Phytoplasma diseases of papaya (*Carica papaya* L.) in Australia: phytoplasma classification, pathology and transmission

DANIEL TREVOR WHITE

A thesis submitted for the degree of Master of Applied Science

to

Central Queensland University, Rockhampton

School of Biological and Environmental Sciences
Faculty of Arts, Health and Sciences

22 June 2001
ABSTRACT

In Australia, phytoplasmas have consistently been associated with the papaya (*Carica papaya* L.) diseases known as papaya dieback (PpDB), yellow crinkle (PpYC) and mosaic (PpM). PpDB is the most economically important of these diseases, followed by PpYC. The investigations presented in this thesis have therefore focused primarily on PpDB.

Analysis of the DNA sequences of the 16S rRNA gene and the 16S-23S rRNA intergenic spacer region (SR) of the PpDB, PpYC and PpM phytoplasmas showed that the PpYC and PpM phytoplasma DNA sequences were identical to each other, but were distinctly different to that of the PpDB phytoplasma. A phylogenetic tree based on 16S rDNA sequences revealed that PpDB is most closely related to the Australian grapevine yellows (AGY) phytoplasma and the *Phormium* yellow leaf (PYL) phytoplasma from New Zealand, forming a distinct group within subclade xii. PpYC and PpM phytoplasmas are most closely related to the tomato big bud (TBB) phytoplasma from Australia, within subclade iii. It was proposed that the PpDB phytoplasma be included in the taxon “*Candidatus* Phytoplasma australiense”, and that the PpYC and PpM phytoplasmas be assigned to a new taxon, “*Candidatus P. australasiense*”.

Histological studies and mapping of phytoplasma distribution using PCR revealed that it is likely that phytoplasma cells are present in very low titre and that, while the plant appears to limit proliferation of the PpDB phytoplasma, this defence response is associated with a rapid decline of the papaya plant. Immature leaf material was sampled weekly for eight months from 60 plants in a commercial papaya plantation, to estimate the minimum time between inoculation and symptom expression of PpDB,
PpYC and PpM. The PpDB phytoplasma was detected by PCR one week prior to, or the same week as, external symptoms were first observed, while phytoplasma DNA was detected between three and eleven weeks prior to expression of PpM symptoms. Examination of lateral shoot regrowth on papaya plants that had recovered from PpDB or were cut back (ratooned) when they initially exhibited PpDB, PpYC or PpM symptoms, revealed that the PpDB phytoplasma did not persist in plants after the initial expression of symptoms. In contrast, the PpYC and PpM phytoplasmas usually persisted in the lower parts of the plant, and then infected the new lateral shoots as they developed.

Dodder (Cuscuta australis R. Brown) was used as a phloem bridge between papaya plants affected by PpDB, PpYC and PpM, and periwinkle (Catharanthus roseus G. Don) plants. "Candidatus P. australasiense", but not the PpDB phytoplasma, was transmitted to periwinkle. The inability to transmit the PpDB phytoplasma corresponds with the view that in papaya, this phytoplasma is likely to be present at low titre, is a highly virulent pathogen, and disrupts phloem function before external disease symptoms are observed.

Based on the results of this study it is recommended that ratooning of PpDB-affected plants and removal of PpYC- and PpM-affected plants are the best strategies currently available for the management of these diseases. Suggestions for future research and disease control strategies are discussed.
TABLE OF CONTENTS

Title page.................................................................................................................. i
Abstract.................................................................................................................... ii
Table of Contents................................................................................................... iv
List of Tables............................................................................................................ viii
List of Figures.......................................................................................................... ix
Acknowledgments.................................................................................................. x
Declaration............................................................................................................... xi
Publications............................................................................................................. xii
Conference Presentations....................................................................................... xiii
Dedication................................................................................................................ xiv

CHAPTER 1

Introduction
1.1 Literature review...................................................................................................... 1
  1.1.1 Papaya............................................................................................................. 1
  1.1.2 Papaya in Queensland....................................................................................... 1
    1.1.2.1 Dieback......................................................................................................... 3
    1.1.2.2 Yellow crinkle.............................................................................................. 5
    1.1.2.3 Mosaic.......................................................................................................... 6
  1.1.3 Phytoplasmas.................................................................................................... 6
    1.1.3.1 Phytoplasma classification.......................................................................... 7
    1.1.3.2 Histopathology and within-plant distribution of phytoplasmas................. 11
    1.1.3.3 Phytoplasma epidemiology......................................................................... 13
    1.1.3.4 Experimental transmission of phytoplasmas............................................ 15
  1.1.4 The etiologies of Australian papaya dieback, yellow crinkle and mosaic....... 16
1.2 Aims and objectives of this thesis......................................................................... 17
CHAPTER 2
Phylogenetic classification of the phytoplasmas associated with papaya dieback, yellow crinkle and mosaic

2.1 Introduction ................................................................. 19
2.2 Materials and methods ..................................................... 20
  2.2.1 Extraction of phytoplasma DNA ...................................... 20
  2.2.2 PCR amplification ...................................................... 20
  2.2.3 DNA sequencing ....................................................... 22
  2.2.4 Comparative sequence analysis ....................................... 23
2.3 Results .............................................................................. 28
  2.3.1 DNA sequences ......................................................... 28
  2.3.2 Phylogenetic analysis of 16S rDNA sequences ....................... 28
  2.3.3 16S rRNA signature sequences ........................................ 30
  2.3.4 Analysis of 16S-23S spacer region DNA sequences .................. 32
2.4 Discussion ......................................................................... 33
  2.4.1 Phytoplasma phylogenetic classification ............................... 33
  2.4.2 PpDB and related strains .............................................. 35
  2.4.3 PpYC, PpM and related strains ........................................ 36
  2.4.4 Origins of Australian phytoplasma strains ............................ 37
  2.4.5 Phytoplasma taxa ......................................................... 38

CHAPTER 3
Histopathology of papaya dieback and within-plant distribution of the associated phytoplasma

3.1 Introduction ....................................................................... 42
3.2 Materials and methods ..................................................... 44
  3.2.1 Plant material: TEM study .............................................. 44
  3.2.2 Plant material: Phytoplasma distribution study ....................... 45
  3.2.3 Microscopy ................................................................. 46
  3.2.4 DNA extraction and PCR .............................................. 46
3.3 Results .............................................................................. 47
  3.3.1 Visualisation of phytoplasma in yellow-crinkle-affected tissue .... 47
  3.3.2 Anatomy of dieback-affected tissue .................................... 47
CHAPTER 4
Estimation of minimum presymptom residency (incubation or lag) period and persistence of phytoplasmas in papaya plants

4.1 Introduction................................................................. 69
4.2 Materials and methods.................................................. 71
4.2.1 Sample collection..................................................... 71
4.2.2 PCR amplification of DNA............................................. 72
4.2.3 RFLP analysis............................................................ 73
4.3 Results........................................................................... 73
4.3.1 Disease incidence - external symptoms.......................... 73
4.3.2 Disease incidence - PCR detection............................... 73
4.3.3 Persistence of phytoplasmas......................................... 74
4.4 Discussion...................................................................... 77
4.4.1 Incubation period – from infection to symptom appearance.. 77
4.4.2 Time of infection......................................................... 79
4.4.3 Persistence of infection................................................ 81
4.4.4 Dual infection............................................................ 83
CHAPTER 5
Transmission of phytoplasmas to the experimental host periwinkle (Catharanthus roseus) using the parasitic vine dodder (Cuscuta australis)

5.1 Introduction ................................................................. 85
5.2 Materials and methods .................................................. 87
5.2.1 Dodder transmission methods ...................................... 87
5.2.2 Dodder transmission of phytoplasma from gerbera .......... 89
5.2.3 Dodder transmission strategies for papaya diseases ......... 89
5.2.4 Nucleic acid extraction and PCR .................................... 91
5.2.5 RFLP analysis of PCR products .................................... 91
5.2.6 Transmission electron microscopy ............................... 92
5.3 Results ............................................................................ 92
5.3.1 Transmission of "Candidatus Phytoplasma australasiense" from gerbera 92
5.3.2 Transmission of phytoplasmas from papaya .................. 93
5.3.3 TEM .......................................................... 98
5.4 Discussion ....................................................................... 101
5.4.1 Transmission of "Candidatus Phytoplasma australasiense" from gerbera 101
5.4.2 Inability to transmit the PpDB phytoplasma .................. 101
5.4.3 Transmission of "Candidatus Phytoplasma australasiense" from papaya 104
5.4.4 Suggestions for future transmission work .................... 105

CHAPTER 6
Summary and conclusions .................................................. 109

REFERENCES ........................................................................... 116
### LIST OF TABLES

1.1. Some key references for genotype-based classifications of phytoplasmas............ 9  
2.1. PCR amplification and sequencing primers.................................................. 21  
2.2. Phytoplasma strains used in this study.......................................................... 24  
2.3. Matrix of direct pairwise similarities between the 16S rDNA sequences........ 31  
2.4. Matrix of direct pairwise similarities between the 16S-23S SR DNA sequences. 34  
3.1. Distribution of laticifer autofluorescence and phytoplasma DNA................. 61  
4.1. PCR detection of dieback and mosaic phytoplasma in young leaf tissue relative to time of appearance of visual symptoms...................................................... 75  
4.2. Presence of phytoplasma in lateral shoot regrowth of papaya trees............... 76  
5.1. Strategies and methods used to transmit phytoplasmas from papaya plants...... 88
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Phylogenetic distance tree of PpDB, PpYC and other phytoplasmas</td>
<td>29</td>
</tr>
<tr>
<td>3.1</td>
<td>Transmission electron micrograph of phytoplasma cells within a mature sieve element of the phloem of minor veins of a yellow crinkle-affected papaya leaf.</td>
<td>48</td>
</tr>
<tr>
<td>3.2</td>
<td>Bright field micrographs of papaya leaf tissue</td>
<td>50</td>
</tr>
<tr>
<td>3.3</td>
<td>Transmission electron micrographs of membrane-bound bodies within cells of phloem tissue of minor leaf veins displaying the “X-Y” water-soaked vein symptom of the papaya dieback disease (adjacent to tissue depicted in Fig. 3.2).</td>
<td>52</td>
</tr>
<tr>
<td>3.4</td>
<td>Transmission electron micrograph of membrane-bound bodies within cells of the phloem of minor leaf veins displaying the “X-Y” water-soaked vein symptom of the papaya dieback disease.</td>
<td>54</td>
</tr>
<tr>
<td>3.5</td>
<td>Transmission electron micrograph of laticifer within phloem tissue of dieback-affected papaya plant.</td>
<td>56</td>
</tr>
<tr>
<td>3.6</td>
<td>Diagrammatic representation of the distribution of laticifer autofluorescence and dieback-associated phytoplasma DNA.</td>
<td>58</td>
</tr>
<tr>
<td>5.1</td>
<td>Rsa I RFLP profiles of P1-P7 PCR products of DNA extracts from gerbera exhibiting virescence and periwinkle infected with phytoplasma from gerbera.</td>
<td>94</td>
</tr>
<tr>
<td>5.2</td>
<td>Shoots of periwinkle plants showing normal flowers on a healthy plant and progressive symptom development in periwinkle plants infected with the phytoplasma transmitted from papaya.</td>
<td>97</td>
</tr>
<tr>
<td>5.3</td>
<td>Rsa I RFLP profiles of P1-P7 PCR products of DNA extracts from transplanted papaya, from which phytoplasma was transmitted, and periwinkles infected with phytoplasma from the transplanted papaya.</td>
<td>99</td>
</tr>
<tr>
<td>5.4</td>
<td>Transmission electron micrographs of phytoplasma cells within sieve elements of veins of virescent flower petals of periwinkle plants infected with “Candidatus Phytoplasma australasiense” transmitted from papaya.</td>
<td>100</td>
</tr>
<tr>
<td>5.5</td>
<td>Transmission electron micrograph of phytoplasma cells within sieve elements of a vein of a virescent flower petal from a gerbera plant infected with “Candidatus Phytoplasma australasiense”.</td>
<td>102</td>
</tr>
</tbody>
</table>
ACKNOWLEDGMENTS

I thank my supervisors, Dr Paul T. Scott and Dr Kerry B. Walsh for their comments and guidance in the preparation of this thesis.

I am especially grateful to Jodie Guthrie for her technical guidance and continual support and encouragement, without which I may not have completed the research or this thesis.

I am also grateful to papaya growers Denis and Heather Hall of Yarwun and Flav Aquilizan of Rockhampton for access to and use of their plantations; Gary Grant for provision of papaya seed; Rob Buckley and Cameron Flower (Queensland Department of Primary Industries, Rockhampton) for sample collection and disease monitoring in the field; Don Gowanlock (The University of Queensland) for his guidance and advice on transmission electron microscopy; Dr Karen Gibb (Northern Territory University) for providing phytoplasma-infected periwinkle plants, dodder and information on PCR primers; Dr Linda Blackall (The University of Queensland) for guidance and use of facilities for the phylogenetic analyses; and Rod Elder (Qld DPI, Rockhampton) and Denis Persley (Qld DPI, Indooroopilly) for their ongoing encouragement.

The research was funded by an Australian Postgraduate Award, Central Queensland University, the Queensland Fruit and Vegetable Growers Association, the Horticultural Research and Development Corporation, Australian Research Council (Small Grant), and an Australian Society for Microbiology Foundation Scholarship.
DECLARATION

I declare that the work presented in this thesis, to the best of my knowledge and belief, is original and my own, with the exception of work presented in Chapters 3 and 4. Chapters 3 and 4 are the results of team efforts of the authors listed in the corresponding publications, of which I estimate my contribution to be approximately 25% and 33%, respectively. Chapters 2, 3 and 4 have been published respectively as the following refereed journal articles:


The material presented in this thesis has not previously been submitted in any form for a degree or diploma at this or any other university or institution of tertiary education.

Daniel Trevor White

Date: 22/6/2001
The following publications resulted from the research presented in this thesis:


[The name "Candidatus Phytoplasma australasiense" as presented in this thesis is the etymologically correct form of the name "Candidatus Phytoplasma australasia" that was proposed in the journal article]


CONFERENCE PRESENTATIONS

The following conference presentations resulted from the research presented in this thesis:


To Jo, my wife and best friend...

...Thank you for being there.