

http://researchspace.auckland.ac.nz

ResearchSpace@Auckland

Copyright Statement

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognise the author's right to be identified as the author of this thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from their thesis.

To request permissions please use the Feedback form on our webpage. <u>http://researchspace.auckland.ac.nz/feedback</u>

General copyright and disclaimer

In addition to the above conditions, authors give their consent for the digital copy of their work to be used subject to the conditions specified on the Library Thesis Consent Form.

The incidence and phylogenetic analysis of viruses infecting New Zealand's native grasses

Catia Delmiglio

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy (Ph. D.) in Biological Sciences, The University of Auckland 2008

Abstract

Grasses form the basis for the meat, dairy, wool and deer industries, which contribute to nearly 50% of New Zealand exports, and are also an important component of natural ecosystems. Worldwide >100 plant viruses infect grass species and even mild and symptomless infections can adversely effect plant populations through reduced reproductive rates and greater susceptibility to environmental extremes. The only previously published study on viruses in New Zealand's natural grasslands found that cereal viruses have invaded the native grass flora of the South Island.

This research provided an extensive survey of New Zealand native grasses, showing that barley yellow dwarf virus diseases (BYDV, Luteoviridae) and Cocksfoot mottle virus (CoMV, Sobemovirus) are widespread in the North and South islands of New Zealand. Significant findings include seven new virus hosts amongst the New Zealand native flora, the first report of BYDV-PAS in New Zealand, detection in Hierochloe redolens of a novel virus in the Luteoviridae family (proposed name BYDV-To), and in Festuca novae-zelandiae a novel dsRNA virus possibly belonging to the Partitiviridae family. New virus host reports in New Zealand include CoMV in Poa anceps, P. cita, F. novae-zelandiae, and Chionochloa rubra; BYDV-PAV and BYDV-PAS in Microlaena stipoides and Dichelacne crinita; BYDV-MAV in P. cita, F. novae-zelandiae and H. redolens; and CYDV-RPV in P. cita and M. stipoides. Molecular techniques for virus detection and identification were developed or improved during this study. Phylogenetic analyses of viral coat protein sequences from native and exotic grass species indicate either frequent or recent virus movement into native ecosystems, and multiple virus introduction events in New Zealand. The likely origins of the virus species are discussed. Two CoMV variants were identified, one of which caused severe necrosis in susceptible cocksfoot cultivars. Reciprocal aphid transmission of BYDV-PAV using cereals and native grasses showed that although transmission to natives was low, the efficiency of transmission from natives to cereals was comparable to that between cereal species, suggesting virus adaptation to the cereal host species.

The findings from this study are discussed in respect to disease management and bio-security in New Zealand, and recommendations are made for future research.

Acknowledgments

First of all I wish to thank Ass. Prof. Michael N. Pearson (SBS, The University of Auckland) for supervising this work with interest, providing helpful advice and being always encouraging and available for discussions. I consider myself very lucky to have had Mike as my PhD supervisor and I consider him a brilliant supervisor and mentor! Thank you Mike!

This study would not have been possible without the assistance of a large number of people and organisations, and I'm truly grateful to anyone that has been involved (even by just forwarding my e-mails to the right people) at any stage of my research. I apologise in advance for not being able to name everybody.

This research, and the presentation of my work at conferences and workshops, would not have been possible without the financial support of several organizations. I would like to thank The University of Auckland and the Agricultural and Marketing Research and Development Trust (AGMARDT) for granting me doctoral scholarships covering a 3-year stipend and annual fees. I'm very grateful to AGMARDT also for covering part of the research costs, and costs related to presenting my work at the Plant Pathology Conference in Australia and at the AGMARD annual seminars. Thanks to The University of Auckland also for lab space, equipment and consumables. I am very grateful also to the Miss E. L. Hellaby Indigenous Grasslands Research Trust for believing in this project, funding the majority of the associated research and field-work costs, and the presentation of the work at their biannual seminars. Without their financial support this work would have remained just a 'nice idea'. I am also very grateful for having received the following grants providing financial assistance to attend conferences and virology workshops: The University of Auckland Graduate Research Fund; School of Biological Sciences Contestable Travel Grant; and the New Zealand Microbiological Society student travel grant.

I would like to express my gratitude to the many people in the School of Biological Sciences for their help during my PhD study. Particularly, to Ian MacDonald and Adrian Turner for their priceless help with photography and microscopy; to Prof. Allan Rodrigo for his valuable time and help in understanding statistical and phylogenetic analyses (and for lending me books which after my extensive use no longer look new); and to Ass. Prof. Brian Murray for helpful discussions. Huge 'special thanks' to Nga Tama for his extensive help with the maintenance of plants in the glasshouses, and assistance with virus inoculations. I am also immensively grateful to all the plant pathology lab members and particularly to Kieren Arthur, Sarah Jane Cowell and Scott Harper who helped in many ways including providing moral support, essential laughter, friendship, and unpaid hard labour. Crushing samples or washing roots till late at night was certainly not fun but they never refused to give a hand, and they continue to offer their help whenever it was needed! Thank you also to Anton Russell for looking after my experimental plants while I was in Italy at my dad's funeral, I truly appreciated your support in that hard time. A big 'thank you' to Dr Jimmy Hatier and Dr Cortwa Hooijmaijers for their wonderful friendship, assistance, and company during the field-work. Although sometimes it was hard work with long hours we certainly had lots of fun..... I will cheer those happy memories for the rest of my life! A huge THANK YOU to Cortwa and Dr Karin Farreyrol also for critically reading early drafts of my thesis, I truly appreciated your comments and encouragement through the writing process (especially considering you are thousands of miles away).

Many Thanks to Ewen Cameron and Mei Nee Lee (Auckland Museum), Rhys Gardner, Colin Ogle, Dr Peter de Lange, and the Auckland Botanical Society members for their assistance with the herbarium work, grass identification, useful information, comments, and discussions. I am also grateful to many staff members of crown research institutes such as Landcare Research, Crop & Food Research, and AgResearch for their assistance; to the Department of Conservation and Auckland Regional Council for permits to access sites and the data from the National Vegetation Survey Database; and to the New Zealand Army for access to their training areas and assistance in finding accommodation in Waiouru. In particular, I wish to express my gratitude to Dr John Fletcher, Ros Lister and Dr Paul L. Guy for provision of virus isolates, useful information and discussions, and warm hospitality and assistance during the field sampling in the South Island. Many thanks to Dr David Teulon and his team for provision of aphids, useful comments on collected aphids, and the best aphid identification keys! Thanks to Dr Barbara Barratt & Colin Ferguson for access and transport to sampling sites; to Nick Singers for helping me find suitable sampling sites and taking me there even though he was really sick and

it was the most horrible and freezing weather; to Dr Richard Leschen and his team for invaluable help with the New Zealand Arthropod Collection, with Coleoptera identification, and helpful discussions; to Jake Overton for helping me find my first sampling sites; and to Dr Nigel Bell, Lee Davis, Dr Kelvin Lloyd, Kevin Sinclair, Moore Kenyon, Dr David Orlovich and Dr Carolyn Malmstrom for taking the time to respond to my numerous questions. Many thanks to all the Department of Conservation staff that collected samples for me or gave me assistance in the field especially in the Northland, Wanganui & Tongariro Conservancies.

I wish to thank also Dr Jacqueline Beggs for letting me join her student's field-trip to Northland, and for her support. Although we got lost in the bush and had to go through steep hills of thick hakea, gorse and sedges (and later discovered that there is 'soft-sand' in New Zealand), I had great fun and feel really privileged to have had the opportunity to know her better and visit such a beautiful and remote part of New Zealand.

Last, but not the least important, are the many landowners which granted me access into their land and showed me genuine 'kiwi hospitality'. A special 'thank you' to you all; and particularly to the Nielsen family, Sim family, Stewards family, John Edwards, and 'Jhonti' for their warm hospitality and interest in my work.

My deepest feelings go to my husband Nick Petraska, all the 'family' (including my dog, Tori), and all my friends for always being there for me, and for all their understanding, support and encouragement over the years (even from 20,000 miles away!!!). *Vi adoro e avete un posto nel mio cuore per l' eternitá!*

Quotes

"In the language of history human beings are said to have domesticated the grasses, but in the language of ecology the grasses might as easily be said to have domesticated the hairless apes". Graham Harvey

"The basis of human proliferation is not our own seed but the seed of grasses". Evan Eisenberg.



Dedication

I wish to dedicate this thesis to my father, Delmiglio Luigi Mario (Gino), and Iannizzi Assunta (my 'second' mother & dear friend). They supported and believed in me, and it was always my desire to make them proud. Unfortunately, they both died in 2006 during my PhD studies, so they are not able to see me reach this important achievement in life. However, they (as well as my mother) will always be alive in my heart, and will continue to give me the strength to persevere with goals I set in life.

Table of contents

Abstract.		i
Acknowle	dgments	ii
Quotes		v
Dedicatio	n	v
Table of c	ontents	vi
List of tak	les	xi
List of fig	ures	.xv
Abbreviat	ionsx	xiii
Chapter 1	. General introduction	1
1.1 A	distinct plant group: the grasses	1
1.2 Gras	ses and human civilization	3
1.3 New	Zealand agriculture	5
1.4 Nativ 1.4.1 M 1.4.2 N	e grasses of New Zealand aori use of native grasses ative grasses and their potential importance in low-input systems	7 7 8
1.5 Virus	diseases of New Zealand's native flora	9
1.6 Virus 1.6.1 T	b diseases of Gramineae (Poaceae) The virus diseases reported in New Zealand grasses	12 . 14
1.7 Aim	and objectives of the research	55
1.8 Impo	rtance of the research	56
1.8 Impo Chapter 2 North Isla	rtance of the research 2. Incidence of virus diseases in native grasses of New Zealan nd	.56 d's 57
1.8 Impo Chapter 2 North Isla	rtance of the research Incidence of virus diseases in native grasses of New Zealan nd	. 56 d's .57 57
1.8 Impo Chapter 2 North Isla 2.1 Ir	rtance of the research Incidence of virus diseases in native grasses of New Zealan nd troduction	. 56 d's .57 . 57
1.8 Impo Chapter 2 North Isla 2.1 Ir 2.2 N 2.2.1 C	rtance of the research Incidence of virus diseases in native grasses of New Zealan nd troduction	. 56 d's .57 . 57 . 57 . 59
1.8 Impo Chapter 2 North Isla 2.1 Ir 2.2 N 2.2.1 C 2.2.2 S	<pre>rtance of the research c. Incidence of virus diseases in native grasses of New Zealan nd troduction aterials and methods hemicals and enzymes election of suitable grass species and sampling sites</pre>	56 d's .57 .57 .59 .59 .59
1.8 Impo Chapter 2 North Isla 2.1 Ir 2.2.1 C 2.2.2 S 2.2.2 S 2.2.2	rtance of the research 2. Incidence of virus diseases in native grasses of New Zealan nd troduction aterials and methods hemicals and enzymes election of suitable grass species and sampling sites P-1 Field Safety	56 d's .57 .57 .59 .59 .59 .61
1.8 Impo Chapter 2 North Isla 2.1 Ir 2.2.1 C 2.2.2 S 2.2.3 2.2.3 C	rtance of the research 2. Incidence of virus diseases in native grasses of New Zealan nd troduction aterials and methods hemicals and enzymes election of suitable grass species and sampling sites 2-1 Field Safety eneral sampling methodology.	56 d's 57 57 59 59 59 59 61
1.8 Impo Chapter 2 North Isla 2.1 Ir 2.2 N 2.2.1 C 2.2.2 S 2.2.3 C 2.2.3 C 2.2.3 C	rtance of the research 2. Incidence of virus diseases in native grasses of New Zealan nd troduction aterials and methods hemicals and enzymes election of suitable grass species and sampling sites P-1 Field Safety eneral sampling methodology B-1 Grass sample collection	56 d's .57 .57 .59 .59 .59 .61 .61 .61
1.8 Impo Chapter 2 North Isla 2.1 Ir 2.2 N 2.2.1 C 2.2.2 S 2.2.3 C 2.2.3 C 2.2.3 C 2.2.3 C 2.2.3 C	rtance of the research 2. Incidence of virus diseases in native grasses of New Zealan nd	56 d's 57 57 57 59 59 59 61 61 61 62 62
1.8 Impo Chapter 2 North Isla 2.1 Ir 2.2 N 2.2.1 C 2.2.2 S 2.2.3 C 2.2.3 C 2.2.3 C 2.2.3 C 2.2.4 D	rtance of the research 2. Incidence of virus diseases in native grasses of New Zealan nd	56 d's 57 57 59 59 59 59 59 59 59 59
1.8 Impo Chapter 2 North Isla 2.1 Ir 2.2 N 2.2.1 C 2.2.2 S 2.2.3 C 2.2.3 C 2.2.3 C 2.2.3 C 2.2.3 C 2.2.3 C 2.2.4 D 2.2.5 S	rtance of the research	56 d's 57 57 59 59 59 59 59 59 59 59
1.8 Impo Chapter 2 North Isla 2.1 Ir 2.2 N 2.2.1 C 2.2.2 S 2.2.3 C 2.2.4 C 2.2.5 S 2.2.6 M	rtance of the research 2. Incidence of virus diseases in native grasses of New Zealan nd	56 d's 57 59 59 59 59 59 59 59 61 61 62 62 62 63 67 68
1.8 Impo Chapter 2 North Isla 2.1 Ir 2.2 N 2.2.1 C 2.2.2 S 2.2.3 C 2.2.3 C 2.2.4 C 2.2.5 S 2.2.6 M 2.2.0	rtance of the research	56 d's 57 57 59 59 59 59 59 59 59 59
1.8 Impo Chapter 2 North Isla 2.1 Ir 2.2 N 2.2.1 C 2.2.2 S 2.2.3 C 2.2.3 C 2.2.4 C 2.2.5 S 2.2.6 M 2.2.6	rtance of the research. P. Incidence of virus diseases in native grasses of New Zealan nd	56 d's 57 57 59 59 59 61 61 61 62 62 63 67 68 68 68 68 68 68 69
1.8 Impo Chapter 2 North Isla 2.1 Ir 2.2 N 2.2.1 C 2.2.2 S 2.2.3 C 2.2.3 C 2.2.3 C 2.2.4 C 2.2.5 S 2.2.6 M 2.2.6	rtance of the research	56 d's 57 57 59 59 59 59 61 61 62 61 62 63 63 63 668 68 68 68 69 69 69
1.8 Impo Chapter 2 North Isla 2.1 Ir 2.2 N 2.2.1 C 2.2.2 S 2.2.3 C 2.2.3 C 2.2.3 C 2.2.4 D 2.2.5 S 2.2.6 M 2.2.0	rtance of the research. 2. Incidence of virus diseases in native grasses of New Zealan nd	56 d's 57 57 59 59 59 59 61 61 61 61 62 63 67 68 68 68 68 69 69 70
1.8 Impo Chapter 2 North Isla 2.1 Ir 2.2 N 2.2.1 C 2.2.2 S 2.2.3 C 2.2.3 C 2.2.3 C 2.2.4 D 2.2.5 S 2.2.6 M 2.2.6 M 2.2.6 M 2.2.6 M 2.2.6 M	rtance of the research. P. Incidence of virus diseases in native grasses of New Zealan nd	56 d's 57 57 59 59 59 59 61 61 61 62 63 67 68 67 68 69 70 70 70
1.8 Impo Chapter 2 North Isla 2.1 Ir 2.2 N 2.2.1 C 2.2.2 S 2.2.3 C 2.2.3 C 2.2.3 C 2.2.3 C 2.2.3 C 2.2.4 D 2.2.5 S 2.2.6 N 2.2.6 N 2.5 N 2.5 N 2.5 N 2.5 N 2.5 N 2.5 N 2.5	rtance of the research e. Incidence of virus diseases in native grasses of New Zealan nd	56 d's 57 57 59 59 59 59 61 61 62 63 67 68 68 68 68 69 69 70 70 70 70
1.8 Impo Chapter 2 North Isla 2.1 Ir 2.2 N 2.2.1 C 2.2.2 S 2.2.3 C 2.2.4 C 2.2.5 S 2.2.6 N 2.2.6 N 2.2.6 N 2.2.6 N 2.2.6 N 2.2.6 N 2.2.6 N 2.2.6 N 2.2.6 N 2.2.6 N 2.2.7 C	rtance of the research e. Incidence of virus diseases in native grasses of New Zealan nd	56 37 57 57 57 59 59 59 59 59 59 59 59
1.8 Impo Chapter 2 North Isla 2.1 Ir 2.2 N 2.2.1 C 2.2.2 S 2.2.3 C 2.2.4 C 2.2.5 S 2.2.6 M 2.2.6 M 2.2.6 M 2.2.6 M 2.2.6 M 2.2.6 M 2.2.6 M 2.2.6 M 2.2.7 C 2.2.7 C 2.2.7 C 2.2.7 C	rtance of the research e. Incidence of virus diseases in native grasses of New Zealan nd	56 377 57 57 59 59 59 59 59 59 59 59
1.8 Impo Chapter 2 North Isla 2.1 Ir 2.2 N 2.2.1 C 2.2.2 S 2.2.3 C 2.2.3 C 2.2.4 C 2.2.5 S 2.2.6 M 2.2.6 M 2.2.6 M 2.2.6 M 2.2.6 M 2.2.6 M 2.2.6 M 2.2.6 M 2.2.7 C 2.2.7 C 2.2.7 C 2.2.7 C 2.2.1 C 2.2.2 S 2.2.2 S 2.2 S 2.2 S 2.2	rtance of the research e. Incidence of virus diseases in native grasses of New Zealan nd	56 d's 57 57 59 59 59 59 59 59 59 59
1.8 Impo Chapter 2 North Isla 2.1 Ir 2.2 N 2.2.1 C 2.2.2 S 2.2.3 C 2.2.3 C 2.2.4 D 2.2.5 S 2.2.6 M 2.2.6 M 2.2.7 M 2.2.7 M 2.2.7 M 2.2.6 M 2.2.6 M 2.2.7 M	rtance of the research e. Incidence of virus diseases in native grasses of New Zealan nd	56 d's 57 57 59 59 59 59 59 59 59 59
1.8 Impo Chapter 2 North Isla 2.1 Ir 2.2 N 2.2.3 G 2.2.3 G 2.2.3 G 2.2.4 D 2.2.5 S 2.2.6 M 2.2.6 M 2.2.6 M 2.2.6 M 2.2.6 M 2.2.6 M 2.2.7 C 2.2.7 C 2.2.8 C 2.2.8 C 2.2.8 C 2.2.9 C 2.2.9 C 2.2.9 C 2.2.9 C 2.2.1 C 2.2.1 C 2.2.1 C 2.2.2 C 2.2.1 C 2.2.2 C 2.2.2 C 2.2.1 C 2.2.2 C	rtance of the research e. Incidence of virus diseases in native grasses of New Zealan nd	56 d's 57 57 59 59 59 59 59 59 59 59
1.8 Impo Chapter 2 North Isla 2.1 Ir 2.2 N 2.2.3 G 2.2.3 G 2.2.3 G 2.2.3 G 2.2.4 D 2.2.5 S 2.2.6 N 2.2.6 N 2.2.6 N 2.2.6 N 2.2.6 N 2.2.6 N 2.2.6 N 2.2.7 G 2.2.7 G 2.2.7 G 2.2.7 G 2.2.1 C 2.2.2 S 2.2.2 S	rtance of the research e. Incidence of virus diseases in native grasses of New Zealan nd	56 37 57 57 57 57 59 59 59 59 59 59 59 59

2	2.2.7-7 Analysis of sequences obtained	73
2.3	Results	73
2.3.	1 Sample collection and observations	
2.3.	2 Overview of ELISA results	80
2.3.	3 Statistical analysis of Cocksfoot mottle virus incidence	84
2.3.	4 Statistical analysis of Luteovirus incidence	84
2.3.	5 Molecular confirmation of potyvirus infection	85
240	inquasion	07
2.4 D	1 Insidence of virus diseases in native grasses in New Zealand's North Island	01
2.4.	A 1-1 Incidence of CoMV in natives	07 87
2	2 4 1-2 Incidence of BYDVs in natives	07 89
2	2.4.1-3 Potvvirus infections in North Island grasses	91
2.5 In	itial conclusions	93
Chapte	r 3. Molecular investigation of Cocksfoot mottle virus in New Zeala	nd's
native a	and exotic grasses	94
2.4		04
3.1	Introduction	94
3.2	Materials and methods	98
3.2.	1 Chemicals and enzymes	98
3.2.	2 Sample selection	98
3.2.	3 South Island sampling	98
3	3.2.3-1 Sample identification, preparation and storage	98
3.2.	4 Detection of CoMV	99
3.2.	5 Primer design for PCR detection of CoMV	99
3.2.	6 Primer evaluation	100
3	3.2.6-1 NUCIEIC ACID EXTRACTION	. 101
3	2.2.6-2 1 Peyerse Transcription using M-MLV reverse transcriptase	101
	3.2.6-2.2 Reverse Transcription using NumerScript TM III reverse transcriptase	101
	3.2.6-2.3 Polymerase chain reaction	101
3	3.2.6-3 RT-PCR of <i>Nad</i> 5	101
3.2.	7 Electrophoresis of PCR products and DNA extraction from gels	102
3.2.	8 Cloning and sequencing	102
3	3.2.8-1 Sequencing	102
3.2.	9 Sequence assembly	103
3.2.	10 Sequence analysis	103
3.3 R	esults	. 105
3.3.	1 Incidence of Cocksfoot mottle virus in the South Island	105
3.3.	2 Molecular confirmation of CoMV detection	108
3	3.3.2-1 Primer design	108
3	3.3.2-2 PCR competency of plant extracts and CoMV RT-PCR tests	108
3.3.	3 Computer analysis of CoMV sequences and phylogenies	112
3 4 Di	iscussion	128
34	1 Incidence of virus diseases in native grasses in New Zealand's South Island	128
3.4.	2 Detection of CoMV by RT-PCR	130
3.4.	3 Molecular diversity of CoMV sequences from New Zealand	131
3.5 Pı	reliminary conclusions	. 134
Chante	or 4 Enidemiological investigations of New Zealand's Cocksfoot m	ottla
virus is		.136
A 1	Introduction	136
-7.1		
4.2	Materials and methods	. 138
4.2.	1 Chemicals and enzymes	138
4.2.	2 Seed sourcing, plant germination and maintenance	. 138
4.2.	3 Internation inoculation method for Control transmission of CoNV using field collection	139 od
4	H2.3-1 Experimental design for the mechanical transmission of Colliv Using field collection of the store and exotic grass species	5u 1∕1∩
11	חופטנפט רומנוער מרוט לאטנוט פרמסט פרטובט	140

4.2.3-2 Experimental design for the mechanical transmission of two CoMV variants on	
selected native species and on cocksfoot cultivars	. 140
4.2.3-3 Experimental design for the mechanical transmission of two CoMV variants on tw	0
cocksfoot cultivars under autumn/spring temperature	. 141
4.2.3-4 Statistical analyses for the growth and symptom data from the mechanical	4.40
transmission of two COMV variants	. 142
4.2.4 Detection of COMV and confirmation of infections	. 143
4.2.5 Agalose gels and DNA extraction from gel	. 147
4.2.0 Single Stranded Conformation Polymorphism (SSCP)	. 147 170
4.2.8 Vector investigation of CoMV	1/19 1/19
4.2.0 Vector Investigation of Coleontera species	140
4.2.8 1 Company of Coleopterd Species	149
4 2 9 Electron Microscopy	150
4.2.9-1 Negative staining Transmission Electron Microscopy	. 150
	450
4.3 Results	153
4.3.1 Mechanical transmission studies.	. 153
	153
4 3 1-2 Mechanical transmission of two CoMV variants on selected native species and on	. 155
cocksfoot cultivars during summer	156
4.3.1-3 Mechanical transmission of two CoMV variants to two cocksfoot cultivars maintair	ned
under a spring/autumn temperature regime	. 157
4.3.1-3.1 Maximum height and number of shoots	. 160
4.3.1-3.2 Necrosis and mosaic development	. 164
4.3.1-3.3 Virus effect on final shoot and root weights	. 168
4.3.1-3.4 Time of onset of symptoms: effect on growth and symptom severity	. 172
4.3.1-3.5 Comparison of data from this experiment to the summer experiment	. 172
4.3.2 Differentiation of New Zealand CoMV variants by SSCP	. 173
4.3.3 Differentiation of New Zealand CoMV variants by restriction digest	. 1/4
	1/6
4.3.4 Investigation of potential Comv vector at one New Zealand site	. 170
4.3.4 Investigation of potential Comv vector at one New Zealand site	177
4.3.4 Investigation of potential CoMV vector at one New Zealand site	177 . 177
4.3.4 Investigation of potential COMV vector at one New Zealand site	177 . 177 . 177 . 177
 4.3.4 Investigation of potential COMV vector at one New Zealand site	177 . 177 . 177 . 177 . 177
 4.3.4 Investigation of potential COMV vector at one New Zealand site	177 . 177 . 177 . 177 . 177 . 178
 4.3.4 Investigation of potential COMV vector at one New Zealand site	177 . 177 . 177 . 177 . 177 . 178 . 179
 4.3.4 Investigation of potential COMV vector at one New Zealand site	177 177 177 177 177 178 178 179 180
 4.3.4 Investigation of potential COMV vector at one New Zealand site	177 . 177 . 177 . 177 . 178 . 179 . 180 . 182
 4.3.4 Investigation of potential COMV vector at one New Zealand site	177 . 177 . 177 . 177 . 177 . 178 . 179 . 180 . 182 . 183
 4.3.4 Investigation of potential COMV vector at one New Zealand site	177 . 177 . 177 . 177 . 177 . 178 . 179 . 180 . 182 . 183 185
 4.3.4 Investigation of potential COMV vector at one New Zealand site	177 177 177 177 177 178 178 179 180 182 183 185
 4.3.4 Investigation of potential CoMV vector at one New Zealand site	177 177 177 177 177 178 179 180 182 183 185
 4.3.4 Investigation of potential CoMV vector at one New Zealand site	177 177 177 177 177 178 179 180 182 183 185 //ow 187
 4.3.4 Investigation of potential Coliviv vector at one New Zealand site	177 177 177 177 177 178 179 180 182 183 185 185 185
 4.3.4 Investigation of potential Coliviv vector at one New Zealand site	177 177 177 177 177 178 179 180 182 183 183 185 <i>Ilow</i> 187
 4.3.4 Investigation of potential Conv vector at one New Zealand Site	177 177 177 177 177 178 179 180 182 183 185 185 185 187 187 191
 4.3.4 Investigation of potential Coliviv vector at one New Zealand site	177 177 177 177 177 178 179 180 182 183 185 185 185 187 187 187 187 191
 4.3.4 Investigation of potential Colliv Vector at one New Zealand site	177 177 177 177 178 179 180 182 183 185 185 185 187 187 187 187 191 191
 4.3.4 Investigation of potential Coliv Vector at one New Zealand site	177 177 177 177 178 179 180 182 183 185 185 185 187 187 187 187 191 191
 4.3.4 Investigation of potential Conv vector at one New Zealand site	177 177 177 177 178 179 180 182 183 185 185 185 187 187 187 191 191 191 191
 4.3.4 Investigation of potential Coliviv Vector at one New Zealand Site. 4.4 Discussion	177 177 177 177 177 178 179 180 182 183 185 185 185 185 187 187 187 191 191 191 191 192 192
 4.3.4 Investigation of potential Conv vector at one New Zealand site	177 177 177 177 177 178 179 180 182 183 185 185 185 185 187 187 187 191 191 191 191 192 192 192
 4.3.4 Investigation of potential Conv vector at one New Zealand site	177 177 177 177 178 177 178 179 180 182 183 185 185 185 185 185 187 187 187 191 191 191 191 192 192 192
 4.3.4 Investigation of potential Conv vector at one New Zealand site	177 177 177 177 178 179 180 182 183 185 185 185 185 185 187 187 187 191 191 191 192 192 192 192 192
 4.3.4 Investigation of potential Coliviv vector at one New Zealand site 4.4 Discussion 4.4.1 Mechanical transmission of CoMV 4.4.1-1 Transmission of CoMV from infected natives and to natives. 4.4.1-2 Transmission of CoMV from infected natives and to natives. 4.4.1-3 Symptom development in CoMV-infected natives. 4.4.1-4 The role of native grasses as reservoir of CoMV. 4.4.1-5 Differences between two CoMV-variants infecting two cocksfoot cultivars. 4.4.2 Differentiation of New Zealand CoMV variants. 4.4.3 CoMV vector investigation. 4.5 Conclusions Chapter 5. Molecular and epidemiological investigations of Barley yedwarf virus in New Zealand's native and exotic grasses. 5.1 Introduction 5.2 Materials and methods 5.2.3 South Island sampling 5.2.3 South Island sampling 5.2.3-1 Sample identification, preparation and storage 5.2.4 Detection of Luteoviruses in South Island samples. 5.2.5 Amplification of Luteoviruses by RT-PCR 5.2.5-3 RT-PCR protocol. 5.2.6 Electrophoresis of PCR products and DNA extraction from gels.	177 177 177 177 177 178 179 180 182 183 185 185 185 185 185 187 187 187 191 191 191 192 192 192 192 192 192
 4.3.4 Investigation of potential Coliviv vector at one New Zealand site 4.4 Discussion 4.4.1 Mechanical transmission of CoMV 4.4.1 Transmission of CoMV from infected natives and to natives. 4.4.1-2 Transmission of CoMV from infected natives and to natives. 4.4.1-3 Symptom development in CoMV-infected natives. 4.4.1-4 The role of native grasses as reservoir of CoMV. 4.4.1-5 Differences between two CoMV-variants infecting two cocksfoot cultivars. 4.4.2 Differentiation of New Zealand CoMV variants. 4.3 CoMV vector investigation. 4.5 Conclusions Chapter 5. Molecular and epidemiological investigations of Barley yeadwarf virus in New Zealand's native and exotic grasses. 5.1 Introduction 5.2 Materials and methods 5.2.1 Chemicals and enzymes 5.2.2 Sample selection for molecular investigations 5.2.3 South Island sampling 5.2.3-1 Sample identification, preparation and storage 5.2.4 Detection of Luteoviruses by RT-PCR 5.2.5-1 Nucleic acid extraction 5.2.5-2 RT-PCR protocol. 5.2.6 Electrophoresis of PCR products and DNA extraction from gels 5.2.7 SSCP analysis of selected samples	177 177 177 177 177 178 179 180 182 183 185 185 185 185 187 187 187 187 191 191 191 191 192 192 192 192 192 194 194
 4.3.4 Investigation of potential Colivi Vector at one New Zealand site. 4.4 Discussion. 4.4.1 Mechanical transmission of CoMV. 4.4.1-1 Transmission of CoMV from infected natives and to natives. 4.4.1-2 Transmission of CoMV from infected natives. 4.4.1-3 Symptom development in CoMV-infected natives. 4.4.1-4 The role of native grasses as reservoir of CoMV. 4.4.1-5 Differences between two CoMV-variants infecting two cocksfoot cultivars. 4.4.1-5 Differences between two CoMV-variants infecting two cocksfoot cultivars. 4.4.2 Differentiation of New Zealand CoMV variants. 4.4.3 CoMV vector investigation. 4.5 Conclusions Chapter 5. Molecular and epidemiological investigations of Barley yea dwarf virus in New Zealand's native and exotic grasses. 5.1 Introduction 5.2 Materials and methods 5.2.1 Chemicals and enzymes 5.2.2 Sample selection for molecular investigations 5.2.3 South Island sampling 5.2.4 Detection of Luteoviruses in South Island samples. 5.2.5 Amplification of Luteoviruses by RT-PCR 5.2.5 Invelic acid extraction 5.2.5 ZRT-PCR protocol 5.2.6 Electrophoresis of PCR products and DNA extraction from gels. 5.2.7 SSCP analysis of selected samples. 5.2.8 Cloning and sequencing	177 177 177 177 177 178 179 180 182 183 185 185 185 185 187 187 187 187 187 191 191 191 191 192 192 192 192 192 192 194 194 195
 4.3.4 Investigation of potential Coliviv vector at one New Zealand site. 4.4 Discussion. 4.4.1 Mechanical transmission of CoMV. 4.4.1-1 Transmission of CoMV from infected natives and to natives. 4.4.1-2 Transmission of CoMV from infected natives. 4.4.1-3 Symptom development in CoMV-infected natives. 4.4.1-4 The role of native grasses as reservoir of CoMV. 4.4.1-5 Differences between two CoMV-variants infecting two cocksfoot cultivars. 4.4.2 Differentiation of New Zealand CoMV variants. 4.4.3 CoMV vector investigation. 4.5 Conclusions . Chapter 5. Molecular and epidemiological investigations of Barley yeadwarf virus in New Zealand's native and exotic grasses	177 177 177 177 177 178 179 180 182 183 185 185 185 185 187 187 187 187 191 191 191 191 192 192 192 192 192 192 192

5.2.10 Primer design for PCR amplification of the coat protein of a distinct luteovirus	195
5.2.11 Sequence analysis	196
5.2.12 Transmission experiments using <i>Rhopalosiphum padi</i>	197
5.2.12-1 Aprild choice experiment using detached leaves	198
5.2.12-2 Aprild choice experiment using whole plants	100
5.2.12-5 Aprild transmission experiments using N. padi	199 es at
one site in Auckland.	201
5.3 Results	. 204
5.3.1 Incidence of luteoviruses in the South Island wild grasses and cereals	204
5.3.2 Confirmation of luteovirus infection by RT-PCR and sequencing	208
5.3.2-1 Multiplex RT-PCR amplification and sequencing from field-collected samples	208
5.3.2-2 RT-PCR amplification from field-collected samples using single primer pairs	213
5.3.2-3 Further molecular characterisation of a distinct luteovirus from 102B1	213
5.3.3 Frigogenetic analysis of New Zealand Interviewses	210
5.3.3-1.1 BYDV-PAV phylogeny	220
5.3.3-1.2 BYDV-PAS phylogeny	221
5.3.3-1.3 BYDV-MAV phylogeny	221
5.3.3-1.4 Phylogeny of isolate To2B1	221
5.3.3-2 Analysis for recombination events	222
5.3.4 Epidemiological investigation of BYDV-PAV using vector experiments	232
5.3.4-1 Aphia choice experiments	232
5.3.5 Investigation of spatial and temporal distribution of BYDV-PAV in <i>Microlaena stipoide</i>	230 ເ 238
	3 200
5.4 Discussion	. 240
5.4.1 Incidence of BYDVs in native grasses in New Zealand's South Island	240
5.4.2 Detection of BTDVS by RT-PCR	241
5.4.2-2 Unexpected fragments from multiplex RT-PCR amplification	242
5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent	RNA-
5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1	RNA- 244
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1 5.4.3 Molecular diversity of BYDV sequences from New Zealand in comparison to oversea 	RNA- 244 }
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1 5.4.3 Molecular diversity of BYDV sequences from New Zealand in comparison to overseas sequences 	RNA- 244 3 245
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1 5.4.3 Molecular diversity of BYDV sequences from New Zealand in comparison to overseas sequences 5.4.3-1 BYDV-PAS 	RNA- 244 3 245 245
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1 5.4.3 Molecular diversity of BYDV sequences from New Zealand in comparison to overseas sequences	RNA- 244 3 245 245 246 248
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1 5.4.3 Molecular diversity of BYDV sequences from New Zealand in comparison to oversear sequences	RNA- 244 245 245 245 246 248 249
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1 5.4.3 Molecular diversity of BYDV sequences from New Zealand in comparison to overseat sequences	RNA- 244 245 245 246 248 249 250
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1 5.4.3 Molecular diversity of BYDV sequences from New Zealand in comparison to overseas sequences	RNA- 244 3 245 245 245 246 248 249 250 251
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1 5.4.3 Molecular diversity of BYDV sequences from New Zealand in comparison to overseas sequences	RNA- 244 245 245 246 246 248 249 250 251 252
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1 5.4.3 Molecular diversity of BYDV sequences from New Zealand in comparison to oversear sequences	RNA- 244 245 245 246 246 248 249 250 251 252 252
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1 5.4.3 Molecular diversity of BYDV sequences from New Zealand in comparison to oversear sequences	RNA- 244 245 245 246 248 249 250 251 252 253 253
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1	RNA- 244 245 245 246 248 248 249 250 251 252 255 257
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1	RNA 244 245 245 245 246 248 249 250 251 252 255 257 257 257
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1	RNA- 244 245 245 246 248 249 250 251 252 255 257 257 259 260
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1	RNA 244 245 245 245 246 248 249 250 251 252 255 257 257 259 260 261
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1	RNA- 244 245 245 246 248 249 250 251 252 253 255 257 257 259 260 . 261
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1	RNA- 244 245 245 246 248 249 250 251 252 253 257 257 259 260 261 262
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1	RNA- 244 245 245 246 248 249 250 251 252 257 257 257 257 259 260 . 261 262 262
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1	RNA- 244 245 245 246 248 249 250 251 252 255 257 257 259 260 261 262 262 262
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1	RNA- 244 245 245 246 248 249 250 251 252 253 255 257 257 259 260 . 261 . 262 . 262 262 263
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1	RNA- 244 245 245 246 248 249 250 251 252 257 257 257 257 259 260 261 262 262 263 264
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1	RNA- 244 245 245 246 248 249 250 251 252 255 257 257 257 259 260 261 262 262 263 264 265
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1	RNA- 244 245 245 246 248 249 250 251 252 255 257 257 259 260 261 262 262 263 264 265 266 265 266
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1	RNA- 244 245 245 246 248 249 250 251 252 253 255 257 257 259 260 261 262 263 264 266 268
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1	RNA- 244 245 245 245 246 248 249 250 251 252 257 257 257 257 257 257 260 261 262 262 263 264 268 268 269
 5.4.2-3 Performance of designed primers for amplification of the partial RNA-dependent polymerase (RdRp) and coat protein of the distinct virus isolate from To2B1	RNA- 244 245 245 246 248 249 250 251 252 257 257 257 257 259 260 261 262 262 263 264 . 265 264 . 266 268 269 270

6.2.5-1 <i>Cocksfoot mottle virus</i> (CoMV) 6.2.5-2 Barley yellow dwarf viruses (BYDVs)	273 277
 6.3 Suggestions for improvements and future research. 6.3.1 Further studies for <i>Cocksfoot mottle virus</i>. 6.3.2 Further studies on lutoviruses 6.3.3 Development of routine detection methods. 6.3.4 Improvements for studies of virus incidence in native grasses 	 280 280 282 283 285
6.4 Concluding remarks	285
Reference List	287
Appendices	313
Appendix 1: Nucleotide sequence alignment for the CoMV partial coat protein 3'UTR.	in and 313
Appendix 2: Nucleotide sequence alignments with different outgroups. A2.1 CoMV alignment with RYMV as outgroup. A2.2 CoMV alignment and Neighbour Joining tree with TNV as outgroup. A2.3 CoMV alignment and Neighbour Joining tree with PEMV as outgroup.	 317 317 321 326
Appendix 3: Amino acid sequence alignment for CoMV partial coat protein ger	ne 331
Appendix 4: Coleopteran species collected from 1 site in Waiouru and us transmission of CoMV	ed for 332
Appendix 5: Graphs on the growth of healthy cocksfoot cv Tekapo and cv grown under summer temperatures (19-39°C), and infected plants of the cv T	/ Kara ekapo
infected with CoMV-NI vs Healthy	336
 infected with CoMV-NI vs Healthy Appendix 6: dsRNA virus sequence analysis and BlastX results (tran nucleotide query to protein database) A6.1 Partial coat protein amino acid alignment of New Zealand's dsRNA virus with selecter Partitiviridae members A6.2 Neighbour Joining bootstrap consensus trees of New Zealand's dsRNA virus with Partitiviridae members A6.3 Sequence identity matrix for the amino acid partial coat protein of New Zealand's dsRNA virus with virus compared to Partitiviridae members 	336 slated 339 ed 345 346 RNA 348
 infected with CoMV-NI vs Healthy Appendix 6: dsRNA virus sequence analysis and BlastX results (tran nucleotide query to protein database) A6.1 Partial coat protein amino acid alignment of New Zealand's dsRNA virus with selecter Partitiviridae members A6.2 Neighbour Joining bootstrap consensus trees of New Zealand's dsRNA virus with Partitiviridae members A6.3 Sequence identity matrix for the amino acid partial coat protein of New Zealand's dsRNA virus with virus compared to Partitiviridae members Appendix 7: Sequence assembly for the partial fusion protein 2 and the ful protein of a distinct luteovirus from sample To2B1 	336 Islated 339 ed 345 346 RNA 348 Il coat 349
 infected with CoMV-NI vs Healthy Appendix 6: dsRNA virus sequence analysis and BlastX results (tran nucleotide query to protein database)	336 Islated 339 ed 345 345 RNA 348 Il coat 349 genies 351
 infected with CoMV-NI vs Healthy	336 Islated 339 ed 345 RNA 346 RNA 348 Il coat 349 genies 351 Ienetic 359
 infected with CoMV-NI vs Healthy	336 Islated 339 ed 345 345 RNA RNA 348 Il coat 349 genies 351 genetic 359 ovirus 361
 infected with CoMV-NI vs Healthy Appendix 6: dsRNA virus sequence analysis and BlastX results (tran nucleotide query to protein database) A6.1 Partial coat protein amino acid alignment of New Zealand's dsRNA virus with selecte Partitiviridae members. A6.2 Neighbour Joining bootstrap consensus trees of New Zealand's dsRNA virus with Partitiviridae members. A6.3 Sequence identity matrix for the amino acid partial coat protein of New Zealand's ds virus compared to Partitiviridae members. Appendix 7: Sequence assembly for the partial fusion protein 2 and the ful protein of a distinct luteovirus from sample To2B1 Appendix 8: Alignment of all sequence including outgroups used for phylog analysis. Appendix 10: Amino acid alignment of the coat protein for selected lute sequences from New Zealand and overseas, produced by Clustal X v1.8. Appendix 11: Percentage differences of selected nucleotide sequences of th protein of luteoviruses 	336 Islated 339 ed 345 RNA 346 RNA 348 Il coat 351 genies 351 genies 359 ovirus 361 e coat 363
 Infected with CoMV-NI vs Healthy Appendix 6: dsRNA virus sequence analysis and BlastX results (tran nucleotide query to protein database) A6.1 Partial coat protein amino acid alignment of New Zealand's dsRNA virus with selecte Partitiviridae members A6.2 Neighbour Joining bootstrap consensus trees of New Zealand's dsRNA virus with Partitiviridae members A6.3 Sequence identity matrix for the amino acid partial coat protein of New Zealand's ds virus compared to Partitiviridae members Appendix 7: Sequence assembly for the partial fusion protein 2 and the fu protein of a distinct luteovirus from sample To2B1 Appendix 8: Alignment of all sequence including outgroups used for phylog analysis Appendix 10: Amino acid alignment of the coat protein for selected lute sequences from New Zealand and overseas, produced by Clustal X v1.8. Appendix 11: Percentage differences of selected nucleotide sequences of th protein of luteoviruses Appendix 12: Full CP nucleotide alignment & AA translation used for recombi analysis. 	336 slated 339 ed 345 RNA 346 RNA 348 Il coat 349 genies 351 enetic 359 ovirus 361 e coat 363 nation 364

Chapter 1

 Table 1.1: Virus species recognised by the International Committee on Taxonomy of

 Viruses that are known to naturally infect at least one grass species. Virus species

 assigned to genera have names in italics, while non-italicised names refer to

 tentative members to the genera or family. Grey-shaded boxes highlights information

 for viruses confirmed as present in New Zealand. Virus species are arranged by

 higher taxonomical order (family or genus unassigned to family) in alphabetical

 order.
 19

 Table 1.2: Virus species not yet recognised by the International Committee on

 Taxonomy of Viruses that are known to naturally infect at least one grass

 species
 46

 Table 1.3: Summary of virus groups (family or genera unassigned to families) with

 species able to infect members of the Gramineae, the number of these found in New

 Zealand, and their genome type.
 53

Chapter 2

Table 2.1: Selected grass species in the Poaceae (Gramineae) family	.60
Table 2.2: List of collection sites	.74
Table 2.3: Apterous aphid nymphs detected on collected grass samples	77
Table 2.4: Summary of ELISA results for each of the plant species tested	.82
Table 2.5: Summary of virus infection for each virus infected plant tribe	.84
Table 2.6: Summary of the statistical values from the Nominal Logistic Regression	n of
CoMV and luteovirus incidence data, for the parameter 'plant species', using the	e α-
level of 0.05	.85

Chapter 3

 Table 3.3a: CoMV sequences from this study and their GenBank accession numbers.
 116

 Table 3.3b: CoMV coat protein sequences downloaded from GenBank used for phylogenetic analysis.
 117

 Table 3.4: Percentage differences (p-distance) between the aligned nucleotide sequences of CoMV coat protein. New Zealand (NZ) groups from phylogenies are highlighted in green shades (NZ main group= Gp1a-lime Gp1b-dark green,) and light blue (South Island only=Gp2).
 123

 Table 3.5: Percentage differences (p-distance) between aligned amino acid (AA) sequences of the CoMV coat protein. New Zealand groups from nucleotide phylogenies are highlighted as per Table 3.4. Orange highlights show that AA sequences from NZ's tussock (nucleotide subgroup Gp1b) and South Island only (Group 2) group together to a Norwegian and Japanese sequence.
 124

Chapter 4

Table 4.1. Plant species and seed (germplasm) accession numbers, used in
mechanical transmission studies of CoMV139
Table 4.2: Summary of CoMV inoculations made from field-collected infected
material145
Table 4.3: Experimental inoculation of two CoMV variants (CoMV-NI and CoMV-SI)
under glasshouse conditions in summer146
Table 4.4: Experimental inoculation of two CoMV variants (CoMV-NI and CoMV-SI)
in a growth cabinet with temperature range of 8 to 16 $^{\rm o}{\rm C}$ (autumn/spring
temperatures)146
Table 4.5: Summary of transmission study from field-collected material and control
inoculations carried out in autumn (April 2005)158
Table 4.6: CoMV-test results for glasshouse experimental inoculations (summer, 19-
Table 4.6: CoMV-test results for glasshouse experimental inoculations (summer, 19-39 °C)
Table 4.6: CoMV-test results for glasshouse experimental inoculations (summer, 19-39 °C)
Table 4.6: CoMV-test results for glasshouse experimental inoculations (summer, 19-39 °C)
Table 4.6: CoMV-test results for glasshouse experimental inoculations (summer, 19-39 °C)159Table 4.7: CoMV-transmission results for growth chamber experiment(autumn/spring temperatures, 8-16 °C)160Table 4.8: Statistical results from the repeated measure analysis performed on
Table 4.6: CoMV-test results for glasshouse experimental inoculations (summer, 19-39 °C)159Table 4.7: CoMV-transmission results for growth chamber experiment(autumn/spring temperatures, 8-16 °C)160Table 4.8: Statistical results from the repeated measure analysis performed onmaximum height and number of shoots, of plants from two cultivars under different
Table 4.6: CoMV-test results for glasshouse experimental inoculations (summer, 19- 39 °C)
Table 4.6: CoMV-test results for glasshouse experimental inoculations (summer, 19- 39 °C)

Chapter 5

Table 5.1: Primers published by Malmstrom and Shu (2004) used for the detection of BYDVs (including CYDV-RPV) by multiplex or single RT-PCR......193
 Table 5.2: Experimental design for aphid transmission from infected native grasses
 and barley: number of each plant species inoculated and source of viruliferous Table 5.4: Multiplex RT-PCR results and sequencing for collected South Island Table 5.5: Luteovirus coat protein nucleotide and amino acid sequences from GenBank selected for primer design and amino acid phylogenies......214 Table 5.6a: Selected forward primers and reverse primers (shaded grey) designed for the amplification of the N-terminal region of the coat protein of a distinct luteovirus from sample To2B1......217 Table 5.6b: Expected product size of selected primers and predicted overlap to Table 5.7: GenBank accession numbers and details of the luteovirus sequences obtained from this study; arranged by latitude from North to South of the sample
 Table 5.8: Accession numbers of Luteovirus coat protein nucleotide sequences
 Table 5.9: Percentage differences (1- similarity $[\pi] \times 100$; top half of table) and raw differences (1- similarity $[\pi]$; bottom half of table) between amino acid sequences of the coat protein of selected luteoviruses. The results for the To2B1 sequence from New Zealand are in grey-shaded cells. Differences within BYDV-MAV, BYDV-PAS,

Chapter 6

Appendix 4

Appendix 9

Appendix 11

Table A11.1: Percentage differences (1- similarity $[\pi]$ x100; top half of table) and raw differences (1- similarity $[\pi]$; bottom half of table) between nucleotide sequences of the coat protein of selected luteoviruses. The results for the To2B1 sequence from New Zealand are in grey-shaded cells. Differences within BYDV-MAV, BYDV-PAS, BYDV-PAV and BYDV-SGV sequences are shaded yellow, green, blue and pink-respectively. The label of New Zealand sequences are in bolded text......**363**

List of figures

Chapter 1

Chapter 2

Figure 2.1: Simplified diagram of the Indirect-ELISA (Enzyme-Linked Immuno-Figure 2.2: Simplified diagram of the Double Antibody Sandwich (DAS) ELISA Figure 2.3: Schematic representation of the Potyvirus genome, with the binding sites and description of the primers (U335/PVI-SP6) used for the detection and Figure 2.4: Satellite image of New Zealand North Island showing the sampling sites and habitat types sampled......75 Figure 2.5: Apterous aphid nymphs detected on sample Jo5 identified as Methopolophium sp. (possibly M. dirhothum). Image depicts details of the head (A), cauda (B), and siphon (C) from a late stage aphid nymph, a young nymph showing progressive pigmentation of antenna segments (D), and details of the base and Figure 2.6: Aphids detected on sample On18 identified as Rapholosiphum sp. (possibly *R. padi*). Image depicts the ventral view of a late stage aphid nymph (A) and a young nymph (B), details of siphon (C), head (D), cauda (E), and antennae (F).....**79** Figure 2.7: Relative percentage incidence of viruses for each grass species based Figure 2.8: 1% TBE-agarose gel showing amplification products from RT-PCR of native and exotic grasses positive for potyviruses by ELISA, using potyvirus primers

Chapter 3

Figure 3.7: Neighbour Joining bootstrap consensus for 1000 replications for the CP of CoMV. The Kimura-2-parameter (K80) was used as model of evolution. Isolates from New Zealand (NZ) are highlighted (pink for South Island and green for North Island) and distinct clades boxed. Symptoms on *Dactylis glomerata* from two NZ isolates are shown in the pictures at the right. A RYMV sequence (GenBank accession NC_001575) was used as outgroup......**118** Figure 3.8: Neighbour Joining bootstrap consensus for 1000 replications for the CP of CoMV. The GTR+I was used as model of evolution. The tree shows the clades of New Zealand (NZ) sequences (boxed) versus overseas sequences. A RYMV sequence (GenBank accession NC_001575) was used as outgroup......**119** Figure 3.9: Maximum parsimony cladogram of CoMV CP nucleotide sequence with bootstrap consensus (1000 replications). Isolates from New Zealand (NZ) are

Chapter 4

CfCP-F1/CfCP-R2) following mechanical inoculation from field collected samples to xvii

the same species and/or to *Dactylis glomerata* (DG), resolved on 1% agarose-TBE. Pa = *Poa anceps*, Fn = *Festuca novae-zelandiae*, DC = *Dichelacne crinita*.....**155**

Figure 4.4: Mean maximum height and standard error for cocksfoot cv Tekapo (top) and cocksfoot cv Kara (bottom) under different virus infection treatments for the period of 6-10 weeks post-inoculation......**162**

Chapter 5

illustrates the position of grids, single plants, and their relative location to each other.

Figure 5.9: Neighbour Joining bootstrap consensus trees (1000 replications) for the nucleotide sequences of the CP of luteoviruses, using the HKY+G (left) and the TVM+G (right) models of evolution. Isolates from New Zealand (NZ) are highlighted (pink for South Island and green for North Island). Isolate To2B1 is marked by a black arrow. PEMV sequences (GenBank accessions: Y09100 and Y09099) were

Appendix 4

Figure A4.1: Selection of insects collected from Waiouru, shown feeding on CoMVinfected cocksfoot pieces (A & B), and accustoming to healthy plants (C & D).....**335**

Appendix 5

Appendix 6

Appendix 7

Figure A7.1: (A) Panel showing length of reverse and forward sequence produced with To2R3 and To2F9, respectively, and overlap with ~400bp sequence already available for To2B1. (B) Close-up of overlapping part of the sequences showing a 100% match, for the region highlighted with a box (broken-lined) in (A)......**349 Figure A7.2:** Final assembled sequence for the To2B1 sample covering part of the RNA-dependent RNA polymerase fusion protein 2 (P2), the full coat protein, and movement protein (encoded through frame-shift at read-through stop codon).....**350**

Appendix 13

SI (Système International) abbreviations are used for chemicals, elements and formulae. Other abbreviations used in the text are listed below.

~	approximately
±	plus-minus
>	greater than
<	less than
≥	greater or equal to
≤	less or equal to
°C	degrees Celcius
μ m	micrometer(s)
μg	microgram(s)
μL	microlitre(s)
aa	amino acid
ACP	antigen coated plate
AIC	Akaike Information Criterion
ANOVA	analysis of variance
AP	alkaline phosphatase
ATG	Army training group
BLAST	Basic Local Alignment Tool
bp	base pairs
BSA	Bovine Serum Albumin
BSMV	Barley stripe mosaic virus
BYDV	Barley yellow dwarf virus
BYDVs	Barley and cereal yellow dwarf virus diseases
cDNA	Complementary deoxyribonucleic acid
СР	coat protein
CMV	Cucumber mosaic virus
CnMoV	Cynosurus mottle virus
CoMV	Cocksfoot mottle virus
cv	cultivar
CYDV	Cereal yellow dwarf virus
DAS	double antibody sandwich
DNA	deoxyribonucleic acid
dNTPs	2'-deoxynucleotide 5'-triphosphate
DoC	Department of Conservation
DsMV	Dasheen mosaic virus
E. coli	Escherichia coli
EDTA	ethylene diamine tetra-acetic acid
ELISA	Enzyme-linked immuno-sorbent assay

g	grams
GLM	general linear model
GPS	Geographical Positioning System
h	hour(s)
HLRTs	Hierarchical Likelihood Ratio Tests
ICTV	International Committee on Taxonomy of
	Viruses
ID	identification
ISEM	immuno-sorbent electron microscopy
Kb	kilobase(s)
L	litre(s)
LINZ	Land Information New Zealand
m	meter(s)
Μ	molar (moles/litre)
MAF	Ministry of Agriculture and Forestry
min	minute(s)
mL	millilitre
ML	Maximum Likelihood
mM	millimolar (millimoles/litre)
mm	millimeter
n/a	not applicable
NCBI	National Centre for Biotechnology
	Information
ng	nanograms
NI	North Island
NJ	Neighbour Joining
nm	nanometer
nt	Nucleotide
NVS	National Vegetation Survey
NZ	New Zealand
NZMS	New Zealand Map Grid
OD	optical density
PCR	Polymerase Chain Reaction
PEMV	Pea enation mosaic virus
pmol	picomoles
RDP	Recombination Detection Programme
RGCV	Ryegrass cryptic virus
RGMV	Ryegrass mosaic virus
RNA	ribonucleic acid
rpm	revolutions per minute
RT	reverse transcription
RYMV	Rice yellow mottle virus
S	second(s)
SBS	School of Biological Sciences (The University
	of Auckland)
SI	South Island

sp	species (singular)
spp	species (plural)
SSCP	Single Strand Conformation Profile
ТВЕ	Tris-Borate-EDTA
TEM	Transmission Electron Microscope
TNV	Tobacco necrosis virus
Tris	Tris(hydroxymethyl)-aminomethane
UV	ultraviolet
v/v	volume/volume
W	Watts
WMTA	Waiouru Military Training Area
WMV	Watermelon mosaic virus
WSMV	Wheat streak mosaic virus
w/v	weight/volume
ZYMV	Zucchini yellow mosaic virus