



Forest Tree Virome as a Source of Tree Diseases and Biological Control Agents

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Abstract

Purpose of Review The collective virome of forest trees can be considered to include not only plant viruses, but also viral communities harbored by all tree-associated organisms. In this review, we will concentrate on reviewing recent developments in the two fields of forest tree virology that have received the most research input during the last 5 years: (1) current knowledge of virus diseases affecting forest trees and their causal agents and (2) fungal viruses (mycoviruses) and properties that are required for utilizing them for biocontrol purposes.

Recent Findings The discovery of plant and mycoviruses has been revolutionized during the last few years due to high-throughput sequencing (HTS). This has altered our view of virus diversity and prevalence, but also their host ranges, transmission routes, and host effects. Utilization of HTS has greatly expanded our knowledge of plant virus diversity and disease etiology in forest trees and revealed the commonness of cross-kingdom transmission events between fungi, oomycetes, plants, and arthropods. Research on mycoviruses has also identified several new mycoviruses that restrict the growth or virulence of forest pathogenic fungi.

Summary Gaining knowledge of the collective virome of forest ecosystems is essential not only for understanding virus evolution and diversity but also for improving our understanding on virus impacts, and our ability for biocontrol-based and environmentally friendly management of viral and fungal diseases that affect economically important plants and beneficial insects, and for preventing possible disease outbreaks in the future. Virus infections play a central role in plant health, but viral symptoms on forest trees remain often unrecognized and may be confused with other biotic or abiotic damages. However, recent studies have revealed previously unknown viruses as causes of forest tree symptoms and suggest that viruses are responsible for far greater economic losses than recognized earlier. However, many knowledge gaps still need to be filled, particularly on the diversity of viruses that infect different species of forest trees, their irregular distribution within the plant, their mode of transmission, epidemiology and choice of hosts also regarding crop plants, their effect on the metabolism of their host tree, and their interaction with other microorganisms. Mycovirus research has already deciphered detailed information on many critical properties that affect utilizing them for biocontrol purposes. Still, more knowledge is needed concerning mycoviral transmission mode and stability in field conditions, the level of host tolerance against mycoviral infection, and the occurrence of interspecies mycovirus transmission in nature, and safety issues related to these topics.

Keywords Plant viruses · Forest pathology, Epidemiology · Forest virome · Mycovirus · Hypovirulence

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Introduction

The health of forest trees can be threatened by various pathogens such as fungi, oomycetes, bacteria, and viruses, as well as insect and nematode pests. According to the current state of knowledge, fungi, oomycetes, and insects are regarded as the most significant damage agents in terms of economic loss caused to the forest industry [1, 2]. But over the last decade studies on plant viruses in forest and urban trees have confirmed the common occurrence of viruses and allow the assumption that these pathogens contribute to loss of vitality and tree damage leading to tree decline [3••]. All the tree-associated organisms harbor their own virus communities (viromes), and the collective tree virome can be considered to include all of these [4••]. From the viewpoint of host tree health, viruses can have two major roles: plant viruses acting as disease agents and viruses of tree pathogens and pests that may act as enemies of enemies, thereby benefiting the tree.

In this review, we will concentrate on reviewing recent developments in the two fields of forest tree virology that have received the most research input during the last 5 years: (1) plant viruses as a source of forest tree diseases and (2) fungal viruses (mycoviruses) as a source

of biocontrol agents. We will focus on recent literature published during the last 5 years (2017 to present), but also refer to earlier reports with essential findings. Finally, we will discuss potential inter-kingdom virus transmission pathways in the forest ecosystem and recommend future research goals.

Plant Viruses in Forest Trees and Agroforestry Systems

The first reports on forest tree viruses were published in 1935 and were based on visual inspection only [5]. After almost a century of efforts in forest virology research, it is apparent that pathogenic viruses seriously deteriorate tree health and can cause emerging infectious diseases. Knowledge on viruses infecting forest trees was significantly expanded when high-throughput sequencing (HTS) studies were employed on several deciduous trees [3••, 4••, 6•]. Based on current knowledge, the 19 most common European forest tree or shrub species are affected by at least 43 different plant virus species (Fig. 1 and Table S1), the majority of which are positive-sense single-stranded ((+) ssRNA) viruses. Most virus species of forest trees are host-specific pathogens. Interestingly, the newly discovered genus *Emaravirus* (order

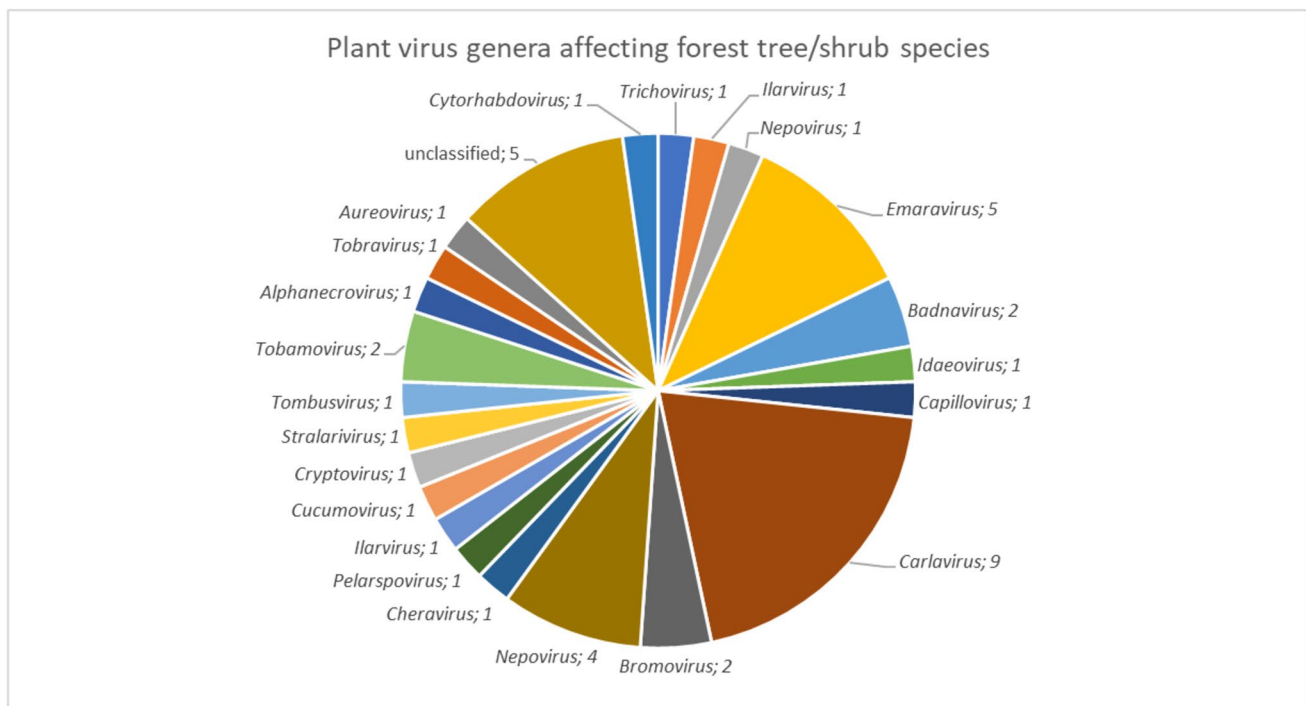


Fig. 1 Diversity of plant virus genera affecting the most common forest tree/shrub species. The names of the virus genera and the number of virus species belonging to these genera are annotated. The diagram is based on the list of plant pathogenic viruses published in Rumbou et al. (2021) [4••], also including two novel viruses discovered later:

the ash shoestring-associated virus (ASaV) in ash and the *Cytorhabdovirus tilliae* in lime. The listed viruses infect the host maple, birch, chestnut, ash, Norway spruce, pine, poplar, oak, elderberry, mountain ash, elm, and beech. Unclassified viruses belong to the families *Caulimo*-, *Amalga*-, *Flexi*-, and *Partitiviridae*

Bunyavirales; family *Fimoviridae*) is represented in the forest tree virome by six new species, and there are also two newly discovered reverse-transcribing dsDNA viruses, one in birch and one in chestnut, both belonging to the *Badnavirus* genus (Table 1). In some forest trees (e.g., whitebeam and lime tree), the virome was examined for the first time only recently [7, 8•]. Within single hosts it was found that multiple virus species or even multiple variants of the same species may be simultaneously present.

Phenotypic Effects on the Hosts and Disease Diagnosis

Symptomatology is the primary tool for viral disease diagnosis. Plant pathogenic viruses may cause various leaf symptoms in deciduous trees, which serve as the initial indication of viruses in trees. Good training on recognizing virus-specific symptoms is needed for differential diagnosis [3••]. Macroscopic symptoms include various patterns on the leaves, like discoloration, leaf chlorosis, and necrosis (Fig. 2) and viruses can affect the size, shape, and quality of the trees, seedlings, fruits, seed, or/and wood [3••, 9–11]. Reduced seed production and twig deformation may occur, and tree growth might be hindered, which could ultimately contribute to the tree's overall decline. There is a range of characteristic virus-related symptoms; however, several symptoms resemble nutrient deficiencies or impact of biotic/abiotic causes. Often, multiple causes interact, making it difficult to attribute symptoms to a specific cause. Symptom observation is particularly challenging in conifers, and coupled with difficulties in extracting total nucleic acids, this results in limited knowledge of the virome in conifers to date. Additionally to the symptomatology, the sampling process of symptomatic samples is highly significant. The selected samples, the sample size, and the timing of the sampling can greatly affect the success or failure of detection assays.

While symptomatology provides the foundation for identifying the causes of plant damage, to determine whether a virus is associated with the expression of particular symptoms, it is necessary to additionally examine a significant number of non-symptomatic samples. In many instances, the viral causal agent of a disease is absent from the virome of non-symptomatic samples. This was observed in infections of trees with emaraviruses, such as *Emaravirus aceris*, *E. populis*, *E. quercus*, and *E. sorbi* and ash shoestring-associated emaravirus, where the non-symptomatic samples were always virus-negative [6•, 7, 12••, 13]. However, this could not be confirmed in birch, where some plant viral genetic material could still be detected in non-symptomatic samples. In the latter case, it becomes difficult to determine the causal agents of a disease or to associate certain viruses with specific symptoms (see specific case studies in the “[Latest Case Studies of Forest Tree Viruses](#)” section).

Latest Case Studies of Forest Tree Viruses

In earlier research, virus-related symptoms in forest trees had quite often been attributed to generalist viruses occasionally detected in affected trees. Latest investigations based on HTS methods have revealed that in many cases the presence of these generalist viruses was not correlated with the disease [4••] (Table 1), whereas certain newly discovered viruses were confirmed to be the causal agents. These novel findings offer a more realistic view on the pathogenic virome of forest trees, and exemplar cases are described below.

Birch leafroll disease (BLRD) causing a severe decline in birch trees was first described in Finland in 2002 (Table 1). BLRD has since been reported in five European countries with varying climates. Diseased trees exhibit leaf symptoms such as vein banding, leaf roll, chlorosis, and subsequent necrosis, causing a loss of vigor and degeneration in the trees (Fig. 2 B1, B2, B3). Before the application of HTS tools, BLR-related symptoms in birch were attributed mainly to cherry leaf roll virus (CLRV) and partly to tobacco necrosis virus and tomato ringspot virus [14]. Lately, HTS analysis revealed a complex virome consisting of both novel and known viruses that correlate with the disease. The novel birch leafroll-associated virus (BLRaV) — associated with symptom appearance — is a reverse-transcribed dsDNA badnavirus that could not be detected with conventional diagnostic methods. Additionally, mixed infections involving other viruses from the group of carla-, ideo-, and capilloviruses, may also play a significant role in the symptom development [15, 16••, 17] or may be present in non-symptomatic samples. HTS-based studies revealed the non-significance of several generalist viruses previously thought to be associated with the disease (Table 1).

Chestnut mosaic disease (ChMD) was first identified in Italy several decades ago, where it was associated with viral symptoms, but it was only recently that a new virus called chestnut mosaic virus (ChMV) was discovered in affected trees, believed to be a strong etiological candidate of ChMD [18]. Studies conducted in France and Central Eastern Italy have shown that ChMV is prevalent in some commercial orchards [18]. ChMV sequences have also been found in *Castanea mollissima* trees in the USA and China, as well as in *C. dentata* trees in the USA [18]. The presence of ChMV has not always been confirmed in diseased trees, though its frequency tends to be higher in symptomatic trees.

The ash shoestring-associated emaravirus (ASaV) was recently found to be associated with the ash shoestring disease in European ash (*Fraxinus excelsior*) and manna ash (*F. ornus*) trees exhibiting chlorotic ringspots, mottle, and leaf deformation such as curling and shoestring symptoms [13] (Fig. 2 D1, D2). The virus could not be previously identified through purification of virus particles and cloning of random cDNA libraries, but was discovered by applying HTS

Table 1 Viruses involved in the etiology of forest tree diseases based on conventional diagnostic tools or high-throughput sequencing (HTS)

Disease	Virus species	Genus, family	Genome organization	Detection by conventional methods	Detection by HTS
Birch leafroll disease (BLRD) in birches (<i>Betula</i> spp.) Symptoms: leaf vein banding, leaf roll, leaf chlorosis, leaf necrosis, tree decline	Birch leafroll-associated virus (BLRaV)	<i>Badnavirus</i> , <i>Caulimoviridae</i>	Monopartite, circular dsDNA	–	+
	Birch capillovirus (BCV)	<i>Capillovirus</i> , <i>Betaflexiviridae</i>	Monopartite, linear (+)ssRNA	–	+
	Birch carlavirus (BiCV)	<i>Carlavirus</i> , <i>Betaflexiviridae</i>	Monopartite, linear (+)ssRNA	–	+
	Birch idaeovirus (BIV)	<i>Idaeovirus</i> , <i>Mayoviridae</i>	Bipartite, linear (+)ssRNA	–	+
	Cherry leaf roll virus (CLRV)	<i>Nepovirus</i> , <i>Secoviridae</i>	Bipartite, linear (+)ssRNA	+	+
	Arabis mosaic virus (ArMV)	<i>Nepovirus</i> , <i>Secoviridae</i>	Bipartite, linear (+)ssRNA	+	–
	Tomato ringspot virus (ToRSV)	<i>Nepovirus</i> , <i>Secoviridae</i>	Bipartite, linear (+)ssRNA	+	–
	Apple mosaic virus (ApMV)	<i>Ilarvirus</i> , <i>Bromoviridae</i>	Tripartite, linear (+)ssRNA	+	–
	Tobacco necrosis virus (TNV)	<i>Alphanecrovirus</i> , <i>Tombusviridae</i>	Monopartite, linear (+)ssRNA	+	–
Chestnut mosaic disease (ChMD) in <i>Castanea sativa</i> Symptoms: mosaic patterns on leaves and leaf deformation	Chestnut mosaic virus (ChMV)	<i>Badnavirus</i> , <i>Caulimoviridae</i>	Monopartite, circular dsDNA	–	+
	Ash shoestring-associated virus (ASaV)	<i>Emaravirus</i> , <i>Fimoviridae</i>	(–)ssRNA, 5 segments	–	+
Shoestring disease in ash (<i>Fraxinus excelsior</i> , <i>F. ornus</i>) Symptoms: chlorotic ringspots, mottle leaf curling, shoestring, might lead to bare branches	Arabis mosaic virus (ArMV)	<i>Nepovirus</i> , <i>Secoviridae</i>	Bipartite, linear (+)ssRNA	+	–
	Cherry leaf roll virus (CLRV)	<i>Nepovirus</i> , <i>Secoviridae</i>	Bipartite, linear (+)ssRNA	+	–
	Tobacco ringspot virus (TRSV)	<i>Nepovirus</i> , <i>Secoviridae</i>	Bipartite, linear (+)ssRNA	+	–
	Tomato ringspot virus (ToRSV)	<i>Nepovirus</i> , <i>Secoviridae</i>	Bipartite, linear (+)ssRNA	+	–
	Tobacco necrosis virus (TNV)	<i>Alphanecrovirus</i> , <i>Tombusviridae</i>	Monopartite, linear (+)ssRNA	+	–
	Tobacco mosaic virus (TMV)	<i>Tobamovirus</i> , <i>Virgaviridae</i>	Monopartite, linear (+)ssRNA	+	–
	Aspen mosaic-associated virus (AsMaV)	<i>Emaravirus</i> , <i>Fimoviridae</i>	(–)ssRNA, 5 segments	–	+
Mosaic disease in aspen (<i>Populus tremula</i>) Symptoms: mosaic, mottle, yellow blotching, variegation, and chloroses on leaves	Poplar mosaic virus (PMV)	<i>Carlavirus</i> , <i>Betaflexiviridae</i>	Monopartite, linear (+)ssRNA	+	–
	Arabis mosaic virus (ArMV)	<i>Nepovirus</i> , <i>Secoviridae</i>	Bipartite, linear (+)ssRNA	+	–
	Tomato black ring virus (ToBRV)	<i>Nepovirus</i> , <i>Secoviridae</i>	Bipartite, linear (+)ssRNA	+	–
	Tobacco necrosis virus (TNV)	<i>Alphanecrovirus</i> , <i>Tombusviridae</i>	Monopartite, linear (+)ssRNA	+	–

Table 1 (continued)

Disease	Virus species	Genus, family	Genome organization	Detection by conventional methods	Detection by HTS
Chlorotic ringspot disease in oak (<i>Ulmus</i> spp.) Symptoms: chlorotic ringspots, chlorotic spots, and mottle, dieback, tree decline	<i>Emaravirus quercus</i> (common oak ringspot-associated virus; CORaV)	<i>Emaravirus</i> , <i>Fimoviridae</i>	(–)ssRNA, 6 segments	–	+
	Tobacco mosaic virus (TMV)	<i>Tobamovirus</i> , <i>Virgaviridae</i>	Monopartite, linear (+)ssRNA	+	–
	Tobacco necrosis virus (TNV)	<i>Alphanecrovirus</i> , <i>Tombusviridae</i>	Monopartite, linear (+)ssRNA	+	–
European mountain ash ringspot disease in <i>Sorbus aucuparia</i> , <i>S. intermedia</i> , <i>Amelanchier</i> spp., <i>Karpatiosorbus</i> × <i>hybrid</i> Symptoms: chlorotic ringspots, mottle, line pattern on leaves	<i>Emaravirus sorbi</i> (European mountain ash ringspot-associated virus; EMARaV)	<i>Emaravirus</i> , <i>Fimoviridae</i>	(–)ssRNA, 6 segments	+	+
Mottle disease in maple (<i>Acer pseudoplatanus</i>) Symptoms: leaf mottle and chlorotic line patterns	<i>Emaravirus aceris</i> (maple mottle-associated virus; MaMaV)	<i>Emaravirus</i> , <i>Fimoviridae</i>	(–)ssRNA, 6 segments	–	+
Viral diseases in elm (<i>Ulmus laevis</i>)	Elm carlavirus (ECV) Symptoms: leaf chlorosis and mottle	<i>Carlavirus</i> , <i>Betaflexiviridae</i>	Monopartite, linear (+)ssRNA	–	+
	Elm mottle virus (EMoV) Symptoms: leaf ringspots	<i>Illavirus</i> , <i>Bromoviridae</i>	Tripartite, linear (+)ssRNA	+	–
Chlorotic leafspot disease in lime (<i>Tilia cordata</i>) Symptoms: chlorotic leafspots	<i>Cytorhabdovirus tiliae</i>	<i>Cytorhabdovirus</i> , <i>Rhabdoviridae</i>	Monopartite, linear (–)ssRNA		+

in diseased ash trees. Other viruses previously detected in symptomatic samples were found to be unrelated to the disease through HTS (Table 1). Lately, new ASaV infections in *F. excelsior* have been confirmed in Switzerland, various locations in Sweden, and Germany [13]. Genetic variability of ASaV was investigated recently and indicated high nucleotide sequence identity (over 92%) [19]. Infections of the second host species, *F. ornus*, were also found in Germany (Hamburg) and Northern Italy (Bolzano) [13]. First investigations on ASaV impact on urban *F. ornus* trees have revealed that virus infection affects the cambium or growth. The structural alterations in woody tissues were non-destructively observed by X-ray micro-tomography (R. Terzano and C. Büttner, personal communication 2023). Performed micro-focused X-ray fluorescence elemental maps of representative areas of the upper surface of virus-infected *Fraxinus* leaves with symptoms demonstrated the differences of elemental distribution maps of K, Ca, Mn, Fe, Cu, and Zn [20]. Furthermore, the widespread presence of novel cytorhabdoviruses was confirmed in two sampled plantations for seed production [21].

A mosaic disease of Eurasian aspen has been observed since 1991 in Norway and since 2009 in Finland. The recently discovered *Emaravirus populi* (prior AsMaV)

from mosaic-diseased Eurasian aspen (*Populus tremula*) was found to be closely associated with leaf symptoms such as mottle, yellow blotching, variegation, and chloroses along veins [7] (Fig. 2E). The virus was confirmed to be the causal agent through its detection in symptomatic, graft-inoculated seedlings and virus-infected scions used for graft inoculation [7] and has a wide geographic distribution in Norway, Finland, and Sweden [7]. Gallmites are suspected as potential vectors after hints from initial experiments [7].

In the 1970s, the “chlorotic ringspot” disease of oaks previously observed in the USA [22] was reported in Europe. The disease etiology was unraveled using HTS technology (Table 1). The novel *Emaravirus quercus* (prior CoRaV) was identified in diseased oak trees and was genetically characterized. Diagnostic tools for reliable molecular detection are provided [6•, 23•] and serological detection was recently developed. Specific antibodies were generated (LOEWE, Sauerlach) based on recent research to create a kit for routine testing purposes for CoRaV. This serological test system is the first to identify a novel tree virus. The disease was found to affect 11–19% of oak seedlings in propagation stations, posing a threat for forests due to the widespread of the disease through infected oak propagation material.

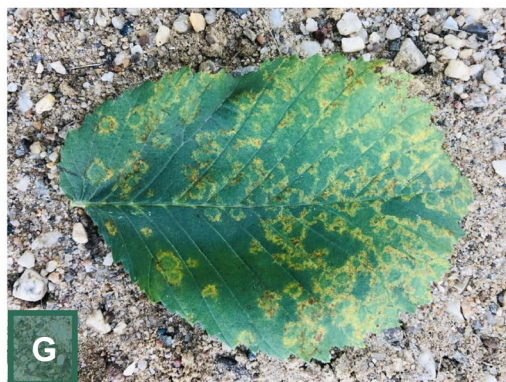
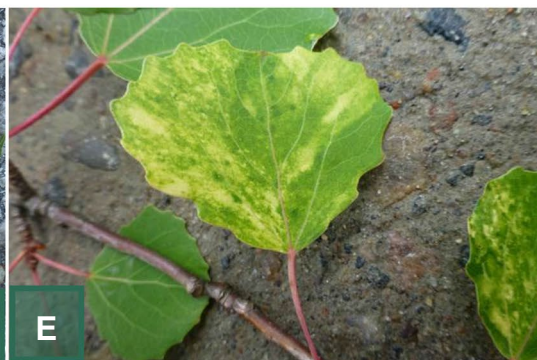
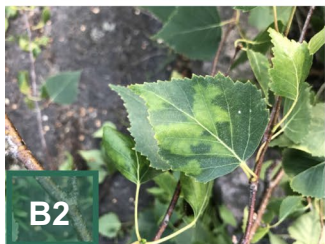
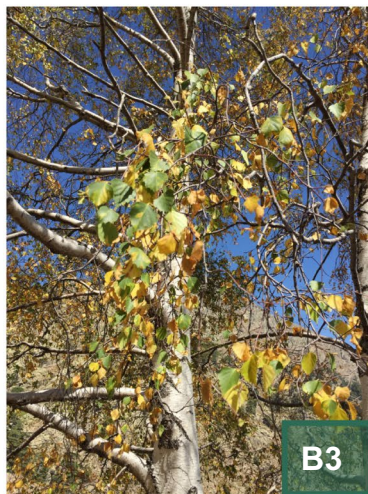


Fig. 2 Symptoms exhibited on virus-infected forest trees. **A1** Oak leaf pattern on virus-infected *Acer pseudoplatanus*. **A2** Mottle, vein, and broad leaf chlorosis on MaMaV-infected *A. pseudoplatanus* leaves. **B1** Complex ringspot pattern on virus-infected *Betula pendula*. **B2** Leaf chlorotic pattern on CLRV-infected *B. pendula*. **B3** Declining *B. pendula* tree due to birch virome. **C** Ringspots and chlorotic spots on CoRaV-infected *Quercus robur*. **D1** Shoestring on ASaV-infected *Fraxinus excelsior*. **D2** Chlorotic ring spots on ASaV-infected *F. excelsior* seedlings in a natural rejuvenation stand. **E** Mottling and mosaic on AsMaV-infected *Populus* sp. **F** Chlorotic line pattern and ringspots on virus-infected *Fagus sylvatica*. **G** Chlorotic and necrotic ringspots on virus-infected *Ulmus laevis*

Other confirmed cases include “European mountain ash ringspot disease,” the main disease affecting *Sorbus aucuparia*, which is caused by *Emaravirus sorbi* (prior EMARaV; Table 1) [24–27] that is widespread in central/northern Europe and England. Recently, EMARaV was detected in new hosts, including serviceberry (*Amelanchier* spp.) in Germany [28], Houston’s whitebeam (*Karpatiosorbus* hybrid) in Finland [29], and Swedish whitebeam (*S. intermedia*) in Sweden [27]. Furthermore, *E. aceris* (prior MaMaV; Table 1) was identified in symptomatic maples in forested areas in Germany [30]. Leaves show mottling and chlorotic line patterns, which turn necrotic under drought conditions (Fig. 2 A1, A2). Vector transmission is assumed by *Aceria macrorhynchus*, but confirming studies are needed. In symptomatic elms, the elm carla virus was found through HTS [31, 32]. This virus and the elm mottle virus [8, 33, 34] are strongly believed to be the two main causes of leaf symptoms (Fig. 2G) and dieback (Table 1). In *Tilia* sp. trees, HTS investigation lately uncovered a new cytorhabdovirus associated with leaf symptoms in Germany [8]. Novel viruses causing leaf symptoms have also been discovered lately in beeches (A. Rumbou, K. Köpke, and C. Büttner; personal communication) (Fig. 2F).

Occasionally, in certain samples analyzed by HTS, short viral sequences, belonging to persistent or latent virus families, have been identified. Examples of such incidences are totiviruses found in BLR-diseased and asymptomatic birch samples [15]. In another symptomatic birch sample, a wide array of double-stranded RNA (dsRNA) viruses resembling toti-, reo-, and partitiviruses as well as (+) ssRNA viruses of the genera *Mitovirus* and *Benyvirus* were detected, while in symptomatic oaks, contigs of partitiviruses were found (C. Büttner and A. Rumbou, personal communication). These findings indicate the presence of a much larger virome than currently known. The role of these viruses in the tree health remains unclear.

Transmission Routes of Plant Viruses in the Forest Ecosystem

Transmission of viruses through vectors (arthropods, nematodes, fungi) is assumed to be the most common mode

of viral transmission in nature [3]. The most important virus vectors are arthropods and Acari seem to be the dominant group of potential arthropod vectors of tree viruses [3]. Gall mites (Eriophyidae) are assumed to transmit *Emaravirus sorbi*, as this virus has been detected in the pear leaf blister mite (*Phytoptus pyri*) [29, 35]. Transmission of viruses through gall mites was studied also in Eurasian aspen [7] and preliminary results for virus transmission through insect vectors in birch are also available [36]. The role of nematodes and fungi as vectors of novel tree viruses needs to be clarified in future investigations [3].

Grafting and mechanical transmission are significant means of horizontal spread of viruses in nurseries, urban green spaces, and seed plantations [3] and have been confirmed as a transmission route for viruses in the families *Betaflexi*, *Bromo-*, *Caulimo-*, *Fimo-*, *Flexi-*, *Poty-*, *Seco-*, *Solemo*, and *Tombusviridae* [3]. Plant viruses can then be introduced from tree nurseries through diseased propagation material such as scions, buds, or chips into the wider environment where they spread and invade neighboring ecosystems through the vectors. The transmissibility of *E. sorbi* by grafting to European mountain ash and of *E. quercus* to oak was reported already in 1995 [37], and recently transmission through grafting has been shown also for *E. populi* in poplar [7], and for the badnavirus BLRaV in birch [16], and for the badnavirus ChMV in chestnut [18]. Natural root grafts between adjacent plants may also occur in forests and orchards. Vertical transmission is not as common in forest tree viruses, but transmission through seed and pollen has been proved for cherry leaf roll virus (CLRV) [38] and tomato black ring virus (ToBRV). Arabis mosaic virus (ArMV) and also the novel *E. quercus* seem to be transmitted only through seed [12].

Additionally, plant viruses from at least seven different genera (including carmo-, cucumo-, diatho-, tobamo-, necro-, potex-, and tombusviruses) have been found in water bodies, such as oceans, streams, rivers, creeks, canals, ditches, ponds, and lakes [3]. It is likely that spread through water plays a significant role in the long-distance dispersal of forest viruses. In addition, virus spread through soil must be equivalently considered. So far there are only very few studies on soil transmission in the context of forest viruses [39], but the risk of viruses spreading in soil and sewage is well documented in agriculture.

Economical Losses

Economic implications due to a forest tree virus disease were first reported in 1939 in the Ohio Valley, USA, when a fatal epidemic occurred among elm trees [40]. The outbreak appears to have been even more serious than that of the Dutch elm disease, as it killed 1000 out of about 1800 elms in one particular locality during 3 years [40]. Losses from

virus diseases are difficult to measure in trees unless they are visibly damaged, or wood quality deficiencies are noticed. Despite these difficulties, estimation of losses is essential to determine the economic threshold for control measures in each virus-host system. While numerical data on the losses from virus diseased forest trees are lacking, current data on symptom appearance, disease dispersal, and epidemic severity provide evidence of this. Moreover, knowledge can be transferred from viral diseases of fruit trees in orchards like pome and stone fruit trees [41–44]. The economic impact of seed transmission should also not be neglected. Barba et al. [41] pointed out that losses are also expected due to poor survival of infected seed and to restricted growth of infected seeds in combination with long-distance spread of seeds.

A well-known example of losses due to forest tree viruses is the cherry leaf roll virus (CLRV) that leads to degeneration in *Betula* sp. and moreover in many species to decline [45]. In birch trees it was also confirmed after many years of monitoring that seedling decline and stepwise tree decline occurs due to virus infection [3••, 16••, 17]. It should also be mentioned that there are suggestions that viruses in birch pollen may affect human health in terms of allergic reactions [46••].

In field conditions, trees cannot be cured of a virus infection and remain infected throughout their lives. Therapy-based methods are available for virus curing for propagative material for fruit trees, but there is no experience on forest trees [47]. This viral inoculum can be further spread through various vertical or horizontal transmission pathways in the ecosystem. Plant viruses in tree nurseries can be particularly damaging, as infected propagative materials serve as a significant source of viral tree diseases that can rapidly spread throughout forest and urban ecosystems. Furthermore, plant viruses can make their hosts susceptible to abiotic and biotic stresses by altering their predisposition [3••].

The novel findings in the field of forest tree virology have raised new questions on the economic and ecological impacts of viral infections. While unraveling the complex virome of birch and the diverse nature of the detected viruses [16••], it remains unclear what is the role of these viruses in disease development, as each virus may interact with or disturb the virome, ultimately causing disease. This is also the case in domesticated tree species such as peach [48] or grapevine [49]. What is the biological significance of such a diverse virome? And how could we establish a correlation between such viral complexes and the appearance of symptoms or how could we differentiate symptomatology in cases of infection by a single virus or by two or more virus species?

Another topical issue is the discovery of the new genus *Emaravirus* involving currently around 24 worldwide emerging plant viruses [12••] and frequently found in forest trees

including oak, maple, European mountain ash, aspen, serviceberry, Houston's whitebeam, and Swedish whitebeam across Europe. Emaraviruses have recorded significant effects on the health of diverse plant species in agricultural, forest, and urban environments [50]. What could be the concrete consequences of the common presence and further spread of this genus on forest health?

Considering the current environmental conditions, the question raises whether the impending climate change may support the spread of forest pathogens and diseases and play a role in the dispersal of forest epidemics. Investigations have shown that higher temperatures, drought stress, and air pollution contribute to increases in viral transmission directly [51] and indirectly via influences on insect vectors [52, 53]. Recent results provide evidence that increased environmental stress may promote viral infections in birch trees and most probably contribute to severe economic losses [3••, 46••]. Furthermore, the pathogen–host–environment interplay may be affected by significant changes in the environment due to deforestation and logging, human encroachment of forests and increased interspecies contacts at the wildlife/agriculture interface. These factors may trigger alterations in the interactions within the holobiont and, consequently, may underlie future outbreaks of diseases [54].

Fungal and Oomycete Viruses as Potential Biocontrol Agents Against Forest Tree Diseases

Forest pathogenic fungi cause devastating losses to the forest industry and natural ecosystems [1, 2]. They are currently controlled by silvicultural practices and biological and chemical control agents [55, 56]. However, the majority of the treatments are prophylactic, and cannot eradicate a pathogen once the infection is already present at the stand. In turn, introducing mycoviruses as antimicrobials that debilitate the growth or dispersal of their host pathogen could be used to mitigate the established disease [57–60].

Cryphonectria hypovirus 1 (CHV-1, representing the species *Alphahypovirus cryphonectriae*) is the textbook example of a mycovirus used as a biocontrol agent [61]. The virus significantly reduces the virulence of its host fungus, *Cryphonectria parasitica*, the causal agent of chestnut blight in field conditions, and the infection seems to be stable, with a predictable outcome. Moreover, CHV-1 is efficiently transmitted to native isolates and continues to spread naturally in Europe. After the discovery of hypovirulence in *C. parasitica*, there has been an expansion in research on viruses that could be used as biocontrol agents against other major fungal forest pathogens, such as *Ophiostoma novo-ulmi* [62], *Gremmeniella abietina* [63], *Fusarium circinatum* [64], *Heterobasidion annosum* and

H. parviporum [65, 66], *Hymenoscyphus fraxineus* [67], *Armillaria* spp. [68], and the fruit tree pathogen *Rosellinia necatrix* [69]. This task requires a substantial amount of virus screening because most mycoviruses known to date are apparently asymptomatic, and some even mediate increased host virulence (hypervirulence) in tree pathogens, as observed in *F. circinatum*, *Cronartium ribicola*, and *Thielaviopsis basicola* [70–73]. However, the discovery of mycoviruses has been revolutionized during the last few years due to HTS. This has not only altered our view of mycovirus diversity and prevalence [74, 75•] but also their host ranges, transmission routes, and host effects. Below, we will discuss critical properties that affect utilizing them for biocontrol of major fungal and oomycete pathogens of forest trees.

Effect of Mycoviruses on Their Fungal Hosts

As a result of extensive virus screening studies, highly diverse virus communities have been revealed in major pathogens affecting forest trees [4••] (Fig. 3; Table S2), including also several cases of debilitation-associated mycoviruses. Different mycoviral isolates belonging to the

same virus species may have contrasting effects on their host fungi, and therefore, the viruses are referred to in this review with abbreviations of their common names as appearing in the cited literature, not by referring to the viral species name as a taxonomic entity. Table 2 lists examples of mycoviruses that alter the phenotype of tree pathogens and their taxonomic classification.

After the discovery of *C. parasitica* hypoviruses, also other hypovirulence-associated viruses have been identified in the chestnut blight fungus, including the mitovirus CpMV-1 and mycoreovirus MyRV1 [76••]. Hypovirulence-associated mitoviruses called d-factors were identified in the Dutch elm disease pathogen (*Ophiostoma novo-ulmi*) already during early decades of mycovirus research [62]. More recent studies have discovered alphapartitviruses that significantly reduce the growth of *Heterobasidion* root rot pathogens: HetPV13-an1 effects were demonstrated both in vitro and in living spruce trees [65] and HetPV15-pal in vitro [77]. The alphapartitvirus RsPV2 has been shown to cause hypovirulence in *Rhizoctonia solani*, the causative agent of damping-off in conifer nursery seedlings and a pathogen of many agricultural crops [78]. Moreover, the fusagravirus CnFGV1 strongly reduces conidiation and in

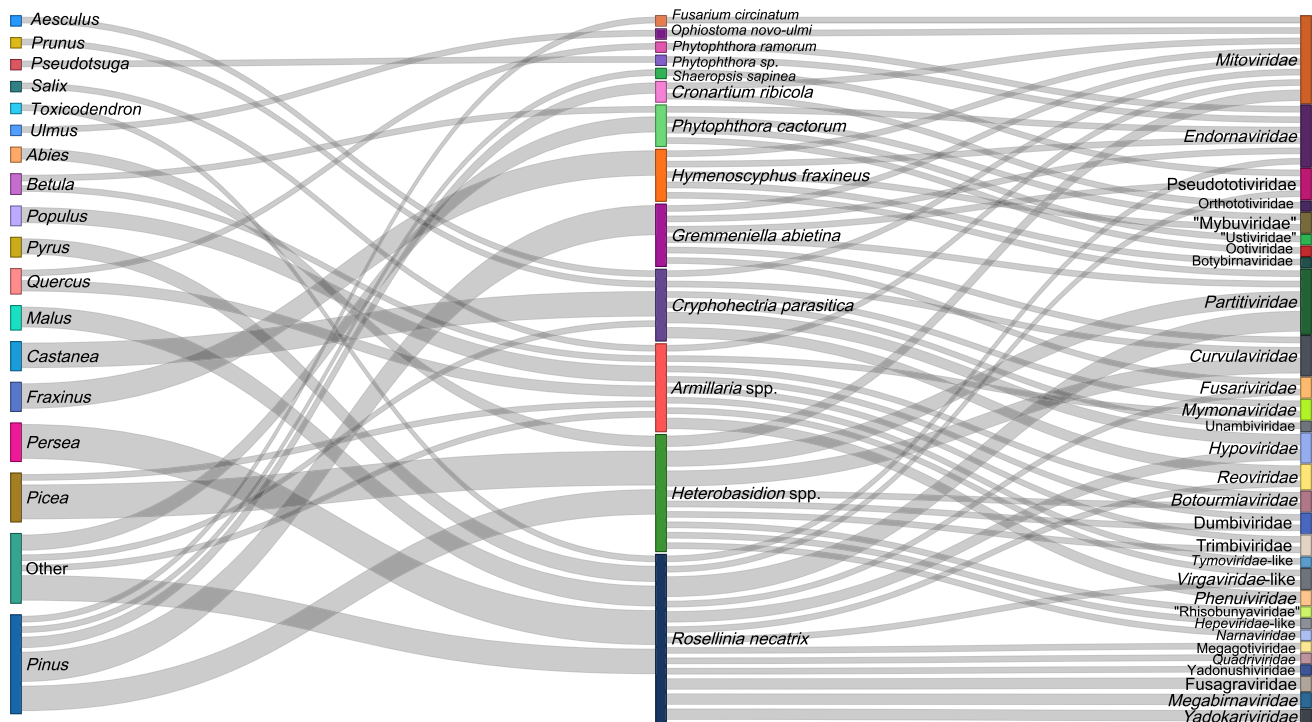


Fig. 3 Sankey diagram summarizing connections among mycovirus families, their fungal and oomycete hosts, and tree species. Host plants designated as “other” include cases from unknown woody or soil sources, re-isolated fungal strains (e.g., isolates of *R. necatrix* originally isolated from *Pyrus* and inoculated on *Malus*), or strains from herbaceous host plants (see Table S2; only the original host tree is considered in the diagram, not the full host range of the path-

ogens). Viral family names are shown in italics for classified families, regular text if suggested in official taxonomical proposals for the International Committee on the Taxonomy of Viruses, and in quotation marks if the name has been proposed in scientific literature but not officially recognized. Viruses affiliated with classified families include both classified species and unclassified viruses with clear taxonomic status based on sequence similarity

Table 2 Examples of mycoviruses that alter the phenotype of tree pathogens

Virus	Order, family, and species	Host	Tree (plant) disease	Virus effect on host	Virus genome	Viral particles
CHV-1 (Cryphonectria hypovirus 1)	Durnavirales, Hypoviridae, Alphahypovirus cryphonectriae	<i>Cryphonectria parasitica</i>	Chestnut blight	Strong hypovirulence (reduced pathogenicity and sporulation, altered gene expression and phenotype)	(+) ssRNA, non-segmented	RNA genome within pleomorphic vesicle, no true particles
Ophiostoma mitoviruses, “d-factors” (Ophiostoma mitoviruses 1a, 1b, 3a, 3b, 4, 5, 6)	Cryppavirales, Mitoviridae, Duamitovirus opno1a, D. opno1b, D. opno3a, D. opno3b, Unuamitovirus opno4, U. opno5, U. opno6	<i>Ophiostoma novo-ulmi</i>	Dutch Elm Disease	Hypovirulence (reduced infectivity in elm trees), debilitated mycelial growth	(+) ssRNA, non-segmented	None (RNA genome in host-derived lipid vesicles)
FcMV1 (<i>Fusarium circinatum</i> mitovirus 1)	Cryppavirales, Mitoviridae, Unuamitovirus fuci1	<i>Fusarium circinatum</i>	Pine pitch cancer	Mildly reduced survival of pine seedlings (possible hypovirulence). Residual effect in <i>Fusarium circinatum</i> transcriptome after virus recovery	(+) ssRNA, non-segmented	None (RNA genome in host-derived lipid vesicles)
HfMV1, HfMV2 (<i>Hymenoscyphus fraxineus</i> mitovirus 1 and 2)	Cryppavirales, Mitoviridae, Unuamitovirus hfr1, and unclassified	<i>Hymenoscyphus fraxineus</i>	Ash dieback	Contrasting effects on fungal growth in vitro, ranging from cryptic to detrimental or beneficial	(+) ssRNA, non-segmented	None (RNA genome in host-derived lipid vesicles)
CpMV1 (<i>Cryphonectria parasitica</i> mitovirus 1)	Cryppavirales, Mitoviridae, Alphahypovirus cryphonectriae	<i>Cryphonectria parasitica</i>	Chestnut blight	Hypovirulence (inhibits canker expansion), small effect on culture morphology	(+) ssRNA, non-segmented	None (RNA genome in host-derived lipid vesicles)
FgV1-DK21 (<i>Fusarium graminearum</i> virus 1 strain DK21)	Durnavirales, Fusariviridae, Alphafusarivirus fusarii	<i>Fusarium boothii</i>	Head blight of wheat	Hypovirulence on <i>Fusarium boothii</i> and in <i>C. parasitica</i> . Decreased growth, increased pigmentation	(+) ssRNA, non-segmented	None
CnFGV1 (<i>Cryphonectria naterciae</i> fusagravirus 1)	Ghabvirales, proposed “Fusagraviridae”	<i>Cryphonectria naterciae</i>	European hornbeam disease	Reduces conidiation and in some cases vegetative growth <i>Cryphonectria carpnicola</i>	dsRNA, non-segmented	Spherical particles
HetPV13-an1 (Heterobasidion partitivirus 13 strain an1)	Durnavirales, Partitiviridae, Alphapartitivirus tredecimheterobasidion	<i>Heterobasidion annosum</i>	Root rot of conifers (<i>Pinus</i> sp. mainly)	Growth debilitation in vitro and in <i>Picea abies</i> trees when transmitted to <i>H. parviporum</i> , altered gene expression	dsRNA, bisegmented	Isometric particle, icosahedral protein capsid

Table 2 (continued)

Virus	Order, family, and species	Host	Tree (plant) disease	Virus effect on host	Virus genome	Viral particles
HetPV15-pa1 (Heterobasidion partitivirus 15 strain pa1)	Dumavirales, Partitiviridae, Alphapartitivirus quindecimheterobasidion	Heterobasidion parviporum	Root rot of <i>Picea</i>	Mycelial growth debilitation in vitro, enhances HetPV13-an1 transmission	dsRNA, bisegmented	Isometric particle, icosahedral protein capsid
RNPV1 (<i>Rosellinia necatrix</i> partitivirus 1-W8)	Dumavirales, Partitiviridae, Betapartitivirus rosellinia	<i>Rosellinia necatrix</i>	White root rot of fruit trees, nut tree crops, broadleaved forest trees	Hypovirulence mediated by co-infection with megabirnavirus RnMBV2 in <i>R. necatrix</i>	dsRNA, bisegmented	Isometric particle, icosahedral protein capsid
RNPV6 (<i>Rosellinia necatrix</i> partitivirus 6 strain W113)	Dumavirales, Partitiviridae, Betapartitivirus, unclassified	<i>Rosellinia necatrix</i>	White root rot of fruit trees, nut tree crops, broadleaved forest trees	Hypovirulence in <i>C. parasitica</i> , asymptomatic infection in the natural host	dsRNA, bisegmented	Isometric particle, icosahedral protein capsid
RsPV2 (<i>Rhizoctonia solani</i> partitivirus 2 strain GD11)	Dumavirales, Partitiviridae, Alphapartitivirus, unclassified	<i>Rhizoctonia solani</i>	Pathogen of crop plants (potato) and conifer seedlings	Reduced mycelial growth and hypovirulence on rice leaves by <i>Rhizoctonia solani</i>	dsRNA, bisegmented	Isometric particle, icosahedral protein capsid
GaRV6 (<i>Gremmeniella abietina</i> RNA virus 6)	Dumavirales, Curvulaviridae, Orthocurvulavirus, unclassified	<i>Gremmeniella abietina</i>	Scleroterris canker on conifers	Reduced growth rates in <i>G. abietina</i> isolates harboring GaRV6 with co-infecting mito- and partitiviruses	dsRNA, bisegmented	Unknown
RnMBV1 (<i>Rosellinia necatrix</i> megabirnavirus 1 strain W779)	Ghabrivirales, Megabirnaviridae, Megabirnavirus ichti	<i>Rosellinia necatrix</i>	White root rot of fruit trees, nut tree crops, broadleaved forest trees	Hypovirulence in <i>R. necatrix</i> and <i>C. parasitica</i> , reduced mycelial growth	dsRNA, bisegmented	Isometric particle, icosahedral protein capsid
RnMBV2 (<i>Rosellinia necatrix</i> megabirnavirus 2-W8)	Ghabrivirales, Megabirnaviridae, Megabirnavirus shi	<i>Rosellinia necatrix</i>	White root rot of fruit trees, nut tree crops, broadleaved forest trees	Hypovirulence mediated by co-infection with partitivirus RnPV1 in <i>R. necatrix</i>	dsRNA, bisegmented	Isometric particles
MyRV1 (<i>Cryphonectria parasitica</i> mycoreovirus 1-9B21)	Reovirales, Spinareoviridae, Mycoreovirus 1	<i>Cryphonectria parasitica</i>	Chestnut blight	Hypovirulence on <i>C. parasitica</i>	dsRNA, 11 genome segments	Double-shelled particles
RnMyRV3 (<i>Rosellinia necatrix</i> mycoreovirus 3-W713)	Reovirales, Spinareoviridae, Mycoreovirus 3	<i>Rosellinia necatrix</i>	White root rot of fruit trees, nut tree crops, broadleaved forest trees	Alters colony morphology and reduces virulence of <i>R. necatrix</i> , reduces virulence of <i>Diaporthe</i> sp., <i>C. parasitica</i> , and <i>V. ceratosperma</i> strains	dsRNA, 12 genome segments	Double-shelled particles

Table 2 (continued)

Virus	Order, family, and species	Host	Tree (plant) disease	Virus effect on host	Virus genome	Viral particles
PcBV1, PcBV2 (<i>Phytophthora cactorum</i> bunyavirus 1 and 2, strain PhF66)	Bunyavirales, unclassified	<i>Phytophthora cactorum</i>	Pathogen of, e.g., strawberry and birch	Viral co-infection reduces hyphal growth and sporangia production and size of <i>P. cactorum</i> . Increased production of elicitors	(-) ssRNA, one known segment	Unknown

Virus classification follows current taxonomy, classified species names are in italics, and names described in pending proposals submitted to the International Committee on the Taxonomy of Viruses are shown in regular text (members of order *Ghabrivirales*, members of family *Partitiviridae*)

some cases vegetative growth of *Cryphonectria carpinicola* involved in the European hornbeam disease [79•]. The original host of CnFGV1 is *Cryphonectria naterciae*, which is hypothesized to be involved in cork oak decline. In turn, in vitro tests suggest that the bunya-like viruses PcBV1 and PcBV2 reduce hyphal growth and sporangia production of the oomycete *Phytophthora cactorum* that infects both agricultural crops and woody ornamentals and birch trees [80]. The above-mentioned cases represent diverse RNA virus families with members residing in host cytosol (most mycoviruses) or mitochondria (*Mitoviridae* members).

The influence of mycoviruses on the host gene expression can be substantial. In *C. parasitica*, CHV-1 can affect the expression of over a thousand fungal genes [81•]. Many of these genes are regulated by the papain-like protease, p29 gene of the virus known to suppress orange pigmentation, sporulation, and laccase accumulation in the host fungus [82]. In the pine pathogen *Heterobasidion annosum*, the alphapartitivirus HetPV13-an1 alters the expression of nearly 700 genes [65]. A virus infection can leave its trace, and the asymptomatic mitovirus FcMV1 causes a residual effect in *Fusarium circinatum*, affecting 14 host genes even in a host spontaneously cured of the virus [83].

The Defenses and Tolerance of Fungal Pathogens Against Viral Infection

Similar to plants and insects, fungi have an intracellular defense system against dsRNA, a hallmark of virus infection, called RNA silencing (RNAi). RNAi is a defense mechanism against both viruses and “genomic parasites” such as transposable elements (TEs), and it can be triggered by endogenous or exogenous dsRNA that will be cleaved to small RNAs of approximately 12–25 nt (reviewed in [60]). In *C. parasitica*, the enzymes participating in RNA silencing have been characterized in detail and their functionality has been examined by generating gene disruptions in the host and testing their effects on mycovirus infection [84]. More recently, production of viral small RNA (vsRNA) was reported in *Heterobasidion* spp. [85], and Muñoz-Adalia et al. [86] demonstrated that *F. circinatum* processes viral RNAs into vsRNA in a similar manner as other plant pathogenic fungi. Certain mycoviruses, most notably CHV-1 and the mycoreovirus MyRV3 of *R. necatrix*, are capable of circumventing the RNAi machinery by producing RNA silencing suppressor proteins [84, 87]. A new research line is aiming to combat plant pathogens or pest insects by utilizing the RNAi system for silencing essential host genes. In forestry, only a few studies have addressed the possibilities of RNAi on pests [88] or diseases [60].

The outcome of a mycovirus-host interaction is not always predictable even among strains of a single host species. For example, HetPV13-an1 induces a strong growth

reduction in some *H. annosum* strains while being practically asymptomatic on others [65, 89]. A recent report also describes that infection by the mitovirus HfMV2 had contrasting effects on the growth of *H. fraxineus* in vitro, ranging from cryptic to detrimental or beneficial outcomes [90]. This type of host tolerance can make the assessment of virocontrol applicability very difficult. Fortunately, in the case of *C. parasitica*, host tolerance seems not to play a major role [91].

It should also be noted that the overall outcome of a virus infection and its suitability for biocontrol purposes can only be reliably assessed in field conditions. For example, the biocontrol success of CHV-1 is evident in practical forestry even though in laboratory conditions certain CHV-1 strains do not mediate growth reduction, and their effects are dependent on temperature and host strain [92]. In a recent study, application of HetPV13-an1 to tree stumps with *Heterobasidion* rot seemed to enhance the control effect obtained by treatments with the commercial biocontrol fungus, *Phlebiopsis gigantea* [93].

Virus Stability and Distribution Within the Host Mycelium

The success of a virocontrol method relies on virus infection stability. Mycovirus infections are typically highly persistent, and we have detected viruses in laboratory stock cultures maintained for decades in the refrigerator (some dating from the 1950s; E. Vainio, personal communication). However, cultures can be cured of viruses in unfavorable conditions such as elevated temperature and prolonged storage if the culture dries out, or during deep freezing [e.g. 68, 83]. Moreover, evidence from virus community analyses in field conditions show that some virus loss occurs in nature during mycelial spread. Tree root pathogens that spread clonally by root contacts or within soil may form very large and long-living clonal individuals, where different sections of the mycelium may be exposed to different conditions and co-existing fungal communities. This may lead to fragmented virus distribution, as observed in the fruit tree pathogens *Helicobasidium mompa* [94] and *R. necatrix* [95], and the conifer root rot fungi *Heterobasidion parviporum* and *H. annosum* [96, 97].

Interestingly, the dispersal of mycoviruses within a fungal mycelium depends on the virus species. Suzuki et al. [76••] found that the hypoviruses CHV-1, CHV-2, and CHV-3 transmitted much more efficiently to established *C. parasitica* cankers than the mycoreovirus MyRV1 or the partitivirus RnPV6 originating from a heterologous host, *R. necatrix*. Earlier research showed that viruses differ in their transmission rates in mycelial colonies of *R. necatrix*, leading to unequal virus distribution [98]. The concentration of

CHV-1 may also vary in different parts of the *C. parasitica* mycelium and on different culturing media [99].

Notably, virus concentration may affect symptom severity, and low-titer infections may be asymptomatic. This has been observed with several viruses infecting ascomycetous pathogens of crop plants, for example, black *Aspergillus* spp. (reviewed in [59]). Also in *H. annosum*, reduced concentration of HetPV13-an1 leads to phenotypic recovery of the host [100].

Mycoviral Co-infections (Mixed Infections)

One factor affecting virus infection stability and outcome is the presence of co-infecting viruses. In the case of mixed virus infections, it may be difficult to identify the causative agent(s) of host debilitation, and virus effects are not necessarily cumulative [101]. For example, HetPV13-an1 reduces the growth rate and alters the gene expression of *H. annosum* when infecting its host alone or with co-infecting mitoviruses HetMV1–3 [65], whereas co-infecting partitiviruses and orthocurvilaviruses may suppress the virus effects, leading to recovery of host growth and morphology [102]. Vice versa, there are cases where host hypovirulence is exclusively mediated by a mixed virus infection and not by the same viruses in single infection, as observed with the megabirnavirus RnMBV2 and partitivirus RnPV1 in *R. necatrix* [103]. In the conifer pathogen *Gremmeniella abietina*, isolates harboring the orthocurvilavirus GaRV6 with co-infecting mito- and partitiviruses were shown to have reduced growth rates [104].

Co-infecting mycoviruses may have an antagonistic, neutral, or synergistic interaction with each other [105•]. Very closely related mycoviruses typically exclude each other, and the ability to co-exist stably in co-infection has been considered a species delimitation criterion, for example, in families *Totiviridae* and *Narnaviridae* [106]. This phenomenon could cause some hindrance for the spread of a virocontrol agent in case highly similar virus variants with less biocontrol potential would be common in the treated host population. In species of *Heterobasidion*, conspecific members of virus families *Curvulaviridae* and *Partitiviridae* have been observed to have an antagonistic relationship [77, 96]. However, even congeneric mycoviruses can stably infect the same fungal strain as found with mitoviruses infecting *O. novo-ulmi* [107] and *F. circinatum* [64, 108], and betapartitiviruses of *R. necatrix* [109]. Interestingly, the victorivirus RnVV1 originally isolated from *R. necatrix* can replicate in *C. parasitica* when co-infected with CHV-1, but not in the virus-free *C. parasitica* strain [110]. Even strictly mutualistic virus-virus associations exist in fungi: the yadokariviruses lack their own capsid proteins but are instead encapsidated by co-infecting yado-nushi viruses [reviewed in 75].

Transmissibility of Mycoviruses Among Fungal Strains (Horizontal Transmission) and Through Spores (Vegetative Transmission)

Mycoviruses are transmitted among fungal strains horizontally through cell-to-cell contacts that are regulated by host processes governing cellular fusions between species (intersterility), between strains (vegetative incompatibility), and between strains with different mating types. Therefore, these processes also greatly affect the success of a virocontrol approach [58]. For example, CHV-1 has spread efficiently in the European *C. parasitica* population due to a low number of introduced host genotypes [61], whereas its spread in North America is limited due to a high number of somatically incompatible host strains originating from multiple introductions of the pathogen from Asia. Zamora et al. [111] used dual fungal cultures to demonstrate that the conversion rates of virulent *C. parasitica* isolates into hypovirulent ones were significantly affected by the vegetative compatibility (vc) type of both the donor and the recipient isolate, and by the interaction between them. However, in some fungal genera, virus transmission between incompatible strains seems to be relatively relaxed. In the ash dieback fungus *H. fraxineus*, the mitovirus HfMV2 can be successfully introduced via co-culturing into conspecific isolates [90]. In *Heterobasidion* sp., many partitiviruses transmit readily between incompatible isolates, and even between congeneric species, although less efficiently [102]. Interestingly, co-culturing of fungal strains has led to cross-species transmission of CHV-1 to *C. japonica* [112], and the fusagravirus CnFGV1 from *Cryphonectria naterciae* to *C. carpnicola* and *C. radicalis*, but not to *C. parasitica* [79•].

The transmissibility of mycoviruses may also be affected by pre-existing viral infections in the mycelium [see 58, 105]. In *R. necatrix*, presence of the mycoreovirus RnMyRV3 restricts the transmission of the partitivirus RnPV1 to the same host strain [98], whereas partitiviruses of *Heterobasidion* sp. may promote each other's transmission [77]. The mechanism behind viruses enhancing each other's transmission may be related to the ability of some viruses to suppress host defense reactions (see above) or host somatic incompatibility reactions, as demonstrated in the soilborne plant pathogen *Sclerotinia sclerotiorum* [113].

Long-range dispersal capability is one of the essential properties of a biocontrol agent — both concerning dispersal success but also risk assessment — and is enabled by vertical virus transmission into sexual asco- or basidiospores. Basidiospores of *H. mompa* have been shown to be virus-free [114], whereas orthocurvulaviruses of *Heterobasidion* spp. [96] and endornaviruses of *R. solani* occur in basidiospores [115]. In ascomycetes, including *C. parasitica*, viruses are not typically transmitted to sexual spores [116].

However, the ascospores of *H. fraxineus* serve as an efficient means of dispersal for mitoviruses [117, 118].

Some short-range virus dispersal may occur via vegetative spores. For example, CHV-1 has very high prevalence in host conidia [91], while CHV-2 is poorly transmitted to conidiospores [119]. In turn, CnFGV1 is readily transmitted to vegetative spores of *C. naterciae* and *C. parasitica* [79•], and FcMV1 and FcMV2-2 in microconidia of *F. circinatum* [120]. Unspecified dsRNA viruses also have been detected in conidia of *Heterobasidion* spp. [121]. To assess putative virocontrol strategies, there is an urgent need to update knowledge about the diversity of viruses present in fungal spores with the aid of HTS.

Virus Transmission Between Fungal Species: Opportunities and Risk Assessment

The vegetative incompatibility barrier is effective in preventing permanent cell-to-cell fusions and virus transmission between different fungal species, but this has been successfully overcome in laboratory conditions by using protoplasts as virus recipients (reviewed in [58]). The fusarivirus FgV1-DK21 associated with hypovirulence on *Fusarium boothii* was transmitted into other *Fusarium* species and into *C. parasitica* and caused hypovirulence in the new hosts [122]. In the same way, purified particles from the fusagravirus CcFGV1 of *C. carpnicola* were used to transfect protoplasts of JS13VF (a Japanese virus-free isolate) as well as *C. parasitica* [123]. This and other artificial conversion strategies such as using viral infectious clones (reviewed in [124•]) could be of great interest for future integrated management of forest pathogens [57].

It has been observed that a heterologous virus may alter the phenotype of its new host species even more than an indigenous virus, probably due to lacking adaptation. Thus, the megabirnavirus RnMBV1 and betapartitivirus RnPV6 originating from *R. necatrix* have been found to cause hypovirulence in *C. parasitica* [125, 126], and even in *Heterobasidion* sp. with common interspecies virus transmission; partitiviruses transmitted from another *Heterobasidion* species cause host growth debilitation more often than indigenous partitiviruses [127]. Therefore, before any virus can be utilized on a large scale as a biocontrol agent, its capability of infecting other fungal species in the environment needs to be assessed. Fortunately, natural transmission of mycoviruses between distantly related fungi is considered to be very uncommon, although a few examples have been described recently in tree root associated fungal communities. Partitiviruses of *H. parviporum* were occasionally detected in the fruiting body tissues of other fungal species inhabiting the same forest stand [128], and Arjona-Lopez and colleagues [129] found that viruses of *R. necatrix* were similar to viruses of distantly related but sympatric fungal

species, such as *Fusarium* spp., and suggested horizontal virus transfer between the soil-inhabitant fungi.

Mycoviruses in Agroforestry Systems and Generalist Plant Pathogens

Above we have concentrated on mycoviruses affecting major forest pathogens, including both indigenous fungi affecting native forest trees (e.g., most *Heterobasidion* spp.) or invasive fungal and oomycete species affecting native forest trees (e.g., *C. parasitica*, *O. novo-ulmi*, *H. fraxinaeus*). Some of these forest pathogens also affect tree species that are utilized in agroforestry; for example, species of *Armillaria* and *Phytophthora* commonly infect poplars, alders, and birches used in silvopasture and riparian forest buffers.

Rosellinia necatrix provides an example of a tree pathogen with a global distribution and a very wide host range including both forest trees and fruit trees such as avocado, apple, and pear. Notably, domesticated crop trees harboring many generalist pathogens may provide new pathways for interspecies virus transmission. For example, in avocado orchards in Spain, a new alphahypovirus tentatively named Entoleuca hypovirus 1 (EnHV1) was detected both in *R. necatrix* and *Entoleuca* sp. A population analysis of EnHV1 strains showed two main clades with members from both fungal species, suggesting intraspecific and interspecific virus transmission in the field [130]. Similarly, in an apple orchard in China, an alphahypovirus named AaHV1 (isolate of *Alphahypovirus alternariae*) from *A. alternata* f. sp. *mali* could replicate and confer hypovirulence in *Botryosphaeria dothidea*, a fungal pathogen causing apple white rot disease [131].

Beyond *R. necatrix*, we will not present a detailed review of mycovirus diversity in pathogens of domesticated crop trees and plantation-grown trees (e.g., rosaceous fruit trees, acacia, citrus, cocoa, eucalyptus, olive, rubber tree, or nut-producing crop trees). Briefly, some of the major pathogens of these tree species include various fungal species of genera *Botryosphaeria*/*Diplodia*, *Colletotrichum*, *Fusarium*, *Monilinia*, *Thielaviopsis*, and *Verticillium*, and oomycetes of genus *Phytophthora* (see [2] and the EPPO global database at <https://gd.eppo.int>). Mycoviral diversity in most of these fungal genera have been compiled thoroughly elsewhere [132••], and HTS-based investigations of mycoviral diversity have recently been conducted, for example, for pathogens of cacao and stone and pome fruits [133–135]. Mycoviruses possibly associated with debilitated host phenotypes have been recently identified in the pome fruit pathogen *Diplodia seriata* [136] and *Colletotrichum gloeosporioides* causing leaf anthracnose in mango trees [137]. Notably, human-mediated transport of plants together with their microbes and viruses may lead to long-range dispersal

of viruses and globally uniform virus communities as seen in *Phytophthora cactorum* [138•].

It should also be noted that nursery seedlings of forest trees are susceptible to many generalist soilborne fungal and oomycete pathogens causing damping-off or root dieback, such as *Alternaria*, *Fusarium*, *Neonectria*, *Rhizoctonia*, *Pythium*, and *Phytophthora* (see Chapter 17 in [1] and [74]), as well as gray mold caused by *Botrytis cinerea*. Recent HTS-based investigations have detected diverse viruses in *B. cinerea* [139•] and also many tree-associated *Phytophthora* microbes such as *P. castaneae* [140] and *P. condilina* [141] (see above for viruses of *P. cactorum* and *P. ramorum*). Considering generalist pathogens with tens or even hundreds of different host species, the possible outcomes of a mycovirus infection in field conditions may be manifold and difficult to predict.

Possibilities for Horizontal Virus Transmission Between Kingdoms

Recent studies have demonstrated cross-kingdom virus transmission events between fungi or oomycetes and plants. This has so far been investigated mostly in agroecosystems, where Cao et al. [142] found that filamentous fungal strains (*Alternaria*, *Lecanicillium*, and *Sarocladium*) isolated from leaf samples of vegetable crops commonly harbored plant viruses. The same authors had earlier identified the plant virus cucumber mosaic virus in *Rhizoctonia solani* isolated from a potato plant [115]. In the case of oomycetes, Mascia et al. [143] showed that diverse plant viruses can replicate and persist in the potato pathogen *Phytophthora infestans* at least temporarily. Vice versa, it has been experimentally demonstrated that fungal partiti- and victoriviruses are able to replicate in plant cells [144]. Moreover, Bian et al. [145•] showed by laboratory infection assays that horizontal transfer of CHV-1 from fungus to plant was enabled by the presence of a pre-existing tobacco mosaic virus infection. In all the above-mentioned studies, the cross-kingdom virus transmission events have been unstable, and the risk of introducing stable virus infections from fungi to host plants seems negligible considering practical biocontrol applications.

At an evolutionary time scale, there is ample evidence of cross-kingdom virus transfer [146••]. Thus, several virus families (for example, *Partitiviridae*, *Endornaviridae*, and *Chrysoviridae*) accommodate both plant and fungal and/or oomycete viruses, and in many cases viruses of plants and plant pathogenic fungi are found intermingled in the same phylogenetic clade [124•, 146••, 147••]. Viral infections may also lead to horizontal gene transfer to the host genome, i.e., endogenization of viral elements [148, 149]. The origin of several endogenized viral elements in plants appears to be

in mycoviruses of pathogenic or endophytic fungi that have at some point colonized the plants [150].

Although insects are the main vectors transmitting plant viruses, studies on viruses associated with forest insects are very scarce. In a few cases, arthropods have been shown to vector or carry mycoviruses, as is the case of SsHADV-1, a ssDNA virus of the *Genomoviridae* family [151] and possibly the unclassified phlegivirus TtV1 of the mycorrhizal fungus *Thelephora terrestris* [152].

There is a long tradition for utilizing insect DNA viruses as biocontrol agents, and many have been already commercialized [see 153]. Concerning forest insects, large dsDNA viruses of family *Baculoviridae* have been successfully used to control populations of the European pine sawfly (*Neodiprion sertifer*), and baculoviruses are also found in lepidopteran species including, e.g., *Lymantria monacha*, *Lymantria dispar*, and *Leucoma salicis* [154]. These DNA viruses are specific to insects, and no spillover to plants or fungi has been reported. However, analysis of RNA viruses by HTS may reveal a very different view, as demonstrated in a groundbreaking study by Shi et al. [155] that described numerous novel RNA viruses in arthropods, many of which were related to plant and mycoviruses. Indeed, the first transcriptomics analyses have revealed some new RNA viruses in the lepidopteran forest pests *Epirrita autumnata* and *Thaumetopoea pityocampa* [156, 157], and also in *I. typographus* [158], in which only dsDNA poxviruses were known earlier. More novel RNA viruses have been recently detected in the European spruce bark beetle, most resembling members of virus families containing also fungal and/or plant viruses, such as *Partitiviridae*, *Narnaviridae*, *Virgaviridae*, *Tombusviridae*, and proposed *Spiciviridae* (E. Vainio, personal communication). Similarly, working with three types of Mediterranean forests several insect-borne viruses from *Picornavirales* order were found in fungi [159]. In the latter work a total of 158 viruses were found in fungi, insects, and trees, some of them not matching known viruses in available databases and others from families *Botourmiaviridae*, *Chrysoviridae*, *Totiviridae*, and *Partitiviridae* with high cross-kingdom transmission potential.

Although our current knowledge is limited, we can conclude that virus flows between species and kingdoms influence the balance of forests and, very often, that of the global ecosystem, including humans. Moreover, forest tree viruses have a high risk of being introduced and established in forests and crops, which results mainly from the wide distribution of susceptible plant germplasm, the often extended host range and the rapid emergence of new genotypes (virus strains or variants). With these precedents, and assuming that RNA virus host jumping can occur at any level of the forest ecosystem, the study of

forests' virome is essential not only for understanding virus evolution and diversity but also for improving our ability to manage and control viral diseases that affect economically important plants and beneficial insects, and to prevent possible outbreaks of plant, animal, and/or human diseases in the future.

Future Prospects and Recommendations

Recent studies have significantly increased our understanding on plant virus diversity and disease etiology in forest trees and identified several new mycoviruses that restrict the growth or virulence of forest pathogenic fungi. These findings offer us hope for better control of forest diseases in the future. However, despite advancements in methodology and virus discovery over the past decade, we are far from being able to fully describe the essential forest virome. Future investigations should focus on studying the proportion of pathogenic, persistent, and mutualistic viruses that infect trees. Knowing the types of viruses that infect forests and urban trees, as well as how they are transmitted and spread, allows for the development of control measures and recommendations. As we look ahead to the future of forest tree production, viruses have to be considered as potential disease agents affecting trees. Symptomatology, knowledge on epidemiology, and reliable diagnostic systems will be essential in meeting phytosanitary requirements and ensuring the growth of healthy trees.

In order to gain a deeper understanding, we recommend the following:

1. Continue determining the essential forest virome in different tree species, forest types and geographical regions.
2. Shifting the research focus from mere diversity analyses to functional studies on effects and disease etiology of the newly discovered viruses.
3. Conducting studies in laboratory and nursery conditions to evaluate the mechanisms of virus-virus interaction.
4. Performing studies in field conditions to assess the true potential of mycovirus biocontrol applications, due to the manifold factors regulating the host-virus interaction.
5. Intensify investigations on the plant virus epidemiology with focus on vector and seed transmission followed by modeling their economic and ecological impact.
6. Investigating potential cross-species and cross-kingdom virus transmission events in forest ecosystems to evaluate the impacts of possible future virus invasions/outbreaks and to ensure safeness of biocontrol strategies.
7. Investigating how climate change is affecting emerging plant virus diseases and the mycovirus-host balance.

The realization of these activities could help the sustainable management of a greater number of forest diseases in the near future.

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