and/or Protocols

Isolation of *Pseudomonas syringae* pv. *actinidiae* from diseased plant samples using a leaf washing protocol:

1. Place kiwifruit leaf in an individual bag and add 10 ml of sterile distilled water. Massage for 5-10 minutes.
2. Take out 100 µl of washing suspension and streak on to a plate of King's B medium supplemented with cyclohexamide.
3. Incubate at 28°C for 2 days.
4. Sub-culture colonies that show Psa colony morphology.

Isolation of *Pseudomonas syringae pv. actinidiae* from canes

1. Surface sterilise cane pieces in 1% sodium hypochlorite for 3 minutes.
2. Rinse three times with sterile distilled water.
3. Take cleaned cane pieces, and using a sterile blade, remove the bark, slice off slivers of wood

4. Place the slivers of tissue in a sterile Petri dish and add about 500 µl of sterile distilled water and cut the slivers into smaller pieces.

5. Take 100 µl of the suspension and streak on King's B medium supplemented with cyclohexamide.

6. Incubate at 28°C for 2 days.

7. Check plates for the presence of Psa by morphology and PCR detection as described below.

Identification of Psa by Polymerase Chain Reaction (PCR):

Isolation of DNA, and the PCR protocol were carried out as described in Rees-George et al. (2010) and Vanneste et al. (2010).

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

1) From cane 1: Psa was detected on the leaf and in the cane piece number 3. The other cane pieces were negative for the presence of Psa.

2) From cane 2: Psa was detected only in the cane piece number 1. All other cane pieces and the leaf were found negative.

3) From cane 3: As for cane 2.

FUTURE RESEARCH STEPS

- This shows that Psa can enter the kiwifruit vine and move rapidly in the canes. How far and how fast it can move will be determined in part using gfp expressing derivatives of Psa. This is a collaboration with Dr. Francesco Spinelli from Bologna University.
- If the ability to cause secondary symptoms is a strain characteristic, then it would be important to determine in an orchard infected by that same strain whether Psa is found in cane tissues of vines showing only leaf spot symptoms.

RECOMMENDATIONS FOR INDUSTRY

Preliminary data indicate that Psa is present in canes of vines where die-back symptoms are observed. This suggests that canes need to be cut and removed when such symptoms exist to reduce the likely spread of the pathogen throughout the remaining vine.

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.

DISCLAIMER

Unless agreed otherwise, The New Zealand Institute for Plant & Food Research Limited does not give any prediction, warranty or assurance in relation to the accuracy of or fitness for any particular use or application of, any information or scientific or other result contained in this report. Neither Plant & Food Research nor any of its employees shall be liable for any cost (including legal costs), claim, liability, loss, damage, injury or the like, which may be suffered or incurred as a direct or indirect result of the reliance by any person on any information contained in this report.

LIMITED PROTECTION

This report may be reproduced in full, but not in part, without prior consent of the author or of the Chief Executive Officer, The New Zealand Institute for Plant & Food Research Ltd, Private Bag 92169, Auckland Mail Centre,
Auckland 1142, New Zealand.

CONFIDENTIALITY

This report contains valuable information in relation to the [Programme Name] programme that is confidential to the business of Plant & Food Research and [Clients name]. This report is provided solely for the purpose of advising on the progress of the [Programme Name] programme, and the information it contains should be treated as "Confidential Information" in accord with the Plant & Food Research Agreement with [Clients name].

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

Scientist

Date:

Bob Fullerton

Science Group Leader, Applied Pathology and Mycology

Date:

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

PROJECT DETAILS

Project Title	2.6 Presence of Psa within tissue of wilting canes of kiwifruit Expt 2
Project Protocol No./ Objective No.	
Project Leader	Joel Vanneste
Research Requested / Contracted by	ZESPRI
Date (Month, Year)	March 2011

KEY QUESTION AND AIM

To ensure that the recommendations to cut vines 10 cm above the graft union when shoot die-back has been observed are supported by some scientific data. Such a measure is needed only if Psa is present in the cane tissue. This project aims to determine whether Psa can be detected in the cane tissues of vines showing secondary die-back symptoms.

METHODOLOGY

1) Experimental Design

Plant material was obtained from an orchard where Psa had been confirmed.

2) Methods and/or Protocols

Isolation of *Pseudomonas syringae* pv. *actinidiae* from diseased plant samples using a leaf washing protocol:

1. Place kiwifruit leaf in an individual bag and add 10 ml of sterile distilled water. Massage for 5-10 minutes.
2. Take out 100 µl of washing suspension and streak on to a plate of King's B medium supplemented with cyclohexamide.
3. Incubate at 28°C for 2 days.
4. Sub-culture colonies that show Psa colony morphology.

Isolation of *Pseudomonas syringae pv. actinidiae* from canes

1. Surface sterilise cane pieces in 1% sodium hypochlorite for 3 minutes.
2. Rinse three times with sterile distilled water.
3. Take cleaned cane pieces, and using a sterile blade, remove the bark, slice off slivers of wood
4. Place the slivers of tissue in a sterile Petri dish and add about 500 µl of sterile distilled water and cut the slivers

into smaller pieces.

5. Take 100 µl of the suspension and streak on King's B medium supplemented with cyclohexamide.
6. Incubate at 28°C for 2 days.
7. Check plates for the presence of Psa by morphology and PCR detection as described below.

Identification of Psa by Polymerase Chain Reaction (PCR):

Isolation of DNA, and the PCR protocol were carried out as described in Rees-George et al. (2010) and Vanneste et al. (2010).

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

1)

FUTURE RESEARCH STEPS

- This shows that Psa can enter the kiwifruit vine and move rapidly in the canes. How far and how fast it can move will be determined in part using gfp expressing derivatives of Psa. This is a collaboration with Dr. Francesco Spinelli from Bologna University.
- If the ability to cause secondary symptoms is a strain characteristic, then it would be important to determine in an orchard infected by that same strain whether Psa is found in cane tissues of vines showing only leaf spot symptoms.

RECOMMENDATIONS FOR INDUSTRY

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.

DISCLAIMER

Unless agreed otherwise, The New Zealand Institute for Plant & Food Research Limited does not give any prediction, warranty or assurance in relation to the accuracy of or fitness for any particular use or application of, any information or scientific or other result contained in this report. Neither Plant & Food Research nor any of its employees shall be liable for any cost (including legal costs), claim, liability, loss, damage, injury or the like, which may be suffered or incurred as a direct or indirect result of the reliance by any person on any information contained in this report.

LIMITED PROTECTION

This report may be reproduced in full, but not in part, without prior consent of the author or of the Chief Executive Officer, The New Zealand Institute for Plant & Food Research Ltd, Private Bag 92169, Auckland Mail Centre,
Auckland 1142, New Zealand.

CONFIDENTIALITY

This report contains valuable information in relation to the [Programme Name] programme that is confidential to the business of Plant & Food Research and [Clients name]. This report is provided solely for the purpose of advising on the progress of the [Programme Name] programme, and the information it contains should be treated as "Confidential Information" in accord with the Plant & Food Research Agreement with [Clients name].

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

Scientist

Date:

Bob Fullerton

Science Group Leader, Applied Pathology and Mycology

Date: