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# Biological control of pathogenic fungi: Can mycoviruses play an important role?

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# Biologická ochrana proti patogenním houbám: budou hrát mykoviry důležitou úlohu?

Tomáš TONKA, Lucie WALTEROVÁ (✉), Vladislav ČURN

Faculty of Agriculture and Technology, Studentská 1668, 370 05 České Budějovice, Czech Republic

✉ Corresponding author: krizol05@zf.jcu.cz

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## ABSTRACT

In the past few years, huge economic and environmental losses have resulted by fungal pathogens in agriculture and food production worldwide. Fungal toxins also pose a threat for food production and animal health. In the last decade, the site-specific fungicides has been used in plant protection, providing highly efficient protection against phytopathogens. However, the use of pesticides boosted problems with resistance in the targeted organisms and increased negative environmental impact of insecticides, herbicides and fungicides. Therefore, new methods are needed to control resistant populations of plant pathogens, including fungi. Mycoviruses are fungal viruses, i.e. viruses infecting fungi. Some mycoviruses affect the growth and virulence of the host fungus and may prevent successful infection of plant host. They have potential to reduce phytopathogens. In this review, the successful examples how mycoviruses alter hypovirulence in well-known phytopathogens are discussed. Mycoviruses could be useful as a one of new next generation pest-control products.

**Keywords:** biological control, fungal viruses, dsRNA, ssRNA, plant protection

## ABSTRAKT

Houbové patogeny způsobují na zemědělských plodinách celosvětově velké ekonomické ztráty. V posledních několika desítkách let se úspěšně používala celá řada fungicidů, které omezovaly vliv houbových infekcí na pěstované plodiny, ovoce, zeleninu a okrasné rostliny. Negativním jevem spojeným s masivním používáním fungicidům je, že celá řada fytopatogenů získala rezistenci k chemickým přípravkům, nehledě na dopad používání pesticidů na necílové organizmy a životní prostředí. Proto v poslední době sílí snaha o nahrazení chemických prostředků na ochranu rostlin prostředky šetrnějšími k životnímu prostředí a lidskému zdraví. Jedním z těchto agens jsou mykoviry. Mykoviry jsou viry, které infikují houby, včetně houbových patogenů. Byla popsána celá řada mykovirů, které negativně ovlivňují virulenci a růst houbových hostitelů, a tím zabraňují k šíření infekce v hostitelské rostlině. Z tohoto pohledu by mohl výzkum mykovirů a jejich použití v ochraně rostlin přinést do budoucna významné výsledky.

**Klíčová slova:** biologická ochrana, houbové viry, dsRNA, ssRNA, ochrana rostlin

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## INTRODUCTION

Viruses are the smallest of all self-replicating organisms and in the basic form they consist of small segment of nucleic acid enveloped in a simple protein shell. They have no metabolism and they invade cells and obligatory parasitise subcellular environments. They most likely infect every organism in nature displaying a huge diversity of structures and life strategies. The only extracellular form of virus life cycle is a virus particle.

It may not be appropriate to write about useful viruses at a time when we do not know if the SARS-CoV-2 epidemic is over, but viruses are not just harmful organisms. Despite the fact, that viral infection of plant, animals and humans can have huge economic and societal impact, it must be mentioned that viruses can also be beneficial.

Viruses, as a part of microbial agents (entomopathogens), are often referred as biopesticides in biological control of insect pests. The description of these viruses is outside the subject of this contribution but is covered in many reviews published in last decades (Hajek, 2004; Hajek et al., 2007; Abd-Alla et al., 2020), We can also mentioned releasing European myxoma virus into the Australian rabbit population in the 1950 where the kill rate of virus was calculated as more 99% (Fenner et al., 1957). The eradication programme using European myxoma virus has been considered to succeed.

Using mycoviruses in biological control has been first described in chestnut blight epidemics in Europe, North America and Japan 50 years ago (Turchetti and Maresi, 1993; Milgroom and Cortesi, 2004). Since that, the interest in fungal viruses considerable increased, and plenty of new mycoviruses are, therefore, described using the new molecular approaches to study viruses in the host. The development of deep sequencing technology (metagenomics) has greatly promoted the discovery of new viruses. Increasing research has supported that mycoviruses are ubiquitous in the kingdom fungi, including edible, entomopathogenic, and phytopathogenic fungi (Herrero et al., 2012; Sutela et al., 2019). The discovery of novel viruses by high-throughput sequencing (HTS) has revealed a remarkable diversity (García-Pedrejas et al., 2019).

In this review, we summarized mycovirus infecting fungi, and focuses on some traits that alter phenotype of the fungal hosts together with mycoviruses of pathogenic fungi as a potential agent in the biological control of serious phytopathogens.

## FUNGAL VIRUSES

Mycoviruses are viruses that infect fungi and multiply inside fungal tissue and cells. Mycoviruses replicate in the cytoplasm but some replicate in mitochondria of host cells (mitovirus) (Göker et al., 2011; Milgroom and Hillman, 2011). In 1948, 'La France disease' of the cultivated mushrooms (*Agaricus bisporus*), characterized by deformed fruiting bodies and yield loss, was reported in Pennsylvania. Similar diseases were described from Europe and Japan. Fourteen years later, this report led to the discovery of fungal viruses (Hollings, 1962). This was the first description of the virus particles inside the fungal host. Fungal viruses (mycoviruses) infect hosts representing different fungal taxa (Herrero et al., 2009, 2013; Nerva et al., 2019).

If there is described approximately 120 000 known fungal species (and many more unknown species still waiting for discovery), it is expected that huge diversity of undescribed mycovirus species occur in nature. It has been estimated that 30 - 80% of fungal species can be infected with mycoviruses (Ghabrial and Suzuki, 2009).

First report about mycoviruses was provided with fungi of economic importance, for example, edible mushroom, and phytopathogenic fungi. For last decades, the description of hundreds mycoviruses showed an increasing trend accelerated by molecular approaches, such as metagenomics and next-generation sequencing (Marzano and Domier, 2016; Nerva et al., 2016; Gilbert et al., 2019).

## HOST AND TRANSMISSION

Although taxonomically diverse, fungal viruses share a cycle of transmission within fungal hosts (Villarreal, 2008). There is no extracellular phase in mycovirus life cycle and horizontal transmission in nature is provided by intracellular hyphal anastomosis (the union of hyphae resulting in cytoplasmic exchange) when the two hyphae are vegetatively compatible (Zhang et al., 2014). The dissemination of fungal spores is a mode of vertical transmission of fungal viruses in nature (Ghabrial et al., 2015; Sutela et al., 2019). Inside the host the cell division spreads the virus. There are no data on extracellular transmission with the exception of *Sclerotinia sclerotiorum* hypovirulence-associated DNA virus 1 (SsHADV-1) that could infect a mycophagous insect, *Lycoriella ingenua*, and use it as a transmission vector (Liu et al., 2016). The same authors described the extracellular transmission of purified SsHADV-1 virus particles that enter host fungal cells on media.

The presence of vector organisms for fungal viruses in nature – nematodes, oribatid mites, mite *Thyerophagus corticalis* - were described in several studies (Hillman et al., 2004; Yaegashi et al., 2013; Simoni et al., 2014; Petrzik et al., 2016). Although there is such evidence of fungal virus transmission vectors, these suggestions have to be more carefully investigated. Novel mycovirus in mycelia of *Rosselinia necatrix*, phytopathogen causing white root rot on apples, during soil incubation has led to the hypothesis that feeding by microarthropods or nematodes may facilitate interspecies transmission of RNA viruses (Yaegashi and Kanematsu, 2015).

Mycoviruses have been reported to infect all major taxonomic groups of fungi. The true fungi, Chytridiomycota, Zygomycota, Basidiomycota and Ascomycota, harbour mycoviruses as well as phytopathogenic oomycetes such as *Phytophthora* sp. or *Pythium* sp. (Gillings et al., 1993; Hacker et al., 2005; Pearson et al., 2009; Herrero et al., 2012). Mycoviruses have also been found in human pathogenic fungi (Kotta-Loizou and Coutts, 2017).

The vegetative incompatibility represents a major barrier to the successful horizontal transmission of fungal viruses between fungal species or strains. The hyphae do not fuse and the programmed cell death is initiated preventing the horizontal transmission of mycovirus (Wu et al., 2017).

The majority of the mycoviruses are associated with latent infections; however, some of the fungal viruses are involved in hypovirulence and can be used in biological control of fungal pathogens (*Cryphonectria parasitica* hypovirus). Some mycoviruses exhibit visible signs of infection, including abnormal colony morphology, growth reduction and can alter pigmentation and sexual reproduction (Jiang et al., 2013; Ghabrial et al., 2015).

## DIVERSITY AND TAXONOMY

In the beginning of the fungal virus research has been percepted that all mycoviruses belong to the dsRNA viruses. It has substantially changed as we have uncovered more about their genome structure and impact on their fungal hosts.

The virus determination is based on the number and size of their genomic segments (Ghabrial and Suzuki, 2009). Mycoviruses harbor different types of genomes, such as double-stranded RNA (dsRNA), single-stranded positive RNA (+ssRNA), single stranded negative RNA (-ssRNA) and circular single-stranded DNA (ssDNA) genome (Ghabrial et al., 2015). Fungal viruses are classified into several viral families, some of them harbour only mycoviruses, some of them, such as in family *Partitiviridae* different viruses infecting plants, animals, and fungi are grouped together. Currently, mycoviruses are classified in dsRNA genome families *Amalgaviridae*, *Chrysoviridae*, *Megabirnaviridae*, *Partitiviridae*, *Polymycoviridae*, *Reoviridae*, *Quadriviridae*, *Totiviridae*, and genus *Botybirnavirus*, in ssRNA+ genome families *Alphaflexiviridae*, *Deltaflexiviridae* and *Gammaflexiviridae*, *Barnaviridae*, *Botourmiaviridae*, *Endornaviridae*, *Hypoviridae*, *Mitoviridae*, *Narnaviridae* or ssRNA- genome family *Mymonaviridae*, and the reverse transcribing *Metaviridae* and *Pseudoviridae*. Only one DNA fungal virus, reported to date, with a circular ssDNA is a member of family *Genomoviridae* (Lefkowitz et al., 2018; Kotta-Loizou, 2021). But dozens of described mycoviruses remain unclassified. Because many new species are currently being described, the list of all mycoviruses on the ICTV (International Committee of Taxonomy of Viruses) web page is updated regularly (ICTV 2021).

Most fungal viruses have small genomes encoding only a few proteins (Ghabrial et al., 2015; Lefkowitz et al 2018). The simplest known are mitoviruses (*Narnaviridae*) encoding only one protein, RdRP (RNA dependent RNA polymerase), required for virus replication (Sutela et al., 2019). Mitoviruses replicate in mitochondria (and members of family *Botourmiaviridae*), other known fungal viruses replicate in cytoplasm. Mitovirus is the simplest positive-strand RNA virus frequently occurs as a coinfection in mycovirus infections (Hillman et al., 2018). Highly similar viruses tend to exclude each other, thereby preventing mixed virus infection (Kashif et al., 2019).

Antagonistic and mutualistic interactions between mycoviruses have been recently reviewed (Hillman et al., 2018). The virus—fungus—plant interactions are divers, involving beneficial, neutral or harmful effects. The tripartite mutualism among host plant, an endophytic fungus and a mycovirus has been described as a fascinating example (Márquez et al., 2007). Curvularia thermal tolerance virus infecting endophytic fungus *Curvularia protuberata* confers heat tolerance not only to endophyte host but also to plant host of the fungus. It has also been reported that the fungi are infected by two or even more non-related viruses containing defective dsRNA (Howitt et al., 2006; Ghabrial and Suzuki 2009). Certain mycoviruses are capable to replicate in taxonomically diverse fungi. Mitoviruses found in *Ophiostoma novo-ulmi* and *Sclerotinia homeocarpa* are the best examples of such viruses (Deng et al., 2003; Sharma et al 2018). Same mycovirus can have different effects on their host depending on ecological conditions (Hyder et al., 2013).

Fungal viruses are not normally associated with clearly defined symptoms. Effects on the development, vegetative growth, and physiological properties of virus infection are main alterations in infected host life cycle. In addition, even asymptomatic infections may affect host organism under stress conditions. In *Heterobasidion* sp. interactions of partitiviruses or related viruses are either beneficial or harmful, depending on virus strain and environmental conditions (Vainio and Hantula, 2016). Some mycoviruses can change phenotype of the infected fungi, can change pigmentation, reduce growth and sporulation of the host and some mycoviruses debilitate disease or reduce virulence of their fungal hosts (Ghabrial et al., 2015).

The evolutionary relationship between fungal viruses and their hosts remains unclear. There are two main hypotheses to explain the origins of mycoviruses (Ghabrial, 1998; Pearson et al., 2009). The first theory assumes that fungal viruses are ancient organisms of unclear origin and evolved in close relationships with fungal hosts. The second theory concludes endophytic origin of mycovirus that they move from the plant host to the endophytic fungus recently.

## HYPOVIRULENCE AS A TOOL FOR BIOLOGICAL CONTROL

Mycovirus-induced hypovirulence is a phenomenon that can occur in virus infected fungi. Hypovirulence is defined as attenuated fungal virulence mediated by virus infection, mitochondrial defects, or mutations in fungal genome (Ghabrial and Suzuki, 2008). In other word, it is a reduction of the virulence of the plant pathogenic fungi either directly, or by debilitation. Hypovirulence of animal fungal viruses has not been described with the exception of the mild hypervirulence of the four segmented dsRNA tetramycovirus-1 in *Aspergillus fumigatus* (Kanhayuwa et al., 2015). Hypovirulence is associated with phenotypic changes like reduced pigmentation, reduced sporulation and growth defects (Hillman et al., 2018). The changes in phenotype are carried out by changes of the fungal gene expression and is induced by means of RNA silencing, altering gene expression and disruption transcriptome (Nuss, 2005; Nuss, 2011). Hypovirulence connected with mycovirus infections and its potential to serve as biological control agents has always been of much interest (Nuss, 2005).

The phenomenon of hypovirulence was discovered in the middle of last century. The chestnut blight fungus *Cryphonectria parasitica* devastated chestnut tree in North America at the beginning of the 20th century has been introduced also in Europe. In the 1950s it was noticed that chestnut trees in some orchards in Italy recovered from fungal infection and *C. parasitica* stopped spreading. The virus isolated from this fungus showed low virulence in laboratory. This report provoked the direction of a biological control strategy and, in addition, biological control program to treat chestnut orchards (Heiniger and Rigling, 1994; Dawe and Nuss, 2001).

Up to date, dozens of mycovirus description of hypovirulence have been reported. Some of these fungi induced serious diseases of plants that are of economically or agriculturally importance (Nuss. 2005, García-Pedrejas et al., 2019).

As mentioned above, the effect of hypovirulence was first described in Chestnut blight pathogen *C. parasitica* strains isolated from poorly pigmented (in contrast to the bright-orange necrotic segments) strains in Europe (Kyrychenko et al., 2018). Cryphonectria hypoviruses 1 - 4 (CHV1 - 4) are grouped in genus Hypovirus in family *Hypoviridae* (Nuss and Hillman, 2011). They infect fungus *C. parasitica* in Europe, Asia, and North America. Strains CHV1 - 3 altered fungal morphology and are transmitted through hyphal contact to infect mycelium and through conidiospores (Ghabrial et al., 2015; Rigling and Prospero, 2017).

The genome organization of CHV1-4 has been reported (Hillman et al., 2004). Infectious cDNA clone of the CHV1-EP713 hypovirus was constructed from RNA. These cDNA copies confer hypovirulence when used to start an infection. cDNA is integrated into the nuclear DNA of *C. parasitica* or bypassed a synthetic copy of hypovirus RNA directly into fungal spheroplasts. These transfected CHV1-EP713 RNA system was introduced in South Africa strain of *C. parasitica* related *C. cubensis* to control Eucalyptus canker disease. Recently, this system has also been introduced in the *Phomopsis* and *Valsa ceratosperma*, fruit tree pathogens, and resulted in decreased infectivity in both pathogens. *C. parasitica* infected with CHV1 hypovirus reduced production of the orange pigment that is produced by virus-free strains. It is a marker to differentiate infected and uninfected fungal strains.

In France the large biocontrol programme during 1960s and 1970s employed hypovirulent *Cryphonectria parasitica* hypovirus strain to control blight in chestnut orchards. Later the hypovirulent strains continued to struggle with chestnut blight in European orchards. These programs has been proved successful reducing extent of the chestnut blight epidemic as a result of a natural spread through European chestnut forests.

*Sclerotinia sclerotiorum* is a plant pathogen of oilseed rape, soybean and numerous vegetable crops widely distributed across the world. It is known as a notorious plant fungal pathogen of phylum Ascomycota and attacks more than 400 species of plants, including Brassicacae, Fabacae and Solanacae (Jiang et al., 2013). *S. sclerotiorum* is well known to produce white mold and causes dark lesions on the stem which develop patches of white fungal growth. It is serious pathogen through sclerotia, overwintering hyphal aggregates which can infect crops in the following seasons. If left untreated, it could potentially infect 100% of crops (Heffer Link et al., 2007). To control *S. sclerotiorum*, fungicides are used to control disease, which leads to many negative consequences on environmental safety, including fungicide resistant isolates.

Hypovirulence and dsRNA viruses in *S. sclerotiorum* were first reported 30 years ago, but without any further interest (Boland, 1992). *Sclerotinia sclerotiorum* hypovirulence-associated DNA virus 1 (SsHADV-1 strain DT-8) has been reported to induce hypovirulence in host fungus as a potential biocontrol agent for *S. sclerotiorum* (Yu et al., 2010). It is a first DNA virus reported to infect fungus. In recent paper it has been showed that SsHADV-1 infected *S. sclerotiorum* fungus acts as an endophyte and reduces growth as compared with virus-free strains (Zhang et al., 2020). The reduced growth effect was confirmed in field experiments, when the hyphal fragment suspension infected with SsHADV-1 have been sprayed on rapeseed plants. In addition, the authors advised that endophytic strain (DT-8) could transmit the SsHADV-1 virus to a vegetatively incompatible virulent strain of *S. sclerotiorum*.

Some other promising hypovirulent virus strains were isolated in last decades. *Sclerotinia sclerotiorum* debilitation-associated RNA virus (SsDRV) was isolated from sclerotia of diseased eggplant *Solanum melongena* (Xie et al., 2006). Virus infected strain grows slowly on PDA medium with abnormal hyphal branching and colony morphology. *Sclerotinia sclerotiorum* hypovirus 1 (SsHV-1) was isolated from rapeseed (*Brassica napus*) in China and is mostly related to CHV2-3 in the family *Hypoviridae* (Xie et al., 2011). *Sclerotinia sclerotiorum* mitovirus 4 (SsMV4) isolated from *S. sclerotiorum* strain AH16 has conferred hypovirulence in host fungus (Ran et al., 2016).

A mycovirus mediated hypervirulent strain of *Leptosphaeria biglobosa* enhanced systematic acquired resistance of B. napus against *L. maculans* (Shah et al., 2020). SsHADV-1 virus infected *S. sclerotiorum* strain exhibits an endophytic lifestyle in rapeseed after having lost their virulence and showed substantial changes in expression changes in genes associated with pathogenicity. The endophytic lifestyle benefits host plant growth by stimulating plant growth and improving resistance to two fungal pathogens of rapeseed with broad host ranges. Small 2-kb genetic element can affect host leading to a three-way mutualistic symbiosis (Zhang et al., 2020).

*Alternaria alternata* is a fungal Ascomycete pathogen that causes leaf spot diseases on various crop plants. It is considered as a weak pathogen that infects dozens of agronomically important plants. Once infecting the host, *A. alteranta* is able to induce spots on leaves, causing defoliation and loss in yield and quality of products. Thus far, number of mycoviruses were described from *Alternaria* spp. (Chen et al., 2017; Xavier et al., 2018; Shamsi et al., 2019). Among them, *Alternaria alternata* chrysovirus 1 has been reported to modify fungus growth (Okada et al., 2018). Some of previously identified mycoviruses from *A. alternata* appeared to alter host growth (Fuke et al., 2011). Recently, *Alternaria alternata* hypovirus 1 (AaHV1) has been described conferred debilitation in *A. alternata* and other plant phytopathogenic fungi (Li et al.. 2019a). AaHV1 is transmitted vertically through conidia with 95% efficiency. The virus infected *A. alternata* isolates clearly grew slower than virus-free isolates indicated that AaHV1 confers hypovirulence. The ability of AaHV1 to attenuate fungal virulence suggests that this hypovirus can be candidate to study virus-fungus interactions as a potential use as a biological control agent for fungal crop diseases.

*Fusarium* is a genus of filamentous fungi of the phyllum Ascomycota. Many members of this genus are known to be a serious phytopathogens. Since exhibits broad host range, this filamentous fungus can infect any crop including maize, rice, wheat and barley (Sharma et al., 2018). For example, *F. graminearum* has a narrow host range and mainly infects the cereals, *F. oxysporum* infects broad range of plants. Fusarium sp. also produce mycotoxins that affect public health (Munkvold, 2017; O'Donnell et al., 2018).

The control of disease is complicated. Excessive growth in the use of fungicides have arised resistant populations of *Fusarium* but the struggle has been made to control disease and minimize serious yield losses. Therefore, utilizing a promising strategy against *Fusarium* using bioagents, such as *Trichoderma* sp., bacteria and yeasts or plant growth promoting rhizobacteria have been tested with different results (Sadfi et al., 2002; Recep et al., 2009; Wachowska et al., 2013; Akrami and Yousefi, 2015). Till date, no effective and environmental friendly programme to control *Fusarium* sp. have been concluded and, thus, the biological control of *Fusarium* sp. disease using mycoviruses may be a promising way.

Up to date, 29 mycoviruses from different species of *Fusarium* have been identified. Four mycoviruses have harmful effect on *Fusarium* sp. phenotypes (Li et al., 2019b). *Fusarium graminearum* hypovirus 2 (FgHV2) reduced mycelial growth and conidia production and also suppress mycotoxin production (Li et al., 2015). *Fusarium graminearum* virus 1, positive ssRNA virus, has been reported to reduce virulence of *F. graminearum*, increase pigmentation and reduce the production of mycotoxin (Chu et al., 2002, 2004; Yu et al., 2009). Reduced mycelial growth, conidia production and virulence in wheat and maize plants are associated with hypovirulence strain *Fusarium graminearum* virus (FgV-ch9) (Darissa et al., 2012). *Fusarium oxysporum* f. sp. dianthi mycovirus 1 (FodV1) altered vegetative growth and virulence of the fungal host (Lemus-Minor et al., 2018).

*Botrytis cinerea* is a pathogenic fungus infecting wide range of ornamental plants, fruits and vegetables. Grey mold is a disease caused with this fungus. Strawberries, raspberries, pears and grapes are major fruits affected with large yield losses. The control of grey mold is often unsuccessful due to emergence of the fungicide resistant isolates. As of other phytopathogenic fungi, mycoviruses have also been described in populations of *Botrytis* sp. (Vilches and Castillo, 1997; Castro et al., 2013; Rodríguez-García et al., 2014). Recently, *Botrytis cinerea* RNA virus 1 (BcRV1), was successfully revealed as the hypovirulent strain of *B. cinerea* (Yu et al., 2015). BcRV1 clearly affected growth of mycelium and significantly decreased pathogenicity of the fungus with accumulation of virus in host cells. The description of BcRV1 is a first step for the further study of mycoviruses for the biological control of grey mold caused by *B. cinerea* strains.

*Rhizoctonia solani* is a soil-borne pathogen causing yield and economic losses in vegetables, field crops, ornamental plants and trees worldwide. In *R. solani* a range of dsRNA and ssRNA viruses have been described up to date and some of them altered host fungal development in the process of hypovirulence (Zheng et al., 2014 ; Zhong et al., 2015; Li et al., 2018; Abdoulaye et al., 2019; Zhang et al., 2021). *Rhizoctonia solani* partitivirus 2 altered size of lesions on rice leaves (Zheng et al., 2014) and transmission of *Rhizoctonia solani* endornavirus 1 resulted in disorders of metabolism in the host and caused hypovirulence (Zheng et al., 2019).

## OTHER FUNGAL PATHOGENS

There are a plenty descriptions of new mycoviruses that influence host fungi through the hypovirulence. A detailed description how the pathogenic fungi are regulated with mycoviruses is outside the scope of this review and was reviewed recently elsewhere (Ghabrial et al., 2015; Muñoz-Adalia et al., 2016; García-Pedrejas et al., 2019). Very often a new description of phytopathogenic fungus infected with virus potentially useful in biological control is reported. For example, Valsa ceratopsperma hypovirus 1 (VcHV1) closely related to SsDHV-1 from fruit tree pathogenic fungus, Valsa ceratosperma confers hypovirulence (Yaegashi et al., 2012). *Cryphonectria parasitica* hypovirus 1 and Mycoreovirus 1 isolated from *C. parasitica* were introduced into *Valsa mali* revealed potential biological control agents for apple Valsa canker disease (Yang et al., 2021). *Rosellinia necatrix* megabirnavirus (RnMBV1) isolated from *R. necatrix* has been confirmed to effectively control plant pathogenic fungal diseases under field and greenhouse conditions and showed significant potential for biological control (Chiba et al., 2009). Forest pathogenic fungi, such as *Phytophthora* sp., *Ophiostoma* sp., *Heterobasidion* or *Armillaria* sp. are reported to harbour mycoviruses to greater or lesser extent (Muňoz-Adalia et al., 2016; Vainio et al., 2018; Linnakoski et al., 2021; Sutela et al., 2021; Walterová et al., 2021).

## FUTURE PERSPECTIVE

There is no doubt that fungal pathogens will continue to acquire resistance to widely used fungicides (Bolton et al., 2005). Mycoviruses would be one of the alternatives to chemical agents. Apparently, many examples of the successful application of mycoviruses in the control of the fungal diseases of plants exist. In all described examples, the result of fungal virus control is mostly accompanied by the vegetative compatibility between infected and uninfected fungal strains/species. There are some obstacles to widely use mycoviruses in biological control, including vegetative incompatibility of the fungal host. The shortest time possible is the demand for farmers for the effective biological control product. Thus, time of successful establishment of the virus population in the plant/fungal pathogen system is crucial. However, the efficiency of viral infection in the field is not likely to fulfil this demand. The vegetative incompatibility between the strains is the main barrier to use them as agents of biological control. In addition, the anastomosis limits the transfer of the mycovirus to virus-free fungal strains. The method how to control crop diseases was to let the virus spread among fungal pathogens and subsequently cause a decline in pathogen virulence in the field (Milgroom and Cortesi, 2004; Zhang and Nuss, 2016).

Some mycoviruses reduce spore production, causing slow mycelial growth or less aggressive invasion in hosts, making virus effective in biocontrol (Nuss, 2005; Milgroom and Hillmanm, 2011). Forest, horticultural and ornamental plants harbour mycoviruses to the greater or lesser extent. Although many of them have not been found to be use for biocontrol purposes, many of them provide new opportunities for research in plant pathology science. The modern scientific approaches, such as metagenomics or next generation sequencing promise the development of new biotechnologies and virus strains could be manipulated to be more virulent and effectively transferred to incompatible hosts. The protoplast virus transfer method appears promising in our path to finding more effective mycoviruses struggle with plant phytopathogens (Nerva et al., 2017).

Virus based biopesticide formulations were used to eradicate rhinoceros beetles in Samoa or serious coniferous insect pests in North America. Gypsy moth nucleopolyhedrovirus (Lymantria disapar NPV) was suggested as a microbial agent early in the 20th century (Hajek et al., 2007). Major success with biological control of invasive pests using viruses occurred in the middle of 20th century against European pine sawfly in North America.

## CONCLUSIONS

Fungal pathogens cause huge crop damages and yield losses around the world. The struggle with pathogenic fungi needs efficient and sustainable strategies to control plant pathogens. More than 200 viral species have been detected in fungi to date but their roles in fungi are largely unknown (Gilbert et al., 2019). Although, there are no reports of mycoviruses killing their host fungi during the fungal life cycle, many fungal viruses significantly reduce the pathogenicity of host fungi and hypovirulence-associated mycoviruses provide unique opportunities to study fungus/plant relationships. Studies involving particular fungal-viral systems have provided interesting insights into virus-virus and virus-host interactions (Hillman et al., 2018).

The control program of serious insect, weeds, pathogens and diseases of economically importance is responding to the need for alternatives to chemical pesticides. Recent developments in our knowledge influenced the development of novel biological agents that are playing and will play an important role in future pest control system. These new biopesticides include, among others, viruses. Biological control represents the next generation of environmentally friendly products with potentially new modes of action aimed at controlling serious plant pathogens. Mycoviruses could be useful as a one of these new next generation pest-control products and, as described above, are a very interesting system to investigate plant/fungus/virus or virus/virus interactions in the case of plant/fungal/virus coinfections.

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