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**Characterisation of
Mycoviruses in the Plant
Pathogenic Fungus,
*Botrytis cinerea***

*A thesis presented in partial fulfilment of the
requirements for the degree of Doctor of Philosophy
in Biological Sciences*

Robyn L.J. Howitt

1998

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ABSTRACT

In recent years a lot of interest has focussed on the role of mycoviruses (fungal viruses) that confer reduced pathogenicity to their fungal hosts. In several plant pathogenic fungi viral double-stranded RNA (dsRNA) elements have been shown to be the agents responsible for this attenuation of virulence.

In New Zealand and other temperate climates the plant pathogenic fungus, *Botrytis cinerea* is an important pathogen of many horticultural crops. Control of this grey mould disease still relies heavily on the use of fungicides to augment cultural practices. Consequently, there is now increasing interest in the use of biological control as an alternative means of control.

This study reports the first large-scale survey of dsRNA elements in *B. cinerea*. Analysis of 200 New Zealand isolates from a range of hosts revealed 72% contained dsRNA, with high variation in dsRNA profiles, both within and between groups of fungal isolates from different hosts. DsRNA has been associated with isometric particles in many different fungi. In this study virus-like particles (VLPs) were observed in three of five dsRNA-containing isolates examined. Morphological types included isometric particles of varying size classes (approx. 30, 35, 40 and 45 nm) and bacilliform particles (approx. 25 x 63 nm). In five isolates apparently lacking dsRNA no isometric or bacilliform particles were found but flexuous rods with a modal length of approx. 720 nm were detected in one of these isolates. Total RNA extraction of the various partially purified VLP preparations gave similar profiles to those obtained from mycelial dsRNA extractions of the same isolates, suggesting that the VLPs comprise encapsidated dsRNA. Comparison of 12 dsRNA-containing and 12 dsRNA-free isolates indicated slight differences between the two groups in radial growth rate, virulence on bean leaves and sclerotial number, but the differences were minor. In summary, while dsRNA was found to be common among *B. cinerea* isolates in this study, the evidence did not reveal any marked phenotypic effects of dsRNA presence.

The putative flexuous rod-shaped virus particles in *B. cinerea* were studied further. Similar particles have been observed in fungi before but none have been characterised at

the molecular level. This study reports the first complete nucleotide sequences of two such mycoviruses, *Botrytis Virus F* (BVF) and *Botrytis Virus X* (BVX), and also the first molecular characterisation of viruses infecting *B. cinerea*. The mycoviruses, which were both present in the same *B. cinerea* isolate, were similar in size and morphology to ssRNA plant viruses of the family Potexviridae. BVF and BVX contained ssRNA genomes of 6827 and 6966 nucleotides respectively, excluding poly(A) tails. Computer analysis of the genomic cDNA sequence of BVF revealed three potential open reading frames (ORFs) encoding putative proteins of 20 kDa (ORF1), 212 kDa (ORF2) and 32 kDa (ORF3). ORF1, which overlapped ORF2 by 467 nt, did not reveal significant homology with known protein sequences. ORF2, which showed significant sequence identity to the replicase proteins of the plant virus families Tymoviridae and Potexviridae, contained a putative opal readthrough codon intermediate between the helicase and RNA-dependent RNA polymerase (RDRP) regions. ORF3 shared amino acid homology with coat proteins of the family Potexviridae. A putative defective RNA (D RNA) of 829 nt was also detected which consisted of a 5' region of the parental genome fused in-frame to the terminal 3' end, complete with poly(A) tail. BVX revealed five potential ORFs encoding putative proteins of 158 kDa (ORF1), 30 kDa (ORF2), 44 kDa (ORF3), 14 kDa (ORF4) and 14kDa (ORF5). ORF1 showed significant amino acid sequence identity to the replicase proteins and ORF3 to the coat proteins of the Potexviridae. The remaining ORFs did not reveal significant homology with known protein sequences.

This is the first report of sequence similarities between mycoviruses and the plant virus families Potexviridae and Tymoviridae. Comparisons of sequence and genome organisation suggest that the mycoviruses BVF and BVX may each belong to a new as yet unassigned genus in the plant virus family Potexviridae. An unexpected result from this study was the high level of identity observed between the derived amino acid sequences of the mycoviruses and plant viruses including a region of ~70% for the RNA-dependent RNA polymerase (RDRP) region of BVX and similar regions in several potex-like viruses of *Allium* spp. This high level of interviral homology has not previously been reported between the plant and fungal kingdoms and is possibly indicative of a recent divergence from a common ancestor. These results suggest that horizontal gene transfer between the plant and fungal kingdoms may occur more

frequently than has been appreciated to date. If so it may play a significant role in viral evolution.

In addition to providing novel phylogenetic information on the relationship between these morphological types of viruses in fungi and in plants, characterisation of these mycoviral genomes provides a possible strategy for incorporating genes into *Botrytis* using viruses as vectors. In this context *Botrytis* strains infected with modified viruses could provide a new approach for the biocontrol of grey mould.

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CONTENTS

ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	v
CONTENTS.....	vii
TABLES.....	xi
FIGURES.....	xii
ABBREVIATIONS.....	xv
CHAPTER 1 - Introduction.....	1
<i>The Host – Botrytis cinerea</i>	<i>1</i>
Taxonomy/Morphology.....	1
Pathology/Infection	3
Noble rot.....	4
Control.....	4
Genetic basis of variation	5
Fungicide resistance	7
<i>Mycoviruses</i>	8
Introduction	8
Taxonomy of VLPs and mycoviruses.....	9
VLPs and mycoviruses associated with altered fungal phenotypes.....	12
Mushroom (<i>Agaricus bisporus</i>) viruses.....	12
Yeast and smut killer systems.....	14
DsRNA viral elements/mycoviruses associated with biological control of plant pathogenic fungi .	17
<i>Cryphonectria parasitica</i>	17
<i>Helminthosporium victoriae</i>	20
<i>Ophiostoma novo-ulmi</i>	21
<i>Sclerotinia</i> sp.	22
<i>Leucostoma personii</i>	23
<i>Rhizoctonia solani</i>	23
<i>Gaeumannomyces graminis</i> var. <i>tritici</i>	25
Mycovirus evolution.....	25
<i>DsRNA and VLPs in Botrytis cinerea</i>	26
<i>Aims of Thesis</i>	27
CHAPTER 2 - Presence of double-stranded RNA and virus-like particles in <i>Botrytis cinerea</i>.....	28
<i>Introduction</i>	28
<i>Materials and methods</i>	29
Media and growth conditions	29
Origin and culture of <i>Botrytis cinerea</i> isolates	30
Fungicide resistance tests	31

Extraction and electrophoresis of dsRNA	31
Confirmation of dsRNA	31
Partial purification of VLPs.....	32
Electron microscopy	32
RNA extraction from partly purified virus preparations.....	32
Physiological measurements.....	33
Results.....	34
Presence of dsRNA in isolates of <i>Botrytis cinerea</i>	34
Analysis of VLPs.....	35
Physiological comparison of selected <i>Botrytis cinerea</i> isolates	38
‘Stop-Start’ growth.....	38
Discussion.....	40
CHAPTER 3 - Characterisation of a flexuous rod-shaped mycovirus, <i>Botrytis Virus F (BVF)</i>.....	42
Introduction.....	42
Materials and methods.....	43
Origin and culture of fungal strain	43
Partial purification of VLPs.....	43
RNA extraction from partly purified preparations	44
cDNA synthesis and cloning	44
PCR amplification	45
Determination of the 5’ end.....	45
Sequencing and sequence analysis	46
Sequences	47
Results.....	47
Isolation of virus-specific cDNA clones.....	47
Genome organisation.....	49
ORF1	55
ORF2	55
ORF3	57
Discussion.....	58
CHAPTER 4 - Characterisation of a second flexuous rod-shaped mycovirus, <i>Botrytis Virus X (BVX)</i>	64
Introduction.....	64
Materials and methods.....	64
PCR amplification	64
Results.....	65
Isolation of virus-specific cDNA clones.....	65
Genome organisation.....	65
ORF1	65
ORF2	71
ORF3	71
ORF4	72
ORF5	73
Discussion.....	73
CHAPTER 5 - Do the mycoviruses BVF and BVX provide evidence for horizontal gene transfer between kingdoms?	77
Introduction.....	77
Materials and methods.....	78

Sequence analysis.....	78
Amino acid sequences	78
Nucleotide sequences.....	79
Results.....	79
Phylogenetic analysis	79
Replicase genes.....	79
Coat protein genes	79
Nucleotide composition.....	80
Replicase genes.....	80
Coat protein genes	80
Monte Carlo analysis.....	80
Discussion.....	80
CHAPTER 6 - General conclusions.....	93
Future directions.....	95
REFERENCES.....	99
APPENDIX 1 <i>Botrytis cinerea</i> isolates selected for dsRNA survey (Chapter 2).....	111
APPENDIX 2 <i>Botrytis cinerea</i> isolates selected for physiological comparison (Chapter 2).....	115
APPENDIX 3 ClustalW alignment of BVF and related plant virus replicase genes (Chapter 3)..	116
APPENDIX 4 Unrooted neighbour joining trees generated from Discalc/Diplomo data for BVF and related plant viruses (Chapter 3). (a) Entire replicase; (b) Methyltransferase region; (c) Helicase region; (d) RDRP region.	126
APPENDIX 5 ClustalW alignment of BVF and related plant virus coat protein genes (Chapter 3).	127
APPENDIX 6 ClustalW alignment of BVX and related plant virus replicase genes (Chapter 4).	129
APPENDIX 7 ClustalW alignment of BVX and related plant virus coat protein genes (Chapter 4).	138
APPENDIX 8 (a) ClustalW alignment of BVX, BVF and related plant virus replicase genes (Chapter 5).....	140
APPENDIX 8 (b) ClustalW alignment of the methyltransferase region of BVX, BVF and related plant virus replicase genes (Chapter 5).....	149
APPENDIX 8 (c) ClustalW alignment of the helicase region of BVX, BVF and related plant virus replicase genes (Chapter 5).	150
APPENDIX 8 (d) ClustalW alignment of the RDRP region of BVX, BVF and related plant virus replicase genes (Chapter 5).	151
APPENDIX 9 Media and solutions.....	152

APPENDIX 10 Glossary of terms..... 154

APPENDIX 11 Publication reporting thesis results (Chapter 2)..... 157

TABLES

Table 1 Mycovirus taxonomy (From Murphy <i>et al.</i> , 1995).....	11
Table 2 Representative mycovirus sequences deposited in the GenBank database.....	13
Table 3 Origin of selected <i>B. cinerea</i> isolates.	30
Table 4 Incidence of dsRNA in <i>B. cinerea</i> isolates from different hosts.....	34
Table 5 Physiological comparison of 24 <i>B. cinerea</i> isolates†	39
Table 6 Details of sequences used in analyses in Chapters 3, 4 and 5.....	48
Table 7 Amino acid sequence % identity between the BVF putative replicase gene and corresponding genes of members of the plant virus families Potexviridae and Tymoviridae. Above diagonal: Entire replicase gene; Below diagonal: Methyltransferase region of replicase (Refer to Table 6 for abbreviations).	56
Table 8 Amino acid sequence % identity between the BVF putative replicase gene and corresponding genes of members of the plant virus families Potexviridae and Tymoviridae. Above diagonal: Helicase region of replicase; Below diagonal: RDRP region of replicase (Refer to Table 6 for abbreviations).	57
Table 9 Amino acid sequence % identity between the BVF putative coat protein gene and corresponding genes of members of the plant virus families Potexviridae and Potyviriidae (Refer to Table 6 for abbreviations).	58
Table 10 Amino acid sequence % identity between the BVX putative replicase gene and corresponding genes of members of the plant virus families Potexviridae and Tymoviridae. Above diagonal: Entire replicase gene; Below diagonal: Methyltransferase region of replicase (Refer to Table 6 for abbreviations).	72
Table 11 Amino acid sequence % identity between the BVX putative replicase gene and corresponding genes of members of the plant virus families Potexviridae and Tymoviridae. Above diagonal: Helicase region of replicase; Below diagonal: RDRP region of replicase (Refer to Table 6 for abbreviations).	73
Table 12 Amino acid sequence % identity between the BVX putative coat protein gene and corresponding genes of members of the plant virus family Potexviridae (Refer to Table 6 for abbreviations).	74
Table 13 Amino acid sequence % identity between regions of various mycoviral, plant viral and plant mitochondrial genomes.....	89
Table 14 RNA base composition of BVF and BVX genomes.....	90

FIGURES

- Figure 1** Schematic representation of the life cycle of *B. cinerea* (Modified from Agrios, 1988). 2
- Figure 2** 'Grey mould' disease. Stem canker on tomato stem developed from earlier flower infection (From Eden *et al.*, 1996b). 3
- Figure 3** Phenotypic variation among *B. cinerea* isolates grown on MEA. Isolate numbers (clockwise from top left): REB205-1, RH100-19, REB226-47, REB200-5, RH104-20, REB171-3. 6
- Figure 4** The arrangement of MBV ORFs with the size indicated by the scale at the bottom of the figure. ORFs 2 & 3 contain sequence motifs typical of plant virus replicase genes and ORF4 shows homology to plant virus coat protein genes. The dotted lines in ORF3 represent sequences upstream of the first methionine (From Revill *et al.*, 1994). 14
- Figure 5** Schematic representation of the genome organisation of the ScV-L-A virus (From Wickner, 1992). 15
- Figure 6** Packaging and assembly model of the ScV-L-A virus resulting in the encapsidation of a single viral (+) strand, which is then converted to dsRNA form within the particle (From Wickner, 1996a). 16
- Figure 7** Schematic representation of L-dsRNA present in hypovirulent *C. parasitica* EP713 (CHV1-713), the type member of the Hypoviridae (From Wickner, 1996a). 19
- Figure 8** Diagram illustrating relative positions of ORFs of *R. solani* M1 dsRNA (From Jian *et al.*, 1998). 24
- Figure 9** Polyacrylamide gel (5%) electrophoresis of dsRNAs extracted from mycelium of isolates of *B. cinerea* by CF-11 chromatography. Lanes are as follows: (1) Rotavirus SA11 dsRNA; (2) dsRNAs from CTV- and TMV-infected tissue; (3) Isolate RH106-3; (4) RH105-21; (5) RH101-21; (6) RH102-3; (7) RH102-5; (8) RH102-19; (9) ICMP 7665; (10) RH104-20; (11) RH101-6; (12) REB200-5; (13) REB171-3; (14) RH101-4; (15) RH102-2; (16) RH103-15; (17) RH105-19; (18) RH100-19. Size of marker dsRNAs in base pairs are indicated at left-hand side of figure. 36
- Figure 10** Electron micrographs of VLPs in partly purified preparations. (a) Isolate RH100-19 showing 35 nm isometric particles and 25 x 63 nm bacilliform particles (arrowed), negatively stained with 2% uranyl acetate, pH 4.0. (b) Enlarged section of (a). (c) Isolate REB205-1 following incubation in CsCl overnight showing 30 and 40 nm isometric particles (note empty virions as indicated by penetration of the negative stain). (d) Isolate REB171-3 showing 45 nm particles. (c) and (d) negatively stained with 2% PTA, pH 4.0. Bars, 100 nm. 36
- Figure 11** PAGE analysis of nucleic acids extracted from mycelium of *B. cinerea* isolates by CF-11 chromatography (lanes 2, 4, 6 and 8) and from corresponding partly purified virus preparations (lanes 3, 5, 7 and 9). Lanes are as follows: (1) Rotavirus marker; (2 and 3) Isolate REB171-3; (4 and 5) RH104-20; (6 and 7) REB205-1; (8 and 9) RH105-19. Size of marker dsRNAs in base pairs is indicated at left-hand side of figure. 37
- Figure 12** PAGE analysis of dsRNAs extracted from different subcultures of *B. cinerea* isolate ICMP 7665 by CF-11 chromatography. Mycelia were derived from spore suspensions prepared at the same time but with differing histories as follows: lane 1 (primary culture ex lyophil; linear thin growth), lane 2 (two transfers from primary culture; after 'stop-start' episodes), lane 3 (one transfer from primary culture; linear thin growth), lanes 4-6 (two transfers from primary culture; lag before slow thin growth). 37

- Figure 13** Electron micrograph of flexuous rod-shaped particles in partly purified preparation of isolate RH106-10, negatively stained with 2% PTA, pH 4.0. Bar, 200nm. 43
- Figure 14** Labelled cDNA, synthesised by reverse transcription using an Oligo(dT)₂₅ primer, from (a) potato virus X (PVX) RNA and (b) mycoviral RNA..... 49
- Figure 15** Schematic representation of the genome organisation and cloning strategy of BVF. (a) Positions of ORFs (boxed) and the size of their putative products are shown. The relative position of the conserved domains of the replicase gene are indicated with coloured boxes. MTR, methyltransferase; HEL, helicase; POL, RNA-dependent RNA polymerase (RDRP). The position of the putative readthrough codon is indicated with a diamond. Yellow boxes represent the portions of the 5' and 3' termini that are found in a putative defective RNA (D RNA) species. (b) D RNA species that has an ORF with the potential to encode a replicase-CP fusion protein. (c) Map of overlapping cDNA clones; the open boxes indicating clones obtained with specific primers..... 50
- Figure 16** The nucleotide sequence of cDNA clones of BVF genomic RNA. Predicted amino acid sequence of the putative ORFs is given in the one-letter code above the nucleotide sequence. (A)_n denotes the 3' poly(A) tail. The conserved GKS (helicase) and GDD (RDRP) motifs are underlined, along with the possible AUU initiation codon for ORF1 (nt 2) and the asterisk denoting the putative readthrough codon (nt 4192)..... 51
- Figure 17** Segments of the coat proteins of BVF and flexuous rod-shaped plant viruses potentially involved in salt bridge formation. * = identical residues • = similar residues. The conserved positively and negatively charged residues (Arg and Asp respectively), proposed to form a salt bridge crucial for the protein structure, are shown in boldface. The distances toward the protein termini, and those between the aligned segments, are indicated by numbers (Modified from Dolja *et al.*, 1991)..... 59
- Figure 18** Schematic representation of the genome organisation and cloning strategy of BVX. (a) Positions of ORFs (boxed) and the size of their putative products are shown. The relative position of the conserved domains of the replicase gene are indicated with coloured boxes. MTR, methyltransferase; HEL, helicase; POL, RNA-dependent RNA polymerase (RDRP). The dashed line in ORF3 indicates the methionine codon that possibly could initiate coat protein synthesis. (b) Map of overlapping cDNA clones; the open boxes indicating clones obtained with specific primers. 66
- Figure 19** The nucleotide sequence of cDNA clones of BVX genomic RNA. Predicted amino acid sequence of the putative ORFs is given in the one-letter code above the nucleotide sequence. (A)_n denotes the 3' poly(A) tail. The conserved GKS (helicase) and GDD (RDRP) motifs are underlined, along with the methionine codon (nt 5831) in ORF3 predicted to initiate coat protein synthesis. ... 66
- Figure 20** Phylograms of replicase genes and internal conserved replicase regions of BVF, BVX and related plant viruses with bootstrap values >50 indicated. (a) Entire replicase; (b) Methyltransferase region; (c) Helicase region; (d) RDRP region (Refer to Table 6 for abbreviations). 81
- Figure 21** Phylogram of coat protein genes of BVX and related plant viruses with bootstrap values >50 indicated (Refer to Table 6 for abbreviations). Figures in brackets represent Monte Carlo scores, quantified as the number of SD above the random expectation..... 82
- Figure 22** Phylogram of coat protein genes of BVF and related plant viruses with bootstrap values >50 indicated (Refer to Table 6 for abbreviations). Figures in brackets represent Monte Carlo scores, quantified as the number of SD above the random expectation..... 82
- Figure 23** Three-dimensional model (NTSYS) based on the replicase % nucleotide composition of the mycoviruses BVF and BVX and related plant viruses. Colours depict genera (Refer to Table 6 for abbreviations). Note: The nucleotide composition data is used to calculate distances between each of the taxa with each distance representing an average relationship based on all data compared. These distances position the taxa in multidimensional space similar to fitting a regression line to a graph in 2D. 83

Figure 24 Three-dimensional model (NTSYS) based on the coat protein % nucleotide composition of the mycoviruses BVF and BVX and related plant viruses. Colours depict genera (Refer to Table 6 for abbreviations). Note: The nucleotide composition data is used to calculate distances between each of the taxa with each distance representing an average relationship based on all data compared. These distances position the taxa in multidimensional space similar to fitting a regression line to a graph in 2D.84

Figure 25 Schematic representation of the genome organisations of plant viruses in the families Potexviridae and Tymoviridae and the mycoviruses BVF and BVX. Coloured boxes represent replicase (blue), coat (yellow) and movement (pink) proteins. Diamonds indicate readthrough codons and asterisks putative initiation codons.86

Figure 26 ClustalW alignment of the conserved RDRP region of replicase genes of BVX, BVF and related plant viruses. * = identical residues • = similar residues (Refer to Table 6 for abbreviations).88

ABBREVIATIONS

(+)	positive strand
A	adenine
aa	amino acid(s)
<i>B. cinerea</i>	<i>Botrytis cinerea</i>
bp	base pair(s)
C	cytosine
cDNA	complementary DNA
CP	coat protein
<i>C. parasitica</i>	<i>Cryphonectria parasitica</i>
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dNTPs	deoxynucleotide triphosphates
DrBr	dicarboximide-resistant, benzimidazole-resistant
DrBs	dicarboximide-resistant, benzimidazole-sensitive
D RNA	defective RNA
DsBr	dicarboximide-sensitive, benzimidazole-resistant
DsBs	dicarboximide-sensitive, benzimidazole-sensitive
dsDNA	double-stranded DNA
dsRNA	double-stranded RNA
EDTA	ethylene diamine tetra-acetic acid
g	acceleration of gravity
G	guanine
GCG	Genetics Computer Group (University of Wisconsin)
h	hour(s)
kb	kilobase(s)

kbp	kilobase pair(s)
kDa, (K)	kilodalton(s)
MEA	Malt extract agar
min	minute(s)
mRNA	messenger RNA
MTR	methyltransferase
nm	nanometre(s)
nt	nucleotide(s)
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
RDRP	RNA-dependent RNA polymerase
RNA	ribonucleic acid
<i>R. solani</i>	<i>Rhizoctonia solani</i>
RT-PCR	reverse transcription-polymerase chain reaction
SDS	sodium dodecyl sulphate
SSC	saline-sodium citrate
T	thymine
TBE	tris-borate
Tris	tris (hydroxymethyl)-amino-methane
U	unit or uracil
v	volume
VC	vegetative compatibility
VLP	virus-like particle
w	weight