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Review

Mycoviruses of filamentous fungi and their relevance to plant pathology

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SUMMARY

Mycoviruses (fungal viruses) are reviewed with emphasis on plant pathogenic fungi. Based on the presence of virus-like particles and unencapsidated dsRNAs, mycoviruses are common in all major fungal groups. Over 80 mycovirus species have been officially recognized from ten virus families, but a paucity of nucleic acid sequence data makes assignment of many reported mycoviruses difficult. Although most of the particle types recognized to date are isometric, a variety of morphologies have been found and, additionally, many apparently unencapsidated dsRNAs have been reported. Until recently, most characterized mycoviruses have dsRNA genomes, but ssRNA mycoviruses now constitute about one-third of the total. Two hypotheses for the origin of mycoviruses of plant pathogens are discussed: the first that they are of unknown but ancient origin and have coevolved along with their hosts, the second that they have relatively recently moved from a fungal plant host into the fungus. Although mycoviruses are typically readily transmitted through asexual spores, transmission through sexual spores varies with the host fungus. Evidence for natural horizontal transmission has been found. Typically, mycoviruses are apparently symptomless (cryptic) but beneficial effects on the host fungus have been reported. Of more practical interest to plant pathologists are those viruses that confer a hypovirulent phenotype, and the scope for using such viruses as biocontrol agents is reviewed. New tools are being developed based on host genome studies that will help to address the intellectual challenge of understanding the fungal–virus interactions and the practical challenge of manipulating this relationship to develop novel biocontrol agents for important plant pathogens.

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INTRODUCTION

Whereas viruses of plants have long been recognized as important components of plant biosystems, viruses of fungi (mycoviruses) have been largely ignored and, apart from a few notable exceptions, their roles in fungi are largely unknown. Although the first definitive record of a mycovirus was published over 40 years ago (Hollings, 1962), our knowledge and understanding of fungal viruses is still in its infancy. Only a few hundred research papers have been published on mycoviruses compared with tens of thousands of publications on plant viruses. Not surprisingly, the number of mycoviruses for which the genome has been fully characterized is small compared with plant and animal viruses. Most publications are concerned with mycoviruses of economically important fungi, such as yeasts, cultivated mushrooms and pathogens of plants and animals. The mycovirus that dominates in the context of plant pathology is the *Cryphonectria hypovirus 1* (CHV1), which has been successfully used as a biological control agent for the chestnut blight pathogen *Cryphonectria parasitica* (Nuss, 2005). Despite the limited information on the incidence and diversity of mycoviruses, it is apparent that viruses and dsRNAs (generally considered indicative of viral infection) occur widely in a diverse range of fungi (Tables 1 and 2). It is probable that they are as widepread as viruses in plants and animals, although interestingly none has so far been characterized in the extensively studied 'model' saprotrophic species *Neurospora crassa*, possibly because of highly efficient gene silencing in that species (Cogoni and Macino, 1999).

For many plant viruses the effects on host plant growth and health are well documented, and although these are sometimes cryptic (non-symptomatic) or latent (expressed only under some conditions) the majority cause discernible symptoms. Whereas our knowledge of mycovirus diversity and roles is slowly but steadily accumulating, our understanding of the interaction between mycoviruses and their hosts is largely limited to a few well-studied, possibly atypical, systems. For the majority of mycovirus–host combinations reported to date, the interactions between the virus and host, and the mode(s) of virus transmis-

Table 1 Summary of formally named and recognized mycoviruses extracted from Fauquet *et al.* (2005) 'Virus Taxonomy: The Eighth Report of the International Committee on Taxonomy of Viruses' (confirmed species in italics, tentative members of genera and unassigned viruses in regular font).

Genome	Family	Genus	Species	Morphology
dsDNA		*Rhizidiovirus*	*Rhizidiomyces virus* (RhiV)	Isometric, 60 nm, not enveloped
ss(+)RNA	*Barnaviridae*	*Barnavirus*	*Mushroom bacilliform virus (MBV)*	Bacilliform, 19 × 50 nm, not enveloped
	Narnavriridae	*Mitovirus*	*Cryphonectria mitovirus 1*	No true particles, virions consist of a nucleoprotein complex, not enveloped
			Ophiostoma mitovirus 3a	
			Ophiostoma mitovirus 4	
			Ophiostoma mitovirus 5	
			Ophiostoma mitovirus 6	
			Gremmeniella mitovirus S1	
			Ophiostoma mitovirus 1a	
			Ophiostoma mitovirus 1b	
			Ophiostoma mitovirus 2	
			Ophiostoma mitovirus 3b	
		Narnavirus	*Saccharomyces 20S RNA narnavirus*	No true particles, nucleoprotein complex, not enveloped
			Saccharomyces 23S RNA narnavirus	
		Unassigned	Rhizoctonia virus M2	No particles
	Unassigned	Unassigned	Botrytis cinerea virus F	Flexuous, ~600–650 nm
			Diaporthe RNA virus	No particles
ss(+)RNA-RT	*Pseudoviridae*	*Hemivirus*	*Candida albicans Tca2 virus*	Isometric to quasi-isometric, 30–40 nm, not enveloped
			Candida albicans Tca5 virus	
			Saccharomyces paradoxus Ty5 virus	
		Pseudovirus	*Saccharomyces cerevisiae Ty1 virus*	Isometric to quasi-isometric, 30–40 nm, not enveloped
			Saccharomyces cerevisiae Ty2 virus	
			Saccharomyces cerevisiae Ty4 virus	
	Metaviridae	*Metavirus*	*Cladosporium fulvum T-1 virus*	Ovoid, 50 nm, irregular, enveloped nucleoprotein complex
			Fusarium oxysporum Skippy virus	
			Saccharomyces cerevisiae Ty3 virus	
			Schizosaccharomyces pombe Tf1 virus	
			Schizosaccharomyces pombe Tf2 virus	
dsRNA	*Chrysoviridae*	*Chrysovirus*	*Helminthosporium victoriae virus 145S*	Isometric, 30–35 nm, not enveloped, multiple components
			Penicillium brevicompactus virus	
			Penicillium chrysogenum virus	
			Penicillium cyaneo-fulvum virus	
			Agaricus bisporus virus 1	
	*Hypoviridae**	*Hypovirus*	*Cryphonectria hypovirus 1*	Pleomorphic vesicles, 50–80 nm
			Cryphonectria hypovirus 2	
			Cryphonectria hypovirus 3	
			Cryphonectria hypovirus 4	
	Partitiviridae	*Partitivirus*	*Agaricus bisporus virus 4*	Isometric, 30–40 nm, not enveloped
			Aspergillus ochraceous virus	
			Atkinsonella hypoxylon virus	
			Discula destructiva virus 2	
			Fusarium poae virus	
			Fusarium solani virus 1	
			Gaeumannomyces graminis virus 019/6-A	
			Gaeumannomyces graminis virus T1-A	
			Gremmeniella abietina RNA virus MS1	
			Helicobasidium mompa virus	
			Heterobasidion annosum virus	
			Penicillium stoloniferum virus S	
			Rhizoctonia solani virus 717	
			Diplocarpon rosae virus	
			Penicillium stoloniferum virus F	
			Phialophora radicicola virus 2-2-A	

Table 1 *continued*

Genome	Family	Genus	Species	Morphology
	Reoviridae	*Mycoreovirus*	*Mycoreovirus-1*	Isometric, ~80 nm, not-enveloped
			Mycoreovirus-2	
			Mycoreovirus-3	
	Totiviridae	*Totivirus*	*Helminthosporium victoriae virus 190S*	Isometric, 30–40 nm, not enveloped
			Saccharomyces cerevisiae virus L-A (L1)	
			Saccharomyces cerevisiae virus L-BC (La)	
			Ustilago maydis virus H1	
			Aspergillus foetidus virus S	
			Aspergillus niger virus S	
			Gaeumannomyces graminis virus 87-1-H	
			Mycogone perniciosa virus	
	Unassigned	Unassigned	Agaricus bisporus virus 1	Isometric, 25 nm, not enveloped
			Allomyces arbuscula virus (AAV)	Isometric, 40 nm, not enveloped
			Aspergillus foetidus virus F (AFV-F)	Isometric, 40–42 nm, not enveloped
			Botrytis cinerea virus-CVg25	Isometric, 40 nm, not enveloped
			Colletotrichum lindemuthianum virus	Isometric, 30 nm, not enveloped
			Fusarium graminearum virus DK21	No virions
			Gaeumannomyces graminis virus 45/101-C	Isometric, 29 nm, not enveloped
			Helminthosporium maydis virus	Isometric, 48 nm, not enveloped
			LaFrance isometric virus	Isometric, 36 nm, not enveloped
			Lentinus edodes virus	Isometric, 39 nm, not enveloped
			Perconia circinata virus	Isometric, 32 nm, not enveloped
	Unassigned	satellite ds-RNAs	M satellites of Saccharomyces cerevisiae L-A virus	Sub-viral NA molecules, require helper virus
			Satellite of Ustilago maydis killer M virus	

*Although there is some evidence to suggest that hypoviruses may have an ssRNA genome (Nuss, 2005), ICTV currently defines them as dsRNA viruses.

sion, are still unstudied. Given that all viruses are obligate parasites it is a reasonable *a priori* assumption that the majority of mycoviruses will have some negative effect(s) on fungal growth or survival. Consistent with this proposal, there is evidence that RNA silencing acts as an antiviral defence mechanism in fungi (Cogoni and Macino, 1999; Segers *et al.*, 2007). Nevertheless, except perhaps in some species such as *Neurospora crassa*, this defence is not fully efficient. Notwithstanding, given the high selective value for a parasite to reduce its impact on the host, many have apparently evolved to a 'steady state' situation of minimal impact. Furthermore, in some instances, mycoviruses may act as extra-chromosomal genes that confer an advantage to the host, as with the killer systems in yeast (Schmitt and Breinig, 2002, 2006).

The primary focus of this review is viruses of filamentous fungi, especially plant pathogens, with emphasis on the molecular characterization of mycoviruses and how this has affected our understanding of their diversity and phylogenetic relationships. We discuss the significance of mycoviruses to plant pathologists, and how molecular technologies are contributing to our understanding of fungal–virus interactions, particularly viral transmission and the phenotypic effects on the fungal host. We follow common mycological usage (Webster and Weber, 2007) in treating both true fungi (kingdom Fungi – Eumycota) and fungal-like groups such as the Oomycota (kingdom Straminipila) in our review. Additional information can be found in the earlier reviews of Ghabrial (1994), Buck (1998), Hillman and Suzuki (2004), Nuss (2005) and Varga *et al.* (2003). We do not discuss virus-like elements such as transposons, and limit discussion of mycoviruses of saprophytic species and yeasts to a few salient points. For reviews of yeast mycoviruses the reader is referred to Wickner (1996), Marquina *et al.* (2002) and Schmitt and Breinig (2002, 2006).

There are several reasons why mycologists in general and fungal plant pathologists in particular should be aware of, and interested in, mycoviruses. At the laboratory level, if the phenotypic characteristics of a fungal isolate are measurably affected by mycovirus infection, for example by loss of vigour or production of non-sporulating sectors in culture (Van Diepeningen *et al.*, 2006), there are important implications for the maintenance of such isolates, and any experimentation using them. At the applied level, there is the potential use of them as expression vectors of non-viral antifungal genes, and especially the opportunity to use them as biological control agents against plant pathogens.

Table 2 Sequenced or partially sequenced mycoviruses not included in Fauquet *et al.* (2005) 'Virus Taxonomy: The Eighth Report of the International Committee on Taxonomy of Viruses'.

Fungal host (virus name)	Genome	Sequence homology	Reference
Botrytis cinerea (virus X)	ssRNA	*Flexivirdae*, *Potexvirus*	Howitt *et al.*, 2006
Botrytis cinerea (debilitation-related virus)	dsRNA	*Narnaviridae*, *Mitovirus (Ophiostoma mitovirus 3b)*	Wu *et al.*, 2007
Botryotinia fuckeliana (Partitivirus 1)	dsRNA	*Partitiviridae*, *Partitivirus*	De Guido *et al.*, 2007
Botryotinia fuckeliana (Totivirus 1)	dsRNA	*Totiviridae*, *Totivirus*	De Guido *et al.*, 2007
Ceratocystis polonica & C. resinifera	dsRNA	*Partitiviridae*	Deng and Bolland 2007
Chalara elegans (RNA virus 1)	dsRNA	*Totiviridae*	Park *et al.*, 2005
Chalara elegans (RNA virus 2)	dsRNA	*Totiviridae*	Park *et al.*, 2005
Chalara elegans	dsRNA	*Narnaviridae*, *Mitovirus*	Park *et al.*, 2006b
Coniothyrium minitans (RNA virus)	dsRNA	*Totiviridae*	Cheng *et al.*, 2003
Cryphonectria nitschkei	dsRNA	*Chrysoviridae*	Liu *et al.*, 2007a,b
Epichloe festucae	dsRNA	*Totiviridae*, *Totivirus*	Romo *et al.*, 2007
Fusarium graminearum (virus-DK21)	dsRNA	*Hypovirus*	Kwon *et al.*, 2007
Gremmeniella abietina	dsRNA	*Partitiviridae*, *Partitivirus*	Tuomivirta and Hantula, 2005
Gremmeniella abietina	dsRNA	*Totiviridae*, *Totivirus*	Tuomivirta and Hantula, 2005
Gremmeniella abietina	dsRNA	*Narnaviridae*, *Mitovirus*	Tuomivirta and Hantula, 2005
Helicobasidium mompa (HmTV1-17 virus)	dsRNA	*Totivirus*	Suzaki *et al.*, 2005
Helicobasidium mompa	dsRNA	*Endornavirus*	Osaki *et al.*, 2006
Helicobasidium mompa (mitovirus 1–18)	dsRNA	*Narnaviridae*, *Mitovirus*	Osaki *et al.*, 2005
Ophiostoma himal-ulmi (partitivirus 1)	dsRNA	*Partitiviridae*, *Partitivirus*	Crawford *et al.*, 2006
Ophistoma minus	dsRNA	*Totiviridae*	Doherty *et al.*, 2007
Ophistoma quercus	dsRNA	*Partitiviridae*	Doherty *et al.*, 2007
Penicillium stolonifera (virus F)	dsRNA	*Partitiviridae*	Kim *et al.*, 2005
Phytophthora sp. (endorna virus 1)	dsRNA	*Endornavirus*	Hacker *et al.*, 2005
Pleurotus ostreatus (oyster mushroom spherical virus)	ssRNA	*Tymovirus*	Yu *et al.*, 2003
Pleurotus ostreatus (virus 1)	dsRNA	*Partitiviridae*, *Partitivirus*	Lim *et al.*, 2005
Rosellinia necatrix (partitivirus 1-W8)	dsRNA	*Partitiviridae*	Sasaki *et al.*, 2005
Sclerophthora macrospora (virus A)	ssRNA	*Novel sequence*	Yokoi *et al.*, 2003
Sclerotinia sclerotiorum (debilitation-associated RNA virus)	ssRNA	*Flexiviridae*, *Allexivirus*	Xie *et al.*, 2006

Note: Totivirus sequences have also been found associated with Amasya cherry disease and Cherry chlorotic spot (Kozlakidis *et al.*, 2006) but the presumed fungal host has not been identified.

DIVERSITY AND INCIDENCE OF MYCOVIRUSES

Taxonomy and genomic organization

Virus nomenclature has had a chequered history. It is now governed by the International Committee for Taxonomy of viruses (ICTV), which recognizes a hierarchical classification including species, genera and families (Fauquet *et al.*, 2005). The 8th ICTV report on virus taxonomy (Fauquet *et al.*, 2005) lists > 90 mycovirus species covering ten viral families, with *c.* 20% of these still unassigned to a genus or in some cases family (Table 1). Although most of those assigned to date are isometric, a variety of other particle morphologies have been observed, including rigid rods, flexuous rods, club-shaped particles, enveloped bacilliform particles and even an example of a herpesvirus-like virus (Kazama and Schornstein, 1972). The paucity of nucleic acid sequence data for many of these makes it difficult to assign them confidently to established virus groups. In addition, there are numerous reports of apparently unencapsidated dsRNAs in fungi. These are usually assumed to be viral in nature, but sequence data are needed before they can be confirmed as viruses and assigned to a virus genus. ICTV convention is that names of species assigned to genera are written in italics but the names of tentative members of genera or unassigned viruses are written in normal text.

At the time of the 7th ICTV report (van Regenmortel *et al.*, 2000) the vast majority of the characterized mycoviruses had double-stranded (ds)RNA genomes with only two single-stranded (ss)RNA mycoviruses having been reported: *Mushroom bacilliform virus* (MBV) in *Agaricus bisporus* (Revill *et al.*, 1999; Tavantzis *et al.*, 1980) and Sclerophthora macrospora virus B (SmVB) from *Sclerophthora macrospora*, the oomycete responsible for downy mildew in gramineous plants (Yokoi *et al.*, 1999). A notable development since that time has been the discovery and characterization of a significant number of ssRNA mycoviruses. The 8th ICTV report (Fauquet *et al.*, 2005) listed 28 ssRNA mycoviruses and

subsequent published reports include the filamentous Sclerotinia sclerotiorum debilitation-associated RNA virus (Xie *et al*., 2006) and Botrytis virus X (Howitt *et al*., 2006), and an isometric virus from *Pleurotus eryngii* (Ro *et al*., 2007). Consequently, ssRNA viruses now account for approximately one-third of the characterized mycoviruses. There is evidence to suggest that hypoviruses may have an ssRNA genome, as summarized by Nuss (2005), who comments 'although hypovirus RNA is found in hyphal extracts as dsRNA, the structural characteristics of the dsRNA are reminiscent of a replicative intermediate or replicative form of a ssRNA virus'. However, to date the ICTV still classifies hypoviruses as dsRNA viruses. A significant taxonomic change in the 8th report is the creation of the family *Chrysoviridae* to accommodate *Penicillium chrysogenum virus* (PcV) and related viruses with four dsRNA segments in the genus *Chrysovirus*, which were previously classified in the family *Partitiviridae*. This followed the molecular characterization of PcV by Jiang and Ghabrial (2004).

Plant pathologists have a long been interested in mycoviruses because of their potential as biological control agents. Also of interest is that several of the more recently described viruses from plant pathogenic fungi show some sequence identity with plant viruses. These include Sclerophthora macrospora Virus B (SmVB) (Yokoi *et al*., 1999) with *Luteoviridae* and *Sobemoviridae*, Sclerophthora macrospora Virus A (SmVA) (Yokoi *et al*., 2003) with *Nodaviridae* and *Tombusviridae*, Oyster mushroom spherical virus (OMSV) (Yu *et al*., 2003) with *Tymoviridae*, Botrytis cinerea virus F (BCVF) (Howitt *et al*., 2001), Botrytis virus X (BVX) (Howitt *et al*., 2006) and Sclerotinia sclerotiorum debilitation-associated RNA virus (SsDRV) (Xie *et al*., 2006) with *Flexiviridae*, and Phytophthora endornavirus 1 (Hacker *et al*., 2005) and a *Helicobasidium mompa* virus (Osaki *et al*., 2006) with *Endornavirus*. Although these mycoviruses are sufficiently distinct from each other and their closest plant virus relatives to be considered as new genera, several of them clearly belong within families that predominently contain plant viruses, for example BCVF, BVX and SsDRV in the *Flexiviridae*. These close phylogenetic relationships raise the possibility that at least some mycoviruses may have originated from plant viruses (see later).

Host range and incidence

The incidence and variability of mycoviruses has most commonly been determined based on the presence of dsRNAs, which have been found in many common filamentous fungi with incidences ranging from a few per cent to 100% (Table 3). All four phyla of the true fungi, Chytridiomycota, Zygomycota, Ascomycota and Basidiomycota, act as hosts to mycoviruses, as do plant pathogenic oomycetes such as *Phytophthora* (Hacker *et al*., 2005; Tooley *et al*., 1989) and *Pythium* (Gillings *et al*., 1993). Mycoviruses are distinct from those viruses that use fungi as vectors because mycoviruses are able to replicate within the fungal host (Rochon *et al*., 2004).

dsRNA profiles can be quite diverse even within isolates of the same fungus. In some cases they show geographical structuring (Liu *et al*., 2007a,b; Park *et al*., 2006a,b; Voth *et al*., 2006) but often they do not (Howitt *et al*., 1995; Rong *et al*., 2001; Tsai *et al*., 2004). Tsai *et al*. (2004) studied the distribution of dsRNA profiles in *Monilinia fructicola* from a single nectarine orchard in Auckland, New Zealand. They found a high diversity in dsRNA profiles between isolates from different blocks of trees, different trees within a block, and even between fruit from the same tree. In most cases it is not known whether multiple and varied dsRNA patterns represent a single virus or a mixture of several viruses, although mixed infections do occur (Chu *et al*., 2004; Howitt *et al*., 2006; Kozlakidis *et al*. 2006). The incidence of one virus may be affected by the presence of other viruses. Romaine and Schlagnhauffer (1995) found that *Mushroom bacilliform virus* (MBV) was present in 60% of *Agaricus bisporus* isolates that were infected with LaFrance isometric virus (LIV), but was present in only 5% in the absence of LIV, suggesting MBV may require 'help' from LIV for transmission.

A curious situation arises with the complex of dsRNA viruses putatively infecting an unknown fungus associated with cherry

Table 3 Examples of dsRNA incidence in fungi.

Fungal species	Percentage isolates with dsRNAs	No. dsRNAs detected	Reference
Aspergillus flavi	13	≤ 9	Elias and Cotty, 1996
Aspergillus spp.	10	≤ 8	Van Diepeningen *et al*., 2006
Botrytis cinerea	72	≤ 8	Howitt *et al*., 1995
Chalara elegans	84	≤ 6	Bottacin *et al*., 1994
Monilinia fructicola	74	≤ 7	Tsai *et al*., 2004
Pyricularia oryzae	70	≤ 12	Hunst *et al*., 1986
Phytophthora infestans	36	≤ 4	Tooley *et al*., 1989
Pythium irregulare	85	≤ 6	Gillings *et al*., 1993
Ustilago maydis (USA)	34	1	Voth *et al*., 2006
Ustilago maydis (Mexico)	100	1	Voth *et al*., 2006

chlorotic rusty spot and Amasya cherry diseases of cherry. These have been identified from sequence data as a mixture of mycoviruses, a species of *Chrysovirus*, a species of *Partitivirus* and a species belonging to the family *Totiviridae* (Coutts *et al.* 2004; Covelli *et al.* 2004, 2008). However, as yet they have not been unequivocally shown to be of fungal origin (Alioto *et al.* 2003; Covelli *et al.* 2008), and indeed could be plant viruses of mycovirus origin. Although partitiviruses are usually regarded as being restricted to fungi, there are other reports suggesting they may be present in plants (Chen *et al.*, 2006; Tzanetakis *et al.*, 2008).

ORIGINS OF MYCOVIRUSES

The vast majority of mycoviruses discovered so far have RNA genomes (Tables 1 and 2). RNA viruses in general are prone to errors within the replication process, with the mutation rate calculated at 10^{-3}–10^{-5} errors per nucleotide per replication cycle (Domingo and Holland, 1997). Consequently, RNA virus populations consist of a complex and dynamic swarm of sequences, sometimes referred to as 'quasi species' (Domingo and Holland, 1997; Van Regenmortel, 2007). Although this may simply be a mechanistic inevitability it may also be evolutionarily advantageous (García-Arenal *et al.*, 2001) as a sequence variant better adapted to a new environment may already exist within the swarm (Schneider and Roossinck, 2001). Despite the great scope for adaptation and evolution arising from the high mutation rate of RNA viruses, for plant viruses, there is generally a trend towards genetic stability especially within homogeneous crop populations (García-Arenal *et al.*, 2001). There is currently insufficient information to address this issue adequately for RNA mycoviruses.

Two main hypotheses have been proposed to explain the origins and evolutionary history of mycoviruses (Ghabrial, 1998). The first, the ancient coevolution hypothesis, proposes that infections are ancient from an unknown source and have coevolved along with their hosts. The second, the plant virus hypothesis, proposes that, at least for plant pathogenic fungi, the viruses have moved relatively recently from the host plant to the fungus.

The ancient coevolution hypothesis

If mycoviruses infected ancestors of their present fungal hosts and have evolved with them to give rise to present-day diversity, it is reasonable to propose that in this coevolutionary relationship there have been reciprocal influences between the host fungus and mycovirus over this time (Voth *et al.*, 2006). This ancestral coevolution hypothesis is based, at least in part, on the proposal that as mycovirus transmission is limited to intracellular mechanisms, horizontal movement of the virus to other fungal hosts is likely to be rare, and thus the association between the host and virus is likely to be ancient (Buck, 1998). Under the coevolution hypothesis mycovirus evolutionary patterns would be expected to follow those of their hosts (Varga *et al.*, 2003). The *Reoviridae*, for example, are a large and biologically diverse family, predominantly animal viruses but including three plant virus genera and the single fungal genus, *Mycoreovirus*. However, the mycoreoviruses show only low to moderate levels of similarity to homologous segments of the closest genus, the mammalian coltiviruses (Hillman *et al.*, 2004; Suzuki *et al.*, 2004; Wei *et al.*, 2003).

The host range of individual mycoviruses would also be limited to single species, or closely related species, where host speciation has occurred following mycovirus infection. Koonin *et al.* (1991) discuss this possibility in relation to the sequence similarity (across five distinct domains) and presumed common ancestry of the chestnut blight hypovirulence-associated dsRNAs and plant potyviruses. They suggest that the most plausible explanation is that a saprophytic or pathogenic fungus acquired an ssRNA virus from a plant followed by loss of the coat protein gene and a shift towards the dominance of the dsRNA replicative form of the virus due to different evolutionary pressures such as horizontal transmission by hyphal anastamosis. However, they also acknowledge that the opposite is possible and that ssRNA plant viruses could have been derived from a dsRNA fungal virus.

Evidence in support of the coevolution hypothesis includes the large number of chromosomal genes needed for replication of killer viruses in *Saccharomyces cerevisiae* (Wickner 1996), the use of mitochondrial genetic translation codes by mitochondrial mycovirues (Park *et al.*, 2006a) and the use of host-encoded enzymes for processing the capsid protein of *Helminthosporium victoriae virus 190S* (Huang *et al.*, 1997). Results from a study of *Ustilago maydis virus-HI* (Umv-H1), from *Ustilago maydis*, the corn smut pathogen of maize, also support this hypothesis. Voth *et al.* (2006) analysed the phylogeography of Umv-H1 populations in the USA and Mexico to determine whether the population dynamics of *U. maydis* and maize influenced the population structure of the virus. They concluded that the ancestral population of Umv-H1 originated in Mexico and that the extant populations of Umv-H1 in the USA today are the result of ancestral founding events, largely unaffected by current trade in maize. Similarly, in a recent survey of BCVF in *Botrytis cinerea* (Arthur, 2007; Arthur *et al.*, 2007), three groups of closely related sequence variants were detected in *Botrytis cinerea* isolates from a range of geographical locations. Two of the groups included isolates from both New Zealand and Europe, probably reflecting anthropogenic co-translocation of the fungus and virus from its northern hemisphere origins to the southern hemisphere (Beever and Weeds, 2004).

The ancient infection hypothesis, reflecting a long period of coevolution, can also account for the apparently symptomless phenotype of many mycovirus infections. However, not all mycovirus associations are consistent with this hypothesis. Some, such as CHV1, have a significant deleterious effect on their host fungus

and their evolutionary relationships do not follow the pattern of their fungal hosts (Carbone *et al*., 2004; Liu *et al*., 2003).

The plant virus hypothesis

Under this hypothesis, the evolution of mycoviruses and their fungal hosts may be incongruent. Evidence in support of this hypothesis has come from sequence comparisons between mycoviruses and plant viruses. For example, the hypoviruses CHV1, CHV2, CHV3 and CHV4, associated with hypovirulence in *Chryphonectria parasitica*, show phylogenetic relatedness to several species of the ssRNA genus *Potyvirus* (Fauquet *et al*., 2005; Linder-Basso *et al*., 2005), while an RNA-dependent RNA polymerase (RdRp) sequence from a *Fusarium graminearum* dsRNA was closely related to those of CHV1, CHV2 and CHV3, and the potyvirus *Barley yellow mosaic virus* (Chu *et al*., 2002; Linder-Basso *et al*., 2005). Xie *et al*. (2006) also point out that several ssRNA mycoviruses associated with debilitation/hypovirulence (including SsDVR) are phylogenetically much closer to positive-strand RNA plant viruses than to the typically avirulent dsRNA fungal viruses. Although a small number of fungal species are known to be vectors of plant viruses, the virions are carried on the outside of the fungus and it is possible that a rare event may have led to their internalization (Varga *et al*., 2003). However, this is not likely to be a common mechanism and it is more probable, especially in the case of fungal plant pathogens, that the fungus becomes infected directly during infection of its plant host.

Three recently described flexuous ssRNA mycoviruses have been classified with plant viruses belonging to the family *Flexiviridae* (Martelli *et al*., 2007). For BCVF (Fauquet *et al*., 2005) (syn. Botrytis virus F—Howitt *et al*., 2001) the putative RdRp most closely matches plant 'tymo' and 'potex' viruses and the putative coat protein gene most closely matches 'potex-like' ssRNA plant viruses. Botrytis virus X (Howitt *et al*., 2006) closely aligns with members of the *Allexivirus* genus, when both the RdRp and the coat protein sequences are analysed, although M. J. Adams (personal communication, 2006) concluded that BVX is likely to be a part of a new genus within the family *Flexiviridae*. SsDRV is a positive-sense ssRNA virus which contains conserved methyl transferase, viral RNA helicase and RdRp domains characteristic of the replicase genes of the 'alphavirus-like' supergroup of positive-strand plant RNA viruses and with significant similarity to potex-like positive-strand plant viruses (Xie *et al*., 2006). *Sclerotinia* and *Botrytis* are closely related fungi and phylogenetic analysis showed that SsDRV is closely related to BCVF and that these two mycoviruses cluster with several allexiviruses including *Garlic virus E* (GVE), *Garlic virus A* (GVA), *Garlic virus X* (GVX) and *Shallot virus X* (ShVX), in the family *Flexiviridae* (Adams *et al*., 2004). However, while most flexivirus genomes contain three to six open reading frames (ORFs), the SsDRV genome contains only a single ORF (193 kDa) which is comparable in size to ORF1 of BCVF and other flexiviruses (150–250 kDa). In common with BCVF (and also BVX) there is no coding sequence for a movement protein, which would be irrelevant within the fungal host, but also there is no coat protein gene and no read-through opal codon in the replicase-coding region (Xie *et al*., 2006). Consequently, these two mycoviruses are sufficiently different from each other and previously recognized flexiviruses to warrant placing them in separate and possibly new genera, in the family *Flexiviridae* (Martelli *et al*., 2007; Xie *et al*., 2006).

Howitt *et al.* (2006) point out that the evolutionary relationship between the fungal pathogen *Botrytis* and the plant genus *Allium* is probably a long one. *Allium* species are susceptible to several allexiviruses, including GVE, GVA, GVX and ShVX, and *B. cinerea* may have coincidentally taken up plant viruses during the infection process. In particular, BVX shows 73% amino acid identity to the RdRp of *Garlic virus A*, which is much higher than that reported between most other mycoviruses and ssRNA plant viruses. This may indicate either a recent recombination event between the two viruses or a relatively recent divergence from a common ancestor.

Overall it is likely that both the 'ancient coevolution' and the 'plant virus' hypotheses are needed to explain the full range of mycovirus diversity that is being revealed. Furthermore, in the case of fungi that infect plants, it is also possible that mycoviruses have moved from their fungal host into the plant host.

VIRUS TRANSMISSION

Mycoviruses have no known extracellular mode of transmission and under natural conditions are reliant on their fungal hosts for intracellular transmission (Buck, 1998). This can occur in two ways, horizontally via protoplasmic fusion and vertically by sporulation. There are no documented examples of intact filamentous fungi being infected with purified mycoviruses, although under experimental conditions protoplasts of several fungi have been infected with purified viruses, or transfer via protoplast fusion. Also, *Saccharomyces cerevisiae* has been successfully transfected with virus-like particles (VLPs) (Schmitt and Breinig, 2002), raising the possibility that this could be a natural route of VLP infection at least in yeasts (Varga *et al*., 2003).

Vertical transmission from the fungal mycelium to spores is a primary means of mycovirus spread, although observed rates vary greatly for different fungus/virus combinations and for different spore types (sexual vs. asexual) of the same fungus. Asexual spores are produced from modified hyphae and mycoviruses are readily transmitted to these spores through the cytoplasm as the spores develop (Buck, 1998). Some authors have concluded that virus transmission through sexual spores is less common and that infection rates are usually lower for those fungi whose sexual stage occurs frequently in the life cycle (Varga

et al., 2003). Although the *c.* 10% incidence of dsRNA viruses in 668 *Aspergillus* isolates (Van Diepeningen *et al.*, 2006) and the 72% incidence for *Botrytis cinerea* (Howitt *et al.*, 1995) support this hypothesis, other results do not. In *Monilinia fructicola*, a fungus in which the sexual stage is commonly produced, Tsai *et al.* (2004) detected dsRNAs in 74% of isolates. Examples of significant rates of transmission through sexual spores include: *Fusarium graminearum* with transmission of dsRNAs through both conidia and ascospores with incidence of up to 100%, in both spore types (Chu *et al.*, 2004); *Heterobasidion annosum* with 10–84% of the germinated basidiospores containing dsRNA (Ihrmark *et al.*, 2004); *B. cinerea*, where Botrytis virus X was detected in 35 and 53% of ascospore progeny where the mycelial (female) parent was the virus recipient and donor, respectively (Tan *et al.*, 2007).

In summary, it appears there are no consistent patterns or differences in rates of virus transmission between sexual and asexual spores, and that transmission of mycoviruses through sexual spores is not a rare event.

Horizontal transmission of mycoviruses between different fungal strains via hyphal anastomosis is a well-established phenomenon and has been used by various workers to transmit mycoviruses experimentally (Suzaki *et al.*, 2005; Xie *et al.*, 2006). Anastomosis occurs when hyphae from different fungal individuals fuse together and genetic and cytoplasmic exchange occurs. Mycoviruses exist within the cytoplasm, and are therefore included in this exchange. Successful anastomosis relies on the two fungi being vegetatively compatible, i.e. in the same vegetative compatibility group (VCG). In ascomycete fungi, this process is controlled by a series of gene loci, variously known as *vic* (vegetative incompatibility) or *het* (heterokaryon incompatibility) loci (Glass and Dementhon, 2006; Saupe, 2000). Conversely, the existence of VCGs in many fungi provides a natural barrier to horizontal transmission of mycoviruses. Although incompatibility has long been known to restrict virus transmission, notably in *Cryphonectria parasitica* (Milgroom and Cortesi, 2004), the strength of the incompatibility reaction affects the rate of transmission in a manner somewhat analogous to the hypersensitive reaction between plants and pathogens. In *C. parasitica*, for example, the effect of individual *vic* genes on transmission is generally additive (Cortesi *et al.*, 2001). Biella *et al.* (2002) speculated that this vegetative compatibility reaction could be influenced by both the virus and the fungus, which may explain the noted phenomenon of asymmetric transmission seen between strains differing in VCGs (Carbone *et al.*, 2004) where, in a pair, transmission can occur in one direction, but does not occur in the reciprocal pairing with the same efficiency.

Many plant viruses infect multiple host species, often from different plant genera or families. In principle it would appear much more difficult for mycoviruses to jump hosts due to the apparent absence of mechanical and vector transmission, but there is evidence for the natural horizontal transmission of mycoviruses between different species, adding an extra dimension to the debate over the origins of some mycoviruses. Liu *et al.* (2003) detected the same variants of CHV1 in different fungal species of *Cryphonectria*, indicative of recent horizontal transmission, and similar viruses have also been detected in different taxa of both ascomycetes and basiomycetes (Ikeda *et al.*, 2005). Van Diepeningen *et al.* (2000) demonstrated the potential for interspecies transmission of mycoviruses between *Fusarium poae* and black *Aspergillus* spp. via protoplast fusion, with infection remaining stable through several rounds of subculturing. However, there is no known natural transmission mechanism for this to occur in field populations.

EFFECTS OF MYCOVIRUSES ON HOST PHENOTYPE

Mycoviruses can confer a range of phenotypes on their fungal hosts, both advantageous and deleterious, but many appear symptomless (cryptic). However, correlation of fungal phenotype with a particular mycovirus is often hampered by the lack of an infectivity assay to connect the two conclusively (McCabe *et al.*, 1999). This problem is compounded by the frequent occurrence of mixed infections of multiple viruses, for example viruses in *Agaricus bisporus* (Romaine and Schlagnhauffer, 1995) and the mixed infection of BVX and BCVF in *Botrytis cinerea* (Howitt *et al.*, 2006).

Symptomless or cryptic infections

Apparently symptomless infections are commonly observed between mycoviruses and their fungal hosts (Buck, 1998), and the widespread nature of mycoviruses and their lack of obvious impact has led many investigators to propose that mycoviruses have no effect on fungal biology. However, the absence of symptoms under one set of conditions does not exclude the possibility that the virus might induce symptoms under some unexplored environmental conditions and, although many mycoviruses produce no obvious phenotypic changes, it is reasonable to assume that many virus infections will have some, albeit small, effect on growth. For example, a comparison of the mycelial growth rate, spore production, and competitive ability of isogenic infected and virus-free lines of *Aspergillus* spp. (Van Diepeningen *et al.*, 2006) revealed small but statistically significant detrimental effects of infection for all strains evaluated. Consequently, the term cryptic is favoured, implying that symptoms can be expressed under some conditions. Although McCabe *et al.* (1999) comment that while it seems odd that so many mycovirus evolutionary lineages have led to the same (apparently) neutral state, virus virulence is ultimately limited by the need for the host to survive and propagate the virus, which produces selective pressure towards viruses that are symptomless or even beneficial to the host.

Hypovirulence and reduced fungal pathogenicity

The economically most important disease due to a mycovirus is La France disease of the non-plant pathogen *Agaricus bisporus* (Hollings, 1962; Ro *et al.*, 2006) caused by La France isometric virus (LIV) (Romaine and Schlagnhauffer, 1995). Other mushroom diseases include Oyster mushroom spherical virus (OMSV) (Yu *et al.*, 2003) and Oyster mushroom isometric virus (Ro *et al.*, 2006). Considering plant pathogenic fungi, there is increasing evidence for the negative effects of mycoviruses on pathogen growth and pathogenicity on plant hosts, although in many cases the evidence is circumstantial and proving causal relationships are often hampered by the lack of genetic manipulation tools (Nuss, 2005). From the purist's point of view, unless purified virus or a nucleic acid copy is able to be introduced into the fungal host to confirm a symptomatic reaction, mycoviruses cannot be said to be disease causing (McCabe *et al.*, 1999), but such formal proof is often difficult to achieve.

The best studied examples of mycoviruses that confer a hypovirulent phenotype on plant pathogenic fungi are the hypoviruses of the Chestnut Blight fungus *Chryphonectria parasitica* (Choi and Nuss, 1992; Nuss 2005). Although the genus *Hypovirus* includes only mycoviruses conferring hypovirulence (Fauquet *et al.*, 2005), other groups of mycoviruses such as the mitoviruses of *Ophiostoma novo-ulmi* (Doherty *et al.*, 2006) can also confer hypovirulent traits. Other examples of negative affects of virus infection in plant pathogenic fungi include decreased growth rate and lack of sporulation of *Diaporthe perjuncta* transfected with Diaporthe RNA virus (Moleleki *et al.*, 2003), attenuation of virulence in *Helicobasidium mompa* infected by the totivirus HmTV1–17 (Suzaki *et al.*, 2005) and reduced germination of basidiospores in dsRNA infected *Heterobasidion annosum* (Ihrmark *et al.*, 2004). Recently, a mitovirus conferring a hypovirulent phenotype has also been identified in *Botrytis cinerea* (Wu *et al.*, 2007) and a hypovirulence-conferring 33-nm isometric dsRNA mycovirus has also been found in this host, causing reduction in sporulation, laccase activity and invasiveness of the fungus (Castro *et al.*, 2003). In view of the vast differences in mycovirus genomes and expression strategies it is not unexpected that hypovirulence is associated with different strategies and different mycovirus groups (Xie *et al.*, 2006).

There is also, perhaps not surprisingly, great variability in reactions between a single host and different viruses or dsRNAs. In a study of *Fusarium graminearum* on wheat, Chu *et al.* (2002) found reduced growth, increased pigmentation, reduced virulence and a 60-fold decreased production of trichothecene mycotoxins associated with a 7.5-kb dsRNA. However, on examining ten additional *F. graminearum* isolates containing 2–4 different dsRNAs of 1.7–10 kb in length, they found no affect on colony morphology. Similarly, Van Diepeningen *et al.* (2006), on examining 668 *Aspergillus* spp. isolates from around the world, found one virus-infected isolate with non-sporulating sectors and reduced growth rate, but also several isolates causing symptomless infections.

Beneficial interactions

In contrast to hypovirulent interactions, there is good evidence that some mycoviruses are benefical to their hosts. Killer phenotyes in yeasts and *Ustilago* represent an extreme form of beneficial interactions. Also, Ahn and Lee (2001) provide strong evidence that a 6.0-kbp dsRNA species in *Nectria radicicola* upregulates virulence in this important root pathogen through perturbing signal transduction pathways. An interesting three-way symbiosis between a mycovirus, the endophytic fungus *Curvularia protuberata*, and panic grass *Dichanthelium lanuginosum* and other plants has been recently recognized (Márquez *et al.*, 2007). Previously, it had been demonstrated that endophytic infection confers thermal tolerance to the fungus and the host plant, allowing both to survive high temperatures. Intriguingly, this tolerance is dependent on the fungus being infected by the dsRNA mycovirus Curvularia thermal tolerance virus (CThTV). However, more subtle beneficial effects occur with other species, and such effects could underlie the widespread occurrence and persistence of mycoviruses in many hosts, given that a parasite limited to vertical transmission is unlikely to persist if it lowers fitness (Fine, 1975). In a detailed study of dsRNA effects on fitness of asexual aspergilli, Van Diepeningen *et al.* (2006) suggest no beneficial effects, at least when tested in the laboratory. In contrast Tan *et al.* (2007) observed small but statistically significant differences in *in vitro* growth rates of BVX-infected and uninfected cultures, with the infected cultures growing more rapidly. If the same difference occurs in the field it may be that virus infection provides an advantage for substrate colonization.

MYCOVIRUSES AS BIOLOGICAL CONTROL AGENTS

Fungal pathogens are a major source of plant disease. Although fungicides have proved immensely successful in controlling many diseases, their use is increasingly threatened by the development of fungicide-resistant strains and public concern about unwanted environmental and human health side-effects. Various biological approaches to plant disease management have been proposed but few are, as yet, widely successful in practice. The potential of mycoviruses as biological control agents of plant pathogenic fungi was first demonstrated for *Chryphonectria parasitica* (Nuss, 1992). However, even when mycoviruses clearly have the ability to reduce the virulence of fungal plant pathogens, given that hyphal interaction is the only known mechanism for transmission between fungal colonies, the vegetative incompatibility shown by many fungal species is a major barrier to their adoption as biological control agents. For example, while CHV1 cDNA was

successfully used as a biological control of *C. parasitica* in Europe it was not as successful in North America because the fungus showed greater genetic diversity with multiple VCGs limiting the spread of the virus (Nuss, 1992). Xie *et al.* (2006) also concluded that it would be very difficult to control *Sclerotinia sclerotiorum* with SsDRV because of vegetative incompatibility, but as a restricted range of VCGs (clones) dominate in some agricultural ecosystems (Hambleton *et al.*, 2002) it may prove possible to produce virus-infected strains to match specifc VCGs. In the case of the closely related *Botrytis cinerea*, however, field strains are much less clonal (Beever and Weeds, 2004) and such matching is likely to be challenging.

The killer phenotype associated with certain dsRNA *Totivirus* species in yeasts such as *Saccharomyces*, *Hanseniaspora* and *Zygosaccharomyces*, and also in *Ustilago maydis*, has been reviewed by Wickner (1996), Marquina *et al.* (2002), Schmitt and Breinig (2002, 2006) and Bruenn (2005). The killer strains excrete a proteinaceous toxin, to which they are immune, but which is lethal to sensitive (non-killer) cells. Killer phenotypes characterized to date are from yeasts or genera such as *Ustilago* with a yeast phase. This may reflect the largely liquid habitat of yeasts in contrast to filamentous fungi. Although the killer phenotype may be useful to beneficial yeasts, by eliminating undesirable competitors, to be useful in controlling plant pathogens it would have to be associated with non-aggressive strains to have any potential as a biological control.

As many mycoviruses appear to have only minimal effect on their host fungi, a complementary approach to biological control might be the use of mycoviruses as gene vectors. Using this approach the mycovirus itself does not need to have a significant deleterious effect on the host but it would require a genomic structure amenable to the incorporation of non-viral genes. The ssRNA mycoviruses belonging to the *Flexiviridae*, BVX, BCVF and SsDRV, are prime candidates for this approach, as the flexivirus *Potato virus X* has been successfully used as a vector for the expression of genes from a range of different sources in plants (Hendy *et al.*, 1999; Kopertekh *et al.*, 2004; Smolenska *et al.*, 1998; Wagner *et al.*, 2004). Although vegetative compatibility would still be an issue it may prove feasible to modify these viruses in such a way as to switch off the incompatible response in the host fungus.

MOLECULAR TECHNIQUES AND THE STUDY OF MYCOVIRUSES

dsRNA profiles have a long been used as a generic method of detecting the presence of virus-like agents in fungi, but in many cases specific identification has been hampered by the inability to detect virus particles and the absence of sequence data. Methods such as virus purification and antiserum production that for many years played a major role in our understanding and detection of plant viruses have been successfully used with some mycoviruses including Oyster mushroom isometric virus (Ro *et al.*, 2006) and *Ustilago maydis virus H1* (Voth *et al.*, 2006), but in general have proved far less successful with mycoviruses. In some instances this is because in the absence of an extracellular route of infection, some mycoviruses can dispense with a protective protein coat resulting in the inability to purify or detect virus particles. Examples of unencapsidated mycoviruses include viruses in the genera *Hypovirus*, *Narnavirus* and *Mitovirus* (Fauquet *et al.*, 2005), Diaporthe RNA virus (Preisig *et al.*, 2000) and SsDVR in *Sclerotinia sclerotiorum* (Xie *et al.*, 2006).

However, the advent of molecular techniques, such as PCR, including protocols for dsRNA templates (Coutts and Livieratos, 2003), and automated sequencing, has substantially increased our knowledge of mycoviruses. One obvious outcome of the ability to detect, clone and sequence mycovirus genomes has been the discovery of a much greater diversity of viruses in fungi, including those with ssRNA genomes. In some instances molecular data will provide an avenue to revisit and more effectively use older technologies such as the production of virus antibodies to synthetic peptides based on genome sequence of virus proteins, as has been achieved for the plant virus *Potato virus Y* (Ounouna *et al.*, 2002). Although dsRNA profiles have been used for several large-scale surveys of mycoviruses (Table 3), the specific identity of the viruses detected is often unknown or uncertain. PCR-based assays such as those used for BVX and BCVF by Arthur (2007) are not only more amenable for large-scale surveys but can also provide greater virus specificity through the use of specific primers and the potential to sequence PCR products. Sensitive and specific PCR-based techniques also allow detailed investigation of virus transmission. Suzaki *et al.* (2005) used PCR for detecting transmission of the totivirus HmTV1–17 between strains of *Helicobasidium mompa* belonging to different mycelial compatibility groups (MCGs) and for DNA fingerprinting to identify the purity of the donor and recipient strains.

Virus-encoded RNAs and proteins can play important roles in determining phenotypic traits such as virulence, sporulation and pigmentation (Ahn and Lee 2001; Park *et al.*, 2004; Segers *et al.*, 2006; Xie *et al.*, 2006) and mycotoxin production (Golubev *et al.*, 2003; Toth *et al.*, 2005), and a range of techniques have been used to investigate their interaction with the fungal host at the cellular and molecular level. For example, Allen *et al.* (2003) developed a *Chryponectria parasitica* cDNA microarray to monitor transcriptional responses to hypovirus infection and were able to detect differentially expressed genes for a range of biological functions, including stress responses, carbon metabolism and transcriptional regulation, changes consistent with a persistent reprogramming of a significant portion of the *C. parasitica* transcriptome. More recently, Segers *et al.* (2007) have demonstrated the occurrence of RNA silencing as an antiviral defence mechanism by disrupting dicer-like genes in *C. parasitica*. The development of

transformation techniques such as DNA-mediated transformation using cDNA infectious clones (Choi and Nuss, 1992), and transfection of fungal spheroplasts with dsRNA (Stanway and Buck, 1984), *in vitro*-transcribed RNA transcripts (Moleleki *et al.*, 2003) or purified virions (Hillman *et al.*, 2004; Sasaki *et al.*, 2007) has allowed comparative studies of genetically identical virus-infected and uninfected cultures (Xie *et al.*, 2006), and experimentation to extend the host range of mycoviruses (Chen *et al.*, 1994), without the obstacles of vegetative incompatability.

THE FUTURE OF MYCOVIROLOGY

The study of mycoviruses has proved technically challenging because of their frequently non-symptomatic nature and lack of infectivity. Nevertheless, it is apparent that the pace of mycovirus research is poised for significant expansion in the next few years, as workers adopt many of the elegant new tools that are becoming increasingly available from molecular studies.

As increasing numbers of mycovirus full genome sequences become available our understanding of phylogenetic relationships and ultimately mycovirus evolution will improve. In particular, new discoveries elucidating the relationships between mycoviruses and plant viruses will inform our understanding of the direction and frequency of cross-kingdom virus movement. Additionally, full genome sequence data are becoming available for some of the target hosts, including *Agaricus bisporus*, *Botrytis cinerea* and *Sclerotinia sclerotiorum*, which, together with *in vitro* methods of transfecting fungal hosts, will expand opportunities to unravel virus–host interactions.

Perhaps the biggest opportunity for mycovirus research is to develop them further as biocontrol agents, to assist in the control of plant pathogens. Such exploitation is highly dependent on knowledge of the behaviour of both host and virus in the field. The population biology of fungi is becoming better understood and although the population studies of mycoviruses are in their infancy, they can be expected to expand rapidly with the increasing availability of virus-specific molecular detection methods. Such developments will underpin biocontrol opportunities and provide new insights into mycovirus biology.

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