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A COMPREHENSIVE REVIEW ON EXPLORING THE SIGNIFICANCE OF MYCOVIRUSES

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A B S T R A C T

Mycoviruses are the viruses that infect fungi. The International Committee on the Taxonomy of Viruses (ICTV) categorizes mycoviruses into 23 viral families. Mycoviruses were discovered in the mushroom. For replication, they depend on their host, which includes plants and animals. The genomes of mycoviruses may contain double-stranded RNA (dsRNA) and single-stranded RNA (ssRNA). The double-stranded RNA genome is more effective at spreading and infecting healthy fungi. They vary in diameter from 25 to 250nm. Some viruses take over the host machinery and cause abnormal growth. They can spread inside cells and form spores without moving proteins. Mycoviruses rely on intercellular transmission via hyphal anastomosis and lack extracellular transmission mechanisms, making it difficult for distinct fungus strains to transmit one another successfully. Mycovirus is significant because its three effects include hypovirulence, hypervirulence, and cryptic effects. The hypovirulence effects of mycovirus are very important because they reduce the host virulence and act as potential biocontrol against the fungi. Cryphonectria parasitica hypovirus 1 (CHV1) were found to have hypovirulent and were categorized as hypovirulent mycoviruses because it reduced the chestnut bight disease. Fusarium oxysporum ourmia-like virus 1 isolated from Fusarium oxysporum f.sp. momordicae, Bitter Gourd was found to be associated with hypovirulence. Mycoviruses that infect plant pathogenic fungi have the ability to reduce their host's virulence, making them potential biocontrol agents against these fungi. The hypervirulence type of mycoviruses increases their pathogenicity. Most of the mycoviruses have cryptic effects. This review focus is on mycoviruses, their effects on hosts, and the possible application of hypovirulence mycoviruses as biological control agents to lessen the fungus's virulence.

Keywords: Mycovirus, hypovirulence, hypervirulence, dsRNA, biocontrol, taxonomy, Virus fungi interactions.

INTRODUCTION

Viruses are microscopic particles that can evade bacterial filters and are invisible under a light microscope. They are small infectious particles that cause disease in all life forms, including plants, animals, bacteria, and fungi (Gkoutselis *et al.*, 2021). They are host-dependent and do not have their own metabolic machinery, however, they do encode proteins that are necessary for their reproduction. Viral infections are present in every kingdom of fungi (Myers *et al.*, 2020). A protein coat

Submitted: February 08, 2024 Revised: May 27, 2024 Accepted for Publication: June 10, 2024 * Corresponding Author: Email: sultanjameel909@gmail.com © 2017 Pak. J. Phytopathol. All rights reserved. frequently, but not always, protects an RNA or DNA's genome. Viral infection produces various types of secondary metabolites, including sterigmatocystin, arugosin, asperthecin, aspertetronin, and ruguloxanthone. They mostly reside in the cytoplasm and occasionally coexist with mitochondria. Mycoviruses are a very diverse class of pathogens that affect a wide variety of organisms, including prokaryotes, fungi, plants, terrestrial and non-terrestrial vertebrates, and invertebrates (Kondo, Botella, & Suzuki, 2022). Morphological changes that vary from highly severe consequences to no impact on their host. These modifications can confer either hypervirulence, which increases fungal virulence, or hypovirulence, which attenuates the host (Deng et al., 2022). Viruses might change how much toxins, metabolites fungi make, and

how these changes might affect interferon production, toxic production, fungal genetics, and plant diseases, they are more critical in environmental health research. Because viruses can alter the metabolism and genetics of fungal cells, their presence in fungi has opened up new avenues for experimental mycology research.

History of mycovirus: Mycovirus has been associated with fungal species; further evidence of mycoviruses was found in ascomycetes. Mycovirus was isolated from the mushroom (Hollings & Stone, 1971). Fungal viruses found in diseased hosts can sometimes cause a disturbance in the host's metabolism. The mycovirus shares some characteristics with plant viruses and animal viruses, but also has distinct characteristics like intracellular transmission, sporulation without protein mobility, which is crucial for the biological cycle in plants and animals, cells that divide and fuse, and a lack of an extracellular route of infection (Deng *et al.*, 2022). loli Kotta Loizou have recognized mycoviruses effects on the host, resulting from disruptions in host cell metabolism or products encoded with the virus (I. Kotta-Loizou & Coutts, 2017).

Mycoviruses originating from plant diseases have gained a significant interest as possible biocontrol agents because of their ability to cause hypovirulence, in the fungi they infect (García-Pedrajas, Cañizares, Sarmiento-Villamil, Jacquat, & Dambolena, 2019). These viruses' capacity to increase in the pathogen's natural populations is critical to their effective use in disease management. Hyphal anastomosis is the main way that mycoviruses are passed from one isolate to another and from parents to children through conidia (J. M. Myers & James, 2022). Artificial transfection techniques have shown that prospective biocontrol mycoviruses often have a broad range of fungal infections (Xie & Jiang, 2014). This increases their potential for infection control outside of their defined area. Another objective of mycovirus studies is to comprehend the complex molecular biology of mycoviruses and the molecular mechanisms underlying their interactions with fungi. With this knowledge, we could alter both the mycovirus and the host to produce combinations with better biological control. The pathogen's lifestyle, the disease's symptoms, and the impacted crops ultimately have a major impact on the particular problems that need to be solved, as well as the creation of biocontrol formulations and delivery systems (Pandit et al., 2022).

Discovered Mycoviruses: Fungal virus are present in all major group of fungi (Ghabrial & Suzuki, 2009). Early in

the 1960s, Hollings considered the "birth of mycovirology" when he discovered three different forms of viral particles in fungus-infected mushrooms, marking the first time that he had linked virus particles to a fungus (Ayllón & Vainio, 2023). The International Committee on Taxonomy of Viruses (ICTV) has discovered more than 168 mycoviruses to date (Hough, Steenkamp, Wingfield, & Read, 2023). Most of the viruses are completely characterized, and a few mycoviruses have been defined for comparability, which furthers our understanding of how they replicate by infecting both plants and mammals. Virus infection causes fungi to exhibit abnormalities and peculiar behaviors. Most benign double-stranded RNA Penicillium species, extracted from fungi, appear to be toxic and harmful to plants (Pandit et al., 2022). To detect the developing fungal viruses in different fungi, extensive research in virology and mycology is required. Researchers continuously discover new pathogenic mycovirus species. (Kondo et al., 2022). Multiple virus presence in the host is one of the intriguing feature of mycoviruses (mixed infections). The Aspergillus foetidus isolate CBS 618.78 exhibits two distinct viral infections: the first is the A. foetidus virus-slow (AfV-S) component (Nazik, Kotta-Loizou, Sass, Coutts, & Stevens, 2021), which is a member of the Totiviridae family; the other is an unidentified mycovirus that results in a six-banded dsRNA profile known as the AfV-fast (AfV-F) component. The fact that most molecularly characterized viruses infect plants and animals exacerbates the lack of knowledge about mycovirus reproduction (J. Wang et al., 2021).

Transmission of Mycovirus: There are many methods of transmission of mycovirus, including horizontal and vertical transmission. Sexual and asexual transmission of mycovirus is the primary mode of fungal virus transmission in fungi (J. M. Myers & James, 2022). Anastomosis and heterokaryosis, two main methods of horizontal transmission, spread the mycovirus across various fungal types (Kondo et al., 2022). Since mycoviruses do not have an extracellular transmission route, they can spread vertically by sporulation or horizontally through anastomosis or heterokaryosis. In anastomosis, various fungus hyphae fuse intimately, causing cytoplasmic and genetic transmission and the presence of the mycovirus in the cytoplasm (Chou, Hsu, Leu, & Reviews, 2022). Anastomosis is more successful in two vegetative-compatible fungi. The sporulation method may be the vertical transmission method. Dowaidar became the first person to show that a variety that had formerly been virus-free might spread virus-like particles (VLPs) (Dowaidar, 2023). Sexual spores can transmit viruses less effectively than asexual spores. *Monilinia fructiocola*, a fungus that undergoes sexual stages, exhibits dsRNA in 74% of its isolates. The transmission rate varies depending on the combination of virus and host, and the host may be a fungus or a plant (Jeger, 2020).

Fungal spores, either sexual or asexual, primarily transmit mycoviruses vertically (Hillman & Milgroom, 2021). Transmission rates, however, can vary depending on the mix of virus and fungus and the type of spore, whether sexual or asexual. Asexual transmission of viruses is more effective than sexual spore transfer (Sun *et al.*, 2023). For example, about 10% of 668 *Aspergillus* isolates had dsRNA viruses, and 68% had *Botrytis cinerea*. For instance, *F. graminearum* demonstrated a 100% occurrence of dsRNA transmission through conidia and ascospores (Ajmal, Hussain, Ali, Chen, & Lin, 2022). *B. cinerea* discovered *Botrytis Virus* X in 35 and 53% of the ascospore offspring. Mycovirus transmission by asexual means is less effective, but it does occur occasionally (Khan, Nerva, & Bhatti, 2023).

There are numerous reports of plant viruses infecting several host species, often from different genera or families. On the other hand, the lack of mechanical and vector transmission makes it difficult for mycoviruses to infect various hosts, yet there have been cases of mycoviruses spreading horizontally between distinct species. The Cryphonectria hypovirus 1 (CHV1) variants were identical in many different species of the Cryphonectria fungus (Romon-Ochoa et al., 2023), the virus is spreading horizontally. Protoplast fusion between some species of Fusarium poae and black Aspergillus spp. could spread the mycovirus (Jacquat et al., 2020). Research has demonstrated that protoplast fusion facilitates the transfer of mycoviruses from one isolate to another. Researchers have successfully used this technique to transfer dsRNA viruses between A. niger, A. tubingensis, A. oryzae, and A. nidulans, as well as between F. poae and A. niger (D. Wang, Iin. Lu. & Chen. 2023).

Mycovirus Classification: The International Committee on the Taxonomy of Viruses (ICTV) reports on more than 23 mycovirus families (De Miccolis Angelini *et al.*, 2022). Five fungi are the primary hosts of the dsRNA families (J. Myers *et al.*, 2020). The mycoviruses are double-stranded dsDNA, single-stranded ssDNA, capsid, and non-capsid. The need for sufficient sequencing information necessitates categorically classifying these viruses into families, groups, and genera. Furthermore, researchers have discovered several unencapsidated dsRNA species in fungi (Sato et al., 2020). The largest family of dsRNA viruses belongs to the Reoviridae family, which has the most diverse host range from plants to animals, protozoa, and fungi (Ali, 2020). Reoviridae possesses icosahedral symmetry with a spherical shape, and the fungal infecting member of the family Reoviridae has 11-12 segments of dsRNA ranging from 732 bp to 4127 bp in size (Liu & Cheng, 2022). Totiviridae is also a family of dsRNA viruses with a non-segmented genome ranging in size from 4.6 to 7.0 kbp that infects fungi and protozoa (Kartali et al., 2021). Chrysovirus was originally part of the Partitiviridae, but now it is part of the Chrysoviridae family (Wu et al., 2023). The chrysovirus genome contains four linear dsRNA elements ranging in size from 2.8 to 3.6 kbp (Umer et al., 2023).

Two open reading frames (ORFs) make up the genome and typically overlap encoding the RNA-dependent RNA polymerase and the coat protein (CP) (Belete et al., 2023). There are also a number of ssRNA mycoviruses, including those in the Hypoviridae family and the Endornaviridae family, which are mainly found in parasitic plants (Bocos-Asenjo, Niño-Sánchez, Ginésy, & Diez, 2022). Replicative forms of dsRNA are not encapsidated, which is an important feature of real virions. Although these viruses are classified as dsRNA viruses, their origin and mechanism are unknown. Replication patterns indicate the presence of ssRNA-containing viruses (Gottipati, McNeme, Tipo, White, & Choi, 2023). The linear genome of hypoviruses spans 9-13 kbp. Endornaviruses consist of a single large dsRNA, ranging from 14 to 17 kbp. The sole non-plant virus in the endornavirus genus is Phytophthora endornavirus (PEV1). The Penicillium chrysogenum virus (PcV) has an icosahedral shape, which was found through biochemical, biophysical, and ultrastructural studies (Daudu, 2022). Satellite or faulty dsRNAs are extra dsRNAs that belong to the Hypoviridae, Narnaviridae, Partitiviridae, and Totiviridae families of viruses (Ye et al., 2023). Under some circumstances, intracellular dsRNA removal or rearrangement can result in the generation of satellite or faulty dsRNAs (Fredericks et al., 2021). Many mycovirus families and genera remain unassigned due to insufficient sequencing data. Important families of the dsRNA and ssRNA mycoviruses are discussed in Table 1.

| dsRNA family | Host | Virus particle morphology | Genome segments | |
|----------------|------------------|---|---------------------------------|--|
| Partitiviridae | Fungi, Plants | Icosahedral capsid protein with 30-40 nm diameter | 2 separately packaged | |
| Chrysoviridae | Fungi | Icosahedral capsid protein, multiple components with 30-40nm diameter | 3-7 packaged separately | |
| Totiviridae | Fungi | Icosahedral capsid protein with 30-40 nm diameter | 1 packaged singly | |
| Reoviridae | Fungi | Icosahedral, one two or three-layered capsid protein 70-90nm diameter | 10, 11, or 12 co-packaged | |
| Hypoviridae | Fungi | Pleomorphic vesicles, no capsid, 50-80nm diameter | 1 unpackaged | |
| SsRNA family | Host | Virus particle morphology | Genome segments | |
| Barnaviridae | Fungi | Bacilliform 19 × 50 | Not enveloped | |
| Narnaviridae | Fungi | No true particles, Virion consists of nucleoprotein complex | Not enveloped | |
| Pseudoviridae | Fungi | Isometric to quasi-30-40nm Non enveloped | | |
| Metaviridae | Fungi | Ovoid, 50nm, irregular | Enveloped nucleoprotein complex | |

Table 1. The dsRNA and ssRNA mycovirus families

(Table 1. is Adapted from (Mertens, 2004) (Fauquet, Mayo, Maniloff, Desselberger, & Ball, 2005)

Significance of Mycovirus: Mycoviruses can develop different morphologies with their fungal hosts, ranging from asymptomatic to hypervirulent (Sato & Suzuki, 2023). Mycoviruses and their hosts typically exhibit asymptomatic infections (Myers *et al.*, 2020). Mycoviruses generate changes in intricate physiological processes, including interactions between the host and viral components, which lead to macroscopic symptoms (Khan *et al.*, 2023). The significance of mycoviruses on host morphology is discussed as follows.

Hypovirulence: There are several instances when fungal virus infection affects fungal development in a hyperactive or hypovirulent manner (Wang et al., 2022). The parasitic Cryphonectria causes hypovirulence. Hypovirus interferes with fungal development (Brusini et al., 2017), such as sexual reproduction and sporulation, making it a great and well-known example that lessen the pathogenicity of the chestnut blight fungus host, C. parasitica. This weakens the fungus and reduces its virulence. Its main host is C. parasitica, CHV1-EP713, which is the first member of the family Hypoviridae (Ćurković-Perica, Ježić, & Rigling, 2022). However, it can also infect and multiply many species of other fungal genera, such as Endothia gyrosa and Valsa ceratosperma. Furthermore, hypoviruses include only mycoviruses that exhibit hypovirulence. The fungus that causes Dutch elm disease, Ophiostoma novo ulmi, is another example of hypovirulence (Wai & Hausner, 2021).

The mitochondria link to the fungal *O. novo ulmi* mycovirus dsRNAs, lowering mitochondrial cytochrome c oxidase activity and ultimately leading to the fungus's respiratory deficit (Shah, 2018). Therefore, the weakened fungus cannot infect elm trees. Helicobasidium momma infected with the *totivirus* HmTV1-17 shows a hypovirulent phenotype,

another example of a fungal virus that makes the host less infectious (Sukphopetch et al., 2021). Mycoviruses have been known to alter the yield and coloration of fruiting bodies in some commercial mushrooms (Song et al., 2020), including the king oyster mushroom, Pleurotus eryngii. This could result in losses in commercial mushroom production. Researchers have recorded up to 50% yield reductions due to a severe mycoviral infection and abnormal fruiting bodies in oyster mushrooms. Therefore, as demonstrated for C. parasitica, mycoviruses can be biological control agents for plant pathogenic fungi (Wang, et al., 2017). Even though mycoviruses can effectively lower the pathogenicity of fungal plant pathogens, vegetative incompatibility, a condition common to many fungal species, makes them much less useful as biological control agents (Tonka et al., 2022). Decreasing a pathogen's capacity to spread disease is known as hypovirulence.

Over the past few years, the importance of mycovirus has increased because of its hypovirulence effects. Mycovirus mediates the pathogenic fungi's virulence. Hypovirulence associated with mycovirus has a significant impact on controlling fungal diseases. Fusarium oxysporum f.sp. momordicae causes Fusarium wilt, an important fungal disease (Wang et al., 2020; Wen et al., 2021) Phylogenetic analysis reveals that the isolation of Fusarium oxysporum ourmia-like virus 1 from Fusarium oxysporum f.sp. momordicae (Wen et al., 2021), Bitter Gourd, is associated with hypovirulence against FoM. Infection with mycovirus alters the physiological processes between the host and virus. For example, infection with Penicillium digitatum virus 1 has hypovirulent effects on *Penicillium digitatum* (Niu et al., 2018). The molecular analysis reveals the presence of two novel mycoviruses, Penicillium digitatum, Polymycovirus 1 and Penicillium digitatum Narna-like virusmycovirusesreducethe1, in P. digitatum strain HS-RH2 (Yang et al., 2018). Thesemycelial growth and on themycelial growth and on theTable 2. mycoviruses describes in literature that triggered hypovirulence in fungal host.mycelial growth and on the

mycoviruses reduce the triazole drug prochloraz in mycelial growth and on the PDA plates (Yang *et al.*, 2018).

| Mycovirus | Genome | Fungal host | Mycovirus Family | Host Plant | Fungal Diseases | References |
|--------------------------|--------|--|---------------------|---|-------------------------------------|--|
| CHV-1 | +ssRNA | Cryphoal parasitica | Hypoviridae | Cascatanea sativa | Chestnut blight | (Chen & Nuss, 1999) (Rigling & Prospero, 2018) |
| CHV-2 | +ssRNA | Cryphoal parasitica | Hypoviridae | Cascatanea sativa | Chestnut blight | (Chen & Nuss, 1999) (Rigling & Prospero, 2018) |
| CHV-3 | +ssRNA | Cryphoal parasitica | Hypoviridae | Cascatanea sativa | Chestnut blight | (Chen & Nuss, 1999) (Rigling & Prospero, 2018) |
| OnuMV | +ssRNA | Ophiostoma Novo-ulmi | Narnaviridae | Ulmus spp. | Dutch elm disease | (Hintz, Carneiro, Kassatenko, Varga, & James, 2013) |
| <i>SsMV</i> -1/ HC025 | +ssRNA | Slerotinia sclerotiorum | Narnaviridae | Glycine max, | White mold | (Rahman <i>et al.,</i> 2020) |
| SsHADV-1 | ssDNA | Slerotinia sclerotiorum | Genomoviridae | Brassica napus, | White mold | (Rahman <i>et al.,</i> 2020) |
| SsHV-1 | +ssRNA | Slerotinia sclerotiorum | Hypoviridae | Lupinus angustifolius | White mold | (Rahman <i>et al.,</i> 2020) |
| SsHV-2 | +ssRNA | Slerotinia sclerotiorum | Hypoviridae | Pisum sativum | White mold | (Rahman <i>et al.,</i> 2020) |
| AaCV-1 | dsRNA | Alternaria alternata | Chrysoviridae | Herbaceous annual plants. | Leaf spots, rots, and blights | (Li <i>et al.,</i> 2022) |
| AaHV-1 | +ssRNA | Alternaria alternata | Hypoviridae | ornamental plants and trees (citrus, apple. | Leaf spots, rots, and blights | (Li <i>et al.,</i> 2022) |
| FgV-ch9 | dsRNA | Fusarium graminearum | Chrysoviridae | Small-grain cereals (wheat and barley) | Fusarium head blight (FHB) | (Dweba <i>et al.,</i> 2017) |
| FgHV-2 | +ssRNA | Fusarium graminearum | Hypoviridae | Small-grain cereals (wheat and barley) | Fusarium head blight (FHB) | (Dweba et al., 2017) |
| FodV-1 | dsRNA | Fusarium Oxysporum f. Sp.dianthi | Chrysoviridae | Dianthus caryophyllus | Carnation disease | (Esmaiel, Al-Doss, & Barakat, 2012) |
| BcMV-1 | +ssRNA | Botrytis cinerea | Narnaviridae | Vegetables and small fruit crops (tomato, raspberry, | Gray mold disease | (Ruiz-Padilla, Rodríguez-Romero, Gómez-Cid, Pacifico, & Ayllón, 2021) |
| RnMBV-1 | dsRNA | Rosellinia necatrix | Megabirnaviridae | Fruit trees (apples, apricots, avocados, cassava, citruses, and Narcissus) | Rosellinia Root rot | (Kondo, Kanematsu, & Suzuki, 2013) |

Hypervirulence: It is possible that mycoviruses help their pathogenic hosts. There are killer and hypervirulent types of the maize smut pathogen, *U. maydis*, and *S.*

cerevisiae (Siscar-Lewin, Hube, & Brunke, 2019). These types release proteins that harm strains of the same or a closely related species. As a result, their interactions are

very useful for species. For example, in *U. maydis*, a satellite dsRNA provides protection to the toxin (Koltin, 2018). Thus, viruses give their hosts a selection advantage by driving out rivals occupying the same biological niche. Mycovirus infection affects varying signal transduction pathways and promotes development (Kotta-Loizou, 2021). Scientists have found that the L1 dsRNA in *N. radicicola* might change the way cAMP signalling pathways work. Scientists are yet to determine the exact mechanism, but dsRNA may alter fungal gene expression by raising cAMP levels (Sarkar *et al.*, 2021), which in turn increases the activity of cAMP-dependent protein kinase (PKA) (Wang, *et al.*,2022).

It is possible for three different viruses to live together in harmony: the mycovirus Curvularia thermal tolerance virus (CThTV) (Afroz, Muzahid-E-Rahman, Akhter, Bhor, & Islam, 2024), the endophytic fungus Curvularia protuberata, and panic grass (Dichanthelium lanuginosum) (Lugtenberg, Caradus, & Johnson, 2016). In this symbiosis, the dsRNA mycovirus infection allows the fungus and plant to withstand high temperatures (Kanhayuwa, Kotta-Loizou, Özkan, Gunning, & Coutts, 2015). Some fungal viruses possess virulence strains that enhance the fungal pathogenicity of the plant-pathogenic fungi and cause diseases. Inoculation of Colletotrichum higginsianum on Arabidopsis plants causes a hypervirulent infection in plants (Olivé & Campo, 2021). Leptosphaeria biglobosa induces a hypervirulent infection in Brassica napus plants (Shah et al., 2020).

In the early studies, mycoviruses were considered unwanted because they targeted industrial mushrooms. Further research indicated that it was also advantageous because most industrially useful crops include them as bioagents for the majority of fungal diseases (Thambugala *et al.*, 2020). These findings suggest that mycoviruses are an efficient biological control agent and call for further research into several variables, including both viral and host properties related DNA virus treated the hypovirulence of *Sclerotinia sclerotiorum* in the rapeseed stem rotting infection, resulting in a decrease in the disease's virulence (Khan *et al.*, 2023). We administered the virus as a suspension containing contaminated hyphal fragments or shards.

Cryptic effects: Most fungal viruses that infect fungi remain asymptomatic, cryptic, or latent, exhibit expression under certain conditions, and serve as biocontrol agents (Tonka *et al.*, 2022). Mycoviruses had no extracellular phase in their life cycle, and there were

no obvious signs or symptoms of their host (Aoki et al., 2009). Mycovirus infections in fungal hosts are typically asymptomatic (Deng et al., 2022). Mycoviruses tend to be widespread and have no discernible effect on their hosts. It is possible for a mycovirus infection to generate changes (García-Pedrajas et al., 2019). For example, disparities in growth rates exist even in the absence of symptoms or obvious phenotypic impacts (van Diepeningen, Varga, Hoekstra, & Debets, 2008). There are instances where mycovirus infections have positive effects as opposed to hypovirulent interactions (Thapa, Roossinck, & microbiology, 2019). Killer fungi strains, which are lethal to strains that do not generate toxin (Peng et al., 2021), are distinguished by the presence of dsRNA elements that encode proteinaceous poisons to which the host is immune. By eradicating rivals occupying the same ecological niche, these viruses provide an advantage to their host.

Mycoviruses as biological control agents: Plant diseases are primarily caused by fungus infections. The primary method of control is the application of fungicides, which has led to the emergence of strains resistant to the chemicals and, more significantly, potentially dangerous consequences for humans and the environment (Lucas, et al., 2015) (Ons, Bylemans, Thevissen, & Cammue, 2020). Mycoviruses possess the potential to serve as biocontrol agents, yet despite numerous attempts, only a handful have proven effective in managing these illnesses (Wagemans et al., 2022). In this process, the majority of fungi that infect plants with the disease are significant actors. Chemical fungicides are the primary strategy for reducing their pathogenicity (Hollomon, 2015). Fungicide-resistant strains have developed because of the prolonged, widespread usage of these persistent fungicides (Corkley, Fraaije, & Hawkins, 2022). More importantly, it has increased the likelihood of potentially harmful side effects for both people and the environment. Few techniques have been used to successfully control these fungi, including mycoviruses, that provide considerable potential as biocontrol agents. The most prominent and effective example of a mycoviruses hypovirulent phenotype is the fungus that causes the chestnut blight (Eusebio-Cope et al., 2015). We recreated the hypovirulence-associated viral RNA in fulllength complementary DNA (cDNA). They transformed the fungi into a potent toxin, and that mutant strain converted the previously suitable mutant strain into a hypovirulent strain. This procedure showed the dsRNA

virus's potential as a biocontrol agent for chestnut blight. Chestnut fungus was successfully eradicated in Europe by the Cryphonectria hypovirus 1 CHV-1 cDNA (Rigling & Prospero, 2018) (Rigling et al., 2018), but not in North America. In 1980, researchers implemented the first method of disease management to combat chestnut rot, using the spore of the fungus C. parasitica, which contained the hypovirus (Kunova et al., 2017). The fungus's greater genetic diversity and several VCGs stopped the virus from propagating (Srinivas et al., 2019). Despite the fact that the dsRNA element significantly reduces pathogenicity, hyphal touch is the only way for CHV-1 to spread, preventing successful transmission (Kondo et al., 2022). The creation of cDNA contagious clones for transformations and other technologies have enabled comparable research of biologically identical, virus-infected, and unaffected cells without being bound by vegetative incompatible clones. Hypovirulence can also be seen in Ophiostoma novo-ulmi, the fungus that causes Dutch elm diseases (Bernier et al., 2015), and Helminthosporium victoriae, the fungus that causes the Victoria blights of cereals by accident (Ćurković et al., 2022). However, discovering the disease's molecular origins in mycoviral systems would open up amazing prospects for cutting-edge biological control strategies to tackle fungus. Another example of a mycovirus with hypervirulent effects is Cryphonectria hypovirus 1, which has hypovirulence effects on its fungal host and was also used as a biocontrol agent in controlling the chestnut blight (Ndifon & Chofong, 2023). Yuhui Niu et al. (2016 hypothesize that *Penicillium digitatum* virus 1 (PdV1) is used as a biocontrol for the citrus green mould (Wang et al., 2020).

The hypovirulent strain's possible lack of fitness presents another difficulty. These obstacles to using mycoviruses for biological control will be removed as research deepens our knowledge of the viruses' interactions with host fungi and the surrounding environment. Usually, the first area of concern when considering the possible field application of mycoviruses is the fungal pathogen's vegetative incompatibility system (Zhang *et al.*, 2020). A complicated VCG structure may hinder the mycoviruses ability to spread to natural fungal isolates (Jacquat *et al.*, 2020). Thus, ongoing research is being done to promote horizontal transmission of mycoviruses. However, other significant variables will affect the introduction of hypovirulence-inducing mycoviruses into the field, their dissemination, and disease management. For example, inquiries: For instance, investigations into the pathogen's life cycle, the disease's characteristics, and the characteristics of the affected crop or crops are crucial factors to consider. While treating apparent symptoms with mycoviruses may be the most straightforward approach, it is only a viable option for a limited range of plant illnesses (You et al., 2019). Furthermore, unlike diseases of woody plants, herbaceous crops harvested at the end of each growing season may not provide an environment conducive to the virus-colonizing pathogen populations in the field. The highly different environments provided by necrotrophic and biotrophic fungal infections can significantly influence the mycoviruses capacity to spread throughout the disease successfully (Sutela et al., 2019). Soil born and airborne infections, depending on the primary agents responsible for disease dissemination and the source of the inoculum (Vainio et al., 2024). Pathogens that persist in resistant structures without a host plant are less likely to spread hypovirulence.

Impact of mycovirus on host: To accurately determine how host viability is impacted by dsRNA mycoviruses, hence it is critical to evaluate identical genetically unaffected and infected species of fungi side-by-side (Vijayraghavan et al., 2023). Either we cure the viral replication in the recipient, or we employ transfection to produce isogenic hosts (infected and uninfected). For fungi with dsRNA infections, a variety of treatments have been discussed in the research, including being exposed to UV light, and warm temperatures and receiving treatments with a range of various compounds, notably cycloheximide emetine. Using cycloheximide, which also prevents the beginning and extension of translations during the process of protein synthesis, to treat infected fungi (Shen et al., 2021), which cures and lowers dsRNA levels, has shown to be the most successful and effective strategy to date. Although generally beneficial, treatments with cycloheximide are not always effective (Pizzol et al., 2021). To yet, dsRNA extracting from collections of treated and unseasoned fungi, examination of the lysates subsequent gel electrophoresis, and ethidium bromide labeling have been the only techniques utilized to gauge the effectiveness of treating fungi of mycovirus infections. A more reliable technique, such as RT-PCR proliferation with virus-specific primers, should be used to detect viral illness (Artika, Wiyatno, Ma'roef, & Evolution, 2020). The potential effects of the fungal virus lead to secondary metabolites, toxic production, and plant disease that increase the importance of mycovirus in environmental health research (Tonka *et al.*, 2022). The virus may influence fungus metabolism and genetics, opening a new door in experimental mycology (Gow *et al.*, 2022).

CONCLUSION

Phytopathogens are detrimental to plant survival because they cause damage to host plants and a decline in crop productivity. Continuous use of synthetic fungicides enhances microbial resistance; moreover, these compounds bioaccumulate in the food chains and have a negative health impact on plants, animals, and humans. We use mycoviruses as biocontrol agents, agents to mitigate fungal diseases, and agents to control economically important crop diseases; this is an emerging approach that continuously explores novel mycoviruses. If we can identify the hypovirulent mycovirus, we can control the fungal diseases. Various crops use the hypovirulence effect of mycoviruses as a biological control strategy to manage fungal diseases. It is even more crucial to find and characterize these mycoviruses in these hosts, as we now know that some of them induce hypovirulence and could serve as biocontrol agents for plant pathogenic fungi. Researchers continue to explore mycoviruses for their potential use as biocontrol agents, with the possibility of solving these difficulties in the future. There remain numerous challenges that require attention. We are currently studying mycoviruses for potential applications as biocontrol agents, and we may solve these challenges in the future.

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