New Insights into Mycoviruses and Exploration for the Biological Control of Crop Fungal Diseases

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Annu. Rev. Phytopathol. 2014. 52:45–68

First published online as a Review in Advance on July 7, 2014

The *Annual Review of Phytopathology* is online at phyto.annualreviews.org

This article's doi: 10.1146/annurev-phyto-102313-050222

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Keywords

mycovirus, hypovirulence, biological control, crop fungal diseases, fungal plant pathogen

Abstract

Mycoviruses are viruses that infect fungi. A growing number of novel mycoviruses have expanded our knowledge of virology, particularly in taxonomy, ecology, and evolution. Recent progress in the study of mycoviruses has comprehensively improved our understanding of the properties of mycoviruses and has strengthened our confidence to explore hypovirulence-associated mycoviruses that control crop diseases. In this review, the advantages of using hypovirulence-associated mycoviruses to control crop diseases are discussed, and, as an example, the potential for Sclerotinia sclerotiorum hypovirulence-associated DNA virus 1 (SsHADV-1) to control the stem rot of rapeseed (*Brassica napus*) is also introduced. Fungal vegetative incompatibility is likely to be the key factor that limits the wide utilization of mycoviruses to control crop diseases; however, there are suggested strategies for resolving this problem.

INTRODUCTION

Mycoviruses, or fungal viruses, are viruses that replicate in fungal cells. Mycoviruses were first discovered from diseased mushroom (*Agaricus bisporus*) and from *Penicillium* spp. that stimulate the production of interferon in mammals. The findings of studies on the take-all decline of cereals, the transmissible disease of *Helminthosporium* (*Cochliobolus*) *victoriae*, and the hypovirulence of the chestnut blight fungal pathogen [*Cryphonectria* (*Endothia*) *parasitica*] led to the discovery of mycoviruses in plant filamentous fungal pathogens in the 1970s. The successful biological control of chestnut blight with hypovirus-mediated hypovirulence has inspired others to seek hypovirulence-associated mycoviruses in other plant fungal pathogens.

French mycologist Jean Grente discovered a hypovirulent strain of *C. parasitica* in healing cankers of chestnut blight. dsRNA (double-stranded RNA) elements were subsequently isolated from all tested hypovirulent strains. However, at that time, it was difficult to prove that dsRNA elements were involved in hypovirulence because many dsRNA-carrying fungal strains were not significantly different from dsRNA-free strains. After the genome sequence of Cryphonectria hypovirus 1 (CHV1) was analyzed and the infectious cDNA clone was available, the cDNA clone was used to successfully convert the hypovirulence of virus-free strains of *C. parasitica* (13, 14, 22, 23, 105). The relationship between mycoviruses and hypovirulence was solidly confirmed, i.e., CHV1 is the cause of *C. parasitica* hypovirulence. This pioneering work was important not only to understand the *C. parasitica*–hypovirus system but also to lead the way for us to study mycoviruses in other fungal plant pathogens.

To date, mycoviruses have been isolated from various fungi, including mushrooms, medical fungi, and plant-pathogenic fungi, and are believed to commonly exist in fungi. More than 250 mycoviruses have been sequenced and registered in the NCBI (National Center for Biotechnology Information) database, and they have been identified from fungi in all major groups of Kingdom Fungi. Mycoviruses often have dsRNA or ssRNA (single-stranded RNA) genomes; however, a DNA virus was recently identified from the fungal plant pathogen *Sclerotinia sclerotiorum*, which suggests that fungi may host both RNA viruses and DNA viruses. Mycoviruses are typically grouped into several families, including *Totiviridae*, *Partitiviridae*, *Chrysoviridae*, *Hypoviridae*, and *Nanoviridae*. Although the families of many mycoviruses cannot be determined, they are phylogenetically related to typical plant viruses and even to animal viruses.

It has been a half century since the first mycovirus was discovered, and mycoviruses remain attractive because of their importance in the biological control of fungal plant diseases and in the understanding of the global ecology and evolution of viruses. There are many previously published reviews regarding the important effect of mycoviruses on plant pathology, virology, mycology, and even ecology (25, 26, 39, 41, 87–93, 98). We have attempted to avoid repeating these previous findings regarding plant fungal mycoviruses and have focused instead on recent findings, which may be important to explore because of the biological control of fungal crop diseases and to extend our knowledge regarding virology.

MYCOVIRUSES IN FUNGAL PLANT PATHOGENS

Mycoviruses in *Cryphonectria parasitica*

Chestnut blight is caused by the ascomycete fungus *C. parasitica* and has been an ecological disaster for the American chestnut (*Castanea dentata*) in both Europe and North America (84). CHV1, which is the hypovirulence-associated mycovirus of *C. parasitica*, was first sequence analyzed in 1991 (105). On the basis of the biological features and the genomic characterization of CHV1, the International Committee on Taxonomy of Viruses (ICTV) established a new family, *Hypoviridae*

(43). *Hypoviridae* contains a single genus of *Hypovirus*, and four virus species that were isolated from *C. parasitica* have been identified as belonging to the genus *Hypovirus*: *Cryphonectria hypovirus 1* (CHV1) (45, 105), *Cryphonectria hypovirus 2* (CHV2) (43, 48), *Cryphonectria hypovirus 3* (CHV3) (34, 107), and *Cryphonectria hypovirus 4* (CHV4) (71). However, the impact of these four virus species on the virulence of *C. parasitica* was different. In addition, three viruses other than hypoviruses were isolated from *C. parasitica*. Cryphonectria parasitica mitovirus 1 (CpMV1), which belongs to the genus *Mitovirus* in the family *Nanoviridae*, was isolated from hypovirulent strain NB631 (95). Cryphonectria parasitica mycoreovirus 1 (CpMyRV1) and CpMyRV2 were isolated from two natural hypovirulent strains, 9B21 and C18, respectively (46).

Mycoviruses in *Sclerotinia sclerotiorum*

S. sclerotiorum is an ascomycetous plant-pathogenic fungus with worldwide distribution that attacks more than 400 species of plant hosts (7). The first hypovirulence-associated dsRNA element was extracted from hypovirulent strain 91; however, this dsRNA element has not been characterized (6). *S. sclerotiorum* hosts various mycoviruses, including dsRNA viruses, ssRNA viruses, and a single-stranded circular DNA virus (53). Sclerotinia sclerotiorum hypovirulence-associated DNA virus 1 (SsHADV-1) is the first DNA mycovirus that was found to infect fungi and confers hypovirulence (130). This mycovirus is related to plant geminiviruses. Sclerotinia sclerotiorum debilitation-associated RNA virus (SsDRV) and Sclerotinia sclerotiorum RNA virus L (SsRV-L) are coinfected in the strain Ep-1PN (72, 122). Both of these mycoviruses have a simple RNA genome that codes for one gene, namely RNA replicase. SsDRV is closely related to viruses in the family *Alphaflexiviridae* and represents a member of the genus *Sclerodarnavirus*. SsRV-L is closely related to the human pathogen hepatitis E virus and to rubi-like viruses. Four mitoviruses have been isolated: two from a US strain and three from a New Zealand strain (55, 121). A partitivirus (SsPV-S) and an unclassified nonsegmented dsRNA virus [Sclerotinia sclerotiorum nonsegmented virus L (SsNsV-L)] were found to coinfect a virulent strain, and the coat protein of SsPV-S shares high similarity to IAA (indole-3-acetic acid)-leucine-resistant protein 2 (ILR2) of *Arabidopsis thaliana* (73). An RNA virus (SsHV1), which is closely related to CHV3 and CHV4 in the family *Hypoviridae*, was identified (123). SsHV1 is the first discovered naturally occurring hypovirus that infects a fungus other than *C. parasitica*. Recently, we isolated and characterized the second hypovirus (SsHV2), which shares the highest sequence identity with CHV1 and CHV2 (Z. Hu, J. Xie, J. Cheng, D. Jiang, Y. Fu, unpublished data). Currently, hypoviruses have also been detected in *Valsa ceratosperma* (VcHV1) and *Fusarium graminearum* (FgHV1) (116, 124). These findings suggest that hypoviruses are more widely distributed among filamentous fungi than previously thought. Many mycoviruses in *S. sclerotiorum* are waiting to be characterized. Zhang et al. (132) characterized the hypovirulent strain XG36-1, which has a transmissible property; however, neither genomic RNA nor DNA was extracted successfully.

Mycoviruses in *Rosellinia necatrix*

White root rot, which is caused by the ascomycete *Rosellinia necatrix* (anamorph: *Dematophora necatrix*), is one of the most destructive diseases of many woody plants. Similar to *S. sclerotiorum*, *Rosellinia necatrix* also hosts various mycoviruses. Mycoviruses in *Reoviridae*, *Partitiviridae*, *Chrysoviridae*, and *Totiviridae* were identified from *R. necatrix* (17, 58). Additionally, three novel mycoviruses that belong to new families were characterized. One is a hypovirulence-associated bipartite dsRNA virus, Rosellinia necatrix megabirnavirus 1 (RnMBV1), which was proposed as a member of the new family *Megabirnaviridae* (18). There are also two four-segment dsRNA viruses, Rosellinia necatrix quadrivirus 1 (RnQV-1) and RnQV-2, which are significantly different

from viruses in *Chrysoviridae* and have been proposed as members of a new family, *Quadriviridae* (69, 70). Interestingly, viruses with ssRNA or DNA genomes have not been isolated from this widespread fungus.

Mycoviruses in *Botrytis* Species

Gray mold disease, which is caused by *Botrytis* spp., is one of the most widespread and destructive fungal diseases of crops and postharvest fruits. Similar to other plant-pathogenic fungi, mycoviruses are prevalent in the *Botrytis* population (12, 49, 97, 115). However, only four viruses, Botrytis virus F (BVF), Botrytis virus X (BVX), Botrytis cinerea mitovirus 1 (BcMV1), and Botrytis porri RNA virus 1 (BpRV1), have been fully sequenced. BVF belongs to *Mycoflexivirus*s in the family *Gammaflexiviridae*, whereas BVX belongs to *Botrexvirus* in the family *Alphaflexiviridae* (56). A mitovirus (BcMV1) was originally isolated from a hypovirulent strain (CanBc-1) in China (119, 120). BcMV1 was supposed to be a strain of *Ophiostoma novo-ulmi mitovirus 3b* (OnuMV3b), which infects *Ophiostoma novo-ulmi*. Recently, BcMV1 was found in 55% of the Spanish *Botrytis cinerea* isolates that contained mycoviruses (97). In *Botrytis porri*, which is a sister species of *B. cinerea*, a novel bipartite dsRNA virus (BpRV1) was isolated from a hypovirulent strain (GarlicBc-72) (118). BpRV1 is significantly different from partitivirus and RnMBV1. Thus, BpRV1 was considered to be a novel type of dsRNA virus.

Mycoviruses in *Fusarium* Species

Fusarium is an important phytopathogenic genus that is widely distributed. Some species are not only responsible for the reduction in both crop yield and quality in cereals, but also produce mycotoxins in cereal crops that can affect human and animal health. To date, mycoviruses have been detected in *F. graminearum*, *Fusarium poae*, *Fusarium solani*, *Fusarium oxysporum*, *Fusarium boothii*, and *Fusarium virguliforme* (19, 79). On the basis of full sequences or partial sequences of *Fusarium* viruses, most of these mycoviruses belong to *Partitiviridae*, *Totiviridae*, or *Chrysoviridae*. Although eleven mycoviruses were identified in *Fusarium* species, only Fusarium graminearum virus 1-DK21 (FgV1) could convert hypovirulence to the host (19, 63, 116). FgV1 is closely related to *Cryphonectria hypovirus* and to *Barley yellow mosaic virus* (63). Recently, a mycovirus was isolated from the strain HN10 of *F. graminearum* (116). This mycovirus, named Fusarium graminearum hypovirus 1 (FgHV1), was closely related to CHV1 and CHV2; however, this mycovirus has a minor effect on mycelial growth rate and conidial production and has no significant effect on virulence and mycotoxin (DON) production.

Viruses in Other Plant-Pathogenic Fungi

The basidiomycete fungus *Rhizoctonia solani* is an important soilborne necrotrophic fungal pathogen. Although various-sized dsRNA factors were detected in natural populations from AG-1 to AG-13, the sequences of only four mycoviruses were characterized. Two dsRNA (M1 and M2; 6.4 kb and 3.6 kb in size, respectively) were isolated from the strain Rhs 1A1 of *R. solani* AG-3. M1 is phylogenetically related to plant Bromoviruses and associated with enhanced vigor and virulence, whereas M2 is related to mitoviruses and associated with hypovirulence (52, 64). The Rhs 717 virus, which was isolated from the strain Rhs 717 of *R. solani* AG-2, was a typical partitivirus (109). RsRV1 was isolated from the strain B275 of *R. solani* AG-1 IA, which has similar genomic organization to the members of *Partitiviridae* but is significantly different from typical members of *Partitiviridae* (135). Rice blast disease, which is caused by the ascomycetous fungus *Magnaporthe oryzae*, is the most destructive rice disease worldwide. Three mycoviruses, MoV1,

MoV2, and MoCV1, were identified in *M. oryzae* (78, 111, 129). MoV1 and MoV2 are in the family *Totiviridae* and cause latent infection, whereas MoCV1 belongs to the family *Chrysoviridae* and not only impairs growth and abnormal pigmentation of the host but also induces macroscopic phenotypic alterations. Two mycoviruses (UvPV1 and UvRV1) were recently isolated from the strain JYH-ZT of *Ustilaginoidea virens*, which causes rice false smut. UvPV1 is a typical member of the genus *Partitivirus*, whereas UvRV1, with two consecutive open reading frames, belongs to the genus *Victorivirus* of the family *Totiviridae* (133, 134).

Although oomycetes are distant phylogenetically from true fungi, these pathogens are customarily considered to be fungi. Oomycetes represent an important group of plant pathogens, including *Phytophthora infestans*, which is notorious for causing the Irish potato famine. Four viruses, namely Phytophthora infestans RNA virus 1 (PiRV-1), PiRV-2, PiRV-3, and PiRV-4, have been isolated and characterized from *P. infestans* (10). Several unclassified viruses were isolated and identified from other species of oomycetes, including the rice downy mildew pathogen *Sclerophthora macrospora* and the sunflower downy mildew pathogen *Plasmopara halstedii* (40, 42, 127, 128). Interestingly, viruses in oomycetes are different from mycoviruses.

INTERACTIONS BETWEEN MYCOVIRUSES AND HOST FUNGI

Interactions between the hypovirulence-associated virus and its host supply an excellent opportunity to identify virus-encoded determinants that are responsible for altering fungal host phenotypes and to understand the molecular basis of fungal biology. Moreover, increasingly, genomic sequences of pathogenic fungi are available and facilitate deciphering pathogen-virus interactions at the molecular level. Previous review articles have described two well-characterized host-virus interaction systems: *C. parasitica*–hypovirus and *H. victoriae*–HvV190S. Using the *C. parasitica*–hypovirus system, researchers around the world have thoroughly studied biological control, virus replication, RNAi response to virus infection, virus transmission and ecology, and virus distribution and diversity (25, 26, 39, 47, 80, 83, 87, 88, 90–93). With the *H. victoriae*–HvV190S system, Ghabrial and his collaborators determined the virion structure of HvV190S and the molecular mechanism of HvV190S translation, and characterized the interaction between HvV190S and its host (11, 30, 32, 36–39, 68). Here, we focus on recent advances of three other host-virus interaction systems: *S. sclerotiorum*–mycovirus, *R. necatrix*–mycovirus, and *F. graminearum*–mycovirus.

Sclerotinia sclerotiorum–Mycovirus Interaction System

Several different mycoviruses (ssRNA, dsRNA, and ssDNA viruses) have been identified in *S. sclerotiorum*. Thus, the *S. sclerotiorum*–mycovirus system provides the opportunity to explore interactions between different types of mycoviruses and *S. sclerotiorum*. SsDRV confers hypovirulence in the strain Ep-1PN (66). Using the *S. sclerotiorum*–SsDRV system, 150 genes were identified that were downregulated in the strain Ep-1PN (67). The genes downregulated by SsDRV represented a broad spectrum of biological functions. Subsequently, the *S. sclerotiorum* integrin-like gene (*SSITL*), which was suppressed in the presence of SsDRV, was further investigated via forward and reverse genetics approaches (136). Targeted silencing of *SSITL* resulted in a significant reduction in virulence and growth rate. In addition, mixed infections by two or more related or unrelated viruses are common in this fungus. Further studies are needed to address whether there is an interaction between coinfecting viruses in the same strain. We have recently identified an ssDNA virus (SsHADV-1) and are establishing a *S. sclerotiorum*–SsHADV-1 interaction system. The thorough investigation of different *S. sclerotiorum*–mycovirus interaction systems might supply new insights or clues regarding virus-host and virus-virus interactions as well as control strategies for Sclerotinia disease.

Rosellinia necatrix–Mycovirus Interaction System

An increasing number of dsRNA viruses have been characterized and many mycoviruses that were mentioned above show great diversity in *R. necatrix*. Moreover, viral transfection systems and a genetics transformation system have been successfully established for this fungus. Those prerequisites provide convenience for the establishment of the *R. necatrix*–mycovirus interaction system. Expanding the experimental host range of five mycoviruses (RnPV1, RnPV2, MyRV3, RnVV1, and RnMBV1) has been accomplished (16, 18, 54, 101, 102). RnPV1 could infect and replicate in *Sordariomycetous* fungi and *Hypocreomycetidae* fungi, whereas MyRV3 infected *Sordariomycetous* fungi but not *Hypocreomycetidae* fungi. Moreover, the newly MyRV3-infected hosts show hypovirulence traits. MyRV3 may have the potential to control other fungal diseases in addition to white root rot disease.

RNA silencing, which is an antiviral defense response, and viruses against host RNA silencing have been well characterized in plant viruses and in the *C. parasitica*–hypovirus system (31, 91). Four viruses (RnPV1, RnPV2, MyRV3, and RnMBV1) were independently introduced into the virus-free *R. necatrix* strain, which contained a constitutively induced RNA silencing of the exogenous GFP (green fluorescent protein) gene (126). MyRV3 could interfere with the dicing of dsRNA into siRNA and has a counter-defense strategy against host RNA silencing. Moreover, the MyRV3 *VP10* gene has RNA silencing suppressor activity in *Nicotiana benthamiana*. Further research will elucidate whether MyRV3 *VP10*, similar to hypovirus p29 of CHV1, acts as a viral suppressor of RNA silencing in its natural host and whether different viruses have a similar mechanism against host RNA silencing in fungi. Most dsRNA mycoviruses, including RnVV1 and RnPV2 in fungi, do not cause any visible abnormal symptoms for host fungi. However, RnVV1 or RnPV2 was introduced into a *Dicer-like 2* (*Δdcl-2*) knockout mutant and a wild-type strain of a non-natural host *C. parasitica*. The RnVV1 or RnPV2-infected *Δdcl-2* mutant strain displayed disease symptoms, but the wild-type strain displayed a normal phenotype (16, 18). Therefore, the life actives of RnVV1 and RnPV2 were suppressed by the antiviral RNA silencing mechanism in *C. parasitica*. Future studies will clarify whether RnVV1 and RnPV2 have similar interactions in their natural host.

Fusarium graminearum–Mycovirus Interaction System

FgV1 is a hypovirulence-associated virus in the *F. graminearum* strain DK21. To identify *F. graminearum* factors that respond to or are involved in mycovirus infection, an *F. graminearum*–FgV1 interaction system was recently established at both proteomic and transcriptional levels. By FgV1 infection, differentially expressed *F. graminearum* proteins were detected; using the 2-DE (2-dimensional gel electrophoresis) technique, 23 proteins of 148 spots that showed altered expression were identified (62). However, the limited number of proteins (23 proteins) was insufficient to obtain a comprehensive understanding of the host response. Subsequently, genome-wide expression differences in FgV1-infected *F. graminearum* at two different time points (36 h and 120 h) were analyzed using a transcriptomic approach (20). In total, 1,775 *F. graminearum* genes were identified that are significantly affected by FgV1 infection. This result indicated that FgV1-DK21 induced the upregulation of a range of genes, including protein synthesis genes that are required for virus replication, cAMP signaling genes that are involved in signal transduction, and genes that are required for transcription. In contrast, genes that are associated with metabolic pathways, which were presumed to play a role in the fungal defense mechanism against viral infection, were initially downregulated at 36 h but were gradually induced at 120 h. In addition, genes that are related to stress responses were upregulated at 120 h, and membrane-associated transporter genes were downregulated at both time points.

The hexagonal peroxisome protein HEX1 was screened using transcriptional and proteomic analyses. The *hex1* deletion mutant displayed reduced virulence and sexual spore accumulation. Interestingly, viral RNA accumulation was significantly decreased in the *hex1* deletion mutant. This result revealed that *hex1* is associated with RNA accumulation of FgV1 in the FgV1-infected host fungus (108).

THE IMPACT OF MYCOVIRUSES ON THE STUDY OF VIRUS EVOLUTION

The discovery of mycoviruses lagged behind the discovery of plant and animal viruses. Most mycoviruses, with a few exceptions, have quite simple genomes; mycoviruses usually have two genes, one encodes the capsid protein and the other encodes replicase. Some mycoviruses have only one gene for RNA replicase. For a long time, the diversity of mycoviruses was unknown, and the importance of mycoviruses in virus evolution was not properly revealed.

Mycoviruses Are Closely Related to Viruses That Infect Other Organisms

Previously, mycoviruses were thought to be dsRNA genomes. Chrysoviruses, totiviruses, and partitiviruses are typical fungal mycoviruses. Other dsRNA viruses, such as reoviruses and endornaviruses, which are usually found in animals and plants, have been isolated from fungi. We now understand that dsRNA viruses are widespread in eukaryotes. For example, Heterobasidion RNA virus 3, Flammulina velutipes browning virus, SsPV-S, and RnPV1 are related to beet cryptic virus 1, carrot cryptic virus, white clover cryptic virus 1, and Vicia cryptic virus, respectively. However, some plant partitiviruses are closely related to typical fungal partitivirus. For example, Primula malacoides virus China/Mas2007 is closely related to *Atkinsonella hypoxylon partitivirus*, which is a model species of fungal partitiviruses (74). We developed an in silico cloning technique to discover novel dsRNA viral sequences from the NCBI expressed sequence tag (EST) database and found 119 novel virus-like sequences that were related to members of *Endornaviridae*, *Chrysoviridae*, *Partitiviridae*, and *Totiviridae* (74). Many of these sequences were identified in cDNA libraries of lineages that included fungi, plants, and animals (74). Thus, these viruses are not solely related to fungi.

Increasingly, ssRNA viruses were isolated and characterized in fungi, and these discoveries provided compelling information regarding virus ecology and evolution. Many fungal positive-stranded RNA viruses are closely related to plant viruses. For example, BVF, BVX, and SsDRV are closely related to plant viruses in *Alphaflexiviridae* and *Gammaflexiviridae*. Additionally, hypoviruses are related to plant potyviruses (59). Diaporthe ambigua RNA virus 1 is closely related to plant viruses in the genus *Tombusvirus* (96), whereas SsRV-L is closely related to the human pathogen hepatitis E virus and rubi-like viruses, such as plant clostero-, beny- and tobamoviruses, as well as to insect omegatetraviruses. The discovery of SsRV-L expanded our knowledge regarding the host range of rubi-like viruses and suggested that ancestral positive-stranded RNA viruses might be of ancient origin and/or might have radiated horizontally among vertebrates, insects, plants, and fungi (72).

A fungal single-stranded circular DNA virus (SsHADV-1) was isolated and identified from a hypovirulent strain of *S. sclerotiorum* (130). This virus is closely related phylogenetically to geminiviruses, but its coat protein has little similarity to that of geminiviruses or to any of the other viruses that were discovered before 2010. The discovery of SsHADV-1 is likely to supply an important clue for studying the origin and evolution of geminiviruses (61, 100). Recently, fungal DNA virus-like sequences were detected in many biotopes. For example, fungal viral DNA-like sequences were found in mosquitoes and dragonflies, mammal fecal matter, endophytic fungi, and

even near atmospheric air and sewage masses (27, 60, 86, 94, 99, 106, 114, 117). This evidence suggested that SsHADV-1 might represent a type of small DNA virus that is widespread in nature.

Various Lineages of Double-Stranded RNA Viruses

Many unclassified dsRNA viruses were frequently isolated from fungi. Three mycoviruses with four-segment dsRNA genomes, RnQV-1 and RnQV-2 from *R. necatrix*, and Amasya cherry disease–associated mycovirus were proposed as a new family, *Quadriviridae*. Alternaria alternata dsRNA mycovirus and Aspergillus mycovirus 341 are significantly different from viruses in *Chrysoviridae* and *Quadriviridae*, although these mycoviruses have four-segment dsRNA genomes. Some novel nonsegmented dsRNA viruses with large genomes were also isolated, including SsNsV-L, Diplodia scrobiculata RNA virus 1, Fusarium graminearum dsRNA mycovirus-3, Grapevine associated totivirus-2, and Phlebiopsis gigantea mycovirus dsRNA 2. These viruses are phylogenetically related to each other but are distant from totiviruses. Phlebiopsis gigantea mycovirus dsRNA 1, Lentinula edodes mycovirus HKB, and Helicobasidium mompa V670 L2-dsRNA virus were also proposed to be nonsegmented dsRNA viruses; however, these viruses are related to RnMBV1, which is a bipartite dsRNA mycovirus. Similarly, Botrytis porri RNA virus 1, which is a bipartite dsRNA virus that is distant from RnMBV1 and other partitiviruses, is closely related to two unclassified insect nonsegmented dsRNA viruses (Spissistilus festinus virus 1 and Circulifer tenellus virus 1) and to Ustilago maydis virus H1, which was grouped in *Totiviridae* (118). We constructed a phylogenetic tree of mycovirus-related dsRNA viruses and found that there are at least ten monopartite, three bipartite, one tripartite, and three quadripartite lineages in the known dsRNA mycoviruses, in addition to mycoreoviruses (75). Additionally, we found that the multipartite lineages have possibly evolved from different monopartite dsRNA viruses (75). Furthermore, we also found that some fungal dsRNA viral lineages have homologs of the core S7 protein of phytoreovirus and suggested that multiple horizontal gene transfer events may have occurred among these dsRNA viruses from different families (75).

The Integration and Endogeneration of Mycoviruses in Eukaryotic Genomes

We found that the amino acid sequence of the coat protein of SsPV-S has the highest amino acid sequence similarity to IAA-leucine-resistant protein 2 (ILR2) of *A. thaliana* (73). Additionally, we further found that the capsid protein and RNA-dependent RNA polymerase genes from totiviruses and partitiviruses have widespread homologs in the nuclear genomes of eukaryotes, including plants, arthropods, fungi, nematodes, and protozoa, which suggested that the direction of these gene transfers is from viruses to eukaryotes and that these transfers are possibly mediated by retrotransposons (73). Chiba et al. (15) also found that the viral genes of fungal partitiviruses horizontally transfer between viruses and plants. These virus fossils on the eukaryotic genomes suggest that partitivirus and totivirus are widespread among eukaryotes and have helped us to understand the evolution of partitiviruses, totiviruses, and other related dsRNA viruses.

Negative-stranded (−)ssRNA viruses have not been reported in fungi experimentally; however, Kondo et al. (57) found genes that were similar to (−)ssRNA viruses in the genome of the pea powdery mildew fungus *Erysiphe pisi* and to viral sequences in the EST of the turf grass dollar spot fungus *Sclerotinia homoeocarpa*. In addition, Kondo et al. (57) found that these putative viruses are distantly related to the modern mononegaviruses and related viruses. Recently, we demonstrated that a (−)ssRNA virus from *S. sclerotiorum* is closely related to bornaviruses and nyaviruses. The properties of this (−)ssRNA virus will be thoroughly studied (L. Liu, J. Xie, J. Cheng, Y. Fu, G. Li, X. Yi, D. Jiang, unpublished data). These discoveries may provide novel insights into the origin and evolution of (−)ssRNA viruses; more importantly, because (−)ssRNA viruses are

dangerous human viruses, the discovery of (−)ssRNA viruses in fungi may supply a convenient and safe way to study the fundamental roles of (−)ssRNA viruses, such as screening antiviral compounds against (−)ssRNA viruses.

HOST RANGE AND TRANSMISSION OF MYCOVIRUSES

Mycoviruses May Have a Wide Host Range

Previously, mycoviruses were believed to have a narrow host range and were limited to host individuals within the same or within closely related natural vegetative compatibility groups (35). However, mycoviruses are not different from the viruses that infect other organisms and may have various host ranges. CHV1 might infect *Cryphonectria* species interspecifically in nature (76, 77), which suggests that *Cryphonectria* spp. are the natural hosts of CHV1. BpRV1, which is a bipartite dsRNA mycovirus, was originally isolated from *Botrytis porri*; however, this virus was also found in *Botrytis squamosa* and in *S. sclerotiorum* (118). *S. sclerotiorum* and *B. porri* share the same family but different genera, and this finding suggested that BpRV1 is likely to infect fungi that belong to the family *Sclerotiniaceae*. Mitovirus BcMV1, which was originally isolated from a *B. cinerea* that grows on rapeseed (*Brassica napus*), shares 95% nucleotide sequence identity with OnuMV3b, which is a mitovirus that was isolated from *O. novo-ulmi*, an elm pathogen (the genus *Ulmus*) (119). Additionally, a mitovirus of *S. homoeocarpa* was identified as *Ophiostoma mitovirus 3a*, which infects *O. novo-ulmi* (28). Because *Botrytis* and *Sclerotinia* are phylogenetically distant from *Ophiostoma*, these mitoviruses most likely have wide host ranges.

Transfection techniques were used to introduce mycoviruses into fungi other than their original hosts. The viral RNA of CHV1, which was transcribed from a full-length cDNA clone in vitro, was introduced into a virus-free strain of *C. parasitica* (13). Then, the hypovirus infectious cDNA was biolistically delivered into *V. ceratosperma* and *Phomopsis* G-type, which share the same order, *Diaporthales*, with *C. parasitica* but belong to different genera (103). Diaporthe RNA virus 1 (DRV1), which is a mycovirus that was isolated from *Diaporthe perjuncta*, was transfected into *Diaporthe ambigua* and *Phomopsis* sp. (85). Virus particles could be used to directly transfect fungi other than their original hosts. A partitivirus, RnPV1-W8 (RnPV1), which was originally isolated from the white root rot fungus *R. necatrix*, was transfected into other sordariomycetous fungi, *Diaporthe* spp., *C. parasitica*, *V. ceratosperma*, and *Glomerella cingulata*, whereas the mycoreovirus MyRV3 could not be transfected into *G. cingulata* (54). Interestingly, a partitivirus (RnPV2), a victovirus (RnVV1), and a megabirnavirus (RnMBV1) of *R. necatrix* could all be successfully transfected into *C. parasitica* (16, 18, 101). Furthermore, the FgV1-DK21 virus, which was originally isolated from *F. boothii*, could be replicated in *C. parasitica*, *Fusarium*, and *Cryphonectria*, which share the same class (*Sordariomycetes*) but belong to different orders. *Fusarium* is in the order *Hypocreales*, whereas *Cryponectria* is in the order *Diaporthales* (65). These studies suggest that these viruses replicate well in the so-called non-nature host fungi and that if the original viral hosts contact these tested fungi, then these viruses should have a chance to replicate in the new hosts.

Similar to other viruses, some mycoviruses may have a narrow host range. We found that SsHADV-1 could transfect *Sclerotinia minor* and *Sclerotinia nivalis* but could not transfect *B. cinerea* and other tested fungi, such as *Coniothyrium minitans*, which is the mycoparasite of *S. sclerotiorum*, and *M. oryzae*, which is the rice blast fungus (131). Because *Botrytis* shares the same family with *Sclerotinia*, we suggest that SsHADV-1 naturally has a narrow host range.

Transmission of Mycoviruses Among Host Vegetative-Incompatible Individuals

The transmission of mycoviruses remains unclear. Until now, no mycovirus vector has been identified, although people believe there should be vectors to transmit mycoviruses. Mycoviruses are

vertically transmitted via host spores, which are primarily asexual spores; rare research has shown that ascospores of a virus-infected strain might be infected by mycoviruses (24, 81, 110) and transmitted horizontally via hyphal anastomosis. When a virus-infected strain contacts a virus-free strain, either hyphal anastomosis or an incompatibility response occurs, and the incompatibility response often leads to programmed cell death (PCD), which limits the transmission of mycoviruses (21).

However, some research showed that a vegetative-incompatibility response between a virus-infected strain and a virus-free strain is not likely to be a hindrance for mycovirus transmission in nature. Although the transmission efficiency of mycoviruses between vegetative-incompatible individuals is not as high as that between vegetative-compatible individuals, transmission between vegetative-incompatible individuals could occur. We found that when a hygromycin-resistant gene (*hph*)-labeled virus-free strain of *S. sclerotiorum* was dual cultured with a vegetative-incompatible virus-infected strain on a plate for up to two weeks, the newly infected strain could be isolated when grown on a hygromycin amended PDA (potato dextrose agar). Brusini & Robin (8) found that the transmission efficiency of CHV1 between two vegetative-incompatible individuals on plates (in vitro) was difficult but was much easier when strains were inoculated on chestnut wood (in situ). Yaegashi et al. (125) inoculated two vegetative-incompatible strains of *R. necatrix*, a virus-free strain and an N10 dsRNA element (mycovirus)-infected strain, on the roots of an apple tree. After two to three years, strains of *R. necatrix* were reisolated from the inoculated tree, and Yaegashi et al. (125) found that the N10 dsRNA element could be isolated from coinoculated trees. Furthermore, Yaegashi et al. (125) also found that these two inoculated strains were infected by novel viruses from an unknown source. Interestingly, those trees that were chosen for inoculation were healthy and did not have any appearance of fungal infection; therefore, there is a possibility that these newly emerged mycoviruses came from other soilborne fungi.

There are possible reasons for the difference in the transmission efficiency between in vitro and in situ experiments. The first reason is that the space of plates is limited, and even when transmission occurs, the converted phenotype is difficult to directly observe. Although viral transmission could occur, the efficiency is not likely to be comparable with that between two vegetative-compatible strains. The second reason is that the environmental conditions of in situ tests are different from that of in vitro tests; *C. parasitica* has to confront the defense reaction from its host and to interact with other microorganisms that grow on its host. Both the host plant and environmental microorganisms may weaken the vegetative-incompatibility response or influence the defense of fungi against viral infection.

The properties of mycoviruses should be important determinants for viral transmission between host vegetative-incompatible groups. Deng et al. (29) found that the transmission of CHV1 among host vegetative-incompatible groups was affected by virus strains; some strains of CHV1 had higher transmission efficiency. One virus with high virulence is likely to reduce the fitness of its fungal host. However, Bryner & Rigling (9) found that virulence of CHV1 has not only costs but also benefits the viral transmission; high-virulence strains of CHV1 have higher transmissibility than medium- or low-virulence strains of CHV1. Urayama et al. (111) found that virus particles of MoCV1 were released into liquid culture, and virus particles in this culture might infect a virus-free strain of *M. oryzae*. Recently, we found that the virus particles of SsHADV-1 could directly infect healthy hyphae of *S. sclerotiorum*. We spread purified virus particles of SsHADV-1 on the surface of PDA in a plate, and then an activating hyphal agar disc was placed on the plate center approximately 3–4 cm away from the virus-particle-spread region. When the colony extended, the newly growing hyphae contacted particles, and when hyphae were infected by viruses, we further found that virus particles of SsHADV-1 could infect the SsHADV-1 host, even on plant leaves (131).

Interspecific Transmission of Mycoviruses

The transmission of mycoviruses from original hosts to other fungal species is often observed. Vainio et al. (112) found that a partitivirus (HetRV3-ec1) of the saprotrophic fungus *Heterobasidion ecrustosum* could infect pathogenic species of the *Heterobasidion annosum* complex and that *H. ecrustosum* anastomosed occasionally with *Heterobasidion abietinum* and *Heterobasidion occidentale*. A partitivirus of *Heterobasidion parviporum* (HetRV4-pa1) could be transmitted to *H. annosum*, and then this virus could spread in the population of *H. annosum* among somatically incompatible individuals in natural conditions (113). Melzer et al. (82) found that a dsRNA element of *S. sclerotiorum* could be transmitted into *S. minor*, which is a sister species of *S. sclerotiorum*, with dual culturing of two species on the same PDA plate. We also found SsDRV could be transmitted to *S. nivalis* via dual culturing. This transmission experiment was conducted on both PDA and lettuce leaves. Leaves were coinoculated with the *S. nivalis* strain Let-19, which was originally isolated from lettuce (66), and SsDRV and SsRV-L coinfected the hypovirulent strain Ep-1PN of *S. sclerotiorum*. Only small lesions were induced by strain Ep-1PN, whereas strain Let-19 could rot whole leaves. Thus, Let-19 had a chance to contact strain Ep-1PN. The inoculated leaves were further incubated to allow sclerotial formation of *S. nivalis*, and then the sclerotia of *S. nivalis* were reisolated to check whether the virus had been transmitted interspecifically. We found the interspecific transmission of mycoviruses occurred on at least 23 leaves among 100 coinoculated lettuce leaves. This finding suggested that interspecific transmission should occur in nature when a mycovirus-free fungus contacts a virus-infected fungus (F. Teng & D. Jiang, unpublished data). However, currently, we do not know the mechanism for interspecific transmission.

Counteraction of Programmed Cell Death by Mycoviruses

The vegetative incompatibility of filamentous fungi is considered to be non-self-recognition to counteract microbial antagonism, particularly against molecular parasites. A vegetative-incompatibility reaction leads to the PCD of contacted fungal cells. If a mycovirus suppresses the host vegetative-incompatibility reaction, then mycoviruses can be transmitted efficiently among host vegetative-incompatible individuals. Biella et al. (3) found that CHV1 could suppress the host vegetative-incompatibility reaction (reduce cell death during heterogenic hyphae contact) and help virus transmission. Shang et al. (104) found that the expression of genes that are involved in PCD in the CHV1-infected strain Ep773 were downregulated. This finding further confirmed that hypoviruses might suppress vegetative-incompatibility reactions. We found that the expression of a gene (DN796016 or SS1G_07434) that codes for ADP-ribosylation factor-like protein, which is involved in apoptosis in the SsDRV-infected strain of *S. sclerotiorum*, was significantly suppressed (67); this gene (ES360483.1) was downregulated in the hypovirus-infected strain Ep713 of *C. parasitica* (104). The coinfection of mycoviruses is a common phenomenon most likely because of the attenuation of the vegetative-incompatibility reaction of host fungi. However, the degree of suppression of the vegetative-incompatibility reaction is likely to be different based on individual mycoviruses and their hosts, and the mechanism of host non-self-recognition system attenuation is unknown.

EXPLORATION OF MYCOVIRUSES FOR THE BIOLOGICAL CONTROL OF CROP DISEASES

In the early 1950s, Italian phytologist Biraghi (4) found the unexpected phenomena that chestnut trees infected by *C. parasitica* were not killed, and the lesions on the stems healed with no outside influence. This finding resulted in the discovery of hypoviruses and of the biological control of

chestnut blight with hypovirulent strains (hypovirus). In 1982, Anagnostakis (1) wrote to *Science* to review their research regarding the use of a hypovirus to control chestnut blight, and she hoped that "if a way can be found to help the spread here of strains of the fungus with controlling agents (hypovirus), it may be possible to save the American chestnut trees in our eastern forests." In 2004, Milgroom & Cortesi (83) presented a critical analysis of the biological control of chestnut blight with hypovirulence and thought that, with few exceptions, biological control had failed almost completely in eastern North America. They believed that the successful biological control of chestnut blight, on a population level, was dependent on the natural spread of viruses and that the characteristics of triple interaction (hypovirus, fungal pathogen, and tree) and environmental factors determined the success or failure (83). Although the complicated pathosystem of chestnut blight in forests could not be easily modified by humans, the exploration of hypoviruses to control chestnut blight successfully in orchards remains worthwhile.

The Advantages of Using Mycoviruses to Control Crop Diseases

Although using mycoviruses (hypoviruses) to control chestnut blight has been common practice for more than 40 years, there are few reports regarding the use of mycoviruses to control fungal crop diseases. The pathosystem in agroecosystems is widely different from that in forests and is also different from that in orchards, where perennial trees are planted. Owing to the high crop density, low species diversity, and unique environmental conditions, crop fields facilitate the growth, reproduction, and transmission of crop pathogens. However, these advantages may also help mycoviruses to establish prevalence in their host populations in fields. Furthermore, the prevalence of mycoviruses in crop fields is established easily if a mycovirus-infected strain of a fungal pathogen is equally applied in fields at the correct time.

Using hypovirulence-associated mycoviruses that control crop fungal diseases may possibly have some advantages. First, once hypovirulence-associated mycoviruses are transmitted to a virulent fungal strain, they quickly inhibit lesion extension because viruses intend to reach the growth region of colonies for replication (5). This property is important because crop fungal diseases often damage or kill plants quickly during the growing season; therefore, quick action to suppress the prevalence of crop diseases is crucial for their successful biological control. Second, the fitness of a hypovirulent strain on crop plants is not likely to be a problem. Because plants are densely covered by the hypovirulent strain via spraying, whether the strain produces spores or other propagation bodies on plants is not important because crops are harvested at the end of the growing season. Third, although hypovirulent strains cannot grow on their hosts as virulent strains do, these strains share a similar niche with virulent strains, and it is likely that hypovirulent strains can grow well on hosts. A good example is the hypovirulent strain of chestnut fungus, which may cause superficial lesions on stems (1). We also found that hypovirulent strains could be frequently isolated from the sclerotia that were collected from diseased rapeseed plants, suggesting that mycovirus-mediated hypovirulent strains are actually growing on the diseased plants (D. Jiang, unpublished data). Fourth, when hypovirulent strains grow on their hosts, they are likely to produce pathogen-associated molecular patterns (PAMPs) and/or effectors that are recognized by their hosts; their hosts produce a defense response that specifically targets the infection of the virulent strain.

Biological Control of Rapeseed Stem Rot Caused by *Sclerotinia sclerotiorum* with SsHADV-1

Here, we show an example of using a mycovirus to control rapeseed stem rot, which is caused by *S. sclerotiorum*. Typically, *S. sclerotiorum* lies dormant with sclerotia under the soil. Under

favorable conditions, the sclerotia germinate to produce and release ascospores into air, and then the ascospores land on the petals and senescent leaves to initiate their infection of rapeseed plants. The infected petals and senescent leaves that drop onto leaves and stems of rapeseed plants result in a contact infection, which kills plants and/or twigs before harvest. New sclerotia are formed on diseased parts of plants at the late stage of infection and drop into the soil, remain in the stubbles, or mix with seeds for dormancy to wait for the next flowering season (7). Experientially, suppression of the contact infection is important for controlling rapeseed stem rot, and many fungicides used to control rapeseed stem rot are suggested to be applied at the full blooming stage of rapeseed plants.

We isolated single-sclerotium strains of *S. sclerotiorum* from sclerotia that were produced on diseased rapeseed plants and screened for hypovirulence-associated mycoviruses from these strains. We found that many mycoviruses could lead to hypovirulence in *S. sclerotiorum*. We then tested the transmission ability between host vegetative-incompatible individuals and found that SsHADV-1 had strong infectivity and that its transmission horizontally was not dependent on hyphal anastomosis of the vegetative-compatible reaction (**Figure 1*a***) (130). We further found that the purified virus particles of SsHADV-1 could infect healthy hyphae of *S. sclerotiorum* (**Figure 1*a***) (131). When the hyphal fragments of the SsHADV-1-infected strain were spread on the surface of rapeseed leaves and then inoculated with a vegetative-incompatible virulent strain of *S. sclerotiorum*, we found that the virulent strain could generate lesions on the sprayed leaves at the beginning, as on nontreated leaves. However, the lesion expansion on sprayed leaves was soon suppressed, and the vegetative-incompatible virulent strain was infected by SsHADV-1.

We applied the hyphal-fragment suspension of the SsHADV-1-infected strain on aerial parts of rapeseed plants at the blooming stage and found that this treatment suppressed both the incidence and the severity of disease generated by the fungal strain on rapeseed plants and also improved the seed yield of rapeseed (131). We further found that SsHADV-1 could be detected in sclerotia that were collected from a hyphal fragment–sprayed field, which suggested that SsHADV-1 indeed transmitted into other vegetative-incompatible individuals.

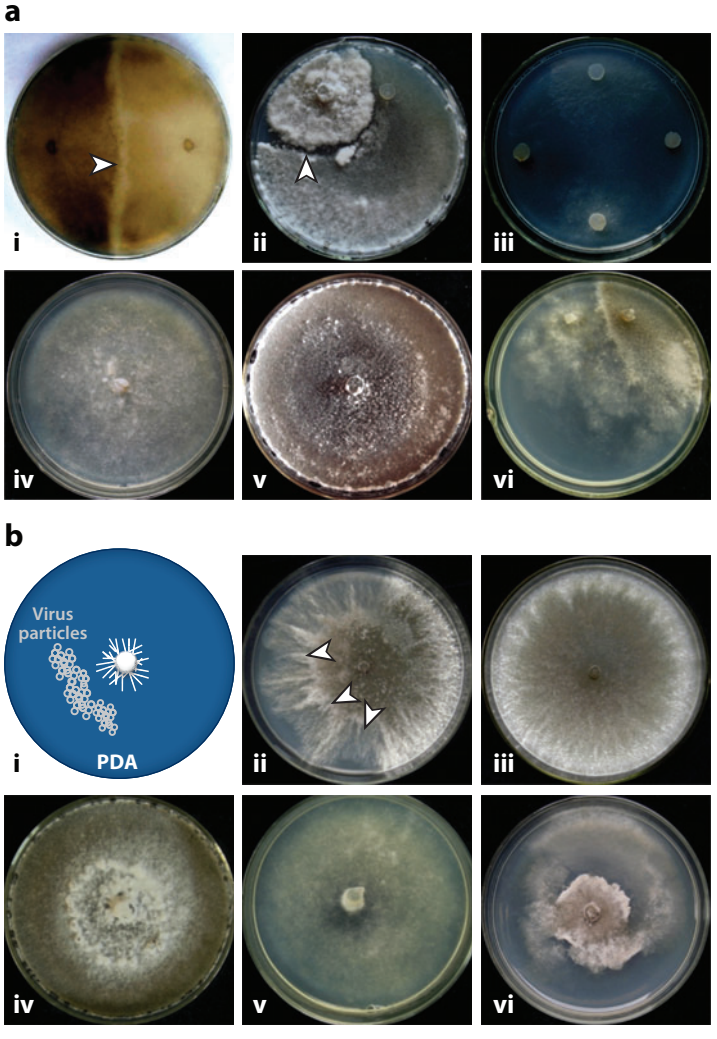
Alternatively, the virus particles can be used to directly control stem rot caused by *S. sclerotiorum*. When virus particles were sprayed on the leaves of *A. thaliana*, a virulent strain of *S. sclerotiorum* was inoculated, and we found that the virus particles on the leaves protected plants against pathogen attack. A virulent strain inoculated on leaves one day ahead of spraying virus particles induced lesions on leaves, but the lesions could not expand (131). However, the infectivity of SsHADV-1 particles on plant leaves was undetectable soon after spraying, although the viral DNA on inoculated leaves could be detected for at least ten days using polymerase chain reaction amplification. This phenomenon suggests that virus particles of SsHADV-1 must be carefully protected when delivered in fields.

The Key Problems in and Strategies for Controlling Crop Fungal Diseases with Mycoviruses

Farmers always require the control of crop diseases in as short a time as possible. If a mycovirus is to be applied, the time for the establishment of the virus population in the field is vital for successful control. However, the efficiency of viral transmission between vegetative-incompatible individuals in the field is not likely to meet this demand. We sprayed hyphal fragments of the hypovirulent strain Ep-1PN, which was infected with SsDRV, on the leaves of rapeseed plants and then inoculated a virulent strain that is incompatible vegetatively with Ep-1PN on these sprayed plants. All plants were killed by the virulent strain. By contrast, when a virulent strain that shared the same vegetative-compatible group with Ep-1PN was used for the challenge inoculation,

the plants survived. There are several possible strategies to resolve the problem of mycovirus transmission in the field.

Screening mycoviruses with strong infectivity. Current discoveries of mycoviruses in fungal plant pathogens have suggested that fungi could host various mycoviruses and that many mycoviruses could convert the hypovirulence of their host. Although only one fungal DNA virus has been isolated at present, we suggested that other fungi could host similar DNA viruses or host other types of DNA viruses with strong infectivity. RNA mycoviruses may also have strong infectivity. For example, Urayama et al. (111) found that chrysovirus-like virus particles that infect rice blast fungus not only affected vegetative growth but also could be secreted from host hyphae and have the ability to infect the host. We isolated a hypovirulence-associated partitivirus from *S. sclerotiorum* with strong infectivity, although the partitiviruses looked like typical mycoviruses with a latent infection property (X. Xiao, Y. Fu, J. Cheng, D. Jiang, S.A. Ghabrial, J. Xie, unpublished data).



Attenuation of the host vegetative-incompatibility reaction by amending chemical compounds. Vegetative-incompatibility reactions lead to PCD and subsequently inhibit viral transmission. Reasonably, the pharmacological suppression of PCD may enhance viral transmission among host vegetative-incompatible individuals. Recently, Ikeda et al. (51) found that zinc compounds could attenuate the heterogenic incompatibility of *R. necatrix*. When vegetative-incompatible strains were dual cultured on the same zinc compound–amended medium, hyphal anastomosis between the tested strains improved and mycoviruses were transmitted through heterogenic hyphal anastomosis. If the PCD mechanism in fungi is carefully studied, other suitable compounds that could not harm either plants or environments may be discovered. Hutchison et al. (50) found that reactive oxygen species (ROS), phosphatidylinositol, and calcium signaling pathways play a role during early heterokaryon (vegetative) incompatibility and during PCD in *Neurospora crassa*. However, homologs to genes that are involved in apoptosis in higher species, such as caspases (metacaspases) and apoptosis-including factor (AIF), were not required. This finding suggests that ROS scavengers are likely to be used to attenuate the vegetative-incompatibility reaction if economically available.

Finding a universal mycovirus donor from a laboratory or from nature. Dr. Donald Nuss recently gave a lecture for the mini-symposium "Viruses of Fungi and Simple Eukaryotes," which was held on September 30, 2013, in honor of Dr. Said Ghabrial on his retirement from the Department of Plant Pathology at the University of Kentucky. During his lecture, Dr. Nuss mentioned the creation of a universal hypovirus donor. Although Dr. Nuss did not point out how to make a universal mycovirus donor, the important thing is to disarm the vegetative-incompatibility reaction. Fedorova et al. (33) suggested that the ancestral PCD machinery is shared by fungi and metazoans. Bidard et al. (2) recently found that there was a significant overlap between regulated genes during incompatibility in *Podospora anserina* and *N. crassa*, indicating similarities in the incompatibility responses in these two species. These findings suggest that among fungi,

Figure 1

Transmission of Sclerotinia sclerotiorum hypovirulence-associated DNA virus (SsHADV-1). (*a*) Virus transmission via hyphal contact between host vegetative-incompatible strains. (*i*) Vegetative-incompatible interaction between a hygromycin-resistance gene labeled strain Ep-1PNA367^{R} (*left*) and virus-free strain DT-8VF (*right*), or (*ii*) between virus-infected strain DT-8 (*left*) and strain Ep-1PNA367^{R} (*right*). Arrows indicate the vegetative-incompatibility interaction zone. (*iii*) Colonies of strain DT-8 (*right*), strain DT-8VF (*left*), and strain Ep-1PNA367^{R} (*top*) as well as a newly infected isolate of strain Ep-1PNA367^{R} (*bottom*) developed in hygromycin-amended PDA (potato dextrose agar) plate (50 μg/ml). Strain DT-8 and newly infected strain Ep-1PNA367^{R} were developed in the plate for 4 days, whereas strain Ep-1PNA367^{R} and strain DT-8VF were developed in the plate for 1 day. (*iv–v*) Colony morphologies of newly infected strain Ep-1PNA367^{R} and virus-free strain Ep-1PNA367^{R} developed on PDA for 7 days. (*vi*) Dual culture between newly virus-infected strain Ep-1PNA367^{R} (*left*) and virus-free strain Ep-1PNA367^{R} (*right*). The virus-infected strain was inoculated 3 days ahead of dual culture, and colonies were dual cultured for 7 days. (*b*) Extracellular transmission via purified virus particles following extracellular application to intact hyphae on PDA plates. (*i*) A diagram of challenging virus particles on PDA plate. (*ii*) Virus-free strain Ep-1PNA367 was infected by SsHADV-1 and sectoring in the colony was observed (*sectors are indicated by arrowheads*). (*iii*) Colony morphology of strain Ep-1PNA367 growing on a PBS (phosphate buffered saline) buffer–treated PDA plate. Colonies were developed on PDA plate with a diameter of 150 mm for 4 days. (*iv–vi*) Colony morphologies of strain Ep-1PNA367, virus-infected strain Ep-1PNA367, and original strain DT-8. All cultures were incubated on a PDA plate with a diameter of 90 mm for 7 days. All strains were incubated at 20°C. The hypovirulence of newly infected strain Ep-1PNA367 or Ep-1PNA367R was further confirmed by virulence tests, and viral genome DNA was extracted successfully from these newly infected strains (see 130, 131 for details). Panel *a* was modified from Reference 130. Panel *b* was modified from Reference 131.

the vegetative-incompatible reactions share similar pathways. Downstream genes that regulate vegetative incompatibility–induced PCD pathways are likely to be modified to create a universal mycovirus donor; thus, the identification of essential genes is important. As mentioned above, some mycoviruses may suppress the host vegetative-incompatibility reaction. If one mycovirus can strongly inhibit the vegetative-incompatibility reaction, then this mycovirus can be used to create a universal donor for virus transmission. In nature, fungi are often coinfected by many mycoviruses (53), which suggests that individual fungal strains could host many viruses. Thus, it is possible that introducing a hypovirulence-associated mycovirus to strains that are infected by a mycovirus could suppress the vegetative-incompatibility reaction. Recently, we found that a mycoreovirus of *S. sclerotiorum* could inhibit the vegetative-incompatibility reaction and help other mycoviruses transmit to virus-free individuals (S. Wu, Y. Fu, J. Xie, J. Cheng, D. Jiang, unpublished data).

Creating vectors for mycoviruses. Viral transmission by vectors may overcome the vegetative-incompatibility barriers and help mycoviruses establish populations in fields quickly. We found the virus-purified particles of SsHADV-1 could directly infect hyphae of *S. sclerotiorum*, suggesting that fungivorous insects or other small animals are likely to transmit SsHADV-1 when these organisms feed on virus-infected colonies and move to virus-free colonies mechanically. The transmission of SsHADV-1 by fungivorous insects has been observed under laboratory conditions but has not yet been found in the field. However, these natural viral vectors are not likely to be practicable because these vectors are not easy to produce and release in fields. Mycoparasitism is a lifestyle that occurs naturally, in which one fungus (mycoparasite) parasitizes another fungus or fungi. Mycoparasites are common, and well-known mycoparasites include *C. minitans*, *Clonostachys rosea*, *Stachybotrys elegans*, *Talaromyces flavus*, and *Trichoderma* spp. Some mycoviruses and mycoparasites may share the same host fungus. For example, *S. sclerotiorum* is the host of both *C. minitans* and SsDRV. It is not difficult to introduce a hypovirulence-associated mycovirus to a mycoparasite using either a transfection technique or a dual-culture technique. If the mycovirus could replicate in the mycoparasite, then the next step is to check whether the mycovirus can be released into the host fungus when the mycovirus-carrying mycoparasite meets the same fungus. As we discussed above, some mycoviruses may have a wide host range. For example, we found that a totivirus could replicate in both *S. sclerotiorum* and its mycoparasite *C. minitans* (S. Liu, J. Xie, J. Cheng, D. Jiang, Y. Fu, unpublished data). Importantly, the hypovirulence-associated mycovirus must not cause a significantly negative impact on the life cycle of the mycoparasite; otherwise, we lose more than we gain.

CONCLUDING REMARKS

Fungi host various mycoviruses, such as dsRNA, ssRNA, and DNA mycoviruses, and are possible reservoirs for mycoviruses. There are benefits to investigating the rich diversity of mycoviruses, including helping to identify suitable mycoviruses for control of fungal plant diseases, understanding global virus origins, evolution, and ecology, and dealing with new emerging viral diseases of plants, animals, and humans.

Mycoviruses may share some general characteristics, but mycoviruses also have distinct specific characteristics. The *Cryphonectria*-hypovirus and *Helminthosporium*-HvV190S systems have been established to study the fundamental roles of mycovirology. Recently, many important studies have been performed using other host-mycovirus systems, such as the *Rosellinia*-mycovirus system, the *Sclerotinia*-mycovirus system, and the *Fusarium*-mycovirus system, which have enriched and expanded our knowledge of mycovirology, particularly regarding the infection, host range, transmission, and evolution of mycoviruses as well as the interaction between mycoviruses and their hosts.

There are many advantages to using hypovirulence-associated mycoviruses to control fungal crop diseases. However, using mycoviruses to control crop diseases has not been previously reported. Most crops are annual plants, and diseases are often quickly prevalent; thus, the highly efficient transmission and quick spread of hypovirulence-associated mycoviruses in fields is required. SsHADV-1 may represent a group of mycoviruses that can be highly efficiently transmitted in fields among host vegetative-incompatible individuals and can be explored to control crop fungal diseases.

SUMMARY POINTS

1. A growing numbers of novel mycoviruses were isolated and characterized from fungal plant pathogens, and many of these mycoviruses are involved in the hypovirulence of their hosts.
2. In addition to the *C. parasitica*–hypovirus and *H. victoriae*–HvV190S systems, three new host-mycovirus systems have been established to investigate the interactions between a mycovirus and its host.
3. Newly discovered mycoviruses have strong impacts on the study of virus taxonomy and virus evolution; key mycovirus genes have been horizontally transferred to eukaryotic genomes.
4. Some mycoviruses have a wide host range, whereas some have a narrow host range. Transfection techniques can be used to expand the host range of mycoviruses. The mycovirus transmission among host vegetative-incompatible individuals and interspecific transmission were frequently observed in nature or in the laboratory. Some mycoviruses are likely to suppress host PCD that is induced by non-self-recognition.
5. The pathosystem in agroecosystems is quite different from that in forests and orchards, where perennial trees are planted. Agroecosystems have a great potential for using hypovirulence-associated mycoviruses to control crop diseases.
6. SsHADV-1 can be used to control stem rot caused by *S. sclerotiorum*. SsHADV-1 has strong infectivity and can transmit and spread among host vegetative-incompatible individuals, and its particles can directly infect healthy hyphae of *S. sclerotiorum*.
7. Fungal vegetative incompatibility is a key factor that limits the use of mycoviruses to control crop diseases. However, several strategies, which may include, but are not limited to, screening mycoviruses with strong infectivity, attenuating the host vegetative-incompatibility reaction by amending chemical compounds, finding a universal mycovirus donor from the laboratory or from nature, and creating vectors for mycoviruses, are suggested to resolve this problem.

FUTURE ISSUES

1. A technique system must be established to explore hypovirulence-associated mycoviruses to control crop fungal diseases. This work may include, but is not limited to, screening hypovirulence-associated mycoviruses with strong infectivity, genetically modifying fungal strains to break the limitation of virus transmission that is caused by the host

vegetative-incompatibility reaction, understanding the ecological properties of virus-infected fungal strains and the mechanisms of virus transmission in fields, understanding the proper time for delivering mycovirus-infected strains in fields, and producing and formularizing virus-infected fungal strains or virus particles for commercial use.

2. The interaction between a mycovirus and its host on plants rather than on culture medium, as well as the tri-interaction among the mycovirus, fungus, and plant, must be understood. It is necessary to study the tri-interaction using different mycovirus-fungus-plant systems because, increasingly, new mycoviruses are being discovered, and it is necessary to understand the mechanisms for latent infection, which are very common in fungi. Because the coinfection of two or more mycoviruses is common in filamentous fungi, it is of interest to understand the simultaneous roles of these mycoviruses in these simple hosts.
3. Humans have to confront newly emerging viruses that may frequently attack crops, animals, or humans, although we do not know where these new viruses originate. Fungi represent a large group of organisms that are a huge bank for virus conservation and evolution. The isolation and characterization of mycoviruses with high-throughput techniques (such as RNAseq) may push the discovery of novel mycoviruses, help us to globally understand virus evolution and ecology, and determine the evolutionary trails of newly emerging viruses.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We apologize to all investigators whose research could not be appropriately cited as a result of space limitations. This research was supported by the National Basic Research Program (2012CB114000), the China National Funds for Distinguished Young Scientists (31125023), the National Natural Science Foundation of China (31101398), the Special Fund for Agro-Scientific Research in the Public Interest (201103016), and the China Agriculture Research System (CARS-13).

We would like to dedicate this review to Dr. Said A. Ghabrial, professor in the Department of Plant Pathology at the University of Kentucky, who did excellent work on mycovirus research for more than 30 years and retired at the end of 2013.

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