



A biocontrol perspective on mycoviruses in fungal pathogen management

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Abstract

Mycoviruses, viruses that infect fungi, have been identified across nearly every fungal taxon. Despite their widespread presence, the ecological effects of mycoviruses remain poorly understood. They can influence the biology of their hosts in various ways, including altering growth, reproduction, and pathogenicity. Their ability to induce either fungal hyper- or hypovirulence and thus regulate general fungal fitness by increasing fungal aggressiveness or, conversely, in extreme cases, converting harmful fungi into beneficial ones, has attracted increasing attention in recent years as a potential means of protecting plants from fungal diseases and pests. Increasing difficulties in controlling fungal diseases, pests and weeds with synthetic chemical pesticides, exacerbated by the emergence of resistance or tolerance to certain active ingredients, and stricter regulatory requirements due to environmental and health concerns, have stimulated interest in alternative approaches. In parallel with the introduction of double-stranded (ds)RNA-based products for crop protection and the fundamental knowledge generated in this field in recent years, the potential use of mycoviruses to control pathogenic fungi appears to be within reach. This review highlights recent advances in the field and emphasizes the potential of mycoviruses as biological control agents (BCAs), with the emphasis on the utilization of mycovirus-induced fungal hypovirulence to control fungi that cause plant diseases and mycovirus-induced fungal hypervirulence to protect plants from fungal hosts such as insect pests or weeds.

Keywords Crop protection · dsRNA · Integrated pest management (IPM) · Mycovirus · Pattern-triggered immunity · RNA interference

Mycoviruses on the rise

Mycoviruses are ubiquitous in the fungal kingdom and have been identified in all taxa, and it is not uncommon for fungi to be infected with two or more unrelated viruses simultaneously (Myers et al. 2020; Hillman and Milgroom

2021). The initial mycovirus discovery was made in the commercially cultivated basidiomycete mushroom *Agaricus bisporus*, which was affected by La France disease (Hollings 1962). This disease originated on a commercial farm in southeastern Pennsylvania (Sinden and Hauser 1950) and subsequently spread to numerous mushroom-growing countries (Dieleman-van Zaayen and Temmink 1968). A variety of symptoms have been linked to the disease, including delayed or misshapen sporophore development, reduced yield, premature veil opening, and accelerated postharvest deterioration. Despite earlier suggestions that viruses may be responsible for certain abnormalities of fungi that apparently did not contain microbial pathogens (Blatný and Pilát, 1957), Hollings was the first to purify and identify several different types of viruses in the abnormal fungal spores, proposing a viral etiology for La France disease (Hollings 1962). However, it was not until 1987 that association of a characteristic double-stranded RNA (dsRNA) pattern with diseased sporophores provided the evidence for the viral etiology in La France disease

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(Wach et al. 1987). Hollings' finding established the field of mycovirolgy and initiated the discovery of the wide range of mycoviruses known today.

The detection of mycoviruses has long been hindered by two significant obstacles. Firstly, some mycoviruses do not manifest obvious symptoms in their fungal hosts, making them difficult to detect. Secondly, the purification of mycoviral particles and the development of a reliable infectivity assay to satisfy Koch's postulates have proven challenging in the past. Today, the advent of cheaper and faster sequencing, coupled with new metagenomic approaches, has led to a rapid increase in the number of virus families and species identified, regardless of the presence of abnormalities in fungal morphology or development (García-Pedrajas et al. 2019; Gilbert et al. 2019). The new techniques have revealed a highly intricate mycovirus diversity, with the number of known families and species rising from 9 and 80, respectively, in 2009 (Pearson et al. 2009) to 23 and 206 in 2022 (Kondo et al. 2022), and to 29 and 262 in 2023 (Villan-Larios et al. 2023) (Fig. 1).

Almost all known mycoviruses have dsRNA or positive sense single-stranded RNA (+ssRNA) genomes (Hough et al. 2023; Contreras-Soto and Tovar-Pedraza 2024; Urayama et al. 2024). Typically, an RNA mycovirus genome is less than 15 kilobases (kb) in length and encodes a maximum of 12 proteins, including an RNA-dependent RNA polymerase (RdRp), which is responsible for replicating viral RNA. Currently, phylogenetic analysis of the nucleotide sequences of RdRp enzymes in RNA viruses is the accepted standard for the correct categorization of mycoviruses. Additionally, the analysis may include other proteins such as coat protein, replication protein, or hypothetical proteins of unknown function (Ruiz-Padilla et al. 2021; Zhao et al. 2023).

While a number of ssDNA mycoviruses have been identified, there is currently no evidence of dsDNA mycoviruses. All are circular replication-associated protein-encoding single-stranded (CRESS) DNA viruses belonging to the family *Genomoviridae* (Myers and James 2022; Urayama et al. 2024). In comparison with RNA viruses, ssDNA mycoviruses have a very small genome, approximately 2 to 2.4 kb in length. Conserved motifs such as the replication-associated protein and/or capsid protein sequences are employed for phylogenetic analysis of these viruses (Varsani and Krupovic 2017; Zhao et al. 2019).

With regard to shape and structural analysis of mycoviruses, the majority of available information pertains to dsRNA viruses, whose genomes are often packaged in icosahedral capsids (Mata et al. 2020). However, they can also exist as ribonucleoprotein complexes that are not encapsulated, as is the case with polymycoviruses (Kanhayuwa et al. 2015). Several other viral forms have been described in the literature, including helical nucleocapsids, as for ssRNA mycoviruses, but also rigid rods, flexible rods, club-shaped particles, enveloped bacilliform particles and herpesvirus-like viruses (Varga et al. 2003; Buck 2018).

While considerable progress has been made in the detection and identification of novel mycoviruses, studies investigating the molecular interaction between a mycovirus and its host and the molecular effects of mycoviruses in a tripartite interaction between virus, pathogenic fungal host and infected plant, especially in natural environments, remain relatively limited. However, there has been a recent resurgence of interest in the area of mycoviruses as biological control agents (BCAs) in alternative and more environmentally friendly plant protection products (PPPs) to control fungal pathogens. The ability of mycoviruses to reduce the

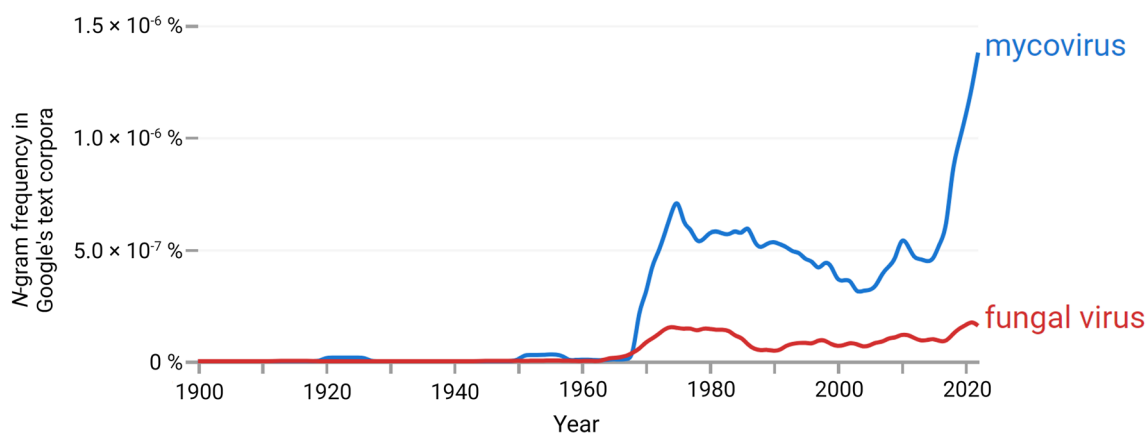


Fig. 1 Frequencies (yearly counts, normalized to the maximum counts; smoothing of 3 and case-insensitive) of two key terms—“mycovirus” and “fungal virus”—found in literature sources printed between 1900 and 2022. The initial publication to mention mycoviruses is typically attributed to the study of Hollings (1962). How-

ever, the term “mycovirus” was established only in the late 1960s and early 70 s. Prior to this, literature, including Hollings' original paper, use the term “virus-like particles” to describe mycoviruses (see also review Bozarth 1972). Analysis performed on <https://books.google.com/ngrams> on Aug, 2024 (Michel et al. 2011)

virulence of their fungal hosts, a phenomenon known as hypovirulence, represents a significant driving force behind their potential as PPPs (Fig. 2). Moreover, some mycoviruses can also induce hypervirulence (increased pathogenicity) in fungi that infect pests or weeds—a mechanism that could also be utilized in plant protection (Fig. 3). This review examines the most recent advances in the study of mycoviruses, with a particular focus on the molecular mechanisms by which they infect and suppress harmful fungi, offering insights into their potential application as BCAs in integrated pest management (IPM) strategies. Finally, it highlights the conditions that currently prevent or hinder the use of mycoviruses in sustainable plant disease management.

Mycoviral propagation strategies

The majority of mycoviruses remain poorly understood with regard to their natural vectors and the underlying mechanisms that facilitate their transmission. This knowledge is of crucial importance for the development of mycoviruses

as BCAs in plant protection, as data on host specificity and on potential effects on non-target organisms are fundamental requirements and criteria for approval of novel active substances and plant protection products (Regulation EU 283/2013 and 284/2013; see also Article 8 of Directive 2009/128/EC, European Commission 2013a, 2013b, 2009). In the natural environment, mycoviruses can spread horizontally through hyphal anastomosis, also known as vegetative fusion, where fungal hyphae from different individuals merge and allow the virus to pass between them. In addition, vertical transmission may occur during the formation of asexual conidia or sexual spores, allowing a virus to be passed directly from parent to offspring through the reproductive process. Thus, the ability and the efficiency of a mycovirus to spread depends heavily on the biology of the host fungus, including its reproductive strategy and ecological factors. Vertical and horizontal transmission ensure that a virus can persist and propagate both within the fungal population and across generations (Ayllón and Vainio 2023; Buivydaite et al 2024). Interestingly, *Sclerotinia sclerotiorum* mycoreovirus 4 (SsMYRV4, dsRNA family *Reoviridae*)

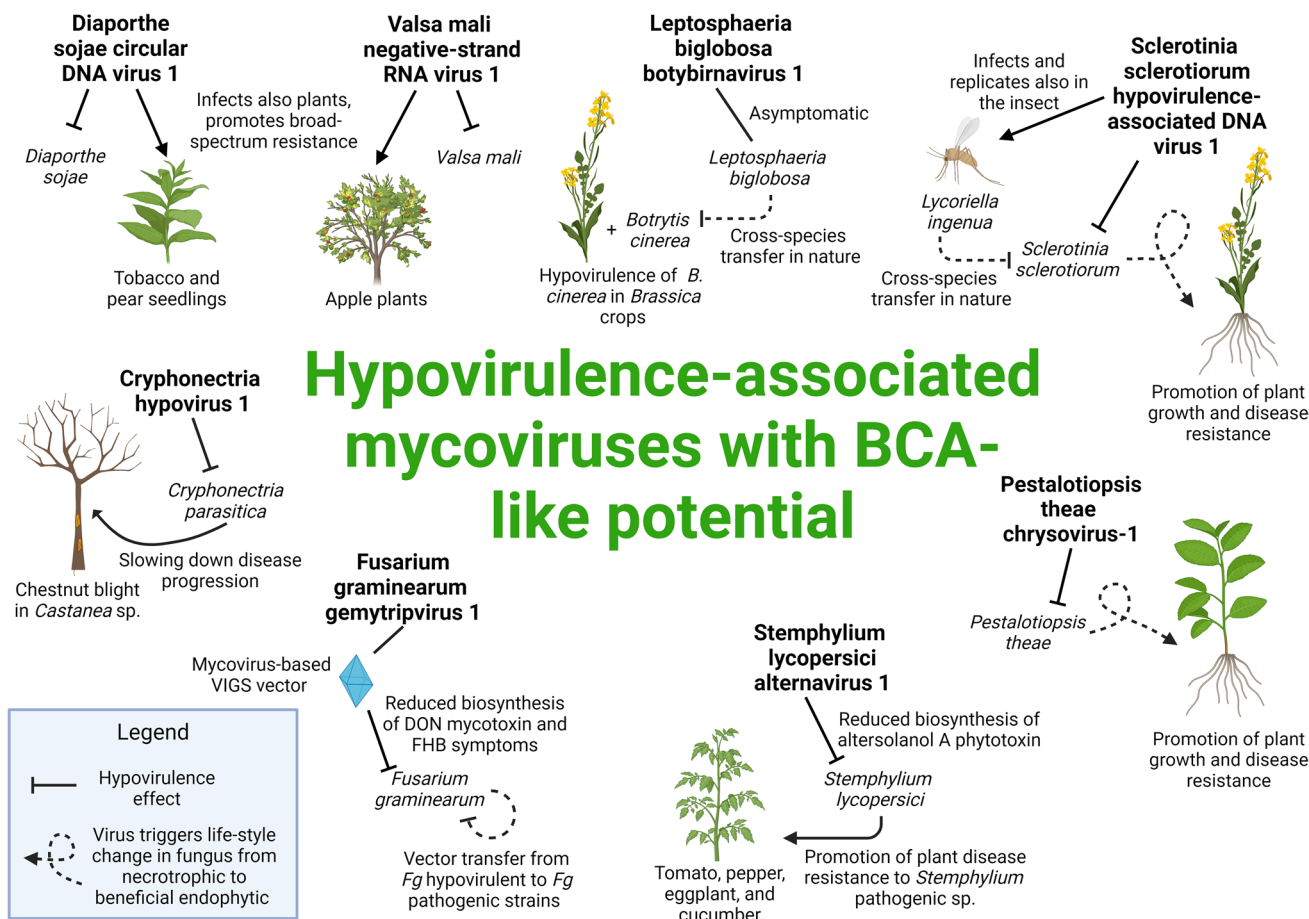


Fig. 2 Representation of known mycoviruses causing hypovirulence with BCA-like potential. Hypovirulence-associated mycoviruses can be used to reduce the virulence of plant-pathogenic fungi

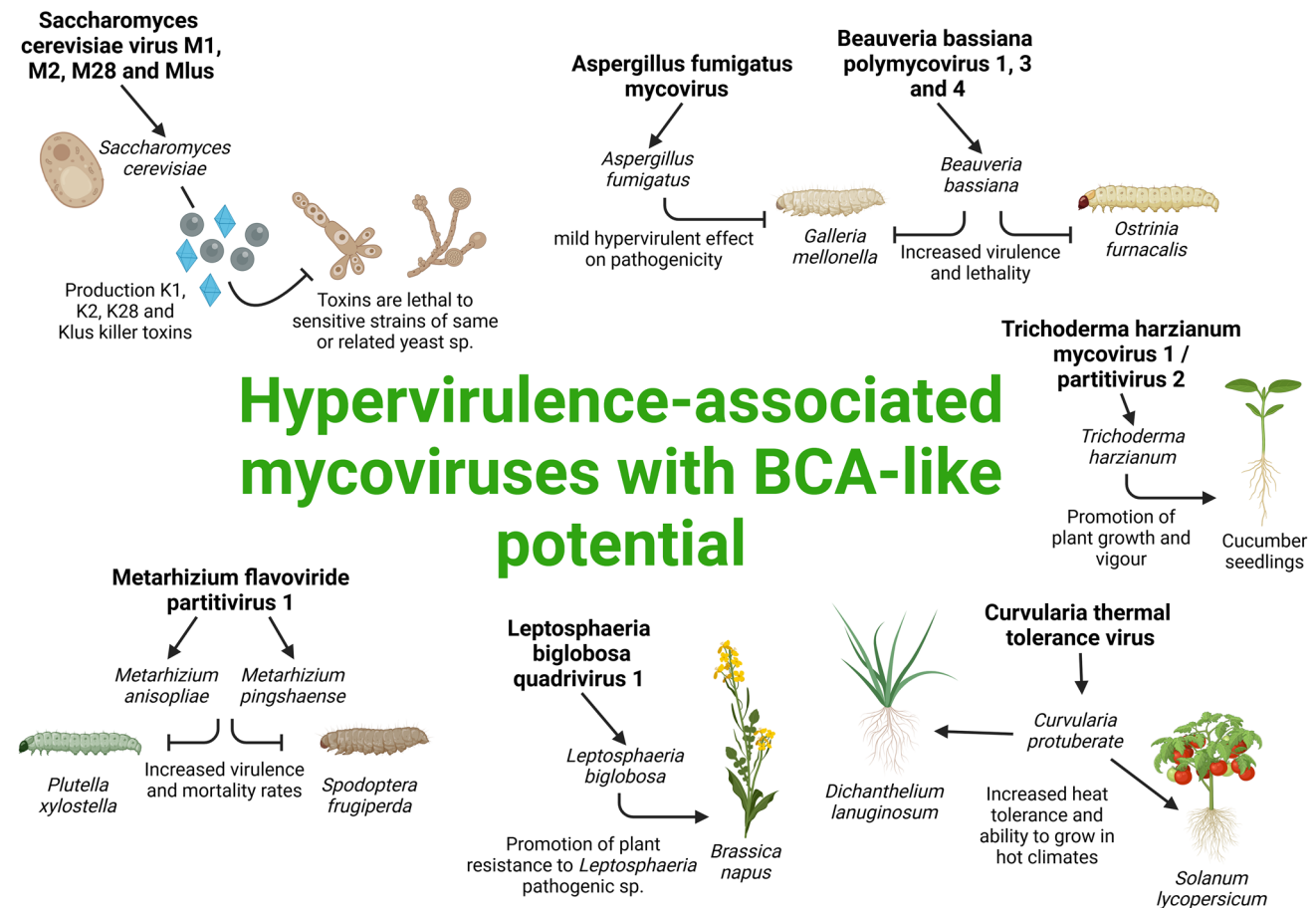


Fig. 3 Representation of known mycoviruses causing hypervirulence with BCA-like potential. Hypervirulence-associated mycoviruses can be used to enhance the growth of beneficial fungi to promote plant

growth and development. They can also be used to increase the virulence of fungi that infect insect pests and weeds

is capable of suppressing host non-self-recognition and thus facilitates the horizontal transmission of unrelated mycoviruses among *S. sclerotiorum* individuals belonging to different vegetative compatibility groups (VCGs), which genetically determine the ability of similar fungal isolates to form hyphal anastomoses or fungal fusion (Wu et al. 2017).

It should be noted that some mycoviruses retain their infectivity outside the host mycelium and are able to spread even to other non-fungal organisms. For example, *Sclerotinia sclerotiorum* hypovirulence-associated DNA virus 1 (SsHADV-1; ssDNA family *Genomoviridae*) is capable of replicating in both its fungal host, *Sclerotinia sclerotiorum*, and its transmission vector, the mycophagous insect *Lycoriella ingenua* (Yu et al. 2010; Liu et al. 2016b; Varsani and Krupovic 2017). Also, the *Valsa mali* negative-strand RNA virus 1 (VmNSRV1; ssRNA family *Phenuiviridae*) is capable of systemically infecting both fungal (*Valsa mali*) and plant (apple trees) hosts (Dai et al. 2023). Another example is the recently identified *Diaporthe sojae* circular DNA virus 1 (DsCDV1; ssDNA family *Genomoviridae*), which

also systemically infects both fungal (*Diaporthe sojae*) and plant (tobacco and pear seedlings) hosts (Wang et al. 2024).

In line with infectivity outside of fungal hosts, many researchers have hypothesized that RNA genome mycoviruses may be transmitted not only by horizontal and vertical spread in their fungal host but may also be adapted for transmission through non-fungal environments. *Penicillium aurantiogriseum* totivirus 1 (PaTV1, dsRNA family *Totiviridae*) and *Penicillium aurantiogriseum* partitivirus 1 (PaPV1, dsRNA family *Partitiviridae*) can replicate in plant protoplasts after artificial transfection without evidence of host adaptation or changes in their nucleotide sequence (Nerva et al. 2017). In addition, *Chrysovirus Magnaporthe oryzae* 1 (MoCV1, dsRNA family *Chrysoviridae*) is released from the mycelia of virus-infected *M. oryzae* into the culture supernatant, although the mechanisms of release are still unknown (Urayama et al. 2010, 2014; Higashiura et al. 2019). Consistent with this notion, purified particles of SsHADV-1 are infectious when applied extracellularly to *Sclerotinia sclerotiorum*. Virus particles isolated from an

infected host can infect the hyphae of virus-free *S. sclerotiorum* when applied to hyphae grown on potato dextrose agar or sprayed on leaves of *Arabidopsis thaliana* and *Brassica napus*, regardless of their specific VCG affiliation. A recent study has shown that the frequency of mycovirus horizontal transmission between incompatible strains of *S. sclerotiorum* is higher *in planta* than *in vitro*. The increased transmission efficiency is associated with elevated proline concentration in the plant following *S. sclerotiorum* infection (Hai et al. 2024). The authors suggest that increased proline levels attenuate the non-self-recognition reaction among fungi by inhibition of cell death, thereby facilitating mycovirus transmission. Field experiments showed that the combined application of hypovirulent *S. sclerotiorum* strains harboring hypovirulence-associated mycoviruses together with exogenous proline confers substantial protection to oilseed rape against virulent *S. sclerotiorum*. This finding provides an interesting new path for a biological control strategy aimed at mitigating fungal diseases in plants by enhancing the efficacy of horizontal mycovirus transmission (Hai et al. 2024). Additionally, it has been postulated that in a natural environment, such as forest or field condition, microarthropods or nematodes may facilitate the spread of virus particles between fungal communities (Brusini and Robin 2013; Petrzik et al. 2016).

Further studies on mycovirus transmission between fungi and other potential intermediate hosts are required to gain a deeper understanding of the diversity and ecology of mycoviruses, which is the basis for the development of mycovirus-based BCAs for plant-pathogenic fungi.

Mycoviruses as BCAs

It is not surprising that agricultural research is investigating mycoviruses with a view to developing them into future potential BCAs. There is currently a polyphonic debate about moving away from the use of synthetic chemicals for crop protection to more “natural” and sustainable bioprotectants—which use living BCAs and non-living, nature-based substances (NBSs; Stenberg et al. 2021; Galli et al. 2024). In this context, some mycoviruses may offer promising solutions for the development of smart and strategic alternatives to synthetic chemical fungicides. Currently, compared to the large number of virus-based BCAs for arthropod control (Holtappels et al. 2021; Wagemans et al. 2022), no mycovirus-based BCA is registered for commercial use in Europe and none is currently under evaluation (EU Pesticides Database, revised in August 2024, European Commission 2024). Infection constraints, especially the supposed lack of an extracellular phase of most mycoviruses, partially explains the absence of mycovirus-based BCA products currently available on the market. Despite recent findings in a few ssDNA mycoviruses,

as a rule, inoculum must be carried in the field and dispersed in the environment via the mycelia or spores of host fungi to be effectively utilized. Consequently, only environments that are already heavily infested with the fungal pathogen can be considered for disease control. One of the most extensively documented examples of a mycoviral-BCA application is the use of the + ssRNA virus *Cryphonectria hypovirus 1* (CHV1) to control the ascomycete *Cryphonectria parasitica*. In the 1970s, CHV1 was employed to reduce the devastating chestnut blight disease infecting American and European chestnut trees (respectively, *Castanea dentata* and *Castanea sativa*) (Turina and Rostagno 2007). CHV-1 induces a hypovirulent phenotype in *C. parasitica* by reducing the parasitic growth and sporulation capacity of the pathogen. This allowed the relevant authorities the opportunity to identify and remove infected plants, thus preventing or slowing down the spreading of the disease (Rigling und Prospero 2018). It is important to note, however, that in comparison with Europe, the genetic variation observed in American *C. parasitica* VCGs, as a consequence of sexual recombination, has historically limited the spread of hypovirus CHV1 between *C. parasitica* fungal strains. This had the effect of hindering control efforts (Pérez-Sierra et al. 2019).

As mentioned in the previous chapter, however, the production and use of stable infectious virus particles for direct application in the field is a key prerequisite for the widespread use of ssDNA mycoviruses as BCAs. The example of SsHADV-1 is promising: when applied to Brassica leaves, the mycovirus can prevent the development of sclerotinia stem rot lesions. SsHADV-1 can reduce disease severity and enhance yield significantly under field conditions. Moreover, SsHADV-1 has a narrow host range, ensuring the avoidance of off-targets; it can infect *S. minor* and *S. nivalis*, but cannot infect or transfect other tested fungi, such as *Botrytis cinerea*. Virus particles are likely to be very stable on the leaves because viral DNA could be detected at 15 dpi on leaves (Yu et al. 2013).

In addition to inducing hypovirulence, mycoviruses have also been shown to be capable of enhancing fungal lifestyles and pathogenicity (hypervirulence), which is of particular interest for pest and weed control, or for enhancing beneficial fungi that promote plant growth. The following chapters discuss viruses from both groups that have been demonstrated to be effective in controlling fungal pathogens. In the next chapters, we provide a summary of the most interesting mycoviruses from an agricultural perspective, with biocontrol-like activity in their fungal or plant hosts.

Mycoviruses causing hypovirulence

The most commonly reported effects associated with the hypovirulence phenotype can be broadly categorized as a reduction in fungal growth rate, an absence of sporulation,

an altered production of pigment and a reduced germination of spores. However, additional symptoms may be produced, such as diminished accumulation of specific fungal metabolites or a general reduction in fungal virulence (*in planta*) (Nuss 2005; García-Pedrajas et al. 2019; Kotta-Loizou 2021; Contreras-Soto and Tovar-Pedraza 2024).

The phenomenon of hypovirulence potentially covers a wide range of applications for controlling pests and diseases (see Fig. 2). In the last decade, many mycoviruses have been discovered that infect a long list of economically important plant pathogens. Several *Fusarium graminearum* mycoviruses reduce the virulence of *F. graminearum* and the production of mycotoxin such as Deoxynivalenol (DON) in Fusarium head blight (Li et al. 2019; Paudel et al. 2022). Reports also describe hypovirulence-associated mycoviruses in *Sclerotinia sclerotiorum* (Pedersen and Marzano 2023; Contreras-Soto and Tovar-Pedraza 2024), *Botrytis cinerea* (Ruiz-Padilla et al. 2021), *Magnaporthe oryzae* (Moriyama et al. 2018), *Cryphonectria parasitica* and *Rosellinia necatrix* (Suzuki 2017). Of note, Yu et al. (2010) have shown that treatment of Arabidopsis and *Brassica napus* plants with the hypovirulence-associated DNA virus SsHADV-1 has a protective effect against *S. sclerotiorum* infection comparable to that of carbendazim, a chemical fungicide commonly used for fungal control. Another interesting report showed that SsHADV-1 can change the lifestyle of its host, *S. sclerotiorum*, from that of a typical necrotrophic pathogen to that of a beneficial endophytic fungus (Zhang et al. 2020; Fu et al. 2024). The SsHADV-1-infected fungal strain DT-8 significantly regulates the expression of rapeseed genes involved in defense, hormone signaling, and circadian rhythm pathways. As a result, plant growth is promoted and disease resistance is enhanced (Zhang et al. 2020). Similar effects were observed in the *Poaceae*, wheat, barley and rice. The SsHADV-1-infected fungal strain DT-8 promotes wheat yield and consistently reduce *Fusarium* head blight and stripe rust (*Puccinia striiformis*) pressure in wheat and blast disease caused by *M. oryzae* in barley and rice (Tian et al. 2020). Another CRESS DNA mycovirus, DsCDV, identified and characterized from the pear-infecting fungus *Diaporthe sojae* shows nearly erasing virulence in its host, conferring broad-spectrum resistance against diverse pathogenic fungi in tobacco and pear seedlings (Wang et al. 2024). Similarly, fungal inoculation experiments with VmNSRV1 showed that this virus can be transmitted bidirectionally between apple plants and *Valsa mali*, and plant viral infection reduced the development of fungal lesions on leaves (Dai et al. 2023). Pestalotiopsis theae chrysovirus-1 (PtCV1, dsRNA family *Chrysoviridae*) has also been linked with the transition of *Pestalotiopsis theae*, a pathogen of tea, from a phytopathogenic to an endophytic lifestyle (Zhou et al. 2021). Inoculation of tea leaves with PtCV1-infected strains resulted in the development of resistance against the pathogenic *P. theae*

counterpart (Zhou et al. 2021). Similarly, Stemphylium lycopersici alternavirus 1 (SIAV1; dsRNA family *Alternaviridae*), which was isolated from the necrotrophic plant pathogen *Stemphylium lycopersici*, causes altered colony pigmentation and hypovirulence by reducing production of the phytotoxin altersolanol A (Liu et al. 2022). Interestingly, Liu and co-workers increased resistance to virulent infection with *Stemphylium* sp. when they expressed SIAV1 open reading frame 3 in *S. lycopersici* and sprayed hyphal suspensions on different host plant species. Finally, Leptosphaeria biglobosa botybirnavirus 1 (LbBV1, dsRNA genus *Botybirnavirus*), which typically asymptotically infects *L. biglobosa*, the causal agent of the blackleg disease (syn. Phoma stem cancer), was observed to transmit in nature to *Botrytis cinerea*, resulting in hypovirulence of the recipient strain in *Brassica* crops (Deng et al. 2022).

Mycoviruses causing hypervirulence

Infection with mycoviruses can also result in an enhanced fungal growth and a higher disease severity in a plant host. Effects can include an increased competitive ability, faster growth rate and increased production of plant host-specific toxins encoded in fungal genomes. A good overview of the subject can be found in recent reviews (Kotta-Loizou 2021; Kondo et al. 2022; Khan et al. 2023). Interesting examples include Puccinia striiformis virus 5 (PsV5; + ssRNA family *Narnaviridae*), which contributes to the full pathogenicity of the wheat stripe rust fungus in wheat and also increases the virulence of other fungal hosts, such as *F. graminearum* (Zhang et al. 2023b). *Alternaria alternata* chrysovirus 1 (AaCV1; dsRNA family *Chrysoviridae*) increases production of fungal AK-toxins in *Alternaria alternate*, resulting in the formation of larger lesions than those induced by the virus-free fungus on Japanese pear leaves (Okada et al. 2018). Similarly, a totivirus and a chrysovirus of the rice blast fungus *Magnaporthe oryzae* drive increased tenuazonic acid production, a non-host-selective mycotoxin that inhibits protein biosynthesis at the ribosomal level, which is particularly important for the establishment of infection by many necrotrophic fungi (Yun et al. 2015), by upregulating transcription of the transcription factor controlling expression of the tenuazonic acid synthase (Ninomiya et al. 2020). A similar phenomenon has been shown in *Aspergillus ochraceus*, where the *Aspergillus ochraceus* virus (AoV, dsRNA family *Partitiviridae*) causes the overproduction of the genotoxic and carcinogenic ochratoxin A, the second most important contaminant of food and feed commodities in Europe (Pfohl-Leszkowicz and Manderville 2012; Nerva et al. 2019). Also, *Saccharomyces cerevisiae* viruses Mlus, M1, M2 and M28 (ScV-M1, M2, M28 and Mlus; dsRNA family *Totiviridae*) are able to modulate secondary metabolite and mycotoxin-like production of the wine yeast *Saccharomyces*

cerevisiae. These “killer strains” produce and secrete killer toxins that are lethal to sensitive strains of the same or related virus-free yeast species (Rodríguez-Cousiño et al. 2011).

However, it would be wrong to assume that all hypervirulence-associated mycoviruses are inherently problematic for agricultural practices. From a farmer's perspective, hypervirulent mycoviruses also offer interesting opportunities, particularly when they increase the severity of fungal infections in macrobial pests or enhance the efficacy of fungal strains used for weed control or to boost agronomical traits (see Fig. 3).

Hypervirulence as a means of enhancing pest control

Entomopathogenic fungi from the genera *Beauveria*, *Metarhizium*, *Isaria* and *Lecanicillium* are already employed as active ingredients in numerous commercial biocontrol products (Lacey et al. 2015). Consequently, mycoviruses that enhance the efficacy of these fungal agents are of interest in the field of biocontrol. To date, this ability has been demonstrated in a limited number of mycoviruses: *Beauveria bassiana* polycyovirus 4 (BbPmV-4; dsRNA family *Polymycoviridae*) enhances the virulence of its host fungus *Beauveria bassiana* against the larvae of the moth *Ostrinia furnacalis* (Kang et al. 2023). Similarly, BbPmV-1 and 3 facilitate fungal growth, pigmentation, sporulation and pathogenicity against the greater wax moth *Galleria mellonella* (Filippou et al. 2021; Kotta-Loizou and Coutts 2017). Also, Guo et al. (2024), after successfully transfecting commercial strains of *Metarhizium anisopliae* and *Metarhizium pingshaense* with *Metarhizium flavoviride* partitivirus 1 (MfPV1, dsRNA family *Partitiviridae*), observed that MfPV1 enhances the virulence of the fungi against two important lepidopteran pests, namely the diamondback moth (*Plutella xylostella*) and the fall armyworm (*Spodoptera frugiperda*) (Guo et al. 2024). Finally, the combination of a partitivirus and another uncharacterized virus infecting *Aspergillus fumigatus* exerts a mild hypervirulent effect on pathogenicity when tested on *Galleria mellonella* (Özkan and Coutts 2015). Other types of mycoviruses have been identified in other entomopathogenic fungi, including *Metarhizium* sp. and *Paecilomyces* sp. However, no discernible impact on fungal fitness or virulence against *G. mellonella* and *Tenebrio molitor* larvae or whitefly *Bemisia tabaci*, respectively, could be observed (Wang et al. 2023; Santos et al. 2017; Azevedo et al. 2000).

Hypervirulence as a means of enhancing weed control

Some plant viruses have been investigated for use as BCAs in weed management, and in 2014, tobacco mild green mosaic virus (TMGMV) strain U2 was approved by the US

Environmental Protection Agency to control the tropical soda apple (*Solanum viarum*), an invasive weed in cattle pastures and natural areas in Florida (Charudattan 2023). With regard to mycoviruses as a means of enhancing mycoherbicidal activity, which is currently a primary area of interest within the broader context of biological control for weed management (Roberts et al. 2022; Basak et al. 2023), the available evidence remains very limited. While some mycoviruses have been identified in *Phoma* (Zheng et al. 2019), *Colletotrichum* (Zhong et al. 2014; Marzano et al. 2016) and *Alternaria* sp. (da Silva Xavier et al. 2018; Wang et al. 2022a), to name just a few of the most promising species with mycoherbicide potential, the majority of these studies in this area have not progressed beyond the identification of the viral particles, without testing for fungal virulence or pathogenicity.

Hypervirulence in plant-growth-promoting fungi

Mycoviruses can also be employed as agents to induce hypervirulence in beneficial fungi that promote plant growth (see Fig. 3). In the fungal genus *Trichoderma*, which is already extensively utilized in agricultural settings as a BCA to prevent soil-borne plant diseases, the presence of *Trichoderma harzianum* mycovirus 1 (ThMV1, + ssRNA family *Partitiviridae*) and *Trichoderma harzianum* partitivirus 2 (ThPV2, dsRNA family *Partitiviridae*) enhances the growth of cucumber plant seedlings (Liu et al. 2019; Wang et al. 2022b). Pagnoni et al. (2023) also list a catalog of new viruses identified in association with a European population subset of *Trichoderma* sp., offering new opportunities for plant protection (Pagnoni et al. 2023).

Leptosphaeria biglobosa quadrivirus 1 (LbQV-1; dsRNA family *Quadriviridae*) enhances biomass production, host colonizing capacity and colony diameter in *Leptosphaeria biglobosa*, a mildly virulent fungus in the *Leptosphaeria* sp. that causes blackleg disease in canola. However, canola leaves previously infected with *L. biglobosa* (which contains LbQV-1) showed increased resistance to the other aggressive pathogen, *L. maculans* (Shah et al. 2020). Another very renowned mycovirus in this context is the *Curvularia thermal tolerance virus* (CThTV; dsRNA family *Curvulariviridae*). It was originally isolated from the endophytic fungus *Curvularia protuberata*, a species in the family *Pleosporaceae* (Márquez et al. 2007). The virus-infected fungus confers heat tolerance to *Dichanthelium lanuginosum* (panic grass) growing in geothermal soils. The study revealed that virus-free fungal isolates were unable to confer heat tolerance to plants. Most importantly for its potential use as a BCA, heat-induced resistance could also be transferred to other plant species, like dunegrass and tomato (Rodríguez et al. 2008). Several CThTV-like viruses have also been identified in ectomycorrhizal fungi (Sutela and Vainio 2020;

Sahin et al. 2021; Guinto and Balendres 2023). The impact of these viruses on fungal and plant abiotic resistance may prove to be of economic importance in agriculture, particularly in relation to their potential use as microbial plant biostimulants, opening up a new target for research.

Molecular interactions between mycoviruses and their fungal hosts impacting biocontrol activity

In order to develop mycovirus-based BCA products with enhanced resilience, further mechanistic analysis is required to gain a more comprehensive understanding of fungal antiviral RNAi and other stress responses and viral defense suppression.

Fungal RNAi pathways mediate control of mycoviruses in their hosts

The molecular changes induced by mycoviruses in their fungal hosts are not well understood (Sato and Suzuki 2023). Mycoviral dsRNA can induce RNA interference (RNAi) (Bocos-Asenjo et al. 2022), an antiviral defense that is conserved in most eukaryotes, including fungi (Fire et al. 1998; Lax et al. 2020; Torri et al. 2022; Jin et al. 2022). With a few exceptions, such as *Ustilago maydis* and *Saccharomyces cerevisiae*, most fungal species possess a functional RNAi machinery, including the key enzymes DICER-LIKE (DCL), ARGONAUTE (AGO) and RNA-DEPENDENT RNA POLYMERASE (RdRp). In the fungus *Neurospora crassa*, viral infection upregulates the transcription of RNAi components (Honda et al. 2020).

Consistent with the notion that the fungal RNAi pathway controls mycovirus pathogenicity, *Cryphonectria parasitica* *dcl2* and *ago2* mutants challenged with CHV1-EP713 exhibited a reduced growth and altered colony morphology and increased levels of viral RNA (Segers et al. 2007; Sun et al. 2009). Similar observations were made with *Colletotrichum higginsianum*, where loss of either DCL1 or AGO1 leads to de-repression of ChNRV1, consequently accumulating at very high levels (Campo et al. 2016). Additional evidence was also reported in *Aspergillus flavus* (Jiang et al. 2023), *M. oryzae* (Nguyen et al. 2018), *F. graminearum* (Yu et al. 2018, 2020) and *S. sclerotiorum* (Mochama et al. 2018; Neupane et al. 2019). In plants, RNAi-based defense against geminiviruses involves complementary posttranscriptional and transcriptional gene silencing mechanisms associated with cytosine methylation of the viral ssDNA (Rodríguez-Negrete et al. 2009; Paprotka et al. 2011). Likewise, RNAi plays an important role against ssDNA genome viruses also in fungi. For example, the circular ssDNA viruses Botrytis gemydayirivirus 1 (BGDaV1), which confers hypovirulence

to its natural host *B. cinerea*, is a target for RNA silencing and genomic DNA methylation, keeping it at very low titers (Khalifa and MacDiarmid 2021). However, although both DCL and AGO proteins are important players in antiviral defense, AGO proteins may be dispensable for antiviral defense in some fungi (Sato et al. 2024). Collectively, these data show that the fungal RNAi machinery is a key factor in understanding mycovirus infections of fungal hosts and exploiting them for crop protection.

It is largely unclear which stress pathways are induced by mycoviruses in their fungal hosts

Of note, a recent report shows that application of exogenous dsRNA, in addition to its RNAi effect, induces several stress responses in fungi and the same phenomenon might happen when dsRNA viruses are replicating in their hosts (Ladera-Carmona et al. 2024). The latter authors show that uptake of dsRNA across a broad size range of 21 to about 3,000 bp by *M. oryzae* conidia induces aberrant germ tube elongation and canonical fungal stress pathways, as evidenced by nuclear accumulation of the stress marker MITOGEN-ACTIVATED PROTEIN KINASE HOG1p and the production of reactive oxygen species (ROS). These data show that the effects of dsRNA on fungi can be diverse and complex, which has implications for the development and use of dsRNA mycoviruses in agricultural practice, especially if they were to be applied exogenously.

Also, largely unexplored is the molecular basis how mycoviruses can evade host RNAi or other immune/stress responses. It has been proposed that RNA silencing suppressors, such as the papain-like protease p29 protein in CHV1, enhance viral RNA accumulation of both homologous and heterologous viruses (Suzuki et al. 2003; Segers et al. 2006; Sun et al. 2006). Other proteins that are capable of suppressing host antiviral RNA silencing include p48, which is required for the initiation but not the maintenance of viral RNA replication, as observed in CHV1 (Deng and Nuss 2008), the autocatalytic protease p24, which suppresses the induction of DCL2 in *C. parasitica* (Aulia et al. 2021), and p20 suppressor with sRNA binding capability in *Fusarium graminearum* hypovirus 1 (FgHV1, + ssRNA family *Hypoviridae*) (Wang et al. 2022c). Interestingly, also the capsid proteins of partitiviruses, such as *Tulasnella partitivirus* 1 and 2 (dsRNA, family *Partitiviridae*), in orchid mycorrhizal fungi have been shown to function as RNAi suppressors both in plants and fungi, although its mechanism remains unknown (Shimura et al. 2022). Further studies are required to explore the antiviral activity of fungal hosts and the RNAi-based counter-defense suppression potential of mycoviruses to develop resilient and effective mycoviral-BCA solutions.

Biotechnological applications of mycoviruses

Currently, novel approaches to control pests and diseases in agriculture include the development of dsRNA-based BCAs able to trigger RNAi in their essential genes. In 2023, the first sprayable dsRNA biocontrol product (Ledprona[®]) targeting a gene in the Colorado potato beetle (*Leptinotarsa decemlineata*) to protect potato crops was registered with the US Environmental Protection Agency (Rodrigues et al. 2021). Concurrently, additional promising tools for the protection of plants against viruses, insects, as well as fungal pathogens and oomycetes based on dsRNA application are also in development (Bocos-Asenjo et al. 2022; Galli et al. 2024). Also, infection by viruses engineered to carry stretches of nucleotide sequence homology with a target gene is used to silence specific genes in a wide range of plant and insect species through virus-induced gene silencing (VIGS) (Dømmes et al. 2019). Densovirus have been investigated as VIGS vectors for the prevention of mosquito-borne diseases, such as malaria, by targeting genes in *Anopheles gambiae* and *Aedes aegypti* (Ren et al. 2008; Liu et al. 2016a). From the perspective of plant protection, VIGS is also interesting for its ability to target insect and fungal genes (Rössner et al. 2022). It seems plausible that, in the future, mycovirus-based VIGS will emerge as a highly effective technique for the specific silencing of fungal genes thus generating hypo- or hypervirulent fungal strains that could be employed to control economically significant pests and diseases. Notably, the feasibility of this strategy has already been demonstrated. Recently, Zhang et al. (2023a) constructed a mycovirus-based VIGS vector targeting several *Fg* endogenous genes utilizing *Fusarium graminearum* gemytripvirus 1 (FgGMTV1, ssDNA family *Genomoviridae*) which converted *F. graminearum* into a hypovirulent strain, resulting in a considerable reduction in the incidence of FHB in wheat. Interestingly, the team also demonstrated a reduction in DON mycotoxin accumulation in the hypovirulent strain and that the VIGS vector could be horizontally transmitted from the hypovirulent to the pathogenic strains under laboratory conditions (Zhang et al. 2023a; see also Fig. 2). An additional strategy to use mycoviruses as BCA is by expression of the mycovirus transcript or genome in the host fungus. This could happen by transfection a synthetic transcript into the host fungus or by expression of a full-length mycoviral cDNA clone from the fungal genome under control of a fungal promoter. Marzano et al. (2015) achieved successful transfection of *S. sclerotiorum* with a recombinant strain of hypovirus SsHV2 using a synthetic transcript. Guo et al. (2024) conducted a transfection of commercial strains of

M. anisopliae and *M. pinghaense* with MFPV1, resulting in a notable increase in mortality rates of larvae of *S. frugiperda* and *P. xylostella* (see also Fig. 3).

These studies offer experimental proof that the introduction of mycoviruses, in the form of a VIGS platform or via nucleotide transfection into (entomopathogenic) fungal species, can result in notable alterations in virulence. This exemplifies the potential of mycoviruses as a synergistic agent to enhance the biocontrol capabilities of fungi against pests.

Integrating mycoviruses into a IPM strategy

Integrated pest management (IPM) aims to control pests and diseases through a combination of methods in which the use of chemical pesticides is limited to what is necessary, with priority given to biological, biotechnological, plant breeding, cultural and agronomic measures. Accordingly, the search for alternatives to synthetic chemical PPPs, including BCAs, and their implementation in crop protection strategies is a key task for agronomic research. However, further reduction of chemical pesticides is a critical and risky strategy in the face of a still growing population, reduced global yields due to climate change and increasing global political crises. To prevent a decline in the efficiency of agricultural production and to protect the health of consumers and users as well as the environment, much more sophisticated and knowledge-based crop protection measures will be needed in the future (Rosa et al. 2022; Goulet et al. 2023; Galli et al. 2024). So far, the employment of chemical PPPs will remain an important part of future agricultural practices to ensure food safety and crop productivity (von Tiedemann 2024). Consequently, the integration of mycoviruses with other disease management strategies, including physical, mechanical, biological, and chemical pest control methods, along with the use of resistant crops, must be a key agronomic strategy. This is also in line with the principles of the IPM approach outlined in Annex III of Directive 2009/128/EC (European Commission 2009), which supports the prevention and/or suppression of pests through a range of options, aiming for a holistic and synergistic effect rather than a single control measure. It is plausible that in the future, the combination of mycoviruses with other (bio)control agents, including beneficial fungi and predatory insects, could enhance the overall effectiveness of pest and disease management strategies, offering a more comprehensive and sustainable solution. While mycoviruses have the potential to directly weaken pathogenic fungi or weaken insect pests indirectly via their fungal hosts, other beneficial organisms used in IPM may contribute by outcompeting these pathogens and pests in the environment, thereby maintaining pathogen and pest pressure at a low level.

Current limitations in the use of mycovirus-based BCAs

As has been demonstrated above, the application of mycovirus-based BCAs could represent a promising approach for the management of fungal plant pathogens, pests and weeds. However, there are still some significant challenges associated with the implementation of these agents.

Regulatory hurdles

Deploying mycoviruses as BCAs involves navigating a complex regulatory landscape. To address potential regulatory issues that may arise during the registration and approval process, it is essential to gain a deeper understanding of the basic biology of mycoviruses. In Europe, the commission Regulations (EU) No 283/2013 and No 284/2013 (European Commission 2013a, 2013b) define the data requirements for the authorization of active substances and plant protection products, respectively, on the EU market, in accordance with Regulation (EC) No 1107/2009 (European Commission 2009). Such information includes detailed data on the identity, physical and chemical properties, and potential effects of the agent on human health, animal health, and the environment. Mycoviruses, being relatively new and less understood as BCAs, pose challenges in meeting the extensive data requirements. Many questions relating to the life and disease cycle, fungal host specificity and the molecular mechanisms underlying parasitism in its host and in the affected crops need to be answered. For the majority of mycoviruses, there are insufficient data on their behavior, interactions, and long-term effects, as the available information is often limited to a small number of cases involving a particular mycovirus, fungus and plant. In addition, the way in which the mycovirus is formulated, stored and the inoculum is dispersed into the environment must also be addressed, as these conditions can vary considerably for airborne or soil-borne fungal pathogens, further complicating the regulatory framework and successful development of mycovirus-based BCAs (Teixidó et al. 2022). Ensuring effective and safe use of mycoviruses requires addressing these variables comprehensively.

Transmission efficiency

Another major challenge often encountered in the study of mycoviruses is their low transmission efficiency. The fungal vegetative incompatibility system, which limits the spread of potentially harmful genetic material within different fungal strains with different VCGs, is usually the first concern for potential field application. Complex fungal VCGs hinder

the transmission of a mycovirus to natural fungal isolates, limiting their spread in natural environments. It is therefore not surprising that horizontal transmission has become an active area of research (for review, see Buivydaite et al. 2024). Of interest in this regard is the mycovirus SsMYRV4, which has the ability to suppress host non-self-recognition and programmed cell death, thereby facilitating horizontal transmission of other co-infecting mycoviruses between *S. sclerotiorum* individuals (Wu et al. 2017). A detailed analysis of the fundamental molecular processes involved could offer new insights into how to optimize horizontal transmission, which could ultimately be harnessed to enhance the efficacy of future mycovirus-based BCAs.

The efficiency of mycovirus dispersal is also strongly influenced by vertical transmission. A number of studies have demonstrated that the transmission rates of mycoviruses to conidia can vary significantly depending on the virus and host strains involved (Lee et al. 2014). In *C. parasitica*, CHV2 infection exhibits poor vertical transmission, which correlates with limited dispersion of the virus within host populations (Hillman and Suzuki 2004). However, commensal interactions between CHV4 and MyRV2 in *C. parasitica* facilitate stable infection and enhanced vertical transmission of MyRV2 (Aulia et al. 2019). A deeper understanding of the mechanisms controlling horizontal and vertical transmission processes is fundamental to improving the mycovirus' ecological fitness and persistence in the ecosystem. The dilemma here is that while broader species efficacy would be interesting from a market perspective to maintain regulatory and commercialization costs, it could also compromise product biosafety.

Biosafety and environmental stability

Due to their intracellular nature, mycoviruses can remain stable under changing environmental conditions. This long-term stability and persistence require careful monitoring and further research to prevent unintended ecological effects. The ability of mycoviruses to mutate, undergo genetic changes and evolve within the fungal host (Wolf et al. 2018) will make it difficult to mitigate any adverse effect. Furthermore, the introduction of mycoviruses into field environments may have unintended consequences, as mycoviruses may also have unwanted effects on non-target organisms. It has been shown that several plant and fungal viruses share a high degree of genetic similarity (Sun et al. 2020; Andika et al. 2023) and that most mycovirus families also have members that infect plant species (King et al. 2018). It is therefore not surprising that cross-kingdom mycoviral infections between fungal and plant hosts are common in nature (Andika et al. 2017; Wei et al. 2019; Bian et al. 2020; Cao et al. 2022; Andika et al. 2023; Dai et al. 2023). The question of whether, and with what consequences a virus can adapt

to both organisms and spread to plant and fungal populations in nature remains unresolved. This is important from a plant pathology perspective as it could lead to unpredictable outcomes such as the emergence of novel plant diseases or unpredictable interactions between mycoviruses and their hosts. Studies of cross-kingdom virus infection in plants and fungi are still in their early stages, and more long-term monitoring is essential for continued assessment of variation in effectiveness and stability of mycovirus-mediated control, and more fungal viruses from different taxa should be tested for infectivity in plant cells.

Conclusion and next steps

Considerable progress has been made in the study of mycoviruses as potential biological agents, and current evidence suggests that mycoviruses could be used in the near future to control a wide range of fungal pathogens by direct infection with mycoviruses to induce hypovirulence, as well as pests and weeds by indirectly inducing hypervirulence of their fungal pathogens. In recent years, a number of mycoviruses have been identified with the potential to control a wide range of pathogens important in food and agricultural production. Moreover, mycoviruses with broad host ranges and the ability to induce hypovirulence, when artificially transferred to heterologous hosts, could be employed in fungi where naturally occurring mycoviruses are lacking or have yet to be identified. Furthermore, several methods to manipulate virus–fungus interactions to enhance their efficacy are being developed.

To unlock the full potential of mycoviruses as effective and sustainable tools in pest management, it is essential to: *i.* identify and characterize new mycoviruses and their role in fungal fitness; *ii.* understand how novel mycoviruses interact with their fungal hosts and the molecular principles that regulate cross-kingdom mycovirus infection; *iii.* monitor the environmental impact of the use of mycoviruses in agriculture in terms of non-target organisms and persistence in the environment; *iv.* develop commercialization and practical application methods that allow scalable methods of formulating and delivering mycoviruses to agricultural fields. In summary, the next steps in mycovirus research require a multifaceted approach that includes further mycovirus discovery, understanding of the molecular mechanisms of action between mycovirus and its host, further studies on the effect of synergistic applications of mycoviruses and other crop protection methods, studies on their environmental safety and practical example of mycovirus-based BCAs in IPM strategies.

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Conflict of interest The authors disclose no conflicts of interest.

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