STUDIES OF SAP-TRANSMISSIBLE VIRUSES OF FLOWERING CHERRIES

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy at Massey University, Palmerston North, New Zealand.

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Six sap-transmitted viruses were identified during a study of 434 flowering cherry trees (*Prunus serrulata* Lindl. *sensu lato*) in the North Island of New Zealand. These included *Prunus* necrotic ringspot ilarvirus strain G (PNRSV-G), apple mosaic ilarvirus (ApMV), flowering cherry virus B (FCVB), strawberry latent ringspot virus (SLRV), prune dwarf ilarvirus (PDV) and flowering cherry virus I (FCVI). Of these, ApMV, FCVB, SLRV and FCVI were new records for this host. FCVB and FCVI are newly described viruses. The most common virus was PNRSV-G (30.6%); the other viruses ranged in incidence from 10.2% (FCVB) to 0.5% (PDV). A further nine viruses were also detected by mechanical transmission, but were not characterized in this study. Repeated sampling of 30 flowering cherry trees during late winter and early spring showed that ELISA was more sensitive for detecting PNRSV-G infection of flowering cherries than sap-transmission.

Three methods for purifying PNRSV-G isolates from flowering cherry were assessed and the best method was one that used ether as a clarification agent. Yields of 5.0 mg/100 g of tissue were obtained. An antiserum was produced to PNRSV-G in New Zealand white rabbits which had a titre in microprecipitin tests of 1/8192. A 338 nucleotide cDNA clone was made to PNRSV-G which hybridised to RNA-3 in Northern analysis.

FCVI had a narrow host range, quasi-isometric particles of c. 26 nm diam., morphologically similar to the particles of ilarviruses, some bullet shaped particles (also characteristic of ilarviruses), four RNA species of 3550, 2800, 2000 and 1050 nucleotides,
and a coat protein of $M_r 30\,000$. These properties indicate that FCVI has affinities with the ilarvirus group, but it differs in host range and symptoms, physical characteristics and serological properties from other members of this group.

FCVB infected both monocotyledons and dicotyledons, but had a limited host range. FCVB has four RNA species of 3900, 2150, 1800 and 800 nucleotides (estimated from denatured dsRNA). Partially purified preparations contained isometric particles about 24nm in diameter. When purified at pH 7.5 FCVB sedimented in sucrose gradients as three UV absorbing components and virus particles appeared to be swollen. At low pH (5.0 or 6.0) or at pH 7.5 with the addition of magnesium ions, FCVB sedimented as a single predominant UV absorbing component and virus particles were not swollen. One major protein band ($M_r 19\,300$) was extracted from partially purified preparations. Based on these features, it is proposed that FCVB is a new member of the bromovirus group. However, serological interrelationships were not detected with antisera to three bromoviruses, brome mosaic virus, broad bean mottle virus and cowpea chlorotic mottle virus.

SLRV was isolated from flowering cherry trees in close proximity to each other in Auckland, New Zealand. The virus was not isolated from any of 390 flowering cherry trees tested from four other regions in the North Island. The virus was identified by host range, particle morphology, RNA and protein content and by serology. This is the first record of SLRV in flowering cherry.

The nucleotide sequence of the 3'-terminal 2427 nucleotides of SLRV RNA-2 were
determined using cDNA clones. The sequence contains a single reading frame terminating at an ochre stop codon 552 nucleotides from a 3'-terminal poly(A) tract. The N-terminal sequences of the two SLRV coat proteins determined by Edman degradation indicated that the larger 43K protein had a N-terminal Gly and the smaller 27K protein was cleaved at a Ser/Gly bond. No homologies were found in amino acid sequences or nucleotide sequences to four comoviruses or six nepoviruses suggesting that SLRV should be placed in a separate plant virus group.
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TABLE OF CONTENTS

TITLE PAGE ......................................................... i

ABSTRACT .............................................................. ii

ACKNOWLEDGEMENTS .................................................. v

TABLE OF CONTENTS ................................................... vi

CHAPTER ONE: GENERAL INTRODUCTION

1.1 Botanical relationships of cherries. .............................. 1
1.2 Study aims and rationale. .......................................... 2
1.3 Incidence and importance of virus and virus-like diseases recorded in flowering cherries

1.4 Incidence and importance of virus and virus-like diseases of other Rosaceae .................................................. 4

1.4.1 Virus-like disorders of cherry ................................. 5
1.4.2 Viruses isolated from cherry ................................. 5
1.4.3 Incidence of PDV and PNRSV in Prunus persica .......... 7
1.4.4 Incidence of ilarviruses in cherry ............................ 9
1.4.5 Incidence of PNRSV-G in rose in New Zealand ........ 9

1.5 Characteristics of viruses and virus groups relevant to the current study ................................................. 9

1.5.1 Characteristics of the I larviruses .......................... 10

1.5.1.1 Serological relationships within the I larvirus group ................................................................. 12

1.5.1.1.1 Subgroup 1 .................................. 13

1.5.1.1.2 Subgroup 2 .................................. 13

1.5.1.1.3 Subgroup 3 .................................. 15

1.5.1.1.4 Subgroup 4 .................................. 17

1.5.1.1.6 Subgroup 6 .................................. 18

1.5.1.1.7 Subgroup 7 .................................. 19

1.5.1.1.8 Subgroup 8 .................................. 19

1.5.1.2 Herbaceous indicator hosts .............................. 19

1.5.1.3 Other viruses closely related to I larviruses ........ 20

1.5.1.3.1 Alfalfa mosaic virus (AMV) .................. 20

1.5.1.3.2 Raspberry bushy dwarf virus (RBDV) ............ 21

1.5.1.3.3 Pelargonium zonate spot virus (PZSV) ........... 21

1.5.1.4 Symptoms and characteristics of I larviruses infecting cherry ....................................................... 22
1.5.1.4.1  Prunus necrotic ringspot virus (PNRSV) .......................... 22
1.5.1.4.2  Prune dwarf virus (PDV) ............... 25
1.5.1.5  Conclusion ........................................... 26
1.5.2  Bromoviruses ........................................... 27
1.5.3  Nepoviruses ........................................... 29
1.5.4  Strawberry latent ringspot virus (SLRV) ................. 29
1.5.4.1  Genomic structure of four nepoviruses .............. 30
1.5.5  Comoviruses ........................................... 31
1.5.5.1  Genomic structure of cowpea mosaic virus (CoMV) ....... 31
1.5.6  Apple chlorotic leafspot virus (ACLSV) .............. 32
1.5.7  Cucumber mosaic virus (CMV) ......................... 33

CHAPTER TWO: MATERIALS AND METHODS

2.1  Indicator plants ............................................. 48
2.1.1  Indicator plants used in survey. ......................... 48
2.1.2  Indicator plants used to determine host range. ...... 50
2.2  Virus maintenance. ......................................... 51
2.3  Biochemicals and reagents. ................................ 51
2.4  Serology. ................................................ 51
2.4.1  Source of antisera. .................................. 51
2.4.2  Preparation of antisera. ................................. 51
2.4.3 Gel diffusion. ............................................. 52
2.4.4 ELISA (Enzyme-linked immunosorbent assay) .......... 52

2.4.4.1 Purification of antiserum. ....................... 54

2.4.4.2 Gamma-globulin conjugation. ............... 55

2.5 Survey. ..................................................... 55

2.6 Mechanical inoculation. .................................. 55

2.7 Electron microscopy. ...................................... 56

2.8 Virus purification. ........................................ 56

2.8.1 Flowering cherry virus B .............................. 57
2.8.2 Strawberry latent ringspot virus. .................... 58
2.8.3 Ilarviruses. .............................................. 59

2.9 Viral coat protein extraction and size determination. ........ 60

2.10 General nucleic acid methods. .......................... 61

2.10.1 Phenol:chloroform extraction of nucleic acids ......... 61
2.10.2 Ethanol precipitation of nucleic acids. ................ 62
2.10.3 Spin dialysis. ........................................... 62
2.10.4 Restriction enzyme digestions. ....................... 63
2.10.5 Non-denaturing gel electrophoresis. .................. 63
2.10.6 Alkaline agarose gels for cDNA. ...................... 64
2.10.7 Gel purification of DNA fragments ..................... 65

2.11 Viral RNA extraction. ..................................... 66

2.11.1 Extraction of RNA from purified virus. ............... 66
2.11.2 Size determination of RNA by formaldehyde
       denaturation electrophoresis. ......................... 67
2.11.3 Double-stranded RNA extraction. ................. 67

2.12 Preparation of cDNA. ........................................ 68
  2.12.1 First strand synthesis. .................................. 69
  2.12.2 Second strand cDNA synthesis. ....................... 69

2.13 Transformation of *Escherichia coli*. ................. 70
  2.13.1 pUC19 cloning. ........................................ 71
    2.13.1.1 Ligation of cDNA to pUC19. ....................... 71
    2.13.1.2 Transformation of *E. coli* strain MC1022. ........ 72
  2.13.1.3 Small-scale Plasmid DNA Isolation from *E. coli* (Minipreps). ......... 73
  2.13.1.4 Large-Scale Plasmid DNA Isolation from *E. coli* (Maxipreps) ........ 74

2.14 Probing RNA immobilised on nitrocellulose. .......... 75
  2.14.1 Northern blotting. ..................................... 75
  2.14.2 Dot-blotting. ......................................... 76
  2.14.3 Random hexamer-primed labelling of DNA. .......... 76
  2.14.4 Hybridisation with nucleic acid immobilised on nitrocellulose. ........... 77

2.15 DNA sequencing. ........................................... 78
  2.15.1 Preparation of Sequencing gel. ...................... 79
  2.15.2 Sequencing reactions. .................................. 80

2.16 Polymerase chain reaction (PCR) amplification. ....... 81
  2.16.1 PCR amplification. ..................................... 81
CHAPTER THREE: SAP-TRANSMISSIBLE VIRUSES IN FLOWERING CHERRY IN NEW ZEALAND

ABSTRACT .................................................................................................................. 86
INTRODUCTION ........................................................................................................... 87
MATERIALS AND METHODS ................................................................................. 88
  Incidence of sap-transmitted viruses in flowering cherry trees .................. 88
  ELISA ...................................................................................................................... 89
  Investigation of types of tissue and time of sampling. ......................... 90
RESULTS .................................................................................................................... 90
  Incidence of sap-transmitted viruses ......................................................... 90
  Investigation of types of tissue and time of sampling ......................... 93
DISCUSSION ............................................................................................................. 94
REFERENCES .......................................................................................................... 97

CHAPTER FOUR: ILARVIRUS PURIFICATION, CHARACTERISATION AND cDNA CLONING

  4.1 Introduction. .................................................................................................... 99
  4.2 Purification. .................................................................................................. 100
    4.2.1 Gardner’s method. .................................................................................. 100
    4.2.2 Uyemoto’s method. ............................................................................... 101
CHAPTER SIX: FLOWERING CHERRY VIRUS B, AN ISOMETRIC VIRUS FROM FLOWERING CHERRY WITH PROPERTIES OF BROMOVIRUSES

SUMMARY ............................................................................................................. 143

INTRODUCTION .................................................................................................. 144
CHAPTER SEVEN: A NEW HOST RECORD: STRAWBERRY LATENT RINGSPOT VIRUS ISOLATED FROM FLOWERING CHERRY

Abstract .................................................. 164

Introduction ............................................. 165

Methods .................................................. 166

Purification .............................................. 166
CHAPTER EIGHT: NUCLEOTIDE SEQUENCING SHOWS THE COAT PROTEINS OF STRAWBERRY LATENT RINGSPOT VIRUS ARE UNRELATED TO THE NEPOVIRUSES AND COMOVIRUSES

References ................................................................. 186

CHAPTER NINE: GENERAL DISCUSSION .................................. 189

REFERENCES ................................................................. 194

APPENDICES

APPENDIX I: FERTILISERS FOR INDICATOR PLANT GROWTH .......... 195

APPENDIX II: REAGENTS AND BUFFERS ................................. 195
APPENDIX III: ABBREVIATIONS FOR VIRUS NAMES .......................... 202

APPENDIX IV ................................................................. 205
  A4.1 Preparation of hydrated calcium phosphate (HCP). .......... 205
  A4.2 Reagents and buffers. ............................................ 205

APPENDIX V ................................................................. 206

APPENDIX VI: Papers submitted or to be submitted to the following journals. ................................. 214

REFERENCES ................................................................. 216